STUDIES ON *PUCCINIA RECONDITA* f. sp. *TRITICI* WITH SPECIAL EMPHASIS ON ADULT PLANT RESISTANCE IN WHEAT

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ABSTRACT

STUDIES ON PUCCINIA RECONDITA f.sp. TRITICI WITH SPECIAL EMPHASIS ON ADULT PLANT RESISTANCE IN WHEAT

Leaf rust (Puccinia recondita f.sp. tritici) of wheat (Triticum aestivum) was widespread in South Africa during 1983, 1984 and 1985 and often reached epidemic levels, especially on autumn-sown spring wheat in the Cape Province. Nine physiologic races were identified during the study period. The most common race was avirulent to the leaf rust differential genes Lr3a, 3bg, 3ka, 11, 16, 20 and 30 and virulent to Lr1, 2a, 2b, 10, 14a, 15, 17, 24. Resistance genes Lr9, Lr19, Lr21 and Lr26 were effective to all isolates tested. Evaluation of wheat genotypes for components of resistance, viz. infection type, latent period, number of uredinia and uredinium size, revealed three phenotypic reaction classes. The first group exhibited negligible resistance, the second was susceptible or moderately susceptible as seedlings but resistant as adult plants while the third group was resistant at all growth stages tested. Adult plant resistance was expressed by hypersensitive or non-hypersensitive reactions and the combination of components conditioning resistance varied. Adult plant resistance conferred by gene Lr22a was characterized by a long latent period, small uredinia, reduced sporulation and an absence of a

differential interaction between components of resistance and different races of Puccinia recondita f.sp. tritici. Numbers of uredinia on flag leaves of RL6044 (Lr22a) were equal to those of a susceptible check, Line E. Lr22a was inherited as a partially recessive gene in crosses with Zaragoza and SST33. Assessment of latent period, number of uredinia and infection type in F_4 and F_5 families homozygous for Lr22a and derived from crosses between RL6044 and Zaragoza or SST33, revealed significantly different levels of resistance between families. Differences were attributed to other genes modifying the expression of Lr22a. Adult plant resistance of Era, Glenlea, RL6044 and Sinton was expressed prior to the fifth-leaf stage. Latent period increased and number of uredinia decreased as each wheat matured. While the latent period of the flag, flag-1 and flag-2 leaf was similar within Era, Glenlea and RL6044, differences between these genotypes occurred. The latent period of flag leaves of Sinton was shorter than that of the two lower leaves. Significantly fewer uredinia developed on the flag-2 leaf of Glenlea. A reduction in temperature from 21 C to 15 C significantly increased latent period in Era, Glenlea and RL6044, and also restricted uredinium size on flag leaves of RL6044. The adult plant resistance of Glenlea crossed with Line E was conferred by two partially recessive genes. Additionally, F_2 to F_5 progenies of this cross exhibited high levels of hypersensitive

seedling resistance at 29 - 31 C to certain isolates. The latter resistance was not conferred by Lr1 or by the LrT2 gene for mature plant resistance in Glenlea. The high-temperature expression of resistance could be due to a second gene for adult plant resistance or to a previously undetected seedling gene.

DECLARATION

I hereby state that the contents of this dissertation, unless otherwise indicated, are my own original work and declare that no part of the dissertation has been submitted for degree purposes to any other university.

Z.A. PRETORIUS

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CONTENTS

CHAPTER 1

OCCURRENCE AND VIRULENCE OF *PUCCINIA RECONDITA* f.sp. *TRITICI* ON WHEAT IN SOUTH AFRICA FROM 1983 THROUGH 1985*

1.1 ABSTRACT

Leaf rust (*Puccinia recondita* f.sp. *tritici*) of wheat (*Triticum aestivum*) was widespread in South Africa during 1983, 1984 and 1985 and often reached epidemic levels. Disease levels were generally high on spring wheat in the Cape Province but severity varied on spring and winter wheat in the Orange Free State, Transvaal and Natal. The leaf rust resistance genes *Lr1*, *Lr2a*, *Lr2b*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr24* and *Lr30* acted differentially to the isolates tested. Nine races were identified during the three year period. A race with avirulence/virulence formula *Lr3a*, *3bg*, *3ka*, *11*, *16*, *20*, *24*, *30/1*, *2a*, *15*, *17* was isolated most frequently (35 %) in 1983. A similar race, but possessing addition-

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* The papers contained in this dissertation were prepared for publication in scientific journals. Therefore, American punctuation and spelling have been used and certain experimental procedures are inevitably repeated in different chapters.

al virulence to Lr24, predominated in 1984 (53 %) and 1985 (66 %). No isolates virulent to resistance genes Lr9, Lr19 and Lr26 were found. Gene Lr21 exhibited low to intermediate infection types to all isolates tested.

1.2 INTRODUCTION

Leaf rust, caused by Puccinia recondita Rob. ex Desm. f.sp. tritici, is important and widely distributed on wheat (Triticum aestivum L.) in South Africa. It occurs annually in most areas, but distribution is influenced by climatic conditions during the growing season. Leaf rust is generally most severe on autumn-sown spring wheat in the winter rainfall regions of the Cape Province, but moist conditions and elevated temperatures during spring also promote leaf rust on winter wheat in the Orange Free State. Irrigated spring wheat throughout South Africa and durum wheat (T. durum Desf.) in the Fish River Valley in the north-eastern Cape Province are also regularly subjected to severe leaf rust epidemics.

Many of the current commercially grown wheat cultivars in South Africa were developed primarily for agronomic characteristics and resistance to stem rust (P. graminis Pers. f.sp. tritici Eriks. & E. Henn.) with little effort on selection for resistance to P. recondita f.sp. tritici. Almost inevitably, all wheat cultivars

officially recommended for cultivation in South Africa (4) were susceptible to the disease during the study period.

The value of determining genetic variation in cereal rust fungi was discussed by Roelfs (80) and race surveys are regularly conducted in Australia (57), Canada (89) and the USA (54). These determinations provide invaluable epidemiological data for use in cooperative breeding programs by identifying isolates that have virulence likely to be important in a geographical area.

The physiologic specialization of P. recondita f.sp. tritici in South Africa was last documented in 1937 by Verwoerd (108) who identified five races according to reactions of eight differential host cultivars (46). The lack of information regarding virulence and epidemiology of the wheat leaf rust pathogen on the sub-Sahara African continent was evident from a recent treatise on the cereal rusts (82). With the exception of a study on physiologic races of leaf rust of wheat in Kenya (34), there are no known records from other central and southern African countries.

Race survey studies on the leaf rust pathogen of wheat were undertaken during 1983 to 1985 to determine the range in pathogen virulence in South Africa and to

identify effective monogenic resistance sources. Additionally, information on survival of inoculum between growing seasons was obtained.

1.3 MATERIALS AND METHODS

1.3.1 Survey

P. recondita f.sp. tritici infected wheat leaves were collected from commercial fields, cultivar evaluation trials and trap nurseries. Extensively surveyed areas were the western, southern and eastern Cape Province, eastern Orange Free State, and the irrigated areas in the eastern and central Transvaal, Natal and northern Cape Province. Collections were also received from Transkei, Lesotho and durum wheat growing areas in the Fish River Valley and occasionally from less representative areas. In the survey results, data from Lesotho and the northern Cape irrigation areas were included with those from the Orange Free State.

Surveys were generally conducted during October to December when wheat crops in the different growing areas were between anthesis and harvest ripeness. In 1985 two additional surveys were conducted in the Cape Province during March and August, respectively, to determine racial identity of inoculum oversummering on volunteer wheat and to monitor leaf rust incidence

during the growing season.

Most samples received from cooperators were collected from wheat rust trap nurseries. The nurseries contained 40 entries and were planted annually at 42 locations throughout South Africa. Included in a nursery were wheat cultivars and lines universally susceptible to both leaf and stem rust as well as genotypes that carry critical genes for resistance.

1.3.2 Inoculum multiplication

Seedlings of Morocco wheat, a susceptible host, were grown in soil in 10-cm plastic pots in a greenhouse. Two days after seedlings had emerged, maleic hydrazide at 10 mg/pot was added as a soil drench to retard plant development and to enhance sporulation of the fungus (97). Urediniospores from each field collection were transferred onto seven-day old Morocco plants which were then placed in darkness in a dew chamber at 19 C for 19 hr; during the last 3 hr of which the plant surfaces were allowed to dry off gradually. Plants were then placed in a greenhouse at 18-24 C where cool-white fluorescent tubes provided supplemental illumination of 9 000 lux for 12 hr daily.

1.3.3 Differential set

An objective of the 1983 survey was to compile a set of wheat genotypes that would differentiate virulence components of the South African population of *P. recondita* f.sp. *tritici*. Preliminary experiments, in which 31 wheat cultivars and lines, each carrying a single (*Lr*) gene for resistance to leaf rust, were inoculated with 83 single uredinium isolates, detected 12 *Lr* genes with clear differential ability. These genes were *Lr1*, *Lr2a*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr24*, *Lr30*.

All of these genes, with the exception of *Lr20* and *Lr24*, are contained in Thatcher wheat isolines. The genes *Lr2b*, *Lr10* and *Lr14a*, also in Thatcher background, were added to the differential set during race classification in 1985. Seed of the Thatcher near-isogenic (RL) lines were obtained from Agriculture Canada, Winnipeg, Canada and seed of the cultivars Thew (*Lr20*) and Agent (*Lr24*) originally from the Plant Breeding Institute, Castle Hill, Australia.

1.3.4 Race testing

Differential hosts were planted in 10-cm plastic pots and a water-soluble fertilizer (28 % N) was added (20 mg/pot) when plants were two days old. Seedlings were

six to eight days old when inoculated, when primary leaves were fully expanded.

Each differential set was inoculated with urediniospores collected from a single uredinium on Morocco. Spores were collected in a gelatine capsule and suspended in Soltrol 130® or Soltrol 170® light mineral oil (Phillips Chemical Company, Borger, Texas). The suspension was atomized onto plants by applying air pressure to an inoculator which was specifically designed to accommodate the capsule containing the inoculum (10). Inoculated plants were kept in a dew chamber as described for Morocco seedlings. After completion of the drying period, plants were placed in a greenhouse at 18-24 C with a 12 hr supplemental photoperiod of 9 000 lux daily.

During 1983, 1984 and 1985, 43, 255 and 330 single uredinium isolates were established, respectively, and each characterized on a differential set. Infection types were allocated on the standard 0-4 scale (97) 10 to 14 days after inoculation. According to this scale, plus or minus signs indicate gradations above or below a distinct infection type; c and n indicate the association of chlorosis and necrosis with a lesion; x indicates a mesothetic reaction; and ; indicates a fleck reaction. Avirulence and virulence characteristics of isolates were determined according to low or

high infection types mediated by Lr genes, infection types below or above 2 indicating avirulence or virulence in the pathogen for a resistance gene. Seedlings of Thatcher near-isogenic lines RL6010 (Lr9), RL6040 (Lr19), RL6043 (Lr21) and of cultivar Kavkaz (Lr26) were inoculated with composite collections of urediniospores each year to identify undetected virulence characteristics. Genes Lr9, Lr19, Lr21 and Lr26 had shown resistance to South African collections of P. recondita f.sp. tritici during preliminary experiments in 1983.

1.4 RESULTS

The collection of 628 isolates of P. recondita f.sp. tritici made in South Africa from 1983 through 1985 was divided into nine pathotypes on the basis of infection types produced on wheat cultivars and lines carrying single Lr genes. Infection types of nine type cultures from the 1985 survey and representative of the spectrum of physiologic specialization identified in South Africa are shown in Table 1.1.

Although Lr2b, Lr10 and Lr14a were not tested as differentials during 1983 and 1984, type cultures from that period corresponded with type cultures from 1985 when tested together on the expanded set of differentials. Thus, isolates 3SA77 and 3SA120; 3SA56, 3SA57 and 3SA122; 3SA73, 3SA78 and 3SA121; 3SA62, 3SA63 and

Table 1.1. Characteristic infection types[a] produced on wheat lines or cultivars that carry different leaf rust resistance genes to nine physiologic races of *Puccinia recondita* f.sp. *tritici* identified in South Africa during 1985

Wheat line or cultivar	*Lr* gene	Leaf rust isolate								
		3SA120	3SA121	3SA122	3SA123	3SA124	3SA125	3SA126	3SA127	3SA128
RL6003	1	4	4	0;	4	0;	0;	4	4	4
RL6016	2a	1c	4	;1c	4	;	2	4	4	;1=c
RL6019	2b	2	4	2=c	4	;cn	3=	4	4	2
RL6002	3a	;	;	3	;	3	3	0;	0;	3
RL6042	3bg	;	;	3	;	;	;1	;	;	x=
RL6007	3ka	;1	;1	;1	;1	3+	3	;	;	3
RL6004	10	3	2	3	2	2+3	2	3	3	4
RL6053	11	2	2	2	2	;1	;1	2	2	4
RL6013	14a	4	x	4	x	4	x	4	4	4
RL6052	15	;c	4	;	4	;	;1cn	4	4	;1=c
RL6005	16	1cn	1cn	3	1cn	2c	3=	1cn	1cn	1cn
RL6008	17	;	;1c	;1+	;1c	;	;1c	4	4	;1c
Thew	20	;	;1	;1	;1	4	4	;	;	4
Agent	24	;	;1cn	;c	4	;1	;	;	4	4
RL6049	30	2	2	2	2	3+	3	;12	;12	3+

[a] Infection types are according to the 0-4 scale (97).

3SA126; 3SA68, 3SA79 and 3SA123; 3SA58 3SA86 and 3SA127; 3SA64, 3SA60 and 3SA128; 3SA75 and 3SA124 evidently belong to the same physiologic races, respectively (Tables 1.2, 1.3 and 1.4). Isolates are numbered according to a system where an accession number is preceded by 3SA, respectively indicating P. recondita f.sp. tritici and South Africa. These isolates could be grouped into six standard races (numbers 6, 10, 14, 50, 58 and 84) by the scheme of the international register of wheat leaf rust races (45) or into four races (numbers 3, 6, 9 and 14) according to the scheme of unified numeration (8, 54). In the present study, international race numbers (8, 45) were not allocated to genetically specialized isolates because current schemes cannot accommodate the South African avirulence/virulence combinations identified. Thus, isolate numbers were used to refer to different races of P. recondita f.sp. tritici in South Africa.

A race (isolate 3SA63) with avirulence/virulence formula Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/Lr1, 2a, 15, 17 was isolated most frequently (34.9 %, Table 1.2) during 1983, and a similar race (isolates 3SA86 and 3SA127), but with additional virulence for Lr24, was isolated most commonly during 1984 (52.9 %, Table 1.3) and 1985 (66.1 %, Table 1.4). A race (isolates 3SA77 and 3SA120) with virulence to Lr1, Lr10 and Lr14a, was isolated once from triticale in 1983 and once from

Table 1.2. Avirulence/virulence combinations and origin of isolates of Puccinia recondita f.sp. tritici characterized on differential leaf rust resistance (Lr) genes in South Africa during 1983

Avirulence/virulence formula	Origin and number of isolates					Total	Percentage	Type culture number
	W and S Cape Prov.	East Cape Prov.	OFS	Tvl	Natal			
	No.	No.	No.	No.	No.	No.	%	
Lr2a, 3a, 3bg, 3ka, 11, 15, 16, 17, 20, 24, 30/1	0	0	1	0	0	1	2.3	3SA77
Lr1, 2a, 3ka, 11, 15, 17, 20, 24, 30/3a, 3bg, 16	3	3	0	0	0	6	14.0	3SA61
Lr3a, 3bg, 3ka, 11, 16, 17, 20, 24, 30/1, 2a, 15	0	0	1	0	3	4	9.3	3SA73
Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/1, 2a, 15, 17	10	0	1	3	1	15	34.9	3SA63
Lr3a, 3bg, 3ka, 11, 16, 17, 20, 30/1, 2a, 15, 24	0	0	4	0	0	4	9.3	3SA68
Lr3a, 3bg, 3ka, 11, 16, 20, 30/1, 2a, 15, 17, 24	1	8	0	0	0	9	20.9	3SA58
Lr2a, 3bg, 15, 16, 17/1, 3a, 3ka, 11, 20, 24, 30	0	0	3	1	0	4	9.3	3SA64
TOTAL	14	11	10	4	4	43		

Table 1.3. Avirulence/virulence combinations and origin of isolates of Puccinia recondita f.sp. tritici characterized on different leaf rust resistance (Lr) genes in South Africa during 1984

Avirulence/virulence formula	Origin and number of isolates					Total	Percentage	Type culture number
	W and S Cape Prov.	East Cape Prov.	OFS	TVL	Natal			
	No.	No.	NO.	No.	No.	No.	%	
Lr1, 2a, 3ka, 11, 15, 17, 20, 24, 30/3a, 3bg, 16	0	1	2	3	3	9	3.5	3SA57
Lr3a, 3bg, 3ka, 11, 16, 17, 20, 24, 30/1, 2a, 15	0	2	6	1	1	10	3.9	3SA78
Lr1, 2a, 3bg, 11, 15, 16, 17, 24/3a, 3ka, 20, 30	0	7	0	0	0	7	2.7	3SA75
Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/1, 2a, 15, 17	8	5	3	11	2	29	11.4	3SA62
Lr3a, 3bg, 3ka, 11, 16, 17, 20, 30/1, 2a, 15, 24	0	2	23	2	6	33	12.9	3SA79
Lr3a, 3bg, 3ka, 11, 16, 20, 30/1, 2a, 15, 17, 24	67	32	13	18	5	135	52.9	3SA86
Lr2a, 3bg, 15, 16, 17/1, 3a, 3ka, 11, 20, 24, 30	3	2	27	0	0	32	12.5	3SA60
TOTAL	78	51	74	35	17	255		

Table 1.4. Avirulence/virulence combinations and origin of isolates of Puccinia recondita f.sp. tritici characterized on differential leaf rust resistance (Lr) genes in South Africa during 1985

Avirulence/virulence formula	Origin and number of isolates					Total	Percentage	Type culture number
	W and S Cape Prov.	East Cape Prov.	OFS	TVL	NATAL			
	No.	No.	No.	No.	No.	No.	%	
Lr2a, 2b, 3a, 3bg, 3ka, 11, 15, 16, 17, 20, 24, 30/1, 10, 14a	0	0	0	0	1	1	0.3	3SA120
Lr3a, 3bg, 3ka, 10, 11, 14a, 16, 17, 20, 24, 30/1, 2a, 2b, 15	0	0	0	0	1	1	0.3	3SA121
Lr1, 2a, 2b, 3ka, 11, 15, 17, 20, 24, 30/3a, 3bg, 10, 14a, 16	0	8	0	5	4	17	5.2	3SA122
Lr3a, 3bg, 3ka, 10, 11, 14a, 16, 17, 20, 30/1, 2a, 2b, 15, 24	0	12	2	0	1	15	4.5	3SA123
Lr1, 2a, 2b, 3bg, 11, 15, 16, 17, 24/3a, 3ka, 10, 14a, 20, 30	0	9	0	0	0	9	2.7	3SA124
Lr1, 2a, 3bg, 10, 11, 14a, 15, 17, 24/2b, 3a, 3ka, 16, 20, 30	0	2	0	0	0	2	0.6	3SA125
Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/1, 2a, 2b, 10, 14a, 15, 17	24	2	4	18	2	50	15.2	3SA126
Lr3a, 3bg, 3ka, 11, 16, 20, 30/1, 2a, 2b, 10, 14a, 15, 17, 24	106	60	9	18	25	218	66.1	3SA127
Lr2a, 2b, 3bg, 15, 16, 17/1, 3a, 3ka, 10, 11, 14a, 20, 24, 30	1	4	3	9	0	17	5.2	3SA128
TOTAL	131	97	18	50	34	330		

Morocco wheat in a trap nursery in 1985.

The surveys indicated differences in the distribution of races. Six races occurred in the Orange Free State, whereas four were identified from the western and southern Cape Province. Drought conditions restricted leaf rust development in the Orange Free State during 1985, but results from the 1984 survey indicated that two races (isolates 3SA79 and 3SA60) predominated on winter wheat (Table 1.4). Spring wheat grown in the Cape Province was severely infected by leaf rust during the entire study period. In 1984 and 1985, spring wheat in this area was also infected by a predominant race (isolates 3SA86 and 3SA127), which comprised 86 % and 81 % of all isolates collected in that region during those years (Tables 1.3 and 1.4).

Eight races were found in the spring and durum wheat production areas of the eastern Cape Province. Two races, typified by isolates 3SA124 and 3SA125 (Table 1.4) were identified only from durum wheat. Again, a single race (isolate 3SA127) was most prevalent in this region during the survey period. Six races were identified from collections made in the Transvaal and Natal during 1984, the races which occurred in the Transvaal were similar to those found in Natal (Tables 1.2, 1.3 and 1.4).

