

THE EXPRESSION OF LEAF RUST RESISTANCE IN WHEAT LINES
CONTAINING *Lr12* AND *Lr13*

By

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List of abbreviations

A. D.	<i>Anno Domini</i>
AP	appressorium
ASSV	aborted substomatal vesicle
CIMMYT	International Maize and Wheat Improvement Center
cm	centimetre(s)
d	day(s)
DNA	deoxyribonucleic acid
d.p.i.	days post-inoculation
e.g.	<i>exempli gratia</i> (for example)
<i>et al.</i>	<i>et alii</i> (and others)
Fig.	figure
f. sp.	<i>forma specialis</i>
g	gram(s)
h	hour(s)
ha	hectare(s)
HCN	host cell necrosis
HI	hypersensitivity index
HMC	haustorium mother cell
h.p.i.	hours post-inoculation
i.e.	<i>id est</i> (that is)
IT	infection type
l	litre(s)
<i>Lr</i>	leaf rust resistance gene
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mol	molar
MR	moderately resistant
MS	moderately susceptible

NPA	nonpenetrating appressorium
R	resistant
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
S	susceptible
t	ton
Tc	Thatcher
v	volume
viz	<i>videlicet</i> (namely)
w	weight
%	percentage
°C	degree Celsius
μ	micro

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We are what we repeatedly do.

Excellence, then, is not an act, but a habit.

Aristotle

INTRODUCTION

Wheat leaf rust, caused by *Puccinia recondita* Roberge ex Desmaz f. sp. *tritici*, is generally regarded as the most common and widely distributed of the wheat rusts (Hiratsuka and Sato, 1982; Knott, 1989a). Although rust has occurred on wheat throughout its evolutionary development, it is presently more damaging because of large areas sown to genetically homogeneous, or closely related cultivars (Samborski, 1985). Leaf rust destroys wheat leaf tissue progressively through the season, often resulting in significant losses in yield and quality.

In South Africa leaf rust occurs annually, but its distribution and severity are determined by the amount of overwintering inoculum, climatic conditions and the susceptibility of the commercially grown cultivars (Pretorius *et al.*, 1987; Pretorius and Le Roux, 1988). Leaf rust occurs in all wheat growing areas in South Africa, but is usually most severe on spring wheat grown in the winter rainfall areas of the Western Cape (Pretorius *et al.*, 1987).

Leaf rust development is favoured by temperatures between 10°C and 20°C and, for germination, an urediospore requires an initial dew period of 100% relative humidity in the dark (Eversmeyer *et al.*, 1988). The germ tube grows towards the stoma where an appressorium and infection peg are formed. Inside the leaf a substomatal vesicle develops from which an infection hypha grows towards a host cell. The hyphal tip is delimited into a haustorium mother cell and the plant cell wall is penetrated to form an intracellular haustorium (Knott, 1989a; Roelfs *et al.*, 1992; Kloppers, 1994). The haustorium extracts nutrients from the host cell to sustain the colony (Harder, 1989), which grows intercellularly (Broers and Jacobs, 1989). In a plant displaying

hypersensitive resistance, host cells may collapse after formation of haustoria (Kloppers, 1994). In a susceptible host, urediospores are formed in the central part of a mature colony and eventually erupt through the upper leaf surface (Broers and Jacobs, 1989). These spores are dispersed mainly by wind and constitute the most important source of inoculum in a developing epidemic.

The typical symptoms of leaf rust are small, round, orange-red pustules, often about 0.2 cm in diameter. The pustules occur mostly on the upper leaf surface and are readily distinguishable from stem rust pustules by their smaller size, round shape, and orange colour. In severe epidemics, almost the entire leaf blade can be covered with pustules. These leaves senesce rapidly and dry out, depriving the plant of much of its photosynthetic area (Knott, 1989a).

Wheat rusts can be controlled by several methods, such as breeding for resistance, the application of fungicides, cultural practices and the eradication of alternate hosts. Genetic resistance is the most cost-efficient method of rust control (Roelfs *et al.*, 1992). In breeding for resistance, the objective is to develop cultivars that will remain resistant for at least the duration of their commercial life-span. Sustainability of resistance is affected by many factors such as the host (e.g., the type of resistance used), the pathogen (e.g., the life cycle of the rust in that area), the environment (e.g., the favourableness of the weather for rust development), and man (e.g., the decision of which cultivar to grow) (Knott, 1989a). Many breeding systems have been, or are being managed depending on the specific situation in an area.

Johnson (1979) proposed and defined the term "durable resistance" as "resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time, in an environment favourable to

a disease or pest". In terms of sustainable wheat production, durable resistance thus contributes to minimising the risk of yield losses due to disease epidemics. Historically, rust pathogens have had little difficulty in producing a mutant for virulence on a particular resistance gene (Luig, 1983). The sexual cycle of leaf rust is not important in areas such as Australia, North-America and South Africa. Therefore, virulence must be produced by either mutation or possibly somatic recombination. If a cultivar carries two or more genes for resistance, two or more mutations have to occur in the pathogen to produce virulence. The chance for mutations arising simultaneously, and the new pathotype becoming established, is considered small. Should the mutations occur stepwise, resistance conferred by individual genes in the genotype may remain effective for several years.

Resistance that is first visible in older plants is called adult- or mature-plant, or post-seedling resistance (Dyck and Kerber, 1985). Dyck *et al.* (1966) identified two *Lr* genes conditioning adult-plant resistance to leaf rust namely, *Lr12* in Exchange and *Lr13* in Frontana. Expression of both genes is influenced by modifying genes (Dyck and Samborski, 1979). Dyck *et al.* (1966) stated that resistance due to *Lr13* becomes effective at about the third leaf stage. More recently, Pretorius *et al.* (1984) showed that *Lr13* can be detected in the primary leaf when seedlings are tested at higher temperatures. *Lr12* is most effective at the flag leaf stage of growth (Dyck *et al.*, 1966).

The value of combinations of *Lr* genes, frequently involving *Lr12* or *Lr13*, has often been reported. Roelfs (1988b) suggested that *Lr12* in combination with *Lr34* might result in higher resistance levels than that conferred by the individual genes. Chinese Spring has shown durable resistance to leaf rust which may be due to the combination of *Lr12* and *Lr34* (Dyck, 1991). In Brazil, Beckman (Van Ginkel and

Rajaram, 1993) made historic crosses with the land race cultivars Alfredo Chavez 6121 and Polysu. From their progeny, resistant lines, one of which was the widely used Frontana and donor of *Lr13*, were selected. Singh and Rajaram (1992) recently showed that the resistance in Frontana is based on four additive genes, including *Lr13* and *Lr34*. Previously, an interaction among the leaf rust resistance genes in Frontana, resulting in a higher level of resistance than is conditioned by any gene alone, was reported (Dyck and Samborski, 1982).

In North America, *Lr13* has provided effective resistance to leaf rust for many years when used singly, or when combined with *Lr34* in hard red spring wheats (Kolmer *et al.*, 1991). Rajaram *et al.* (1988) emphasised the importance of *Lr13* in gene combinations for the attainment of durable resistance to leaf rust in CIMMYT cultivars. The importance of multigenic resistance involving *Lr13* is, furthermore, illustrated by the cultivars Manitou and Chris. Both cultivars possess *Lr13* and derived their resistance to leaf rust from Frontana (Dyck *et al.*, 1966). The cultivars were released at about the same time, and were grown commercially in Canada and the United States, respectively (Kolmer *et al.*, 1993). The rust population quickly developed virulence on Manitou, which was protected by *Lr13* and *Lr22b*, whereas Chris was protected by at least one additional gene and has remained resistant in Mexico, South Africa, United Kingdom and South America (McIntosh *et al.* 1995). Virulence to *Lr13* became common following the release of cultivars with this gene (Kolmer, 1989).

The resistance of many Australian wheats can be explained on the basis of gene combinations including *Lr13* (McIntosh, 1992). The wheat rust survey carried out in 1989-90 by the University of Sydney Plant Breeding Institute demonstrated that although virulence to *Lr13* was present in the pathogen population, cultivars with a

combination of *Lr13* and *Lr1* (Dollarbird, Hartog, Lowan, Suneca and Sunfield), or *Lr13* and *Lr2a* (Miskle), were resistant to the pathotypes isolated. The effectiveness of gene combinations involving *Lr13* in Australia has been verified for several wheats considered susceptible in other countries, such as the Indian cultivars WL711(*Lr13+Lr14a*) and Sonalika (*Lr13+Lr14a*), the Canadian cultivar Manitou (*Lr13+Lr22b*), and the CIMMYT-derived cultivar Inia 66 (*Lr13+Lr17+Lr14a*) (McIntosh, 1992).

Roelfs (1988b) mentioned that durable resistance in the wheat leaf rust pathosystem can be attributed to combinations of *Lr12* and/or *Lr13* with *Lr34*. This type of resistance has not always been adequate, but in general provides sufficient protection of the crop against leaf rust. The objective of this study was to determine if *Lr12* interacts with *Lr13* to condition improved levels of adult-plant resistance to *P. recondita* f. sp. *tritici*. Resistance expressed by wheat lines containing both *Lr12* and *Lr13* was assessed by quantitative studies of microscopic and macroscopic components of resistance. The reaction of these lines to leaf rust in the field was also determined.

LITERATURE REVIEW

INTRODUCTION

Rust fungi occur in most wheat growing areas of the world and probably cause more crop losses than any other group of plant pathogens (Samborski, 1985). Three rust species are pathogenic on wheat. Brown or leaf rust is caused by *Puccinia recondita* Roberge ex Desmaz f.sp. *tritici* Eriks. and Henn., black or stem rust by *P. graminis* Pers.:Pers. f. sp. *tritici*, and yellow or stripe rust is caused by *P. striiformis* Westend. (Knott, 1989a). Their importance from area to area depends primarily on climate and the degree of resistance in the predominant cultivars (Saari and Prescott, 1985).

According to early records, wheat has historically been affected by blight, blasting and mildew. It is now assumed that some of these disorders referred to diseases caused by rust fungi (Roelfs *et al.*, 1992). These records also stated that Aristotle (484-322 B.C.) wrote of rust being produced by "warm vapours". Theophrastus reported that rust was more severe on cereals than legumes (Roelfs *et al.*, 1992). Excavations in Israel have revealed urediospores of stem rust dating back to 1300 B.C. (Kislev, 1982). Roelfs *et al.* (1992) also noted that the Italians, Fontana and Tozzetti, provided the first detailed reports of wheat stem rust in 1767. In 1797 Persoon, a Dutch scientist, made the first significant efforts towards the classification of fungi (Schafer *et al.*, 1984) and named the organism causing wheat stem rust *P. graminis* (Roelfs *et al.*, 1992). Leaf rust was not distinguished from stem rust in the early records. However, by 1815 de Candolle had shown that leaf rust was caused by a distinct fungus and described it as *Uredo rubigo-vera* (Roelfs *et al.*, 1992). Since the

early records, the pathogen underwent several name changes before Cummins and Caldwell (1956) recommended the current nomenclature of *P. recondita* f. sp. *tritici*.

Average losses due to leaf rust are estimated at approximately 10% (Roelfs *et al.*, 1992). Yield losses higher than 40% were, however, reported by Dubin and Torres (1981). Rust fungi are of interest not only for the economic losses they cause, but also for their highly specialised relationships with host plants. This specialisation is evident from complex life cycles, a mode of nutrition which essentially prevents culturing them away from their hosts, and an extreme form of host-specificity. Leaf rust incited by *P. recondita* f. sp. *tritici*, is internationally recognised as probably the most important rust in common wheat (*Triticum aestivum* L.)(Hiratsuka and Sato, 1982; Samborski, 1985).

TAXONOMY

Rust fungi belong to the highly specialised order Uredinales, and constitute one of the largest groups in the Basidiomycetes (Littlefield and Heath, 1979; Hiratsuka and Sato, 1982). About 5000-6000 species have been recognized (Hiratsuka and Sato, 1982). Rusts are included in the subclass Heterobasidiomycetidae (Hiratsuka and Sato, 1982). The term rust is generally applied both to the pathogen and the disease it inflicts. Most rusts are obligate parasites on ferns or seed plants and are host specific or pathogenic to a group of related host species (Hiratsuka and Sato, 1982). A close relationship exists between the rusts and their hosts and they are, therefore, considered to have evolved in close association. Rust-host relationships are considered very important in determining the phylogeny and origin of rusts (Hiratsuka and Sato, 1982). The polymorphic nature and variable life cycles of rusts, together with

their host specificity, have often restricted the use of certain morphological or other characteristics for taxonomy and classification. One problem in rust classification is that certain morphological characters are very similar in different rusts, but at the same time, other characters are very different in obviously closely related rusts. According to Hiratsuka and Sato (1982), Peterson defined this phenomenon as the "reticulate nature of taxonomic characters". For example, the morphology of urediospores of species from distinct genera such as *Puccinia*, *Cronartium* Fr. and *Ravenelia* Berk. is very similar, but their teliospore morphology differs considerably (Savile, 1984). Because of these distinctions separate keys for rust classification were developed by Savile (1984).

Traditionally, rusts are divided into the families Melampsoraceae and Pucciniaceae (Hiratsuka and Sato, 1982) according to certain teliospore characteristics (Arthur and Cummins, 1962). The teliospores of Melampsoraceae are more or less indefinite and sessile, single or grouped within the tissues of the host, or compacted laterally and united into columns or layers. Teliospores of Pucciniaceae are usually well circumscribed and pedicelled or sessile, free or fascicled, but not united laterally, except when born on a stalk (Arthur and Cummins, 1962).

DISTRIBUTION AND ECONOMIC IMPORTANCE

According to Chester (Broers and Jacobs, 1989) leaf rust occurs wherever wheat is grown. It is also the most common and widely distributed of all cereal rusts. Although rust has occurred on wheat throughout its evolutionary development, it is probably causing more damage now because of the large areas planted to genetically

homogeneous, or closely related cultivars (Samborski, 1985). *Puccinia recondita* f. sp. *tritici* destroys leaf tissue progressively through the season, resulting in a reduced number of kernels, shrivelled grain and lower mass and protein content (Knott and Dvorak, 1976; Martens and Dyck, 1989).

NORTH AMERICA

Wheat leaf rust occurs annually in varying amounts over most wheat-growing areas of the United States (Long *et al.*, 1988). The fungus overwinters in the southern plains of the United States, and each year during late spring and early summer the urediospores are carried to the northern plains and prairie provinces by the southerly winds (Roelfs, 1989; Kolmer, 1992). In the eastern prairies of Canada, yields of susceptible cultivars are reduced by 5-15% annually (Samborski, 1985). According to Samborski and Peterson (1960), extensive losses can occur if the disease becomes severe before flowering. According to Chester (in Kolmer, 1989), losses as high as 50-95% can be experienced if moderate to heavy infection occurs before the wheat has headed. In recent years, however, losses due to leaf rust have generally been prevented through the use of resistant cultivars (Knott, 1989a). In 1984, estimated wheat yield losses due to leaf rust were as high as 5% in California and New York and averaged 1.4% on winter wheat and 0.6% on spring wheat in the other states (Long *et al.*, 1986). In 1986, leaf rust was severe with estimated yield losses up to 8% in Kansas, with an average yield loss of 4.9% on winter wheat and 1.1% on spring wheat (Long *et al.*, 1988). Since susceptible alternate hosts have not been found in North America, leaf rust is believed to reproduce there only by asexual means (Samborski, 1985).

Surveys determining the occurrence and distribution of wheat leaf rust are conducted annually in the United States (Roelfs *et al.*, 1982; Long *et al.*, 1986; Long *et al.* 1988) and Canada (Kolmer, 1989; 1991; 1994).

MEXICO

According to CIMMYT (International Maize and Wheat Improvement Center), leaf rust is the most important wheat disease in Mexico (Samborski, 1985). Wheat is intensively cultivated in isolated areas across Mexico where irrigation is available (Roelfs, 1985). Populations of leaf rust are highly variable in northwestern Mexico and therefore a widely cultivated cultivar has seldom remained resistant for longer than two years (Dubin and Torres, 1981). It appears that the rust overwinters in the mountainous highlands and, with favourable conditions, moves northeast into the southern United States and northwestward into the major production areas in Sinaloa and Sonora (Roelfs, 1985). Similar pathogen races exist in South Texas and Mexico, but these differ from races occurring in the central and north central areas of the United States (Roelfs *et al.*, 1982). In 1976-1977 a severe leaf rust epidemic occurred on the widely grown cultivar Jupateco 73 in northwestern Mexico, and severe crop losses were prevented by fungicide application (Dubin and Torres, 1981).

SOUTH AMERICA

Leaf rust is common in South America where it has caused significant losses (Saari and Prescott, 1985). In 1982 yield losses of 18 and 20% were reported by Da Luz (1984) in Brazil. Breeding for leaf rust resistance has been done for many years and South American cultivars have frequently been used as sources of resistance in other

countries (Knott, 1989a). South America is divided into two subzones by the Andes mountains. The western zone is characterised by tremendous variation in elevation and by primitive agriculture in many areas. The southeastern subzone includes the main wheat-growing areas of the pampas of Argentina, Paraguay and Brazil. The subzones are generally isolated from one another by the Andes but interchange of spores may occur in the south (Knott, 1989a). In Chile losses between 9.5 and 67% have been reported (Cortazar *et al.*, 1989). In 1985 a destructive epidemic in the entire wheat producing area in Uruguay were caused by a new race of *P. recondita* f. sp. *tritici* first observed in 1982 (German *et al.*, 1986). Yield losses, sometimes exceeding 50%, were greatest in late-sown crops (German *et al.*, 1986).

EUROPE, NORTH AFRICA AND ASIA

Leaf rust is commonly found in western Europe but losses are generally slight (Saari and Prescott, 1985). Knott (1989a) reported that leaf rust is widespread and has recently been responsible for yield losses in the former U.S.S.R. According to Dwurazna as cited by Samborski (1985), leaf rust is the most damaging wheat disease in eastern Europe, causing an average yield reduction of 3-5%. It is endemic in the dry delta of Egypt, where irrigation provides suitable moisture for rust development (Saari and Wilcoxson, 1974). Leaf rust has been considered one of the most destructive diseases of spring wheat in Egypt (Stewart *et al.*, 1977). The disease occurs frequently in the northern part of the Indian subcontinent where its damaging potential qualifies it as the most important rust disease of wheat (Saari and Prescott, 1985). In the Far East leaf rust is widespread, but causes limited damage. Although leaf rust is found in most areas of Southeast Asia, it is considered relatively unimportant (Knott, 1989a).

AUSTRALIA AND NEW ZEALAND

According to Luig (1985) the importance of leaf rust has been underestimated in Australia. Wheat is grown in the southwest and in a larger area in the southeastern regions of Australia (Knott, 1989a). Field surveys to assess variability in *P. recondita* f.sp. *tritici* were conducted by Waterhouse from 1921 to 1951, but only two biotypes were isolated until 1946 (Luig, 1985). In the northern parts of New South Wales and southern parts of Queensland, favourable conditions frequently lead to an abundance of the disease, and susceptible cultivars may be defoliated, whereas cool temperatures during the late winter and early spring retard disease development in southern New South Wales, Victoria and in South Australia (Luig, 1985). In Western Australia, prevailing dry conditions often prevent serious outbreaks of leaf rust (Luig, 1985).

In 1945, a new strain of *P. recondita* f.sp. *tritici* virulent to *Lr23* was detected on the cultivar Gabo cultivated in northern New South Wales and Queensland (Luig, 1985). In 1958, Watson and Luig detected virulence for *Lr3* and *Lr15* (Luig, 1985). Nine pathotypes of *P. recondita* f. sp. *tritici* were identified during 1988 and 12 during 1989 (Park and Wellings, 1992). Cultivars with *Lr13* have remained resistant to all Australian pathotypes (Luig, 1985). However, Park and McIntosh (1994) reported virulence for *Lr12* and *Lr13* in Australia during the period 1989 to 1992. Virulence for *Lr12* probably originated in Queensland and/or in northern New South Wales following the release of the cultivar Timgalen in 1967 (Park and McIntosh, 1994).

A limited amount of wheat is grown in New Zealand, and although it is separated from Australia by about 2000 km across the Tasman Sea, spores are regularly transported, presumably by air currents, from eastern Australia (Luig, 1985). In 1989, two pathotypes previously recorded in Australia, were detected for the first time in New

Zealand (Park and Wellings, 1992). In addition, two new pathotypes were detected in New Zealand (Park and Wellings, 1992). In New Zealand leaf rust can be serious when the weather is favourable, but the disease is well controlled by resistant cultivars (Knott, 1989a).

SOUTHERN AFRICA

This zone includes the sub-Saharan countries and south-western part of the Arabian peninsula (Saari and Prescott, 1985). In general wheat is grown on a limited basis throughout this zone and is often geographically isolated, thus restricting the exchange of spores between regions. Most wheat is produced in Ethiopia and South Africa.

Hailu (in Kebede *et al.*, 1994) considered Ethiopia a major producer of wheat in sub-Saharan Africa. In this region leaf rust is regarded as a disease of economic importance (Geleta and Tanner, 1994). Epidemics of rusts in wheat were reported in Ethiopia as early as 1908 (Wanyera, 1994). Although the occurrence and intensity of leaf rust in Ethiopia varies from year to year, losses as high as 75% have been reported in some areas (Bechere *et al.*, 1994). Leaf rust usually does not cause economic yield losses on wheat when it occurs in low to medium altitude areas such as Endebess, Njoro and Eldoret (Owuocha *et al.*, 1994). In Tanzania (Kuwite, 1994), and in the mid-altitude areas of Uganda (Wagoire *et al.*, 1994), leaf rust is an important disease and therefore a limiting factor to wheat production.

In Zambia wheat is the second most important food crop after maize and is grown under irrigated or rainfed conditions (Muyanga, 1994). Rainfed wheat is mainly grown by small scale farmers in the northern province. Leaf rust is considered the second most important disease of rainfed wheat in Zambia and remains a threat to

wheat production (Mukwavi, 1994). Pretorius and Purchase (1990) identified pathotypes 3SA126, 3SA135 and 3SA137 from Zambian samples. Pathotypes 3SA126 and 3SA137 were also detected in South Africa whereas 3SA135 from Zambia appeared related to 3SA122 in South Africa (Pretorius and Le Roux, 1988). In Malawi wheat is grown under dryland conditions by smallholder farmers, and regular summer rainfall with prolonged periods of mist and light showers during winter favour leaf rust epidemics (Pretorius and Purchase, 1990). Pathotypes 3SA126, 3SA132, 3SA133 and 3SA134 were detected in samples obtained from Malawi (Pretorius and Purchase, 1990).

Leaf rust is presently the most important disease in Zimbabwe, especially at the lower altitudes (Mtisi and Mashiringwani, 1994). In other areas rust is relatively unimportant because it occurs towards the end of the growing season (Mtisi and Mashiringwani, 1994). According to Pretorius and Purchase (1990) soft-white spring wheat is grown under irrigation during the cool, dry winter and leaf rust is present in most seasons. Pathotypes 3SA126 and 3SA137 were identified in Zimbabwe during 1986 by Pretorius and Purchase (1990).

In South Africa leaf rust occurs annually in most wheat growing areas (Pretorius *et al.*, 1990), but its distribution and severity are determined by the amount of oversummering inoculum, climatic conditions and the susceptibility of commercially grown cultivars (Pretorius *et al.*, 1987, Pretorius and Le Roux, 1988). Leaf rust occurs in the Free State if environmental factors are favourable for infection and disease development, but is usually most severe on spring wheat grown in the winter rainfall areas of the Western Cape (Pretorius *et al.*, 1987). Since annual wheat leaf rust surveys were introduced in 1983, 15 pathotypes have been detected (Pretorius *et al.*,

1987; Pretorius and Le Roux, 1988; Pretorius *et al.*, 1990).

MORPHOLOGY

DEFINITION AND TERMINOLOGY OF SPORE STATES

The basic terminology for the spore states of rust fungi was recommended by de Bary and Tulasne in the middle of the last century, but has since been modified by urediniologists such as Arthur in 1905 and 1925, Cunningham in 1930, Cummins in 1959, Azbukina in 1970, and Hiratsuka in 1973 and 1975 (Hiratsuka and Sato, 1982).

Terminology of spore states is based on morphology and ontogeny. The morphological system emphasises the morphology of spores as the basis for defining states, whereas the ontogenic system emphasises positions of the spore states in the life cycle rather than recognisable morphological entities (Hiratsuka and Sato, 1982). Numerous names have been proposed for each spore state. Variations for each spore state, with the most commonly accepted term listed first, are: spermatia (in spermogonia), picniospores (in pycnia), pycnidiospores (in pycnidia); aeciospores (in aecia), aecidiospores (in aecidia); urediniospores (in uredinia), urediospores (in uredia or uredosori); teliospores (in telia), teleutospores (in teleutosori); basidiospores (on basidia), and sporidia (on promycelia) (Hiratsuka and Sato, 1982).

Spermatia

Spermatia (singular=spermatium) are formed in spermogonia usually on the upper side of leaves and are small, hyaline, single-celled spores, contained in a sugar-rich nectar which oozes from spermogonia. The infectious or sexual function of spermatia as

gametes in heterothallic rusts was revealed by Craigie with *P. graminis* and *P. helianthi* Schwein. (Hiratsuka and Sato, 1982). Buller, according to Hiratsuka and Sato (1982), found that nectar containing spermatia attracts several insects and is therefore important in cross-fertilization of haploid pustules of the opposite mating type.

Aeciospores

Aeciospores are nonrepeating vegetative spores formed in aecia (singular=aecium) as the result of dikaryotization. They are commonly associated with spermatia. Aecia are conventionally divided into five types: aecidioid, peridermioid, roestelioid, caeomoid and uredinoid. These types correspond to the morphology of the five imperfect genera *Aecidium* Pers.:Pers., *Peridermium* (Link) J.C. Schmidt & Kunze, *Roestelium*, *Caeoma* Link. and *Uredo* Pers.:Pers., respectively. The first three types of aecia have surrounding wall structures called peridia, constituted of specialised spores. These aecia are reasonably distinct in morphology, but are difficult to define and separate purely on morphological bases (Hiratsuka and Sato, 1982).

Urediospores

Urediospores (summer spores, red rust spores) are spiny or warty, reddish brown and are borne on stalks or in chains in uredia (singular=uredium). They are defined as vegetative spores, usually formed on dikaryotic mycelium, and are repeatedly produced on a host plant during the growing season of the latter. The uredial state is the most destructive spore state of rusts as exemplified by, e.g., wheat stem rust (*P. graminis*), coffee leaf rust (*Hemileia vastatrix* Berk. & Broome) and poplar leaf rust (*Melampsora medusae* Thuem.) (Hiratsuka and Sato, 1982).

Teliospores

Teliospores (winter spores, black rust spores) are one or multi-celled, formed in a telium and may be produced on pedicels as a single layer or as a multilayer of cells, or in chains with or without peridial cells (Hiratsuka and Sato, 1982).

Basidiospores

Basidiospores are produced on basidia which usually divide transversely into four cells, forming one sporidium from each cell at the tip of the sterigma. Two-celled basidia are often produced in species of various genera (Hiratsuka and Sato, 1982).

INFECTION PROCESS

Successful entry of a rust fungus into the host depends on the development of specialised structures of the germ tube. These infection structures, e.g. the appressorium, infection peg, substomatal vesicle and infection hypha, are characteristic for each rust species (Littlefield and Heath, 1979). The formation of these structures requires special stimuli, which may be induced by contact with the leaf surface (Wolf, 1982).

Prepenetration stages in the development of wheat leaf rust are germination, germ tube growth and formation of an appressorium over a stoma (Jacobs, 1989a). Differences in urediospore germination and appressorium formation in the wheat leaf rust interaction have not been related to the presence of resistance genes (Jacobs, 1989a). Poyntz and Hyde (1987), however, reported a difference between susceptible and resistant genotypes in spore germination, but not in appressorium formation. No significant differences in spore germination and appressorium formation were reported

between susceptible and slow leaf-rusting genotypes (Gavinlertvatana and Wilcoxson, 1978; Chang and Line, 1983; Lee and Shaner, 1984), or between near-isogenic lines with hypersensitive resistance genes to wheat leaf rust (Plotnikova *et al.*, 1985). It is believed that germination and germ tube growth require less stimuli than appressorium formation (Goodman *et al.*, 1986).

Spore germination is an essential phase in the propagation and survival of biotrophic fungi, which under normal conditions, depend on their hosts. Germination is the transformation of a rust spore from a resting to an active state (Teng and Bowen, 1985). Germination of an urediospore on a wheat leaf is favoured by 100% relative humidity, a dark period, and temperatures ranging between 10°C and 20°C (Roelfs *et al.*, 1992). Four stages of urediospore germination have been recognised: a) hydration and swelling; b) dissolution of the cellwall pore plug and appearance of the germ tube; c) growth of the germ tube; and d) differentiation of the infection structures, e.g. appressorium, infection peg, substomatal vesicle, infection hypha and haustorium (Wolf, 1982). Chemical and physical stimuli have been shown to affect growth of the germinating rust spore (Staples and Macko, 1984; Hoch and Staples, 1987).

Inhibitors

Fungal spores in dense populations, either in pustules or in suspension, do not germinate or germinate only at a reduced rate (Staples and Macko, 1984). This effect might be the result of self-inhibition by substances present in the spores or produced by them. Self-inhibition is valuable to the rusts since it prevents premature germination of spores in pustules. The action of these inhibitors is restricted to the first 10-20 min, when, following swelling and hydration of the urediospore, the germtube extrudes

through the cell wall. The germ tube is no longer sensitive to inhibitors once it is outside the cell wall (Wolf, 1982). Germination of urediospores is also inhibited by continuous irradiation (Sharp *et al.*, 1958; Givan and Bromfield, 1964; Lucas *et al.*, 1975; Staples and Macko, 1984). Temperature and air ions, e.g. lead, have also been shown to inhibit the germination of urediospores (Staples and Macko, 1984).

Stimulators

The regulation of urediospore germination depends on endogenous stimulators. One of the first compounds described by French and as a stimulator for fungal spore germination was *n*-nonanol obtained from *P. graminis* f. sp. *tritici* (Wolf, 1982). According to Staples and Macko (1984), Rines *et al.* had previously isolated the same compound from *P. recondita*. Some compounds which act as stimulators of germination have been found in natural products such as essential oils and perfumes. Therefore, it is possible that similar, but unidentified substances from host plants may contribute to the regulation of spore germination in biotrophic fungi on plant surfaces (Wolf, 1982). According to Macko *et al.* (in Staples and Macko, 1984) stimulants overcome the self-inhibition that prevails in dense populations of spores without reacting to the self-inhibitor molecule.

Germ tube growth and formation of an appressorium

The germ tube grows towards the stoma and forms an appressorium over the stomatal opening (Littlefield and Heath, 1979). A penetration peg is then formed to penetrate the stoma (Knott, 1989a; Roelfs *et al.*, 1992; Kloppers, 1994). Germ tube growth has been found to be parallel to the short axis of the leaf (Johnston, 1934; Staples and Macko,

1984). Jacobs (1989b) found that the majority of germ tubes grew at right angles to veins and others grew directly to a nearby stoma, not following lines parallel to the short or long axis of the leaf. Depending on temperature, a dew period of at least six hours might be necessary for achieving successful infection (Roelfs *et al.*, 1992).

Vegetative growth

Inside the leaf a substomatal vesicle is formed from which an infection hypha grows towards a host cell (Knott, 1989a; Roelfs *et al.*, 1992). Hyphae are septate and hyaline. The gametophytic mycelium hyphae are generally haploid and monokaryotic, whereas the hyphae of sporophytic mycelia are mostly dikaryotic. Berkson (Hiratsuka and Sato, 1982) reported that although clamp connections occur in a few species, they generally do not occur in dikaryotic hyphae of rusts. Both kinds of hyphae grow between host cells (intercellular) obtaining nutrients from the host cells by specialised, intracellular structures called haustoria (Hiratsuka and Sato, 1982).

In leaf rust the initial step in haustorium formation occurs when a hyphal tip contacts an appropriate host cell and differentiates into a haustorium mother cell (Harder, 1989; Knott, 1989a; Roelfs *et al.*, 1992; Kloppers, 1994). A haustorium was defined by Bushnell (1972) as : "... a specialized organ which is formed inside a living host cell as a branch of an extracellular (or intercellular) hypha or thallus, which terminates in that host cell, and which probably has a role in the exchange of substances between host and fungus." Haustoria may range from small spherical "simple" structures to large, multilobed structures which nearly fill their host cells (Harder, 1989). Comparison of haustoria showed that morphological characters had some taxonomic value in the Pucciniaceae (Hiratsuka and Sato, 1982).

Sporulation

The primary hypha develops rapidly into a branched, multicellular network of mycelium and after a brief period of growth new urediospores, erupting through the upper leaf surface, are formed in the central part of a mature colony (Broers and Jacobs, 1989). The leaf rust fungus typically produces small, round, orange-red pustules, often about 0.2 cm in diameter on leaf surfaces (Knott, 1989a). Pustules occur mostly on the upper leaf surface and are readily distinguishable from stem rust pustules on leaves by their smaller size, circular shape, and orange colour (Roelfs *et al.*, 1992). The surface layer of spores may darken but it can be wiped off to reveal the true colour (Knott, 1989a). In severe epidemics, almost the entire surface of the leaf blades can be covered with pustules. These leaves senesce rapidly and dry out, depriving the plant of much of its photosynthetic area (Knott, 1989a). Orange-coloured uredial pustules are followed by grey to black telial sori.

LIFE CYCLE OF RUSTS

According to Hiratsuka and Sato (1982) three basic types of life cycles are recognized in rust fungi. Depending on the spore states, life cycles are macrocyclic, demicyclic or microcyclic. The macrocyclic cycle includes all spore states as described above, the demicyclic cycle lacks the uredial state, whereas the microcyclic cycle lacks the aecial and the uredial states. In all three cycles, the spermogonial state may be absent. Many rust species are heteroecious, completing their life cycle on two different kinds of plants, but some are autoecious (monoecious), having all spore forms on a single host species (Schafer *et al.*, 1984). Rusts with a complete life cycle have all five

different spore forms (Hiratsuka and Sato, 1982).

Puccinia recondita f. sp. *tritici* is a macrocyclic rust with a sexual cycle involving teliospores, basidiospores and aeciospores, and an asexual cycle constituted by urediospores (Samborski, 1985). The asexual stage occurs on wheat and related grasses and the sexual stages on an alternate host (Knott, 1989a).

HOSTS

PRIMARY HOSTS

The primary host of wheat leaf rust is *T. aestivum* (Roelfs *et al.*, 1992). The fungus also infects *T. turgidum* L. in the Mediterranean and Middle East, Ethiopia and India where durum wheats are extensively cultivated (Roelfs *et al.*, 1992). It is of minor importance on *T. monococcum* L., *T. dicoccum*, and *T. speltoides* (Tausch) Gren. ex Richter (Roelfs *et al.*, 1992). According to Skovmand *et al.* (1984) wheat leaf rust seems to be a major threat to triticale (*XTriticosecale* Wittmack), the crop derived from the man-made cross between wheat and rye.

ACCESSORY HOSTS

Several species of grasses are attacked by *P. recondita* but the one serving as the functional host in nature for the forma specialis *tritici* is not known (Roelfs *et al.*, 1992). Many grasses can be artificially inoculated, but may not be infected in the field. Wild or weedy species of the genera *Triticum* and *Aegilops* (= *Triticum*), and the related species of *Agropyron* and *Secalis* (Roelfs *et al.*, 1992), are potential hosts for wheat leaf rust. Azbukina reported *Agropyron repens* L. as a host, whereas Casulli and Siniscalco (in Roelfs *et al.*, 1992) reported certain *Agropyron* spp. in southern Italy to

be infected by a wheat and *Thalictrum*-infecting rust. *Triticum (Aegilops) cylindrica* L. has been reported as a host for leaf rust in North America, but the races isolated from this host differed from those on adjacent wheat plants (Long *et al.*, 1988).

Volunteer or self-sown wheat is the most common noncrop host, growing in fallow fields, along the edges of fields and roads, as weeds in a second crop, and as a cover crop under orchards and along irrigation canals (Roelfs *et al.*, 1992). These plants serve as the major sources of initial inoculum throughout much of the world where autumn or winter wheat is sown (Roelfs *et al.*, 1992).

ALTERNATE HOSTS

The sexual stages of the life cycle of leaf rust occur on alternate hosts such as *Thalictrum* L. spp. in Portugal (Young and D'Oliveira, 1982), *Anchusa italica* Retz. in Portugal (Roelfs *et al.*, 1992) and Morocco (Ezzahiri *et al.*, 1992) and *Isopyrum fumarioides* L. in Siberia (Johnson *et al.*, 1966; Wahl *et al.*, 1984). Most alternate hosts belong to the Ranunculaceae and Boraginaceae families (Roelfs *et al.*, 1992). A report by Young and Browder (1965) suggested that the rusts might be acquiring the ability to infect some *Thalictrum* L. spp. in America, an important alternate host genus in Europe. Other alternate hosts reported include *Clematis* spp. in Italy and in the Soviet Far East (Samborski, 1985). Infected *Thalictrum thunbergii* D.C. was found by Yamada *et al.* near wheat fields in Japan, but may not be the primary source of inoculum for wheat as occurs elsewhere (Roelfs *et al.*, 1992). In most areas, however, the alternate hosts do not appear to play an important role in the life cycle through initiating early infections in the spring (Samborski, 1985). In some of the Mediterranean areas the sexual cycle is considered important in the production of new combinations

of virulence through genetic recombination (Anikster and Wahl, 1979; Samborski, 1985).

DISEASE CONTROL

Wheat rusts can be controlled by several methods, none of which is completely satisfactory on its own. The earliest attempts to control wheat rust involved religious practices (Roelfs *et al.* 1992). These ceremonies existed as early as 1000 B.C., continued into the first century A.D., and apparently varied between areas. In the early 1600s, Worldridge (in Roelfs, 1985) recommended pulling a rope over the grain to reduce dew deposition on plants. This practise continued until the 1900s in some areas, while in France, according to Roelfs (in Knott, 1989a), the laws required barberry eradication in 1660. Today, rusts are primarily controlled by either genetic resistance or the use of chemicals, and to a lesser extent by cultural methods (Knott, 1989a).

CULTURAL METHODS

Cultural methods of rust control are aimed at breaking the life cycle of the fungus at a critical stage such as overwintering or oversummering (Roelfs, 1985). In the USA cultural methods depend largely on the use of early-maturing cultivars and the planting of spring wheats. In Australia, Farrer developed early-maturing wheat cultivars to escape the damage caused by rust diseases (McIntosh, 1976). In the USA early planting may increase the chance for infection during autumn and subsequent overwintering of the rust in milder climates (Roelfs, 1985). Most of the environmental

conditions that favour wheat also favour rust development (Roelfs, 1985). Zadoks and Bouwman (1985) highlighted the importance of the green bridge in carrying the disease from one crop to the next. When some growers plant early and others late, the green bridge is extended (Roelfs *et al.*, 1992). An effective measure for preventing epidemics resulting from endogenous inoculum, is removing the green bridge with tillage or herbicides (Roelfs *et al.*, 1992). In areas where rusts overwinter, the destruction of volunteer wheats and susceptible grasses several weeks before planting reduces the inoculum density, and therefore delays the initial infection (Roelfs, 1985). When both spring and winter wheat are grown in the same area, the separation of these crops by space or another non-susceptible crop can delay disease spread between fields (Roelfs, 1985).

ERADICATION OF ALTERNATE HOSTS

Destroying alternate hosts interrupts the life cycle of rust fungi, limits their diversity, and prevents the production of early-spring inoculum (Wiese, 1987). Eradication of the alternate hosts has four main effects: 1) delaying the onset of rust, which in the absence of alternate hosts, depends on wind-blown urediospores; 2) reducing the initial inoculum which often is heavy around alternate hosts, but light when it depends on airborne urediospores travelling long distances; 3) decreasing the number of pathogenic races by excluding sexual recombination; and 4) stabilising the pathogen population by reducing the number of races found each year and the frequency of changes (Knott, 1989a). A successful alternate host eradication programme was launched for stem rust control in northern Europe (Roelfs *et al.*, 1992) and in the north-central states of the USA (Roelfs *et al.*, 1978).

CHEMICAL CONTROL

Studies on chemical control of cereal rusts began in the last century, but Dickson (Samborski, 1985) concluded it was not economically viable. Rowell (1968) stated that organic compounds and mixtures of inorganic salts plus dithiocarbamate fungicides showed considerable promise. The recent introduction of systemic fungicides increased interest in chemical control of wheat leaf rust (Samborski, 1985). Bayleton (triadimefon) (Buchenauer, 1982) and Indar (fenbuconazole or 4-butyl-4H-1,2,4,-triazole) (Dubin and Torres, 1981) are particularly effective against leaf rust. These compounds are valuable because they can be applied as a seed dressing or foliar spray. Usually one application is sufficient, but depending on the chemical, weather, and the length of the growing season, two or more applications may be necessary (Samborski, 1985). Chemicals are expensive and there is an added cost of application. Their use is only economical where intensive cereal management is practised and the fungicide applied also controls other diseases (Knott, 1989a). Chemicals have been successfully used in Europe where high yields (6-7 t/ha) were achieved (Buchenauer, 1982). Chemical control is especially valuable when new races of leaf rust develop and resistant cultivars are not available (Samborski, 1985). In this regard chemicals were successfully used in the irrigated Yaqui and Mayo Valleys of Mexico to control the 1977 leaf rust epidemic on the predominantly grown cultivar Jupateco 73 (Dubin and Torres, 1981). Wheat was sprayed with Indar or Bayleton and new resistant cultivars were released before the next growing season (Dubin and Torres, 1981). In the eastern and southern United States fungicides have been applied against leaf rust when yields were expected to exceed 2 t/ha (Roelfs *et al.*, 1992). In Brazil and Paraguay, chemicals are used to control an array of diseases on wheat expected to yield 1 t/ha or higher (Roelfs

et al., 1992). Fungicides are usually important in the less developed countries, but may not be readily available. Farmers cannot afford to buy chemicals or equipment to apply them, and therefore it may not be a viable method for rust control in these countries (Knott, 1989a).

BIOLOGICAL CONTROL

Suppression

The use of biocontrol agents to suppress infection by foliar pathogens in cereals is limited (Levy *et al.*, 1988). Inhibition of germination of leaf rust urediospores by isolates of *Pseudomonas* spp. or *Bacillus* spp. was reported by Buskova (Levy *et al.*, 1988). Levy *et al.* (1988) found that the application of two isolates of fluorescent pseudomonads to leaves of wheat seedlings prior to inoculation with *P. recondita* f. sp. *tritici* reduced symptom expression. This reduction was ascribed to the ability of biocontrol agents to produce compounds which suppress the development of fungal pathotypes (Levy *et al.*, 1988).

Hyperparasitism

Cunningham (1967) reported that *Sphaerellopsis filum* (Biv.-Bern. ex Fr.) Sutton (= *Darluca filum*) was, of the many hyperparasites of rust fungi in the tropics, most commonly observed. Surveys by Kranz (in Buchenauer, 1982) on the natural distribution of *S. filum* in Guinea and Kenya indicated that between 0-99% of urediosori of all rusts studied were infested with this hyperparasite. *S. filum* forms clumps of shiny black spherical pycnidia, producing two-celled conidia, among the spores in uredial sori. Pycnidia seemed to inhibit the development of teliospores. The fungus may also

parasitise pycnial, aecial and telial spore states. Von Shroeder and Hassebrauk (in Buchenauer, 1982) observed direct penetration of urediospores of *P. recondita* by *S. filum* without formation of adsorption structures. Penetration of the urediospores was presumably through mechanical and enzymatic processes. Cell content was disorganised after penetration and the cytoplasmic constituents and cell wall material were considered to serve as nutrients for the invading fungal hyperparasite (Buchenauer, 1982). Bean and Rambo (1968) showed differences in nutrient requirements between mycelial and conidial isolates of *S. filum*. The optimum temperature for production of conidia was 30°C and spores remained viable after five months of dry storage under outdoor conditions, and the germination rate of conidia of *S. filum* was significantly stimulated in the presence of urediospores of *P. recondita* (Bean and Rambo, 1968). Artificial inoculation experiments by Fedorintchik (in Buchenauer, 1982) showed that under favourable environmental conditions for *S. filum*, 98% of the leaf rust sori were damaged or destroyed. In greenhouse experiments *S. filum* often causes severe damage, but under field conditions rust epidemics occur even in the presence of the hyperparasite. Therefore, biological control of *P. recondita* by *S. filum* seems unlikely.

