Virulence in *Puccinia recondita* f. sp. *tritici* Isolates from Canada to Genes for Adult-Plant Resistance to Wheat Leaf Rust

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ABSTRACT

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Sixty-seven isolates of *Puccinia recondita* f. sp. *tritici* collected in Canada were tested for virulence to adult plants of Thatcher near-isogenic wheat lines with leaf rust resistance genes *Lr13, Lr22a, Lr34, Lr35,* and *Lr13* and *Lr34* combined. All of the isolates had low infection type to the Thatcher lines with *Lr22a* and *Lr35*. All isolates had lower infection type and lower rust severity on the Thatcher line with *Lr34* compared with Thatcher. The isolates were polymorphic for virulence to the Thatcher line with *Lr13*; many isolates were completely virulent to this line, and other isolates produced very low or intermediate avirulent infection type. On the Thatcher line with *Lr13* and *Lr34* combined, many isolates had infection type and rust severity similar to the Thatcher line with *Lr34*, while other isolates had lower infection type and rust severity compared with the single-gene lines with *Lr13* or *Lr34*. Fifteen isolates with low, intermediate, and high infection type to adult plants with *Lr13* were tested for infection type on seedling plants of the Thatcher lines with resistance genes *Lr13, Lr22a, Lr34, Lr35,* and *Lr37,* and on Thatcher lines with *Lr13* paired with seedling resistance genes. Most isolates were completely virulent to seedling plants with *Lr13* and *Lr22a*. Plants with *Lr37* expressed seedling resistance to all isolates tested. Seedling plants with *Lr34* had lower infection types to all isolates compared with Thatcher. Seedlings with *Lr35* had high infection types to most isolates, with varying amounts of chlorosis. The Thatcher lines with *Lr13* plus seedling resistance genes were most resistant to the isolates that had very low infection types on adult plants with *Lr13* and intermediate infection types on plants with seedling resistance genes. Genes *Lr22a, Lr35,* and *Lr37* offer additional sources of highly effective leaf rust resistance in wheat.

Additional keywords: specific virulence, *Triticum aestivum*

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Virulence surveys of cereal rust fungi have traditionally used differential host lines that express resistance in the primary leaves of seedling plants. Wheat leaf rust (*Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. & E. Henn.) virulence phenotypes can be characterized on host seedling differential sets within 35 days from receipt of the rust collection. In the annual survey of physiologic specialization in *P. recondita* f. sp. *tritici* in Canada, more than 250 single-uredinial isolates are tested for virulence on differential sets within a 2-month period (12,15). Inoculation of spores from single uredinia onto differential sets of seedling plants minimizes the amount of rust required and also the amount of time and space required to grow the differential sets. Surveys of wheat leaf rust in Canada using seedling differentials have been very useful in describing virulence variation, geographic distribution of virulence phenotypes, and how leaf rust phenotypes change in response to host selection (11,14,15).

The most durable leaf rust resistance in wheat (*Triticum aestivum*) is conditioned by adult-plant resistance genes (1,23). Genes *Lr13* and *Lr34* are important in North American spring wheats, in breeding programs in South America (16), and in the CIMMYT program (24). However, the frequency of *P. recondita* f. sp. *tritici* isolates with virulence to either of these genes has not been directly measured in leaf rust virulence surveys conducted in North America (12,15,20). Large-scale testing of *P. recondita* f. sp. *tritici* isolates for virulence to adult-plant genes requires more greenhouse space, more inoculum of the individual survey isolates compared with testing on seedling differentials, and 2 to 3 months for the plant to reach the flag leaf stage, when resistance is most effective. Genes *Lr22a* and *Lr35* are adult-plant resistance genes that were derived from species related to wheat (5,10). These genes have not yet been widely deployed in wheat cultivars and are sources of highly effective leaf rust resistance.

Gene *Lr13* was shown previously to interact with other leaf rust resistance genes in cultivars and near-isogenic lines to condition a higher level of resistance than expressed by lines with the single genes (13,25). *Lr13* enhances the effect of certain seedling resistance genes that by themselves confer an intermediate infection type (13). Since these tests were conducted with seedling plants, the effect of the *Lr13* adult-plant infection type for each isolate on the degree of interaction for resistance was not determined. The objective of this study was to characterize a representative collection of *P. recondita* f. sp. *tritici* isolates from Canada for virulence to adult plants of near-isogenic lines with resistance genes *Lr13, Lr22a, Lr34, Lr35,* and *Lr13* and *Lr34* combined. Information on specific virulence to adult-plant resistance genes in a large representative collection of *P. recondita* f. sp. *tritici* isolates will provide an estimate of the frequency of isolates in the general population with virulence to these genes and hence their potential effectiveness in breeding programs. Selected isolates were further tested to determine the effect of isolates with different infection type to adult plants with *Lr13* on interaction for enhanced resistance between *Lr13* and seedling resistance genes. Seedling plants with *Lr22a, Lr34, Lr35,* and *Lr37* were also tested with selected isolates to determine if these genes expressed any discernible resistance under normal ambient greenhouse conditions.