Table 1.5. Origin and number of isolates of *Puccinia recondita* f.sp. *tritici* virulent to leaf rust resistance genes in South Africa during 1983, 1984 and 1985

Resistance gene	Western and Southern Cape Province			Eastern Cape Province			Orange Free State			Transvaal			Natal			Frequency of virulence gene in population (%)		
	1983	1984	1985	1983	1984	1985	1983	1984	1985	1983	1984	1985	1983	1984	1985	1983	1984	1985
Lr1	11	78	131	8	43	78	10	72	18	4	32	45	4	14	30	86.0	93.7	91.5
Lr2a	11	75	130	8	41	74	6	45	15	3	32	36	4	14	29	74.4	81.2	86.1
Lr2b[a]	-	-	130	-	-	76	-	-	15	-	-	36	-	-	29	-	-	86.7
Lr3a	3	3	1	3	10	23	3	29	3	1	3	14	0	3	4	23.3	18.8	13.6
Lr3bg	3	0	0	3	1	8	0	2	0	0	3	5	0	3	4	14.0	3.5	5.2
Lr3ka	0	3	1	0	9	15	3	27	3	1	0	9	0	0	0	9.3	15.3	8.5
Lr10[a]	-	-	131	-	-	9	-	-	16	-	-	50	-	-	32	-	-	72.1
Lr11	0	3	1	0	2	4	3	27	3	1	0	9	0	0	0	9.3	12.5	5.2
Lr14a[a]	-	-	131	-	-	83	-	-	16	-	-	50	-	-	32	-	-	94.5
Lr15	11	75	130	8	41	74	6	45	15	3	32	36	4	14	29	74.4	81.2	86.1
Lr16	3	0	0	3	1	10	0	2	0	0	3	5	0	3	4	14.0	3.5	5.8
Lr17	11	75	130	8	37	62	1	16	13	3	29	36	1	7	27	55.8	64.3	81.2
Lr20	0	3	1	0	9	15	3	27	3	1	0	9	0	0	0	9.3	15.3	8.5
Lr24	1	70	107	8	36	76	7	63	14	1	20	27	0	11	26	39.5	78.4	75.8
Lr30	0	3	1	0	9	15	3	27	3	1	0	9	0	0	0	9.3	15.3	8.5
Number of isolates tested	14	78	131	11	51	97	10	74	18	4	35	50	4	17	34			

[a] Genes Lr2b, Lr10 and Lr14a were not tested during 1983 and 1984.

The frequency of virulence genes in the population of P. recondita f.sp. tritici for specific Lr genes is presented in Table 1.5. Virulence for Lr1, Lr2a, Lr2b, Lr10, Lr14a, Lr15, Lr17 and Lr24 was common. Avirulence or virulence to Lr2a was always associated with avirulence or virulence to Lr15 and similar associations existed among Lr3ka, Lr20 and Lr30 in the isolates tested. Virulence to Lr3bg, Lr3ka, Lr11, Lr16, Lr20 and Lr30 was not common.

Inoculation of seedlings carrying genes Lr9, Lr19, Lr21 and Lr26 with urediniospore composites of field collections did not reveal virulence for these genes. Lr9 and Lr19 exhibited infection type 0; whereas Lr21 and Lr26 exhibited infection types 2c and ;1=c, respectively. Inoculation of seedlings with Lr genes other than those used in the differential set indicated no avirulence in the isolates tested for Lr14b, Lr23 and Lr25.

In the overseasoning component of the study, infected wheat leaves were collected from various parts of South Africa each month beginning in September 1983 and continuing through December 1985. Uredinia were common on wheat leaves in the summer rainfall regions from January to May. During June, leaf rust occurred on early sown wheat in the irrigated areas of the Transvaal and Transkei. From July through September infections occurred sporadically on wheat in the Cape Province,

north-eastern Orange Free State and Transvaal.

Unusually wet conditions in the southern and eastern Cape Province during the summer of 1984/1985 favored development of volunteer wheat. Severe leaf rust infection was observed on plants in the eastern Cape Province where several fields under minimum tillage ensured an abundance of volunteer plants in a wide range of growth stages. However, no volunteer plants were found in the western Cape Province.

From surveys of the southern and eastern Cape Province during March 1985, when the annual wheat crop in that region had not yet been sown, 33 isolates of P. recondita f.sp. tritici were established. These isolates yielded three races with virulence characteristics similar to those of isolates 3SA126, 3SA127 and 3SA128 (Table 1.4). Two races, similar to isolates 3SA123 and 3SA128 were also identified from concurrent collections made from volunteer winter wheat in the eastern Orange Free State. Samples collected during June through August 1985, when several spring wheat fields in the southern Cape Province as well as experimental plots in the Transvaal already were heavily infected with leaf rust, revealed races with avirulence/virulence combinations similar to isolates 3SA122, 3SA126, 3SA127 and 3SA128. Survey results obtained during March and August 1985 are combined with results for the October

and December period (Table 1.4).

1.5 DISCUSSION

Observations on the epidemiology of *P. recondita* f.sp. *tritici* in South Africa confirmed continuous asexual uredinial cycles. Main foci of oversummering inoculum were located in the summer rainfall areas, but precipitation in the southern and eastern Cape Province during December 1984 and January 1985 provided enough host plants for survival of *P. recondita* f.sp. *tritici*.

According to Anikster (3) and Samborski (88), the heteroecious nature of wheat leaf rust is validated in various parts of the world by aecial infections on *Anchusa* spp. (Boraginaceae) and on *Thalictrum* spp., *Clematis* spp. and other genera belonging to the Ranunculaceae. A number of *Anchusa*, *Thalictrum* and *Clematis* species occur widespread throughout South Africa and are either indigenous or introduced (M. Welman, Botanical Research Institute, Pretoria: *personal communication*). *A. capensis* is commonly found in South Africa and was described as an aecial host for leaf rust of rye in the United States (5). Large populations of *A. azurea* also occur adjacent to wheat fields annually in the western Cape Province. However, no aecial infections on any of these hosts have been observed or reported in South Africa, despite the frequent produc-

tion of teliospores on mature wheat leaves.

Certain epidemiological trends in race distribution and the effect of cultivars on occurrence of races in different agroecological areas were evident. Most races of P. recondita f.sp. tritici occurred throughout the country. However, three of the nine identified races were not widespread. Although durum and bread wheat cultivars are grown interchangeably in the Fish River Valley and leaf rust collections from both crops were analysed, two races were isolated only from durum cultivars. Furthermore, a race with virulence to Lr1, Lr10 and Lr14a (isolate 3SA120, Table 1.4) was isolated twice only during the three year study period.

Differential fitness and selection among races of the South African population of P. recondita f.sp. tritici was emphasized by the frequent isolation of one race during consecutive surveys of the Cape Province in 1985. The common occurrence of this race, which was virulent to Lr1, Lr2a, Lr2b, Lr10, Lr14a, Lr15, Lr17 and Lr24, could partly be ascribed to the extreme susceptibility of all spring wheat cultivars recommended in South Africa. However, this race is avirulent to the local winter wheat cultivars Betta, SST102 and Karee, which accordingly acted as selective hosts for a different race (similar to isolate 3SA128) in the Orange Free State. Similarly, the spring wheat cultivar

Zaragoza was selective for another race (isolate 3SA122), found mostly in irrigated areas in the Transkei, Natal and Transvaal. The genes for resistance to leaf rust in the current spring and winter wheat cultivars are not known. Comparison of present races with those described by Verwoerd (108) is not possible because different differential hosts were used in the two studies. However, if we do not consider Brevit, Loros and Mediterranean because these were not included as differential hosts in the present study, race 15 identified in 1937 (108) corresponds with isolates 3SA122 and 3SA124 and race 91 corresponds with isolate 3SA128. Harder (34) identified five races in Kenya, but the lack of avirulence in the East African isolates for Lr10 or Lr17 and the lack of virulence for the gene in Aniversario (Lr3ka), distinguished those races from the current population of P. recondita f.sp. tritici in South Africa.

Data collected from 1983 through 1985 suggested genes Lr9, Lr19, Lr21 and Lr26 would be most useful for developing cultivars resistant to P. recondita f.sp. tritici in South Africa. Ideally, a combination of these genes should be incorporated into new cultivars.

CHAPTER 2

COMPONENTS OF RESISTANCE IN WHEAT INFECTED WITH *PUCCINIA RECONDITA* f.sp. *TRITICI*

2.1 ABSTRACT

Expression of resistance to *Puccinia recondita* f.sp. *tritici* was determined after inoculation of a selection of 22 winter wheat, adult-plant-resistant and susceptible cultivars and lines at two stages of plant development. Inoculation of primary leaves with 19 pathogenically different isolates of wheat leaf rust indicated genes for seedling resistance in 13 host genotypes. Wheat hosts were grouped in three classes according to phenotypic expression of host-pathogen interaction. Four cultivars and lines in the first category expressed little or no resistance at any growth stage tested. Latent period, uredinium density, uredinium size and infection type were determined on flag leaves and indicated that 10 host cultivars and lines in the second class, despite seedling susceptibility, possessed some level of adult plant resistance. Measurement of components showed that adult plant resistance in wheat to leaf rust may be expressed by hypersensitive or non-hypersensitive reaction types and that the combination of components mediating such resistance may vary. The third class contained eight host cultivars and lines which exhibited hypersensitive, growth stage-unrelated resistance. Latent period

and density of uredinia were negatively correlated in the experiments conducted, and this relationship varied considerably between individual cultivars or lines. This study showed that resistance to P. recondita f.sp. tritici, comparable to adult plant resistance conferred by known genes, may be identified in randomly selected wheat germplasm collections.

2.2 INTRODUCTION

Maintaining stable resistance in breeding lines is an important objective in all programs developing rust-resistant cereal cultivars. A variety of resistance sources are available to cereal breeders and investigations of host resistance mechanisms have provided numerous reports on theory, concepts, terminology and genetics of host : pathogen interactions (37, 43, 55, 61, 105, 106, 107).

Resistance identified in seedlings and characterized by infection types has commonly been exploited in the breeding of cultivars resistant to the cereal rusts. This type of resistance is generally conferred monogenically and the genes are incorporated either individually or in combination with other genes for resistance into agronomically adapted plant genotypes. Because of the well-documented ephemeral nature of single gene resistance (61), alternative strategies

have been advocated for improvement of cultivars. Techniques for identifying components of resistance and the inheritance of slow rusting or partial resistance in wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) to different rust pathogens have been investigated (70, 79, 109).

Adult plant resistance (APR) is a recognized, but less studied type of resistance in wheat infected with Puccinia recondita Rob. ex Desm. f.sp. tritici (80). This resistance is typified by seedlings being susceptible while adult plants are resistant. Leaf rust resistance (Lr) genes Lr12, Lr13, Lr22a and Lr22b are associated with APR (80), indicating that APR frequently may be of the monogenic type.

Incomplete information on the level and stability of APR against leaf rust in wheat has probably discouraged breeders from using it more extensively. Most studies have investigated inheritance of APR (7, 21, 25, 27), while components of APR in different wheat host : leaf rust pathogen interactions have not been clearly delineated. Roelfs (80) mentioned the effects of host growth stage and nutrition, plant age, inoculum density, temperature and light as prospective fields of study to determine the value of APR. Stability of APR might also depend on the effect of different races of P. recondita f.sp. tritici. Different infection types

produced by APR genes Lr12, Lr13 and Lr22b to different races have been reported (19, 25). APR expression and inheritance in several other small grain host : pathogen associations have also been studied (33, 35, 38, 50, 65, 102, 110).

In this study, a randomly selected collection of winter wheat cultivars and lines were evaluated for the presence of genes for seedling resistance and for components of resistance in the adult plant stage. The objectives of this research were to determine which components acted singularly or collectively during resistance expression.

2.3 MATERIALS AND METHODS

2.3.1 Inoculation of seedlings

Seedlings of eight cultivars and nine lines from the South African winter wheat breeding program, a cultivar and three lines reported to possess APR conditioned by single resistance genes and a susceptible check line (Table 2.1), were inoculated with 19 different Cereal Rust Laboratory (CRL) isolates of P. recondita f.sp. tritici. Isolates originated from the United States, Mexico and China and have differential virulence for resistance genes: Lr1, Lr2a, Lr2c, Lr3a, Lr3ka, Lr9, Lr10, Lr16, Lr17, Lr18 and Lr24.

Urediniospores of each isolate were suspended in light mineral oil (Soltrol 170®, Phillips Chemical Company, Specialty Chemicals Division, Borger, Texas) and atomized (10) onto primary leaves of 10 seven-day-old plants per cultivar or line. After a 16 hr dew period at 18 C in darkness, followed by 3 hr of gradual drying, seedlings were placed in a greenhouse at 18-25 C where natural light was supplemented with light emitted from cool-white fluorescent tubes at 11 000 lux for 12 hr daily. Infection types were scored on a 0 to 4 scale (97) 12 days after inoculation.

2.3.2 Inoculation of adult plants

Seed of the winter wheat cultivars and lines (Table 2.1) were vernalized at 4 C for 35 days. Germlings were planted along with the APR and susceptible checks in soil in 10-cm pots (one plant per pot) and grown to maturity in a greenhouse at 18-25 C with supplementary light at 11 000 lux for 12 hr daily. A water-soluble fertilizer (23:19:17 NPK) was applied three times (0.4 g/pot) during the course of the experiment. Maturation of the wheat genotypes was not synchronized due to inherent differences in growth habits, therefore plants were inoculated in three sets when all plants in a maturity set were at stage 13 on the Romig scale (15, see Appendix for description of scale).

Flag leaves were inoculated with isolate CRL 82-MGC-OQ of P. recondita f.sp. tritici with the aid of the Andres inoculation device (2). This isolate was selected because in previous experiments with wheat seedling : leaf rust isolate combinations it was pathogenic to a large range of wheat genotypes. Isolate CRL 82-MGC-OQ was characterized by the avirulence/virulence formula: Lr2a, Lr2c, Lr3ka, Lr9, Lr16, Lr17, Lr18/Lr1, Lr3a, Lr10, Lr24. A suspension of 0.2 mg urediniospores per ml Soltrol 170® oil was sprayed onto adaxial leaf surfaces, placed 160 mm from the inoculator nozzle. Air pressure was set to raise the nozzle 300 mm/sec while air from another source (41.4 kPa), discharged the spore suspension. Prior to placing plants in a dew chamber where relative humidity was maintained by nebulizers, paper clips were attached to leaf tips to correctly position inoculated surfaces for uniform dew deposition. A 16 hr dew period at 22 C was followed by 3 hr of drying before plants were returned to the greenhouse regime described for seedling evaluation.

2.3.3 Measurement of components of resistance

Areas on the inoculated leaf surfaces were marked and inspected regularly for erumpent uredinia. Numbers of erumpent uredinia were recorded daily at 08h00 and 20h00 until no more appeared. Infection type (97),

reaction type and size of uredinia on flag leaves were visually determined 13 days after inoculation. Uredinial sizes were specified according to a 0 to 8 scale where 0= no sporulation, but visible infection site; 2= minute uredinia; 4= small uredinia; 6= intermediate uredinia and 8= large uredinia. According to a scale indicating elliptical areas, uredinia smaller than 1 mm^2 were regarded as minute and larger than 4 mm^2 as large.

Flag leaves were then detached and the areas in which uredinia were counted were measured with a leaf area meter (model LI-3000, Lambda Instruments Corporation, Lincoln, Nebraska). Numbers of uredinia were divided by leaf area (cm^2) to obtain uredinium density. Latent period was calculated for each cultivar or line according to the linear regression method described by Andres (1) and indicated the time (hr) between commencement of the dew chamber cycle and eruption of 40 % of the final number of primary uredinia.

2.3.4 Data analysis

Four, six and seven flag leaves per cultivar or line were inoculated in the three maturity sets, respectively. Each experiment was arranged in a randomized complete block design. Data were evaluated by analysis of variance and differences among means were tested for

significance using Tukey's procedure (100). Coefficients of correlation (r) between latent period and number of uredinia for each of the three sets were calculated and tested for significance.

2.4 RESULTS AND DISCUSSION

Infection types on seedling and flag leaves and reaction type and uredinium size on flag leaves to isolate CRL 82-MGC-OQ of *P. recondita* f.sp. *tritici* are shown in Table 2.1. Presence of genes for resistance in seedlings of the wheat cultivars and lines tested is indicated by whether isolates were virulent or avirulent to the respective hosts (Table 2.1). Latent period and density of uredinia for each host in the three sets of inoculations are shown in Table 2.2.

In characterizing resistance of wheat cultivars to leaf rust, Milus and Line (64) identified seven classes of resistance after measuring four components in both seedling and adult plants. Phenotypically, these classes included susceptibility, hypersensitivity and varying degrees of slow rusting; the latter also including APR. Similar variation in host : pathogen interactions was observed in our study and host cultivars and lines were classified into three classes on the basis of the reactions of seedlings and adult plants (Table 2.1).

Table 2.1. Responses of primary and flag leaves of South African winter wheat cultivars and lines, adult-plant-resistant and susceptible check lines to Puccinia recondita f.sp. tritici in the greenhouse

Cultivar or Line per response class	Seedling reaction			Adult plant reaction to isolate CRL 82-MGC-OQ		
	No. of isolates		Infection type[a] to isolate CRL 82-MGC-OQ	Flag leaf infection type[a]	Reaction type[b]	Uredinium size[c]
	Virulent	Avirulent				
Susceptible						
Line E	19	0	4	4	S	8
Scheepers 69	19	0	4	3c	MS-S	8
SNK108	19	0	4	3c	MS	8
Wilge	16	3	4	4	S	8
Adult-plant-resistant[d]						
Thatcher (Lr22b)	19	0	4	4	S	8
RL6044 (Lr22a)	19	0	4	1+	MS	4
RL6011 (Lr12)	19	0	4	;1cn	MR-MS	4
CT263 (Lr13)	19	0	4	2cn	MR-MS	4
Belinda	19	0	4	4	S	8
SNK104	19	0	4	2	MR-MS	4
SWP2-29	18	1	4	4	S	8

Table 2.1 (continued). Responses of primary and flag leaves of South African winter wheat cultivars and lines, adult-plant-resistant and susceptible check lines to Puccinia recondita f.sp. tritici in the greenhouse

Cultivar or Line per response class	Seedling reaction			Adult plant reaction to isolate CRL 82-MGC-OQ		
	No. of isolates		Infection type[a] to isolate CRL 82-MGC-OQ	Flag leaf infection type[a]	Reaction type[b]	Uredinium size[c]
	Virulent	Avirulent				
Flamink	16	3	4	3c	MS-S	6
T79/12	12	7	3	3	S	6
T81/1	2	17	4	3	S	6
Resistant as seedlings and adult plants[e]						
T80/13	5	14	;1	;12	MS-S	0-6
T81/6	3	16	;1c	;1=c	MR-MS	0-4
Betta	1	18	;1	;1	MR	0-2
Karee	1	18	2+	;1+	MR-MS	0-4
T78/10	1	18	2+	;1+	MR-MS	0-4
T80/1	1	18	2	;1++	MR-MS	2
T81/8	0	19	;1c	;1+c	MR	0-2
T81/13	0	19	;1	;1	R-MR	0-2

[a] Seedling and flag leaf infection types were scored on a 0-4 scale (97).

[b] S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant.

Table 2.1 (continued). Responses of primary and flag leaves of South African winter wheat cultivars and lines, adult-plant-resistant and susceptible check lines to Puccinia recondita f.sp. tritici in the greenhouse

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[c] Uredinium sizes were estimated on a 0-8 scale: 0 = no sporulation but visible flecking; 2 = minute uredinia; 4 = small uredinia; 6 = intermediate uredinia; 8 = large uredinia.

[d] Cultivars and lines in this class exhibited hypersensitive or non-hypersensitive adult plant resistance.

[e] Resistant as seedlings and adult plant to isolate CRL 82-MGC-OQ.

Table 2.2. Mean latent period and uredinium density on flag leaves of South African winter wheat cultivars and lines, adult-plant-resistant and susceptible check lines inoculated with isolate CRL 82-MGC-OQ of Puccinia recondita f.sp. tritici in the greenhouse

Cultivar or Line[1/]	Latent period[2/] (hr) ± standard deviation	Number[2/] of uredinia/ cm^2 flag leaf surface ± standard deviation
First maturity set		
Karee	262 ± 6.5 b	4.6 ± 1.8 abcd
T80/1	262 ± 6.6 b	4.0 ± 2.5 abc
T81/13	249 ± 13.2 b	1.5 ± 1.3 a
Wilge	194 ± 11.9 c	9.5 ± 5.1 bcde
T80/13	192 ± 16.9 c	7.0 ± 2.4 abcde
T79/12	187 ± 0.9 c	11.1 ± 3.7 de
Flamink	181 ± 9.7 c	10.6 ± 1.7 cde
T81/1	179 ± 15.1 c	6.2 ± 2.2 abcde
Line E	177 ± 3.5 c	12.2 ± 2.4 e
Second maturity set		
Betta	269 ± 5.5 a	5.9 ± 0.7 a
Karee	263 ± 14.3 ab	4.8 ± 2.7 a
T78/10	251 ± 5.0 ab	7.1 ± 1.8 a
T81/6	248 ± 11.5 b	6.2 ± 2.1 a
T79/12	196 ± 3.3 de	6.1 ± 0.9 a
SNK108	183 ± 2.2 e	9.0 ± 0.8 ab
Flamink	183 ± 10.1 e	10.4 ± 1.4 ab
Line E	182 ± 6.6 e	14.7 ± 3.5 b

Table 2.2 (continued). Mean latent period and uredinium density on flag leaves of South African winter wheat cultivars and lines, adult-plant-resistant and susceptible check lines inoculated with isolate CRL 82-MGC-OQ of Puccinia recondita f.sp. tritici in the greenhouse

Cultivar or Line[1]	Latent period[2] (hr) ± standard deviation	Number[2] of uredinia/ cm^2 flag leaf surface ± standard deviation
Third maturity set		
Betta	262 ± 4.4 a	5.0 ± 2.1 a
RL6044 (Lr22a)	260 ± 9.7 a	8.7 ± 2.1 ab
T81/8	229 ± 5.1 b	3.6 ± 2.3 a
SNK104	217 ± 12.1 bc	4.7 ± 2.2 a
SWP2-29	216 ± 4.5 bc	9.1 ± 3.0 ab
CT263 (Lr13)	210 ± 5.8 c	5.6 ± 2.6 a
RL6011 (Lr12)	209 ± 9.9 c	6.6 ± 3.2 a
Belinda	199 ± 7.9 cd	6.7 ± 2.8 a
Line E	190 ± 8.6 d	14.2 ± 8.6 b
Scheepers 69	189 ± 11.5 d	8.3 ± 2.4 ab
Thatcher	183 ± 8.5 d	4.8 ± 3.1 a

[1] Cultivars and lines were inoculated in three sets when all plants in a set were at stage 13 on the Romig scale (15).