Induced resistance

Plants inoculated or treated with nonpathogens, avirulent races of pathogens, heat-inactivated pathogens and high molecular weight substances of virulent agents, often show resistance to disease caused by fungal pathogens. Wheat seedlings inoculated with urediospores of oat crown rust (*P. coronata* Corda) prior to inoculation with leaf rust showed a reduced number of pustules and a different infection type (Johnston and

Huffman, 1958). Pustules were smaller than those on plants inoculated with leaf rust only. Johnston and Huffman (1958) assumed that the mechanism was partly due to killing and plugging of stomata. Cheung and Barber (1972) demonstrated induced resistance in wheat to a virulent race of *P. graminis*, which after inoculation with an avirulent race, resulted in a reduction of 80% in the number of pustules. Induced resistance in beans to *Uromyces phaseoli* (Pers) Wint. and in *Antirrhinum majus* L. to *P. antirrhini* Diet & Holw was reported by Yarwood (1956). The number of pustules produced by a virulent strain of *U. phaseoli* was reduced by 33% after pre-inoculation with an avirulent strain. Littlefield (1969) demonstrated reductions in both number and size of pustules in similar experiments with flax (*Linum usitatissimum* L.) and *M. lini* (Ehrenb.) Lev. Johnson and Allen (1975) demonstrated induced resistance with *P. striiformis* in wheat and verified the findings of Cheung and Barber (1972) that induced resistance includes an inducible active process which is not solely due to blocking of stomata.

The opposite situation, induced susceptibility, was described by Bahamish and Wood (1985) in wheat infected with two isolates of *P. recondita* f. sp. *tritici*. Manners and Gandy (1954) cited and confirmed two early reports by Johnston (1934) and Roberts (1936) that infection by powdery mildew increased disease caused by *P. recondita*.

RESISTANCE

The most important measure of control of the cereal rusts has been through the use of resistant cultivars (Johnson, 1981a). Resistance should be understood as any genetically determined characteristic of a host plant that in any way limits the damage

produced by disease (Browder, 1985).

Physiologic specialisation and pathogenic variation

Many rusts show physiological specialisation, exemplified by the existence within a species of numerous strains or races, that look alike, but differ in their ability to attack varieties of a single species (Anikster, 1984). Many physiologic races of *P. recondita* f. sp. *tritici* exist. Mains and Jackson (1926) demonstrated that physiologic races are stable taxons that differ in virulence on particular host lines. They selected 11 lines as a standard set of differential hosts on which to identify leaf rust cultures (Roelfs *et al.*, 1992). All cultures that gave the same pattern of virulence and avirulence on the standard differentials were considered to be a single physiologic race. They could, however, differ in virulence on other host lines or in other characters (Knott, 1989a). Three of these lines were eventually discarded and the remaining eight were internationally accepted as standard differentials (Roelfs *et al.*, 1992). More recently, Long and Kolmer (1989) proposed a new race nomenclature system for *P. recondita* f. sp. *tritici* in North America in which physiologic races are identified on the basis of their pathogenicity on a set of 12 differentials (viz. *Lr1*, *2a*, *2c*, *3*, *3ka*, *9*, *11*, *16*, *17*, *24*, *26*, and *30*).

Race surveys Since the discovery of pathogenic variability within the *forma specialis* of leaf rust, race surveys have been carried out in all the major wheat growing areas of the world. In most cases a survey is done by intensive sampling in cultivated fields (Knott, 1989a). Initially the primary purpose was descriptive, i.e. to determine the range of variability within each of the wheat rusts (Knott, 1989a). As breeding for rust

resistance developed, race surveys provided essential information to determine the direction of breeding programmes (Schafer and Long, 1988; Roelfs *et al.*, 1992). A major objective was to detect new, highly virulent pathogen phenotypes before they became a threat to the crop (Knott, 1989a). Knowledge of the occurrence of either new genes for virulence, or new combinations of genes, is essential for determining the resistance necessary in breeding programmes (Schafer and Long, 1988). Potentially dangerous races could be used to evaluate potential parents and to select for resistance in the progeny of hybrids (Knott, 1989a).

Physiologic specialisation in South Africa was documented by Verwoerd (1937) who identified five races according to the reactions produced on eight differential host cultivars. Survey studies were undertaken by Pretorius *et al.* (1987) during 1983 to 1985 and nine races were identified. In a survey conducted during 1986 seven pathotypes, six of which had previously been described, were identified (Pretorius and Le Roux, 1988). Pathotype 3SA134 (notation of the Small Grain Institute, Bethlehem, South Africa) was isolated for the first time in South Africa (Pretorius and Le Roux, 1988). During the 1987 survey, virulence to *Lr26* was detected for the first time in South Africa from a uredial sample (pathotype 3SA140) collected near Riviersonderend (Pretorius and Le Roux, 1988). In 1987 seven pathotypes were detected, but pathotype 3SA134 was not isolated (Pretorius and Le Roux, 1988). During 1988 eleven pathotypes were identified, including pathotypes 3SA137, 3SA141, 3SA142 and 3SA143, which were characterised for the first time in South Africa (Pretorius *et al.*, 1990). Pathotype 3SA137 was previously detected in Zimbabwe (Pretorius and Purchase, 1990). With the inception of rust research at the University of the Orange Free State, pathotypes of *Puccinia recondita* f. sp. *tritici* maintained in the Department

of Plant Pathology were assigned UVPrt numbers.

Virulence An isolate of the pathogen is referred to as virulent only in relation to a specific host or host gene (Luig, 1985). It is the ability of the pathogen to overcome a specific gene for resistance (Roelfs *et al.*, 1992). Gordon and Welsch (in Martens, 1985) studied the inheritance of pathogenicity in *P. graminis* f. sp. *avenae*, and found avirulence to be dominant. Johnson (in Martens, 1985) showed virulence in oat stem rust to be dominant and controlled by two pairs of complementary genes. Green (1965) concluded that avirulence in oat stem rust was governed by single dominant genes. Several studies on the inheritance of virulence in leaf rust were done at the Agriculture Canada Research Station in Winnipeg and at North Dakota State University. Generally, virulence were found to be controlled by single recessive genes (Samborski and Dyck, 1968; 1976; Haggag *et al.*, 1973; Statler, 1977; 1979; Statler and Jones, 1981). Knott (1989a) stated that virulence in the leaf rust fungus is more often dominant than in the stem rust pathogen.

Genetic basis of resistance

The genes for leaf rust resistance are named after the first letters (*Lr*) of the common name for the disease. Numbers are used to specify specific genes and lowercase letters designate alleles (Knott, 1989a). Resistance to leaf rust may show several types of expression and modes of inheritance (Knott and Yadav, 1993).

Race-specific and race-nonspecific resistance Two types of disease resistance in plants, vertical and horizontal, were proposed by Vanderplank (1963). Vertical

resistance was defined as being effective against some races but ineffective against others. It is therefore race-specific and is relatively simply inherited (Dyck and Kerber, 1985). In race-specific resistance an interaction between genotypes of the host and pathogen occurs (Knott, 1989a). Vanderplank (1963) defined horizontal resistance as being "evenly spread against all races of the pathogen". Therefore, no differential interaction occurs between genotypes of the host and pathogen and the resistance is race-nonspecific (Knott, 1989a). Race-nonspecific resistance is often polygenically determined (Knott, 1989a). The terms horizontal and vertical arose from the figures used to illustrate the two types of resistance, but the terms specific and non-specific are more descriptive of how the resistance functions (Mackenzie, 1991). The concept of vertical or race-specific resistance is clear, but nonspecific (horizontal) resistance has been subjected to considerable dispute. Several theoretical models for specific and nonspecific resistance were developed (Knott, 1989a). These models illustrated the difficulty in distinguishing between specific (vertical) and nonspecific (horizontal) resistance. Hypersensitive or moderate resistance, and resistance due to genes with an additive or cumulative effect, have been shown to be race-specific (Dyck and Kerber, 1985).

Adult-plant and seedling resistance Resistance that is first visible in older plants is called adult-, mature-plant or postseedling resistance (Dyck and Kerber, 1985). According to Denissen (1993) Zadoks characterised adult-plant resistance as a resistance which is not expressed in the seedling stage but develops with advancing plant age. Conversely, seedling resistance is easily detectable in primary leaves and subsequently through all developmental stages of growth. A summary of characterised

Lr genes, their chromosome location and source are given in Table 1. The genes *Lr12*, *13*, *22a*, *22b*, *34*, *35*, and *37* have been described as adult-plant resistance genes (Roelfs *et al.*, 1992). Robinson (1976) stated that adult-plant resistance is of the horizontal type, but race-specificity has been found for several of the adult-plant genes for resistance including *Lr12* and *Lr13* (Dyck and Kerber, 1985).

The genes *Lr1*, *2a*, *2b*, *2c*, *3a*, *3bg*, *3ka*, *9*, *10*, *15 - 21*, *23 - 26*, *28 - 30*, *32*, *33*, *36*, *38*, *41* and *42* are clearly expressed in primary leaves under normal testing conditions. Virulence to most of these genes has been reported on a global basis (Roelfs *et al.* 1992). Despite the apparent durability of *Lr19*, virulence has recently been confirmed in Mexico (Huerta-Espino and Singh, 1994) and Russia (Krupnov *et al.*, 1995). For certain gene combinations, however, e.g. *Lr9 + Lr24*, no virulence has been detected (Roelfs *et al.*, 1992).

Durability due to gene combinations The controversies associated with the concept of nonspecific (horizontal) resistance led Johnson (1979; 1981b; 1983) to propose the term "durable resistance". Johnson (1981b) defined durable resistance as "resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time, in an environment favourable to a disease or pest". The advantage of this concept is that it describes what has actually been observed but does not imply any underlying cause or genetic basis. Basically, there are two possible reasons for resistance being durable. Firstly, the pathogen is unable to develop virulence or the virulent races are not competitive with the prevalent avirulent races. Secondly, the virulent races do not, for some reason, come into contact with the resistant host (Knott, 1989a).

Table 1. Named genes for leaf rust resistance, genome locations and source as described by Roelfs *et al.* (1992)

<i>Lr</i> gene	Genome location	Source
1	5DL	Malakof
2a	2DS	Webster
2b	2DS	Carina
2c	2DS	Brevit
3	6BL	Democrat
3bg	6BL	Bage
3ka	6BL	Klein Aniversario
9	6BL	<i>Triticum umbellulatum</i>
10	1AS	Lee
11	2A	Hussar
12	4A	Exchange
13	2BS	Frontana
14a	7BL	Hope
14b	7BL	Bowie
15	2DS	Kenya 1-12E-19-J
16	2BS	Exchange
17	2AS	Klein Lucero
18	5BL	Africa 43
19	7DL	<i>Agropyron elongatum</i>
20	7AL	Thew
21	1DL	<i>T. tauschii</i>
22a	2DS	<i>T. tauschii</i>
22b	2DS	Thatcher
23	2BS	Gabo
24	3DL	<i>A. elongatum</i>
25	4A β	Rosen rye
26	1BL-1RS	Imperial rye
27	3BS	Gatcher
28	4BL	<i>T. speltoides</i>
29	7DS	<i>A. elongatum</i>

Table 1 (cont.). Named genes for leaf rust resistance, genome locations and source as described by Roelfs *et al.* (1992)

<i>Lr</i> gene	Genome location	Source
30	4BL	Terenzio
31	4A β	Gatcher
32	3D	<i>T. tauschii</i>
33	1BL	PI58458
34	7D	Terenzio
35	2B	<i>T. speltoides</i>
36	6BS	<i>T. speltoides</i>
37	2AS	<i>T. ventricosum</i>
38	2AL	<i>A. intermedium</i>
39	2DS	<i>T. tauschii</i>
40	1D	<i>T. tauschii</i>
41	1D	<i>T. tauschii</i>
T3		Terenzio
Exch		Exchange
B		Brevit

Interactions between wheat and wheat rusts are complex and the durability of resistance may be affected by several factors. A rust pathogen may have great difficulty to produce a mutant for virulence on a particular gene (Luig, 1983). Resistance may therefore be due to a specific gene combination for which corresponding virulence does not occur in the pathogen (Roelfs *et al.*, 1992). Secondly, the absence of the sexual cycle determines that new gene combinations must be produced either through mutation or possibly somatic recombination (Knott, 1989a). If a cultivar carries two or more genes for resistance for which the rust population in that area lacks the corresponding genes for virulence, two or more simultaneous mutations have to occur in the rust population to produce virulence (Roelfs *et al.*, 1992). The probability of such a mutant arising and becoming established is small (Johnson, 1983; Schafer *et al.* 1984). Alternatively, the virulent rust genotype has to evolve in two or more steps over time (Knott, 1989a).

Green and Campbell (1979) suggested that wheat cultivars with durable resistance to stem rust in Canada contained more genes for resistance and different gene combinations than those that became susceptible. Therefore, breeders used the accumulation or pyramiding of resistance genes into a single host genotype as a method of attaining durable resistance (Pederson, 1988; Van Ginkel and Rajaram, 1993). Johnson (1981b) noted that although resistance in some Canadian wheat cultivars is controlled by as many as five race-specific genes, it does not automatically result in durable resistance. He also noted that the genetic basis of durable resistance may vary and that there is no reason to assume that it should be complex.

Pederson (1988) mentioned that to confirm resistance gene combinations, individual genes must be recognised in progeny tests, either by selecting pathotypes

virulent to one of the genes, or by identifying genotypes with increased resistance compared to their parents. Genes usually act independently when deployed in combination in a single genotype, exhibiting the infection type of the most effective gene (Dyck and Kerber, 1985; Roelfs, 1988a). However, if two genes both condition resistance but not immunity, it may be possible to identify the gene combination by looking for increased resistance (Knott, 1989a). Interactions between leaf rust resistance genes thus occurs, and this phenomenon has been defined as the resulting improvement in resistance, relative to the respective genes (Samborski and Dyck, 1982). Interactions among resistance genes in wheat rust pathosystems have frequently been demonstrated (Schafer *et al.* 1963; Sharp *et al.*, 1976; Samborski and Dyck, 1982; Ezzahiri and Roelfs, 1989; German and Kolmer, 1990; Kolmer, 1992; Kolmer *et al.*, 1993). According to Knott (1989a) knowledge of the genes that are to be combined is essential in the methodology of gene pyramiding. This strategy involves the identification of different sources of resistance and incorporation of these genes into high-yielding, well adapted wheat cultivars (Roelfs *et al.*, 1992).

The genes *Lr13* and *Lr34* are present singly, or combined in many cultivars displaying durable resistance to leaf rust world-wide (Roelfs, 1988a). Kolmer (1992) found that *Lr13* conditioned higher levels of resistance when paired with the seedling genes *Lr2a*, *Lr3ka*, *LrB*, *Lr11*, *Lr16*, *Lr18*, *Lr21*, *Lr30* or *Lr33*. Kolmer *et al.* (1993) also found that combinations of *Lr13* and *Lr16*, and *Lr13* and *Lr34*, expressed similar levels of resistance as the highly resistant cultivars Columbus (*Lr13+Lr16*) and Era and Chris (*Lr13+Lr34*). Previously, Samborski and Dyck (1982) reported a similar interaction between *Lr13* and *Lr16* to produce an incompatible reaction to leaf rust. German and Kolmer (1992) showed that the combination of *Lr13* and *Lr34* in a Thatcher (Tc)

background was more resistant than the single gene lines *Tc/Lr13*, and *Tc/Lr34*. Krupnov *et al.* (1995) reported that from 10 gene combinations tested, only four, viz *Lr23+Lr26*, *Lr13+Lr26*, *Lr23+Lr26+Lr34* and *Lr26+?*, were effective in Russia. A summary of wheat cultivars that remained resistant to leaf rust over a number of years, including their origin, year released, probable source of resistance and *Lr* gene(s) is given in Table 2. This information strongly emphasises the importance of *Lr* gene combinations in durability of resistance. According to Rieger *et al.* (1976), the interaction between complementary genes produces an effect qualitatively distinct from the effects of any of those genes separately. Confirmed instances of complementary genes in the wheat leaf rust pathosystem, where both genes must be present for the expression of resistance, are rare. *Lr27* and *Lr31* were identified by Singh and McIntosh (1984) as complementary genes for rust resistance.

Rate-reducing resistance Certain forms of resistance (e.g. slow rusting, partial resistance) influence the rate of epidemic development by retarding disease progress (Vanderplank, 1963). The opposite would be forms of resistance that reduce the amount of initial inoculum without any affect on the rate of disease development (Mackenzie, 1991). Partial resistance was described by Parlevliet (1978) as resistance "characterized by a reduced rate of epidemic development in spite of a susceptible infection type". Slow rusting in wheat to *P. recondita* has been identified in a number of wheat cultivars (Caldwell *et al.*, 1970; Ohm and Shaner, 1976; Statler *et al.*, 1977; Gavinlertvatana and Wilcoxson, 1978; Kuhn *et al.*, 1978; Shaner *et al.*, 1978; Milus and Line, 1980; Shaner and Finney, 1980). Ohm and Shaner (1976) showed that slow leaf-rusting was less effective after flowering. Latent period, receptivity (number of uredia

Table 2. Wheat cultivars that have remained resistant to leaf rust for a number of years (Roelfs *et al.*, 1992).

Name	Origin	Released	Probable source	
			of resistance	<i>Lr</i> gene(s)
Americano 44d	Uruguay	1918	Land variety	Unknown
Atlas 66	USA	1948	Froncosa	13,+
Chris	USA	1965	Frontana	13,34,+
Centenario	Uruguay	1633	Americano 44d	1,+
Ciano F67	CIMMYT	1967	Chris	13,+
Era	USA	1970	Frontana	10,13,34,+
Froncosa	Brazil	1934	Alfredo Chaves ^a	13,+
Frontana	Brazil	1943	Froncosa	13,34,T3,+
Fronteira	Brazil	1934	Alfredo Chaves ^a	13,+
Gage	USA	1963	Unknown	3,+
Klein Aniversario	Argentina	1945	Americano 44d	13,3ka,+
Klein Cometa	Argentina	1942	Americano 44d	13,+
Klein Lucero	Argentina	1950	Americano 44d	17,+
Klein Progreso	Argentina	1937	Americano 44d	13,+
Klein Rendidor	Argentina	1954	Americano 44d	13,+
Klein Titan	Argentina	1925	Americano 44d	13,3ka,+
Klein Vencedor	Argentina	1925	Americano 44d	13,+
La Prevision 3	Argentina	1935	Americano 44d	13,34,+
La Prevision 25	Argentina	1937	Americano 44d	13,34,+
La Prevision 32	Argentina	1935	Americano 44d	13,34,+
Minter	USA	1949	Unknown	Unknown
Pavon F76	CIMMYT	1976	Ciano F67'S'	1,10,13,+
Redcoat	USA	1960	Surpreza	13,+
Sinvalocho MA	Argentina	1936	Americano 44d	13,+
Sturdy	USA	1960	Sinvalocho MA	12,34
Surpreza	Brazil	1934	Alfredo Chaves ^a	13,+

^a Land variety

per unit leaf area), uredium size, and spore production have been considered important components of rate reducing resistance (Parlevliet and Kuiper, 1977; Shaner and Finney, 1980; Kulkarni *et al.*, 1982; Niks, 1982; Broers, 1989; Broers and Jacobs, 1989; Jacobs, 1989b). Singh *et al.* (1991) observed considerable variation in components of slow rusting in CIMMYT bread wheats.

Wheat genotypes displaying slow leaf rusting usually express longer latent periods, smaller uredia, and fewer uredia per unit leaf area in comparison to susceptible cultivars (Ohm and Shaner, 1976; Broers, 1989; Singh *et al.*, 1991; Das *et al.*, 1993; Knott and Yadav, 1993). Partial resistance not only seems to be responsible for a reduction of mycelium growth, but also leads to abortion of infection structures compared to the growth in a susceptible genotype (Jacobs, 1989b). Jacobs (1989b) reported that latent period and infection frequency did not appear to be under the same genetic control in wheat genotypes partially resistant to *P. recondita* f. sp. *tritici*. Partial resistance is assumed to be durable (Kuhn *et al.*, 1978; Bjarko and Line, 1988; Broers, 1989), because it is inherited polygenically (Parlevliet, 1988) or oligogenically (Broers and Jacobs, 1989) and is temperature insensitive (Parlevliet, 1975). Broers and Jacobs (1989) and Jacobs and Broers (1989) concluded that two to three genes control partial resistance to *P. recondita* f. sp. *tritici* in wheat cultivars they analysed. Gavilertvatana and Wilcoxson (1978) estimated that, depending on the host cultivar, three to 21 genes controlled slow leaf rusting. No case of erosion or loss of partial or quantitative resistance has been reported (Parlevliet, 1993).

Inheritance of resistance Working with yellow rust of wheat, Biffen (Knott, 1989a) provided evidence of monogenic disease resistance in 1905. The findings of discrete,

heritable differences in disease response created doubts about the 1903 bridging-host theory of Ward, which suggested that a pathogen could gradually adapt to a resistant host by passing through taxonomically intermediate hosts (Schafer *et al.*, 1984). The confusion, resulting from the occurrence of physiologic races of pathogens, varying effects of environment on the disease, polygenic inheritance, and the bridging-host theory, was debated for several years before agreement was reached that Mendel's genetic principles were applicable to resistance against cereal rusts (Schafer *et al.*, 1984).

Gene-for-gene concept According to Barret (1985), Flor was the first person to study both the inheritance of pathogenicity in a pathogen and the inheritance of disease reaction in its host. Using flax rust (caused by *M. lini* (Ehrenb.) Desmaz.) and its flax host (*L. usitatissimum* L.), he proved that if a cultivar carried a single gene for resistance, virulence in the pathogen was also conditioned by a single gene (Flor, 1942). Therefore, if resistance in a cultivar was conditioned by two genes, virulence on that cultivar was also conditioned by two genes. Person *et al.* (1962) defined the gene-for-gene concept as follows: "A gene-for-gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population, and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized." Studies on the inheritance of resistance to leaf rust by Dyck and Samborski (1968) in a number of the standard differential cultivars, and on the inheritance of virulence in the pathogen (Samborski and Dyck, 1968; Statler, 1977; 1979) have clearly supported the gene-for-gene theory.

A gene for resistance to wheat rust has no selective advantage unless the pathogen carries the corresponding gene for avirulence, and a gene for virulence has no selective advantage if the host does not carry the corresponding gene for resistance (Knott, 1989a). Most genetic analyses have shown that resistance to rust in wheat is controlled by single dominant genes and virulence in the pathogens is controlled by corresponding recessive genes. Resistance and avirulence are generally dominant, thus probably resulting from the production of active gene products (Barret, 1985). A common theory is that incompatibility (resistance) is a recognition phenomenon involving an active gene product from a resistant host and an active gene product from an avirulent pathogen. Recognition of a pathotype as avirulent requires that the infected plant contains a disease resistance gene and that the complementary avirulence gene occurs in the invading pathogen. If either associate lacks the respective functional allele, recognition and resistance do not occur and the plant becomes diseased (Keen, 1990). A recessive gene for susceptibility in a host can mutate to a dominant gene for resistance during evolution (Knott, 1989a). Mutations from recessiveness to dominance producing an active product are likely to be rare (Barret, 1985).

Standard terminology for host-pathogen interactions was needed. Therefore, Loegering and Powers (1962) proposed that the character of the host be termed its reaction, which could either be resistant or susceptible; the character of the pathogen is its pathogenicity, which could either be virulent or avirulent; and the interaction results in an infection type which may be low (resistance) or high (susceptibility).

Genetic linkage Different resistance genes are frequently clustered and alternative functional alleles often occur at resistance gene loci. Additional recombination may occur between tightly linked resistance genes (Shepherd and Mayo, 1972; Islam *et al.*, 1989), such as when transfers from alien sources of resistance to *P. recondita* f. sp. *tritici* (Gill *et al.*, 1985; Moseman *et al.*, 1985; Valkoun *et al.*, 1986; Dyck and Lukow, 1988; Manisterski *et al.*, 1988) are made. Linked, deleterious genes are, however, often transferred as well (Knott, 1989b). In some cases the resistance was not expressed in the new background due to genomic interactions or dilution at the higher ploidy level (Quinones *et al.*, 1972; The and Baker, 1975; Chevre *et al.*, 1989; Bai and Knott, 1992; Rubiales *et al.*, 1992). The *Sr25* gene for stem rust resistance is linked with *Lr19* and a gene for yellow flour colour (Knott, 1980; McIntosh, 1988), and the gene *Lr24* has always been associated with the presence of *Sr24*. The genes *Lr34* and *Yr18* (a gene for yellow rust resistance) are associated with the distinctive *Ltn* gene for leaf tip necrosis (Dyck, 1991; Singh, 1992). According to McIntosh (1992), Bariana found that VPM1 wheat and its derivatives, Hyak and Madson, carry the linked genes *Sr38*, *Yr17* and *Lr37* on chromosome 2A. According to Dyck and Lukow (1988) the chromosome segment associated with the transferred genes *Lr29* and *Lr37* for rust resistance may also carry genes for increased protein. The leaf rust resistance gene *Lr26* is associated with the genes *Yr9*, *Sr31* and *Pm8* on the 1BL/1RS chromosome translocation. All genetic linkages have been summarised by McIntosh *et al.* (1993). Linkages between genes for rust resistance and genes controlling other characters can be useful when identifying the presence of particular genes for rust resistance in unknown genotypes (Knott, 1989a).

Sources of resistance

The pool of potentially useful genes available to wheat breeders has shrunk because of the replacement of highly variable land races by higher-yielding, pure-line varieties (Sears, 1981; Gill *et al.*, 1985). Considering genetic variability for wheat leaf rust resistance, *T. aestivum* has limited potential as a source of new or unused genes (Martens and Dyck, 1989). Many varieties carry single genes for resistance which increase the potential of disease epidemics caused by virulent pathotypes (Feldman, 1988; Porceddu *et al.* 1988). Furthermore the diversity and the ability of *P. recondita* f. sp. *tritici* to overcome these monogenic sources of leaf rust resistance in wheat require an endless search for new and effective genes (Kloppers *et al.*, 1995).

Transfer of rust resistance from alien species An important reservoir of resistance genes exists among the relatives of cereal crops for wheat improvement (Gerechter-Amitai and Loegering, 1977; Gill *et al.*, 1985; Moseman *et al.*, 1985; Valkoun *et al.*, 1986; Dyck and Lukow, 1988; Manisterski *et al.*, 1988). The incorporation of these genes through induced homoeologous recombination may yield genotypes with new desirable alleles and more favourable gene combinations (Kushner and Halloran, 1984). Although *T. aestivum* has been successfully crossed with some genera of the tribe Triticeae (Pienaar, 1990), transfer of valuable genes from alien species was limited to *Triticum* species, some *Aegilops* species, *Secale cereale*, and two species of *Agropyron* (Valkoun *et al.*, 1990).

A high degree of homology exists between the chromosomes of *T. aestivum* and its diploid progenitors *T. monococcum* and *T. tauschii*. Therefore, the introduction of genes e.g. for disease resistance, from these diploid species to hexaploid wheat, is

possible (Valkoun *et al.*, 1990). Repeated crossing-overs during backcrosses with the recurrent parent, *T. aestivum*, could result in the removal of unwanted linkage with primitive characteristics (Stam and Zeven, 1981). Unfortunately, hybridisations between hexaploid wheat and its A and D genome donors are difficult because of incompatibility (Valkoun *et al.*, 1990). This limitation can be overcome, however, by bridging crosses with tetraploid wheat (Dyck and Kerber, 1970; Kerber and Dyck, 1973; McIntosh *et al.*, 1984.)

Success of transferring a character to wheat from related species depends on the crossability of the species and the fertility of the hybrid, the pairing ability of the chromosomes of the two species and the genetic complexity of the character (Knott, 1989a). Direct hybridisation enables rapid gene transfer to productive cultivars of *T. aestivum* (Valkoun *et al.*, 1990). Other techniques applicable to wide crosses in cereals are premature pollination, hybridising agents, embryo rescue and culture (Mujeeb-Kazi and Kimber, 1985), reciprocal crosses (Knott, 1989b), and chromosome doubling (Mujeeb-Kazi and Asiedu, 1990).

The first example of the transfer of leaf rust resistance to a cereal crop from related species was provided by McFadden (Dyck and Kerber, 1985) who crossed the highly disease-resistant tetraploid wheat Yaroslav emmer with Marquis, resulting in the selections Hope and H-44. Leaf rust genes transferred from wild relatives to wheat cultivars are *Lr9*, *Lr19*, *Lr21*, *Lr22a*, *Lr24*, *Lr26*, *Lr28*, *Lr29*, *Lr32*, *Lr35*, *Lr36*, *Lr37*, *Lr38*, *Lr39*, *Lr40*, and *Lr41* (Table 1) (Roelfs *et al.*, 1992).

Expression of resistance

In wheat, variation in the expression of resistance due to plant age, growth stage or age

of leaf tissue has often been demonstrated (Dyck and Samborski, 1979; Sunderwirth and Roelfs, 1980; Gustafson and Shaner, 1982; Qayoum and Line, 1985; Pretorius *et al.*, 1988a; Jacobs and Kiriswa, 1993). Furthermore, disease development can be influenced dramatically by the environment (Dyck and Johnson, 1983; Tomerlin *et al.*, 1983; Pretorius *et al.*, 1984; Browder, 1985; Pretorius *et al.*, 1988a).

Environmental influence Williams and Johnston (1965) reported that difficulties in race identification could be due to the reactions of hosts and pathogens changing under different environmental conditions, particularly temperature. However, temperature sensitivity can help identifying certain genes that are masked when in combination with other genes. This is particularly useful when combining or pyramiding genes into a single cultivar (Dyck and Kerber, 1985). The expression of many *Lr* genes is affected by temperature (Dyck and Johnson, 1983; Tomerlin *et al.*, 1983; Pretorius *et al.*, 1984; Browder, 1985; Pretorius *et al.*, 1988a). Some genes become inactive at low temperatures, whereas others become ineffective at high temperatures (Kaul and Shaner, 1989). This principle was used by Pretorius *et al.* (1988b) to locate a high-temperature seedling resistance gene in the cultivar Glenlea. The practical use of some of the genes ineffective at high or low temperatures may be limited to cultivars grown in temperate climates (Dyck and Kerber, 1985). Johnson and Schafer (1965) reported that several wheat lines were more resistant at lower temperatures than at higher temperatures. In a review article, Browder (1985) concluded that the expression of parasite: host relationships depends on their interaction in a specific environment.

Certain adult-plant resistance genes are highly influenced by background genotype, temperature, growth stage, light and even sometimes cytoplasmic factors

(Dyck and Kerber, 1985; Kaul and Shaner, 1989). For efficient transfer and use of adult-plant resistance genes, test conditions and races must be well defined (Gupta and Saini, 1993). This was demonstrated for the adult-plant resistance gene *Lr13* which can be detected in adult-plant or seedling stages using different races and temperatures (Gupta and Saini, 1993). Pretorius *et al.* (1984) demonstrated that *Lr13* can be identified in wheat seedlings with certain isolates at high temperatures (25.5°C). Singh and Rajaram (1991) noted that *Lr13* conditioned an infection type X to some pathotypes at 18-22°C. Statler and Christianson (1993) also found that resistance in some lines with temperature sensitive genes was stable only with certain isolates of *P. recondita* f. sp. *tritici*. Drijepondt *et al.* (1991) reported that lines with the genes *Lr33*, *LrT3*, and *Lr34* expressed race-specific characteristics at different temperatures.

Hypersensitivity The hypersensitive response, characterised by rapid death of invaded and neighbouring cells and resulting in macroscopic lesions at infection sites (Tani *et al.*, 1974), is an active defence system occurring in most of the higher plants in response to pathogens (Keen, 1990). This mechanism is typical of major-genic and race-specific resistance for host plants of rust fungi (Heath, 1981a; Heath, 1982; Niks and Dekens, 1991). The use of cultivars that carry genes for hypersensitive resistance has been one of the most effective and economical means of controlling rusts on cereals (Lee and Shaner, 1985a).

Early collapse and death of the host cells at the infection site prevent the further growth of fungal hyphae (Dyck and Kerber, 1985). Tani *et al.* (1974) reported that fungal development was inhibited before the formation of the first haustorium and before host cell necrosis occurred. According to Samborski and Peterson (1960) the

necrotic areas associated with resistance may involve the extensive destruction of leaf tissue around each infection site. Cultivars exhibiting this type of resistance can therefore suffer substantial yield losses due to destruction of photosynthetic tissue. Brown *et al.* (1966) and Ogle and Brown (1971) concluded from their experiments with wheat stem rust that hypersensitivity-induced necrotic tissue does not necessarily inhibit growth of the rust. They found that the extent of necrosis in invaded tissue was not generally correlated with the area colonized. Mayama *et al.* (1975) supported the hypothesis that a necrotic hypersensitive response does not determine the incompatible reaction but that it is only an incidental stress symptom to the disease. Moreover, susceptibility does not imply that there are no necrotic cells associated with infection sites. A few necrotic host cells are usually associated with these colonies, presumably as a result of a general stress reaction (Samborski *et al.*, 1977). Major-gene resistance to cereal rust diseases has in general been non-durable (Knott and Yadav, 1993). The short-lived nature of hypersensitive resistance has, therefore, led to a search for alternative forms of resistance (Nelson, 1978; Wilcoxson, 1981).

Components of resistance MICROSCOPIC COMPONENTS Histological research has shown that the mechanism of resistance in diploid wheat to wheat leaf rust could be either prehaustorial or posthaustorial (Niks and Dekens, 1991). Prehaustorial resistance indicates that sporeling development is arrested before the formation of haustoria (Niks and Dekens, 1991). Usually normal haustorium mother cells develop, but at the site of cell wall penetration a papilla is induced (Niks and Dekens, 1991). Prehaustorial resistance is very common in nonhost interactions (Heath, 1981a, Heath, 1982; Elmhirst and Heath, 1987). Posthaustorial resistance suggests that the fungus

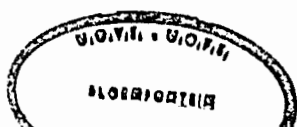
is stopped after the formation of at least one haustorium (Niks and Dekens, 1991). The cells containing the haustoria usually die due to the hypersensitive response. Major genic, race-specific resistance to rust fungi is mostly posthaustorial (Heath, 1981a; Heath, 1982).

It has been debated that prehaustorial resistance is caused by general defence mechanisms in the plant (Heath, 1982). The presumably durable character of nonhost resistance suggests that prehaustorial resistance may be difficult to overcome (Heath, 1981b; Heath, 1982). Furthermore, due to the absence of basic compatibility between the host plant and pathogen, prehaustorial resistance may be longlasting (Parlevliet and Kievit, 1986; Niks and Dekens, 1991). The results of Zhang *et al.* (1993) indicated that prehaustorial resistance to wheat leaf rust was controlled by one recessive major gene, whereas the posthaustorial resistance appeared to be governed by one (semi)-dominant major gene or two dominant complementary genes.

Previous histological studies showed no significant differences in spore germination on resistant or susceptible hosts (Zimmer, 1965; Heath, 1974; Lee and Shaner, 1984). Successful infection depends, among other factors, on spore germination and the location of a stoma near the germ tube (Heath, 1981a). The process of infection seems to involve responses to the leaf topography which might result in the "directional growth" of the germ tube towards the stoma, and the resulting "recognition" of a stoma for appressorium formation (Littlefield and Heath, 1979). It is likely that significant resistance to rust infection might be conferred by "incorrect" pre-penetration behaviour mediated by the surface features of the leaf (Littlefield and Heath, 1979). Less appressorium formation was reported for *P. coronata* f. sp. *avenae* on the sheaths and penduncles, than on the leaves of normally susceptible oats

(Kochman and Brown, 1975). Furthermore, a host-genotype effect was noted on penetration of wheat by *P. recondita* (Brown and Shipton, 1964; Heagle and Moore, 1970; Russel, 1977). Only a few histological studies have been done on pre-penetration behaviour. These studies suggested that adult-plant resistance to *P. striiformis* (Mares and Cousen, 1977), slow rusting of *P. recondita* (Ohm and Shaner, 1976; Gavinlertvatana and Wilcoxson, 1978; Shaner *et al.*, 1978; Shaner and Finney, 1980; Lee and Shaner, 1984), and hypersensitive resistance to *P. recondita* of wheat (Romig and Caldwell, 1964) were not associated with a pre-penetration phenomenon (Heath, 1982).

The actual process of penetration into resistant tissue showed no relation between a reduction in penetration frequency and the degree or type of resistance displayed by the plant (Zimmer, 1965; Ohm and Shaner, 1976; Gavinlertvatana and Wilcoxson, 1978; Shaner *et al.*, 1978; Shaner and Finney, 1980; Heath, 1982; Lee and Shaner, 1984). Infection structures such as the substomatal vesicle, infection hypha and haustorium mother cell developed before the first haustorium invaded a host cell (Heath, 1982) and are, therefore, independent of different resistance types. Abortive penetration was described by Kochman and Brown (1975) and included nonpenetrating appressoria and aborted substomatal vesicles. The inhibition of colony establishment between the formation of appressoria and haustorium mother cells was described by Parlevliet and Kievit (1986) as nonpenetrating appressoria and aborted substomatal vesicles. The formation of substomatal vesicles, infection hyphae and the first haustorium mother cell of *P. recondita* f. sp. *tritici* was unaffected by the presence of the resistance genes *Lr9*, *20* and *28* in wheat (Southerton and Deverall, 1989). Niks (1987) also found that nonpenetrating appressoria and aborted substomatal vesicles as



components played an unimportant role in resistance of barley to *P. hordei* and *P. recondita*.

Resistant hosts often produce at least one haustorium, no matter which infection type eventually develops (Heath, 1982). Some investigations have shown deleterious effects on the fungus during the growth of the infection hypha (Zimmer, 1965; Niks, 1982). A few examples of prehaustorial responses were noted, e.g. the collapse of wheat mesophyll cells adjacent to infection hyphae of *P. graminis* f. sp. *tritici* that have not formed haustoria (Martin *et al.*, 1977); the disorganisation of mesophyll cells near unhealthy-looking infection hyphae of *P. glumarum* (= *P. striiformis*); and the deposition of irregular electron-opaque material along the walls of mesophyll cells of oat leaves next to inhibited infection hyphae of *P. coronata* f. sp. *avenae* (Heath, 1982).

It has been shown with light microscopy that posthaustorial necrosis is usually recognised by the browning, collapse or dye intake of the affected cell (Heath, 1982). Staining with Uvitex 2B (Niks and Dekens, 1991) revealed bright, blue-fluorescing appressoria and haustorium mother cells, whereas the necrotic cells, with a different filter combination, were fluorescing bright yellow. Necrosis of the invaded cells in some interactions might begin soon after the first haustorium is initiated (Rohringer *et al.*, 1979; Niks and Dekens, 1991), whereas in other types of resistance e.g. slow-rusting (Gavinlertvatana and Wilcoxson, 1978) and adult-plant resistance, cells may not become necrotic until several days after inoculation by which time numerous haustoria may have been formed (Mares and Cousen, 1977; Rohringer *et al.*, 1979). Non-invaded cells adjacent to invaded cells may also become necrotic and in some incompatible interactions, a diffusible toxin may be the cause of extensive areas of necrosis beyond the limits of fungal invasion (Mares and Cousen, 1977). The

involvement of such a toxin in host necrosis was shown by Jones and Deverall (1977) in host-rust interactions. Regardless of whether necrosis at the infection site involves cells with or without haustoria, macroscopic lesions develop when tissue damage is extensive. The size and appearance of the lesions vary with different race-cultivar combinations (Heath, 1982). The fungus may remain alive after host necrosis, but this does not mean that it continues growing. There are several reports of host cell death accompanied by the cessation or a reduction in the fungal growth rate (Jones and Deverall, 1977; Rohringer *et al.*, 1979). There are also examples of "ordinary" cultivar resistance, adult-plant resistance and slow rusting where reduced fungal growth can be detected before the beginning of necrosis (Mares and Cousen, 1977; Gavinlertvatana and Wilcoxson, 1978; Rohringer *et al.*, 1979).

Although cell death is usually the most common response of a resistant host to rust infection, it does not occur in all incompatible interactions and there may be other ways in which resistant plants respond to infection (Heath, 1982). Tani *et al.* (1974) showed that host cell necrosis played an insignificant role in the expression of resistance to *P. coronata avenae* on oats. It should be emphasised that the infection sites are rarely identical in any host-rust pathogen combination (Mares and Cousen, 1977; Rohringer *et al.*, 1979). The degree of fungal growth depended on the race-cultivar combination and varied from little or no intercellular growth to production or attempted production of uredia (Heath, 1982; Lee and Shaner, 1984). According to Lee and Shaner (1984), a critical colony size is necessary before uredia are formed. Fungal growth is commonly accompanied by morphological host responses, although these are usually seen after initiation of the first haustorium. Changes in host morphology may be seen in the absence of any recognisable effect on infection

structures, for example the development of hemispherical sediments of callose in mesophyll cells of cowpea adjacent to infection hyphae of *U. phaseoli* var. *vignae* which have normal ultrastructure (Heath and Heath, 1971).

Papillae are wall appositions deposited in localized areas between the plasmalemma and the cell wall at the site of attempted penetration (Aist, 1976). Papillae are heterogeneous in texture and staining properties at the ultrastructural level (Bushnell and Berquist, 1975; Aist, 1976). De Bary was the first to suggest that papillae may function in disease resistance after he noticed the co-occurrence of penetration failure and papillae formation (Smart, 1991). Several studies have correlated resistance (the cessation of fungal growth) with papilla formation in a wide range of host-parasite interactions (Akai *et al.*, 1968; Aist and Israel, 1977a; b; Mayama and Shishiyama, 1978; Aist *et al.*, 1979; Heath, 1979; Ride and Pearce, 1979; Sherwood and Vance, 1980; Allen and Friend, 1983; Coffey and Wilson, 1983). The inducement of papillae is a typical prehaustorial resistance mechanism (Niks and Dekens, 1991) and their presence as a resistance mechanism in cereals to rust diseases has been well documented (Niks, 1986; Jacobs 1989c;d; Niks and Dekens, 1991). Many papillae contain callose which is a substance forming cylinders surrounding the connecting strands in the sieve areas in the phloem (Smart, 1991). At times the sieve plate becomes completely covered by this substance. Mangin found in 1910 that the chemical nature of callose was related to cellulose because the hydrolytic product of callose was glucose and in 1947 Salmon found that it was proteinaceous in nature (Vidhyasekaran, 1988). Callose appears rapidly in the cell walls of parenchyma cells when wounded and becomes visible by fluorescent staining with decolourised aniline blue (Vidhyasekaran, 1988). According to Vidhyasekaran

(1988) callose is the first substance formed in papillae. He demonstrated the presence of callose 10 to 12 hours after inoculation of reed canary grass with the nonpathogen, *Helminthosporium avenae*. His time course study indicated that papillae were synthesized early during attempted penetration. Callose secures or plugs wounds rapidly. Vidhyasekaran (1988) reported that the invasion of root tissue by *Phytophthora cinnamomi* Rands resulted in the production of papillae in resistant maize, but not in susceptible lupin. The papillae showed only callose and no lignin. The callose was found to contain glucan(s) with 1,3-1 and 1,4- β -glucosidic linkages (Vidhyasekaran, 1988).

Whether the fungus is affected before or after haustorium formation seems to be controlled by the specific interaction between pathogen race and host cultivar (Heath, 1982). Both pre- and posthaustorial resistance mechanisms have been described for wheat infected by *P. recondita* f. sp. *tritici* (Heath, 1981a; Heath, 1982; Elmhirst and Heath, 1987; Niks and Dekens, 1991).

MACROSCOPIC COMPONENTS Characterisation of components is important because a combination of such components presumably confers more stable resistance than that based on a single component (Russel, 1978). Parlevliet and van Ommeren (1975) considered latent period to be the most important component. Vanderplank (1963) defined latent period as the time between spore deposition and uredium eruption. The processes of deposition, germination, fungal penetration, and growth within plant tissue until the appearance of uredia are thus included in this period (Teng and Bowen, 1985). Eyal and Peterson (1967) found a decrease in latent period with increasing temperatures on wheat inoculated with five races of *P. recondita*. The growth rate of

leaf rust was strongly influenced by greenhouse temperature (Denissen, 1993). Pretorius *et al.* (1994) noted that the latent period of leaf rust was influenced by host genotype and temperature. Although differences in latent period were not large, it was influenced by growth stage and leaf position (Pretorius *et al.*, 1988a). A long latent period has also been associated with partial resistance to leaf rust (Lee and Shaner, 1985a; Broers and Jacobs, 1989; Jacobs and Broers, 1989).

The inheritance of a longer latent period in cultivars expressing slow rusting was described using Mendelian ratios (Kuhn *et al.*, 1980; Lee and Shaner, 1985a;b). The inheritance of a longer latent period in Suwon 92 is controlled by two partially recessive genes (Kuhn *et al.*, 1980), whereas Lee and Shaner (1985a) reported that one to three genes, depending on the cross, controlled latent period in several other wheat cultivars. Kuhn *et al.* (1980) reported that latent period was negatively correlated with uredium size and suggested that long latent period and small uredium size were possibly controlled by genes in common. This negative correlation was confirmed by Lee and Shaner (1985a).