[2] Values followed by different letters within sets of inoculations and columns differ significantly at P=0.05 according to Tukey's procedure for comparison of means (100).

The susceptible class included Line E, Scheepers 69, Wilge and SNK108. Line E was the susceptible check in this study, and Scheepers 69, Wilge and SNK108 similarly showed negligible resistance. Wheat cultivars and lines in the second class were characterized by APR, although infection types of seedlings indicated little or no resistance (Table 2.1). Moreover, Belinda and Thatcher appeared susceptible as adult plants as well, but showed a significant reduction in the number of uredinia per cm^2 flag leaf surface. Studies by Modawi *et al.* (66) have indicated that fewer uredinia developed on Thatcher seedlings than on Wichita when inoculated with a specific culture of *P. recondita* f.sp. *tritici*, but not when infected with other cultures. However, Thatcher has consistently behaved as a fast rusting cultivar in field experiments where it was subjected to natural leaf rust epidemics (99). Gene *Lr22b* is associated with hypersensitive APR in Thatcher and is effective only to one leaf rust race (19). Therefore, from present evidence and other work (66), it appears that Thatcher might possess non-hypersensitive resistance to different races of *P. recondita* f.sp. *tritici*. Tests with a mixture of races would most probably mask the expression of genes in Thatcher. A similar, single-component APR was expressed as an increased latent period in line SWP2-29 despite the indication of susceptibility by other determinations (Tables 2.1 and 2.2).

No indication of seedling resistance was observed on RL6011 (Lr12), CT263 (Lr13), RL6044 (Lr22a) or SNK104, but components measured on flag leaves indicated effective APR in each (Table 2.1). All components indicated APR in RL6011, CT263 and SNK104, whereas APR expressed by RL6044 was not associated with a significant reduction in numbers of uredinia. Duration of latent period mediated by Lr22a was significantly longer than that of other APR lines or SNK104, and was comparable with latent period of entries exhibiting low infection types at all stages tested (Table 2.2). Additionally, uredinia on flag leaves of RL6044 were not associated with the same amount of necrosis and chlorosis as with uredinia on flag leaves or RL6011 and CT263.

APR of Flamink, T79/12 and T81/1 was evidenced only by restricted uredinial development (Table 2.1). The conflicting values for numbers of uredinia on T79/12 in repeated inoculations (Table 2.2) could possibly be ascribed to genetic heterogeneity. Genes for seedling resistance were indicated for several host genotypes described in the second category (Table 2.1) and although ineffective or moderately effective to isolate CRL 82-MGC-OQ, those genes could have conferred residual levels of resistance apparent in adult plants. The responsible factors remain to be elucidated. Corresponding differences in expression of partial resistance in wheat lines with defeated genes for resistance

to powdery mildew have been reported (67). Vanderplank (107) considered the levels of resistance that remain when vertical resistance is lost, to be of the horizontal type.

The third class of host plant response contained eight cultivars and lines (Table 2.1). These genotypes were resistant according to all measurements at both growth stages. As seedlings, Karee and T78/10 produced infection type 2+, but showed effective APR. According to seedling data from this study, they appeared to possess Lr24 in addition to other resistance factors. Line T80/13 was also classified as resistant according to its low seedling and flag leaf infection types, but latent period and number of uredinia calculated from the sporulating lesions did not differ from that of Line E. It seems that variation not only occurs for hypersensitive and non-hypersensitive host responses, but also within. Scheibe's rule, as discussed by Vanderplank (107), could be applied to Karee and T78/10 because moderate resistance in seedlings changed to highly effective APR. Contradictions to the rule were observed in the second class, where seedling susceptibility subsequently changed to APR.

Races virulent to the genes Lr12, Lr13 and Lr22b, which are associated with APR, exist (19, 25). In view of the numerous examples of differential interaction

reported for APR in cereal rust literature, Vanderplank (106) was of the opinion that APR is vertical and should not be confused with horizontal resistance. Therefore, exploitation of vertical genes with effects conditioned by ontogenic change should only be considered when other sources of resistance have been depleted (107).

The gradation of plants with resistance expressed as hypersensitive or non-hypersensitive responses in this study suggested that different components of resistance exist within APR. Such variability within rust resistance types seems common since Heath (39) concluded that each host : pathogen interaction is unique when studied histologically. However, the potentially valuable variation within the phenonenon of APR to wheat leaf rust would only be worth exploiting if the stability of different components to different races is established.

Coefficients of correlation ($\underline{r}$) between latent period and numbers of uredinia were -0.667, -0.707 and -0.644 for the three sets of experiments, respectively. The inverse relationship between these components was significant at $\underline{P}$=0.05 for each maturity set. The host genotypes tested in this study provided evidence that latent period and numbers of uredinia are not always under the same genetic control. A lack of correlation

between components of resistance to stripe rust, P. striiformis West., in barley, has also been found (69). However, Asher and Thomas (6) demonstrated a close relationship between latent period and colony density in barley infected with powdery mildew. They suggested a common genetic control for components of resistance in that specific host : pathogen association.

It is recommended that primary screening programs emphasizing APR, should use latent period and flag leaf infection type as selection criteria. Both components appear stable and are descriptive of the potential of the host plant to resist colonization by the leaf rust fungus and to retard ensuing disease progress.

CHARACTERIZATION OF ADULT PLANT RESISTANCE TO LEAF RUST OF WHEAT CONFERRED BY THE GENE *LR22A*

3.1 ABSTRACT

Inoculations of wheat line RL6044 at early stages in plant development established that the gene *Lr22a* could be identified by infection types produced on the third and fourth leaf of the same plant, but not on the newly emerged third leaf. With Cereal Rust Laboratory isolates of *Puccinia recondita* f.sp. *tritici*, the mean latent period determined on flag leaves of RL6044 was 40.0 hr greater than that of Line E, a susceptible line. Uredinium density was not reduced, but the mean uredinium size on flag leaves of RL6044 was about 70 % less than on Line E. On flag leaves of RL6044 inoculated with South African isolates the mean difference in latent period between RL6044 and Line E was 134 hr. Total urediniospore production until 19 days after inoculation was about 89 % lower on RL6044 than on the susceptible cultivar Morocco. The absence of an interaction between individual resistance components of *Lr22a* and different races of the wheat leaf rust pathogen suggests that this gene may be useful for managing epidemics of *P. recondita* f.sp. *tritici*.

3.2 INTRODUCTION

Species of the Gramineae related to bread wheat (Triticum aestivum L.) have been investigated as sources of stable and durable resistance to the rusts of wheat (96). There have been several successful transfers of genes for resistance to stem rust (Puccinia graminis Pers. f.sp. tritici Eriks. & E. Henn.) and leaf rust (P. recondita Rob. ex Desm. f.sp. tritici) from species related to wheat (11, 22, 49, 62, 96). Although some of these resistance genes have limited value in cultivar development because of their association with genes that produce undesirable characteristics (62), new resistance genes should continue to enhance our potential to manage the rust pathogens of wheat.

Kerber and Dyck (47) and Dyck and Kerber (21) reported the development of two synthetic hexaploid wheat lines (2n=42= AABBDD) each with a different leaf rust resistance gene derived from Aegilops squarrosa (2n=14= DD). In the first study, Tetra Canthatch (the extracted AABB tetraploid component of the common wheat cultivar Canthatch), was combined with A. squarrosa var. meyeri (accession number RL5289) and a gene for seedling resistance, Lr21, was transferred from the wild species (83). In the second study, Tetra Canthatch was crossed with A. squarrosa var. strangulata (accession number

RL5271) to produce another synthetic hexaploid line that possessed gene Lr22, a gene for adult plant resistance to leaf rust (83). After the development of a Thatcher backcross line, RL6044 (Thatcher*7// Canthatch/A. squarrosa var. strangulata RL5271), the gene Lr22 was designated Lr22a (19). Lr22a is at the same locus as Lr22b, another gene for adult plant resistance in Thatcher, but Lr22b was not retained in developing RL6044 (19).

Previous studies on Lr22a indicated that the gene produced an intermediate host response (21, 25). Pathogen races highly virulent to Lr22a may exist or may come into existence, but the fact that the gene does not completely suppress the pathogen suggests it may condition a stable type of resistance. Such stability could confer an increased level of durability (43).

The objectives of this study were to characterize the expression of resistance conditioned by gene Lr22a as influenced by plant growth stage and age of leaf tissue, and to determine the effect of different races of P. recondita f.sp. tritici on latent period, number of uredinia on leaves, uredinium size and urediniospore production.

3.3 MATERIALS AND METHODS

3.3.1 Effect of CRL isolates of P. recondita f.sp. tritici

Seed of RL6044 and a susceptible check line, Line E W3498 (Gabo*3/Charter//Little Club/Indian H.) were planted in methyl bromide-treated soil in 10-cm plastic pots. After emergence, plants were thinned to one per pot and kept in a greenhouse at 18-25 C. Daylight was supplemented for 12 hr per day with light from cool-white fluorescent lamps emitting 11 000 lux. A water-soluble fertilizer (23:19:17 NPK) was applied three times during the course of the experiment at a rate of 0.4 g/pot.

Plants were inoculated with an Andres inoculation device (2) 55 days after planting when flag leaves of main tillers were fully extended and heads had just emerged [Romig growth stage 12 (15)]. Extra tillers were removed the day before inoculation. Plants were inoculated with fresh urediniospores of P. recondita f.sp. tritici suspended in light mineral oil (Soltrol 170®, Phillips Chemical Company, Borger, Texas) at a concentration of 0.2 mg spores/ml of oil. Six isolates of the pathogen were studied; they possessed differential virulence to the following wheat leaf rust resistance genes: Lr1, Lr2a, Lr2c, Lr3a, Lr3ka, Lr9,

Lr10, Lr16, Lr17, Lr18 and Lr24. Avirulence/virulence characteristics of the isolates are given in Table 3.1.

The inoculation device was adjusted to maintain 160 mm between the discharge nozzle and the adaxial surface of the flag leaves. After inoculation, paper clips were attached to tips of the leaves to uniformly position their adaxial surfaces for even dew formation in a dew chamber. Plants were kept in the chamber for 19 hr in darkness at 22 C; during the last 3 hr leaves were allowed to dry off gradually. Plants were then transferred to a growth chamber programmed to maintain 21 C during the day and 19 C at night. A 14 hr daylength of 8 600 lux light intensity was provided by fluorescent lamps.

On each flag leaf, an area covering about two-thirds of the leaf and extending a minimum of 20 mm from the terminal and proximal end of the leaf, was marked with a water-resistant marker. These areas were inspected daily and when uredinia became visible, those within the marked area were counted at 08h00 and 20h00 each day until the logarithmic phase of uredinium eruption had passed. A final count of uredinia was made 24 hr later. Latent period was calculated by linear regression as the number of hours after inoculation when 40 % of the uredinia were visible (1).

Table 3.1. Virulence characteristics of Cereal Rust Laboratory (CRL) and South African (SA) isolates of *Puccinia recondita* f.sp. *tritici* used in experiments measuring the resistance components of the adult plant resistance gene *Lr22a*

Isolate	Avirulence/virulence combination[1/]
CRL 82-PHD-IV	Lr2a, 9, 16, 18, 24/1, 2c, 3a, 3ka, 10, 17
CRL 82-FLD-DZ	Lr1, 2a, 10, 16, 17, 18, 24/2c, 3a, 3ka, 9
CRL 82-CGB-OV	Lr1, 2a, 2c, 3ka, 9, 16, 17, 18, 24/3a, 10
CRL 82-TBL-MX	Lr3ka, 9, 10, 16, 17, 24/1, 2a, 2c, 3a, 18
CRL 82-MJB-MCV	Lr2a, 2c, 3ka, 9, 17, 18, 24/1, 3a, 10, 16
CRL 80-KGB-DJ	Lr1, 3ka, 9, 16, 17, 18, 24/2a, 2c, 3a, 10
3SA57	Lr1, 2a, 3ka, 11, 15, 17, 20, 24, 30/3a, 3ka, 16
3SA58	Lr3a, 3ka, 3bg, 11, 16, 20, 30/1, 2a, 15, 17, 24
3SA60	Lr2a, 3bg, 15, 16, 17/1, 3a, 3ka, 11, 20, 24, 30
3SA62	Lr3a, 3ka, 3bg, 11, 16, 20, 24, 30/1, 2a, 15, 17
3SA78	Lr3a, 3ka, 3bg, 11, 16, 17, 20, 24, 30/1, 2a, 15

[1/] Different differential sets were used to characterize CRL and SA isolates of wheat leaf rust.

Infection types (97) were recorded 15 days after inoculation. Flag leaves were then detached and the area of the portion of the leaf on which uredinia had been counted was determined with a leaf area meter (model LI-3000, Lambda Instruments Corporation, Lincoln, Nebraska). The number of uredinia per cm^2 of flag leaf surface was calculated. Twelve leaves, representing six replicates, were studied for each wheat line and leaf rust isolate combination in determining latent period and density of uredinia.

The size of uredinia was estimated by measuring two diameters of five randomly selected uredinia per leaf. For each wheat line : rust isolate combination, measurements were taken from images of four leaves that had been photographed at a known magnification. From these measurements the size of a uredinium was calculated using the formula, area = π AB/4, where A = length and B = width.

3.3.2 Effect of plant age on resistance due to Lr22a

Seed of RL6044 were planted in soil in 10-cm plastic pots and grown (four plants/pot) in a greenhouse at 19-23 C. Daylight was supplemented with light from cool - white fluorescent lamps at 9 000 lux for 12 hr each day. Three consecutive plantings were made at weekly intervals. Each planting consisted of three

replicates. Plants were inoculated on the same day, when they were 16, 23 and 30 days old. Plants 30 days old displayed a well developed fourth leaf and third and second leaves. Plants 23 days old had their third, second and first leaves. Those 16 days old had only their second and first leaves.

Plants were inoculated with a South African isolate of P. recondita f.sp.tritici (isolate 3SA78, Table 3.1). Fresh urediniospores (0.5 mg/ml) were suspended in light mineral oil (Soltrol 130®) and atomized onto leaves according to procedures outlined by Browder (10). Plants were placed in a dew chamber in darkness at 19 C for 19 hr and during the last 3 hr they were allowed to dry off. They were then transferred to a greenhouse set at 19-23 C with 12 hr supplemental illumination of 9 000 lux daily. Twelve days after plants were placed in the greenhouse infection types were recorded on the second, third and fourth leaves of 30-day-old plants; on the third, second and first leaves of 23-day-old plants and on the second and first leaves of 16-day-old plants.

3.3.3 Effect of South African isolates of P. recondita f.sp. tritici

Plants of RL6044, Line E and the leaf-rust-susceptible wheat cultivar Morocco were grown in soil in 15-cm pots

in a plastic greenhouse in which temperatures ranged from 2 to 30 C. Three weeks after planting and weekly thereafter for the duration of the experiment, a water-soluble fertilizer (6.5:2.7:13.0 NPK) was applied as a soil drench at a rate of 0.5 g/pot.

Flag leaves of RL6044 and Line E that were of similar age and appearance were inoculated with the Andres device 104 days after planting when plants were at growth stage 15 based on the Romig scale (15). Four South African isolates of P. recondita f.sp. tritici (isolates 3SA57, 3SA58, 3SA60 and 3SA62) were selected for their differential virulence to the following wheat leaf rust resistance genes: Lr1, Lr2a, Lr3a, Lr3ka, Lr3bg, Lr11, Lr15, Lr16, Lr17, Lr20, Lr24 and Lr30 (Table 3.1).

Each isolate was sprayed onto four leaves each of RL6044 and Line E at a concentration of 0.2 mg urediniospores/ml of Soltrol 130® oil. Twenty additional flag leaves each of RL6044 and Morocco were similarly inoculated with isolate 3SA62. Inoculated plants were incubated in a dew chamber at 19 C for 19 hr, during the last 3 hr of which they were allowed to dry off. They were then placed in a greenhouse at 19-23 C with daylight supplemented with 9 000 lux from fluorescent lamps for 12 hr each day.

Leaves were inspected daily at 08h00 and uredinia within designated areas were counted. The latent period for RL6044 and Line E infected with the different isolates was calculated as described in the first experiment (section 3.3.1). Infection types were recorded 14 days after plants were inoculated.

Urediniospores were collected from upper leaf surfaces of Morocco and RL6044 infected with isolate 3SA62 using a laboratory pump with fixed vacuum capacity. A cyclone collector (10) to which gelatine capsules of known weight were attached, was connected to the vacuum pump. The capsules were left open for one hr after collection to air-dry the spores and then the capsules and spores were weighed. Collection from Morocco was initiated seven days after inoculation and continued until secondary sporulating uredinia formed (19 days after inoculation). Collection from RL6044 was on days 13, 16 and 19 after inoculation because sporulation was slight. Primary uredinia were counted and urediniospore yield was expressed as mg spores produced per uredinium for each collection. The mass of spores collected from 20 leaves represented 10 replicates in data analysis.

Experiments 3.3.1 and 3.3.3 were arranged as randomized complete block designs and the data were analysed for variance. Tukey's procedure for comparison of means

was applied where analysis of variance indicated significant differences (100).

3.4 RESULTS

3.4.1 Effect of CRL isolates of P. recondita f.sp. tritici

Mean latent periods differed because of the different genotypes of the wheat lines, the pathogen isolates and the interaction of the two (Table 3.2). The mean latent period of RL6044 was significantly longer (40.0 hr) than that of Line E. Mean latent period of pathogen isolate CRL 82-PHD-IV was less than for isolates CRL 82-TBL-MX and CRL 82-CGB-OV, whereas that for other isolates was intermediate between these two extremes. The interaction of pathogen isolates and wheat genotypes was statistically significant ($P=0.05$) probably because of shorter mean latent periods of isolates CRL 82-CGB-OV and CRL 82-TBL-MX on Line E, without a corresponding reduction on RL6044.

Mean number of uredinia on flag leaves (Table 3.3) varied because of pathogen isolates ($P=0.05$) and the interaction of wheat lines with isolates ($P=0.05$) but not because of the wheat lines. Isolate means indicated that CRL 82-PHD-IV produced significantly fewer uredinia/cm^2 leaf surface than isolate CRL 82-TBL-MX,

Table 3.2. Mean latent period (hr) measured on the flag leaves of RL6044 (Lr22a) and Line E (susceptible check) inoculated at Romig growth stage 12 with six Cereal Rust Laboratory (CRL) isolates of Puccinia recondita f.sp. tritici

	Latent period (hr)[1/]		
CRL isolate	RL6044	Line E	Isolate Mean
82-PHD-IV	192 a	149 b	171 X
82-FLD-DZ	181 a	145 bcd	163 XY
82-CGB-OV	181 a	135 cd	158 Y
82-TBL-MX	181 a	134 d	158 Y
82-MJB-MCV	181 a	148 bc	165 XY
80-KGB-KJ	181 a	146 bcd	164 XY
Line mean	183 A	143 B	

[1/] Tukey's procedure (100) was applied to compare differences for line x isolate ($P=0.05$), isolate ($P=0.05$) and line ($P=0.01$) means. Values, within each set of means, followed by different letters differ significantly from each other.

Table 3.3. Mean number of uredinia per cm^2 leaf surface on flag leaves of RL6044 (Lr22a) and Line E (susceptible check) inoculated at Romig growth stage 12 with six Cereal Rust Laboratory (CRL) isolates of Puccinia recondita f.sp. tritici

	Uredinia/cm^2 flag leaf surface[1/]		
CRL isolate	RL6044	Line E	Isolate mean
82-PHD-IV	9.8 b	10.9 ab	10.4 X
82-FLD-DZ	14.1 ab	10.6 ab	12.4 XY
82-CGB-OV	11.2 ab	12.5 ab	11.9 XY
82-TBL-MX	14.9 ab	13.3 ab	14.1 X
82-MJB-MCV	11.6 ab	15.4 a	13.5 XY
80-KGB-KJ	10.5 ab	11.6 ab	11.1 XY
Line mean	12.0 A	12.4 A	

[1/] Tukey's procedure (100) was applied to compare differences for line x isolate ($P=0.05$), isolate ($P=0.05$) and line ($P=0.01$) means. Values, within each set of means, followed by different letters differ significantly from each other.

whereas other isolate means were intermediate between these two. The interaction was significant probably because the differences in uredinia/cm^2 between RL6044 and Line E for isolates CRL 82-FLD-DZ and CRL 82-MJB-MCV were larger than for the other isolates.

The mean size of uredinia (Table 3.4) produced by the six CRL isolates on RL6044 and Line E differed significantly only because of the wheat lines (P=0.01). Uredinia on RL6044 were 70.7 % smaller than those on Line E. Isolate means indicated that the isolates produced uredinia of about equal size on the same line.

3.4.2 Effect of plant age on resistance due to Lr22a

Infection types produced on leaves of line RL6044, when plants were of different ages, are shown in Table 3.5. Resistance to *P. recondita* f.sp. *tritici* due to gene *Lr22a* was evident on the fourth leaf of plants that were 30 days old at inoculation as well as on the older leaves of these 30-day-old plants. Resistance was not obvious on plants that were 23 or 16 days old at inoculation, but there was some indication of the resistance gene on second and third leaves of 23-day-old plants. Resistance was not detectable in 16-day-old plants or on the first leaf of plants 23 days old.

Throughout all other experiments with CRL and SA

Table 3.4. Mean size (mm^2) of uredinia on flag leaves of RL6044 (Lr22a) and Line E (susceptible check) inoculated at Romig growth stage 12 with six Cereal Rust Laboratory (CRL) isolates of Puccinia recondita f.sp. tritici

	Uredinium size (mm^2)[1]		
CRL isolate	RL6044	Line E	Isolate mean
82-PHD-IV	0.116	0.440	0.278
82-FLD-DZ	0.141	0.391	0.266
82-CGB-OV	0.132	0.547	0.340
82-TBL-MX	0.151	0.409	0.280
82-MJB-MCV	0.131	0.484	0.308
80-KGB-KJ	0.150	0.530	0.340
Line mean	0.137 A	0.467 B	

[1] Tukey's procedure (100) was applied to compare differences for line x isolate (P=0.05), isolate (P=0.05) and line (P=0.01) means. Values, within each set of means, followed by different letters differ significantly from each other. Analysis of variance indicated that the interaction between isolates and lines was not significant at P=0.05.

Table 3.5. Effect of plant age on infection types[a] on different leaves of wheat line RL6044 infected with Puccinia recondita f.sp. tritici[b]

Age of plants at inoculation (days)	Infection type/leaf			
	First	Second	Third	Fourth
16[c]	4	4	-	-
23[d]	4	3+	3	-
30[e]	-	2+3	2+	1+

[a] Infection types according to a 0-4 scale (97).

[b] Isolate 3SA78.

[c] Third and fourth leaves not yet developed at inoculation.

[d] Fourth leaf not yet developed at inoculation.

[e] First leaf became chlorotic before infection types could be determined.

isolates of the pathogen, RL6044 exhibited a 1+ infection type on flag leaves. This infection type was characterized by small uredinia associated with a slight amount of chlorosis.

3.4.3 Effect of South African isolates of P. recondita f.sp. tritici

Mean latent period was 134 hr longer in RL6044 than in Line E (Table 3.6). The length of the latent periods did not differ significantly with the pathogen isolates nor the interaction between isolates and lines.

Urediniospore production on RL6044 and Morocco was estimated by collecting spores at different time intervals after inoculation (Fig. 3.1). Urediniospores were collected from a mean of 162 ± 31 uredinia per flag leaf of RL6044 and 149 ± 24 uredinia per flag leaf of Morocco. Spore yield on RL6044 began 13 days after inoculation whereas that on Morocco began after 7 days. Nineteen days after inoculation Morocco had yielded a mean of 0.0269 mg spores per uredinium and RL6044 0.0028 mg spores per uredinium.

DISCUSSION

According to Dyck and Samborski (25), gene Lr22a had, in 1979, not yet been incorporated into a commercial

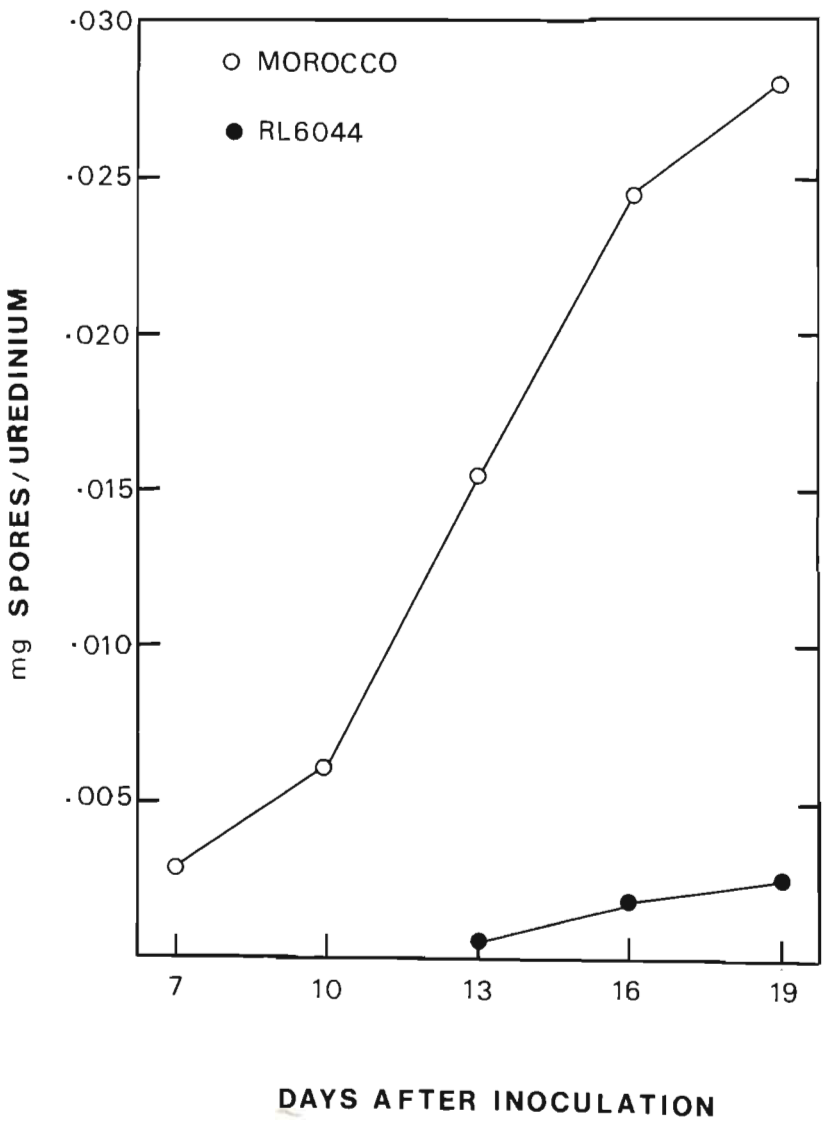
Table 3.6. Duration of latent period (hr) measured on flag leaves of RL6044 (Lr22a) and Line E (susceptible check) inoculated at Romig growth stage 15 with four South African isolates of Puccinia recondita f.sp. tritici

	Latent period (hr)[1]		
SA isolate	RL6044	Line E	Isolate mean[1]
3SA57	299	147	223
3SA58	304	167	236
3SA60	298	174	236
3SA62	280	155	218
Line mean[2]	295 A	161 B	

[1] Isolate x line and isolate means did not differ significantly according to analysis of variance.

[2] Line means were significantly different at P=0.01 according to Tukey's procedure (100).

Figure 3.1. Cumulative urediniospore yield rate (mg/uredinium on wheats RL6044 (Lr22a) and Morocco (susceptible check) seven to 19 days after inoculation with isolate 3SA62 of Puccinia recondita f.sp. tritici. Tukey's procedure (100) indicated significant differences at P=0.01 between total spore yield of RL6044 and Morocco 19 days after inoculation.


·030
·025
·020
·015
·010
·005
mg SPORES/UREDINIUM
○ MOROCCO
● RL6044
7
10
13
16
19
DAYS AFTER INOCULATION

wheat cultivar. As far as the present author is aware, this situation remains unchanged today. Characterization of the resistance components in the present study suggests that the gene has great potential as a source of resistance to leaf rust of wheat, particularly because it is effective against different races of the pathogen. The partially dominant mode of inheritance of Lr22a (21), and the distinctive infection type, provide evidence that the gene could easily be incorporated into adapted wheat genotypes. If the gene continues to condition slow rusting, its potential usefulness will be considerable, especially because advances in selection of slow rusting progeny are likely to be more rapid with a monogenically than with a quantitatively inherited trait. It is probable that the high density of uredinia on leaves of line RL6044 has tended to mask the potential effectiveness of the gene during routine screening in the past.

Because Lr22a is a single gene it is possible that a virulent race of P. recondita f.sp. tritici could evolve. However, the expression of this gene suggests that selection pressure to produce such a race would not be unduly strong. Unfortunately, because of apparently limited exploitation of Lr22a, evidence for neither its durability, nor for the amount of protection it can provide in the field, is available. Greenhouse evaluation of the gene in the present study,

suggests it would be valuable in breeding programs.

Epidemiologically, Lr22a may be useful in reducing the rate of rust development because it conditions a long latent period, smaller uredinia, and reduced spore production. Despite the fact that Lr22a is associated with high numbers of uredinia, the other components would contribute to retard progress of epidemics by restricting the logarithmic increase of inoculum and disease.

Other studies have indicated that slow rusting in wheat or barley genotypes is associated with low numbers of uredinia produced on leaf surfaces (44, 72, 94, 95). This was not the case in this study, clearly indicating that the relationship between resistance components in a particular host : pathogen interaction should be established before utilizing one component for screening purposes. In the present study, latent period appeared to be a useful stable component, irrespective of differences in aggressiveness in different races. The effectiveness of resistance, as expressed in duration of latent period, appears to be enhanced in plants as they grow older. More information is needed to quantify this effect, however.

The fact that Lr22a confers resistance only in post-seedling growth stages, classifies this gene as an

adult-plant-resistance gene. Other genes, such as Lr12 and Lr13, also mediate adult plant resistance to wheat leaf rust, but their associated low infection types indicate that hypersensitivity is involved and differential interaction with races has been reported (25). Another gene for adult plant resistance in the wheat line PI 250413 was considered to condition horizontal resistance, since it was resistant to all races tested and the presence of uredinia indicated that resistance was incomplete (25). From the latter study and the work presented here it is clear that resistance mechanisms and their expression are often unique, and no generalizations with regard to resistance categories may be made without detailed studies of the interactions involved. In the adult-plant-resistance category, similarities in expression of resistance exist but the phenotypic characterization and stability of resistance components to different races of the pathogen undoubtedly vary in wheat genotypes.

When gene Lr22a was originally transferred from the diploid donor parent (A. squarrosa line RL5271) to the synthetic hexaploid (line RL5404), the level of resistance was reduced somewhat (21). The infection type in adult plants of line RL5271 is 0; whereas in line RL5404 it is 1+. According to Dyck and Kerber (21), the gene is slightly less effective in the synthetic hexaploid line because it is sensitive to its genetic

background. Consequently, the exploitation of Lr22a in a breeding program may require a careful evaluation of progenies to select those with superior degrees of resistance.

CHAPTER 4

INHERITANCE AND EXPRESSION OF RESISTANCE CONFERRED BY GENE LR22A TO LEAF RUST IN TWO SPRING WHEAT CROSSES

4.1 ABSTRACT

The spring wheat cultivars Zaragoza and SST33 were crossed with line RL6044, a Thatcher backcross line with gene Lr22a for adult plant resistance to Puccinia recondita f.sp. tritici. Evaluation of the F_1, F_2 and F_3 generations from both crosses indicated that Lr22a was inherited as a partially recessive, single gene. This finding differs from previous genetic studies in which the gene acted partially dominantly. Quantitative assessment of latent period and uredinium numbers in randomly selected F_4 families homozygous for gene Lr22a, indicated significantly different levels of resistance. Although infection types denoted the presence of Lr22a, certain F_4 families exhibited a latent period statistically equal to that of the susceptible check. Other F_4 families showed resistance superior to that of RL6044. Since within and between family variation for infection type commonly occurred and genetic differences between families were implied by the genotypic variances for latent period and number of uredinia, several genes modifying the expression of Lr22a appeared to be present. Evaluation of selected F_5 families from both crosses indicated that genotypes with levels of adult plant resistance superior to those of

the parents could be developed. Enhanced levels of resistance were not caused by the combination of Lr22a with other known, but ineffective genes for hypersensitive seedling resistance in Zaragoza or SST33 or by the growth rate of plants in families segregating for maturity.

4.2 INTRODUCTION

The gene Lr22a confers adult plant resistance (APR) in wheat (Triticum aestivum L.) against leaf rust [Puccinia recondita Rob. ex Desm. f.sp. tritici (19)]. Greenhouse studies on components of this resistance (Chapter 3) indicated that Lr22a mediates a slow rusting type of resistance that is characterized by a long latent period, small uredinia and reduced sporulation. Evidence that the resistance conditioned by Lr22a is stable to different races of the leaf rust pathogen has also been presented (19, see Chapter 3 in this thesis).

Dyck and Kerber (21) determined that Lr22a is inherited in a partially dominant mode; thus suggesting that the gene could successfully be manipulated in breeding programs. Although races virulent to Lr22a may evolve, or may already exist, the combination of Lr22a with other genes for resistance may enhance the potential usefulness of this resistance gene.

Selection for APR conferred by Lr22a in breeding programs should be conducted carefully. Dyck and Kerber (21) found that the resistance of Lr22a decreased when it was transferred from the diploid parent Aegilops squarrosa var. strangulata to a synthetic hexaploid wheat, appearing sensitive to its genetic background.

Resistance conferred by the wheat stem rust resistance gene Sr22 also decreased when the gene was first transferred from T. monococcum (2n = 14) to T. durum (2n = 28) and then into T. aestivum [(2n = 42) 48]. It is clear that genetic background and environment may have significant effects on the expression of resistance (61). Such non-allelic gene interactions associated with resistance of wheat to rust diseases have often been reported (21, 27, 36, 40, 52, 56, 63, 78). These studies generally attributed the gradation in levels of resistance to modifying genes.

Knott and Green (51) observed that the number of backcrosses of wheat lines that carry stem rust (P. graminis Pers. f.sp. tritici Eriks. & E. Henn.) resistance genes, to a recurrent parent, influenced the expression of Sr genes. Furthermore, Dyck and Samborski (24) found that alleles of Lr2 were more effective in a Thatcher wheat background than in Red Bobs.

However, resistance levels do not necessarily de-

crease when genes are transferred to other backgrounds. Dvorak (18) transferred leaf rust resistance from five accessions of A. speltoides to wheat and reported that resistance was diluted only in lines derived from one hybrid. Similarly, the levels of APR conferred by a certain gene transferred from wheat line PI 250413, could be recovered in offspring derived from the resistant parent (25).

In the present study, line RL6044 (Lr22a) was crossed with two South African spring wheat cultivars. The objectives were to study the mode of inheritance, the variation in the expression of resistance due to apparent background effects, and the feasibility of developing breeding lines with superior levels of resistance conditioned by Lr22a.

4.3 MATERIALS AND METHODS

4.3.1 Inheritance of Lr22a

Wheat line RL6044 (Thatcher*7//Tetra Canthatch/Aegilops squarrosa var. strangulata) was used as the male parent in crosses with the spring wheat cultivars Zaragoza (Mengavi/8156) and SST33 (SST3*/3/Flameks*3/Langdon 398*2//Langdon 357/Stewart 464). Both cultivars carry genes for seedling resistance to P. recondita f.sp. tritici, but are susceptible to one or more South Afri-

can races. Inheritance of gene Lr22a was investigated in the F_1, F_2 and F_3 generations of both crosses and in the F_2 of the first backcross to SST33.

Three to eight plants of the different generations were grown per pot containing 5 kg of soil in a greenhouse at 19-25 C. A 14 hr photoperiod of 14 500 lux was provided daily by 250 W high-pressure mercury tungsten lamps. Three weeks after planting and at weekly intervals thereafter for the duration of the experiment, a water-soluble fertilizer (6.5:2.7:13.0 NPK) was applied as a soil drench at a rate of 0.5 g/pot.

Progenies of the cross between Zaragoza and RL6044 were inoculated with isolate 3SA57 of P. recondita f.sp. tritici and progenies of the cross between SST33 and RL6044 were inoculated with isolate 3SA86. Parental genotypes were included with each inoculation. Each population was inoculated according to the method of Browder (9) when plants were between 40 and 61 days old. Most plants were at growth stage 13 (15) at the time of inoculation, but some plants were at late-tillering, boot or heading stages of growth. Freshly collected urediniospores were suspended in Soltrol 130® light mineral oil (Phillips Chemical Company, Borger, Texas) at 0.5 mg spores/ml and atomized onto plants. Plants were kept at room temperature for one to 3 hr after inoculation to allow oil to evaporate from plant

surfaces. They were then placed in a dark dew chamber at 19 C for 19 hr. Plants were returned to the greenhouse when moisture on leaves had gradually dried off.

When populations were too large to be accommodated in the dew chambers during an incubation cycle, inoculations were carried out on consecutive days. Inoculated plants were maintained in a greenhouse at 19 -25 C with a 14 hr photoperiod of 14 500 lux before they were evaluated for segregation of Lr22a, 13 to 16 days after inoculation.

In the F_1 generation, 12 plants of the Zaragoza x RL6044 cross and 33 plants of the SST33 x RL6044 cross were evaluated. In the F_2 generation, segregation ratios were determined among 536 plants of the Zaragoza x RL6044 cross and among 486 plants of the SST33 x RL6044 cross. Inheritance of Lr22a in the F_2 of the first backcross to SST33 was assessed on 377 plants. Sixty F_1-derived single-seed descent F_3 families, consisting of 12 to 27 plants per family, were evaluated for the Zaragoza cross and 48 F_3 families, consisting of 10 to 22 plants per family, were evaluated for the SST33 cross. Progeny tests of 16 resistant and 16 susceptible F_2 plants from each cross were also conducted on 10 to 19 plants per F_3 family.

4.3.2 Expression of Lr22a in F_4 families

From the progeny tests conducted in the F_3 generation, plants with APR of both crosses were grown to maturity and harvested. Twenty of these F_4 families from the Zaragoza x RL6044 cross and 18 F_4 families from the SST33 x RL6044 cross were randomly selected and five plants per family were grown in soil in 5-kg pots. Plants were grown in a greenhouse with a 12 hr day/night temperature regime of 20-22/12-14 C, respectively. Fertilization of plants was as described in the inheritance study. Thirty-nine days after planting, foliage was trimmed and the fourth leaf of each of five plants per family was exposed. These leaves were inoculated three days later with the aid of the Andres inoculation device (2). Plants from the Zaragoza x RL6044 and SST33 x RL6044 crosses as well as five plants of each of the parental genotypes at similar growth stage, were inoculated with isolates 3SA57 and 3SA86 of *P. recondita* f.sp. *tritici*, respectively. During inoculation, leaves were taped 160 mm from the inoculator nozzle, which was set to rise 330 mm/sec. During the upward movement of the nozzle, a suspension of 0.2 mg fresh urediniospores per ml Soltrol 130® oil was sprayed onto the adaxial surface of a leaf. Air pressure regulating the discharge of inoculum was kept at 31.05 Kpa. The carrier oil on leaves was allowed to evaporate for 3 hr before plants were placed in a dark

dew chamber at 19 C for 19 hr; during the last three hours in the chamber plants were allowed to dry off. They were then placed in a greenhouse at 18-20 C where daylight was supplemented with 9 000 lux provided by cool-white fluorescent tubes for 12 hr each day.

On each inoculated leaf, a 50 mm long portion approximately in the middle of the leaf, was marked with a water-resistant marker. These areas were inspected daily and when uredinia became visible, those within the marked area were counted. Counting continued until no more primary uredinia appeared. The latent period was calculated by linear regression for each plant as the number of hours after inoculation when 40 % of the uredinia were visible as erumpent structures (1).

Infection types were determined 15 days after inoculation on a 0 to 4 scale (97). Leaves were then detached and the area of the portion of the leaf on which uredinia had been counted was determined with a leaf area meter (model LI-3100, Lambda Intruments Corporation, Lincoln, Nebraska). The number of uredinia per cm^2 leaf surface was calculated.

Twenty days after the inoculation described above, regrowth of plants, of families that did not exhibit within - family variation for Lr22a infection type, was reinoculated according to the method of Browder (9).

Families of the Zaragoza cross were inoculated with isolate 3SA86 and families of the SST33 cross with isolate 3SA57. These isolates are avirulent to Zaragoza and SST33, respectively, and inoculations were conducted to determine in which lines genes for resistance to P. recondita f.sp. tritici, other than Lr22a, had been retained. F_2 populations of both crosses were also tested in the seedling stage with isolates 3SA86 and 3SA57 to determine the mode of inheritance of the genes for resistance in Zaragoza and SST33. The number of days to heading was recorded for each F_4 plant to assess the relationship between growth period and expression of APR; the fourth leaves of early-maturing plants would have been physiologically older at the time of initial inoculation than the fourth leaves of late-maturing plants.

A second experiment was carried out as described above with different sets of adult-plant-resistant F_4 families derived from the same F_3 population. Latent period, number of uredinia, infection types and number of days to heading were determined as before. In addition, inoculations were made to indicate the presence of ineffective genes derived from the susceptible parents. The fifth leaf on each of five plants of 11 F_4 families per cross were inoculated 45 days after planting. The leaf-rust-susceptible wheat, Morocco, was inoculated along with the parental wheats. Inocu-

lum was urediniospores of isolates 3SA57 and 3SA86 retrieved from liquid nitrogen and heat-shocked at 45 C for 6 min prior to inoculation. Percentage germination of urediniospores of each isolate was determined after 3.5 hr and 6.5 hr by placing 1 ml of the spore-oil suspension onto water-agar medium and incubating in darkness at 21 C. Two random samples of 100 spores per isolate were microscopically examined and a spore was considered germinated when the length of the germ tube was twice spore width.

4.3.3 Expression of Lr22a in F_5 families

Eight seeds harvested from four F_4 families per cross that exhibited different levels of resistance according to latent period, were planted along with the parents in soil in 5-kg pots. Four plants, each from a different family, were grown randomly per pot in a greenhouse at 19-25 C and the fifth leaf of each plant was inoculated 46 days after planting. Plants were inoculated with fresh urediniospores, incubated and assessed for latent period, number of uredinia and infection type as described for experiments with F_4 families.