Uredium size is a sensitive criterion of adult-plant resistance because it has been correlated with slow rusting (Ohm and Shaner, 1976). Differences between resistant and susceptible wheat genotypes emphasise its importance as a component of resistance (Pretorius *et al.*, 1988a). Statler and Jones (1981) determined that pustule length, but not width or area, varied between genotypes. Uredium size is also influenced by temperature, e.g. lines containing *Lr34* developed smaller uredia at low temperatures than at high temperatures (Pretorius *et al.*, 1994).

Uredium density is an important criterion of adult-plant resistance. Pretorius *et al.* (1994) found that uredium density controlled by *Lr34* was not significantly influenced

by temperature. The number of pustules were, however, affected by host developmental stage, host genotype, and the interaction of the two (Pretorius *et al.*, 1988a). Denissen (1993) found no influence of temperature on uredium density in the seedling stage, but in adult plants a significant temperature x genotype interaction occurred. In the barley-*P. hordei* pathosystem, uredium densities varied between partially resistant and susceptible cultivars (Parlevliet and Kuiper, 1977). Similar results for uredium density were obtained in wheat leaf rust studies (Ohm and Shaner, 1976; Milus and Line, 1980; Singh *et al.*, 1991; Das *et al.*, 1993). Drijepondt and Pretorius (1989) found that uredium numbers were more affected by differences in leaf age than the other macroscopic components.

Breeding for resistance

The development of resistant cultivars is the most cost-effective method of biological control of the rusts (Knott, 1989a). The use of resistant cultivars adds no extra cost to farmers because there are no chemicals to buy or additional cultural operations to carry out (Hogenboom, 1993). Although resistant cultivars were known as early as 1841, breeding for resistance did not become common until the 1900's (Roelfs, 1985). The rediscovery in 1900 of Mendel's laws of segregation (Klug and Cummins, 1994) and independent assortment of genes inspired genetic evaluation of plant resistance data (Schafer *et al.*, 1984). The first report was by Rowland Harry Biffen in 1905 at Cambridge. He crossed the stripe rust-susceptible wheat cultivar Red King with the resistant cultivar Rivet. All F_1 plants were susceptible. In the F_2 generation he found 195 infected and 64 rustfree plants, fulfilling a 3:1 prediction of Mendelian genetics, with susceptibility being dominant (Knott, 1989a). In 1907 he also found that resistance

and susceptibility were independent of other plant characteristics (Knott, 1989a). Within a few years several examples of Mendelian inheritance in wheat were found (Knott, 1989a). One of the first successes in resistance breeding was the transfer of stem rust resistance from emmer to bread wheat by McFadden in 1930. This resulted in the cultivar Thatcher, which is still used in wheat breeding (Roelfs, 1985).

In most wheat-producing countries breeding for resistance to the rusts seems to be a never-ending, repeating cycle. The resistant cultivars are developed and released, their production increases rapidly, and new, virulent races of the rust appear. Thereafter, the breeding cycle is repeated (Knott, 1989a). According to Kilpatrick (in Roelfs *et al.*, 1992) this failure of resistance over the short term has led to a boom-and-bust syndrome. This is typical of resistance based on a single host gene rendered ineffective by shifts in pathogen virulence (Wiese, 1987). Therefore, wheat breeders are interested in types of resistance and methods of resistance management that will result in long-lasting control of rusts (Knott, 1989a). Multigenic resistance (controlled by combinations of specific genes) may be stable for years (Wiese, 1987). A series of diverse cultivars used in North America has been undamaged by leaf rust for more than 30 years (Roelfs, 1978).

The success of breeding is affected by many factors such as the host (e.g., the type of resistance used), the pathogen (e.g., the life cycle of the rust in the area), the environment (e.g., the favourableness of the weather for rust development), and man (e.g., germ plasm selection) (Knott, 1989a). Many breeding systems have been or are being directed depending on the specific situation in that area.

Breeding systems PEDIGREE SYSTEM The standard pedigree system has been used

in several breeding programmes for rust resistance (Knott, 1989a). The name "pedigree system" refers to the fact that pedigrees of each line are maintained during the breeding process (Moreno-González and Cubero, 1993). The basic pedigree system can be modified in various ways for specific objectives, particular situations and available resources (Knott, 1989a). According to Bingham and Lupton (1987), wheat breeders at the Plant Breeding Institute, Cambridge, England use a pedigree system with intensive selection and early generation yield and quality testing. About 2000 plants are grown from each cross (\pm 1200 crosses made) in F_2 , with a total of about two million plants in this generation. From these, approximately 50 are selected on the basis of leaf rust resistance and agronomic characters in the ensuing generations.

BULK SYSTEM The early generations are planted in bulk at normal seeding rates and the material is allowed to evolve through natural selection. For rust resistance, the system can be modified by growing the bulks in a nursery. An early, heavy rust epidemic will cause shrivelling of the seeds produced on susceptible plants. When a bulk sample of seeds are planted these shrunken seeds should have reduced viability and produce weaker, less competitive plants. The speed of evolution towards resistance will depend on the severity of rust epidemics (Knott, 1989a). McFadden (in Knott, 1989a) used this method for selecting plump, bread wheat type kernels from disease resistant plants subjected to a heavy rust epidemic.

BACKCROSSING The use of backcross breeding to transfer genes for resistance into a cultivar is particularly useful if an otherwise desirable cultivar lacks rust resistance (Knott, 1989a). Backcross lines containing new resistance genes could be released

as new cultivars when existing lines become susceptible (Johnson and Lupton, 1987). Genes for specific rust resistance are often dominant and can be backcrossed into a recurrent parent. Plants are tested with the appropriate rust race each generation and resistant ones selected for a further cycle of backcrossing (Bingham and Lupton, 1987). With recessive genes, a cross and a backcross are made and the progeny selfed to recover the resistance. Another backcross can be made and the process repeated (Knott, 1989a).

When resistance is dominant, clearly identifiable and simply inherited, its inheritance may be determined directly from the segregation within F_1 plants from a backcross. Normally it is desirable or even essential to test F_2 families from the backcross. By determining the number of genes for resistance, it is necessary to decide whether each family is segregating or susceptible. If only one gene is involved, the ratio will be 1 segregating :1 susceptible family, for two genes 3:1 and for three genes 7:1. The segregation patterns within segregating families often provide additional information on the type of gene action involved (Knott, 1989a).

If a single gene is involved, infection types on the F_1 and backcross F_1 plants will indicate whether the gene is dominant or recessive. If two linked genes are involved, the segregating families will be of three types depending on whether they are segregating for the first gene, the second gene, or both genes. Whether the three types of families can be distinguished depends on whether the genes are dominant or recessive and whether they produce clearly distinguishable infection types. The situation becomes more complex if the genes show incomplete dominance (Knott, 1989a). When three genes are involved, there are seven types of segregating backcross F_2 families, three segregating for one gene, three segregating for two genes,

and one segregating for all three genes. It is unlikely that all seven types can be identified. Nevertheless, the ratio of segregating to susceptible families and the observed segregation within families should indicate the number of genes involved (Knott, 1989a).

Quantitative resistance controlled by several genes is more difficult to transfer by backcrossing. A cross and a backcross are made, the material selfed and selection carried out for several generations until the resistance has been recovered. Thereafter, two further backcrosses are made followed by selfing and selection. The number of required backcrosses depends on how completely the recurrent parent must be reconstituted. Two to three backcrosses are usually adequate to produce lines that are morphologically comparable to the recurrent parent (Bingham and Lupton, 1987).

An obsolete recurrent parent can justifiably be used when backcrossed only once or twice. This system of partial or incomplete backcrossing is used by the CIMMYT wheat breeding programme in Mexico to transfer resistance genes into a desirable genetic background. Backcrossing is a conservative procedure and the basic objective is to change one character in an otherwise acceptable cultivar. Many of the current Canadian cultivars are backcross derivatives of Thatcher (Knott, 1989a).

Selection strategies

The success of breeding for disease resistance depends on an appropriate method of selection (Johnson, 1992). Screening for resistance under field conditions is representative of commercially grown crops and relatively inexpensive (Niks *et al.*, 1993). In greenhouses the environmental conditions are highly controlled but the situation is less representative of actual growing conditions (Niks *et al.*, 1993). In

controlled environments, furthermore, factors such as inoculum type and distribution can be manipulated. It is also possible to screen plants independent of the growing season (Niks *et al.*, 1993).

Screening can be done by assessing disease severity on leaves. These ratings are based on the modified Cobb scale (Peterson *et al.*, 1948) and are usually accompanied by a host reaction type. Four reaction types, viz resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) are usually distinguished (Peterson *et al.*, 1948). Scoring of reaction types to leaf rust can be done on a designated leaf or the whole plant. In the field, leaf rust severity is usually evaluated by means of a single observation on the flag leaf. Flag leaf infections are generally a reflection of earlier disease development and yield loss is most closely related to severity on the uppermost leaf (Seck *et al.*, 1985). Several factors such as variation in plant development among cultivars or breeding lines, interplot interference, the amount of inoculum, time of assessment, and environmental conditions influence severity ratings in the field. These factors should therefore be taken into account to interpret the possible overestimation or underestimation of resistance (Niks *et al.*, 1993).

When plants are screened for resistance to leaf rust under controlled-environment conditions, an infection type scale (Table 3) is often used (Roelfs 1988b). Infection types 0 to 2 are usually considered to indicate resistance and 3 to 4 susceptibility.

Molecular markers According to Kochert (1994), Botstein *et al.* suggested that genetic maps could be constructed by using pieces of chromosomal DNA as direct

Table 3. Host response and infection type descriptions used in wheat leaf rust evaluation (Roelfs 1988b).

Host response (class)	IT ^a	Disease symptoms
Immune	0 low	No uredia or macroscopic sign of infection
Nearly immune	; low	No uredia, but necrotic or chlorotic flecks
Very resistant	1 low	Small uredia with necrotic border
Moderately resistant	2 low	Small to medium uredia with chlorosis or necrosis
Heterogeneous	X low	Random distribution of variable-sized uredia
Heterogeneous	Y low	Variable-sized uredia, decreasing in size with distance from the leaf tip
Heterogeneous	Z low	Variable-sized uredia, decreasing in size with distance from the leaf base
Moderately susceptible	3 high	Medium-sized uredia
Susceptible	4 high	Large uredia without chlorosis or necrosis

^a The infection types (ITs) are often refined by modifying characters as follows: =, uredia at lower size limit; -, uredia somewhat smaller than normal; +, uredia somewhat larger than normal; ++, uredia at the upper size limit; C, more chlorosis than normal; and N, more necrosis than normal for the infection type.

markers for the segregation pattern of chromosome segments. The first DNA markers were produced by restriction enzyme digestion (Kochert, 1994). Thereafter, it was determined that restriction fragments from a given chromosomal locus usually varied in size (or length) in separate individuals of the same or different species. These differences were named restriction fragment length polymorphisms (RFLPs). RFLP has been shown to be a powerful tool for establishing linkage relationships to important qualitative and quantitative genes (Paterson *et al.*, 1991). Random amplified polymorphic DNA (RAPD), which is based on random priming and used almost exclusively in plant molecular mapping, has also been applied in finding new markers linked to a specific locus (Burr, 1994).

Near-isogenic lines differing in specific traits have been successfully used to isolate RFLP markers for the gene determining that specific trait. This approach has been successful in detecting markers for several important disease resistance genes, e.g. the *Tm-2a* virus resistance gene in tomato (Yang and Tanksley, 1989), the *Ml-a* (Schüller *et al.*, 1992) and the *rpg4* genes in barley (Borovkova *et al.* 1995), the *Ht1* gene in maize (Bentolila *et al.* 1991), the *Pc91* and *Pc92* genes in oats (Rooney *et al.*, 1994), *Pm3* (Hartl *et al.* 1993), *Lr9* (Schachermayr *et al.*, 1994), and *Lr9*, *Lr19*, *Lr24* and *Lr32* (Autrique *et al.*, 1995) in wheat. The RAPD technique has been successfully utilized to identify a molecular marker for the disease resistance genes *Pg3* (Penner *et al.*, 1993a) and *Pc68* in oats (Penner *et al.*, 1993b), whereas biochemical markers were used to indicate the linkage between the *Lr19* leaf rust resistance gene and the gene *Ep-D1d* which encodes endopeptidase phenotype EP-D1d (McMillan *et al.*, 1993).

The success of molecular markers depends largely on naturally occurring polymorphisms (Burr, 1994). The low level of DNA polymorphism in certain crop

species, such as *T. aestivum* (Kam-Morgan *et al.*, 1989), is the greatest barrier to the use of RFLP and RAPD technology for marker-aided selection in plant breeding (Ohm and Mackenzie, 1992). Devos and Gale (1992) found that the level of polymorphism in wheat, obtained with RAPD analysis, is as low as with conventional RFLP analysis and therefore not worthwhile for the construction of linkage maps.

Linked RFLP markers can be used to identify leaf rust resistance genes and to generate new combinations in breeding populations, especially when virulence is lacking (Autrique *et al.*, 1995). Markers can allow the breeder to identify and evaluate genealogies of important lines as well as to estimate the relation among breeding material (Tanksley *et al.*, 1989). Combining genes is a principal strategy for long term rust control (Johnson, 1983) and because genetic mechanisms thereof are poorly understood, mapping the different genes for resistance by RFLPs may provide a tool for detecting genes and their contribution to durable resistance (Autrique *et al.*, 1995). DNA markers can therefore significantly accelerate breeding and may be useful in identifying the desired progeny that possess multiple gene combinations in gene pyramiding (Mackenzie, 1991).

MATERIALS AND METHODS

VIRULENCE OF SOUTH AFRICAN LEAF RUST PATHOTYPES TO *Lr12* AND *Lr13*

WHEAT GENOTYPES AND GROWING CONDITIONS

To assess the potential of *Lr12* and *Lr13* in breeding for leaf rust resistance in South Africa, the adult-plant expression of these genes to different pathotypes of *P. recondita* f. sp. *tritici* was determined. Five seeds of the wheat lines RL6011 (Thatcher*6/Exchange [*Lr12*]) (Dyck and Kerber, 1970), CT263 (Thatcher/*Lr13*) (Pretorius *et al.* 1984), and the leaf rust-susceptible recurrent parent Thatcher, were planted per 1-liter-capacity pot in sterilised soil, respectively. To allow inoculation with different pathotypes, 14 sets of host plants were planted. Plants were grown in a leaf rust-free air conditioned glasshouse cubicle where a day/night temperature cycle of $26.5 \pm 1.2^{\circ}\text{C}/16.8 \pm 0.8^{\circ}\text{C}$ was maintained. Natural daylight was supplemented with $120 \mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetically active radiation emitted by cool-white fluorescent tubes, arranged directly above plants, for 14 h each day. Seven days after planting, and three times weekly thereafter, 50 ml of 3 g/l hydroponic nutrient solution (6.5-2.7-13 N-P-K plus micro-elements), were added as a soil drench per pot. Fertilisation continued until the experiment was terminated.

INOCULATION AND INCUBATION

Prior to inoculation, leaf rust pathotypes (Table 4) were multiplied in isolation on seedlings of selective wheat hosts. Emerging seedlings planted for rust multiplication were drenched with 50 ml per 10-cm plastic pot of a 0.3 g/l maleic hydrazide solution

Table 4. Avirulence/virulence^a combinations of pathotypes of *Puccinia recondita* f.sp. *tritici* used for inoculation of trials

Pathotype	Leaf rust resistance (<i>Lr</i>) genes ^b	Selective hosts ^c
UVPrt2	<i>Lr1,2a,2b,3ka,11,15,17,20,24,26,30/2c,3a,3bg,10,14a,16</i>	Zaragoza
UVPrt3	<i>Lr3a,3bg,3ka,10,11,14a,16,17,20,26,30/1,2a,2b,2c,15,24</i>	Agent
UVPrt4	<i>Lr1,2a,2b,3bg,11,15,16,17,24,26/2c,3a,3ka,10,14a,20,30</i>	Thew
UVPrt5	<i>Lr1,2a,3bg,10,11,14a,15,17,24,26/2b,2c,3a,3ka,16,20,30</i>	Thew
UVPrt6	<i>Lr3a,3bg,3ka,11,16,20,24,26,30/1,2a,2b,2c,10,14a,15,17</i>	RL6052
UVPrt7	<i>Lr3a,3bg,3ka,10,11,14a,16,17,20,24,26,30/1,2a,2b,2c,15</i>	RL6052
UVPrt8	<i>Lr3a,3bg,3ka,11,16,20,26,30/1,2a,2b,2c,10,14a,15,17,24</i>	Agent
UVPrt9	<i>Lr2a,2b,3bg,15,16,17,26/1,2c,3a,3ka,10,11,14a,20,24,30</i>	Karee
UVPrt10	<i>Lr3a,3bg,3ka,10,11,16,20,24,26,30/1,2a,2b,2c,14a,15,17</i>	RL6008
UVPrt12	<i>Lr3a,3bg,3ka,11,16,20,24,30/1,2a,2b,2c,10,14a,15,17,26</i>	RL6078
UVPrt13	<i>Lr3a,3bg,3ka,11,16,20,30/1,2a,2b,2c,10,14a,15,17,24,26</i>	RL6078
UVPrt14	<i>Lr2a,2b,3a,3bg,3ka,11,15,16,17,20,24,26/1,2c,10,14a,30</i>	RL6049
UVPrt15	<i>Lr2a,2b,3a,3bg,3ka,11,15,16,17,20,26/1,2c,10,14a,24,30</i>	Agent
UVPrt16	<i>Lr1,2a,2b,3bg,3ka,10,11,14a,15,16,17,20,26/2c,3a,24,30</i>	Agent

^a Avirulence/virulence characteristics determined at 18-24 °C.

^b South African leaf rust differentiating genes.

^c Selective hosts on which pathotypes were increased.

to retard plant development and enhance sporulation (Knott, 1989a). Seven-day-old seedlings were spray-inoculated (approximately 1mg spores /ml oil) with single pustule-derived isolates suspended in light mineral oil (McSherry and Harris, Wedmore, Somerset, UK) and kept in darkness in a dew-simulation chamber at 18-21°C for 16 h. Seedlings were then placed in isolation cabinets on a glasshouse bench at 18-25°C, with 14 h of supplemental fluorescent light of 120 $\mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetically active radiation emitted by cool-white fluorescent tubes, arranged directly above plants.

Flag leaves of plants were spray-inoculated with freshly harvested spores of each of the South African pathotypes UVPrt2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, and 16 of *P. recondita* f. sp. *tritici*. Urediospores were suspended with the aid of Tween 20 in distilled water (1 mg spores/ml) and applied to adaxial leaf surfaces using a spray nozzle and compressed air propellant. After inoculation, plants were incubated in the dark in a dew simulation chamber for 16 h at 20-23°C.

POST-INOCULATION MAINTENANCE OF ADULT PLANTS

Upon removal from the chamber, plant surfaces were allowed to dry for 2 h at room temperature in fan-circulated air before plants were transferred to a glasshouse and maintained as described for the pre-inoculation period. Infection types were determined according to the 0 to 4 scale 15 days post-inoculation (d.p.i.) (Roelfs, 1988b).

SELECTION OF LINES CONTAINING *Lr12+Lr13* GENE PAIRS

DETECTING *Lr13* IN SEEDLINGS

To identify families homozygous for *Lr13*, seeds of 52 CT263 x RL6011 and 52 Thatcher x CT263 F₃ families were planted (45 seeds/10 cm pot) in a 1:1 v/v sterilised soil/peatmoss mixture, and grown at continuous 20°C in a growth chamber. To test for possible expression of *Lr12* in seedlings of the F₃ lines, 49 Thatcher x RL6011 F₃ families were included. A 14 h daylength of 200 $\mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetically active radiation was provided by fluorescent tubes and incandescent bulbs arranged 30 cm above plants. All genetic stocks were previously developed as part of an ongoing wheat leaf rust research programme in the Department of Plant Pathology, University of the Orange Free State.

Seven days after planting, the primary leaves of CT263 x RL6011, Thatcher x CT263 and Thatcher x RL6011 families were spray-inoculated with freshly harvested spores of pathotype UVPrt2 (Table 4) of *P. recondita* f. sp. *tritici* suspended (1 mg spores/ml) in light mineral oil. On seedlings, UVPrt2 is avirulent to *Lr13* and virulent to *Lr12* at 25°C. After inoculation, seedlings were allowed to dry for at least 1 h to prevent damage to leaves saturated with oil. Thereafter, the plants were placed in the dark in a dew simulation chamber at 20-24°C for 16 h. After incubation, plant surfaces were dried for 2 h at room temperature in fan-circulated air, transferred to leaf rust-free growth chambers and maintained at 25°C. The lighting in the growth chambers was similar to that describe for the pre-inoculation period.

Disease reactions were rated according to a 0 to 4 rust infection type scale (Roelfs, 1988b) nine d.p.i. Segregation ratios were determined and tested for

monogenic inheritance of resistance using the chi-square test.

DETECTING *Lr12* IN ADULT PLANTS

To identify the families homozygous for *Lr12*, 30 seeds of each CT263 x RL6011 and Thatcher x RL6011 F₃ family were planted (15 seeds/1-liter-capacity pot) in sterilised soil. Thatcher x CT263 F₃ families were included to detect possible residual expression of *Lr13*. Glasshouse temperatures, supplemental illumination and fertilisation of plants were similar to the procedures described previously.

The flag leaves of CT263 x RL6011 and Thatcher x RL6011 plants were spray-inoculated with freshly harvested spores of pathotype UVPrt13 (Table 4) of *P. recondita* f. sp. *tritici* suspended (1 mg spores/ml) in distilled water. Thatcher x CT263 F₃ families were similarly inoculated with UVPrt13 to investigate potential residual effects of *Lr13*. On adult plants, UVPrt13 is avirulent to *Lr12* and virulent to *Lr13*. Inoculation and incubation procedures were similar to those described earlier. Infection types were determined according to the 0 to 4 scale 15 d.p.i. and the distribution of resistant and susceptible classes tested for monogenic inheritance using the chi-square test.

CT263 x RL6011 F₃ families homozygous for both *Lr12* and *Lr13* were identified and retained for further investigations. Similarly, one Thatcher x CT263, and one Thatcher x RL6011 F₃ family, homozygous for either *Lr13* or *Lr12*, were selected.

SEEDLING REACTION OF GENE COMBINATION LINES

GENOTYPES, GROWING CONDITIONS AND INOCULATION PROCEDURES

To determine infection types of the CT263 x RL6011 gene combination lines,

approximately 10 seeds of each of the F₃ families 3, 9, 19 and 40 were planted in 10-cm pots containing a 1:1 v/v sterilised soil/peatmoss mixture. The selected Thatcher x CT263 and Thatcher x RL6011 families, CT263, RL6011 and Thatcher were also included. All seedlings were grown at continuous 20°C in a growth chamber. A 14 h daylength of 200 $\mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetically active radiation was provided by fluorescent tubes and incandescent bulbs arranged 30 cm above plants.

Seven days after planting the primary leaves of two replicate sets were spray-inoculated as described previously with freshly harvested spores of the South African pathotypes UVPrt2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, and 16 of *P. recondita* f. sp. *tritici* (Table 4). After a 16 h dew chamber cycle at 20-22°C, one set was transferred to a growth chamber at 15°C and the other to a similar chamber at 25°C. The lighting in the growth cabinets was the same as described for the pre-inoculation period. Disease reactions were rated according to a 0 to 4 rust infection type scale (Roelfs 1988b) 10 d.p.i. for plants kept at 25°C and 14 d.p.i. for those at 15°C.

ADULT-PLANT REACTION OF GENE COMBINATION LINES

MICROSCOPIC COMPONENTS OF RESISTANCE

Wheat genotypes, growing conditions and inoculation procedures

The expression of histological components of resistance to pathotypes UVPrt2 and UVPrt13 was studied in the wheat lines listed in Table 5. Two seeds of each line were planted in 1-liter- capacity plastic pots containing sterilised soil. All treatments were replicated three times and the entire experiment was repeated in a similar, replicated study. Plants were maintained in leaf rust-free, air conditioned glasshouse cubicles.

Table 5. Host genotypes used to study the expression and inheritance of the *Lr12* and *Lr13* genes for resistance to *Puccinia recondita* f.sp. *tritici*

Genotypes	<i>Lr</i> gene(s)
Thatcher (Tc)	Susceptible control
RL6011 ^a	<i>Lr12</i>
CT263 ^a	<i>Lr13</i>
CT263 x RL6011	
Family 3 F ₃	<i>Lr12,Lr13</i>
Family 9 F ₃	<i>Lr12,Lr13</i>
Family 19 F ₃	<i>Lr12,Lr13</i>
Family 40 F ₃	<i>Lr12,Lr13</i>
Tc x RL6011 F ₃	<i>Lr12</i>
Tc x CT263 F ₃	<i>Lr13</i>

^a Near-isogenic Thatcher lines

Temperatures during the first experiment were $14.0 \pm 0.5^{\circ}\text{C}$ (night) and $23.7 \pm 2.5^{\circ}\text{C}$ (day), whereas during the second experiment they were $15.2 \pm 0.5^{\circ}\text{C}$ (night) and $27.4 \pm 1.3^{\circ}\text{C}$ (day).

Freshly harvested urediospores were suspended in light mineral oil. A standardised concentration for each of pathotypes UVPrt2 and UVPrt13 (40×10^4 urediospores/ml oil) was prepared using a haemocytometer and light microscope ($\times 100$). A 3-cm-long area on the adaxial surface of each of six flag leaves per genotype, never closer than 2 cm to either the base or the tip of the leaf, was marked and inoculated using a modified, vertically spraying inoculation device (Andres and Wilcoxson, 1984). A control system, allowing electronic activation and termination of the sources of air pressure required during inoculum application, was fitted to the apparatus. This improved reproducibility of uniform spore deposition between sprays. Using lacquer-coated glass slides, calibration of the inoculation device revealed that 99 ± 4 spores of UVPrt2, and 112 ± 12 spores of UVPrt13, were deposited per square centimetre in the first experiment. In the second experiment, 138 ± 4 spores of UVPrt2, and 129 ± 6 of UVPrt13 were applied. Microscopic examination of spore suspension droplets on 2% water agar plates, incubated in the dark at 20°C for 3 h, indicated a minimum of 99.5% germination for pathotypes UVPrt2 and UVPrt13 in both experiments.

Plants were gradually dried for at least 2 h under fan-circulated air at room temperature to prevent damage to oil saturated leaves. Thereafter, plants were placed in the dark in a dew-simulation chamber for 16 h at $19\text{--}22^{\circ}\text{C}$ (experiment 1), and $20\text{--}23^{\circ}\text{C}$ (experiment 2). Following removal of plants from the chamber, foliage was gradually dried for 2 h in fan-circulated air at room temperature before they were

transferred to a glasshouse. Plants were maintained in a 6.5 m² air-conditioned glasshouse cubicle where mean temperatures of 14.0 ± 0.5°C (night) and 23.7 ± 2.5°C (day) (first experiment), and 15.2 ± 0.5°C (night) and 27.4 ± 1.3°C (day) (second experiment) were maintained. Illumination and plant nutrition were as described previously.

Fluorescence microscopy

Sample preparation and staining Two leaves per pathotype/host combination were sampled at 64 and 240 h post-inoculation (h.p.i.) and cut into 1-2 cm long segments. Leaf segments were cleared and fixed in ethanol:dichloromethane (3:1 v/v) + 0.15% trichloroacetic acid for 24 h. Specimens were then washed twice in 50% ethanol for 15 min, twice for 15 min in 0.05 M sodium hydroxide, and rinsed three times with water before being submerged in Tris[hydroxymethyl]aminomethane/hydrochloric acid (pH 5.8) and stained for 5 min in 0.1% diethanol (Uvitex 2B, Ciba-Geigy AG) (Niks and Dekens, 1991) in the preceding buffer. This was followed by rinsing four times with water and washing with 25% aqueous glycerol for 30 min. Stained leaf sections were then stored in 50% glycerol containing a trace of lactophenol to prevent deterioration of fungi and drying of material.

Microscopic examination Leaf sections were used as whole mounts for fluorescence microscopy (Rohringer *et al.*, 1977; Kuck *et al.*, 1981). Observations on 20 randomly selected infection sites on each of five leaf segments were carried out at X100 or X400 with a Nikon Optiphot epifluorescence microscope. The filter combinations UV-1A (excitation filter 330-380 nm and barrier filter 420nm) for fungal structures, and B-2A

(excitation filter 450–490 nm and barrier filter 520 nm) for autofluorescence measurements were used. Only infection sites where appressoria had formed over stomata were studied to determine the proportion of sites where substomatal vesicles, infection hyphae and haustorium mother cells occurred. Parlevliet and Kievit (1986) classified 'abortive penetration' (AP) as sporelings that did not develop beyond the appressorial or substomatal vesicle phases. In the present study all nonpenetrating appressoria (NPA) and atypical substomatal vesicles (aborted substomatal vesicles [ASSV]) were considered aborted. All fungal structures except haustoria fluoresced a bright light-blue colour. Haustorium mother cells fluoresced extremely brightly. Host cells fluorescing an orange-yellow colour were considered necrotic, whereas unaffected healthy cells did not fluoresce (Rohringer *et al.*, 1977). Measuring of the hyphal length from the substomatal vesicle to either the hyphal tip, or initiation of a haustorium mother cell, was done with a calibrated eyepiece micrometer at X400 magnification, whereas the number of haustorium mother cells was counted at X100 magnification and confirmed at X400 where necessary.

Two dimensions of fungal colonies and of the necrotic leaf area were measured at X100 with a calibrated eyepiece micrometer and corresponding areas (mm^2) calculated according to the formula $\pi \times \text{length} \times \text{width}$. A hypersensitivity index was calculated by dividing the necrotic leaf area (mm^2) with the corresponding colony size (mm^2). Uredium formation was calculated on the leaf sections sampled 240 h.p.i. as the percentage infection sites that resulted in sporulating colonies. Uredium size was determined in a similar manner as for colony size. Coalescing colonies were excluded from measurements.

Phase contrast microscopy

Sample preparation and staining Two leaves per pathotype/host combination were sampled at 64 and 240 h.p.i. and cut into 1-2 cm long segments. Leaf segments were cleared and fixed in ethanol:dichloromethane (3:1 v/v) + 0.15% trichloroacetic acid for 24 h before they were boiled for 5 min in a 0.03% solution of Trypan blue in lactophenol:ethanol (1:2 v/v). Specimens were then cleared by immersing them for 24 h in a saturated solution of chloral hydrate (5:2 w/v) and storing in 50% glycerol with a trace of lactophenol. To study cell wall appositions, specimens were transferred through a series of 80% (30 min), 90% (30 min) and 100% (2 x 30 min) ethanol for dehydration. Thereafter they were stained with a saturated solution of picric acid in methyl salicylate for 5 min (Niks, 1986). Stained leaf segments were mounted with adaxial sides upwards in methyl salicylate under cover slips sealed with nail varnish.

Microscopic examination Leaf sections were used as whole mounts for phase contrast microscopy. Screening of leaf segments for detection of infection sites were conducted at X100. Detailed observations of haustoria, and cell wall appositions visible as luminous structures, were conducted at X1000 (oil immersion). All colonised stomata observed in three leaf sections were studied by counting the number of haustoria and bright cell wall appositions in contact with hyphal tips or haustorium mother cells.

MACROSCOPIC COMPONENTS OF RESISTANCE

Wheat genotypes, growing conditions and inoculation procedures

The effect of the interaction between *Lr12* and *Lr13* on three components of resistance

(latent period, uredium density, and uredium size) to pathotypes UVPrt2 and UVPrt13 was studied. Two seeds of each of the genotypes listed in Table 5 were planted in 1-liter-capacity plastic pots containing sterilised soil. All treatments were replicated three times and the entire experiment was repeated in a similar, replicated study. Plants were grown in leaf rust-free, air-conditioned glasshouse cubicles. During the first and second experiments day/night temperature variation of $20.8 \pm 1.1^\circ\text{C}/13.2 \pm 0.3^\circ\text{C}$, and $26.2 \pm 0.5^\circ\text{C}/14.2 \pm 0.4^\circ\text{C}$, was recorded.

The adaxial surface of six flag leaves per wheat genotype was quantitatively inoculated with freshly harvested spores of either pathotype UVPrt2 or UVPrt13. All inoculation and incubation procedures were similar to those described for the measurement of microscopic components. Calibration of the inoculation device showed that 108 ± 2 spores of UVPrt2 and 107 ± 3 of UVPrt13 were deposited per square centimetre in the first experiment. In the second experiment, 162 ± 20 spores of UVPrt2 and 172 ± 8 of UVPrt13 were applied. Microscopic examination of spore suspension droplets on 2% water agar plates, incubated in the dark at 20°C for 3 h, indicated a minimum of 98% germination for pathotypes UVPrt2 and UVPrt13 in both experiments. On completion of the dew chamber and subsequent drying cycle, plants were returned to the glasshouse. Mean post-inoculation temperatures of $13.2 \pm 1.1^\circ\text{C}$ (night) and $20.8 \pm 1.1^\circ\text{C}$ (day) were recorded during the first experiment, and $15.2 \pm 0.5^\circ\text{C}$ (night) and $27.4 \pm 1.3^\circ\text{C}$ (day) during the second experiment. Illumination and plant nutrition were as described previously.

Components of resistance

Components of resistance were measured where uredia of pathotypes UVPrt2 and

UVPrt13 developed.

Latent period The inoculated area was inspected daily, and when uredia became visible as erumpent structures, those within the marked area were counted. Counting continued each day until the logarithmic phase of primary uredium eruption had passed. A final count was made 2 d later. The log number of uredia visible within the specified area at each observation was plotted against time (h). Using the linear regression equation, latent period was then calculated as the number of hours after inoculation when 40% of primary uredia had erupted (Andres, 1982).

Uredium size Four leaves per treatment were detached 14 d.p.i. and photographed at known magnification on colour slide film. Uredium size was estimated by measuring two diameters of 10 randomly selected but non-coalescing uredia per leaf with a digital micrometer from projected slide images. From these measurements, uredium size (mm^2) was calculated using the formula $\pi \times \text{length} \times \text{width}/4$.

Uredium density The remaining leaves were then excised and the area of the portion on which uredia had been counted on all leaves was determined using a conveyer area meter (model CI-251, CID Inc., Moscow, Idaho, USA). The uredium density was calculated as the number of uredia per cm^2 leaf area.

Infection types Disease reactions were rated according to the 0 to 4 infection type scale (Roelfs, 1988b) 14 d.p.i. for both pathotypes on flag leaves of plants.

FIELD REACTION OF GENE COMBINATION LINES

The wheat genotypes Thatcher, RL6011, CT263, Tc x CT263 F₄, Tc x RL6011 F₄, and each of the F₄ families 3, 9, 19 and 40 of the CT263 x RL6011 cross were evaluated for leaf rust reaction type and severity in a field trial at Greytown (Natal, South Africa) during 1994. The trial was planted on 26 May 1994. Plots consisted of one 2-m row (120 seeds/row) per entry, planted within the annual rust nursery of the Department of Plant Pathology, UOFS. The plot was surrounded by two rows of Morocco, a highly susceptible cultivar serving as a leaf rust spreader. Prior to planting, during seedbed preparation, fertiliser was placed in the soil below designated rows at a rate of 50 kg nitrogen per hectare.

Pathotypes UVPrt2, 3, 9 and 13 (Table 4) of *P. recondita* f.sp.*tritici* were used as inoculum. Plants were spray-inoculated on 20 September 1994 with a concentrated inoculum suspension of water and Tween 20, containing urediospores of each pathotype. Plots were irrigated to supplement rainfall and to ensure favourable conditions for vigorous growth and leaf rust development. Leaf rust severity and reaction type were estimated on 8, 18 and 29 November 1994 according to the descriptions of Stubbs *et al.* (1986).

EXPERIMENTAL DESIGN AND DATA ANALYSES

Analyses of data obtained in the determination of both the microscopic and macroscopic components of resistance were done with SOLO (BMDP Statistical Software Inc., Los Angeles, CA), using the procedure for a general linear model. A completely randomised design was used for data analysis. Standard deviations were

calculated as a measure for distinguishing treatment means. For each component, data from the first and second experiments were combined if experiment-by-treatment interaction was not significant, and if experiments, according to analysis of variance, were similar.

RESULTS

VIRULENCE OF SOUTH AFRICAN LEAF RUST PATHOTYPES TO *Lr12* AND *Lr13*

The flag leaf responses of Thatcher, RL6011 and CT263 are presented in Table 6. Thatcher was susceptible (infection type [IT] 3⁺⁺) to all pathotypes except UVPrt3, 7 and 10 to which it produced a moderately resistant reaction (IT 2). On RL6011 a resistant IT (;1) was observed for pathotype UVPrt13 and a susceptible IT (3⁺) for UVPrt2. A moderately resistant reaction (IT 2⁺⁺) was observed on RL6011 for pathotype UVPrt5, whereas resistant IT's (;1 to ;1⁺⁺) were produced by UVPrt3, 4, 6, 7, 8, 9, 10, 12, 15, and 16. On CT263 a susceptible IT (3) was observed for UVPrt13 and a resistant IT (;1^{cn}) with UVPrt2. Susceptible reactions (IT's 3 to 3⁺) were observed on CT263 for UVPrt6, 8, 12 and 13, whereas resistant IT's (;-;1^{cn} to ;1^{++c}) were observed for UVPrt3, 4, 5, 7, 9, 10, 14, 15, and 16.

SELECTION OF LINES CONTAINING *Lr12+Lr13* GENE PAIR

SEGREGATION RATIOS

Monogenic inheritance of *Lr12* and *Lr13* was indicated by a 1:2:1 among-family segregation ratio and 3:1 within-family segregation pattern in those F₃ families segregating for resistance (Table 7).

INFECTION TYPES

Seedlings of the Thatcher x RL6011 F₃ families inoculated with UVPrt2 showed IT's of

Table 6. Infection types^a produced by South African pathotypes of *Puccinia recondita* f.sp. *tritici* on flag leaves of Thatcher (leaf rust-susceptible), RL6011 (Thatcher/Lr12) and CT263 (Thatcher/Lr13)

Pathotypes	Thatcher	RL6011	CT263
UVPrt2	3 ^{**}	3 ⁺	;1 ^{-cn}
UVPrt3	2	;1 ^{-cn}	;1 ^{cn}
UVPrt4	3 ^{**}	;1 ^{ct}	;1 ^{cn}
UVPrt5	3 ^{**}	2 ^{**}	;1 ^{-cn}
UVPrt6	3 ^{**}	;1 ⁺	3 ⁺
UVPrt7	2	;1 ^{-cn}	;1 ^{cn}
UVPrt8	3 ^{**}	;1 ⁺	3 ⁺
UVPrt9	3 ^{**}	::1	;1 ^{-c}
UVPrt10	2	;1 ^{-c}	;1 ^{++c}
UVPrt12	3 ^{**}	;1 ^{**}	3 ⁺
UVPrt13	3 ^{**}	;1	3
UVPrt14	3 ^{**}	x ⁺	;1 ^{-cn}
UVPrt15	3 ^{**}	;1 ^c	;1 ^{tc}
UVPrt16	3 ^{**}	;1	;1 ^{-c}

^a Infection types were recorded 15 d.p.i. according to a 0 to 4 scale (Roelfs, 1988b).

Table 7. Segregation ratios observed in F₃ seedling and adult progenies derived from crosses involving lines RL6011 (*Lr12*), CT263 (*Lr13*) and Thatcher (Tc)

Growth stage	Cross	Gene- ration	Pathotype	Number of plants ^a or families ^b			Ratio	χ^2
				Resistant	Segregating	Susceptible		
Seedlings	Tc x CT263	F ₃ ^a	UVPrt2	716		191	3:1	7.515 ^c
	CT263 x RL6011	F ₃ ^a	UVPrt2	572		162	3:1	3.359
	Tc x CT263	F ₃ ^b	UVPrt2	13	29	10	1:2:1	1.038
	CT263 x RL6011	F ₃ ^b	UVPrt2	10	24	18	1:2:1	2.769
Adult plants	Tc x RL6011	F ₃ ^a	UVPrt13	438		157	3:1	0.611
	CT263 x RL6011	F ₃ ^a	UVPrt13	444		159	3:1	0.601
	Tc x RL6011	F ₃ ^b	UVPrt13	10	20	19	1:2:1	4.959
	CT263 x RL6011	F ₃ ^b	UVPrt13	12	24	16	1:2:1	0.923

^a Within-family segregation.

^b Among-family segregation.

^c Deviated significantly (P<0.01) from expected ratio.

3⁻ to 3⁺⁺. No evidence of seedling resistance was detected. IT's on the CT263 x RL6011 and Thatcher x CT263 F₃ families inoculated with UVPrt2 varied from resistant (X^{cn}) to highly susceptible (3⁺⁺). In adult plants inoculated with UVPrt13, the resistant families of Thatcher x RL6011 exhibited IT's of ;1^{cn} to 2⁺⁺ whereas the IT's of susceptible families for Thatcher x CT263 were 3 to 3⁺. Segregates in CT263 x RL6011 F₃ families inoculated with UVPrt13 displayed IT's from 1^{cn} to 3⁺ (Fig. 1). No indication of residual effects of the individual genes were observed when progenies were tested in a growth stage/pathotype combination negating typical expression of either *Lr12* or *Lr13*. Four CT263 x RL6011 F₃ families (3, 9, 19 and 40), homozygous for both *Lr12* and *Lr13*, were identified.

SEEDLING REACTION OF GENE COMBINATION LINES

IT's produced by the 14 selected leaf rust pathotypes on Thatcher and genotypes containing *Lr12* and/or *Lr13* at two different temperatures, are shown in Table 8 (15°C), Table 9 (25°C) and Fig. 2. Thatcher and RL6011 were susceptible (IT 3 to 3⁺⁺) to all isolates at both temperatures. CT263 was susceptible (IT 3^c to 3⁺⁺) to all isolates tested at 15°C, but a high level of resistance was expressed (IT X^{-cn} to X^{cn}) to UVPrt2, 4, 9, 14, 15, and 16 at 25°C. On the *Lr12* and *Lr13* combination lines, IT's produced at 15°C by UVPrt2, 4, 9, 14, and 16 were slightly lower than those of CT263 and RL6011. However, these lines were not necessarily more resistant than Tc/13-22, the Thatcher line carrying only *Lr13* (Table 8). At 25°C, differences between the CT263 x RL6011 lines and their parents were more pronounced, especially for pathotypes UVPrt2, 3, 6, 7, 8, 9, 12, 13 and 15 (Fig. 2). Similar to the results obtained at 15°C,

Fig. 1 Infection types produced by UVPrt13 of *Puccinia recondita* f. sp. *tritici* on flag leaves of (A) CT263 x RL6011 F₃ family 1; and (B) CT263 x RL6011 F₃ family 40, 14 days post-inoculation.

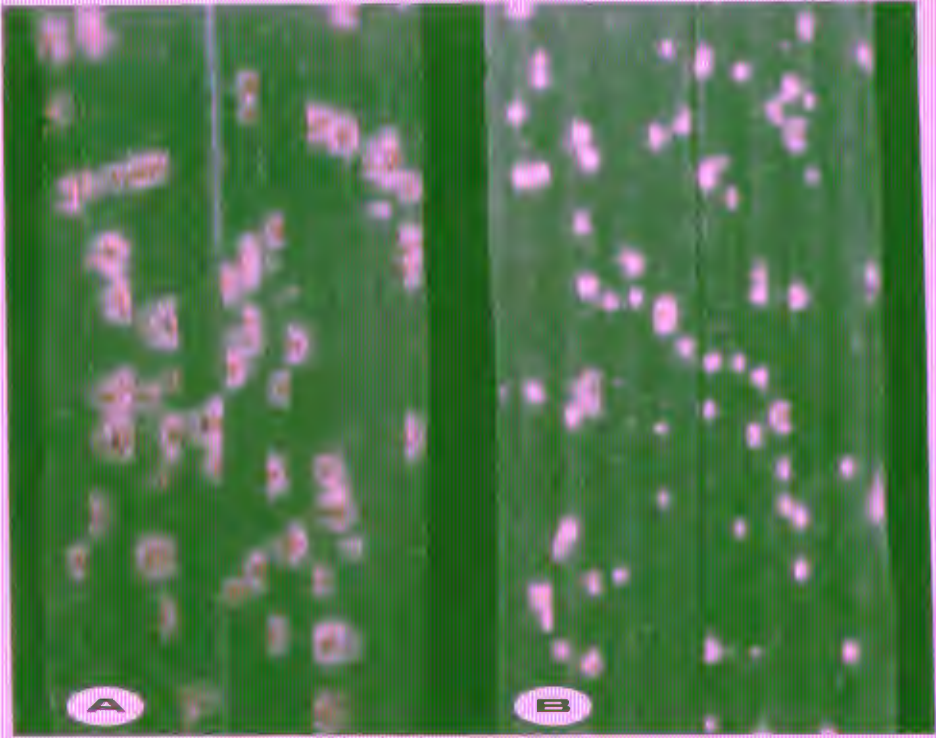


Table 8. Infection types^a produced at 15°C by selected pathotypes of *Puccinia recondita* f. sp. *tritici* on primary leaves of Thatcher ([Tc] leaf rust-susceptible) wheat and genotypes containing *Lr12* and/or *Lr13*

Pathotype	Tc	RL6011	CT263	13/12-3	13/12-9	13/12-19	13/12-40	Tc/12-16	Tc/13-22
UVPrt2	3 ⁺⁺	3 ⁺⁺	3 ^c	3 ^c	2*3	2*3	2*3	3 ⁺⁺	2
UVPrt3	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ⁺⁺	3 ⁺⁺	3 ^c
UVPrt4	3 ⁺⁺	3 ⁺⁺	3 ^c	2	2	2	2	3 ⁺⁺	2 ^{cn}
UVPrt5	3	3 ⁺	3 ^c	3	3	3	3	3 ⁺⁺	2*3
UVPrt6	3 ^c	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c
UVPrt7	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ⁺⁺	3 ⁺⁺	3 ^c	3 ⁺⁺	3 ⁺⁺	3 ^c
UVPrt8	3 ^c	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c
UVPrt9	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c	3 ⁺	3 ^c	2*3	3 ⁺⁺	3
UVPrt10	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺
UVPrt12	3 ^c	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c
UVPrt13	3 ^c	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c	3 ^c
UVPrt14	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ^c	3 ^c	3 ⁺⁺	3 ^c
UVPrt15	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ⁺	3 ⁺	3 ^c	3 ⁺	3 ⁺⁺	3 ^c
UVPrt16	3 ⁺⁺	3 ⁺⁺	3 ⁺⁺	3 ^c	3 ^c	2*3	2*3	3 ⁺⁺	3 ^c

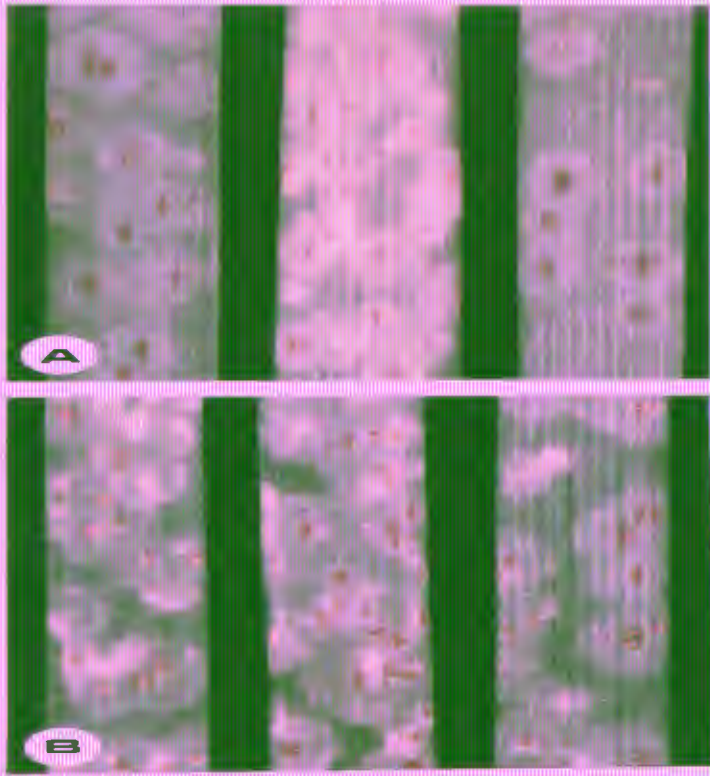
^a Infection types according to a 0 to 4 scale (Roelfs, 1988b) were recorded 14 d.p.i. on primary leaves of plants seven days old at inoculation.

Table 9. Infection types^a produced at 25°C by selected pathotypes of *Puccinia recondita* f. sp. *tritici* on primary leaves of Thatcher ([Tc] leaf rust-susceptible) wheat and genotypes containing *Lr12* and/or *Lr13*

Pathotype	Tc	RL6011	CT263	13/12-3	13/12-9	13/12-19	13/12-40	Tc/12-16	Tc/13-22
UVPrt2	3 ⁺⁺	3 ⁺⁺	X ^{-cn}	X ^{-cn}	X ^{-cn}	;1 ^{-cn}	X ^{-cn}	3 ⁺⁺	;1 ^{-cn}
UVPrt3	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ^c	2 ⁺³	2 ⁺³	2 ⁺³	3 ⁺	3 ^c
UVPrt4	3 ⁺	3 ⁺	X ^{-cn}	X ^{-cn}	X ^{-cn}	X ^{-cn}	X ^{-cn}	3 ⁺	X ^{-cn}
UVPrt5	3	3	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	3	2 ⁺³
UVPrt6	3	3	3	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	3	2 ⁺³
UVPrt7	3 ⁺⁺	3 ⁺⁺	3 ⁺	3 ^c	3 ^c	2 ⁺³	3 ^c	3 ⁺⁺	3 ^c
UVPrt8	3	3	3 ⁻	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	3	3 ⁻
UVPrt9	3 ⁺	3 ⁺	X ^{cn}	;1 ^{-cn}	;1 ^{-cn}	;1 ^{-cn}	;1 ^{-cn}	3 ⁺	X ^{-cn}
UVPrt10	3	3 ⁻	3 ⁻	3 ⁻	3 ⁻	3 ⁻	3 ⁻	3	3 ⁻
UVPrt12	3	3 ⁺	3 ⁺	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	3 ⁺	3 ⁻
UVPrt13	3	3	3 ⁻	2 ⁺³	2 ⁺³	2 ⁺³	2 ⁺³	3	3 ⁻
UVPrt14	3 ⁺⁺	3 ⁺	X ^{cn}	X ^{cn}	X ^{cn}	X ^{-cn}	X ^{-cn}	3 ⁺⁺	X ^{cn}
UVPrt15	3 ⁺	3 ⁺	X	X ^{cn}	X ^{cn}	X ^{-cn}	X ^{cn}	3 ⁺	X
UVPrt16	3 ⁺	3 ⁺	X ^{cn}	X ^{cn}	X ^{cn}	X ^{-cn}	X ^{-cn}	3 ⁺	X ^{-cn}

^a Infection types according to a 0 to 4 scale (Roelfs, 1988b) were recorded 10 d.p.i. on primary leaves of plants seven days old at inoculation.

Fig. 2 Infection types produced at 25 °C by pathotype UVPrt2 of *Puccinia recondita* f. sp. *tritici* on primary leaves of (A) RL6011 (left), CT263 (centre) and Thatcher (right); and of (B) CT263 x RL6011 F₃ family 12, 14 days post-inoculation.



resistance was enhanced when compared to the parents, but not when compared with line Tc/13-22 (Table 9).

ADULT-PLANT REACTION OF GENE COMBINATION LINES

GREENHOUSE STUDIES

Microscopic components of resistance

Histological assessments Abortive penetration was defined as NPA (Fig. 3) and ASSV (Fig. 4). The percentage of infection units on the flag leaves of adult plants where the fungus failed to successfully penetrate and establish a colony was significantly ($P < 0.05$) influenced by host genotype, pathotype and their interaction. The two experiments were significantly ($P < 0.05$) different at both 64 h.p.i. and 240 h.p.i. Most monogenic and combination lines had significantly more aborted infection units of both pathotypes compared to the susceptible control, Thatcher (Figs. 5 and 6). In the first experiment sampled at 64 h.p.i., and both experiments at 240 h.p.i., more infection units of pathotype UVPrt13 appeared to abort on the four combination lines than on the parents or single gene lines (Figs. 5 and 6). Differences among the four combination lines and their parents were not as pronounced for UVPrt2 (Figs. 5 and 6). In general, the second experiment showed a higher percentage AP (64 h.p.i. = 63.4% and 240 h.p.i. = 59.9%) than the first experiment (64 h.p.i. = 58.6%, 240 h.p.i. = 55.1%).

Nonpenetrating appressoria A lower percentage NPA was recorded in the first experiment (64 h.p.i. = 20.9% and 240 h.p.i. = 19.7%) than in the second experiment (64 h.p.i. = 36.4% and 240 h.p.i. = 39.3%). In both 64 h.p.i. samplings, the combination

Fig. 3 Nonpenetrating appressorium of (A) pathotype UVPrt13 on RL6011 flag leaves 240 hours post-inoculation and (B) of pathotype UVPrt2 of *Puccinia recondita* f. sp. *tritici* on CT263 x RL6011 F₃ family 19, 64 hours post-inoculation. Abbreviations used: U=urediospore; G=germtube; AP=appressorium; SSV=substomatal vesicle; H= hypha; S=stoma. Scale bar represents 50 µm for (A) and 10 µm for (B).

Fig. 4 Aborted substomatal vesicles in flag leaves 64 hours after inoculation of (A) CT263 with pathotype UVPrt13 and (B) CT263 x RL6011 F₃ family 40 with pathotype UVPrt2 of *Puccinia recondita* f.sp. *tritici*. Abbreviations used: U=urediospore; G=germtube; AP=appressorium; SSV=substomatal vesicle; H=hypha; S=stoma. Scale bar represents 50 µm for (A) and 10 µm for (B).

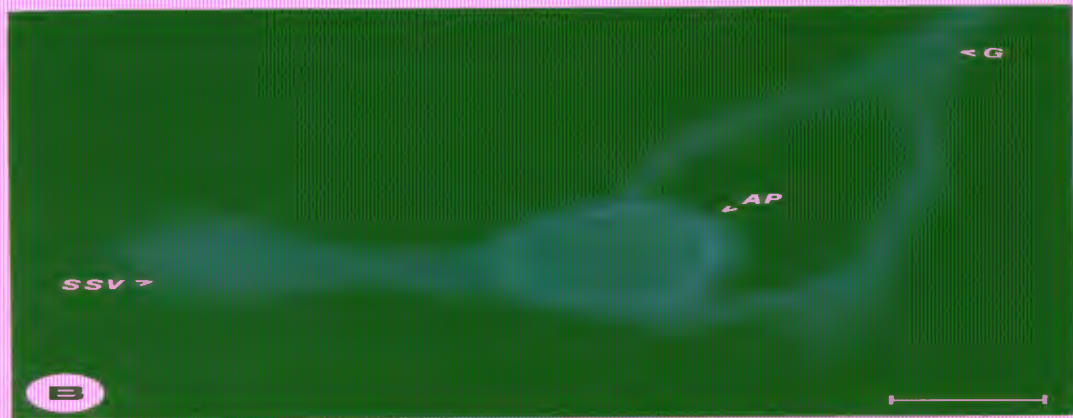
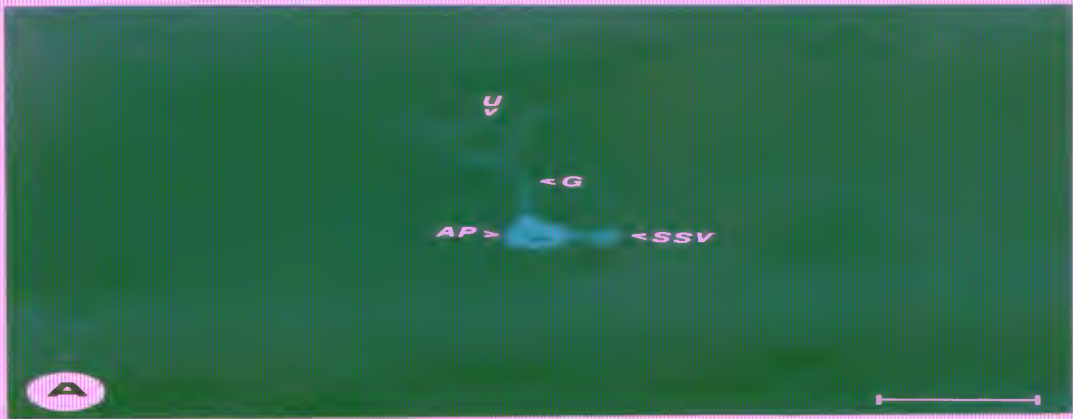
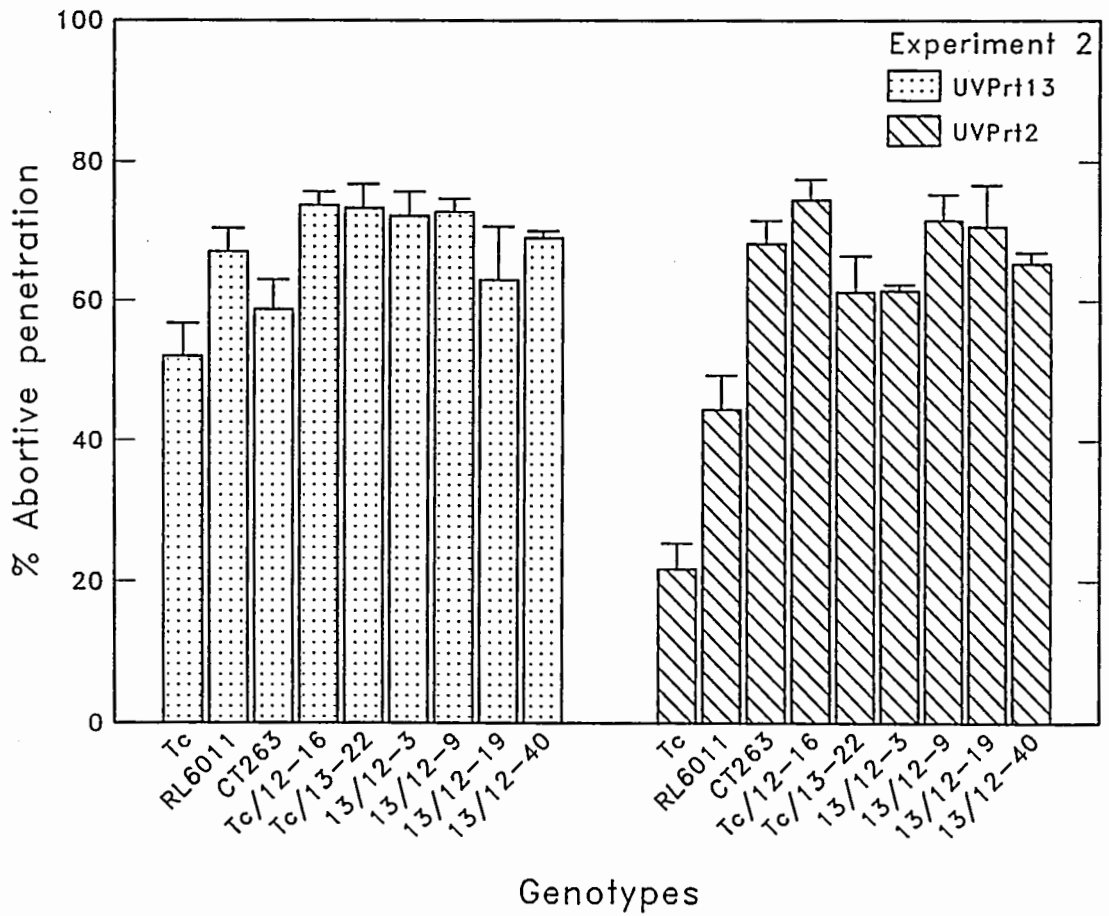
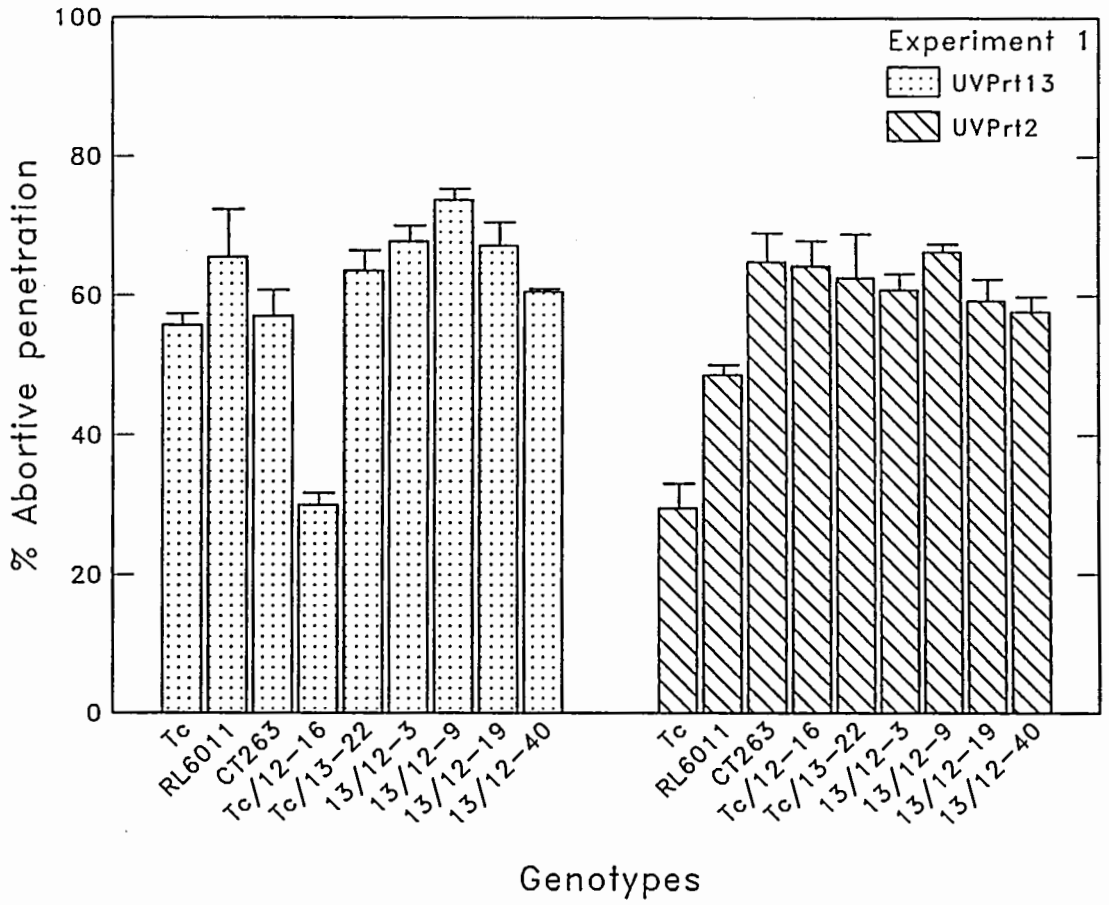


Fig. 5 Percentage abortive penetration determined histologically 64 hours post-inoculation on Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. Plants were inoculated with pathotypes UVPrt13 and UVPrt2 of *Puccinia recondita* f.sp. *tritici*. Error bars represent positive standard deviations.



lines supported fewer NPA of pathotype UVPrt13 than the parents (Tables 10 and 11). Similar results were obtained in the second experiment 240 h.p.i. In both experiments 64 h.p.i., the combination line *13/12-40*, inoculated with UVPrt2, showed a lower percentage NPA than RL6011. The combination lines inoculated with UVPrt13 were compared with CT263 and the single gene line *Tc/13-22*, whereas those infected with UVPrt2 were compared with RL6011 and the single gene line *Tc/12-16*. In the first 240 h.p.i. sampling, the combination lines *13/12-3*, *13/12-9* and *13/12-40* supported fewer NPA of pathotype UVPrt2 than RL6011 and *Tc/12-16*, whereas in the second sampling all four combination lines supported fewer NPA than RL6011, but not than *Tc/12-16*.

Aborted substomatal vesicles A higher percentage ASSV was observed in the first than in the second experiment (Tables 10 and 11). At 64 h.p.i. in the first experiment more ASSV of pathotype UVPrt13 were encountered on the combination lines than on CT263 (Table 10) and the same results were obtained at 240 h.p.i. in the second experiment (Table 11). In both experiments sampled at 64 h.p.i., the lines *13/12-3*, *13/12-9*, and *13/12-19* showed a lower percentage ASSV than RL6011 and *Tc/12-16* with pathotype UVPrt2. In both 240 h.p.i. samplings the combination lines, except for *13/12-19* in the second experiment, supported a higher percentage ASSV of UVPrt2 than RL6011.

Colony development Only infection sites where haustorium mother cells occurred were classified as colonies (Fig. 7).

Number of haustorium mother cells (HMC) 64 h.p.i. Results obtained in the two

Table 10. Histological components^a of resistance conferred by *Lr12* and *Lr13* alone and in combination to *Puccinia recondita* f.sp. *tritici* as determined in the first experiment

Histol. comp.	Pathotype	Sampling time ^b	Thatcher	RL6011	CT263	13/12 fam 3 ^c	13/12 fam 9 ^c	13/12 fam 19 ^c	13/12 fam 40 ^c	Tc/12 fam 16	Tc/13 fam 22
Abortive penetration											
NPA ^d	UVPrt2	64 h.p.i.	28.0±6.7	14.0±1.8	15.8±5.4	15.1±0.8	16.3±2.3	18.6±2.6	13.6±1.4	10.7±0.3	19.8±5.5
		10 d.p.i.	20.3±28.8	19.2±3.0	15.8±0.8	9.4±3.9	8.5±0.5	45.6±4.5	4.0±3.6	10.7±5.6	0.9±1.5
	UVPrt13	64 h.p.i.	29.9±10.8	23.6±3.9	37.4±3.8	22.9±3.7	26.1±3.0	19.2±3.5	24.3±3.5	16.8±8.3	24.4±4.0
		10 d.p.i.	31.9±16.4	20.0±9.6	35.5±8.3	12.1±5.0	23.2±3.5	21.2±4.8	24.8±10.1	17.6±8.9	32.6±8.2
ASSV ^e	UVPrt2	64 h.p.i.	72.1±6.7	86.0±1.8	84.2±5.4	84.9±0.8	83.7±2.3	81.4±2.6	86.4±1.4	89.3±0.3	80.17±5.5
		10 d.p.i.	79.7±28.8	80.8±3.0	84.2±0.8	90.6±3.9	91.5±0.5	54.4±4.5	96.0±3.6	89.3±5.6	99.1±1.5
	UVPrt13	64 h.p.i.	70.1±10.8	76.4±3.9	62.6±3.8	77.1±3.7	74.0±3.0	80.8±3.5	75.7±3.5	83.2±8.3	75.6±4.0
		10 d.p.i.	68.1±16.4	80.0±9.6	64.5±8.3	87.9±5.0	76.8±3.5	78.8±4.8	75.2±10.1	82.4±8.9	67.4±8.2

^a Means ± standard deviation.

^b Histological components of resistance were determined 64 h post-inoculation (h.p.i.) and 10 days post-inoculation (d.p.i.).

^c F₃ families, homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

^d Nonpenetrating appressoria (NPA) as a percentage of all infection sites showing abortive penetration.

^e Aborted substomatal vesicles (ASSV) as a percentage of all infection sites showing abortive penetration.

Table 11. Histological components^a of resistance conferred by *Lr12* and *Lr13* alone and in combination to *Puccinia recondita* f.sp. *tritici* as determined in the second experiment

Histol. comp.	Pathotype	Sampling time ^b	Thatcher	RL6011	CT263	13/12 fam 3 ^c	13/12 fam 9 ^c	13/12 fam 19 ^c	13/12 fam 40 ^c	Tc/12 fam 16	Tc/13 fam 22
Abortive penetration											
NPA ^d	UVPrt2	64 h.p.i.	18.2±5.8	30.8±4.7	36.4±4.6	36.12±4.8	33.4±5.0	37.4±6.2	26.5±11.0	31.1±5.1	25.8±3.2
		10 d.p.i.	30.6±7.8	48.3±5.4	19.4±3.4	31.7±2.1	23.6±10.6	23.2±8.2	41.9±8.5	20.9±3.2	20.8±6.0
	UVPrt13	64 h.p.i.	46.2±12.4	50.0±2.7	46.9±4.2	41.3±7.7	37.7±6.0	40.0±9.0	40.7±2.0	43.2±5.2	34.2±4.5
		10 d.p.i.	44.9±9.6	62.4±2.3	63.0±3.5	45.5±3.1	57.6±6.6	23.1±10.6	49.3±4.0	50.6±7.2	50.6±10.4
ASSV ^e	UVPrt2	64 h.p.i.	81.9±5.8	69.2±4.7	63.6±4.6	63.9±4.8	66.6±5.0	62.6±6.2	73.5±11.0	68.9±5.1	74.3±3.2
		10 d.p.i.	69.4±7.8	51.8±5.4	80.6±3.4	68.3±2.1	76.4±10.6	76.8±8.2	58.1±8.5	79.1±3.2	79.2±6.0
	UVPrt13	64 h.p.i.	53.8±12.4	50.4±2.7	53.1±4.2	58.6±7.8	62.3±6.0	60.1±9.0	59.3±2.0	56.8±5.2	65.8±4.5
		10 d.p.i.	55.1±9.6	37.6±2.3	37.0±3.5	54.5±3.1	42.4±6.6	76.9±10.6	50.7±4.0	49.4±7.2	49.4±10.4

^a Means ± standard deviation.

^b Histological components of resistance were determined 64 h post-inoculation (h.p.i.) and 10 days post-inoculation (d.p.i.).

^c F₃ families, homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

^d Nonpenetrating appressoria (NPA) as a percentage of all infection sites showing abortive penetration.

^e Aborted substomatal vesicles (ASSV) as a percentage of all infection sites showing abortive penetration .

Fig. 7 Infection site displaying haustorium mother cells of pathotype UVPrt2 in flag leaves of CT263 x RL6011 F₃ family 19, 64 hours post-inoculation (A)(x100) and (B)(x400). Abbreviations used: AP=appressorium; SSV=substomatal vesicle; IH=infection hypha; HMC=haustorium mother cell. Scale bar represents 50 μm for (A) and 10 μm for (B).

experiments differed significantly ($P < 0.05$). Fewer HMC's developed in line 13/12-40 than the parents for both pathotypes (Table 12). When the experiment was repeated, a reduced HMC count in line 13/12-40 inoculated with UVPrt13 could not be confirmed. Fewer HMC's of pathotype UVPrt13 developed in the four combination lines than in CT263 in the first experiment, whereas only 13/12-3, 13/12-9 and 13/12-19 supported fewer HMC's in the second experiment. The lines 13/12-19 and 13/12-40 inoculated with UVPrt2 produced notably fewer HMC's than RL6011 and Tc/12-16 in both experiments, whereas 13/12-9 supported the same number of HMC's in the first, and fewer in the second experiment. The numbers of HMC's on both parents were slightly higher for the avirulent pathotypes.

Colony size 64 h.p.i. Measurements of colony size (Fig. 8) were significantly ($P < 0.05$) different between the two experiments. In general smaller colonies were observed in the first (0.0022 mm^2) than in the second experiment (0.0030 mm^2). Means indicated that colony size was statistically ($P < 0.05$) similar for pathotypes and genotypes, respectively. Colony size was significantly ($P < 0.05$) influenced by the interaction of host genotype and pathotype. A reduced susceptibility to leaf rust in the combination lines was not observed for any of the pathotypes.

240 h.p.i. Determination of colony size indicated a significant ($P < 0.05$) difference between the two experiments (Fig. 9). Colony size was significantly influenced by host genotype, pathotype and their interaction. Both pathotypes produced larger colonies in the flag leaves of Thatcher and parents than in the combination lines. Colonies in the combination lines inoculated with UVPrt2 were smaller than those in Tc/12-16. Colonies of UVPrt13 in the combination lines were smaller than in Tc/13-22 in the first

Table 12. Number^a of haustorium mother cells of two pathotypes of *Puccinia recondita* f. sp. *tritici* observed 64 hours post-inoculation per infection site in flag leaves of adult Thatcher (leaf rust-susceptible) wheat plants and genotypes containing *Lr12* and/or *Lr13*

Pathotype	Thatcher	RL6011	CT263	13/12 fam 3 ^b	13/12 fam 9 ^b	13/12 fam 19 ^b	13/12 fam 40 ^b	Tc/12 fam 16	Tc/13 fam 22
First experiment									
UVPrt2	2.3±0.2	3.5±0.1	4.0±0.3	4.1±0.4	3.5±0.3	2.3±0.1	2.2±0.2	2.6±0.2	2.1±0.2
UVPrt13	3.3±0.4	3.6±0.7	4.0±0.4	2.9±0.4	2.7±0.3	3.5±0.2	1.9±0.3	4.8±0.5	3.6±0.4
Second experiment									
UVPrt2	9.8±1.8	7.4±0.7	6.6±0.9	6.8±0.4	4.9±0.5	3.5±0.2	3.5±0.4	5.7±0.7	8.0±0.5
UVPrt13	8.5±0.4	7.7±0.3	5.3±0.4	3.7±0.6	2.6±0.5	6.0±0.3	8.2±0.8	2.5±0.2	4.0±0.6

^a Means ± standard deviation.

^b F₃ families, homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

Fig. 8 Colony size (mm²) of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. *tritici* measured 64 hours post-inoculation in Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. Error bars represent positive standard deviations.

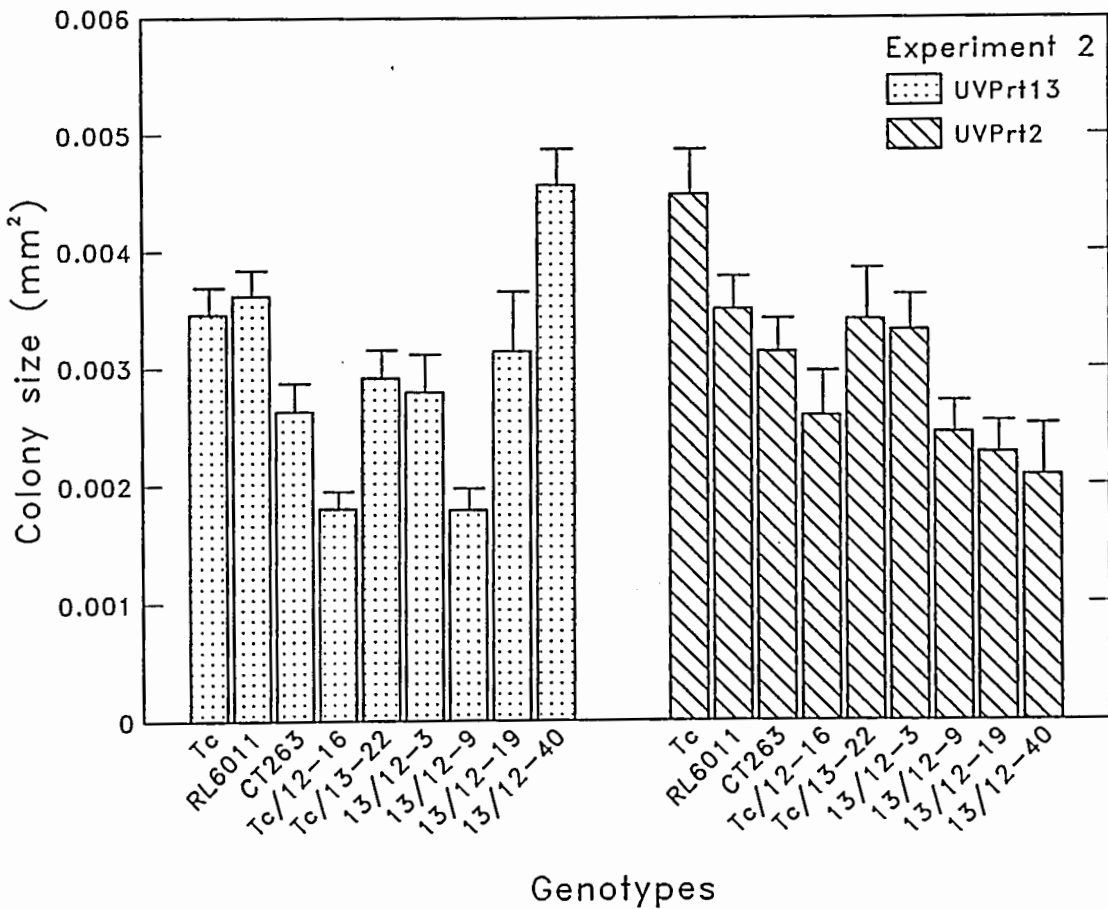
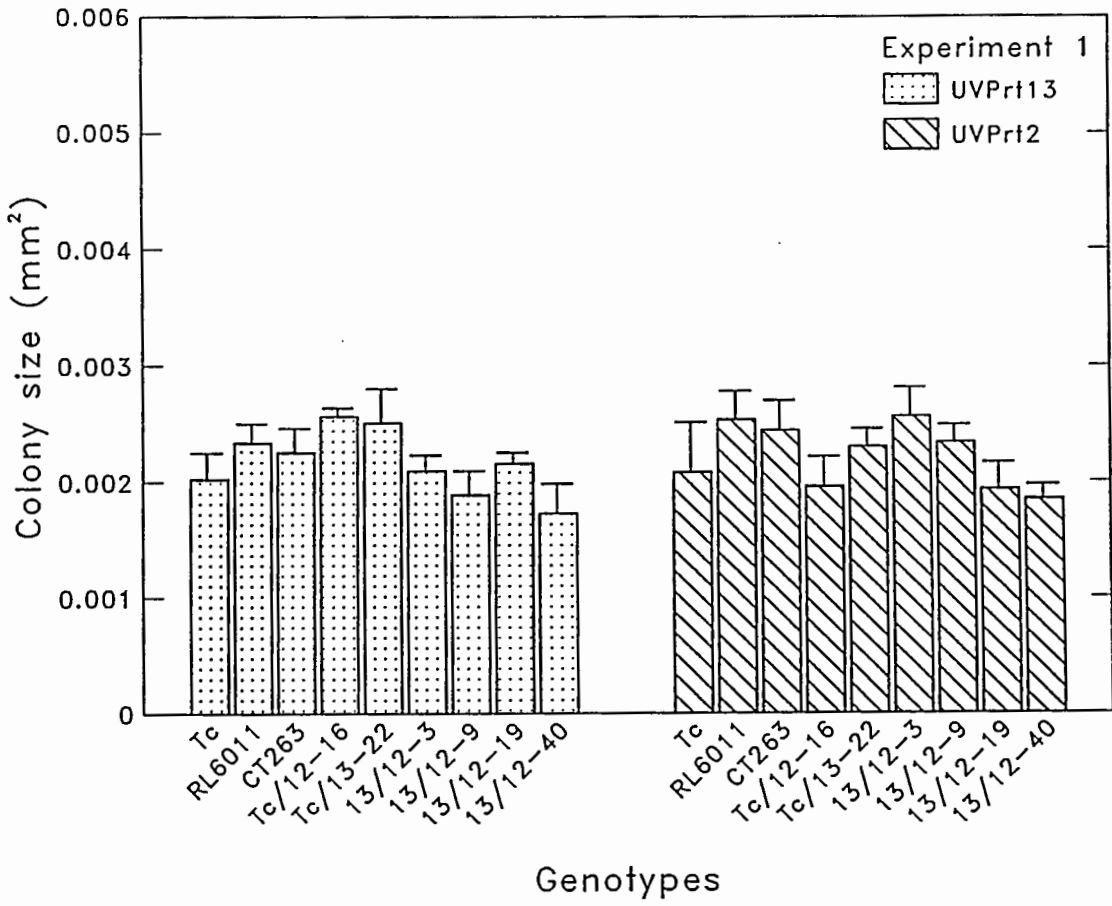
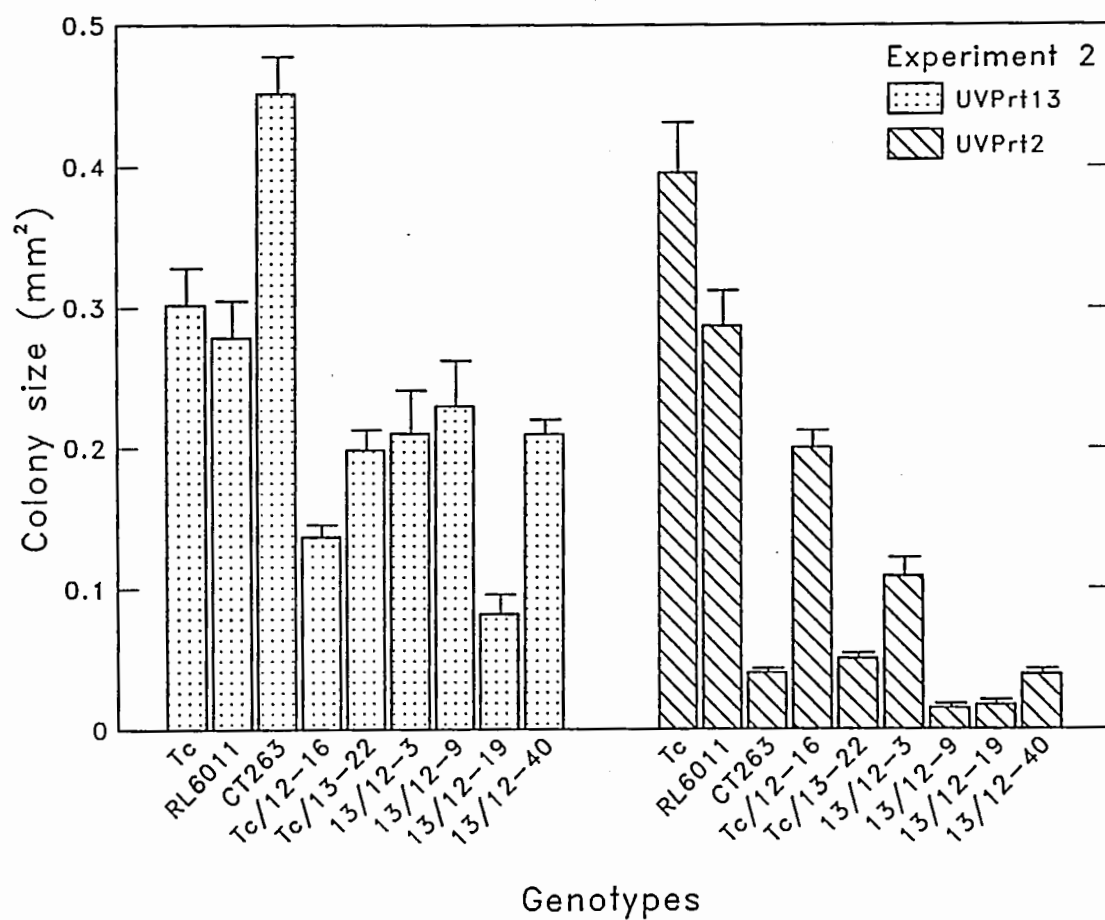
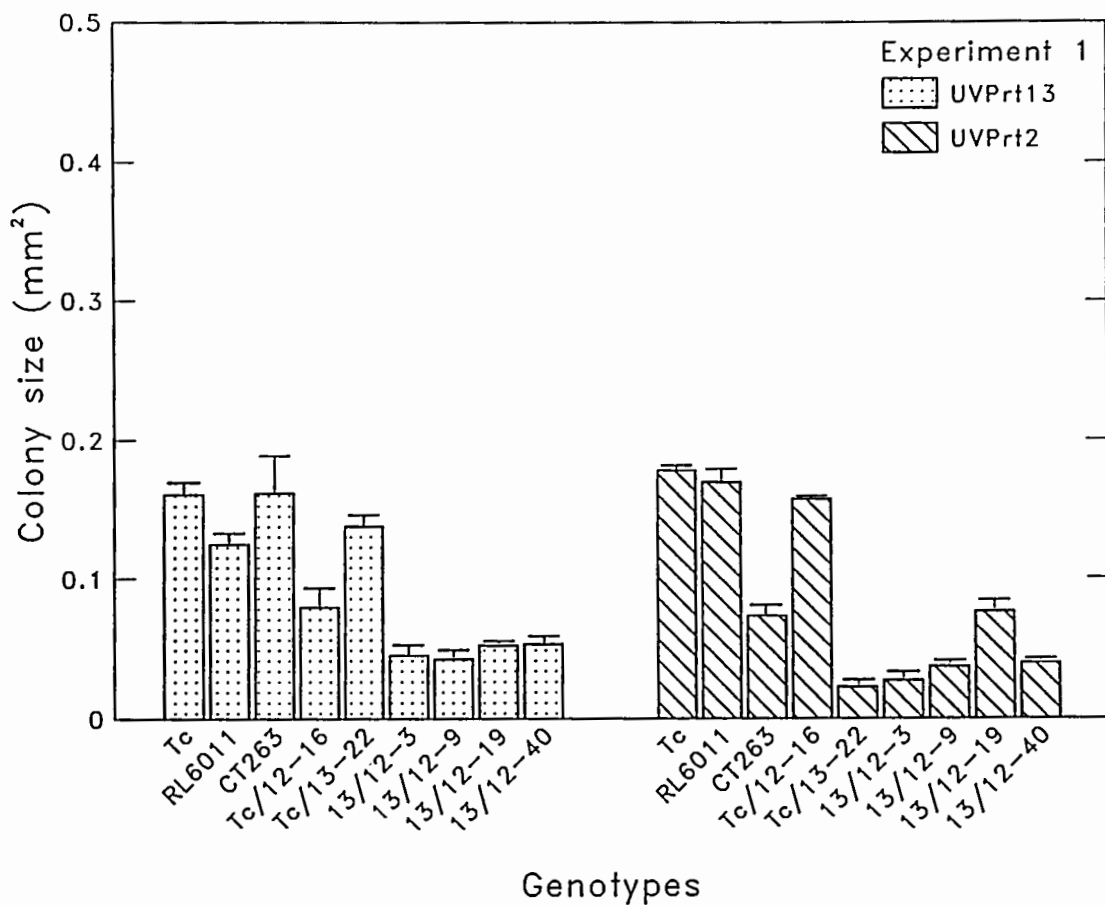


Fig. 9 Colony size (mm²) of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. *tritici* measured 240 hours post-inoculation in Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. Error bars represent positive standard deviations.



experiment.

Host cell necrosis (HCN) 64 h.p.i. Percentage infection sites associated with HCN (Fig. 10) differed significantly ($P < 0.05$) between the two experiments. In the second experiment, an overall average of 19.3% of the colonies were associated with HCN, whereas in the first experiment, the overall average was 17.6% (Table 13). The number of colonies associated with HCN was significantly influenced by host genotype, pathotype and their interaction. HCN was more regularly associated with the single gene lines Tc/13-22 and Tc/12-16 than with the *Lr12 + Lr13* combination lines. However, it was more severe and occurred more frequently in the combination lines than in CT263 or RL6011.

240 h.p.i. Results obtained in the two experiments were statistically similar and were pooled (Table 13). The number of colonies associated with HCN was significantly ($P < 0.05$) influenced by host genotype, pathotype and their interaction. With pathotype UVPrt13, HCN occurred more frequently in the four combination lines than in the parents (Table 13). No marked differences were recorded among the four combination lines and the single gene lines Tc/13-22 and Tc/12-16.

Necrotic area 64 h.p.i. Assessment of necrotic leaf area (mm^2) (Fig. 10) did not differ significantly ($P < 0.05$) between experiments and data were therefore pooled (Fig. 11). Although necrotic leaf area was not significantly ($P < 0.05$) influenced by pathotype effects it was significantly influenced by host genotype and the interaction with pathotype. Larger necrotic areas were observed in the four combination lines than in Thatcher. In leaves colonised by UVPrt2 the necrotic areas in lines 13/12-3, 13/12-9

Fig. 10 (A) A colony of pathotype UVPrt13 of *Puccinia recondita* f. sp. *tritici* and (B) associated host cell necrosis at the same flag leaf infection site (240 hours post-inoculation)(x100) on CT263 x RL6011 F₃ family 19.

and 13/12-19 were larger than in RL6011 and Tc/12-16. With UVPrt13 the necrotic area in 13/12-9, 13/12-19 and 13/12-40 leaves were significantly larger than CT263. Only line 13/12-9 showed a necrotic leaf area larger than Tc/13-22.

240 h.p.i. Results from the two experiments were significantly ($P < 0.05$) different. Analysis of variance showed that necrotic leaf area was influenced ($P < 0.05$) by host genotype, pathotype and their interaction. In Thatcher necrotic leaf areas induced by UVPrt13 and UVPrt2 were notably smaller than in the four combination lines (Fig. 12). In both experiments conducted with UVPrt13, the necrotic leaf areas in the four combination lines were smaller than in RL6011, and larger than in CT263. With pathotype UVPrt2 the necrotic leaf areas associated with the combination lines were smaller than CT263, and larger than RL6011 in the first experiment only.

Hypersensitivity index (HI) **64 h.p.i.** This index differed between experiments ($P < 0.05$), with smaller values obtained in the second experiment (Fig. 13). The HI was significantly influenced by pathotype, host genotype and their interaction. HCN occurred more regularly and was more severe in tissues colonised by UVPrt2 in 13/12-9, 13/12-19 and 13/12-40 than in Thatcher and the parents (Fig. 13). The HI of UVPrt13 was higher on 13/12-3 and 13/12-9.