4.3.4 Data analysis

In the inheritance study, the probability by which the observed ratios approximated expected ratios, was cal-

culated according to Chi-square values (100). Latent period and number of uredinia data from the F_4 and F_5 families were analysed for variance according to a completely randomized design. F_4 data were analysed per experiment per cross and two analyses were carried out on data sets. In the first analysis, data from all F_4 families of a cross within an experiment, but not from the checks, were included. From these one-way analyses of variance, the ratio of the genotypic variance to the total variance was calculated if the variation between families was significant at $\underline{P} = 0.05$. In the second analysis of variance, families within crosses and experiments that did not exhibit variation among plants for Lr22a infection types were analysed together with the parental checks. The square root transformation (100) was applied to the numbers of uredinia in all experiments. Tukey's procedure (100) was also applied to means for latent period and number of uredinia to reveal statistical differences ($\underline{P} = 0.05$) between families and checks when analysis of variance had indicated significant variation.

4.4 RESULTS

Phenotypic expression of the high and low reactions of seedling and adult plants of Zaragoza, SST33, RL6044 and Thatcher wheats to isolates 3SA57 and 3SA86 are shown in Table 4.1. The avirulence/virulence formulae

of isolates are also presented in Table 4.1.

4.4.1 Inheritance of Lr22a

Segregation ratios for the gene Lr22a are given in Table 4.2 and ratios for the seedling genes in Zaragoza and SST33 are given in Table 4.3. Evaluation of F_1 plants indicated that Lr22a behaved recessively in both crosses. This was confirmed by the 1:3 ratio of resistant to susceptible plants obtained in the F_2 and backcross F_2 generations. In both crosses, infection types produced by plants that carried Lr22a ranged from a ;1 to 2++. Y-reactions (13), with larger uredinia occurring more abundantly towards the apex than towards the base of a leaf, were commonly observed among resistant F_2 plants of the Zaragoza x RL6044 cross.

Tests with progenies of resistant F_2 plants showed that all plants were homozygous for Lr22a. In tests with progenies of susceptible F_2 plants, they segregated into resistant and susceptible classes, but among plants from the cross SST33 x RL6044 the expected 1:5 ratio was not significant (Table 4.2). In tests with F_3 populations advanced by single-seed descent without selection in the F_2, the F_3 families segregated into homozygous resistant, segregating and homozygous susceptible classes (Table 4.2). Monogenic inheritance of resistance was confirmed by significant 1:2:1 ratios in

Table 4.1. Infection types of parental cultivars and lines to isolates 3SA57 and 3SA86 of Puccinia recondita f.sp. tritici

Cultivar or Line	Isolate	
	3SA57[a/]	3SA86[b/]
Primary leaf		
SST33	xcn	4
Zaragoza	4	0;
RL6044	4	4
Flag leaf		
SST33	2cn	4
Zaragoza	4	;
RL6044 (Lr22a)	1+	1+
Thatcher	4	4

a/ Avirulence/virulence combination: Lr1, 2a, 2b, 3ka, 9, 10, 11, 15, 17, 20, 21, 24, 29, 30/2c, 3a, 3bg, 14a, 14b, 16, 23, 25, 27.

b/ Avirulence/virulence combination: Lr3a, 3bg, 3ka, 9, 11, 16, 21, 29, 30/1, 2a, 2b, 2c, 10, 14a, 14b, 15, 17, 23, 24, 25, 27.

Table 4.2. Segregation ratios for the wheat leaf rust resistance gene Lr22a in post-seedling plants in the F_1, F_2, BC_1F_2 and F_3 generations of the crosses Zaragoza x RL6044 and SST33 x RL6044

Generation	Cross [a]	Number of plants or families			Total no. of plants	Expected ratio	χ^2	P
		Res.	Segr.	Susc.				
F_1	Zaragoza x RL6044	0		12	12			
	SST33 x RL6044	0		33	33			
F_2	Zaragoza x RL6044	121		415	536	1:3	1.682	0.25-0.10
	SST33 x RL6044	129		357	486	1:3	0.617	0.50-0.25
BC_1F_2	SST33 x RL6044	106		271	377	1:3	1.953	0.25-0.10
F_3 (progeny tests)	Zaragoza x RL6044							
	Susc. F_2	39		218	257	1:5	0.411	0.75-0.50
	Res. F_2	259		0	259	1:0		
	SST33 x RL6044							
	Susc. F_2	27		232	259	1:5	7.264	<0.01
	Res. F_2	258		0	258	1:0		
F_3 (SSD) [b]	Zaragoza x RL6044	13	33	14	1305	1:2:1	0.633	0.75-0.5
	SST33 x RL6044	11	28	9	856	1:2:1	1.5	0.50-0.25

[a] Zaragoza x RL6044 progenies were inoculated with isolate 3SA57 and SST33 x RL6044 progenies with isolate 3SA86.

[b] SSD = single-seed descent F_3 families.

Table 4.3. Segregation ratios for genes for seedling resistance to Puccinia recondita f.sp. tritici in the F_2 generation of crosses involving the spring wheat cultivars Zaragoza and SST33

Cross	Isolate	Number of Plants		Expected ratio	χ^2	P
		Resistant	Susceptible			
Zaragoza x RL6044	3SA86	393	72	3:1	22.458	<0.001
SST33 x RL6044	3SA57	348	158	3:1	10.459	<0.001

both crosses. Variation in infection types, similar to those observed among F_2 plants, was apparent between and within F_3 families.

Assuming that Lr22a is not expressed in primary leaves (see Chapter 3), evaluation of F_2 seedling plants from both crosses indicated that Zaragoza and SST33 each possess a dominant gene for hypersensitive resistance to P. recondita f.sp. tritici (Table 4.3). However, the observed segregation ratios deviated from significant 3:1 fits. Resistant F_2 seedlings from the cross Zaragoza x RL6044 produced a range of infection types from ; to x . These infection types were higher than the 0; reaction of Zaragoza (Table 4.1). Resistant F_2 seedlings from the cross SST33 x RL6044 also showed a range of infection types from ;1cn to xcn. In the seedling stage SST33 produced mesothetic reactions associated with chlorosis and necrosis to isolate 3SA57 and was susceptible to isolate 3SA86 whereas line RL6044 was susceptible (infection type 4) to both isolates (Table 4.1).

4.4.2 Expression of Lr22a in F_4 families

Analyses of variance for latent period and number of uredinia, without selection or parental genotypes, are presented in Table 4.4. Between family variation was significant at P = 0.01 in all experiments except for

number of uredinia in the SST33 x RL6044 cross in the second experiment. The genotypic variance in the two experiments for latent period was, respectively, 53.4 % and 56.1 % for the Zaragoza cross and respectively 46.9 % and 39.5 % for the SST33 cross (Table 4.5). For number of uredinia, the genotypic variance in the two experiments were 38.6 % and 33.2 % for the Zaragoza cross and 29.0% for the SST33 cross in the first experiment (Table 4.5).

Tables 4.6 and 4.7 show the mean latent period, number of uredinia and infection types of those families that did not produce variable reactions. The number of days to heading as well as the presence of genes for seedling resistance are also indicated. In the cross between Zaragoza and RL6044, means of the first experiment indicated that latent period varied from 170 to 233 hr, despite all plants being homozygous for Lr22a (Table 4.6). Latent period of line RL6044 and Zaragoza were 201 and 150 hr, respectively. Number of uredinia varied from 1.7 to 3.1 per cm^2 leaf surface. Infection types ranged from ;1 to 2. Seven families were homozygous for the resistance gene from Zaragoza in addition to Lr22a. The resistance gene in Zaragoza presumably is Lr3a derived from Mengavi (79), one of the parents of Zaragoza. The possibility of Lr3a being present in the experimental materials was indicated by a 0; to ; adult plant infection type to isolate 3SA86. Plants

Table 4.4. Analysis of variance for latent period and number of uredinia measured in two experiments with F_4 families homozygous for gene Lr22a for resistance to Puccinia recondita f.sp. tritici in two crosses

Resistance component	Source of variation	Cross					
		Zaragoza x RL6044			SST33 x RL6044		
		DF	MS	F	DF	MS	F
FIRST EXPERIMENT							
Latent period							
	Between families	19	1459.28	6.718**	14	1211.429	5.409**
	Within families	80	217.205		60	223.948	
Number of uredinia							
	Between families	19	0.813	4.148**	14	0.624	3.04**
	Within families	80	0.196		60	0.205	
SECOND EXPERIMENT							
Latent period							
	Between families	10	1011.501	7.399**	10	773.156	4.268**
	Within families	44	136.711		44	181.165	

Table 4.4 (continued). Analysis of variance for latent period and number of uredinia measured in two experiments with F_4 families homozygous for gene Lr22a for resistance to Puccinia recondita f.sp. tritici in two crosses

Resistance component	Source of variation	Cross					
		Zaragoza x RL6044			SST33 x RL6044		
		DF	MS	F	DF	MS	F
Number of uredinia							
	Between families	10	0.362	3.481**	10	0.103	0.80
	Within families	44	0.104		44	0.128	

** = Significant at P=0.01.

Table 4.5. Percentage genotypic variance for latent period and number of uredinia measured in two experiments with F_4 families from two crosses homozygous for gene Lr22a for resistance to Puccinia recondita f.sp. tritici

Resistance component	Cross	
	Zaragoza x RL6044	SST33 x RL6044
First experiment		
Latent period	53.4	46.9
Number of uredinia	38.6	29.0
Second experiment		
Latent period	56.1	39.5
Number of uredinia	33.2	-[a]

[a] Between family variation was non-significant.

Table 4.6. Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross Zaragoza x RL6044[1], growth period and indication of additional genes for resistance to Puccinia recondita f.sp. tritici

Cultivar or line/ experiment	Latent period (hr)	Uredinia/ cm^2 leaf surface[2]	Infection type[3]	Additional leaf rust resistance gene[4]	Days to heading[5]
First experiment[6]					
A21R	233 a	2.6 abc	1+	*	65-87
A34R	232 a	2.5 abc	1	*	75-96
A14R	212 ab	1.7 a	1+	-	87
A28R	206 abc	1.9 ab	1+	-	89
A17R	206 abc	2.9 c	1+	*	65-75
A10R	201 bcd	2.3 abc	1+	*	65-75
RL6044	197 bcde	2.7 bc	1+	-	85
A18R	193 bcde	2.8 bc	1+	*	84-95
A5R	190 bcde	2.7 bc	;1	-	70-88
A37R	183 cde	3.1 c	1+	*	89
A8R	176 def	2.8 bc	1+	-	93
A39R	174 def	2.3 abc	2	*	77-82
A36R	170 ef	3.0 c	1++	-	73-87
Zaragoza	150 f	2.4 abc	4	*	77
Means	195	2.6			

Table 4.6 (continued). Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross Zaragoza x RL6044 [1], growth period and indication of additional genes for resistance to Puccinia recondita f.sp. tritici

Cultivar or line/ experiment	Latent period (hr)	Uredinia/ cm^2 leaf surface [2]	Infection type [3]	Additional leaf rust resistance gene [4]	Days to heading [5]
Second experiment [6]					
A29R	226 a	2.1 ab	1	*	65-79
A3R	222 ab	1.6 a	1	*	72-74
A15R	218 ab	2.2 ab	1	-	83
A27R	217 ab	1.7 a	1	*	65-73
RL6044	206 ab	2.2 ab	1+	-	84
A11R	204 abc	2.0 ab	1	*	62-65
A16R	203 abc	2.0 ab	1+	*	73-92
A19R	202 bc	2.2 ab	1+	*	77-95
A23R	202 bc	1.9 ab	1+	*	77-95
A30R	202 bc	2.2 ab	1+	-	74-79
A32R	182 cd	2.5 b	1+	*	91-97
Morocco	169 d	2.4 b	4	-	68
Zaragoza	163 d	1.7 a	4	*	76
Means	201	2.1			

[1] RL6044 is a Thatcher backcross line with gene Lr22a.

Table 4.6 (continued). Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross Zaragoza x RL6044 [1], growth period and indication of additional genes for resistance to Puccinia recondita f.sp. tritici

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[2] Square root transformation was applied to number of uredinia (100).

[3] Infection types on 0-4 scale (97).

[4] * indicates presence of Lr3a gene for low reaction to P. recondita f.sp. tritici in a family.

- indicates absence of Lr3a.

[5] Range indicates within family variation for number of days to heading.

[6] Values followed by different letters in a column differed significantly at P=0.05 according to Tukey's procedure (100).

Table 4.7. Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross SST33 x RL6044[1], growth period and indication of additional genes for resistance to *Puccinia recondita* f.sp. *tritici*

Cultivar or line/ experiment	Latent period (hr)	Uredinia/cm^2 leaf surface[2]	Infection type[3]	Additional leaf rust resistance gene[4]	Days to heading[5]
First experiment[6]					
D12R	233 a	2.4 a	1+	*	64-106
D20R	231 a	3.3 ab	1+	-	88
D34R	226 ab	3.3 ab	1+	*	86
D6R	224 ab	3.3 ab	1+	-	87
D16R	209 abc	3.3 ab	1+	-	83-92
D15R	204 bc	3.3 ab	1+	-	89
D7R	203 bc	3.2 ab	1+	-	88
RL6044	190 cd	2.9 ab	1+	-	85
D17R	188 cd	3.6 b	1+	*	85
D39R	188 cd	3.4 b	1++	-	93-100
D3R	188 cd	3.3 ab	2	-	90
SST33	169 d	2.9 ab	4	*	66
Means	204	3.2			

Table 4.7 (continued). Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross SST33 x RL6044[1/], growth period and indication of additional genes for resistance to Puccinia recondita f.sp. tritici

Cultivar or line/ experiment	Latent period (hr)	Uredinia/cm^2 leaf surface[2/]	Infection type[3/]	Additional leaf rust resistance gene[4/]	Days to heading[5/]
Second experiment[6/]					
D31R	233 a	3.4	1	-	85
D28R	222 ab	3.3	1	-	90
D29R	220 ab	3.1	1+	*	88
D5R	212 bc	3.3	1+	-	86-96
D26R	207 bc	3.3	1+	*	88
RL6044	205 bc	3.3	1+	-	85
D32R	198 c	3.4	1+	*	75
Morocco	166 d	3.6	4	-	66
SST33	142 e	3.0	4	*	66
Means	201	3.3			

1/ RL6044 is a Thatcher backcross line with gene Lr22a.

2/ Square root transformation was applied to number of uredinia (100).

3/ Infection types on 0-4 scale (97).

Table 4.7 (continued). Duration of latent period (hr), number of uredinia per cm^2 leaf surface and infection type measured on adult-plant-resistant F_4 families from the cross SST33 x RL6044 [1], growth period and indication of additional genes for resistance to Puccinia recondita f.sp. tritici

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[4] * indicates presence of an unknown, dominant gene for low reaction to P. recondita f.sp. tritici in a family.

- indicates absence of gene.

[5] Range indicates within family variation for number of days to heading.

[6] Values followed by different letters in a column differed significantly at P=0.05 according to Tukey's procedure (100). Means for numbers of uredinia in the second experiment were not compared because of non-significant variation among families.

that did not possess Lr3a showed typical Lr22a reactions. Segregation for maturity resulted in heading of plants from 65 to 96 days after planting.

In the second experiment with Zaragoza x RL6044 F_4 families, mean latent period ranged from 182 to 226 hr (Table 4.6). Number of uredinia varied from 1.6 to 2.5 per cm^2 leaf area and infection types varied from 1 to 1+. The latent period of RL6044 was 206 hr and that of Zaragoza and Morocco 163 and 169 hr, respectively. Eight families possessed Lr3a and heading was completed 62 to 97 days after planting.

Similar variation in components of resistance occurred in F_4 families of the cross between SST33 and RL6044 (Table 4.7). In the first experiment, means indicated that latent period varied from 188 to 233 hr, despite homozygosity for Lr22a. Latent period of RL6044 was 190 hr and that of SST33 was 169 hr. Number of uredinia ranged from 2.4 to 3.6 and infection types 1+, 1++ and 2 were observed. Three families additionally expressed the hypersensitive resistance of SST33. Heading was completed between 64 and 100 days after planting. In the second experiment, latent period varied between 198 and 233 hr and number of uredinia between 3.1 and 3.4; the latter difference being non-significant. Latent period of RL6044 was 205 hr and that of Morocco and SST33 166 and 142 hr, respectively.

Infection types ranged from 1 to 1+ and three families also expressed SST33 seedling resistance to isolate 3SA57. Heads were produced 66 to 96 days after planting.

In all F_4 experiments, the number of uredinia per unit leaf area of RL6044 did not statistically vary from that of the susceptible parent. Although some families from the Zaragoza cross produced significantly fewer uredinia, they did not necessarily exhibit the longest latent period (Table 4.6).

Different infection types for Lr22a were produced among plants of several F_4 families. The range of infection types as well as latent period and untransformed number of uredinia associated with the extreme infection types per family are presented in Table 4.8. Apparently the lowest infection type was associated with a longer latent period and with fewer uredinia although exceptions occurred (Table 4.8).

Infection types observed among families listed in Table 4.8 often were of the Z type (13) where the susceptible type uredinia are predominantly distributed towards the base of the leaf. Some plants in three families of the SST33 x RL6044 cross produced no or a negligible number of uredinia, a situation which precluded calculation of latent period or number of uredinia. In the Zaragoza

Table 4.8. Range of latent period (hr) and number of uredinia/cm^2 leaf surface determined on single plants of F_4 families with Lr22a from the crosses Zaragoza x RL6044 and SST33 x RL6044, but segregating for expression of infection type

Family/ cross	Infection type[a] range	Latent period[b] range	No. of uredinia[b] range
FIRST EXPERIMENT			
Zaragoza x RL6044			
A4R	z;1 z1 ;1 1+	177 - 216	10.3 - 6.6
A6R	z1+ 1++	174 - 230	10.5 - 6.6
A9R	1+ 1++	194 - 228	16.6 - 7.9
A13R	;1n ;1+ 1+	205 - 217	10.9 - 1.9
A25R	;1 1+	182 - 212	10.6 - 5.7
A33R	z;1 1+	172 - 203	12.3 - 4.4
A35R	1n 1 1+	150 - 202	6.8 - 3.4
A38R	;1n 1+	177 - 199	6.4 - 2.5
SST33 x RL6044			
D1R	;1 1+	202 - 222	14.8 - 1.9
D4R[c]	;n ;1n 1+	227 -	10.4 -
D9R	z;1 z;1+ z2 1 1+	144 - 236	9.0 - 3.6
D11R[c]	; ;1= 1+	208 -	7.2 - 0.8
D14R[c]	;1= ;1 1++	208 -	7.9 - 0.9
D19R	z;1 1+	193 - 283	11.8 - 3.9
D22R	;1 1 1+	209 - 239	10.4 - 8.1
D35R	1++ 2	161 - 212	12.5 - 10.7

Table 4.8 (continued). Range of latent period (hr) and number of uredinia /cm^2 leaf surface determined on single plants of F_4 families with Lr22a from the crosses Zaragoza x RL6044 and SST33 x RL6044, but segregating for expression of infection type

Family/ cross	Infection type [a] range	Latent period [b] range	No. of uredinia [b] range
SECOND EXPERIMENT			
Zaragoza x RL6044			
A7R	;1 1	227 - 253	2.9 - 3.1
SST33 x RL6044			
D24R	1 1+	217 - 229	10.0 - 7.5
D25R	1 1++	201 - 219	14.8 - 7.8
D27R	;1 1+	201 - 238	9.8 - 11.2
D33R	1+ 2+	166 - 233	11.9 - 13.7
D38R	1 1+	210 - 230	14.1 - 7.3

[a] Infection types on 0-4 scale (97). Z indicates predominant distribution of larger uredinia towards the leaf base; n indicates necrosis and "+" or "-" indicate gradation above or below an established infection type class.

[b] Extreme latent period and number of uredinia values correspond with infection types, indicating lowest and highest levels of resistance. Uredinium numbers are untransformed values.

[c] No uredinia or too few developed to determine latent period or numbers of uredinia/cm^2 leaf surface.

cross, duration of latent period varied between 150 and 253 hr in different families whereas the range was between 144 and 283 hr for plants from the cross SST33 x RL6044.

Comparison between isolates for germination showed that 84 % of the spores of isolate 3SA57 and 88 % of the spores of isolate 3SA86 had germinated after 3.5 hours. After 6.5 hours, 98 % and 97 % of the urediniospores of the isolates had germinated, respectively.

4.4.3 Expression of Lr22a in F_5 families

Mean latent period, number of uredinia and range of infection types determined on eight plants per F_5 family derived from resistant and moderately resistant F_4 plants are shown in Table 4.9. Variable infection types occurred within all families, except D12R. According to means for latent period, families A21R, A34R and D12R showed the expected superior levels of resistance in the F_5 generation. In the SST33 cross, families D17R and D3R exhibited latent periods statistically similar to that of D12R, although they were not selected as such in the F_4. The range of infection types observed among plants of family D17R suggested that different genes mediate components of resistance and were still segregating in D17R. Although only a limited number of plants could be evaluated, no D4R

Table 4.9. Latent period, number of uredinia and infection types determined on F_5 families[1] selected from the crosses Zaragoza x RL6044 and SST33 x RL6044

Cultivar or line/ cross	Latent period (hr)	Number[2] of uredinia/cm^2 leaf surface	Infection type[3] range
Zaragoza x RL6044			
A34R	239 a	3.9 ab	1 1+
A21R	235 a	3.9 ab	1 1+ 1++ 2
RL6044	225 a	4.4 b	1+
A36R	187 b	4.1 ab	1+ 1++
A32R	183 b	4.0 ab	1+ 1++ 2
Zaragoza	144 c	3.5 a	4
Means	202	4.0	
SST33 x RL6044			
D12R	241 a	3.8 ab	1+
D17R	239 a	3.8 ab	;1 ;1+ 1+ 2
RL6044	230 a	4.6 b	1+
D4R	228 a	3.8 ab	1+ 2
D3R	219 a	4.3 ab	1++ 2 2+
SST33	178 b	3.5 a	4
Means	223	4.0	

[1] Single plant selections evaluated in the F_4 generation were advanced and tested in the F_5.

Table 4.9 (continued). Latent period, number of uredinia and infection types determined on F_5 families selected from the crosses Zaragoza x RL6044 and SST33 x RL66044

2/ Square root transformation was applied to numbers of uredinia (100).

3/ Variation for infection type observed among eight plants per F_5 family.

plants with fleck reactions only were detected. Latent period of all respective families differed significantly from Zaragoza or SST33. According to latent period, families A36R and A32R appeared less resistant than RL6044.

Significantly more uredinia developed on leaves of RL6044 than on leaves of Zaragoza or SST33. Family means for number of uredinia were intermediate between those for the parents.

4.5 DISCUSSION

Data obtained in this study indicated a reversal of dominance for the gene Lr22a (21) as well as significant quantitative differences in expression of resistance among adult-plant-resistant families. Susceptibility of F_1 plants, and significant approximation of recessive gene behavior in the F_2 generation indicated that, in the present study, Lr22a was not phenotypically expressed when in the heterozygous condition. This finding differs from the reported partially dominant behavior of Lr22a (21, 25, 28).

The degree of dominance of a host gene for resistance to disease may be influenced by genetic background (21, 23, 24, 27, 51, 56, 58, 74), the genetic constitution of the pathogen (29, 41, 42, 55, 93, 101), temperature

(59, 60, 107), growth rate of the host plant (107) or by a combination of some of these factors (78).

Dyck and Kerber (21) observed that resistance conferred by Lr22a was reduced after backcrossing to Thatcher wheat. They suggested that expression of the gene was altered by the effects of the genetic background. In their experiments, plants of segregating F_3 lines showed a gradation of infection types and they assumed that plants with intermediate reactions were heterozygous for Lr22a. In crosses evaluated in the present study, similar variation in reaction to leaf rust was observed, but all progeny tests of resistant F_2 plants indicated their homozygosity for the Lr22a gene. Thus the variable expression of infection types is the result of other factors influencing gene expression, rather than gene dosage in heterozygotes.

Moreover, Dyck and Kerber (21) were of the opinion that, if the Lr22a donor line (RL5404) used in their study possessed genes modifying the reaction of Lr22a, variation would have occurred among F_3 lines. They found no obvious variation in greenhouse evaluations, though some was observed in the field. In the present study, within and between family variation for infection type commonly occurred in the F_3 families of both crosses, suggesting that modifier genes are present.

Several other examples of dominance reversal in the wheat : leaf rust association have been reported. Dyck and Samborski (23) found that the cultivars Prelude and Thatcher influenced the dominance of the Lr2 alleles for resistance to P. recondita f.sp. tritici. Furthermore, the Lr2 alleles in both Webster and Carina were highly effective in a Red Bobs background but their expression was variable in a Thatcher background. In another study (24) the Lr2 alleles were, in general, most effective in a Thatcher background, intermediate in Prelude and least effective in Red Bobs. The gene Lr13 for APR to wheat leaf rust segregated with partial dominance in crosses of Thatcher with Frontana, but recessively in crosses of Thatcher with Manitou, which has APR transferred from Frontana (27). Selections from the Thatcher x Frontana cross were also more resistant than selections from the Thatcher x Manitou cross. Both crosses were inoculated with the same race and both Frontana and Manitou plants used in these crosses displayed no seedling resistance to this race.

Data reviewed by Loegering (55) indicated a reversal in dominance of gene Lr9 in Transfer, when it was evaluated in a Thatcher cross. Lr9 was dominant to an isolate homozygous for avirulence, but segregated recessively when tested with a heterozygous isolate. In the study reported here, it was not possible to relate dominance reversal of Lr22a to the pathogen, because the

genetic constitution of the isolates of P. recondita f.sp. tritici used is not known.

Non-genetic environment has also been shown to affect dominance of resistance genes. The degree of both resistance conferred and dominance of gene Lr18 increased with decreasing temperatures (59). McIntosh and Dyck (60) previously had demonstrated that the gene Lr23 was recessive in some crosses and partially dominant in others. However, the degree of dominance increased following exposure at elevated temperatures.

It is apparent that phenotypes resulting from interactions between genes for avirulence in a pathogen and those for resistance in a host could be modified by unknown genes in both and by environmental effects. Evidence presented indicates that gene Lr22a in line RL6044 is associated with genes that modify the reaction of Lr22a. Similar variation was noted when the gene was transferred into two genetically different backgrounds.

The retention of donor genes other than the target gene is a common phenomenon in developing sets of near-isogenic lines by backcrossing (111). In the barley leaf rust system, Parlevliet and Kuiper (71) found that the cultivar Cebada Capa possessed genes, mediating long latent period, in addition to the Pa7 gene for

hypersensitive resitance to P. hordei (Otth).

From the data presented in this study, it is clear that the development of cultivars with Lr22a resistance should not be based on infection types alone. Selected F_5 families generally reacted as expected and confirmed that selections for long latent period could successfully be conducted among genotypes to develop breeding lines with high levels of resistance. Within - family variation for infection type and the indication of genetic factors responsible for continuous variation in latent period, raised the question of whether the same genes were modifying the expression of components of resistance as well as the degree of dominance of Lr22a.

A lower infection type was not always associated with a long latent period or fewer uredinia, although such a relationship occurred in certain families. Falconer (32) stated that continuous variation for a character could either be the result of the simultaneous segregation of genes affecting that character or it could arise from non-genetic causes. Variable phenotypes may be associated with monogenic resistance if non-heritable variance exceeds heritable variance (107). Since the genotypic variances suggested genetic differences among F_4 families, it is hypothesized that a number of genes are involved in the modification of the expression of Lr22a and that certain combinations are neces-

sary to confer a low infection type, increased latent period and reduced number of uredinia.

Because the expression studies were conducted at relatively early stages of plant development, greater differences in latent period could have been expected if more mature plants had been used. Enhanced levels of resistance were not associated with the occurrence of genes for hypersensitive seedling resistance. Superior levels of hypersensitive resistance have been reported when different genes for resistance to leaf rust are combined (90, 92).

Growth rate of plants probably was not responsible for the variation observed in the quantitative assessment of resistance. Early maturing plants were not necessarily the most resistant nor were late maturing plants the most susceptible. The effect of the Zaragoza and SST33 backgrounds on Lr22a resistance could not be compared directly because different isolates of the pathogen were used. However, resistance expression appeared similar in both crosses. Although germination of isolates 3SA57 and 3SA86 was equal, and both, on a basis of infection type, were fully virulent to Zaragoza and SST33, respectively, the isolates differed in the number of uredinia produced on the common host, Morocco.

EVALUATION OF COMPONENTS OF ADULT PLANT RESISTANCE TO *PUCCINIA RECONDITA* f.sp. *TRITICI* IN WHEAT IN THE GREENHOUSE

5.1 ABSTRACT

Pathogen race, growth stage, leaf position and temperature effects on components of resistance to *Puccinia recondita* f.sp. *tritici* in wheat (*Triticum aestivum*) genotypes were studied. Adult plant resistance conferred by the genes *Lr12* and *Lr13*, and that of Era and Sinton, interacted differentially with races of the pathogen. Inoculation of adult-plant-resistant genotypes, when plants were of different ages, indicated that resistance of the wheats studied, although mediated by different genetic factors, was usually expressed prior to the fifth-leaf stage. Expression of resistance in adult plants of Glenlea and line RL6044 (*Lr22a*) was stable to pathogen variability. Inoculation of Era, Glenlea, Sinton and RL6044 plants at growth stages 4 to 18 on the Romig scale showed that latent period increased and uredinium numbers decreased as each wheat matured. No significant differences in latent period determined on the flag, flag-1 and flag-2 leaves of the same plant were observed for Era, Glenlea and RL6044. However, leaf age should be considered in assessing adult plant resistance because the latent period in flag leaves of Sinton was shorter than that

determined for the flag-1 and flag-2 leaves. Evaluation of sequentially formed leaves of the same plant showed that less uredinia developed on the flag-2 leaf of Glenlea than on the penultimate and flag leaves. The latent period of Era, Glenlea and RL6044 was significantly longer at 15 C than at 21 C but temperature did not affect Sinton to the same degree. Uredinium size was a sensitive criterion of adult plant resistance. Estimation of uredinium size on flag leaves of Era, Glenlea, Sinton and RL6044 showed that uredinium development in line RL6044 was significantly restricted at 15 C.

5.2 INTRODUCTION

Variation in expression of resistance due to plant age, age of leaf tissue or to differences in growth stage (16, 25, 33, 76, 77, 85, 102) has been demonstrated in many host : pathogen associations. Furthermore, in the cereal rusts, disease development can be influenced by the environment (12, 20, 30, 75, 76, 103, 104).

Virulence of leaf rust, caused by Puccinia recondita Rob. ex Desm. f.sp. tritici, for most Lr genes for resistance in wheat (Triticum aestivum L.) has been reported (11, 54, 89). Therefore, few characterized and universally effective genes remain for use in developing wheat cultivars highly resistant to leaf

rust. Milus and Line (64) and Shaner and Finney (94) demonstrated, however, that slow rusting or adult plant resistance (APR), could readily be identified in wheat germplasm collections. Studies investigating the genetic nature of slow rusting or APR in wheat to leaf rust have often indicated monogenic or oligogenic inheritance of such resistance (7, 25, 27, 53), thus implying that these forms of resistance can be manipulated with relative ease in breeding programs. An indication of their value could be obtained by studying the effects of environment and host growth stage on levels of resistance. Characterization of components of resistance is important, since the resistance of plants with more than one type of resistance presumably is more stable than that based on a single component (84). Some sources of APR appear stable to different races of P. recondita f.sp. tritici and the suggested combination of APR with other genes for resistance would considerably enhance its potential (25).

In the present study, the APR of certain wheat genotypes was characterized in terms of reaction to races, onset of resistance expression, plant growth stage, leaf position and temperature effects.

5.3 MATERIALS AND METHODS

5.3.1 Wheat genotypes, inoculation and incubation

For experiments with adult wheat plants, seed of the cultivars and lines Era [Lr10, Lr13, + (81)], Glenlea [Lr1, LrT2, + (28)], Sinton [Lr10, Lr13, + (81)], Manitou [Lr13 (88)], Neepawa [Lr13 (88)], RL6044 [Lr22a (25)], RL6011 [Lr12 (25)] and of leaf-rust-susceptible checks Morocco, Line E and SST33 were planted in pots containing 5 kg of soil. Eight consecutive plantings were made at 14-day intervals and plants were grown (three/pot) in a plastic greenhouse at 2-30 C. A water-soluble fertilizer (6.5:2.7:13.0 NPK) was applied as a soil drench (0.5 g/pot) three weeks after planting, and weekly thereafter, for the duration of the experiments.

The consecutive plantings permitted selection of plants of similar appearance and growth stage of each cultivar or line for evaluation in a specific experiment. Leaves of plants were quantitatively inoculated with the aid of an Andres inoculation device (2). The device was adjusted to maintain 160 mm between the inoculum discharge nozzle and adaxial leaf surface. Air pressure, controlling both upward speed of the inoculator and discharge of inoculum, was kept at fixed settings during all experiments. In the

experiments determining seedling reactions to different races and defining initial expression of host resistance, plants were inoculated according to the methods described by Browder (10).

Preparation of inoculum was standardized by using a suspension of 0.2 mg fresh urediniospores per ml Soltrol 130® light mineral oil (Phillips Chemical Company, Borger, Texas) in all experiments. After inoculation, leaves were allowed to dry for at least one hr before plants were placed in a dew chamber where they were kept in darkness at 18-20 C for 19 hr. During the last 3 hr in the chamber leaves were allowed to dry off gradually before plants were placed in a greenhouse.

5.3.2 Reaction to different races of P. recondita f.sp. tritici

Four flag leaves and duplicate sets of seven-day-old seedlings of each of Era, Glenlea, Manitou, Neepawa, Sinton, RL6044, RL6011 and Line E plants were inoculated with isolates 3SA57, 3SA58, 3SA60 and 3SA62 (see Table 5.1 for avirulence/virulence combinations). For inoculation all adult plants were at growth stage 13 on the Romig scale (15). After completion of the infection process in the dew chamber, sets of seedlings were placed in two greenhouse compartments at 17-19 C and

Table 5.1. Avirulence/virulence combinations of isolates of *Puccinia recondita* f.sp. *tritici*

Isolate	Resistance (*Lr*) genes
3SA57	Lr1, 2a, 2b, 3ka, 11, 15, 17, 20, 24, 30/3a, 3bg, 10, 14a, 16
3SA58, 3SA86	Lr3a, 3bg, 3ka, 11, 16, 20, 30/1, 2a, 2b, 10, 14a, 15, 17, 24
3SA60	Lr2a, 2b, 3bg, 15, 16, 17/1, 3a, 3ka, 10, 11, 14a, 20, 24, 30
3SA62	Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/1, 2a, 2b, 10, 14a, 15, 17

24-26 C. Adult plants were placed in a greenhouse at 20-24 C. Additional illumination of 9 000 lux was provided in all greenhouse compartments for 12 hr daily by cool-white fluorescent tubes. Infection types (97) on seedlings were recorded nine and 12 days after inoculation and on flag leaves 14 days afterwards.

5.3.3 Expression of post-seedling resistance

Seeds from single-plant selections of Era, Glenlea, Sinton, RL6044 and SST33, were planted in 5 kg of soil in plastic pots (three seeds/pot, four replicate pots/wheat). Plants were grown in a greenhouse at 18-24 C and four consecutive plantings were made at weekly intervals. When plants were 10, 17, 24 and 31 days old, they were inoculated on the same day with isolate 3SA86 (Table 5.1) of *P. recondita* f.sp. *tritici*. A water-soluble fertilizer (6.5:2.7:13.0 NPK) was applied (0.5 g/pot) three times during the growing period of plants 31 days old at inoculation, twice to the 24-day-old plants and once to plants 17 days old at inoculation.

Of plants 31 days old, Glenlea displayed its sixth, Sinton and SST33 their fifth and Era and RL6044 their fourth leaf. Of plants 24 days old, Glenlea, Era, RL6044 and SST33 displayed their fourth and Sinton its third leaf. Of plants 17 days old, Glenlea, Sinton,

RL6044 and SST33 displayed their third and Era its second leaf. Ten days after planting, wheats displayed only their primary leaf, except Glenlea, which showed a partially developed second leaf.

After incubation, plants were transferred to a greenhouse at 23-25 C with normal daylight supplemented by a 12 hr cycle of 9 000 lux emitted by fluorescent tubes. Infection types (97) were determined 12 days after inoculation.

5.3.4 Quantitative measurement of components of resistance

On each quantitatively inoculated leaf, an area was marked covering about two-thirds of the leaf, but excluding the extreme basal and distal parts. These areas were inspected daily after inoculation and when uredinia became visible as erumpent structures, those within the marked area were counted. Counting continued at 08h00 each day until the logarithmic phase of uredinium eruption had passed. A final count was made 48 hr later. Latent period was then calculated according to the linear regression method (1) as the number of hours between initiation of the incubation period and visibility of 40 % of the primary uredinia as erumpent structures. After infection types (97) had been scored, leaves were detached and the area of the

portion on which uredinia had been counted, was determined with a leaf area meter (model LI-3100, Lambda Instruments Corporation, Lincoln, Nebraska). Number of uredinia/cm^2 leaf surface was calculated.

Uredinium size was estimated by measuring two diameters of randomly selected but non-coalescing uredinia. Measurements were taken from images of four leaves that had been photographed at a known magnification. From these measurements, uredinium size was calculated using the formula: Area = πAB/4, where π = pi, A = length and B = width.

5.3.5 Growth stage and leaf position effects

Glenlea, Era, Sinton, RL6044 and Morocco plants, at Romig growth stages 4, 7, 8, 11 and 18, were selected to determine the effect of growth stage on resistance expression. The most recently emerged, but fully unfolded leaf of each plant, at a specified growth stage, was inoculated on the same day with isolate 3SA62. After incubation, plants were transferred and maintained in a greenhouse set at 20-24 C with 12 hr additional light (9 000 lux emitted by fluorescent tubes) daily. Five leaves for each host : growth stage combination were evaluated for latent period and number of uredinia. Infection types were recorded 17 days after inoculation.

To investigate the effect of sequentially formed leaves of the same plant on APR, the flag, flag-1 and flag-2 leaves of Glenlea, Era, Sinton and RL6044 plants at Romig growth stage 12, were inoculated on the same day with isolate 3SA62. Latent period and number of uredinia were determined on eight leaves per position per host. Values were then randomly paired to represent four replicates. Infection types were recorded 15 days after inoculation. Greenhouse temperature and illumination were similar as described for the growth stage experiment.

5.3.6 Temperature effects

Flag leaves of Glenlea, Era, Sinton, RL6044 and Morocco (Romig growth stage 13) were inoculated with isolate 3SA62. Five inoculated plants of each cultivar or line were maintained in each of two greenhouse compartments, one set at 14-16 C and the other at 20-22 C with 12 hr supplemental lighting of 9 000 lux each day. Latent period and number of uredinia were determined as before. Uredinium size was determined 18 days after inoculation from eight pustules on each of four infected leaves per host per temperature. In a second temperature experiment, a flag leaf on each of 10 plants of Era and SST33 at Romig stage 13 was inoculated with isolate 3SA86. Five plants of each cultivar were maintained in two greenhouse compartments, one at

21-23 C and the other at 16-18 C. Latent period and number of uredinia were calculated.

5.3.7 Data transformation and statistical analysis

The square root transformation was applied to number of uredinia/cm^2 leaf area (100). Data for latent period, number of uredinia and uredinium size were analysed for variance as factorial experiments arranged in completely randomized designs. Tukey's procedure was applied to host genotype, growth stage, leaf, temperature and interaction means to test significance of differences at $P=0.05$ (100). Comparisons were made only when analysis of variance had indicated a factor as a significant source of variation.

5.4 RESULTS

5.4.1 Reaction to different races of P. recondita f.sp. tritici

Infection types produced on seedling and flag leaves by the four races of *P. recondita* f.sp. *tritici* confirmed the adult plant resistance of Era, Glenlea, Manitou, Neepawa, Sinton, RL6011 and RL6044 (Table 5.2). Each adult-plant-resistant wheat, except line RL6044, displayed variation in response to the test races. Gene *Lr13* was apparent in seedlings of Manitou and Neepawa

Table 5.2. Infection types produced by four isolates of Puccinia recondita f.sp.tritici on primary and flag leaves of wheat cultivars and lines reported to possess adult-plant-resistance

Cultivar or line	Infection type[a]/Isolate/Leaf											
	3SA57			3SA58			3SA60			3SA62		
	Primary		Flag	Primary		Flag	Primary		Flag	Primary		Flag
	18 C	25 C		18 C	25 C		18 C	25 C		18 C	25 C	
Manitou (Lr13)	3+	x	;1c	4	4	3+	3+	x	;1c	4	4	3+
Neepawa (Lr13)	3+	x	;1c	4	4	3+	3+	x	;1c	4	4	3
Era (Lr10, 13, +)	;1	;12c	0;	3+	2++	1++3	x	x+	0;	3	3+	1++3
Sinton (Lr10, 13, +)	2c	;12c	;	3	3+	1++3	3	2+	o;	3	4	1++3
Glenlea (Lr1, LrT2, +)	0;	0;	;	4	4	;1++	3	;12+	;1	4	4	;1++
RL6011 (Lr12)	4	4	3	4	4	;12c	4	4	;	4	4	2c
RL6044 (Lr22a)	4	4	1+	4	4	1+	4	4	1+	4	4	1+
Line E	4	4	4	4	4	4	4	4	4	4	4	4

[a] Infection types (97) on primary leaves were determined at 18 C and 25 C and those on flag leaves at 22 C.

when tested at 25 C with isolates 3SA57 and 3SA60, but not at 18 C. Era and Sinton, also possessing Lr13, were highly resistant as adult plants but moderately resistant as seedlings at both temperatures to isolates 3SA57 and 3SA60. Isolates virulent on seedlings of Era and Sinton produced only small uredinia on flag leaves (infection type 1++3). Glenlea was resistant at all growth stages to isolate 3SA57, which lacks virulence for Lr1. Resistance of Glenlea to the other three isolates was expressed as low to intermediate infection types on flag leaves. Infection type ;12+ was observed on primary leaves of Glenlea at 25 C after inoculation with isolate 3SA60. Line RL6011 displayed large uredinia on flag leaves infected with isolate 3SA57, but hypersensitive reactions to the other isolates. Gene Lr22a in RL6044 was expressed as a 1+ infection type on flag leaves to all isolates tested.

5.4.2 Expression of post-seedling resistance

Infection types produced on leaves of Era, Glenlea, Sinton, RL6044 and SST33, when plants were of different ages, are shown in Table 5.3. In Glenlea resistance was evident only on the fifth and sixth leaves of plants that were 31 days old at inoculation, but on the fifth leaves the infection type varied from 2c to 3++ among plants. There was some evidence of intermediate infection types on the second leaf of Era plants 17

Table 5.3. Infection types[a/b] produced by isolate 3SA86 of *Puccinia recondita* f.sp. *tritici* on different leaves of adult-plant-resistant wheat cultivars and lines after inoculation at four stages of plant development

Age of plants at inoculation (days)	Leaf	Cultivar or Line				
		Glenlea	Era	Sinton	RL6044	SST33 (check)
31	First	-	-	-	-	-
	Second	-	-	-	-	-
	Third	3++	3	2-2+3	2+3	4
	Fourth	3++	2c-3	2c-3++	1++2	4
	Fifth	2c,3++	-	2c-3	-	4
	Sixth	x	-	-	-	-
24	First	4	-	-	-	-
	Second	3	2+3	-	4	4
	Third	3++	2+3	2+3	3++	4
	Fourth	3++	3+	-	3=	4
17	First	4	3	3++c	3++	4
	Second	4	2+3	3++c	4	4
	Third	4	-	3++	4	4
10	First	4	3	3++	4	4
	Second	4	-	-	-	-

[a/] Infection types according to 0-4 scale (97).