240 h.p.i. Index values obtained in the second experiment 240 h.p.i. were smaller than those at 64 h.p.i. (Fig. 14). In leaves colonised by UVPrt2 HCN occurred more commonly and was more severe than with UVPrt13. With both pathotypes the combination lines displayed higher HI values than the parents. The occurrence and severity of HCN in colonies were greater on leaves sampled at 240 h.p.i. compared to 64 h.p.i. This association was especially noticeable in colonies of both pathotypes in

Fig. 12 Necrotic area (mm²) measured 240 hours after inoculating flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*, with pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici*. Error bars represent positive standard deviations.

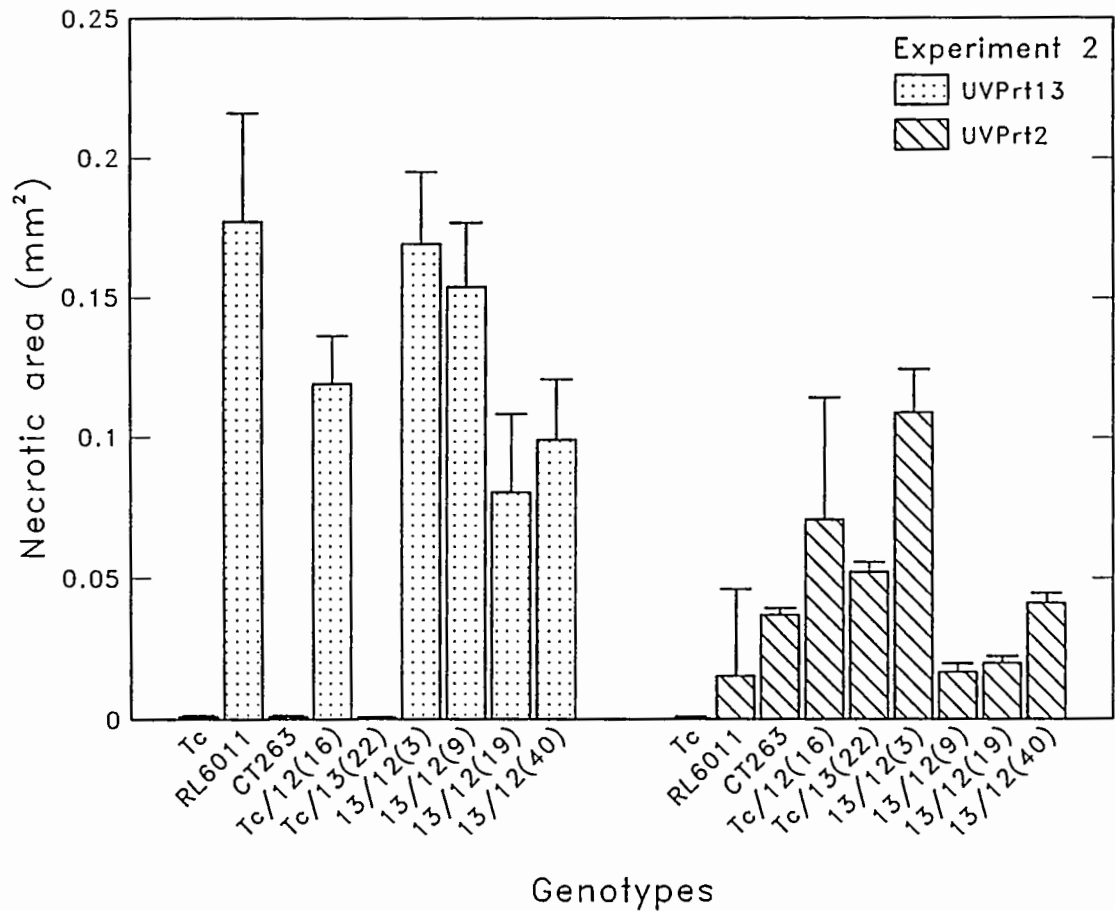
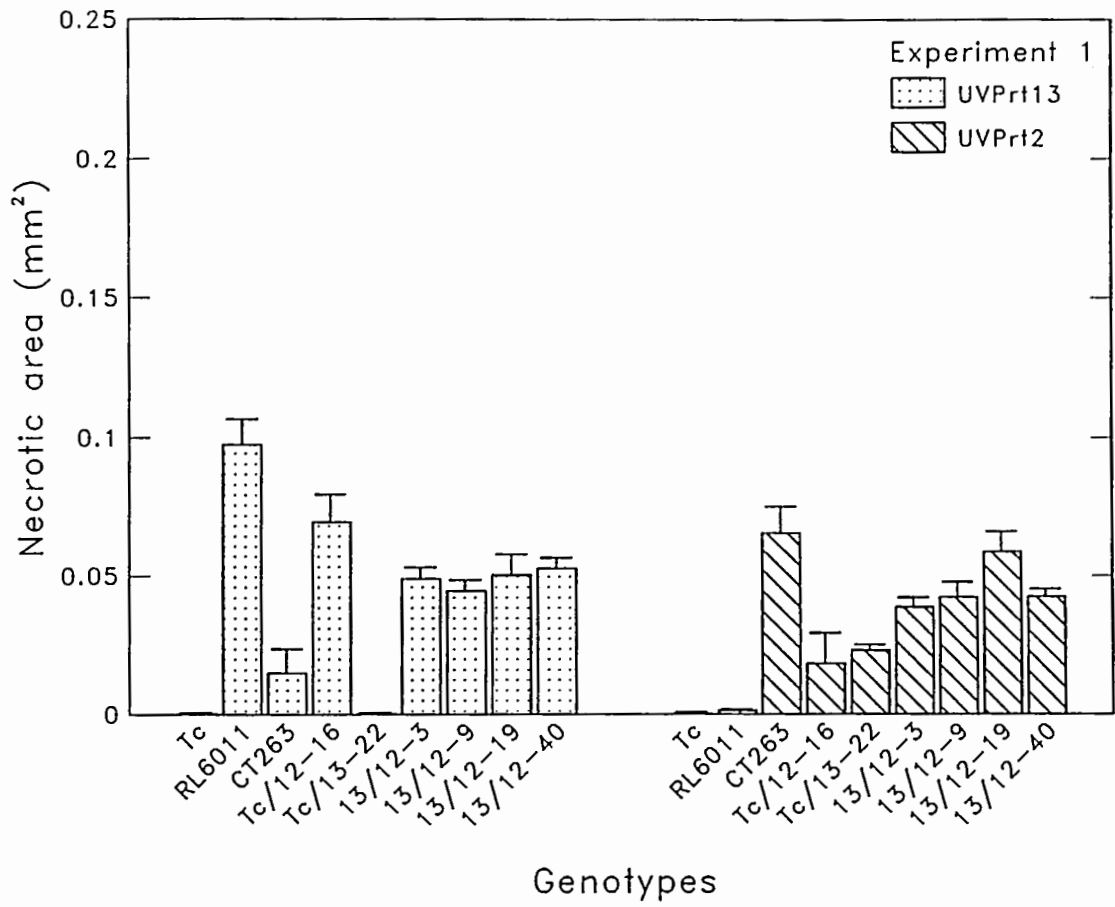


Fig. 13 A hypersensitivity index determined 64 hours post-inoculation on flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. The index was obtained by expressing the area of host cell necrosis as a fraction of the colony of either pathotype UVPrt2 or UVPrt13 of *Puccinia recondita* f. sp. *tritici*. Error bars represent positive standard deviations.

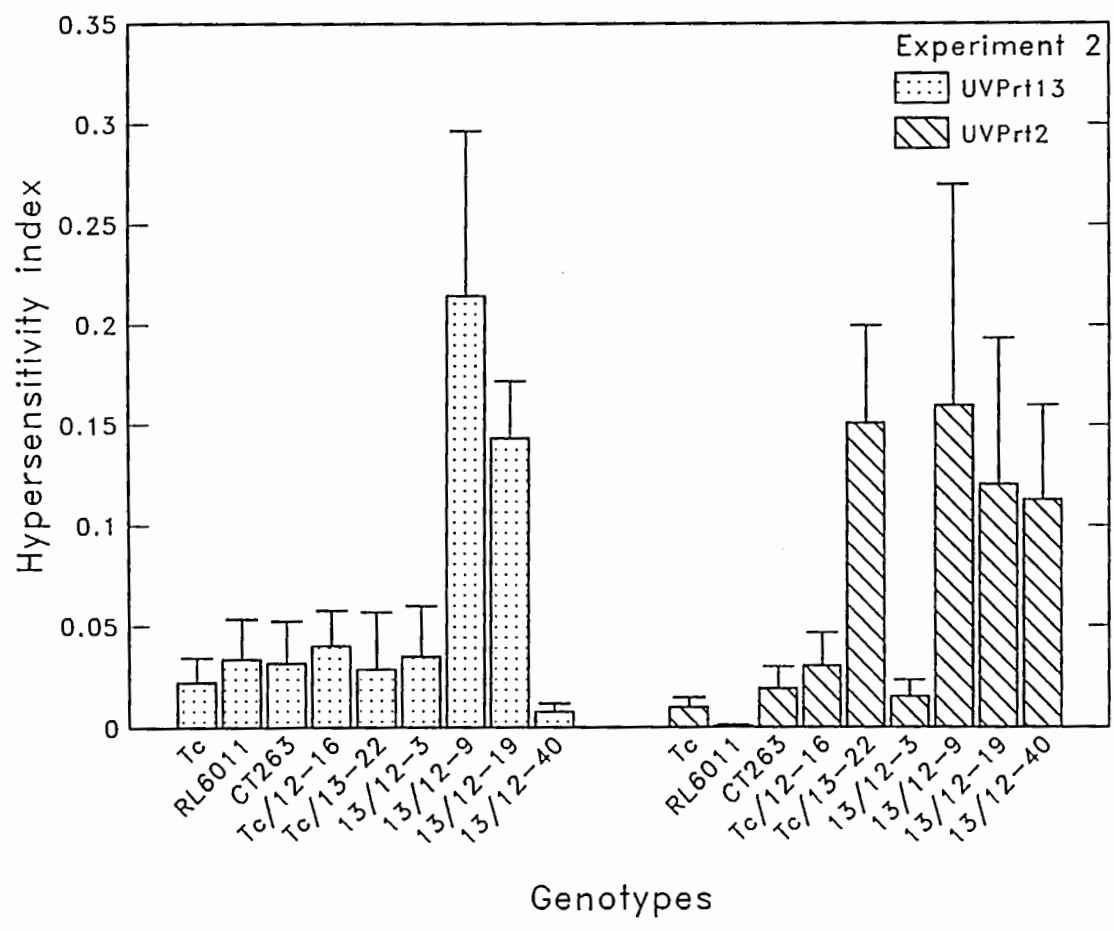
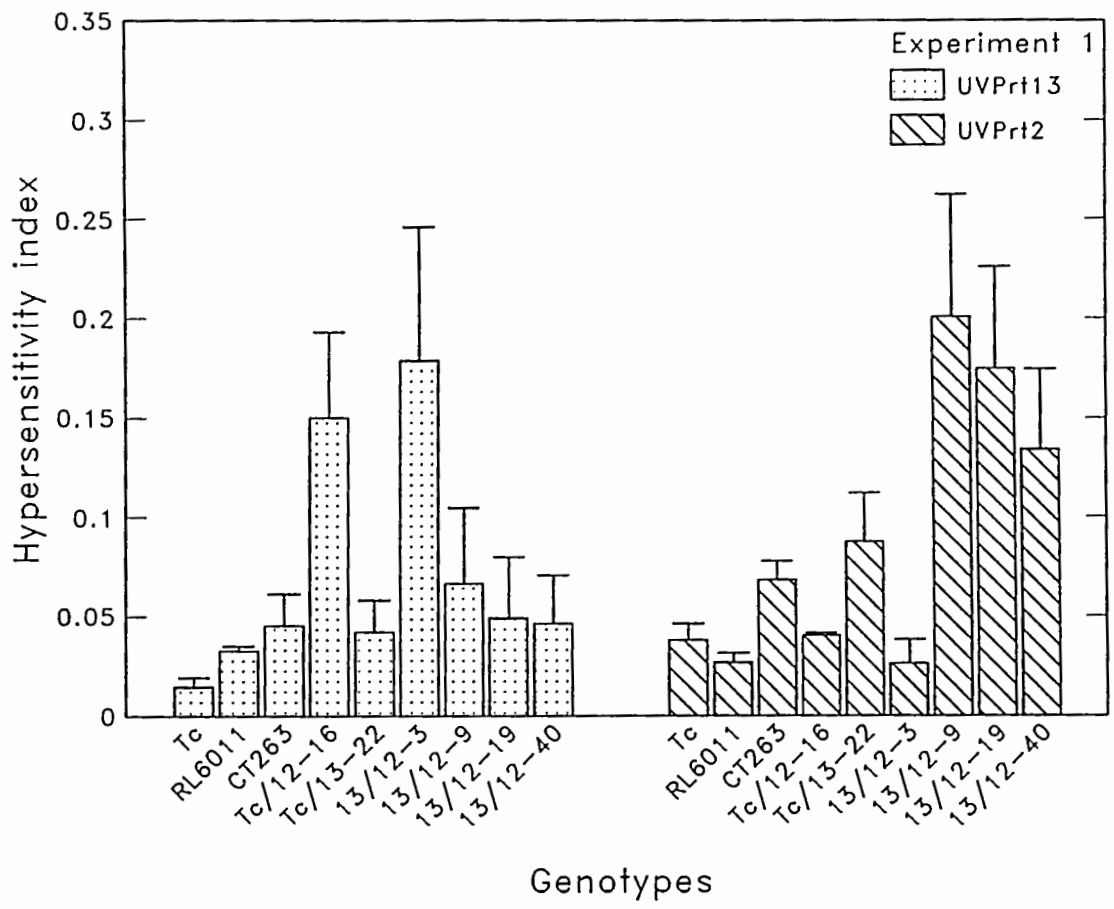
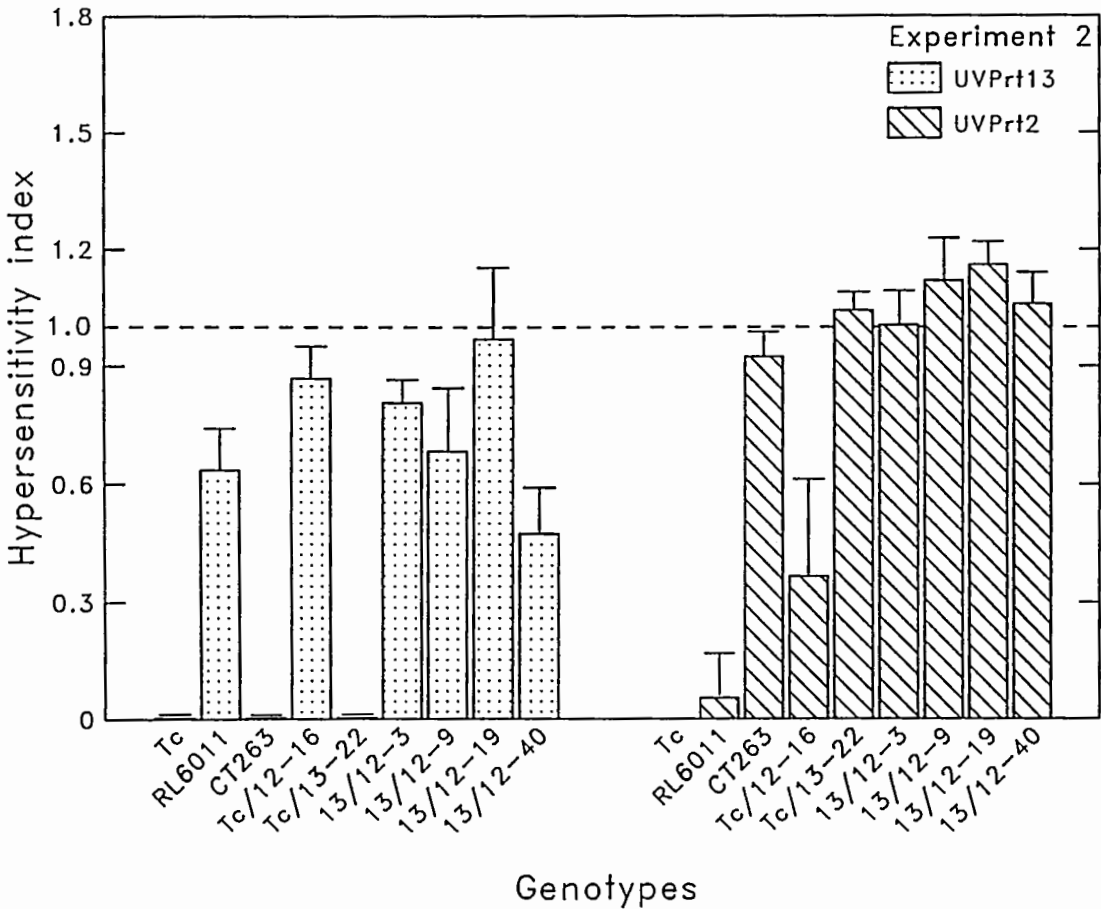
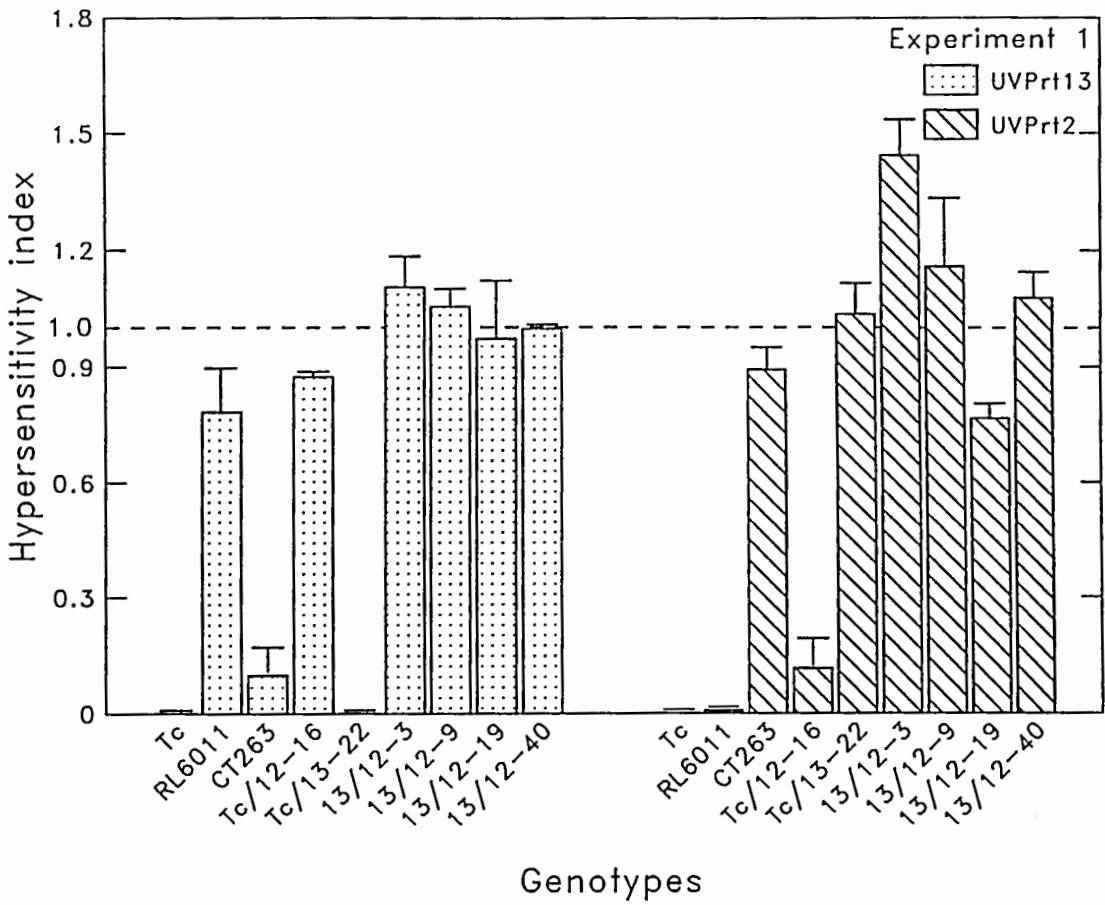


Fig. 14 A hypersensitivity index determined 240 hours post-inoculation on flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. The index was obtained by expressing the area of host cell necrosis as a fraction of the colony area of either pathotype UVPrt2 or UVPrt13 of *Puccinia recondita* f. sp. *tritici*. Error bars represent positive standard deviations.



the four combination lines. For RL6011 and Tc/12-16 it was apparent only for pathotype UVPrt13 and CT263, and for Tc/13-22 inoculated with pathotype UVPrt2 (Figs. 13 and 14).

Uredium formation 240 h.p.i. Formation of uredia, calculated as the percentage infection sites that resulted in sporulating colonies (Fig. 15), was not analysed for variance due to zero values in the data set. More infection sites produced uredia in the second experiment than in the first experiment (Table 14). The data obtained suggested that UVPrt13 sporulated more promptly than UVPrt2 on the gene combination lines.

Uredium size 240 h.p.i. Due to the failure of infection sites to develop into sporulating uredia on many lines, uredium size (Fig. 16) was not analysed for variance. In the first experiment uredium size was determined only for UVPrt13 on CT263 (0.0099 mm²) and Tc/13-22 (0.0077 mm²), and for UVPrt2 on Thatcher (0.0095 mm²) and RL6011 (0.0192 mm²). The results obtained in the experiments showed that more colonies sporulated and that the uredia were also slightly larger in the second experiment. From sizes determined for UVPrt13 in the second experiment, the combination lines that allowed sporulation appeared more resistant than the parents (Fig. 16).

Phase contrast microscopy The cell wall appositions (Figs. 17, 18 and 19) and haustoria (Fig. 20) were studied at 64 h.p.i. only. No detailed observations were possible on leaves sampled 240 h.p.i. because extensive fungal growth and cell collapse prevented quantification of parameters at the later sampling stage.

Fig. 15 Urediospores produced by a colony of pathotype UVPr2 of *Puccinia recondita* f.sp. *tritici* 240 hours post-inoculation on Thatcher (x100). Abbreviation used : U=urediospore.

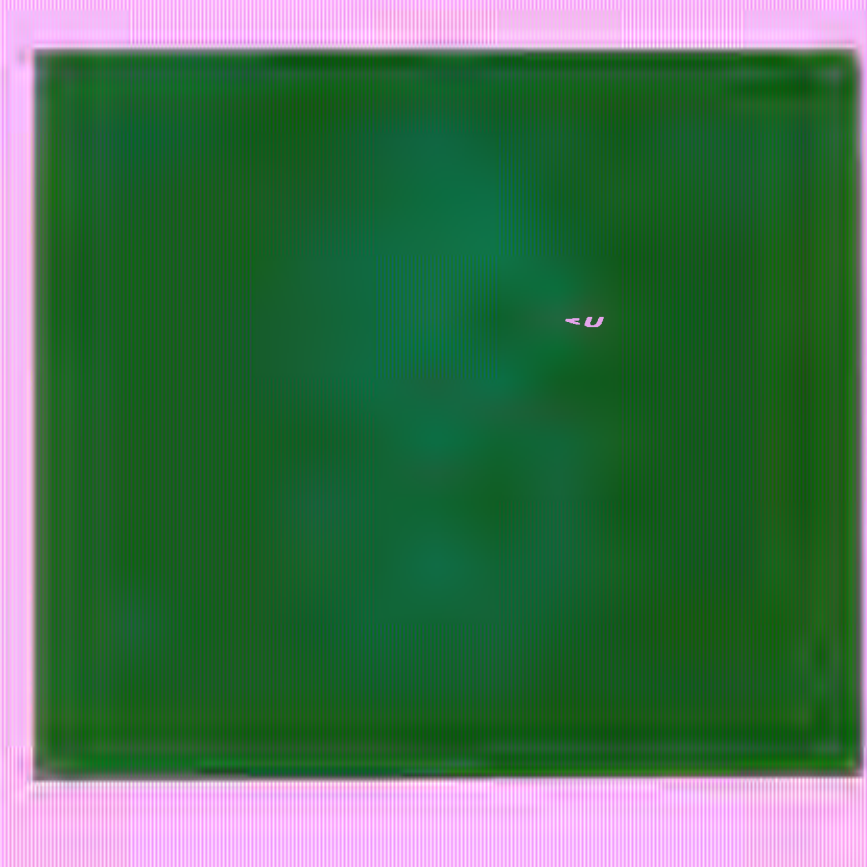


Table 14. Percentage^a of infection sites culminating in sporulating colonies on flag leaves of Thatcher (leaf rust-susceptible), RL6011, CT263 and their derivatives inoculated with pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici*.

Pathotype	Sampling time ^b	Thatcher	RL6011	CT263	13/12 fam 3 ^c	13/12 fam 9 ^c	13/12 fam 19 ^c	13/12 fam 40 ^c	Tc/12 fam 16	Tc/13 fam 22
First experiment										
UVPrt2	10 d.p.i.	10.8±6.9	6.9±3.7	0.0±0.0	0.8±1.3	0.0±0.0	4.6±1.3	0.0±0.0	3.3±3.1	0.0±0.0
UVPrt13	10 d.p.i.	1.1±1.9	3.0±5.2	5.9±5.8	0.0±0.0	0.0±0.0	0.0±0.0	4.7±4.1	0.0±0.0	17.9±4.9
Second experiment										
UVPrt2	10 d.p.i.	84.5±3.1	86.7±5.3	0.0±0.0	28.4±5.3	0.9±2.0	0.0±0.0	0.9±1.2	43.0±26.4	1.9±4.2
UVPrt13	10 d.p.i.	48.3±19.5	63.5±9.1	81.1±6.1	46.4±15.5	63.6±5.0	10.4±12.5	52.9±24.2	45.8±6.6	46.6±13.1

^a Means ± standard deviation.

^b Histological components of resistance were determined 64 h post-inoculation (h.p.i.) and 10 days post-inoculation (d.p.i.).

^c F₃ families, homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

Fig. 16 Uredium size (mm^2) measured on flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13* 240 hours after inoculation with pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f.sp. *tritici* . Error bars represent positive standard deviations.

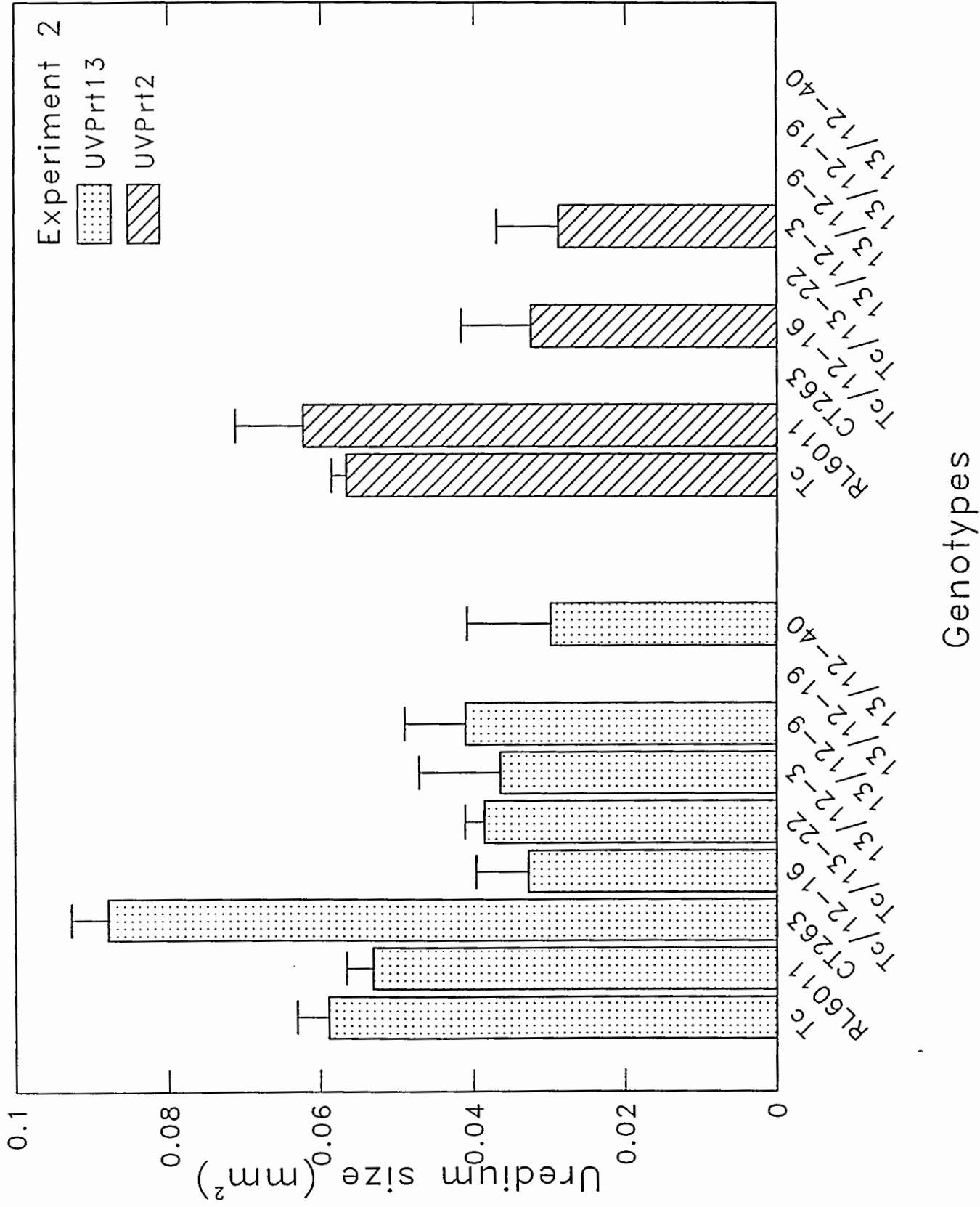


Fig. 17 Hyphal growth and papilla formation in flag leaves stained with picric acid and methyl salicylate in (A) Thatcher and (B) CT263 x Thatcher F₃ family 22 inoculated with pathotype UVPrt2 of *Puccinia recondita* f.sp. *tritici*. Abbreviations used: S=stoma; SSV=substomatal vesicle; IH=infection hypha; HMC=haustorium mother cell; P=papilla; HN=haustorium neck; H=haustorium. Scale bar represents 2.5 μ m.

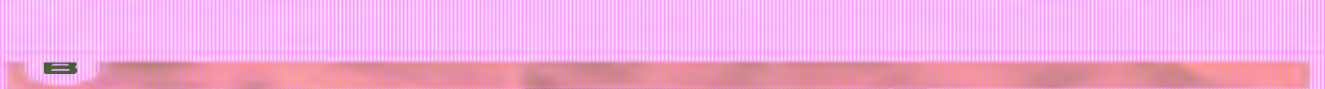
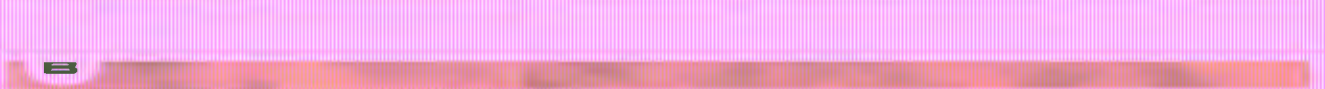
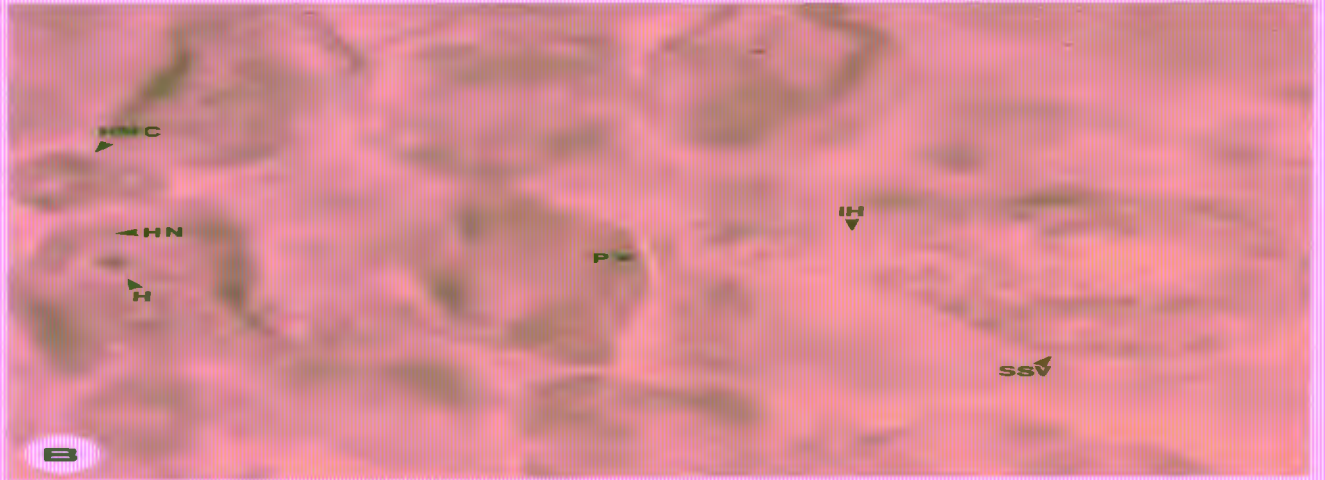
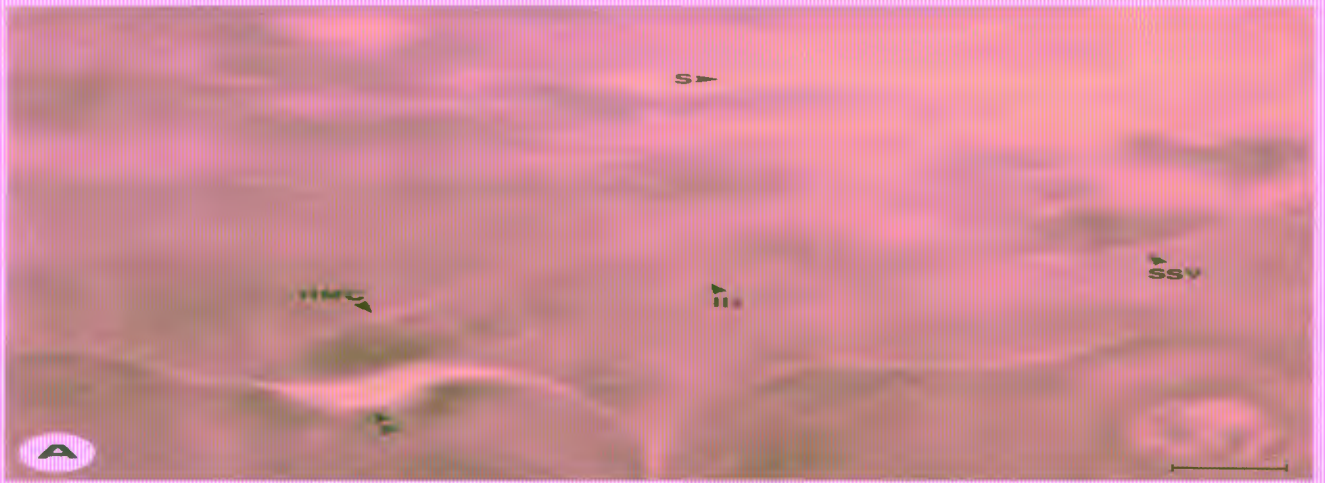
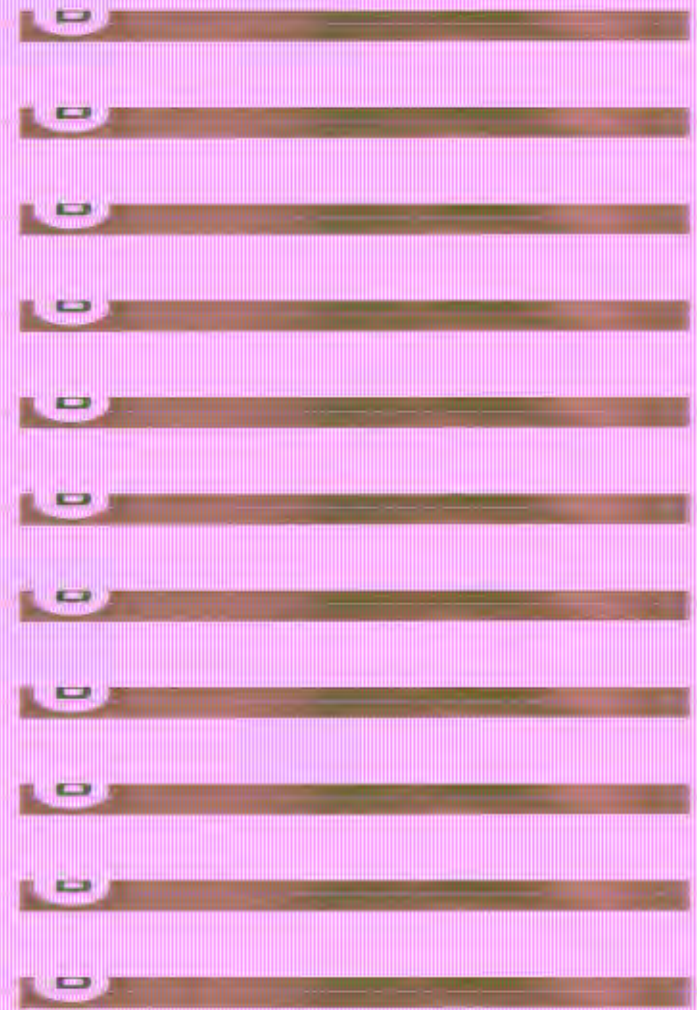
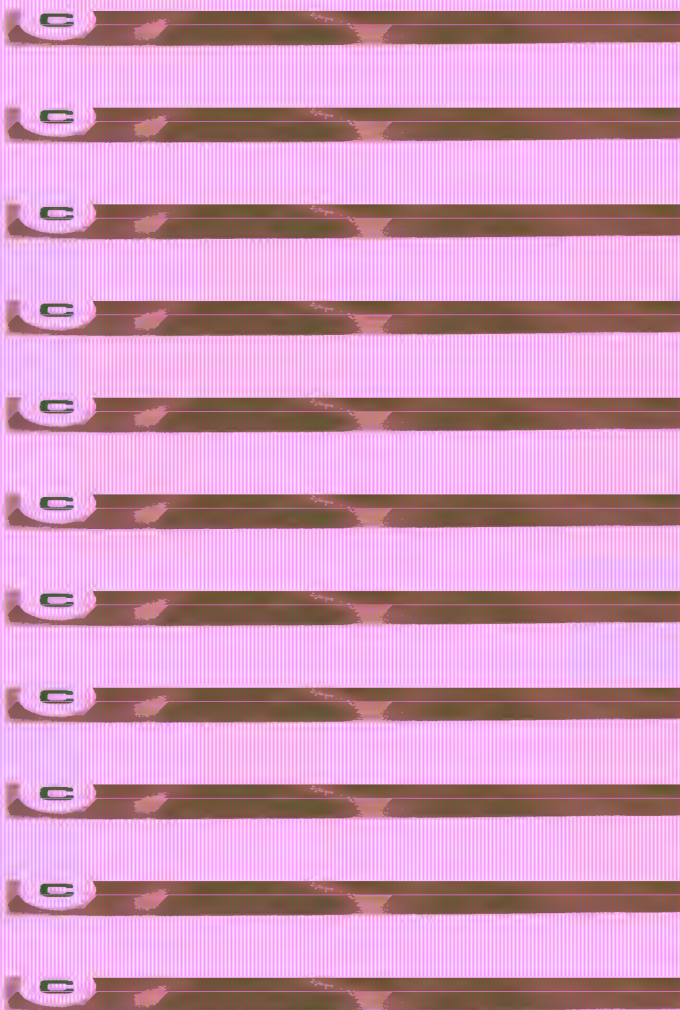
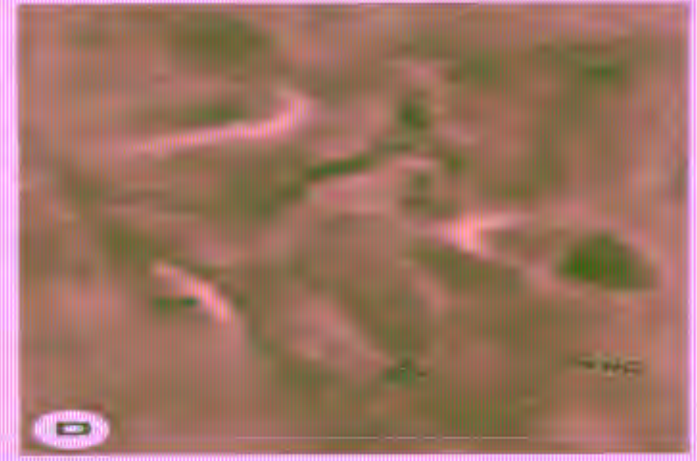
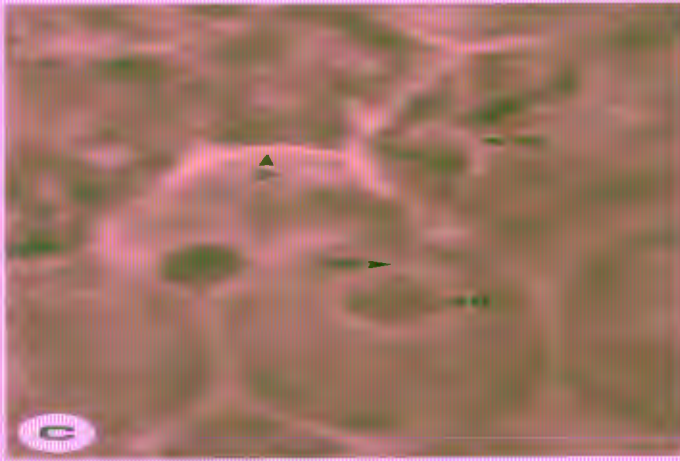
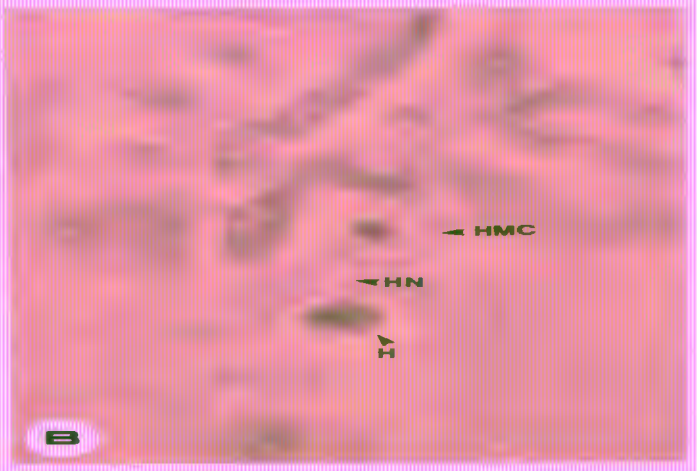
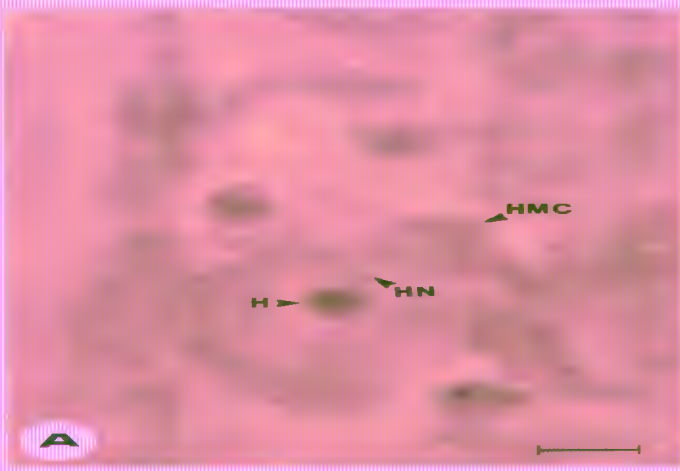


Fig. 18 Different types of papillae in flag leaves stained with picric acid and methyl salicylate 64 hours post inoculation in (A) Thatcher ;(B,C) CT263 x RL6011 F₃ family 19 inoculated with UVPrt2, and (D) CT263 x RL6011 F₃ family 3 inoculated with UVPrt13 of *Puccinia recondita* f.sp. *tritici*. Abbreviations used : HMC=haustorium mother cell; P=papilla. Scale bar represents 2.5 µm.

Fig. 19 Encapsulated infection peg, partially encapsulated and encapsulated haustorium in host cells of (A) Thatcher inoculated with UVPrt2 ; (B) RL6011 inoculated with UVPrt2 and (C) CT263 x RL6011 F₃ family 3 inoculated with pathotype UVPrt13 of *Puccinia recondita* f.sp. *tritici*. Abbreviations used : S=stoma; SSV=substomatal vesicle; IH=infection hyphae; P=papilla; PP=penetration peg; HN=haustorium neck; H=haustorium. Scale bar represents 2.5 µm.