[b/] - indicates leaves chlorotic or not yet developed.

days old at inoculation, although resistance was expressed more clearly in the fourth leaf at 31 days. In Sinton, resistance was first expressed in the third leaf of plants 24 days old at inoculation, but chlorosis was associated with large uredinia on the first and second leaves of plants 17 days old at inoculation. Infection types typical of Lr22a were observed on the fourth leaves of RL6044 plants 31 days old at inoculation, while some indication of resistance was observed on the third leaves of the same plants, as well as on the fourth leaves of 24-day-old plants. SST33 leaves were susceptible at all stages of plant development evaluated.

5.4.3 Growth stage and leaf position effects

Latent period and numbers of uredinia on terminal leaves of RL6044, Era, Sinton, Glenlea and Morocco inoculated with isolate 3SA62 at five growth stages are shown in Tables 5.4 and 5.5. Latent period varied significantly on a basis of growth stage, host effect and due to the interaction of the two. Means ranged from 251 hr for growth stage 18 to 194 hr for growth stage 4. Host genotype means indicated that, on the basis of latent period, RL6044 was most resistant and that it differed significantly from the other cultivars, which among themselves were similar. Latent period means of the adult-plant-resistant wheats were

Table 5.4. Latent period[1/](hr) of isolate 3SA62 of *Puccinia recondita* f.sp. *tritici* determined on terminal leaves of adult-plant-resistant wheat cultivars and lines at different growth stages

Growth stage (Romig scale)	Cultivar or Line					Growth stage mean[1/]
	RL6044	Era	Sinton	Glenlea	Morocco[2/]	
18	326 a	232 def	251 de	275 bc	169 ij	251 A
11	341 a	218 fg	222 f	254 cd	168 ij	241 B
8	325 a	213 fg	229 ef	226 f	167 ij	232 C
7	319 a	224 f	216 fg	195 gh	154 j	222 D
4	282 b	182 hi	178 hi	183 hi	147 j	194 E
Cultivar or Line mean[1/]	319 A	214 C	219 BC	227 B	161 D	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among growth stage or cultivar or line means is valid only within the respective column or row.

[2/] Susceptible check.

Table 5.5. Number[1/2/] of uredinia/cm^2 leaf surface of isolate 3SA62 of *Puccinia recondita* f.sp. *tritici* determined on terminal leaves of adult-plant-resistant wheat cultivars and lines at different growth stages

Growth stage (Romig scale)	Cultivar or Line					Growth stage mean [1/]
	RL6044	Era	Sinton	Glenlea	Morocco	
18	2.7 abcdef	2.3 abcd	1.7 a	2.3 abcd	4.0 ghi	2.6 A
11	2.1 abcd	2.3 abcd	3.2 defgh	1.8 a	3.9 ghi	2.7 A
8	3.1 cdefgh	2.6 abcde	2.0 abc	3.8 fghi	4.5 i	3.2 BC
7	3.0 bcdefg	2.0 abc	1.9 ab	3.5 efghi	3.7 efghi	2.8 AB
4	3.0 bcdefg	3.7 efghi	2.6 abcde	4.2 hi	3.1 cdefg	3.3 C
Cultivar or Line mean [1/]	2.8 BC	2.6 AB	2.3 A	3.1 C	3.8 D	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among growth stage or cultivar or line means is valid only within the respective column or row.

[2/] Square root tranformed values.

significantly higher when compared to that of Morocco. Considering RL6044, Era and Sinton individually, growth stage 4 differed from the other growth stages, the latter, amongst themselves, varying little statistically. In Glenlea, growth stages 4 and 7 were similar and differed from growth stages 8, 11 and 18 in which latent period increased progressively.

The number of uredinia/cm^2 leaf area varied with host developmental stage, host genotype, and the interaction of the two. Means indicated that plants at growth stages 11 and 18 were more resistant than plants at the other growth stages tested. Morocco produced most uredinia/cm^2 leaf area and Sinton fewest. According to cultivar and line means, fewer uredinia developed on adult-plant-resistant hosts than on Morocco. Growth stage did not affect number of uredinia on leaves of RL6044, but in Era, significantly more uredinia were produced on leaves of plants at growth stage 4 than on leaves of plants at other growth stages. In Sinton, all growth stages evaluated reacted the same except for growth stage 11, when plants were more susceptible. Glenlea plants at growth stages 11 and 18 were more resistant than plants at growth stages 4, 7 and 8.

Infection types on terminal leaves of wheats at different developmental stages varied. Glenlea consistently produced mesothetic infection types at all

stages evaluated, and the amount of chlorosis associated with lesions was more pronounced at stages 11 and 18. Uredinia on leaves of RL6044 were smaller at growth stages 11 and 18 (infection type 1+) compared with infection type 1++ at the other stages. Era also exhibited a mesothetic infection type at growth stages 4, 7 and 8, but a more susceptible reaction type (1++3) at stages 11 and 18. Sinton showed similar 1++3 infection types at growth stages 8, 11 and 18, susceptible reactions at growth stage 4 and intermediately sized uredinia associated with chlorosis (2+3c) at growth stage 7. Morocco exhibited infection type 4 in all tests.

The effects of sequentially formed leaves of adult plants on latent period and uredinium numbers are shown in Tables 5.6 and 5.7. Latent period and number of uredinia/cm^2 varied on a basis of leaf position, host genotype and the interaction of the two. Means indicated that latent period was most prolonged in the flag-1 leaf and that RL6044 and Era, which behaved similarly, differed from Sinton and Glenlea, which also were similar. Latent period differences among leaves of RL6044, Era and Glenlea plants were not significant, respectively. The latent period measured on flag leaves of Sinton was markedly shorter than that of the lower leaves.

Table 5.6. Latent period[1/] (hr) of isolate 3SA62 of Puccinia recondita f.sp. tritici determined on the flag, flag-1 and flag-2 leaves of adult-plant-resistant wheat cultivars and lines

Leaf	Cultivar or Line				Leaf mean[1/]
	RL6044	Era	Sinton	Glenlea	
Flag	246 ab	251 ab	182 d	220 bc	225 B
Flag-1	250 ab	250 ab	239 ab	226 abc	241 A
Flag-2	252 a	240 ab	236 ab	203 cd	233 AB
Cultivar or Line mean[1/]	249 A	247 A	219 B	217 B	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among leaf or cultivar means is valid only within the respective column or row.

Table 5.7. Number[1/2/] of uredinia/cm^2 leaf surface of isolate 3SA62 of Puccinia recondita f.sp. tritici determined on the flag, flag-1 and flag-2 leaves of adult-plant-resistant wheat cultivars and lines

Leaf	Cultivar or Line				Leaf
	RL6044	Era	Sinton	Glenlea	mean[1/]
Flag	2.6 cd	1.7 abc	3.0 d	2.3 bcd	2.4 A
Flag-1	2.2 bcd	1.5 ab	2.9 d	1.8 abc	2.1 AB
Flag-2	2.8 d	1.5 ab	2.6 cd	1.2 a	2.0 B
Cultivar or Line mean[1/]	2.5 A	1.6 B	2.8 A	1.8 B	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among leaf or cultivar means is valid only within the respective column or row.

[2/] Square root transformed values.

Means for uredinium numbers indicated that fewest uredinia developed on the flag-2 leaf. Fewer uredinia developed on leaves of Era and Glenlea than on leaves of RL6044 and Sinton. Infection types produced on the flag-2 leaf of Glenlea (;2), RL6044 (1) and Sinton (1++3c) were lower than infection types produced on flag and flag-1 leaves. In Era, infection type 1++3 was observed on the flag leaf while the lower leaves both produced infection type 2+3.

5.4.4 Temperature effects

Latent period varied due to differences in temperature, host genotype and the interaction of the two (Table 5.8). Mean latent period of plants kept at 15 C was 69 hr longer than that of plants at 21 C. According to the cultivar and line means, latent periods of RL6044 and Era were similar, but longer than those of Sinton and Glenlea, which were similar. The latent period of Morocco was significantly shorter than that of the other wheats. Interaction means indicated that the low temperature effect was most pronounced on Era, Glenlea and RL6044. No significant difference between the two temperatures was observed for Sinton. Even the latent period measured for the susceptible Morocco was increased by 59 hr in the low temperature environment.

In an experiment where Era and SST33 plants were

Table 5.8. Latent period[1/] (hr) of isolate 3SA62 of Puccinia recondita f.sp. tritici determined on flag leaves of three adult-plant-resistant wheat cultivars and one line at two temperatures

Cultivar or Line	Latent period		Cultivar or Line mean[1/]
	21 C	15 C	
RL6044	249 bc	334 a	292 A
Era	221 cd	339 a	280 A
Sinton	196 de	217 cd	207 BC
Glenlea	198 de	263 b	231 B
Morocco[2/]	165 e	224 bcd	195 C
Temperature mean[1/]	206 B	275 A	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among cultivar or line or temperature means is valid only within the respective column or row.

[2/] Susceptible check.

inoculated with isolate 3SA86, the latent period of Era plants grown at 17 C was increased by 135 hr compared to plants grown at 22 C (Table 5.9). The latent period of SST33 did not differ significantly between temperature regimes.

The number of uredinia determined at 21 and 15 C showed that the test wheats, as a group, were not significantly affected by temperature (Table 5.10). However, differences due to host genotypes were observed. According to the cultivar and line means, significantly more uredinia developed on flag leaves of RL6044 and Morocco than on flag leaves of Era and Glenlea. Sinton differed from Morocco only. Temperature similarly had no significant effect on the density of uredinia on leaves of Era and SST33 (Table 5.9), but means showed that Era was more resistant than SST33.

Temperature means further indicated that the size of uredinia on flag leaves of the wheats studied, as a group, was not affected (Table 5.11). However, size of uredinia varied with the individual wheats tested. They were smallest on RL6044 and largest on Morocco. Although temperature means were about equal, larger uredinia were produced on Morocco at 15 C than at 21 C while the uredinia on RL6044 were significantly smaller at 15 C than at 21 C. RL6044, Era and Sinton produced uredinia of similar dimensions, but those on leaves of

Table 5.9. Latent period[1/](hr) and number[1/]of uredinia/cm^2 leaf surface of isolate 3SA86 of Puccinia recondita f.sp. tritici determined on flag leaves of the wheat cultivars Era and SST33[2/]at two temperatures

Cultivar	Temperature		Cultivar
	22 C	17 C	mean[1/]
LATENT PERIOD			
Era	259 b	394 a	327 A
SST33	200 c	236 bc	218 B
Temperature mean[1/]	230 A	315 B	
NUMBER OF UREDINIA[3/]			
Era	2.7 ab	2.2 a	2.5 A
SST33	3.7 b	3.5 b	3.6 B
Temperature mean[1/]	3.2 A	2.9 A	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among cultivar or temperature means is valid only within the respective column or row.

[2/] Susceptible check.

[3/] Square root transformed values.

Table 5.10. Number[1/2/] of uredinia/cm^2 leaf surface of isolate 3SA62 of Puccinia recondita f.sp. tritici determined on flag leaves of three adult-plant-resistant wheat cultivars and one line at two temperatures

Cultivar or Line	Number of uredinia/cm^2		Cultivar or Line mean[1/]
	21 C	15 C	
RL6044	2.3 cd	2.4 cd	2.4 BC
Era	2.0 abcd	1.4 ab	1.7 A
Sinton	1.6 abc	2.2 bcd	1.9 AB
Glenlea	1.9 abcd	1.2 a	1.6 A
Morocco[3/]	2.3 cd	2.7 d	2.5 C
Temperature mean[1/]	2.0 A	2.0 A	

[1/] Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Comparison among cultivar or line or temperature means is valid only within the respective column or row.

[2/] Square root transformed values.

[3/] Susceptible check.

Table 5.11. Uredinium size[1/] (mm^2) of isolate 3SA62 of *Puccinia recondita* f.sp. *tritici* determined on flag leaves of three adult-plant-resistant wheat cultivars and one line at two temperatures

Cultivar or Line	Uredinium size		Cultivar or Line mean[1/]
	21 C	15 C	
RL6044	0.175 bc	0.083 a	0.129 A
Era	0.178 bc	0.142 ab	0.160 A
Sinton	0.175 bc	0.163 abc	0.169 A
Glenlea	0.283 d	0.243 cd	0.263 B
Morocco[2/]	0.438 e	0.628 f	0.533 C
Temperature mean[1/]	0.250 A	0.252 A	

[1/]Values followed by different letters differed significantly at $P=0.05$ according to Tukey's procedure (100). Comparison among cultivar or line or temperature means is valid only within the respective column or row.

[2/]Susceptible check.

Glenlea were larger than on the other adult-plant-resistant wheats.

5.5 DISCUSSION

In this study, APR was expressed as a partial or incomplete form of resistance. Gradation between levels of APR appears to depend on the relative contributions of various components of resistance or on the interaction between such components. Not all components functioned in all adult-plant-resistant host genotypes evaluated. Glenlea, compared with the other wheats tested exhibited a low infection type, intermediate latent period and fewer uredinia on post-seedling leaves. RL6044 showed a moderately susceptible infection type, high density of uredinia, but extremely long latent period and restricted uredinium size. Furthermore, on this line, latent period was lengthened and uredinium size decreased at 15 C. At 21-22 C, Era showed effective levels of APR which markedly increased when inoculated plants were maintained at 15-17 C. APR of Sinton was expressed by reduced uredinium size in the greenhouse, but resistance seemed directly conditoned by the age of leaf tissue and growth stage.

Monogenic APR functions similarly to hypersensitive seedling resistance. Resistance conferred by Lr12 and

Lr13 in mature plants was completely lost in the presence of races with matching virulence. This loss supports Vanderplank's view (107) that interaction between host and pathogen in vertical resistance is not altered by ontogenic change in the host. In the present study, cultivars that possessed seedling resistance as well as factors for APR appeared more resistant in terms of the components measured than those depending on monogenic APR alone.

Flag leaf infection types of Era and Sinton to isolates 3SA57 and 3SA60 indicated high levels of resistance. Seedling infection types of Manitou and Neepawa at 25 C showed that both isolates were avirulent to Lr13, a gene present in both Era and Sinton. However, isolates virulent to seedlings of Era and Sinton could not produce fully susceptible reactions on flag leaves, thus indicating the presence of APR factors besides Lr13. Ezzahiri and Roelfs (31) have reported that Era possesses two complementary genes for APR.

APR of Era is undoubtedly complex. In the seedling stage, resistance conferred by Lr13 in Manitou is more effective at 25.5 C than at 18.1 C (75). However, seedlings of Era were, in contrast to the Lr13 reaction, more resistant at the lower temperature regime (75). In the present study, adult plants of Era similarly were more resistant at 15-17 C than at 21-22

C on a basis of latent period.

In Glenlea, Dyck et al. (28) have identified the resistance genes Lr1, LrT2, plus another gene for APR, allelic or closely linked with Lr13. The low seedling infection types of Glenlea to isolate 3SA60 at 25 C suggested that either Glenlea has genes effective in the seedling stage additional to Lr1, or that 3SA60 can detect the APR genes at an early stage in plant development in a specific environment.

According to Dyck et al. (27), Lr13 in Manitou was effective at the third leaf stage while Samborski and Ostapyk (91) found that APR in the cultivar Exchange was not fully expressed until after flag leaf emergence. Post-seedling resistance in some wheat cultivars to stem rust (P. graminis Pers. f.sp. tritici Eriks. & E. Henn.) became apparent between the third and fifth-leaf stages (17) and onset of resistance was not affected by different pathogen races. APR to stem rust of wheat, mediated by the gene Sr2, was detectable as early as the second node stage (102). In the present study, the onset of expression of APR varied with host genotype, but was evident in all resistant hosts between the primary and fifth-leaf stages. The association of chlorosis with susceptible type reactions on the first leaf of Sinton plants that were 17 days old at inoculation, but not on first leaves of

plants 7 days younger, emphasized the importance of leaf tissue age in expression of resistance. A mesothetic infection type response was also observed on the same leaf of both Era and Sinton plants. The reaction of RL6044 was in agreement with that described in Chapter 3. In Glenlea, variable infection types between plants were apparent. Because plants within a cultivar were considered genetically almost identical, variation observed may be attributed to environmental influences. Variable reaction types among individual plants of adult-plant-resistant wheat line PI 250413 have also been reported (25).

Although differences were not large, the effects of growth stage and leaf position on latent period could be detected. The latent period of line RL6044 appeared unaffected by growth stage while the latent period of the other adult-plant-resistant wheats gradually increased as the plants matured. On the basis of latent period, the resistance expressed by Era, Sinton and Glenlea plants, inoculated at growth stage 4 on the Romig scale, was not effective. The shorter latent period expressed by flag leaves of Sinton, compared to the flag-1 and flag-2 leaves on the same plant can be ascribed to age of leaf tissue. Sinton was slow in maturing and plants were inoculated at the first indication of awns protruding from the boot. At that stage flag leaves of Sinton were physiologically younger and

greener than flag leaves of other wheats, although growth stage was similar. However, the growth stage experiment indicated that flag leaves of Sinton plants at flowering expressed effective APR. Ohm and Shaner (68) also reported wheat flag leaves to be less resistant to P. recondita f.sp. tritici than the flag-1 and flag-2 leaves. Leaf position was similarly important in evaluating wheat cultivars for resistance to powdery mildew (33) and to stripe rust (76). Cultivars could not be distinguished when mildew severities were determined on flag leaves, but distinct differentiation was obtained when the two leaves below the flag leaf were evaluated (33). In wheat cultivars infected with stripe rust, flag leaves produced higher infection types than the lower leaves when evaluated at reduced temperatures (76); differences were ascribed to the flag leaves being greener and not as hardened as the older leaves. The flag leaf is an important photosynthate source for developing heads (73) and due to translocation a flag leaf may not have sufficient reserves to express maximum levels of resistance.

In the present study, flag leaves supported more uredinia/cm^2 leaf surface than did the lower leaves. However, variation between leaves was not large, except for Glenlea which supported considerably fewer uredinia on the flag-2 leaf. Number of uredinia were not as sensitive as latent period to growth stage differences.

From present greenhouse evidence and other studies (68, 98, 99) it can thus be concluded that APR in wheat to leaf rust is best assessed on a sub-terminal leaf of plants between Romig growth stages 11 and 18. A loss of resistance at post-anthesis stages has also been reported (14, 91) and should be taken into consideration in evaluations.

Browder (12) stated that rust pathogen : cereal host specificity mechanisms are adapted to specific environmental conditions. Such specificity was observed especially in Era and RL6044, but the components affected were not the same in these genotypes. Temperature clearly did not affect number of uredinia, but restricted the rate of uredinium development. Temperature is also a significant source of variation in the latent period of wheat infected with P. recondita f.sp. tritici (30, 103, 104). Uredinium size was a sensitive criterion of APR and the marked differences observed between adult-plant-resistant and susceptible hosts emphasized its importance as a component of resistance. According to the temperature study, uredinium size appeared inversely related to latent period, but this relationship was not significant.

EXPRESSION AND INHERITANCE OF RESISTANCE TO *PUCCINIA RECONDITA* f.sp. *TRITICI* IN THE WHEAT CULTIVAR GLENLEA

6.1 ABSTRACT

Determinations of latent period and infection type produced by isolate 3SA62 of *Puccinia recondita* f.sp. *tritici* on the flag and flag-1 leaves of Glenlea (*Triticum aestivum*) at plant growth stages 11 and 18 on the Romig scale, showed that the genes for adult plant resistance could, for experimental purposes, be detected in the boot stage. In a genetic study conducted in the F_1, F_2 and F_3 generations derived from the cross between Line E (leaf-rust-susceptible) and Glenlea, a dominant gene for seedling resistance and two recessive genes for adult plant resistance to *P. recondita* f.sp. *tritici* were indicated. Seedling tests with Glenlea and Line E x Glenlea progenies at 29-31 C revealed that Glenlea has seedling resistance in addition to that conferred by *Lr1*. Expression of the gene(s) for high-temperature seedling resistance in Glenlea was much more pronounced in progenies of the cross between Line E and Glenlea than in the donor parent. Isolates detecting the temperature-sensitive gene were virulent to *LrT2* (a gene for adult plant resistance in Glenlea) in the seedling stage under similar conditions. Thus, the seedling resistance of Glenlea at 29-31 C is either mediated by the second

gene for adult plant resistance, which has been reported as allelic or closely linked with Lr13, or it may be a previously undetected resistance factor. Seedling identification of the gene(s) conditioning resistance to P. recondita f.sp. tritici in Glenlea is valuable to breeding programs aimed at developing wheat genotypes with levels of durable resistance similar to that of Glenlea.

6.2 INTRODUCTION

Resistance to leaf rust (Puccinia recondita Rob. ex Desm. f.sp. tritici) should be an objective of every wheat (Triticum aestivum L.) breeding program. Leaf rust is probably the most important rust disease of wheat world-wide (88) and is extremely severe in South Africa. In view of the restricted number of single genes universally resistant to P. recondita f.sp. tritici, efforts to provide resistant cultivars should be centered on the adult-plant type of resistance because this resistance has been durable in cultivars such as Era (31, 81) and Glenlea (86, 87).

In Canada, the effective resistance against P. recondita f.sp. tritici characteristic of the cultivar Glenlea has been associated with resistance genes Lr1, LrT2 and a gene allelic or closely linked to Lr13 (28).

The incorporation of more than one gene for resistance to leaf rust in a wheat genotype may result in enhanced levels of resistance (90). Thus, the identification and characterization of an effective combination such as that of Glenlea is important for the exploitation of resistance in other genetic backgrounds.

The objectives of this study were to evaluate the resistance of seedlings and adult plants of Glenlea to South African isolates of P. recondita f.sp. tritici, and to study the inheritance and expression of this resistance for utilization in South African wheat breeding programs. The seedling experiments were conducted to determine whether the genes associated with adult plant resistance in Glenlea (28) could be detected in a way similar to that for Lr13 in other genetic backgrounds (75).

6.3 MATERIALS AND METHODS

6.3.1 Pathogen isolates and inoculation procedures

Eight South African isolates of P. recondita f.sp. tritici were used in this study (Table 6.1). The isolates were maintained on Morocco wheat seedlings in separated compartments in a greenhouse. When experiments were to be made, freshly collected urediniospores suspended in Soltrol 130® light mineral oil (Phillips

Table 6.1. Avirulence/virulence combinations of isolates of Puccinia recondita f.sp. tritici used to study the expression and inheritance of resistance to leaf rust in the wheat cultivar Glenlea

Isolate	Leaf rust resistance (Lr) genes[1/]
3SA57, 3SA122	Lr1, 2a, 2b, 3ka, 11, 15, 17, 20, 24, 30/3a, 3bg, 10, 14a, 16
3SA62, 3SA126	Lr3a, 3bg, 3ka, 11, 16, 20, 24, 30/1, 2a, 2b, 10, 14a, 15, 17
3SA121	Lr3a, 3bg, 3ka, 10, 11, 14a, 16, 17, 20, 24, 30/1, 2a, 2b, 15
3SA123	Lr3a, 3bg, 3ka, 10, 11, 14a, 16, 17, 20, 30/1, 2a, 2b, 15, 24
3SA127	Lr3a, 3bg, 3ka, 11, 16, 20, 30/1, 2a, 2b, 10, 14a, 15, 17, 24
3SA128	Lr2a, 2b, 3bg, 15, 16, 17/1, 3a, 3ka, 10, 11, 14a, 20, 24, 30

[1/] South African leaf rust differential genes.

Chemical Company, Borger, Texas) were used as inoculum. Flag leaves on which latent period was determined were inoculated with the Andres inoculation device (2) while flag leaves of plants in the inheritance study and primary leaves in seedling experiments were inoculated according to the methods of Browder (9, 10). A standard suspension of 0.2 mg urediniospores/ml oil was used in all inoculations.

One hr after inoculation of seedlings and 3 hr after inoculation of adult plants, when the oil had evaporated from leaves, the plants were placed in a dew chamber in darkness at 18-20 C for 19 hr. During the last 3 hr in the chamber, leaves were allowed to dry off gradually before placement in a greenhouse where daylight was supplemented with 9 000 lux emitted by cool-white fluorescent tubes for 12 hr each day.

6.3.2 Resistance of adult plants of Glenlea

Glenlea plants were grown in soil in plastic pots (three plants/pot) in a plastic greenhouse at 2-30 C. Three plantings at 10-day intervals were made to provide plants of different maturity stages when inoculations were done. A water-soluble fertilizer (6.5:2.7:13.0 NPK) was applied as a soil drench (0.5 g/pot) three weeks after planting, and weekly thereafter, for the duration of the experiment. The

flag and penultimate leaves of the main tiller of eight plants at each of growth stages 11 and 18 on the Romig scale (15) were inoculated on the same day. Adaxial surfaces of leaves were inoculated (2) with South African isolate 3SA62 (Table 6.1) of P. recondita f.sp. tritici. Inoculated plants were kept in a greenhouse at 20-24 C. Isolate 3SA62 was studied because of its widespread occurrence in South Africa and because it is virulent to seedlings of Glenlea.

Marked areas on each inoculated leaf were inspected daily, and when uredinia were erumpent those within the marked areas were counted. Counting continued daily until no more primary uredinia appeared. The latent period was calculated as the number of hours between the iniation of the incubation period and the visibility of 40 % of the uredinia as erumpent structures (1).

Data were analysed for variance according to a completely randomized design and the differences among means were tested for significance at P=0.05 according to Tukey's procedure (100).

6.3.3 Resistance of seedlings of Glenlea

Three different tests were conducted to evaluate the resistance of Glenlea seedlings in comparison with seedlings of cultivars and lines that possess different

resistance genes. The seedlings were produced in a room at 25 C and illuminated by about 2 000 lux natural daylight. Inoculation of seven-day old seedlings was as outlined above. Infection types (97) were scored eight to 11 days after inoculation.

In the first seedling experiment, the resistance of Glenlea was compared to that of Era [Lr10, Lr13, + (81)], Manitou [Lr13 (88)], Sinton [Lr10, Lr13, + (81)] and Line E (susceptible check). Seedlings were inoculated with isolates 3SA121, 3SA122, 3SA123, 3SA126, 3SA127 and 3SA128 (Table 6.1) and kept at 17-19 C and 30-32 C in two greenhouse compartments, respectively.

In the second experiment, seedlings derived from the cross between Line E and Glenlea were evaluated for resistance to isolates 3SA121 or 3SA127 of P. recondita f.sp. tritici. Seedlings were grown from remnant seeds of an inheritance study (described later in this chapter). Seedlings of the F_2, F_3 and F_4 generations were tested at 30-32 C. One test with F_3 seedlings was conducted at 15-17 C. The genotypes Glenlea, Line E, Thatcher and a Thatcher backcross line (RL6003) with gene Lr1, were included in each inoculation.

In the third seedling experiment, the resistance of Glenlea was compared with that of Thatcher backcross lines with genes LrT2, LrT3, LrT2 + LrT3, four families

from Line E x Glenlea F_5 progenies (lines H9, H10, H11 and H12) and with Line E, Thatcher and Manitou (Lr13). LrT2 was included in this study because it conditions resistance in Glenlea. LrT3 was tested because it has been reported (26) to enhance the effect of LrT2 in certain combinations. The Thatcher near-isogenic lines were supplied by Dr. P.L. Dyck, Agriculture Canada, Winnipeg, Canada. Seven-day old seedlings were inoculated with isolates 3SA121 (avirulent to Glenlea at 31 C) and 3SA127 (virulent to Glenlea at 31 C) of P. recondita f.sp. tritici and kept at 16-18 C and 28-30 C until infection types were scored.

6.3.4 Inheritance of resistance

Glenlea was crossed as the male parent with leaf rust susceptible wheat Line E. Forty-nine adult plants of the F_1 generation were inoculated at Romig growth stage 13 (15) and the reaction of flag leaves was recorded 14 days later. Two hundred F_2 plants were evaluated 14 days after inoculation of flag leaves for genes that conditioned adult plant resistance in Glenlea. Plants from both F_1 and F_2 generations were grown before and after inoculation in a greenhouse at 20-24 C with additional illumination of 9 000 lux provided for 12 hr each day by fluorescent tubes.

One hundred and ninety-eight F_2-derived F_3 families

were additionally tested. The F_3 plants were grown in a greenhouse at 15-21 C and due to segregation for maturity and limited dew chamber space, eight inoculations were carried out over a period of 21 days. F_1, F_2 and F_3 plants were inoculated with isolate 3SA62 of P. recondita f.sp. tritici. Flag leaves of plants of the F_2 and F_3 generations were inoculated when most plants were between late-boot and flowering stages [Romig scale 11-16 (15)]. In all inoculations, Line E and Glenlea were included as checks. Plants were fertilized as described for the latent period study. Inoculated F_2 and F_3 plants were placed in a greenhouse at 15-21 C until evaluation of reaction types.

One hundred and eighty F_2 seedlings from the cross between Line E and Glenlea were tested for resistance to isolate 3SA57 (Table 6.1). Inoculated seedlings were maintained in a greenhouse at 19-23 C with illumination as described above until the ratio of resistant to susceptlible plants was determined 11 days after inoculation.

Chi-square values for F_2 and F_3 ratios were calculated (100) and the probability that observed ratios resembled expected ratios was determined.

6.4 RESULTS

6.4.1 Resistance of adult plants of Glenlea

Infection type x (;12+c) was produced on each flag and penultimate leaf tested. Mean latent period was somewhat shorter in flag leaves than in penultimate leaves (Table 6.2) but the difference was not statistically significant. Mean latent period was significantly shorter in leaves of plants in the boot than in the anthesis stage (Table 6.2).

6.4.2 Resistance of seedlings of Glenlea

Infection types produced at 18 C and 31 C on Glenlea and other wheat genotypes that possess different genes for resistance are shown in Table 6.3. Isolate 3SA126 produced infection types 3 to 4 on seedlings of each of the wheats at both temperatures, indicating that this isolate is virulent to the genes evaluated. The other isolates tested produced infection types that indicated seedling resistance depending on the temperature of testing and the genotype of the wheat host. Manitou was susceptible at 18 and 31 C to all isolates, except to isolates 3SA122 and 3SA128, which were avirulent to Lr13 at the higher temperature. Era was resistant to all isolates tested at 31 C except to isolate 3SA126. The low reaction produced by isolates 3SA122

Table 6.2. Latent period[1/](hr) of isolate 3SA62 of Puccinia recondita f.sp. tritici in the flag and flag-1 leaves of Glenlea plants inoculated at two growth stages

Leaf	Growth stage		Leaf means[1/]
	Boot	Anthesis	
Flag	220 b	233 ab	227 A
Flag-1	226 ab	238 a	232 A
Growth stage means[1/]	223 B	236 A	

[1/]Values followed by different letters differed significantly at P=0.05 according to Tukey's procedure (100). Growth stage means should not be compared with leaf means.

Table 6.3. Seedling infection types of adult-plant-resistant wheat genotypes at two temperatures to six isolates of Puccinia recondita f.sp. tritici

Cultivar or Line	Infection type/Isolate/Temperature											
	3SA121		3SA122		3SA123		3SA126		3SA127		3SA128	
	18 C	31 C	18 C	31 C	18 C	31 C	18 C	31 C	18 C	31 C	18 C	31 C
Era (Lr10, 13, +)	;	;c	3	;c	;c	;c	3	3	3	;12	3	;c
Glenlea (Lr1, LrT2, +)	3+	;12	0;	0;	3+	;12	3+	3+	3+	3	3++	;12
Manitou (Lr13)	4	3	4	;1cn	4	3+	4	4	4	4	4	;1cn
Sinton (Lr10, 13, +)	3	;c	2+3	;cn	2-	;1c	3+	3+	4	4	3+	2=
Line E (check)	3++	3++	3++	3++	3++	3++	3++	3++	3++	3++	3++	3++

and 3SA128 on Era was attributed to Lr13 whereas the low reactions produced by isolates 3SA121 and 3SA123 were attributed to Lr10, Lr13 and additional resistance factors. Isolate 3SA127 was virulent to Era at 18 C but avirulent at 31 C, indicating an unknown gene for high-temperature seedling resistance. Sinton, also possessing Lr10 and Lr13, was susceptible to isolate 3SA121 and only moderately resistant to isolate 3SA123 at 18 C, thus differing from Era. Isolates 3SA122 and 3SA128 indicated the presence of Lr13 in Sinton when tested at 31 C. Sinton was susceptible to isolates 3SA126 and 3SA127 at both temperatures. Evidently Era and Sinton are not genetically similar though they have some genes for resistance in common. Glenlea was resistant at 31 C to isolates 3SA121, 3SA122, 3SA123 and 3SA128 and susceptible to isolates 3SA126 and 3SA127. It was susceptible at 18 C to all isolates except 3SA122, which lacks virulence to Lr1.

The seedling plants derived from F_2, F_3 and F_4 seed of the cross between Line E and Glenlea indicated segregation for resistance to isolates 3SA121 and 3SA127 (Table 6.4). Because the number of F_2 plants and F_3 families tested was statistically insufficient, conclusions on the genetics of seedling resistance could not be made. However, resistance observed at 31 C in seedlings derived from the cross involving Line E and Glenlea was much more pronounced in some progeny than

in Glenlea. Line E, Thatcher and RL6003 (Lr1) did not show low infection types to isolates 3SA121 or 3SA127 at 16 C or 31 C. Isolate 3SA121 produced infection types ;1, ;12, 3 and 3+ on F_2 seedlings evaluated at 31 C. Isolate 3SA127, virulent to Glenlea at 31 C, produced the low infection types $\underline{x}^{=}$, $\underline{2}^{-}$ and 2+3 on individual plants in eight of the 24 F_3 families tested at 31 C with this culture. Isolate 3SA121, avirulent to Glenlea at 31 C, produced low infection types in the range ;c to 2+3 in 17 of the 21 F_3 families tested at this temperature. Isolate 3SA121 produced high infection types on Glenlea at 16 C, but low infection types in the range ;1 to 2+3 were observed in 14 of the 25 F_3 families evaluated at the lower temperature. In the F_4 generation tested at 31 C with isolate 3SA121, low infection types (;n to x2) were observed in seven of the eight families evaluated. Isolate 3SA127 depicted low infection type ;12c at 31 C in four out of eight different F_4 families.

In the test designed to determine whether the high-temperature seedling resistance of Glenlea was due to genes LrT2, LrT3 or Lr13, isolate 3SA121 produced low infection types at 29 C only on Glenlea and lines H9, H10 and H11 (Table 6.5). At 17 C, isolate 3SA121 was virulent to all seedlings evaluated, except line H11 which exhibited an intermediate reaction. All the cultivars and lines tested were susceptible to isolate

Table 6.4. Detection of seedling resistance at two temperatures to isolates 3SA121 and 3SA127 of *Puccinia recondita* f.sp. *tritici* in the F_2, F_3 and F_4 generations of the cross between Line E and Glenlea

Isolate	No. of plants or families per generation		Temp.	Ratio			Total no. of plants
				Res.	Segr.	Susc.	
3SA121	F_2	34	31 C	11		23	34
	F_3	21	31 C	3	14	4	627
	F_3	25	16 C	6	8	11	638
	F_4	8	31 C	5	2	1	237
3SA127	F_3	24	31 C	0	8	16	680
	F_4	8	31 C	4	0	4	201

Table 6.5. Infection types produced by isolates 3SA121 and 3SA126 of *Puccinia recondita* f.sp. *tritici* at two temperatures on primary leaves of lines derived from the wheat cultivar Glenlea and of lines with the genes *LrT2* and *LrT3*

Cultivar or Line	Isolate/Temperature			
	3SA121		3SA126	
	17 C	29 C	17 C	29 C
Glenlea	3-	;12c	3-	3
Line E	3++	3+	3+	3++
Thatcher	3+	3+	3	3++
Manitou (*Lr13*)	3+	3	3++	3++
Line 897 (*LrT2*)	3+	3	3+	3
RL6058 (*LrT2*)	3	3	3+	3
Line 896 (*LrT3*)	3	3	3+	3
RL6050 (*LrT2* + *T3*)	3	3	3	3
Line H9	3++	;12=c	4	3++
Line H10	3+	;1=c	3+	3++
Line H11	2+3	;1=cn	3+	3++
Line H12	3++	3++	3++	3++

3SA126 at both temperatures (Table 6.5).

6.4.3 Inheritance of resistance

The segregation for seedling resistance to isolate 3SA57 of P. recondita f.sp. tritici in a F_2 population derived from the cross between Line E and Glenlea indicated a single dominant gene (Table 6.6). Infection types of the resistant plants were 0; or ;c.

The segregation for adult plant resistance in Glenlea to isolate 3SA62 was studied in the F_1, F_2 and F_3 generations of the Line E x Glenlea cross (Table 6.6). In the F_1 population the plants were all susceptible. In the F_2 population the observed ratio indicated segregation of two recessive genes for adult plant resistance. The infection types on the flag leaves of plants displaying resistance were ;c, ;1c, and 2c. Z-reactions were common. With this reaction the larger uredinia are produced towards the base of the leaf (13). In the F_3 population the segregation of the genes for resistance was again indicated (Table 6.6). Of the 198 F_3 families evaluated, 92 were homozygous resistant, eight homozygous susceptible and 98 families segregated for adult plant resistance. Resistant F_3 plants were characterized by flag leaf infection types in the range ; to Z4. Again, Z-reactions were common. Reactions of Glenlea and Line E were resistant (x) and susceptible

Table 6.6. Segregation ratios of genes for resistance to isolates 3SA57 and 3SA62 of *Puccinia recondita* f.sp. *tritici* in F_1, F_2 and F_3 progenies of the cross between line E and Glenlea

Generation	Isolate	Number of plants or families			Total no. of plants	Expected ratio	χ^2	P
		Res.	Segr.	Susc.				
Seedlings								
F_2	3SA57	139		41	180	3:1	0.474	0.50-0.25
Adult plants								
F_1	3SA62	0		49	49			
F_2	3SA62	79		121	200	7:9	1.468	0.25-0.10
F_3	3SA62	92	98	8	3461	7:8:1	1.890	0.50-0.25

(3++), respectively.

6.5 DISCUSSION

The resistance of Glenlea to South African isolates of P. recondita f.sp. tritici was evaluated because this cultivar has been useful in the management of the leaf rust pathogen in Canada for many years (28, 86). Glenlea has considerable potential for use as a source of adult plant resistance in South Africa and furthermore, genes conditioning resistance in Glenlea were readily transmitted to its progeny in crosses with Line E.

The segregation of a single dominant gene for resistance to isolate 3SA57 in seedlings derived from Glenlea agrees with the report of Dyck et al. (28). Data from the present study do not identify the gene but Canadian studies indicated that the gene involved is probably Lr1 (28). Moreover, in the latter report (28) Glenlea showed negligible seedling resistance in addition to that conferred by Lr1. In the study with South African isolates of P. recondita f.sp. tritici, extremely high levels of seedling resistance in Glenlea derivatives were observed. Detection of this resistance depended on the isolate used and temperature. Although it was most readily detected at 29-31 C, some Line E x Glenlea families exhibited seedling resistance at 16-17 C. Furthermore, Line E x Glenlea progenies displayed seed-

ling resistance at 31 C to isolate 3SA127 despite the fact that this isolate is virulent to Glenlea at 31 C. Apparently the gene for high-temperature seedling resistance is inhibited in Glenlea since its expression in the Line E background was much more pronounced.

The adult plant resistance of Glenlea was conferred by two recessive genes. Determination of latent period and infection type on sequentially formed terminal leaves of plants at two growth stages indicated that the adult plant resistance of Glenlea, as expressed by flag leaves, is stable. Therefore, evaluation of adult plant reaction of plants between the boot and flowering stages was appropriate for greenhouse assessment of the genetic nature of resistance in Glenlea to P. recondita f.sp. tritici. Dyck et al. (28) also found two genes for adult plant resistance in Glenlea, but the genes segregated in a dominant manner in crosses with lines RL6011 (Lr12) and RL6044 (Lr22a). Such a reversal in dominance for genes for resistance to leaf rust has been reported (23).

The relationship between the genes for adult plant resistance in Glenlea and the gene for high-temperature seedling resistance is not clear. Infection type studies showed that the South African isolates of P. recondita f.sp. tritici tested could not detect LrT2, a gene previously reported to be present in Glenlea (28).

Although LrT2 is considered to be a gene for adult plant resistance in Glenlea (28), it has been detected in the seedling stage under certain conditions (26). The temperature-sensitive gene identified in the present study was detected in a way similar to that reported for the effects of Lr13 (75). However, isolates 3SA121 and 3SA123 are both virulent to Lr13 at 31 C, but avirulent to Glenlea at the same temperature. Similarly, Dyck et al. (28) reported that the second gene for adult plant resistance in Glenlea was allelic to, or closely linked with Lr13. Although genetic evidence was not provided in the present study, the temperature-sensitive gene in Glenlea appears be the same gene described earlier as linked or allelic to Lr13 (28).

Complementary effects between genes for resistance to P. recondita f.sp. tritici have been reported for the pairs LrT2 and LrT3 (26) and Lr13 and Lr16 (90). Therefore, the highly effective and durable resistance to leaf rust in Glenlea could be ascribed to the combination of LrT2 with the gene linked or allelic to Lr13. The characterization of this combination is of considerable value to the South African wheat breeding program. By using appropriate isolates of P. recondita f.sp. tritici, selection for the temperature-sensitive gene in crosses with LrT2 could be conducted in the seedling stage. Superior levels of adult plant resistance due to complementary gene effects could

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Appendix Table 1. Romig scale (15) for assessing development of wheat plants

Scale	Description
1	One shoot
2	Beginning of tillering
3	Tillers formed, leaves often twisted spirally
4	Beginning of the erection of pseudo-stem, leaf sheath beginning to lengthen
5	Pseudo-stem (formed by sheaths of leaves) strongly erected
6	First node of stem visible at the base of shoot
7	Second node of stem formed, next-to-last leaf just visible
8	Last leaf visible, but still rolled up; head beginning to swell
9	Ligule of last leaf visible
10	Boot stage, sheath of last leaf completely grown out, head swollen but not yet visible
11	Awns just showing
12	Heading: 1/4 of heading completed
13	Heading: 1/2 of heading completed
14	Heading: 3/4 of heading completed
15	Heading: 95 % of heads out of sheath
16	Beginning of flowering

Appendix Table 1 (continued). Romig scale (15) for assessing development of wheat plants

Scale	Description
17	Flowering complete to top of head
18	Flowering complete to base of head
19	Kernels near middle of head 1/8 formed
20	Kernels near middle of head 1/4 formed
21	Kernels near middle of head 1/2 formed
22	Kernels near middle of head 3/4 formed
23	Kernels fully formed, contents watery
24	Early milk
25	Milk
26	Late milk
27	Early dough
28	Mid dough: kernel soft but dry
29	Late dough: kernel hard but not ripe
30	Ripe
31	Harvest