Fig. 20 Haustoria of pathotype UVPrt13 of *Puccinia recondita* f.sp. *tritici* in host cells of (A) RL6011; (B) CT263 x RL6011 F₃ family 3; (C) a young haustorium of UVPrt2 adjacent to a necrotic cell in CT263 x RL6011 F₃ family 3 and (D) a mature haustorium of UVPrt2 in a necrotic cell of the latter line. Abbreviations used : HMC=haustorium mother cell; HN=haustorium neck ; MH=mature haustorium; NC=necrotic cell. Scale bar represents 2.5 µm.



Cell wall appositions The morphology of the cell wall appositions varied considerably. Most of these structures consisted of a narrow, luminous zone slightly wider in the centre opposite the haustorium mother cell (Fig. 17). Usually the length of the zone exceeded its width. A few cell wall appositions showed a thick central part opposite the haustorium mother cell, sharply decreasing towards both ends (Fig. 18). Sporadically, an encapsulated peg was observed, and on rare occasions, partially-encapsulated or encapsulated haustoria (Fig. 19), or a haustorium in a cell displaying a wall apposition, were observed (Fig. 20).

Number of papillae per colony The results were combined (Fig. 21) since the two experiments did not differ and no experiment x pathotype, or experiment x host genotype interactions occurred. The only significant ($P < 0.05$) difference observed was between the host genotypes. Fewer papillae were observed in Thatcher leaves compared to the other genotypes. In RL6011, UVPrt13 infection sites displayed more papillae than in the other genotypes.

Number of haustoria per colony The number of haustoria per colony ($P < 0.05$) differed between the two experiments. More haustoria were observed in the second (overall average 1.33) compared to the first experiment (overall average 0.80) (Fig. 22). In the second experiment more haustoria of both pathotypes developed in line 13/12-3 than in the other genotypes. No haustoria could be detected in leaf segments of line 13/12-19 inoculated with pathotype UVPrt13 in the first experiment due to oil damage and consequent cell collapse.

Fig. 21 Number of papillae per infection site of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici* in flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*, 64 hours post-inoculation. Error bars represent positive standard deviations.

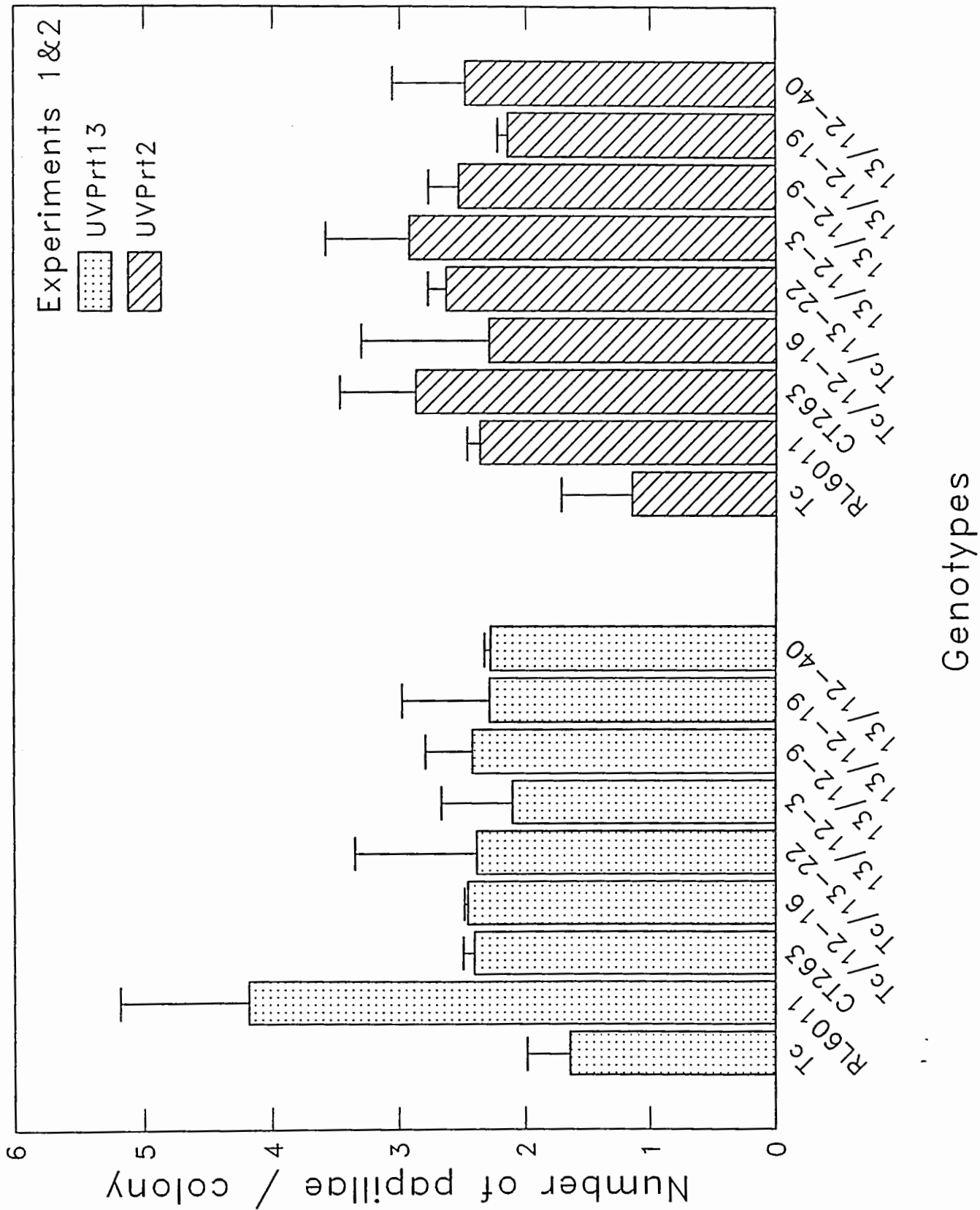
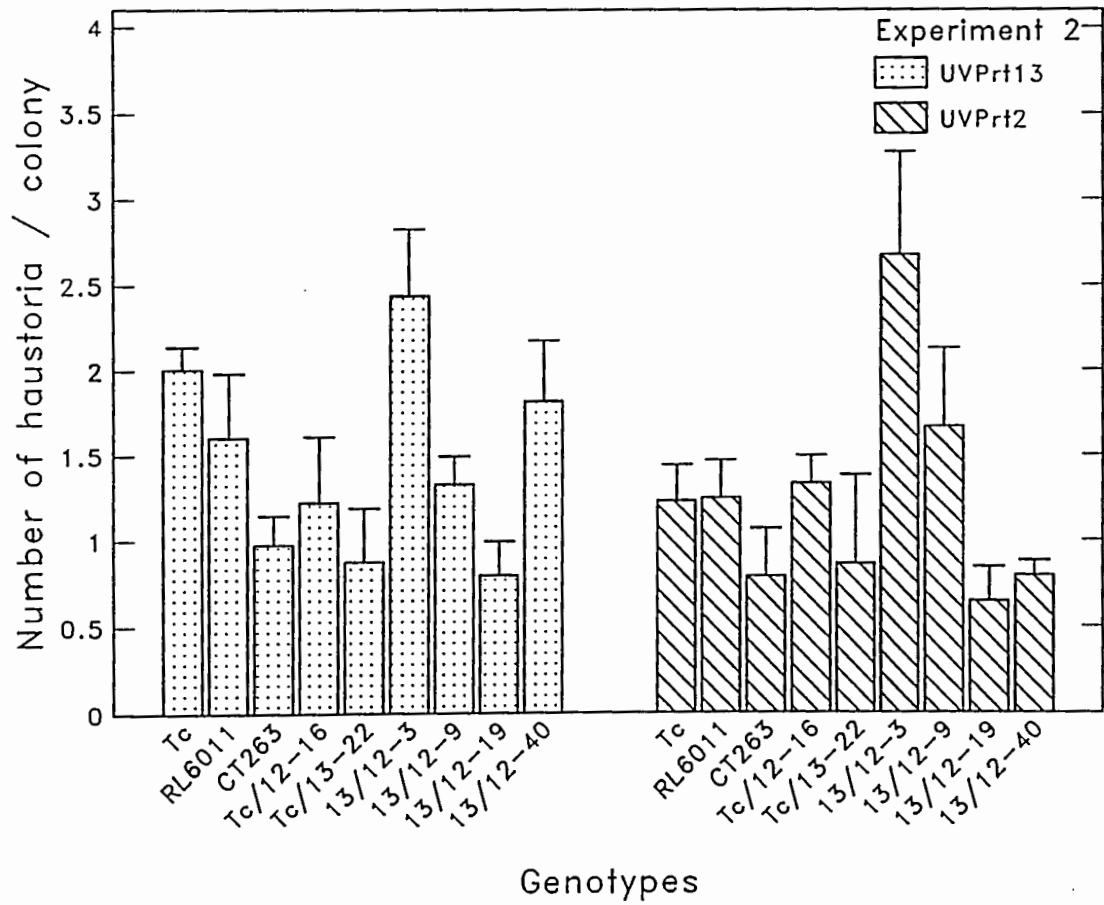
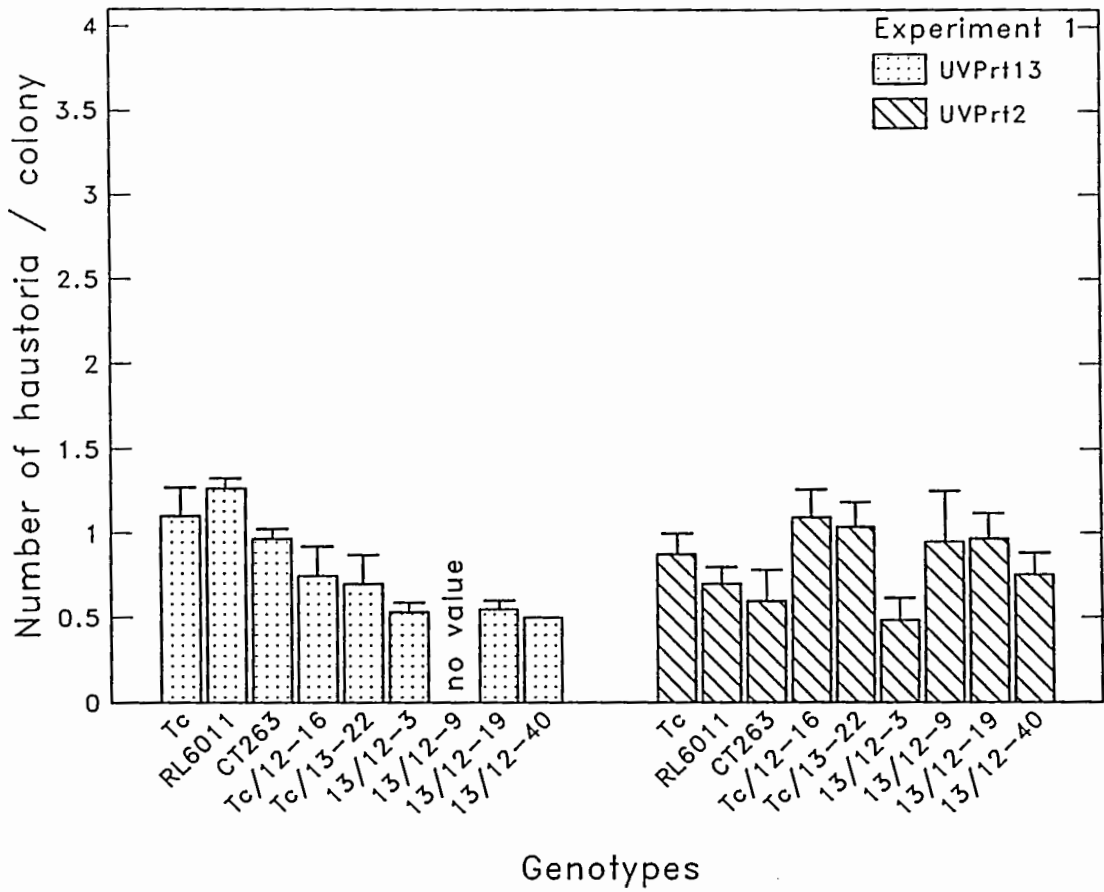


Fig. 22 Number of haustoria per infection site of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f.sp. *tritici* in flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*, 64 hours post-inoculation. Error bars represent positive standard deviations.



Number of papillae per haustorium The ratio of papillae per haustorium (Fig. 23) in the two experiments differed statistically ($P < 0.05$). The ratio for the two pathotypes was similar, but the experiment x host genotype, and pathotype x host genotype interactions were significantly different. Thatcher showed the lowest ratio of papillae per haustorium for both pathotypes. In the first experiment no data were obtained for 13/12-19/UVPrt13 due to oil damage to leaves during inoculation.

Macroscopic components of resistance

Infection types The IT's of Thatcher, RL6011, CT263, the four F_3 families homozygous for both *Lr12* and *Lr13*, and two single gene lines Tc/12-16 and Tc/13-22 to pathotypes UVPrt13 and UVPrt2 are presented in Table 15. The flag leaf responses of CT263, RL6011 and 13/12-9 to UVPrt2 and CT263, RL6011, and 13/12-40 to UVPrt13 are presented in Figs. 24 and 25, respectively. Thatcher was susceptible to both pathotypes (IT 3⁺⁺). Highly resistant IT's were observed on flag leaves of lines carrying both *Lr12* and *Lr13*. These IT's ranged from 0; to ;1 (Figs. 24 and 25) except for 13/12-9 which displayed a 2⁻ IT to UVPrt13 in the second experiment. When inoculated with UVPrt2 in the first experiment, the combination line 13/12-9 showed a more resistant IT (0;-;1^{-cn}) in comparison with the resistant parent [CT263 (IT ;;-;1^{-cn})]. This was confirmed for UVPrt2 in the second experiment when lines 13/12-3 and 13/12-9 showed more resistant IT's than the resistant parent [CT263 (IT ;;1^{-cn})]. With UVPrt13, only line 13/12-40 displayed a more resistant IT (;1) than the resistant parent RL6011 (IT ;1⁺) in the second experiment.

Latent period Latent period of leaf rust was significantly ($P < 0.05$) influenced by host

Fig. 23 Number of papillae for each haustorium of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f.sp. *tritici* in flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*, 64 hours post-inoculation. Error bars represent positive standard deviations.

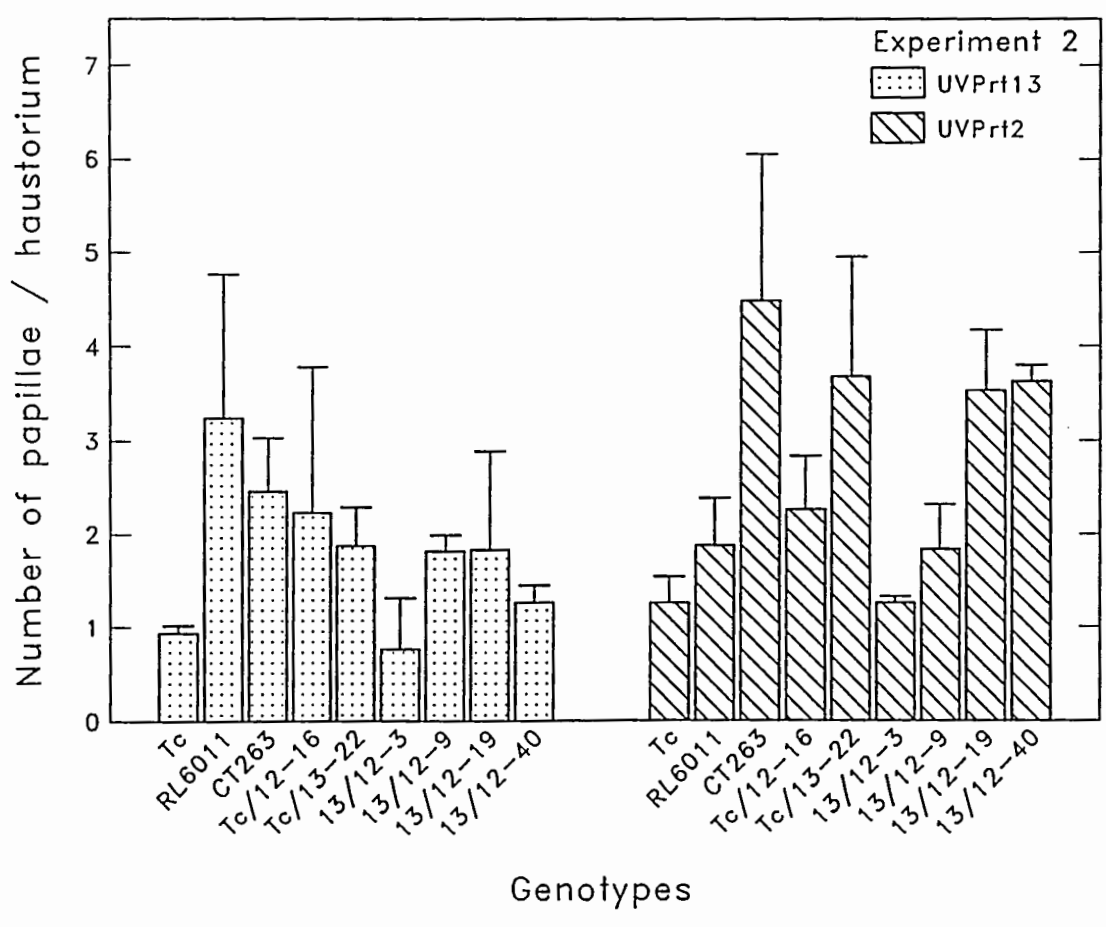
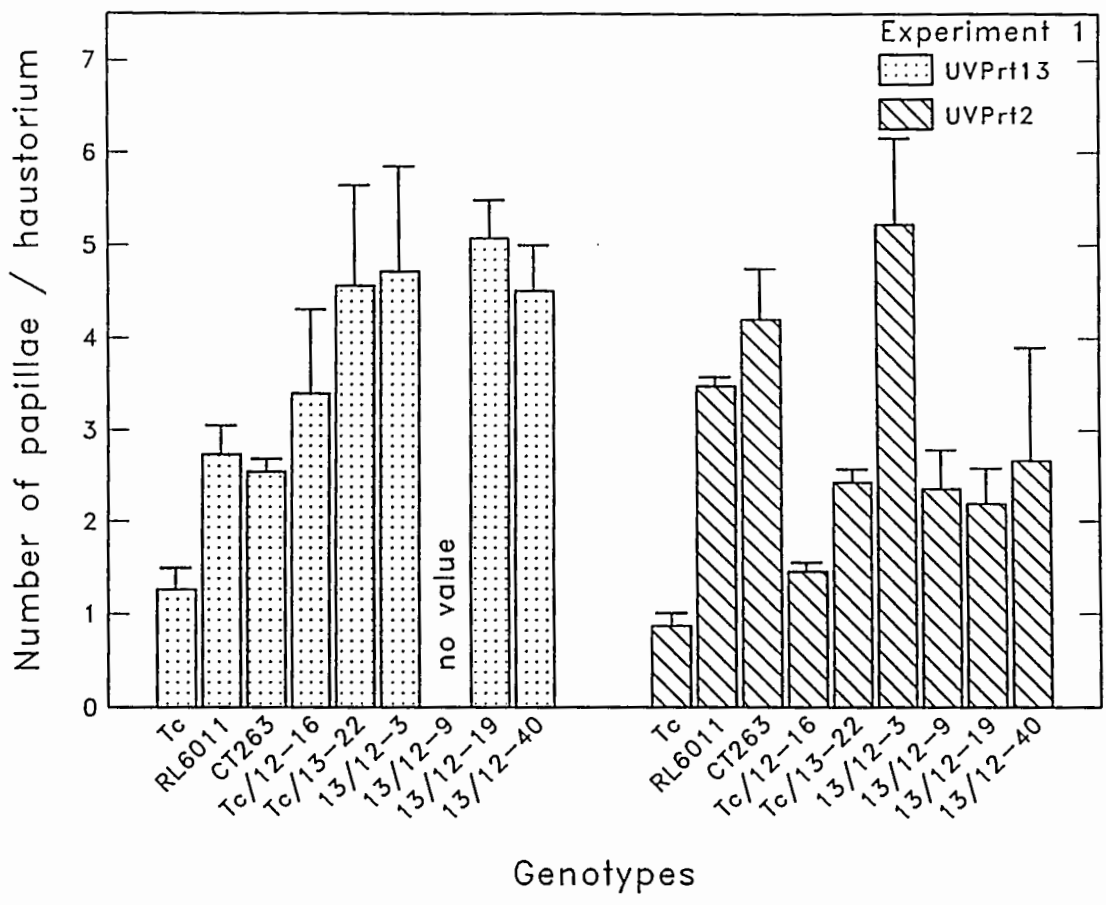


Table 15. Infection types^a produced by pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici* on flag leaves of Thatcher ([Tc] leaf rust-susceptible) wheat and genotypes containing *Lr12* and/or *Lr13*

Pathotype	Thatcher	RL6011	CT263	13/12 fam 3 ^b	13/12 fam 9 ^b	13/12 fam 19 ^b	13/12 fam 40 ^b	Tc/12 fam 16	Tc/13 fam 22
First experiment									
UVPrt2	3 ⁺⁺	3 ⁺⁺	;1 ^{-cn}	;1 ^{-cn}	0;-;1 ^{-cn}	;1 ^{cn}	;1 ^{-cn}	3 ⁺⁺	;1 ^{cn}
UVPrt13	3 ⁺⁺	;1 ⁺⁺	3 ⁺⁺	;1 ⁺⁺	;1 ⁺⁺	;1 ⁺⁺	;1 ⁺⁺	2	3 ⁺⁺
Second experiment									
UVPrt2	3 ⁺⁺	3 ⁺⁺	;1 ^{-cn}	;1 ^{-cn}	;1 ^{-cn}	;1 ^{cn}	;1 ^{cn}	3 ⁺⁺	;1 ^{-cn}
UVPrt13	3 ⁺⁺	;1 ⁺	3 ⁺⁺	;1 ⁺	2 ⁻	;1 ⁺	;1	;1 ⁺⁺	3 ⁺⁺

^a Infection types according to a 0 to 4 scale (Roelfs, 1988b) were recorded 14 d.p.i. on flag leaves of adult plants.

^b F₃ families, homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

Fig. 24 Infection types produced by pathotype UVPrt2 of *Puccinia recondita* f. sp. *tritici* on flag leaves of (A) CT263, (B) RL6011 and (C) CT263 x RL6011 F₃ family 9, 14 days post-inoculation.

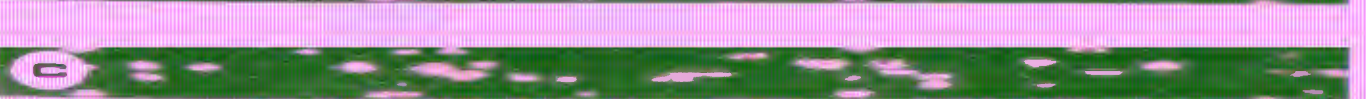
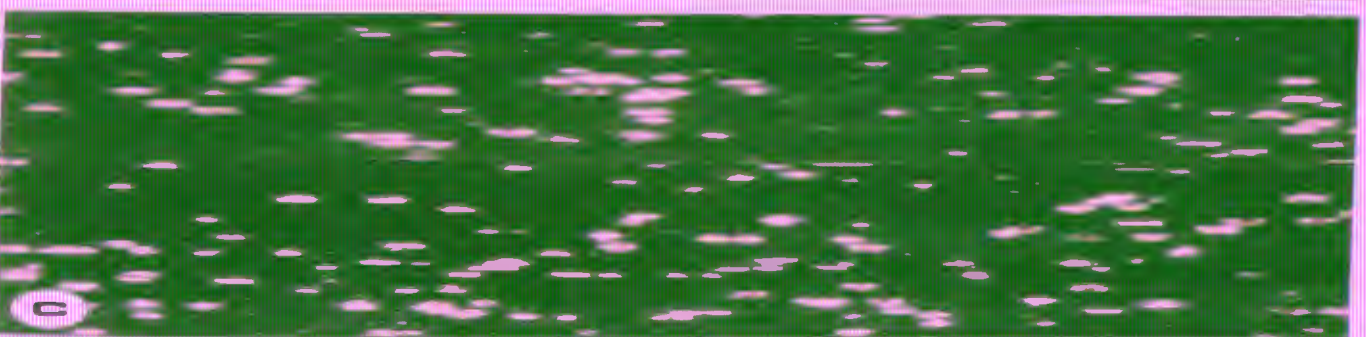
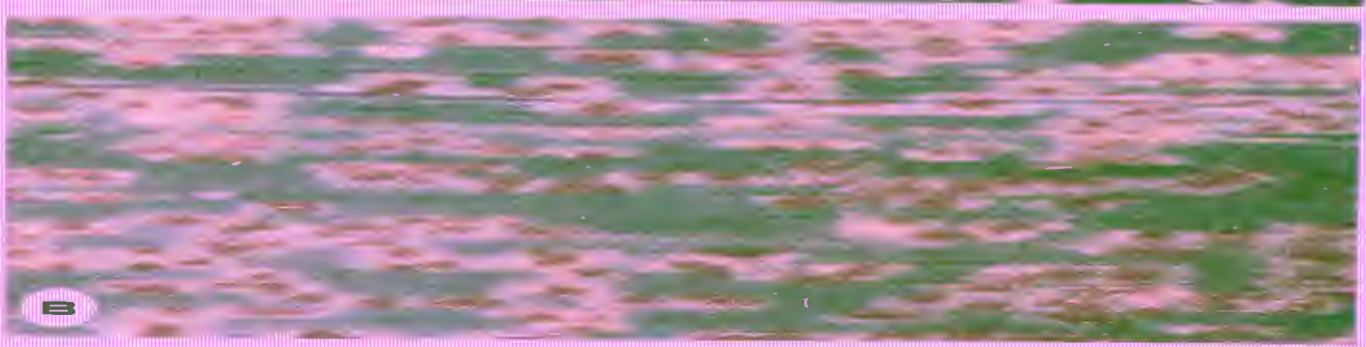
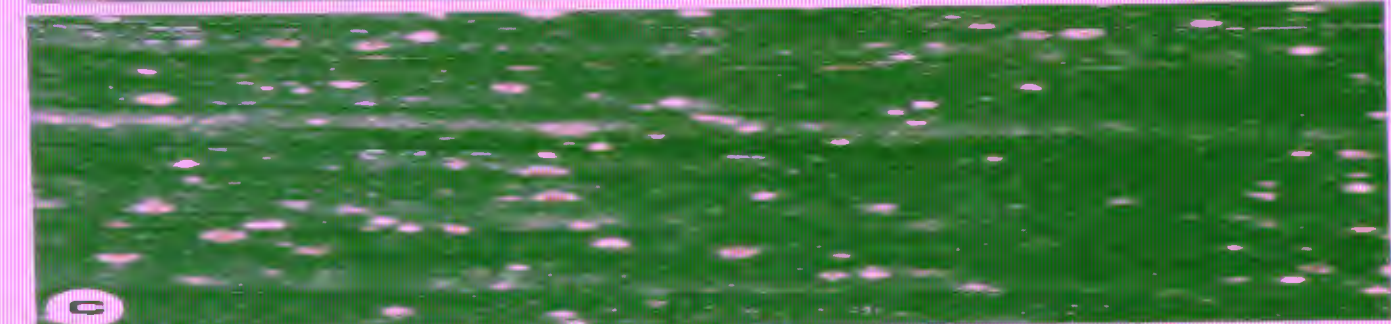
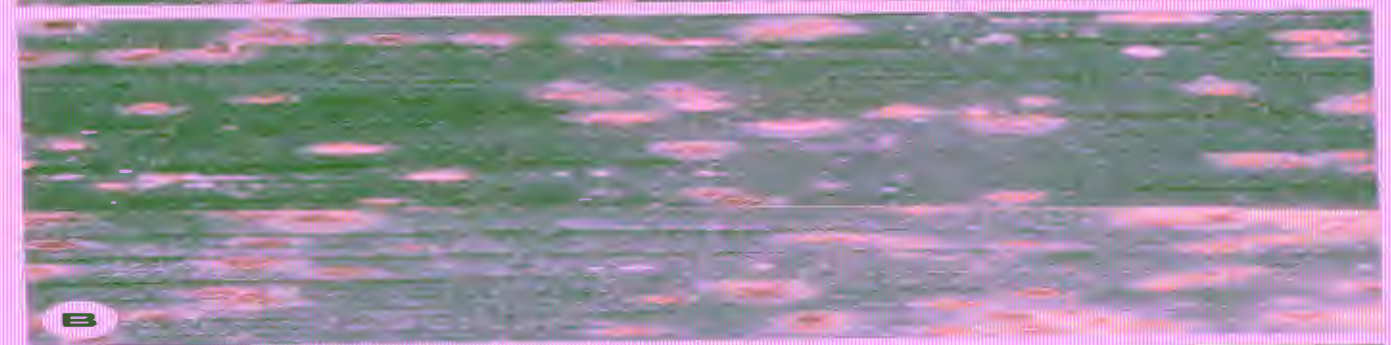


Fig. 25 Infection types produced by pathotype UVPrt13 of *Puccinia recondita* f. sp. *tritici* on flag leaves of (A) RL6011, (B) CT263 and (C) CT263 x RL6011 F₃ family 40, 14 days post-inoculation.



genotype, pathotype and their interaction. The two experiments were significantly different with shorter latent periods observed in the second experiment. In the first experiment only line *13/12-19* showed a significant improvement of resistance (i.e. longer latent period) to both pathotypes when compared to the parents or the single gene lines *Tc/12-16* and *Tc/13-22* (Table 16). Latent periods determined in the other gene combination lines were statistically similar to, or marginally different from Thatcher, the parents, or single gene lines. Furthermore, no indication of an increased latent period of leaf rust due to the combination of *Lr12* and *Lr13* was obtained in the second experiment. The increased resistance observed previously for *13/12-19* was not confirmed.

Uredium density In the first experiment, the mean number of uredia was consistently less ($P < 0.05$) than in the second experiment (Fig. 26). The number of uredia was significantly influenced by pathotype, host genotype and their interaction. It was visibly higher on Thatcher than on the other genotypes. Variation in uredium density was observed between the combination lines. Considering both pathotypes, *13/12-3* and *13/12-9* supported less uredia compared to the other lines. In both experiments the line *Tc/12-16*, inoculated with UVPrt2, displayed a lower density than RL6011. Similar results were observed for *Tc/13-16* inoculated with UVPrt13 in the first, but not in the second experiment. The differences were more apparent in the second experiment when lines were inoculated with higher inoculum densities.

Uredium size The mean size of uredia was significantly ($P < 0.05$) different between the two experiments. Uredium size was significantly influenced by pathotype, host

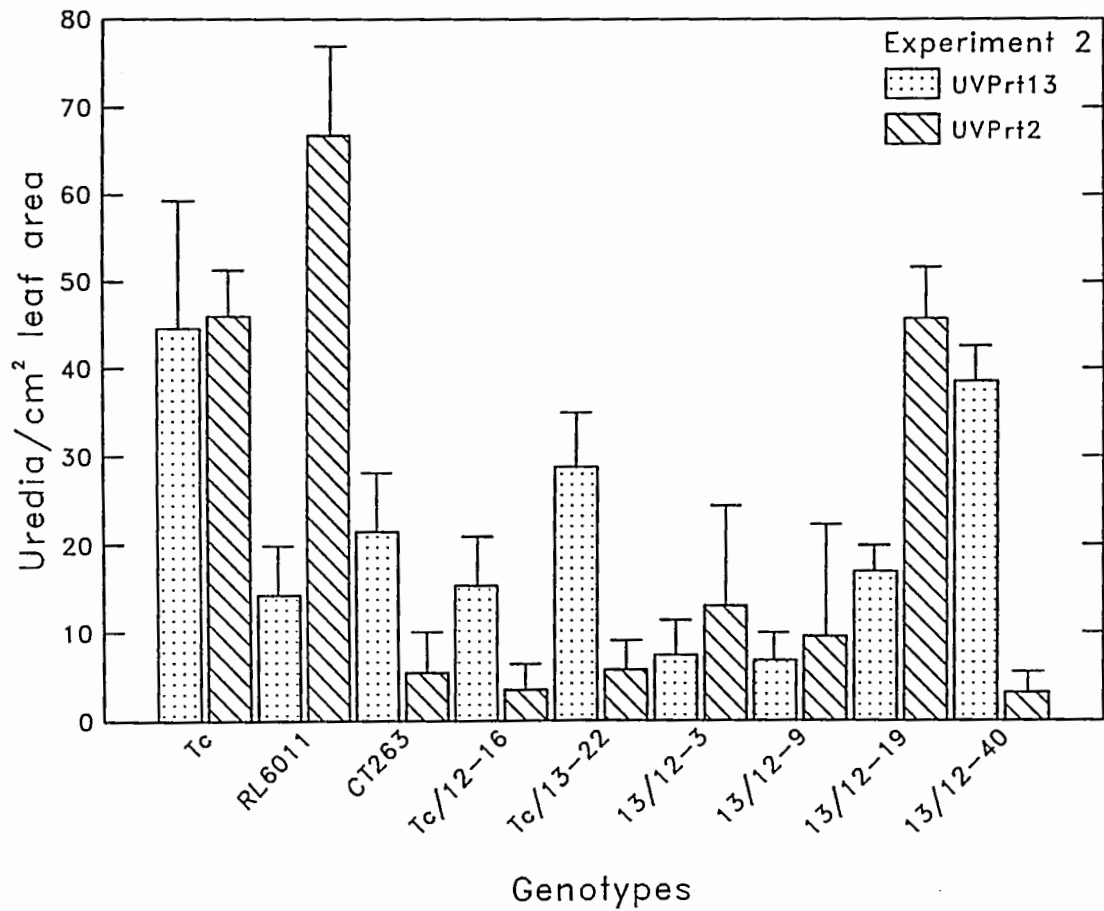
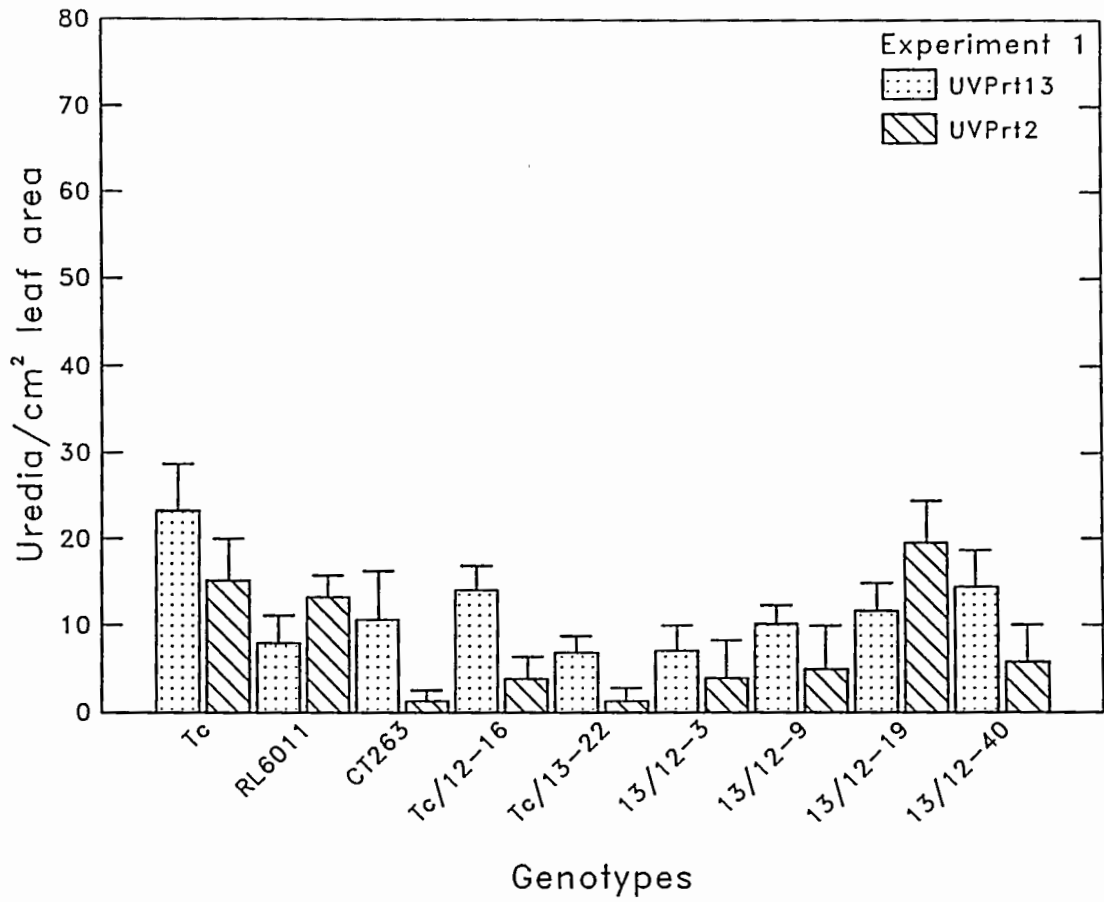
Table 16. Latent period^a of pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici* determined on flag leaves of Thatcher (leaf rust-susceptible) wheat and plants containing *Lr12* and/or *Lr13*

Pathotype	Latent period (h)								
	Thatcher	RL6011	CT263	13/12 fam 3 ^b	13/12 fam 9 ^b	13/12 fam 19 ^b	13/12 fam 40 ^b	Tc/12 fam 16	Tc/13 fam 22
First experiment									
UVPrt2	295±11	274±11	288±14	257±13	265±8	301±7	273±10	272±8	290±44
UVPrt13	290±5	296±8	305±14	294±13	307±5	351±25	311±6	287±8	285±8
Second experiment									
UVPrt2	236±3	223±5	249±12	240±20	226±2	223±8	236±10	233±12	250±3
UVPrt13	237±4	262±17	246±11	266±12	284±6	260±14	224±5	260±8	223±5

^a Means ± standard deviation. Latent period was calculated as the time (h) between inoculation and visibility of 40 % of the primary uredia as erumpent structures.

^b F₃ families homozygous for *Lr12* and *Lr13*, derived from the cross CT263 x RL6011.

Fig. 26 Uredium density (number of pustules/cm² leaf area) measured 14 days post-inoculation on flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. Plants were inoculated with pathotypes UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici*. Error bars represent positive standard deviations.



genotype and their interaction. Evidence for a reduced pustule size was found in lines *13/12-3*, *13/12-9*, and *13/12-40* inoculated with UVPrt2, but no significant restriction of uredium size could be demonstrated for UVPrt13 (Fig. 27). Uredia produced by UVPrt2 on the single gene line *Tc/12-16* were smaller than on the parent RL6011. A similar effect, but not as pronounced, was found for CT263 and *Tc/13-22* inoculated with UVPrt13.

Field reaction of gene combination lines

The flag leaf responses of Thatcher, the backcross lines CT263, RL6011, and the F_4 lines *13/12-3*, *13/12-9*, *13/12-19* and *13/12-40* to leaf rust are presented in Table 17. The response of CT263, RL6011 and *13/12-40* are displayed in Fig. 28. Thatcher, RL6011, CT263 and the single gene lines were fully susceptible and defoliated by leaf rust. Flag leaves of the four combination lines showed little evidence of disease. The terminal rust reaction type was moderately resistant to moderately susceptible on the four combination lines and visual severity ratings did not exceed 20%.

Fig. 27 Uredium size (mm^2) measured 14 days post-inoculation on flag leaves of Thatcher, RL6011, CT263, Tc/12-16, Tc/13-22 and four lines containing *Lr12* and *Lr13*. Plants were inoculated with UVPrt2 and UVPrt13 of *Puccinia recondita* f. sp. *tritici*. Error bars represent positive standard deviations.

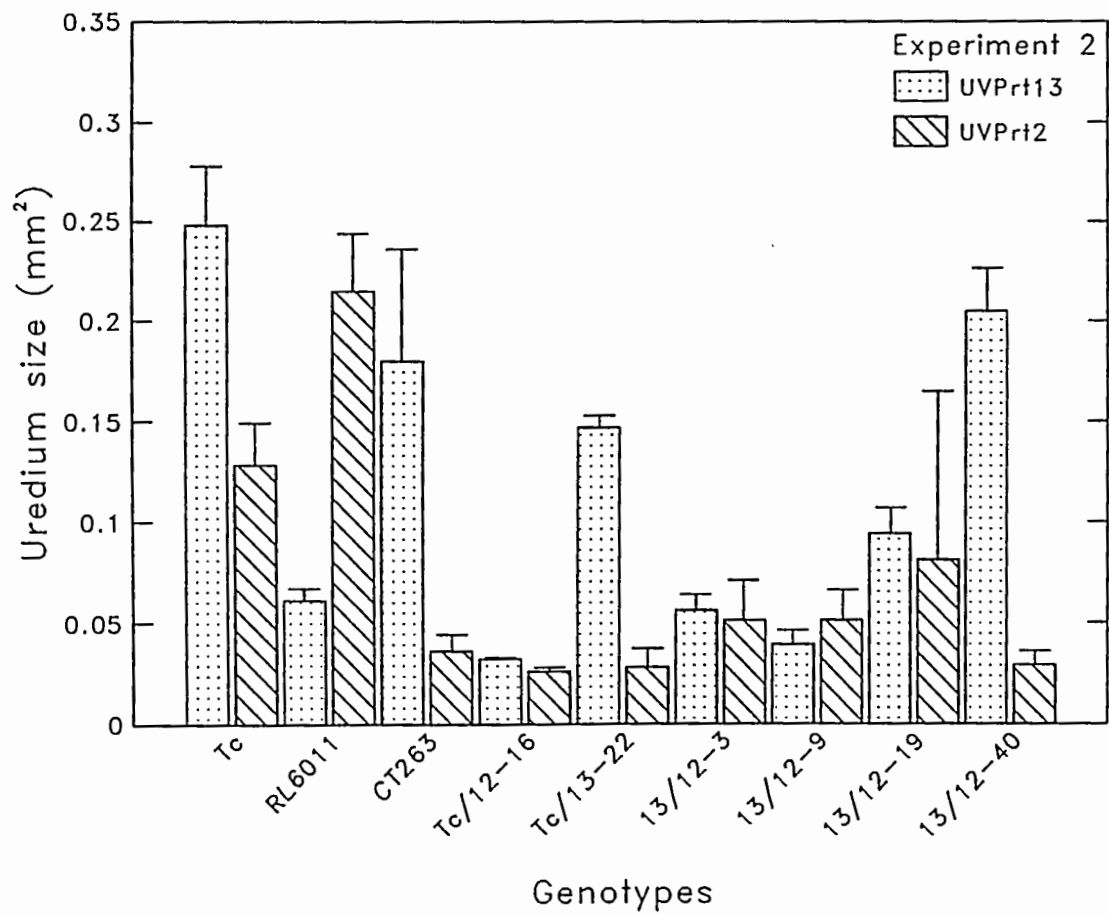
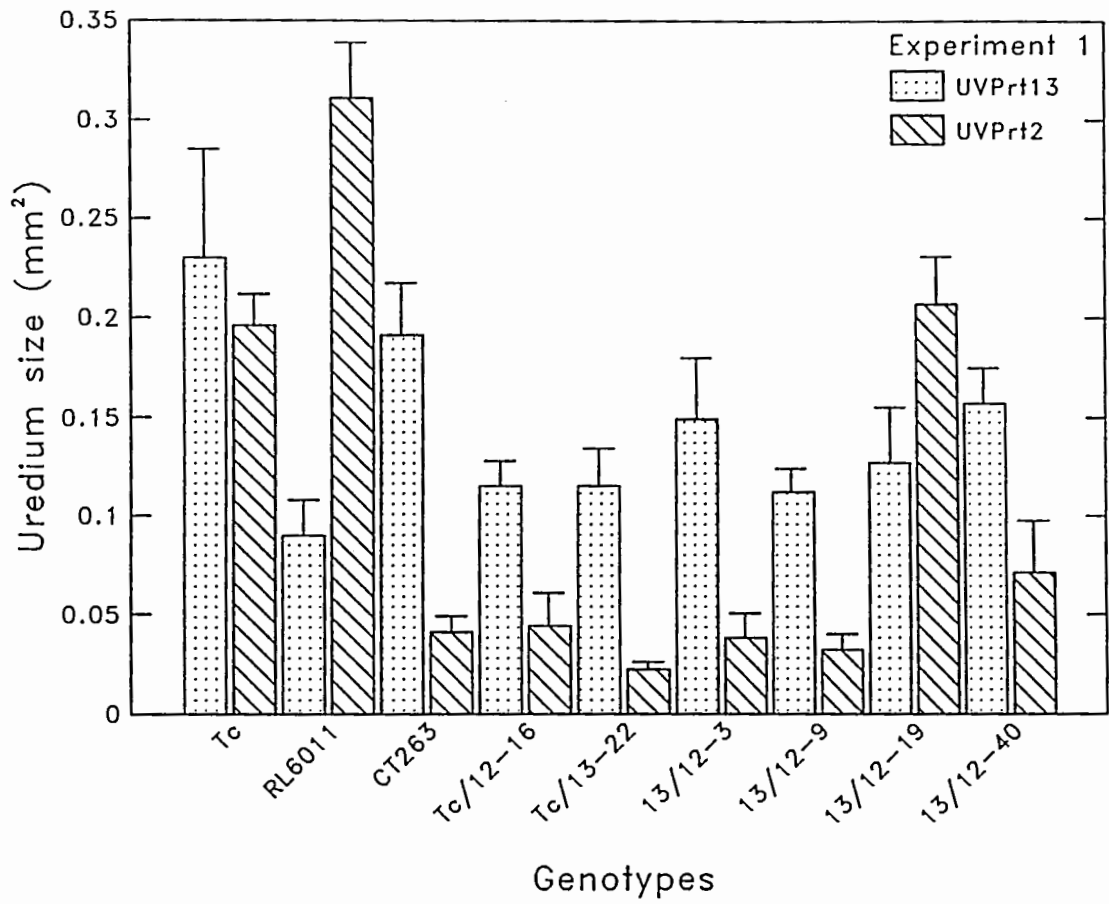


Table 17. Leaf rust severity and reaction type^a determined in the field on flag leaves of Thatcher ([Tc] leaf rust-susceptible) wheat and genotypes containing *Lr12* and/or *Lr13*

Genotype	Severity and reaction type ^a		
	49 d.p.i.	59 d.p.i.	70 d.p.i.
Tc	90S	_ ^b	_ ^b
RL6011	10S	80S	_ ^b
CT263	30S	90S	_ ^b
CT263 x RL6011			
F ₄ family 3	0	15MR	20MR-MS
F ₄ family 9	0	15MR	20MR-MS
F ₄ family 19	0	10MR	20MR-MS
F ₄ family 40	0	10MR	20MR-MS
Tc x CT263 F ₄	10S	70S	_ ^b
Tc x RL6011 F ₄	40S	80S	_ ^b

^a Leaf rust severity and reaction type (S = susceptible; MS = moderately susceptible; MR = moderately resistant) were determined according to Stubbs *et al.*, (1986). Plots were inoculated with pathotypes UVPrt2, 3, 9 and 13 of *Puccinia recondita* f. sp. *tritici*.

^b Defoliated by leaf rust.

Fig. 28 Leaf rust severity on flag leaves of (A) CT263 (*Lr13*), (B) RL6011(*Lr12*) and (C) CT263 x RL6011 F₃ family 40 (*Lr12+Lr13*) in the field during 1994.



DISCUSSION

By characterising the resistance conferred by the genes *Lr12* and *Lr13*, this study contributes to information currently available regarding the effectivity and management of sources of resistance to wheat leaf rust in South Africa. According to the flag leaf reactions, RL6011 was resistant to all pathotypes of *P. recondita* f. sp. *tritici* tested except UVPrt2. *Lr12* is therefore effective to those pathotypes predominantly isolated during previous surveys of pathogenic variability in *P. recondita* f. sp. *tritici* in South Africa (Pretorius *et al.*, 1987; Pretorius and Le Roux, 1988; Pretorius *et al.*, 1990). The effectiveness of *Lr12* against the majority of local leaf rust pathotypes can probably be ascribed to the fact that *Lr12* has not been used in wheat breeding in South Africa. Virulence to *Lr12* has been reported from elsewhere (Samborski, 1983; 1984; 1986; Stoyanov, 1987; Sorokina *et al.*, 1991; Singh and Gupta, 1992; Park and McIntosh, 1994), emphasising the nondurability of the gene when exposed singly in commercial cultivars.

Lr13 was effective against all pathotypes except UVPrt 6, 8, 12 and 13. These pathotypes are considered to be related and differ only with respect to avirulence or virulence to *Lr24* and *Lr26*. The common occurrence of pathotypes with these virulence characteristics in South Africa (Pretorius *et al.*, 1990; B.D. van Niekerk, Small Grain Institute, South Africa, pers. comm. 1995) implies that *Lr13* alone has no value in breeding for leaf rust resistance locally. However, the importance of *Lr13* in genotypes known to possess durable resistance has been shown (Samborski, 1983; Luig, 1985; Rajaram *et al.*, 1988; Roelfs, 1988a; McIntosh, 1992; Kolmer *et al.*, 1993). In view of its potential to interact with other *Lr* genes, thereby enhancing the level of

resistance (Samborski and Dyck, 1982; Kolmer, 1992; Roelfs *et al.*, 1992; Kolmer *et al.*, 1993; Kloppers, 1994; Krupnov *et al.*, 1995), *Lr13* may still be of value when managed appropriately. The phenomenon of resistance enhancement due to gene combinations has also been demonstrated in genotypes containing *Lr34* (German and Kolmer, 1992). In a comprehensive study, Kolmer (1992) showed that gene pairs involving *Lr13* were often more resistant than any of the lines containing the genes singly. In this regard, combinations of *Lr13* with either *Lr2a*, *Lr3ka*, *LrB*, *Lr11*, *Lr16*, *Lr18*, *Lr21*, *Lr30* or *Lr33* produced the highest levels of resistance in seedlings. In the field, combinations between *Lr13* and, respectively, *Lr16*, *Lr17*, *Lr30* and *LrB*, were the most effective over two seasons. Kolmer (1992) did not test lines containing both *Lr12* and *Lr13*.

Lr12 or *Lr13* could easily be detected in adult plants. Present results showed that both genes were inherited dominantly, and expressed clearly defined resistance phenotypes to several pathotypes. Using UVPrt2, *Lr13* was also detectable in primary leaves when plants were evaluated at higher temperatures. Detection of *Lr* genes in seedlings is highly desirable when breeding for resistance. Expression of resistance during early growth stages allows extensive screening in controlled environments, without costly and time-consuming field experiments. This result is in accordance with a previous report (Pretorius *et al.*, 1984) that *Lr13* is expressed in seedlings under certain environmental conditions. The temperature sensitivity of *Lr13* was emphasised by the fact that detection of the gene at 15°C was not possible. Although the IT's produced by UVPrt2, 4 and 5 on CT263 were associated with chlorosis, the large pustules negated confident confirmation of *Lr13*. An interesting observation was that pathotypes UVPrt3, 7 and 10, which were all avirulent on CT263, did not detect *Lr13* in seedlings at 25°C. Since these pathotypes also appeared avirulent to Thatcher

which carries the *Lr22b* gene for adult-plant resistance (Dyck, 1979), the low reaction of flag leaves of CT263 might not have been conferred by *Lr13*. Since CT263 is a Thatcher backcross line with *Lr13*, it has been assumed that *Lr22b* was retained (Park and McIntosh, 1994).

In general it appeared that gene interaction in seedlings was not readily detected, that it was pathotype-specific, and that factors associated with *Lr13* alone could have been responsible for an apparent enhancement of resistance. Several of the *Lr12+Lr13* combination lines produced seedling IT's that were lower than those of the parents. This was, however, not consistent over all pathotypes, temperatures or combination lines. Furthermore, this "enhancement" of resistance was often not visible when the combination lines were compared with the monogenic line Tc/13-22. At 15°C, for example, all four combination lines produced IT 2 to pathotype UVPrt4, whereas the RL6011 and CT263 parents exhibited IT's 3⁺⁺ and 3[°], respectively. The 2^{cn} IT exhibited by Tc/13-22 showed, however, that the improvement of resistance in the two-gene lines cannot be ascribed to an interaction between *Lr12* and *Lr13*, but rather genetic factors associated with *Lr13*. Similar results were obtained with other pathotypes at 25°C. Testing of the lines at this temperature showed an enhancement of resistance to UVPrt9.

During infection of a host plant, growth of fungal structures can be interrupted at several phases. In principle, each of these phases can be affected by the action of resistance genes (Niks, 1982). Considering the microscopic components, effects of *Lr12* and/or *Lr13* resistance on the prepenetration stages, preventing the fungus from establishing an infection site, were not determined. Although inhibition of prehaustorial growth has been reported for several species of rust fungi including *P. recondita* f. sp.

tritici (Heath, 1982), histological studies of the wheat leaf rust interaction have seldom indicated an active resistance mechanism inhibiting urediospore germination or appressorium formation (Jacobs, 1989a). Similarly, Lee and Shaner (1984) concluded that neither hypersensitive nor slow-rusting resistance had an effect on the prepenetration stages of *P. recondita* f. sp. *tritici* on wheat. Southerton and Deverall (1989) showed, furthermore, that the formation of substomatal vesicles, infection hyphae and the first haustorium mother cell of a leaf rust isolate avirulent for *Lr9*, *Lr20* and *Lr28* was unaffected by the presence of these genes. In his study with resistance of barley to *P. hordei* and *P. recondita*, Niks (1987) found that nonpenetrating appressoria and aborted substomatal vesicles were unimportant in resistance.

Results from the present study showed that inhibition of fungal growth in wheat lines containing *Lr12* and *Lr13* alone or in combination, was activated to a certain degree before haustoria were formed. This was evident from data obtained for nonpenetrating appressoria and aborted substomatal vesicles, previously defined as abortive penetration by Parlevliet and Kievit (1986). At both sampling times and in both replicated experiments, the percentage infection sites of pathotype UVPrt2 classified as aborted were significantly higher on the *Lr* lines than on the susceptible control Thatcher. For UVPrt13 similar, but less prominent differences were observed between the combination lines and Thatcher. With this pathotype, differences were best seen at 240 h.p.i. From the data presented in Tables 10 and 11 it was evident that aborted penetration resulted mainly from the arrestation of substomatal vesicles, rather than nonpenetrating appressoria. The misclassification of infection sites according to their phase of penetration is possible, especially where the first haustorium mother cell is concealed directly beneath the brightly fluorescing appressorium. Infection sites with

only one haustorium mother cell were, however, frequently observed. It is therefore unlikely that misclassified infection sites accounted for large differences in abortive penetration between genotypes.

Infection sites where the first haustorium mother cell was visible were considered a colony. Determination of colony size 240 h.p.i. indicated that dimensions were significantly influenced by host genotype, pathotype and their interaction. At this sampling time, colonies in the combination lines were generally smaller than in the parents, but not necessarily smaller than those in the monogenic line Tc/13-22. Results were, furthermore, not consistent over experiments. Analyses of variance showed that the two experiments were significantly different for most histological parameters determined. This can be attributed to differences in spore deposition during inoculation of lines in the respective experiments, as well as higher glasshouse temperatures experienced in the second experiment. The determination of colony size 64 h.p.i. produced inconclusive results with little variation among lines. The difference between the two sampling times can be explained by the fact that colonies were more developed 240 h.p.i. and that the interaction effects between *Lr* genes apparently only become functional during more advanced stages of disease development.

The hypersensitive response, characterised by rapid death of invaded and neighbouring cells, resulting in macroscopic lesions at the infection sites (Tani *et al.*, 1974), is an active defence system occurring in most higher plants in response to pathogens (Keen, 1990). There remains uncertainty as to whether hypersensitivity is the result or cause of disease resistance in plants, and several pathosystems have been studied to support both arguments (Mayama *et al.*, 1975; Heath, 1976). Heath (1976) stated that the role of hypersensitivity varies from one host-pathogen interaction

to another, which might explain the differences in hypersensitive responses. Host cell necrosis features prominently in the description of posthaustorial resistance, a mechanism where at least one haustorium is formed before fungal growth is arrested. This mechanism is typical of major-genic, race-specific and usually non-durable resistance of plants to rust fungi (Heath, 1981a; 1982; Niks and Dekens, 1991). Susceptibility does not imply that no necrotic cells are associated with infection sites. A few necrotic host cells usually occur within the colonised tissue, presumably as a result of a general stress reaction (Samborski *et al.*, 1977). This was confirmed in the present study by necrotic leaf tissue observed in the susceptible control Thatcher.

Host cell necrosis was more frequently associated with infection sites, specifically of UVPrt2, in the combination lines than in the parents. This prominence in hypersensitivity was more apparent at the 64 h.p.i. sampling time than at 240 h.p.i.. Niks and Dekens (1987) concluded that the onset of hypersensitivity is variable. The reaction may be induced at an early, intermediate or late stage and may be associated with complete or incomplete inhibition of sporulation. Considering the mean necrotic area surrounding an infection site, variable results were obtained. At 64 h.p.i. necrosis was generally more severe in the combination lines than in the parents. The two pathotypes also evoked different hypersensitive responses in the individual combination lines. At 240 h.p.i. in the first experiment, the combination lines displayed smaller necrotic areas to both pathotypes than the most resistant parent. This reduction in hypersensitivity due to the combination of *Lr12* with *Lr13* was not confirmed for all lines with paired genes in the second experiment. Using pathotype UVPrt13, some lines responded similarly to the resistant parent whereas others exhibited less necrosis. With UVPrt2, larger necrotic areas were detected in line 13/12-

3 than in the resistant CT263 parent. The hypersensitivity index, calculated as the necrotic leaf area divided by colony size, was strongly influenced by pathotype. Index values obtained at both sampling times indicated that host cell necrosis was more severe following infection of the combination lines with UVPrt2. These values thus confirmed the observations that hypersensitivity resulted more regularly after inoculation with UVPrt2. At 240 h.p.i. the necrotic area exceeded the actual colony dimensions in several two-gene lines, whereas this was seldom observed with UVPrt13. The higher index values determined for combination lines in comparison with their parents suggested that an interaction between *Lr12* and *Lr13* influenced hypersensitivity. However, the monogenic line Tc/13-22 responded similarly to the digenic lines in some of these treatments, as well as for other components of resistance, e.g. uredium size (Fig. 16). It is therefore not clear if the interaction occurred between *Lr13* and *Lr12*, or between *Lr13* and other modifying genes.

Prehaustorial resistance, defined as the arrestation of fungal development prior to haustorium formation (Niks and Dekens, 1991), has been shown to be common in nonhost interactions (Heath, 1981a; 1982). A typical prehaustorial resistance mechanism is the inducement of papillae at the contact planes between the haustorium mother cell and host cell (Niks and Dekens, 1991). De Bary was the first to suggest that papillae may function in disease resistance after he noticed the co-occurrence of penetration failure and papillae formation (Smart, 1991). Several studies have correlated resistance (the cessation of fungal growth) with papilla formation in a wide range of host-parasite interactions (Akai *et al.*, 1968; Aist and Israel, 1977a; b; Mayama and Shishiyama, 1978; Aist *et al.*, 1979; Heath, 1979; Ride and Pearce, 1979; Sherwood and Vance, 1980; Allen and Friend, 1983; Coffey and Wilson, 1983). The

presence of papillae as a resistance mechanism in cereals to rust diseases has also been well documented (Niks, 1986; Jacobs 1989c;d; Niks and Dekens, 1991). Niks and Dekens (1991) were of the opinion that diploid wheat with high levels of prehaustorial resistance can be a valuable source of nonhypersensitive, durable resistance. At present, however, no data have been presented that prehaustorial resistance in wheat to leaf rust is actually durable.

Observations in this study showed that young haustoria became more visible in host cells after staining with trypan blue, picric acid and methyl salicylate than with trypan blue only. The morphology of the cell wall appositions varied considerably. Most of these structures consisted of a narrow, luminous zone showing a central thickening opposite the haustorium mother cell. A few cell wall appositions showed a thick central part, sharply decreasing towards both ends of the luminous structure. Occasionally an encapsulated peg, and on rare occasions partially-encapsulated or encapsulated haustoria, were observed. Cells displaying both a wall apposition and haustorium were also observed. Differences in the number of cell wall appositions per infection site between the combination lines and their parents were small and no experiment x pathotype x host interactions occurred. The only significant difference observed was between host genotypes where fewer papillae occurred in Thatcher than in the other lines. When the fungus is not able to form the first haustorium, it often lacks the ability to continue penetration attempts and is aborted. Some infection hyphae, however, were capable of branching after a failed first attempt to form a second haustorium mother cell (Fig. 19A). The fate of the infection structure appears to be determined by the presence of cell wall appositions opposite the haustorium mother cell. It is therefore of utmost importance for the pathogen to form the first

haustorium, enabling the established colony to overcome barriers subsequently encountered at cell walls (Jacobs, 1989d).

According to Jacobs (1989c), the genotype of host plants clearly influenced the number of haustoria in seedlings. In the present study, however, the number of haustoria observed per colony did not indicate any clear, repeatable differences between lines. Jacobs (1989d) mentioned that cells often collapse after penetration of the cell wall and the formation of a haustorium. Only a few collapsed (necrotic) cells displaying haustoria and cell wall appositions were observed (Fig. 20D). According to Jacobs (1989d) a higher number of collapsed cells were associated with aborted infection structures compared to established colonies. This suggested that posthaustorial collapse of cells may contribute to the abortion of infection structures. Since the number of collapsed cells was not determined in the present study, pre- or posthaustorial resistance due to cell wall appositions or cell collapse could not be confirmed. The total number of haustoria per barley leaf rust colony has been estimated at 10^4 (Kneale and Farrer, 1985). If this estimate is, according to Jacobs (1989d), applied to leaf rust, it indicates that one in a thousand penetration attempts is hindered by cell wall appositions. Jacobs (1989d) concluded that differences in colony size between susceptible and partially resistant wheats were not caused by the formation and presence of cell wall appositions. Niks (1986) reported, however, a correlation between cell wall appositions and the level of partial resistance in barley to leaf rust. In this study the assessment of the number of papillae per haustorium indicated that the susceptible Thatcher showed the lowest ratio. This suggested an association between the *Lr* gene lines and the occurrence of papillae.

The common occurrence of host cell necrosis observed during histological

examinations was also reflected in the examination of macroscopic components. Infection types indicating a high degree of resistance to leaf rust were observed on the flag leaves of lines carrying both *Lr12* and *Lr13*. These low IT's resulted from the fact that the experiments were conducted with pathotypes avirulent to either *Lr12* or *Lr13*. Considering qualitative evaluation of the resistance phenotype, and with the exception of individual plants in families 13/12-3 (UVPrt2, both experiments) and 13/12-9 (UVPrt2, second experiment), IT's did not reveal resistance enhancement in the two-gene lines. The IT of line 13/12-9 to UVPrt13 (second experiment) suggested a lower level of resistance when compared with the parents. From these tests it appeared that resistance improvement was more easily detected in host-pathotype interactions involving avirulence for *Lr13* rather than *Lr12*.

The value of quantifying macroscopic components of resistance is the description of rate-reducing factors, e.g. an extended latent period, and fewer and smaller pustules, despite an apparent susceptible host reaction. German and Kolmer (1992) emphasised the necessity of resistance component studies, especially in quantifying the expression of resistance gene combinations. The use of individual components to describe resistance has often been studied (Broers and Wallenburg, 1989; Drijepondt and Pretorius, 1989; Jacobs and Kiriswa, 1993; Pretorius *et al.*, 1994). In studying *Lr34*-resistance, Pretorius *et al.* (1994) found that at low temperatures (17.5°C) different inoculum densities had negligible effects on resistance components, whereas at 24.6°C all components indicated a reduced level of resistance. In a previous study with *Lr26*, Pretorius and Kemp (1990) showed that uredium density was not influenced by temperature but that uredium size was restricted by higher temperatures. In the barley leaf rust pathosystem, Parlevliet and Kuiper (1977) found

that partially resistant and susceptible cultivars reacted differently to varying inoculum densities. Latent period and infection frequency in wheat cultivars partially resistant to *P. recondita* f. sp. *tritici* appeared not to be under the same genetic control (Jacobs, 1989d). Broers and Wallenburg (1989) also suggested that uredium density is controlled by a different mechanism than that controlling latent period and uredium size. In their study temperature had a different effect on uredium density than on the other components. Das *et al.* (1993) stated that latent period and uredium size are preferable as components, because of their relative insensitivity to environmental variation.

Since most plants produced sporulating pustules, measurement of the above mentioned components was possible. In the first experiment, only line 13/12-19 showed a longer latent period compared to the parents. This could be interpreted as a significant improvement of resistance towards both pathotypes. This improvement was, however, not confirmed in the second experiment. Latent period did not reveal large differences in leaf rust reaction between susceptible and resistant lines and was therefore not considered appropriate for quantifying improvement of resistance in the lines studied. Considering both pathotypes, lines 13/12-3 and 13/12-9 supported fewer uredia when compared with the other genotypes. The combination lines varied in uredium density, especially in the second experiment when 13/12-19 and 13/12-40 were less resistant than the others according to this component. Based on the data obtained in the second experiment, lines containing *Lr12* were significantly more receptive to leaf rust than *Lr13* when confronted with a virulent pathotype. According to the size of the macroscopically measured uredia, no clear indication of resistance enhancement due to the combination of *Lr12* and *Lr13* was seen. Uredia on these lines

were generally not smaller than the resistant parent. Kloppers (1994) demonstrated that a decrease in uredium size and extension of latent period was evidence of an enhancement of resistance due to the gene combination *Lr13+Lr34*. Similar to uredium density, pustule size measurements showed that RL6011 was more susceptible than CT263 or Thatcher in compatible interactions.

High yield potential, acceptable quality characteristics and effective disease control in the field are important attributes of new, productive wheat cultivars (Johnson and Lupton, 1987). The single gene lines were highly susceptible to the pathotypes in the field and were completely defoliated by leaf rust. Resistance expressed under field conditions by the combination lines resulted from the absence of a pathotype virulent to both *Lr12* and *Lr13* and can thus be ascribed to monogenic resistance. Tests with a pathotype virulent to both genes would have been more valid in evaluating whether the genes interacted under field conditions. All four lines displayed 20% leaf rust severity accompanied by an intermediate reaction type. In view of the extremely high inoculum density created in this trial, the field performance of these lines was considered excellent. In a similar field experiment with lines containing *Lr13+Lr37* and *Lr13+Lr34*, Kloppers (1994) found that the resistance in the combination lines was dramatically improved when compared to the monogenic parents.

Data obtained in this study were not conclusive in suggesting pronounced resistance enhancement as a result of pairing *Lr12* and *Lr13*. The assumption of durable leaf rust resistance associated with *Lr12+Lr13* could thus not be proven through demonstrating a novel resistance mechanism associated with this specific *Lr* gene combination.

CONCLUSIONS

1. Virulence exists for both *Lr12* and *Lr13* in the South African population of *Puccinia recondita* f. sp. *tritici*. The use of these monogenic sources of resistance to leaf rust should be discouraged.
2. *Lr12* and *Lr13* are easily detected in flag leaves of wheat plants using appropriate avirulent pathotypes. Furthermore, *Lr13* is clearly expressed in primary leaves of wheat seedlings tested at 25°C.
3. Pathotypes UVPrt3, 7 and 10 of *P. recondita* f. sp. *tritici* were avirulent to *Lr22b* in Thatcher. These pathotypes are thus not suitable for the detection of *Lr* genes in Thatcher near-isogenic lines in which *Lr22b* has been retained.
4. In adult plants, more infection sites of pathotype UVPrt2 than UVPrt13 aborted on *Lr* gene lines than on the leaf rust-susceptible cultivar Thatcher. Arrestation of substomatal vesicles was more important than nonpenetrating appressoria as a mechanism of aborted penetration.
5. Host cell necrosis associated with infection sites occurred more frequently and was more extensive for pathotype UVPrt2 than UVPrt13.
6. Based on the ratio of papillae per haustorium, the occurrence of cell wall appositions was more frequent in lines containing *Lr* genes than in Thatcher.
7. The morphology of cell wall appositions varied considerably.
8. Resistance enhancement in seedlings or adult plants containing both *Lr12* and *Lr13* could not decisively be confirmed on a basis of infection types. Genetic factors associated with *Lr13* alone appeared to influence the infection types of the combination lines. Similarly, quantification of the macroscopic components

of resistance, viz. latent period and the number and size of uredia, was not conclusive in revealing resistance enhancement due to the combination of *Lr12* and *Lr13*.

9. According to the density and size of pustules, line RL6011 (*Lr12*) was significantly more susceptible than CT263 (*Lr13*) when infected with a virulent pathotype.
10. In the absence of a pathotype virulent to both *Lr12* and *Lr13*, the combination lines were resistant in the field.
11. Results did not indicate a novel mechanism of leaf rust resistance associated with the combination of *Lr12* and *Lr13* in a Thatcher background.

SUMMARY

Monogenic resistance in wheat (*Triticum aestivum* L.) to leaf rust, caused by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*, has generally not been durable. One strategy of attaining durable resistance to rust diseases of wheat is the combination of several resistance genes in a single genotype. Interactions among *Lr* genes have been defined as the combination of two or more genes resulting in higher resistance levels than that conferred by the genes individually. It has been suggested that *Lr12* and *Lr13*, both adult-plant resistance genes, in combination with other genes, form the basis of durable resistance in several cultivars. In this study, the assumption that *Lr12* and *Lr13* may interact to condition improved resistance to leaf rust, was investigated.

Four Thatcher (Tc) F₃ lines (13/12-3, 13/12-9, 13/12-19 and 13/12-40), homozygous for both *Lr13* and *Lr12*, were selected and compared with the parents (CT263 [=Tc*Lr13*] and RL6011 [=Tc*Lr12*]), the single gene lines Tc/13-22 and Tc/12-16, and Thatcher. In addition to infection type studies in seedlings and adult plants, lines were compared according to several histological and macroscopic components of resistance, as well as disease ratings in the field.

Flag leaf infection type studies showed that *Lr12* is effective against most pathotypes of *P. recondita* f. sp. *tritici* occurring in South Africa. Conversely, *Lr13* is ineffective against the dominant pathotypes, implying that the gene has no value as a monogenic source of resistance. Both *Lr12* and *Lr13* were inherited dominantly. Based on the fact that several pathotypes are avirulent to these genes, they should be manipulated with relative ease in local breeding programmes directed at utilising these sources in combination with other *Lr* genes.

Considering the microscopic components, effects of *Lr12* and/or *Lr13* resistance on the prepenetration stages were not determined. Results of aborted penetration, consisting of nonpenetrating appressoria and aborted substomatal vesicles, showed that inhibition of fungal growth in wheat lines containing *Lr12* and/or *Lr13*, was activated, to a certain degree, before haustoria were formed. Determination of colony size 240 hours after inoculation indicated that fungal colonies in the combination lines were generally smaller than in the parents, but not necessarily smaller than those in the monogenic line Tc/13-22. Host cell necrosis was more frequently associated with infection sites, specifically of UVPrt2, in the combination lines than in the parents. Hypersensitivity index values (calculated by dividing the area of leaf necrosis with the area of the respective colony), indicated that host cell necrosis was more severe following infection of the combination lines with UVPrt2. Quantification of cell wall appositions showed that fewer papillae occurred in Thatcher than in the other host genotypes. The number of haustoria observed per colony did not indicate any clear, repeatable differences between lines.

The common occurrence of host cell necrosis observed during histological examinations was also reflected in the macroscopic components. Infection types on the flag leaves of lines carrying both *Lr12* and *Lr13* often displayed chlorosis and necrosis. These ratings on primary and flag leaves, as well as the quantitative components including latent period, uredium density and uredium size, did not indicate clear differences between the digenic lines and the most resistant parent. In the absence of a pathotype virulent to both genes, the combination lines were resistant in the field. Field tests with a pathotype virulent to both *Lr12* and *Lr13* would have been more valid in evaluating whether the genes interacted.

Data obtained were not conclusive in suggesting pronounced resistance enhancement due to combining *Lr12* with *Lr13*. Therefore, the assumption of durability, resulting from a novel resistance mechanism conditioned by this combination, was not confirmed.

SAMEVATTING

Enkelgeen-weerstand in koring (*Triticum aestivum* L.) teen blaarroes, veroorsaak deur die swam *Puccinia recondita* Rob. Ex. Desm. f. sp. *tritici*, is dikwels nie volhoubaar nie. Een metode om genetiese bestandheid teen roessiektes meer standhoudend te maak is die kombinering van verskeie weerstandsgene in 'n enkel genotipe. Interaksies tussen blaarroesweerstandgene (*Lr* gene) word algemeen gedefinieer as die kombinasie van twee of meer gene wat gevolglik 'n hoër vlak van weerstand bemiddel as die gene individueel. Vroeëre literatuur suggereer dat *Lr12* en *Lr13* (beide is volwasseplantweerstandsgene) in kombinasie met ander weerstandsgene die basis vorm van standhoudende weerstand teen blaarroes. In hierdie studie is die aanname ondersoek dat die interaksie tussen *Lr12* en *Lr13* verhoogde weerstand teen blaarroes tot gevolg het.

Vier Thatcher (Tc) F₃ lyne (13/12-3, 13/12-9, 13/12-19 en 13/12-40), homosigoties vir beide *Lr12* en *Lr13*, is geselekteer en met die ouers (CT263[=Tc*Lr13*] en RL6011[=Tc*Lr12*]), die enkelgeenlyne Tc/13-22 en Tc/12-16, en Thatcher vergelyk. Behalwe vir die bepaling van infeksietipes, is verskeie histologiese en makroskopiese weerstandskomponente gekwantifiseer. Siekteramings is ook in die veld gedoen.

Vlagblaarinfeksietipes het getoon dat *Lr12* effektief is teen meeste Suid-Afrikaanse patotipes van *P. recondita* f. sp. *tritici*. Die mees dominante patotipes is egter virulent tot *Lr13*. Beide *Lr12* en *Lr13* is dominant oorgeërf en die voorkoms van verskeie avirulente patotipes het getoon dat die gene met relatiewe gemak in plaaslike koringveredelingsprogramme gerig op *Lr* geenkombinerings gebruik sou kon word.

Met betrekking tot die mikroskopiese komponente van weerstand in lyne met

Lr12 en/of *Lr13*, spesifiek ten opsigte van nie-penetrende appressoria en aborterende substomatale vesikels, het die studie getoon dat swamgroeï tot 'n sekere mate reeds voor haustoriumvorming geïnhibeer is. Die bepaling van koloniegroottes het getoon dat swamontwikkeling beperk is in geenkombinasielyne in vergelyking met die ouers, maar nie noodwendig meer as in die enkelgeenlyn Tc/13-22 nie. Gasheerselnekrose was ook meer algemeen geassosieer met infeksiepunte van veral UVPrt2 in die kombinasielyne as in die ouers. Die hipersensitiwiteitsindeks (berekend deur die nekrotiese blaararea met die koloniegrootte te deel) het aangetoon dat gasheerselnekrose meer prominent was in plante wat met UVPrt2 geïnokuleer is. Geen verskille in die tipe en hoeveelheid selwandverdikkings (-versperrings of papillas) kon tussen die kombinasielyne en hul ouers getoon word nie. Minder verdikkings is egter in die vatbare kontrole, Thatcher, waargeneem. Die bepaling van die aantal haustoria per infeksiepoint het ook geen beduidende en herhaalbare verskille tussen lyne getoon nie.

Die algemene voorkoms van nekrose soos histologies waargeneem is ook met die bepaling van makroskopiese komponente bevestig. Chlorose en nekrose was algemeen geassosieer met infeksietipes op vlagblare van die kombinasielyne. Infeksietipes tydens beide saailing- en volwasseplantgroeistadia, sowel as die kwantitatiewe weerstandskomponente latente periode, urediumdigtheid en urediumgrootte, het geen betekenisvolle verskille tussen die enkelgeen- en kombinasielyne aangetoon nie. In die afwesigheid van 'n patotipe met virulensie teenoor beide gene, was die kombinasielyne weerstandbiedend in die veld. Studies met 'n patotipe met sodanige virulensie-eienskappe sou waarskynlik meer waardevol gewees het in die meting van potensiële interaksie tussen *Lr12* en *Lr13*.

Met hierdie studie is dus getoon dat geen oortuigende verhoging in blaarroesweerstand aan die kombinasie van *Lr12* met *Lr13* toegeskryf kon word nie. Dus kon die aanname, dat standhoudende weerstand wat geassosieer word met hierdie *Lr* geenkombinasie die gevolg is van 'n unieke bestandheidsmeganisme, nie bewys word nie.

REFERENCES

- Aist, J.R. 1976. Papillae and related wound plugs of plant cells. *Annual Review of Phytopathology* 14:145-163.
- Aist, J.R. and H.W. Israel. 1977a. Timing and significance of papilla formation during host penetration by *Olpidium brassicae*. *Phytopathology* 67: 187-194.
- Aist, J.R. and H.W. Israel. 1977b. Papilla formation: Timing and significance during penetration of barley coleoptiles by *Erysiphe graminis hordei*. *Phytopathology* 67: 455-461.
- Aist, J.R., H. Kunoh and H.W. Israel. 1979. Challenged appressoria of *Erysiphe graminis* fail to breach preformed papillae of a compatible barley cultivar. *Phytopathology* 69: 1245-1250.
- Akai, A., H. Kunoh and M. Fukutomi. 1968. Histochemical changes in the epidermal cell wall of barley leaves infected with *Erysiphe graminis hordei*. *Mycopathologia Mycologia Applicata* 35: 175-180.
- Allen, F.H.E. and J. Friend. 1983. Resistance of potato tubers to infection by *Phytophthora infestans*: A structural study of haustorial encasement. *Physiological Plant Pathology* 22: 282-292.
- Andres, M.W. 1982. Latent period and slow rusting in the *Hordeum vulgare* L. *Puccinia hordei* host-parasite system. Ph.D thesis. University of Minnesota, St. Paul.
- Andres, M.W. and R.D. Wilcoxson. 1984. A device for uniform deposition of liquid-suspended urediniospores on seedling and adult cereal plants. *Phytopathology* 74: 550-552.
- Anikster, Y. 1984. The formae speciales. Pages 115-130 in W.R. Bushnell and A.P. Roelfs, eds., *The Cereal Rusts*, Vol. I. Origins, specificity, structure, and physiology. Academic Press, Orlando.
- Anikster, Y. and I. Wahl. 1979. Coevolution of the rust fungi on *Gramineae* and *Liliaceae* and their hosts. *Annual Review of Phytopathology* 17: 367-403.
- Athur, J.C. and G.B. Cummins. 1962. *Manual of the rusts in United States and Canada*. Hafner Publishing Company, New York.

- Autrique, E., R.P. Singh, S.D. Tanksley and M.E. Sorrels. 1995. Molecular markers for four leaf rust resistance genes introgressed into wheat from wild relatives. *Genome* 38: 75-83.
- Bahamish, H.S. and R.K.S. Wood. 1985. Induction of susceptibility and resistance of wheat leaves by preinoculation with high and low virulence races of *Puccinia recondita* f. sp. *tritici*. *Phytopathologische Zeitschrift* 113: 97-112.
- Bai, D. and D.R. Knott. 1992. Suppression of rust resistance in bread wheat (*Triticum aestivum* L.) by D-genome chromosomes. *Genome* 35: 276-282.
- Barret, J. 1985. The gene-for-gene hypothesis: parable or paradigm. Pages 215-224 in D. Rollinson and R.M. Anderson eds., *Ecology and Genetics of Host-Parasite interactions*. Linnean Society, London.
- Bean, G.A. and G.W. Rambo. 1968. Factors affecting growth and survival of the mycoparasite *Darluca filum*. *Phytopathology* 58: 883.
- Bechere, E., T. Tessema, M. Hulluka and Y. Andenow. 1994. Breeding durum wheat for resistance to leaf and stem rust in Ethiopia. Pages 125-132 in Daniel L. Danial, ed., *Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa*. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Bentolilla, S., C. Guitton, N. Bouvet, A. Sailland, S. Nykaza and G. Freyssinet. 1991. Identification of an RFLP marker tightly linked to the *HT1* gene in maize. *Theoretical and Applied Genetics* 82: 393-398.
- Bingham, J. and F.G.H. Lupton. 1987. Production of new varieties : an integrated research approach to plant breeding. Pages 487-538 in F.G.H. Lupton, ed., *Wheat Breeding : its scientific basis*. Chapman and Hall, London.
- Bjarko, M.E. and R.F. Line. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology* 78 : 457-461.
- Borovkova, I.G., B.J. Steffenson, Y. Jin, J.B. Rasmussen, A. Kilian, A. Kleinhofs, B.G. Rosnagel and K.N. Kao. 1995. Identification of molecular markers linked to the stem rust resistance gene *rpg4* in barley. *Phytopathology* 85: 181-185.
- Broers, L.H.M. 1989. Influence of development stage and host genotype on three components of partial resistance to leaf rust in spring wheat. *Euphytica* 44: 187-195.

- Broers, L.H.M. and Th. Jacobs. 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat II. Number of segregating factors and evidence for transgressive segregation in F₃ and F₅ generations. *Euphytica* 44: 207-214.
- Broers, L.H.M. and S.C. Wallenburg. 1989. Influence of post-infection temperature on three components of partial resistance in wheat to wheat leaf rust. *Euphytica* 44: 215-224.
- Browder, L.E. 1985. Parasite:host:environment specificity in the cereal rusts. *Annual Review of Phytopathology* 23: 201-222.
- Brown, J.F. and W.A. Shipton 1964. Relationship of penetration to infection type when seedling wheat leaves are inoculated with *Puccinia graminis tritici*. *Phytopathology* 54: 89-91.
- Brown, J.F., W.A. Shipton and N.H. White. 1966. The relationship between hypersensitive tissue and resistance in wheat seedlings infected with *Puccinia graminis tritici*. *Annals of Applied Biology* 58: 279-290.
- Buchenauer, H. 1982. Chemical and biological control of cereal rusts. Pages 247 - 279 in K.J. Scott and A.K. Chakravorty, eds., *The rust fungi*. Academic Press, London.
- Burr, B. 1994. Some concepts and new methods for molecular mapping in plants. Pages 1-7 in R.L. Phillips and I.K. Vasil, eds., *DNA-based markers in plants*. Kluwer Academic Publishers, Dordrecht.
- Bushnell, W.R. 1972. Physiology of fungal haustoria. *Annual Review of Phytopathology* 10: 151-176.
- Bushnell, W.R. and S.E. Berquist. 1975. Aggregation of host cytoplasm and the formation of papillae and haustoria in powdery mildew of barley. *Phytopathology* 65: 310-318.
- Caldwell, R.M., J.J. Roberts and Z. Eyal. 1970. General resistance ("slow rusting") in *Puccinia recondita* f. sp. *tritici* in winter and spring wheats. *Phytopathology* 60: 1287. (Abstr.)
- Chang, K.F. and R.F. Line. 1983. Urediospore germination and growth of *Puccinia recondita* f. sp. *tritici* in leaves of resistant and susceptible wheat cultivars. *Phytopathology* 73: 816. (Abstr.)

- Cheung, D.S.M. and H.N. Barber. 1972. Activation of resistance of wheat to stem rust. *Transactions of the British Mycological Society* 58: 333-336.
- Chevre, A.M., J. Jahier. and M. Trottet. 1989. Expression of disease resistance genes in amphiploid wheat - *Triticum tauschii* (Coss.) Schmal. *Cereal Research Communications* 17: 23-29.
- Coffey, M.D. and U.E. Wilson. 1983. An ultrastructural study of the late blight fungus *Phytophthora infestans* and its interaction with the foliage of two potato cultivars possessing different levels of general (field) resistance. *Canadian Journal of Botany* 61: 2669-2685.
- Cortazar, S.R., A.I. Ramirez., E.E. Hache., M.O. Moreno and B.F. Riveros. 1989. The leaf rust (*Puccinia recondita*) in the north central zone of Chile in 1986. *Agricultura Technica Santiago* 49: 41-44. (CAB Abstracts, AN 901136397).
- Cummins, G.B. and R.M. Caldwell. 1956. The validity of binomials in the leaf rust fungus complex of cereals and grasses. *Phytopathology* 46: 81-82.
- Cunningham, J.L. 1967. Natural enemies of rust fungi in the tropics. *Phytopathology* 57: 645. (Abstr.)
- Da Luz, W.C. 1984. Yield losses caused by fungal foliar wheat pathogens in Brazil. *Phytopathology* 74: 1403-1407. (Abstr.)
- Das, M.K., S. Rajaram, W.E. Kronstadt, C.C. Mundt, and R.P. Singh. 1993. Associations and genetics of three components of slow rusting in leaf rust of wheat. *Euphytica* 68: 99-109.
- Denissen, C.J.M. 1993. Components of adult plant resistance to leaf rust in wheat. *Euphytica* 70: 1131-140.
- Devos, K.M. and M.D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. *Theoretical and Applied Genetics* 84: 567-572.
- Drijepondt, S.C. and Z.A. Pretorius. 1989. Greenhouse evaluation of adult-plant resistance conferred by the gene *Lr34* to leaf rust of wheat. *Plant Disease* 73: 669-671.
- Drijepondt, S.C., Z.A. Pretorius, and F.H.J. Rijkenberg. 1991. Expression of two wheat leaf rust resistance gene combinations involving *Lr34*. *Plant Disease* 75: 526-528.
- Dubin, H.J. and E. Torres. 1981. Causes and consequences of the 1976 - 1977 wheat

- leaf rust epidemic in Northwest Mexico. *Annual Review of Phytopathology* 19: 41-49.
- Dyck, P.L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. *Canadian Journal of Genetics and Cytology* 19: 711-716.
- Dyck, P.L. 1979. Identification of the gene for adult-plant leaf rust resistance in Thatcher. *Canadian Journal of Plant Science* 59: 499-501.
- Dyck, P.L. 1991. Genetics of adult-plant resistance in 'Chinese Spring' and 'Sturdy' cultivars. *Crop Science* 31: 309-311.
- Dyck, P.L. and R. Johnson. 1983. Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita*. *Canadian Journal of Plant Pathology* 5: 229-234.
- Dyck, P.L. and E.R. Kerber. 1970. Inheritance in hexaploid wheat of adult-plant leaf rust resistance derived from *Aegilops squarrosa*. *Canadian Journal of Genetics and Cytology* 12: 175-180.
- Dyck, P.L. and E.R. Kerber. 1985. Resistance of the Race-specific Type. Pages 469-500 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- Dyck, P.L. and O.M. Lukow. 1988. The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. *Canadian Journal of Plant Science* 68: 633-639.
- Dyck, P.L. and D.J. Samborski. 1968. Genetics of resistance of leaf rust in the common wheat varieties Webster, Loros, Brevit, Carina, Malakof and Centenario. *Canadian Journal of Genetics and Cytology* 16: 323-332.
- Dyck, P.L. and D.J. Samborski. 1979. Adult plant leaf rust resistance in PI250413, an introduction of common wheat. *Canadian Journal of Plant Science* 59: 329-332.
- Dyck, P.L. and D.J. Samborski. 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. *Canadian Journal of Genetics and Cytology* 24: 273-283.
- Dyck, P.L., D.J. Samborski and R.G. Anderson. 1966. Inheritance of adult plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. *Canadian Journal of Genetics and Cytology* 8: 665-671.
- Elmhirst, J.F. and M.C. Heath. 1987. Interactions of the bean rust and cowpea rust fungi with species of the *Phaseolus-Vigna* plant complex. I. Fungal growth and

- development. *Canadian Journal of Botany* 65: 1096-1107.
- Eversmeyer, M.G., C.L. Kramer and Z.M. Hassan. 1988. Environmental influences on the establishment of *Puccinia recondita* infection structures. *Plant Disease* 72: 409-412.
- Eyal, Z. and J.L. Peterson. 1967. Uredospore production of five races of *Puccinia recondita* Rob. ex. Desm. as affected by light and temperature. *Canadian Journal of Botany* 45: 537-540.
- Ezzahiri, B., S. Diouri and A. P. Roelfs. 1992. *Anchusa italica* as an alternate host for wheat leaf rust in Morocco. *Plant Disease* 76:1185.
- Ezzahiri, B. and A. P. Roelfs. 1989. Inheritance and expression of adult plant resistance to leaf rust in Era wheat. *Plant Disease* 73: 549-551.
- Feldman, M. 1988. Cytogenetic and molecular approaches to alien gene transfer in wheat. Pages 23-32 in T.E. Miller and R.M.D. Koebner, eds., *Proceedings of the Seventh International Wheat Genetics Symposium*. Cambridge, England, July 13-19, 1988. Bath Press, Bath, Avon.
- Flor, H.H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32: 653-669.
- Gavinlertvatana, S. and R.D. Wilcoxson. 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f. sp. *tritici* and host parasite relationships. *Transactions of the British Mycological Society* 71: 413-418.
- Geleta, B. and D.G. Tanner. 1994. Status of cereal production and pathology research in Ethiopia. Pages 42-50 in Daniel L. Danial, ed., *Breeding for disease resistance with emphasis on durability*. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Gerechter-Amitai, Z. K. and W.Q. Loegering. 1977. Genes for low reactions to *Puccinia graminis tritici* in *Aegilops* and *Triticum*. *Crop Science* 17: 830-832.
- German, S., T. Abadie and C. Perea. 1986. Epidemic to leaf rust in wheat cultivar La Paz INTA. *Investigaciones Agronomicas, Centro de Investigaciones Agricolas "Alberto Boerger"*, Uruguay 7: 75-77. (CAB Abstracts, AN 902302167).
- German, S and J.A. Kolmer. 1990. Effect of *Lr* gene combinations on resistance to wheat leaf rust. *Phytopathology* 79: 1216. (Abstr.)

- German, S and J.A. Kolmer. 1992. Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theoretical and Applied Genetics* 84: 97-105.
- Gill, B.S., H.C. Sharma, W.J. Raupp, L.E. Browder, J.H. Hatchett, T.L. Harvey, J.G. Moseman and J.G. Wainnes. 1985. Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, Hessian fly and greenbug. *Plant Disease* 69: 314-316.
- Givan, C.V. and K.R. Bromfield. 1964. Light inhibition of urediospore germination in *Puccinia recondita*. *Phytopathology* 54: 116-117.
- Goodman, R.N., Z. Kiraly and K.R. Wood. 1986. The biochemistry and physiology of plant disease. University of Missouri Press, Columbia.
- Green, G.J. 1965. Stem rust of wheat, rye and barley in Canada in 1964. *Canadian Plant Disease Survey* 45: 23-29.
- Green, G.J. and A.B. Campbell. 1979. Wheat cultivars resistant to *Puccinia graminis* in western Canada: their development, performance, and economic value. *Canadian Journal of Plant Pathology* 1:3-11.
- Gupta, A.K., and R.G. Saini. 1993. Leaf rust resistance in wheat. Pages 235-237 in Th. Jacobs and J.E. Parlevliet, eds., *Durability of disease resistance*. Kluwer Academic Publishers, Dordrecht.
- Gustafson, G.D. and G. Shaner. 1982. The influence of plant age on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72: 746-749.
- Haggag, M.E.A, D.J. Samborski, and P.L. Dyck. 1973. Genetics of pathogenicity in three races of leaf rust on four wheat varieties. *Canadian Journal of Genetics and Cytology* 15: 73-82.
- Harder D.E. 1989. Rust fungal haustoria - past, present, future. *Canadian Journal of Plant Pathology* 11: 91-99.
- Hartl, L., H. Weiss, F.J. Zeller and A. Jahoor. 1993. Use of RFLP markers for the identification of alleles of the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 86: 959-963.
- Heagle, A.S. and M.B. Moore. 1970. Some effects of moderate adult resistance to crown rust of oats. *Phytopathology* 60: 461-466.
- Heath, M.C. 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust-*Uromyces phaseoli* var. *vignae*.

- Physiological Plant Pathology 4: 403-414.
- Heath, M.C. 1976. Hypersensitivity, the cause or the consequence of rust resistance. *Phytopathology* 66: 935-936.
- Heath, M. 1979. Partial characterization of the electron-opaque deposits formed in the non-host plant, French bean, after cowpea rust infection. *Physiological Plant Pathology* 15: 141-148.
- Heath, M.C. 1981a. Resistance of plants to rust infection. *Phytopathology* 71:971-974.
- Heath, M.C. 1981b. A generalized concept of host-parasite specificity. *Phytopathology* 71: 1121-1123.
- Heath, M.C. 1982. Host defense mechanisms against infection by rust fungi. Pages 221-245. *in* K.J. Scott and A.K. Chakravorty, eds., *The rust fungi*. Academic Press, London.
- Heath, M.C., and I.B. Heath. 1971. Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. *Physiological Plant Pathology* 1: 277-287.
- Hiratsuka, Y. and S. Sato. 1982. Morphology and taxonomy of rust fungi. Pages 1-36 *in* K.J. Scott and A.K. Chakravorty, eds., *The rust fungi*. Academic Press, London.
- Hoch, R.C. and R.C. Staples. 1987. Structural and chemical changes among the rust fungi during appressorium development. *Annual Review of Phytopathology* 25: 231-247.
- Hogenboom, N.G. 1993. Economic importance of breeding for disease resistance. Pages 5-9 *in* T.H. Jacobs and J.E. Parlevliet, eds., *Durability of disease resistance*. Kluwer Academic Publishers, Dordrecht.
- Huerta-Espino, J. and R.P. Singh. 1994. First report of virulence to wheat with leaf rust resistance gene *Lr19* in Mexico. *Plant Disease* 78: 640.
- Islam, M.R., K.W. Shepherd and G.M.E. Mayo. 1989. Recombination among genes at the L group in flax conferring resistance to rust. *Theoretical and Applied Genetics* 77: 540-546.
- Jacobs, Th. 1989a. Germination and appressorium formation of wheat leaf rust on susceptible, partially resistant and resistant wheat seedlings and other

- Graminae. *Netherlands Journal of Plant Pathology* 95: 65-71.
- Jacobs, Th. 1989b. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Pages 19-26 *in* L.H.M. Broers and Th. Jacobs eds., *Histological, genetical and epidemiological studies on partial resistance in wheat to wheat leaf rust*. Ponsen & Looijen, Wageningen.
- Jacobs, Th. 1989c. Haustorium formation and cell wall appositions in susceptible and partially resistant wheat and barley seedlings infected with wheat leaf rust. *Journal of Phytopathology* 127: 250-261.
- Jacobs, Th. 1989d. The occurrence of cell wall appositions in flag leaves of spring wheats, susceptible and partially resistant to wheat leaf rust. *Journal of Phytopathology* 127: 239-249.
- Jacobs, Th. and L.H.M. Broers. 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. I. Estimation of gene action and number of effective factors in F_1 , F_2 and backcross generations. *Euphytica* 44: 197-206.
- Jacobs, Th. and F.K.L. Kiriswa. 1993. The relation between the developmental stage of wheat and barley and the latency period and infection frequency of leaf rust. *Cereal Research Communications* 21: 195-200.
- Johnson, R. 1979. The concept of durable resistance. *Phytopathology* 69: 198-199.
- Johnson, R. 1981a. Durable disease resistance. Pages 55-63 *in* J.F. Jenkyn and R.T. Plumb, eds., *Strategies for control of cereal diseases*. Blackwell, Oxford.
- Johnson, R. 1981b. Durable resistance: definition of, genetic control, attainment in plant breeding. *Phytopathology* 71: 567-568.
- Johnson, R. 1983. Genetic background of durable resistance. Pages 5-26 *in* F. Lamberti, J.M. Waller and N.A. van der Graaff, eds., *Durable resistance in crops*. Plenum, New York.
- Johnson, R. 1992. Past, present and future opportunities in breeding for disease resistance, with examples from wheat. *Euphytica* 63: 3-22.
- Johnson, R. and D.J. Allen. 1975. Induced resistance to rust diseases and its possible role in the resistance of multiline varieties. *Annals of Applied Biology* 80: 359-363.
- Johnson, R. and F.G.H. Lupton. 1987. Breeding for disease resistance. Pages 369-

- 418 in F.G.H. Lupton, ed., *Wheat Breeding : its scientific basis*. Chapman and Hall, London.
- Johnson, L.B. and J.F. Schafer. 1965. Identification of wheat leaf rust resistance combinations by differential temperature effects. *Plant Disease Reporter* 49: 222-224.
- Johnson, T. 1934. A tropic response in germ tubes of urediospores of *Puccinia graminis tritici*. *Phytopathology* 24: 80-82.
- Johnson T., G.J. Green and D.J. Samborski. 1966. The world situation of the cereal rusts. *Annual Review of Phytopathology* 79: 183-200.
- Johnston, C.O. 1934. Effects of mildew infection on the response of wheat leaf tissues normally resistant to leaf rust. *Phytopathology* 24: 1045-1046.
- Johnston, C.O. and M.D. Huffman. 1958. Evidence of local antagonism between two cereal rust fungi. *Phytopathology* 48: 69-70.
- Jones, D.R. and B.J. Deverall. 1977. The effect of the *Lr20* resistance gene in wheat on the development of leaf rust, *Puccinia recondita*. *Physiological Plant Pathology* 10: 275-284.
- Kam-Morgan, L.N.W., B.S. Gill and S. Muthukrishnan. 1989. DNA restriction length fragment length polymorphisms: a strategy for genetic mapping of D-genome of wheat. *Genome* 32: 724-732.
- Kaul, K. and G. Shaner. 1989. Effect of temperature on adult plant resistance to leaf rust in wheat. *Phytopathology* 79: 391-394.
- Kebede, T., B. Geleta, B. Yai, and A. Badebo. 1994. Status of wheat rusts in the major producing regions of Ethiopia. Pages 180-184 in Daniel L. Danial, ed., *Breeding for disease resistance with emphasis on durability*. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Keen, N.T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. *Annual Review of Genetics* 24: 447-463.
- Kerber, E.R. and P.L. Dyck. 1973. Inheritance of stem rust resistance transferred from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location in the gene involved. *Canadian Journal of Genetics and Cytology* 15: 397-409.

- Kislev, M.E. 1982. Stem rust of wheat 3300 years old found in Israel. *Science* 216: 993-994.
- Kloppers, F.J. 1994. Characterisation of resistance conferred by selected *Lr* genes with emphasis on histopathology, leaf rust development and associated quality attributes in wheat. Ph. D. Thesis, University of the Orange Free State, Bloemfontein.
- Kloppers, F.J., Z.A. Pretorius and D. Van Lill. 1995. Field evaluation of leaf rust severity, yield loss and quality characteristics in near-isogenic wheat lines with *Lr29*, *Lr35* or *Lr37*. *South African Journal of Plant and Soil* 12: 55-58.
- Klug, W.S and M.R. Cummings. 1994. Concepts of genetics. Macmillan College Publishing Company, New York.
- Kneale, J. and J.F. Farrer. 1985. The localization and frequency of haustoria in colonies of brown rust on barley leaves. *New Phytologist* 101: 495-505.
- Knott, D.R. 1980. Mutation for a gene for yellow pigment linked to *Lr19* in wheat. *Canadian Journal of Genetics and Cytology* 22: 651-654.
- Knott, D.R. 1989a. The wheat rusts - Breeding for resistance, Springer - Verlag, Berlin.
- Knott, D.R. 1989b. The effect of transfers of alien genes for leaf rust resistance on the agronomic and quality characteristics of wheat. *Euphytica* 4: 65-72.
- Knott, D.R. and J. Dvorak. 1976. Alien germ plasm as a source of resistance to disease. *Annual Review of Phytopathology* 14: 211-235.
- Knott, D.R. and B. Yadav. 1993. The mechanism and inheritance of adult plant leaf rust resistance in 12 wheat lines. *Genome* 36: 877-883.
- Kochert, G. RFLP technology. 1994. Pages 8-38 in R.L. Phillips and I.K. Vasil eds., DNA-based markers in plants. *Advances in cellular and molecular biology of plants*. Vol. 1. Kluwer Academic Publishers, Dordrecht.
- Kochman J.K. and J.F. Brown. 1975. Development of stem and crown rust fungi on leaves, sheaths, and penduncles of oats. *Phytopathology* 65: 1404-1408.
- Kolmer, J.A. 1989. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1988. *Canadian Journal of Plant Pathology* 11: 431-434.
- Kolmer, J.A. 1991. Phenotypic diversity in two populations of *Puccinia recondita* f. sp. *tritici* in Canada during 1931-1987. *Phytopathology* 81: 311-315.

- Kolmer, J.A. 1992. Enhanced leaf rust resistance in wheat conditioned by resistance gene pairs with *Lr13*. *Euphytica* 61: 123-130.
- Kolmer, J.A. 1994. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1992. *Canadian Journal of Plant Pathology* 16: 61-63.
- Kolmer J.A., P.L. Dyck., and A.P. Roelfs. 1991. An appraisal of stem rust and leaf rust resistance in North American spring wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. *Phytopathology* 81: 237-239.
- Kolmer, J.A., S.E. German, and P.L. Dyck. 1993. Resistance gene combinations that condition enhanced resistance to *Puccinia recondita*, leaf rust of wheat. Page 330 (Abstr.) in Th. Jacobs and J. E. Parlevliet, eds., *Durability of disease resistance*. Kluwer Academic Publishers, Dordrecht.
- Krupnov, V.A., S.A. Voronina, S.N. Sibikeev, V.A. Elesin and Yu. E. Sibikeva. 1995. Virulence of leaf rust in bread wheat to *Lr19*. *Annual Wheat Newsletter* 41: 165.
- Kuck, K.H., R. Tiburzy, G. Hänssler and H.-J. Reisener. 1981. Visualization of rust haustoria in wheat leaves by using fluorochromes. *Physiological Plant Pathology* 19: 439-441.
- Kuhn, R.C., H.W. Ohm and G.E. Shaner. 1978. Slow leaf rusting resistance in wheat against twenty-two isolates of *Puccinia recondita*. *Phytopathology* 68: 651-656.
- Kuhn, R.C., H.W. Ohm and G.E. Shaner. 1980. Inheritance of slow leaf-rusting resistance in Suwon 85 wheat. *Crop Science* 20: 655-659.
- Kulkarni, R.N., V.L. Chopra and D. Singh. 1982. Relative importance of components affecting the leaf rust progress. *Theoretical and Applied Genetics* 62: 205-207.
- Kushner, U. And G.M. Halloran. 1984. Transfer of high kernel weight and high protein from tetraploid wheat (*Triticum turgidum dicoccoides*) using homologous and homoeologous recombination. *Euphytica* 33: 249-255.
- Kuwite, C.A. 1994. Breeding for disease resistance in wheat and barley in Tanzania. Pages 51-54 in Daniel L. Danial, ed., *Breeding for disease resistance with emphasis on durability*. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Lee, T.S. and G. Shaner. 1984. Infection processes of *Puccinia recondita* in slow- and

- fast-rusting wheat cultivars. *Phytopathology* 74: 1419-1423.
- Lee, T.S. and G. Shaner. 1985a. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. *Phytopathology* 75: 636-643.
- Lee, T.S. and G. Shaner. 1985b. Transgressive segregation of length of latent period in six slow leaf-rusting wheat cultivars. *Phytopathology* 65: 631-632.
- Levy, E., Z. Eyal and I. Chet. 1988. Suppression of *Septoria tritici* blotch and leaf rust on wheat seedling leaves by pseudomonads. *Plant Pathology* 37: 551-557.
- Littlefield, L.J. 1969. Flax rust resistance induced by prior inoculation with a avirulent race of *Melampsora lini*. *Phytopathology* 59: 1323-1328.
- Littlefield, L.J. and M.C. Heath. 1979. Ultrastructure of rust fungi. Academic Press, New York.
- Loegering, W.P. and H.R. Powers. 1962. Inheritance of pathogenicity in a cross of physiological races 111 and 36 of *Puccinia graminis* f. sp. *tritici*. *Phytopathology* 52: 547-554.
- Long, D.L. and J.A. Kolmer. 1989. A North America system of nomenclature for *Puccinia recondita* f. sp. *tritici*. *Phytopathology* 79: 525-529.
- Long, D.L., J.F. Schafer and A.P. Roelfs. 1986. Virulence and epidemiology of *Puccinia recondita* f. sp. *tritici* in the United States in 1984. *Plant Disease* 70: 395-397.
- Long, D.L., J.F. Schafer and A.P. Roelfs. 1988. Virulence of *Puccinia recondita* f. sp. *tritici* in the United States in 1986. *Plant Disease* 72: 22-24.
- Lucas, J.A., R.E. Kendrick and C.V. Givan. 1975. Photocontrol of fungal spore germination. *Plant Physiology* 56: 847-849.
- Luig, N.H. 1983. A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Parey, Berlin.
- Luig, N.H. 1985. Epidemiology in Australia and New Zealand. Pages 301-328 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- Mackenzie, D.R. 1991. Progress in plant disease resistance research. *FAO Plant Protection Bulletin* 39: 147-154.
- Mains, E.B. and H.S. Jackson. 1926. Physiologic specialization in the leaf rust of wheat *Puccinia triticina* Erikss. *Phytopathology* 16: 89-120.

- Manisterski, J., A. Segal, A.A. Levy and M. Feldman. 1988. Evaluation of Israeli *Aegilops* species for resistance to wheat leaf rust. *Plant Disease* 72: 941-944.
- Manners, J.G. and D.G. Gandy. 1954. A study of the effects of mildew infection on the reaction of wheat varieties to brown rust. *Annals of Applied Biology* 41: 393-404.
- Mares, D.J. and S. Cousen. 1977. The interaction of yellow rust (*Puccinia striiformis*) with winter wheat cultivars showing adult plant resistance: macroscopic and microscopic events associated with the resistant reaction. *Physiological Plant Pathology* 10: 257-274.
- Martens, J.W. 1985. Oat stem rust. Pages 103-129 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- Martens, J.W. and P.L. Dyck. 1989. Genetics of resistance to rusts in cereals from a Canadian perspective. *Canadian Journal of Plant Pathology* 11: 78-85.
- Martin, C.D., L.J. Littlefield, and J.D. Miller. 1977. Development of *Puccinia graminis* f. sp. *tritici* in seedling plants of slow rusting wheats. *Transactions of the British Mycological Society* 68: 161-166.
- Mayama, S., J.M. Daly, D.W. Rehfeld and C.R. Daly. 1975. Hypersensitive response of near-isogenic wheat carrying the temperature sensitive *Sr6* allele for resistance to stem rust. *Physiological Plant Pathology* 7: 35-47.
- Mayama, S. and J. Shishiyama. 1978. Localized accumulation of fluorescent and UV-absorbing compounds at penetration sites in barley leaves infected with *Erysiphe graminis hordei*. *Physiological Plant Pathology* 13: 347-354.
- McIntosh, R.A. 1976. Genetics of wheat and wheat rusts since Farrer : Farrer Memorial Oration 1976. *Journal of the Australian Institute of Agricultural Science* 42: 203-216.
- McIntosh, R.A. 1988. Catalogue of gene symbols for wheat. Pages 1225-1323 in T.E. Miller and R.M.D. Koebner, eds., *Proceedings of the Seventh International Wheat Genetics Symposium*. Cambridge, England, July 13-19, 1988. Bath Press, Bath, Avon.
- McIntosh, R.A. 1992. Pre-emptive breeding to control wheat rusts. *Euphytica* 63: 103-113.

- McIntosh, R.A., P.L. Dyck, T.T. The, J. Cusick and D.L. Milne. 1984. Cytogenetical studies in wheat XIII, *Sr35* - a third gene from *Triticum monococcum* for resistance to *Puccinia graminis tritici*. *Zeitschrift für Pflanzenzüchtung* 92: 1-14.
- McIntosh, R.A., G.E. Hart and M.D. Gale. 1993. Catalogue of gene symbols for wheat. Pages 1333-1500 in Z.S Li and Z.Y. Xin, eds., *Proceedings of the Eighth International Wheat Genetics Symposium*, Beijing, China, July 20-25, 1993. China Agricultural Sciencetech Press, Beijing.
- McIntosh, R.A., C.R. Wellings and R.F. Park. 1995. *Wheat rusts. An atlas of resistance genes*. Kluwer Academic Publishers, Dordrecht.
- McMillan, D.E., J.W. Johnson and J.J. Roberts. 1993. Linkage between endopeptidase *Ep-D1d* and the gene conferring leaf rust resistance (*Lr19*) in wheat. *Crop Science* 33: 1201-1203.
- Milus, E.A. and R.F. Line. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheats. *Phytopathology* 70: 167-172.
- Moreno-González, J. and J.I. Cubero. 1993. Selection strategies and choice of breeding methods. Pages 280-313 in M.D. Hayward, N. O. Bosemark and I. Romagosa, eds., *Plant Breeding: Principles and prospects*. Chapman and Hall, London.
- Moseman J.G., E. Nevo, Z.K. Gerechter-Amittai, M.A. Elmorshidy and D. Zohary. 1985. Resistance of *Triticum dicoccoides* collected in Israel to infection with *Puccinia recondita tritici*. *Crop Science* 25: 262-265.
- Mtisi, E. and N.A. Mashiringwani. 1994. An overview of wheat production in Zimbabwe with emphasis on some phytopathological aspects. Pages 55-59 in Daniel L. Danial, ed., *Breeding for disease resistance with emphasis on durability*. *Proceedings of a regional workshop for Eastern, Central and Southern Africa*. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Mujeeb-Kazi, A. and R. Asiedu. 1990. Wide hybridization - potential of alien genetic transfer for *Triticum aestivum* improvement. Pages 111-127 in Y.P.S. Bajaj, ed., *Biotechnology in agriculture and forestry* 13. Wheat. Springer-Verlag, Berlin.
- Mujeeb-Kazi, A. and G. Kimber. 1985. The production, cytology and practicality of wide hybrids in the Triticeae. *Cereal Research Communications* 13: 111-124.

- Mukwavi, M.V. 1994. Breeding for disease resistance in rainfed wheat in Zambia. Pages 143-145 in Daniel L. Danial, ed., Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Muyanga, S. 1994. Production and research review of small cereals in Zambia. Pages 60-64 in Daniel L. Danial, ed., Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Nelson, R.R. 1978. Genetics of horizontal resistance to plant diseases. Annual Review of Phytopathology 16: 359-378.
- Niks, R.E. 1982. Early abortion of colonies of leaf rust *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.
- Niks, R.E. 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley. Physiological and Molecular Plant Pathology 28: 309-322.
- Niks, R.E. 1987. The importance of abortive stoma penetration in the partial resistance and nonhost reaction of adult barley and wheat plants to leaf rust fungi. Euphytica 36: 725-731.
- Niks, R.E., and R.G. Dekens. 1987. Histological studies on the infection of triticale, wheat and rye by *Puccinia recondita* f. sp. *tritici* and *Puccinia recondita* f. sp. *recondita*. Euphytica 36: 275-285.
- Niks, R.E., and R.G. Dekens. 1991. Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid wheat seedlings. Phytopathology 81: 847-851.
- Niks, R.E., P.R. Ellis and J.E. Parlevliet. 1993. Resistance to parasites. Pages 422-447 in M.D. Hayworth, N.O. Bosemark, I. Romagosa, eds., Plant breeding: principles and prospects. Chapman and Hall, London.
- Ogle, H.J. and J.F. Brown. 1971. Quantitative studies of the postpenetration phase of infection by *Puccinia graminis tritici*. Annals of Applied Biology 67: 309-319.
- Ohm, S.He.H. and S. Mackenzie. 1992. Detection of DNA sequence polymorphisms among wheat varieties. Theoretical and Applied Genetics 84: 573-578.

- Ohm, H.W. and G.E. Shaner. 1976. Three components of slow leaf rusting at different growth stages in wheat. *Phytopathology* 66: 1356-1360.
- Owuocha, J.O., J.M. Pinto and S. Wekesa. 1994. Resistance of wheat cultivars for the three rust species in Kenya. Pages 133-138 in Daniel L. Danial, ed., Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Park R.F. and R.A. McIntosh. 1994. Adult plant resistances to *Puccinia recondita* f. sp. *tritici* in wheat. *New Zealand Journal of Crop and Horticultural Science* 22: 151-158.
- Park, R.F. and C.R. Wellings. 1992. Pathogenic specialisation of wheat rusts in Australia and New Zealand in 1988 and 1989. *Australasian Plant Pathology*. 21: 61-69. (CAB Abstracts, AN 922323695)
- Parlevliet, J.E. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. V. Analysis of the components of partial resistance in 8 barley cultivars. *Euphytica* 27: 33-39.
- Parlevliet, J.E. 1978. Race-specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei*. *Netherlands Journal of Plant Pathology* 84: 121-126.
- Parlevliet, J.E. 1988. Strategies for the utilization of partial resistance for the control of cereal rusts. Pages 48-62 in N.W. Simmonds and S. Rajaram, eds., Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico D.F.
- Parlevliet, J.E. 1993. What is durable resistance, a general outline. Pages 23-40 in T.H. Jacobs and J.E. Parlevliet, eds., Durability of disease resistance. Kluwer Academic Publishers, Dordrecht.
- Parlevliet, J.E. and C. Kievit. 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. I. Effect of partial resistance and plant stage. *Euphytica* 35: 953-959.
- Parlevliet, J.E. and H.J. Kuiper. 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. *Euphytica* 26: 249-255.
- Parlevliet, J.E. and A. Van Ommeren. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro plot tests and latent

- period. *Euphytica* 24: 293-303.
- Paterson, A.H., S.D. Tanksley and M.E. Sorrells. 1991. DNA markers in plant improvement. *Advances in Agronomy* 46: 39-90.
- Pederson, W.L. 1988. Pyramiding major genes for resistance to maintain residual effects. *Annual Review of Phytopathology* 26: 369-378.
- Penner, G.A., J. Chong, M. Levesque-Lemay, S.J. Molnar and G. Fedak. 1993a. Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. *Theoretical and Applied Genetics* 85: 702-705.
- Penner, G.A., J. Chong, C.P. Wight, S.J. Molnar and G. Fedak. 1993b. Identification of an RAPD marker for the crown rust resistance gene *Pc68* in oats. *Genome* 36: 818-820.
- Person, C., D.J. Samborski and R. Rohringer. 1962. The gene-for-gene concept. *Nature* 194: 561-562.
- Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust intensity of leaves and stem cereals. *Canadian Journal of Research (C)* 26: 496-500.
- Pienaar, R. de V. 1990. Wheat x *Thinopyrum* Hybrids. Pages 167-217 in Y.P.S. Bajaj, ed., *Biotechnology in agriculture and forestry* 13. Wheat. Springer-Verlag, Berlin.
- Plotnikova, L.Y., B.G. Reiter, L.Yu and L.V. Meshkova. 1985. Race specific resistance and ectophyte stage development of brown rust. *Mikologiya i Fitopatologiya* 19: 334-347. (CAB Abstracts, AN 861316666).
- Porceddu, E., C. Ceoloni, D. Lafiandra, O.A. Tanzarella and G.T. Scarascia Mugnozza. 1988. Genetic resources and plant breeding: problems and prospects. Pages 7-22 in T.E. Miller and R.M.D. Koebner, eds., *Proceedings of the Seventh International Wheat Genetics Symposium*. Cambridge, England, July 13-19, 1988. Bath Press, Bath, Avon.
- Poyntz, P. and P.M. Hyde. 1987. The expression of partial resistance of wheat to *Puccinia recondita*. *Journal of Phytopathology* 120: 136-142.
- Pretorius, Z.A., and G.H.J. Kemp. 1990. Effects of growth stage and temperature on components of resistance to leaf rust in wheat genotypes with *Lr26*. *Plant Disease* 74: 631-635.

- Pretorius, Z.A., F.J. Kloppers, and S.C. Drijepondt. 1994. Effects of inoculum density and temperature on three components of leaf rust resistance controlled by *Lr34* in wheat. *Euphytica* 74: 91-96.
- Pretorius, Z.A., and J. Le Roux. 1988. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1986 and 1987. *Phytophylactica* 20: 349-352.
- Pretorius, Z.A., J. Le Roux and S.C. Drijepondt. 1990. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1988. *Phytophylactica* 22: 225-228.
- Pretorius, Z.A. and J.L. Purchase. 1990. Virulence characteristics of wheat leaf rust in Zimbabwe, Zambia and Malawi. *Phytophylactica* 22: 141-142.
- Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson. 1987. Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa from 1983 through 1985. *Plant Disease* 71: 1133-1137.
- Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson. 1988a. Effect of growth stage, leaf position and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f. sp. *tritici*. *Plant Pathology* 37: 36-44.
- Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson. 1988b. Temperature-specific seedling resistance and adult plant resistance to *Puccinia recondita* f. sp. *tritici* in the wheat cultivar Glenlea. *Plant Disease* 72: 439-442.
- Pretorius, Z.A., R.D. Wilcoxson, D.L. Long, and J.F. Schafer. 1984. Detecting wheat leaf rust resistance gene *Lr13* in seedlings. *Plant Disease* 68: 585-586.
- Qayoum, A. and R.F. Line. 1985. High-temperature adult plant resistance to stripe rust of wheat. *Phytopathology* 75: 1121-1125.
- Quinones M. A., E.N. Larter and D.J. Samborski. 1972. The inheritance of resistance to *Puccinia recondita* in hexaploid triticale. *Canadian Journal of Genetics and Cytology* 14:495-505.
- Rajaram, S., R.P. Singh and E. Torres. 1988. Current CIMMYT approaches in breeding wheat for rust resistance. Pages 101-118 in N.W. Simmonds and S. Rajaram, eds., *Breeding strategies for resistance to the rusts of wheat*. CIMMYT, Mexico, DF.
- Ride, J.P. and R.B. Pearce. 1979. Lignification and papilla formation at sites of

attempted penetration of wheat leaves by non-pathogenic fungi. *Physiological Plant Pathology* 15: 79-92.

Rieger, R., A. Michaelis and M.M. Green. 1976. *Glossary of genetics and cytogenetics: Classical and Molecular*. Springer-Verlag, Berlin.

Roberts, F.M. 1936. The determination of physiologic forms of *Puccinia triticina* Erikss. in England and Wales. *Annals of Applied Biology* 23: 271.

Robinson, R.A. 1976. *Plant Pathosystems*. Springer - Verlag, Berlin.

Roelfs, A.P. 1978. Estimated losses caused by rust in small grain cereals in the United States: 1918-1976. *Miscellaneous Publication*. United States Department of Agriculture 1363: 1-85.

Roelfs, A.P. 1985. Wheat and rye stem rust. Pages 4-37 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.

Roelfs, A.P. 1988a. Resistance to leaf and stem rust in wheat. Pages 10 - 22 in Simmonds N.W. and S. Rajaram, eds., *Breeding strategies for resistance to the rusts of wheat*. Mexico, D.F.: CIMMYT.

Roelfs, A.P. 1988b. Genetic control of phenotypes in wheat stem rust. *Annual Review of Phytopathology* 26: 351-367.

Roelfs, A.P. 1989. Epidemiology of the cereal rusts in North America. *Canadian Journal of Plant Pathology* 11: 86-90.

Roelfs, A.P., D.L. Long and D.H. Casper. 1982. Races of *Puccinia graminis* f. sp. *tritici* in the United States and Mexico in 1980. *Plant Disease* 66: 205-207.

Roelfs, A.P., R.P. Singh and E.E. Saari. 1992. *Rust Diseases of Wheat: Concepts and methods of disease management*. Mexico, D.F.: CIMMYT.

Rohringer R., W.K. Kim and D.J. Samborski. 1979. A histological study of interactions between avirulent races of stem rust and wheat containing resistance genes *Sr5*, *Sr6*, *Sr8*, or *Sr22*. *Canadian Journal of Botany* 57: 324-331.

Rohringer, R., W.K. Kim, D.J. Samborski and N.K. Howes. 1977. Calcofluor, an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. *Phytopathology* 67: 808-810.

Romig, R.W. and R. Caldwell. 1964. Stomatal exclusion of *Puccinia recondita* by bread wheat peduncles and sheaths. *Phytopathology* 54: 214-218.

- Rooney, W.L., H.W. Rines and R.L. Philips. 1994. Identification of RFLP markers linked to crown rust resistance genes *Pc 91* and *Pc 92* in oat. *Crop Science* 34: 940-944.
- Rowell, J.B. 1968. Chemical control of the cereal rusts. *Annual Review of Phytopathology* 6: 243-262.
- Rubiales, D., J. Ballesteros and A. Martin. 1992. Resistance to *Septoria tritici* in *Hordeum chilense* x *Triticum* spp. amphiploids. *Plant Breeding* 109: 281-286.
- Russel, G.E. 1977. Varietal differences in formation of substomatal vesicles by *Puccinia striiformis* in winter wheat. *Phytopathologische Zeitschrift*: 88: 1-10.
- Russel, G.E. 1978. Plant breeding for pest and disease resistance. Butterworths, London.
- Saari, E.E. and J.M. Prescott. 1985. World distribution in relation to economic losses. Pages 259-298 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- Saari, E.E. and R.D. Wilcoxson. 1974. Plant disease situation of high-yielding dwarf wheats in Asia and Africa. *Annual Review of Phytopathology* 12: 49-68.
- Samborski, D.J. 1983. Occurrence and virulence of *Puccinia recondita* in Canada in 1982. *Canadian Journal of Plant Pathology* 5: 194-196.
- Samborski, D.J. 1984. Occurrence and virulence of *Puccinia recondita* in Canada in 1983. *Canadian Journal of Plant Pathology* 6: 238-242.
- Samborski, D.J. 1985. Wheat leaf rust. Pages 39-59 in A.P. Roelfs and W.R. Bushnell eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- Samborski, D.J. 1986. Occurrence and virulence of *Puccinia recondita* in Canada in 1985. *Canadian Journal of Plant Pathology* 8: 436-438.
- Samborski, D.J. and P.L. Dyck. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. *Canadian Journal of Genetics and Cytology* 10: 24-32.
- Samborski, D.J., and P.L. Dyck. 1976. Inheritance of virulence in *Puccinia recondita* on six backcross lines of wheat with single genes for resistance to leaf rust. *Canadian Journal of Botany* 54: 1666-1671.
- Samborski, D.J. and P.L. Dyck. 1982. Enhancement of resistance to *Puccinia*

- recondita* by interactions of resistance genes in wheat. Canadian Journal of Plant Pathology 4: 152-156.
- Samborski, D.J., W.K. Kim, R. Rohringer, N.K. Howes and R.J. Baker. 1977. Histological studies on host-cell necrosis conditioned by the *Sr6* gene for resistance in wheat to stem rust. Canadian Journal of Botany 55: 1445-1452.
- Samborski, D.J. and B. Peterson. 1960. Effect of leaf rust on the yield of resistant wheats. Canadian Journal of Plant Science 40: 620-622.
- Savile, D.B.O. 1984. Taxonomy of the cereal rust fungi. Pages 79 -114 in W.R. Bushnell and A.P. Roelfs, eds., The Cereal Rusts, Vol. I. Origins, specificity, structure, and physiology. Academic Press, Orlando.
- Schachermayr, G., H. Siedler, M.D. Gale, H. Winzeler, M. Winzeler and B. Keller. 1994. Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theoretical and Applied Genetics 88: 110-115.
- Schafer, J.F., R.M. Caldwell, F.L. Patterson and L.E. Compton. 1963. Wheat leaf rust resistance combinations. Phytopathology 53: 569-573.
- Schafer J.F. and D.L. Long. 1988. Relations of races and virulences of *Puccinia recondita* f. sp. *tritici* to wheat cultivars and areas. Plant Disease 72: 25-27
- Schafer, J.F., A.P. Roelfs, and W.R. Bushnell. 1984. Contribution of early scientists to knowledge of cereal rusts. Pages 3-38 in W.R. Bushnell and A.P. Roelfs, eds., The Cereal Rusts, Vol. I. Origins, specificity, structure, and physiology. Academic Press, Orlando.
- Schüller, C., G. Backes, G. Fishbeck and A. Jahoor. 1992. RFLP markers to identify the alleles on the *Mla* locus conferring powdery mildew resistance in barley. Theoretical and Applied Genetics 84: 330-338.
- Sears, E.R. 1981. Transfer of alien genetic material to wheat. Pages 75-89 in L.T. Evans and W.J. Peacock eds., Wheat science-today and tomorrow. Cambridge University Press, Cambridge.
- Seck, M., P.S. Teng and A.P. Roelfs. 1985. The role of wheat leaves in grain yield and leaf rust losses. Phytopathology 75: 1299. (Abstr.)
- Shaner, G.E. and R.E. Finney. 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70: 1183-1186.
- Shaner, G.E., Ohm H.W. and R.E. Finney. 1978. Response of susceptible and slow

- leaf rusting wheats to infection by *Puccinia recondita*. *Phytopathology* 68: 471-475.
- Sharp, E.L., B.K. Sally and G.A. Taylor. 1976. Incorporation of additive genes for stripe rust resistance in winter wheat. *Phytopathology* 66: 794-797.
- Sharp, E.L., C.G. Schmitt, J.M. Staley and C.H. Kingsolver. 1958. Some critical factors involved in establishment of *Puccinia graminis* var. *tritici*. *Phytopathology* 48: 469-474.
- Shepherd K.W. and G.M.E. Mayo. 1972. Genes conferring specific plant disease resistance. *Science* 175: 375-380.
- Sherwood, R.T. and C.P. Vance. 1980. Resistance to fungal penetration in *Gramineae*. *Phytopathology* 70: 273-279.
- Singh, R.P. 1992. Genetic association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in bread wheats. *Crop Science* 32: 874-878.
- Singh, R.P. and A.K. Gupta. 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. *Plant Disease* 76: 489-491.
- Singh, R.P. and R.A. McIntosh. 1984. Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. *Canadian Journal of Genetics and Cytology* 26: 723-735.
- Singh, R.P., T.S. Payne and S. Rajaram. 1991. Characterization of variability and relationships among components of partial resistance to leaf rust in CIMMYT bread wheats. *Theoretical and Applied Genetics* 82: 674-680.
- Singh, R.P. and S. Rajaram. 1991. Resistance to *Puccinia recondita* f. sp. *tritici* in 50 Mexican bread wheat cultivars. *Crop Science* 31: 1472-1479.
- Singh, R.P. and S. Rajaram. 1992. Genetics of adult-plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. *Genome* 35: 24-31.
- Skovmand, B., P.N. Fox and R.L. Villareal. 1984. *Triticale* in commercial agriculture: progress and promise. *Advances in Agronomy* 37: 1-45.
- Smart, M.G. 1991. The plant cell wall as a barrier to fungal invasion. Pages 47-66 in G.T. Cole and H.C. Hoch eds., *The fungal spore and disease initiation in plants and animals*. Plenum Press, New York.
- Sorokina, G.K., T.P. Alekseeva, Zh.G. Naskidashvili and Z.V. Sikharulidze. 1991. Age-related resistance of wheat to brown rust. *Seletsiya I Semenovodstvo Moskva*

6: 19-20. (CAB Abstracts, AN 921695453).

- Southerton, S.G. and B.J. Deverall. 1989. Histological studies of the expression of the *Lr9*, *Lr20* and *Lr28* alleles for resistance to leaf rust in wheat. *Plant Pathology* 38: 190-199.
- Stam, P. and A.C. Zeven. 1981. The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. *Euphytica* 30: 227-238.
- Staples, R.C. and V. Macko. 1984. Germination of urediospores and differentiation of infection structures. Pages 255-289 in W.R. Bushnell and A.P. Roelfs, eds., *The Cereal Rusts, Vol. I. Origins, specificity, structure, and physiology*. Academic Press, Orlando.
- Statler, G.D. 1977. Inheritance of virulence of culture 73-47 *Puccinia recondita*. *Phytopathology* 67: 906-908.
- Statler, G.D. 1979. Inheritance of pathogenicity of culture 70 - 1, race 1, of *Puccinia recondita tritici*. *Phytopathology* 69: 661-663.
- Statler, G.D., and T. Christianson. 1993. Temperature studies with wheat leaf rust. *Canadian Journal of Plant Pathology* 15: 97-101.
- Statler, G.D., and D.A. Jones. 1981. Inheritance of virulence and uredial color and size in *Puccinia recondita tritici*. *Phytopathology* 71: 652-655.
- Statler, G.D., J.E. Watkins and J. Nordgaard. 1977. General resistance displayed by three hard red spring wheat (*Triticum aestivum*) cultivars to leaf rust. *Phytopathology* 67: 759-762.
- Stewart D.M., A.H. Kamel and R.E. Heiner. 1977. A source of combined resistance in spring wheat to stem, leaf and stripe rust in Egypt. *Plant Disease Reporter* 61: 926-930.
- Stoyanov, I. 1987. Brown rust on wheat in North Bulgaria for the period 1979-1985. *Cereal Rusts Bulletin* 15: 72-78. (CAB Abstracts, AN 891131582).
- Stubbs, R.W., J.M. Prescott, E.E. Saari, and H.J. Dubin. 1986. *Cereal disease methodology manual*. Mexico D.F. CIMMYT, Mexico.
- Sunderwirth, S.D. and A.P. Roelfs. 1980. Greenhouse evaluation of the adult plant resistance of *Sr2* to wheat stem rust. *Phytopathology* 70: 634-637.
- Tani, T., H. Yamamoto, T. Onoe and N. Naito. 1974. Initiation of resistance and cell

- collapse in the hypersensitive reaction of oat leaves against *Puccinia coronata avenae*. *Physiological Plant Pathology* 7: 231-242.
- Tanksley, S.D., N.D. Young, A.H. Paterson and M.W. Bonnierbale. 1989. RFLP mapping in plant breeding: new tools for an old science. *Technology* 7: 257-264.
- Teng, P.S., and K.L. Bowen. 1985. Disease modeling and simulation. Pages 435-466 in A.P. Roelfs and W.R. Bushnell, eds., *The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control*. Academic Press, Orlando.
- The, T.T. and E.P. Baker. 1975. Basic studies relating to the transference of genetic characters from *Triticum monococcum* to hexaploid wheat. *Australian Journal of Biological Science* 28: 189-199.
- Tomerlin, J.R., M.G. Eversmeyer, C.L. Kramer and L.E. Browder. 1983. Temperature and host effects on latent and infectious periods and on urediospore production of *Puccinia recondita* f. sp. *tritici*. *Phytopathology* 73: 414-419.
- Valkoun, J., J. Dostál, and D. Kucerová. 1990. *Triticum x Aegilops* Hybrids through embryo culture. Pages 152-166 in Y.P.S. Bajaj, eds., *Biotechnology in agriculture and forestry* 13. Wheat. Springer-Verlag, Berlin.
- Valkoun, J., J D. Kucerová and P. Bartos. 1986. Transfer of leaf rust resistance from *Triticum monococcum* L. to hexaploid wheat. *Zeitschrift für Pflanzenzüchtung* 96: 271-278.
- Van Ginkel, M., and S. Rajaram. 1993. Breeding for durable resistance to diseases in wheat: An international perspective. Pages 259 - 272 in Th. Jacobs and J. E. Parlevliet, eds., *Durability of disease resistance*. Kluwer Academic Publishers, Dordrecht.
- Vanderplank, J.E. 1963. *Plant diseases: Epidemics and control*. Academic Press, New York.
- Vanderplank, J.E. 1991. The two gene-for-gene hypotheses and a test to distinguish them. *Plant Pathology* 40: 1-3.
- Verwoerd, L. 1937. Die physiologische rasse van *Puccinia triticina* Eriks. wat in Suid-Afrika voorkom. *South African Journal of Science* 33: 648-652.
- Vidhyasekaran, P. 1988. *Physiology of disease resistance in plants*. Vol II. CRC Press, Inc. Boca Raton.

- Wagoire, W.W., W.O. Vogel and R. Little. 1994. Towards the identification of stable wheat varieties for Uganda. Pages 150-158 in Daniel L. Danial, ed., Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Wahl, I., Y. Anikster, J. Manisterski and A. Segal. 1984. Evolution at the center of origin. Pages 30-77 in W.R. Bushnell and A.P. Roelfs, eds., The Cereal Rusts, Vol. I. Origins, specificity, structure, and physiology. Academic Press, Orlando.
- Wanyera, R. 1994. The status of rust diseases of wheat in Kenya. Pages 222-225 in Daniel L. Danial, ed., Breeding for disease resistance with emphasis on durability. Proceedings of a regional workshop for Eastern, Central and Southern Africa. Njoro, Kenya, Oct. 2-6, 1994. CIP-DATA Koninklike Bibliotheek, Den Haag.
- Wiese, M.V. 1987. The compendium of wheat diseases. Second edition. APS Press. Minnesota.
- Wilcoxson, R.D. 1981. Genetics of slow rusting in cereals. *Phytopathology* 71: 989-993.
- Williams, E. and C.O. Johnston. 1965. Effect of certain temperatures on identification of physiologic races of *Puccinia recondita* f. sp. *tritici*. *Phytopathology* 55: 1317-1319.
- Wolf, G. 1982. Physiology and biochemistry of spore germination. Pages 151 - 178 in K.J. Scott and A.K. Chakravorty, eds., The rust fungi. Academic Press, London.
- Yang, N.D. and S.D. Tanksley. 1989. RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theoretical and Applied Genetics* 77: 353-359.
- Yarwood, C.E. 1956. Cross protection with two rust fungi. *Phytopathology* 46: 540-544.
- Young, H.C. and L.E. Browder. 1965. The North American 1965 set of supplemental differential wheat varieties for identification of races of *Puccinia recondita tritici*. *Plant Disease Reporter* 49: 308-311.
- Young, H.C. Jr. and B. D'Oliveira. 1982. A further study of race populations of *Puccinia recondita* f. sp. *tritici*. *Garcia de Orta, Estudos Agronomicos* 9: 1-2, 37-

51. (CAB Abstracts, AN 851637942).

Zadoks, J.C. and J.J. Bouwman. 1985. Epidemiology in Europe. Pages 329-369 in A.P. Roelfs and W.R. Bushnell, eds., The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology and control. Academic Press, Orlando.

Zhang, H.S., R.E. Niks, R.G. Dekens, and H.H. Lubbers. 1993. Inheritance of resistance to wheat leaf rust (*Puccinia recondita*) in four accessions of diploid wheat. Page 358 in Th. Jacobs and J. E. Parlevliet, eds., Durability of disease resistance. Kluwer Academic Publishers, Dordrecht.

Zimmer, D.E. 1965. Rust infection and histological response of susceptible and resistant safflower. *Phytopathology* 55: 296-301.