MATERIALS AND METHODS

***P. recondita* f. sp. *tritici* isolates.** Fifty-six isolates from the 1994 survey (17) were used in this study. The isolates from 1994 were chosen based on their three-letter virulence phenotype designation to the 12 seedling differentials in the *Prt* nomenclature (19). Isolates from 27 different virulence phenotypes collected in 1994 were tested. Multiple isolates of the most common virulence phenotypes were included to determine if variation for virulence to the adult-plant resistance genes occurred within them. Phenotypes MBR (nine isolates tested), MFM (eight isolates tested), and TDG (four isolates tested) were the three most common in Manitoba and Saskatchewan in 1994. Three isolates of PBL, the most common phenotype in Ontario and Quebec in 1994, were included. Seven isolates from the 1989 survey in Canada (12) were included, as were four isolates

Table 1. Year of collection, wheat host of origin[a], isolate number, virulence phenotype[b], and infection type[c] for adult plants of Thatcher near-isogenic wheat lines with *Lr13* and *Lr13,34* of single uredinial isolates of *Puccinia recondita* f. sp. *tritici* collected in Canada

Year	Host	Isolate number	Virulence phenotype	TcLr13 March	TcLr13 June	TcLr13,34
1954	Spring wheat	56-1	BBB	;	;1+	0;/ TR[d]
1959	Spring wheat	59-1	MBB	;	;	0;/ TR
1959	Spring wheat	59-2	SBD	;	;	0;/ TR
1964	Spring wheat	64-1	CHB	;	;	0;/ TR
1989	Spring wheat	89-1	KBG	2+	;12–	0;/ TR
1989	Spring wheat	89-2	MBB	;	;	0;/ TR
1989	Spring wheat	89-3	MBG	;	12–	23/ 5
1989	Spring wheat	89-4	MDB	;12	4	0;/ TR
1989	Spring wheat	89-5	TBB	2+n	12	0;/ TR
1989	Spring wheat	89-6	TBG	;1	12	0;1–/ TR
1989	Spring wheat	89-7	TDG	;11+	12	0;1/ TR
1994	QW572	178-1	CBB	4	22+	22+/ 5
1994	CDC Teal (*Lr1,13,34*)	87-1	KBG	4	4	23/ 5-10
1994	Little Club	139-1	KBG	4	4	23/ 10
1994	Winter wheat	63-3	KDG	4	4	0;/ TR
1994	Belvedere	147-1	LBB	;1	12+	12/ 10
1994	Columbus (*Lr13,16*)	115-1	MBB	4	4	23/ 20
1994	Red Bobs	156-1	MBG	4	4	23+/ 30
1994	Spring wheat	183-1	MBG	4	4	23+/ 40
1994	Columbus (*Lr13,16*)	57-1	MBQ	2	33+	;12–/ 3
1994	Spring wheat	98-2	MBR	4	4	;12/ TR
1994	Winter wheat	68-1	MBR	2++	12	23/ 5
1994	Columbus (*Lr13,16*)	128-1	MBR	4	4	23/ 10-20
1994	Genesis (*Lr13*)	17-1	MBR	4	4	12/ TR
1994	Spring wheat	76-1	MBR	4	4	;12/ TR
1994	Katepwa (*Lr13*)	4-1	MBR	4	4	;1/ TR
1994	AC Domain (*Lr10,12,16,34*)	41-1	MBR	2++	4	;1– / TR
1994	Kenya Farmer	45-1	MBR	2++	12	; / TR
1994	Little Club	48-1	MBR	4	4	; / TR
1994	Spring wheat	101-1	MCB	4	4	0;/ TR
1994	Neepawa (*Lr13*)	19-1	MCR	4	4	33+/ 20
1994	BW 186	56-1	MCR	4	4	3/ 30
1994	Spring wheat	106-1	MDB	22–	23	;12–/ 5
1994	Little Club	34-1	MDG	;1	12-	;1/ TR
1994	CDC Teal (*Lr1,13,34*)	87-2	MDM	4	4	23+/ 10
1994	Spring wheat	75-1	MDM	4	4	33+/ 30
1994	Winter wheat	84-1	MDR	2	12	;1/ 3
1994	Glenlea (*Lr1,34*)	43-1	MDR	2	;12–	;12-/ TR
1994	Glenlea (*Lr1,34*)	31-2	MDR	2–	;12–	;1-/ TR
1994	Spring wheat	89-5	MFB	2++	4	0; / TR
1994	Spring wheat	94-1	MFM	4	4	23+/ 40
1994	Spring wheat	73-1	MFM	2++	22+	;1/ TR
1994	Spring wheat	71-1	MFM	2++	22+	22+/ 10
1994	Roblin (*Lr1,10,13,34*)	143-2	MFM	2++	22+	23/ 10
1994	Glenlea (*Lr1, 34*)	3-1	MFM	2++	4	;1/ TR
1994	Spring wheat	67-1	MFM	2++	22+	;2/ 3
1994	Roblin (*Lr1,10,13,34*)	12-1	MFM	2++	2+3	;12–/ 3
1994	Roblin (*Lr1,10,13,34*)	51-1	MFM	2++	4	;1/ TR
1994	Glenlea (*Lr1,34*)	118-1	NBB	;1	;	0;/ TR
1994	Klein Titan (*Lr3ka*)	152-1	PBL	4	4	23+/ 20
1994	Max	224-1	PBL	4	4	23+/ 20
1994	Winter wheat	212-1	PBL	4	4	23+/ 50
1994	QW572	178-2	PBR	2	12	23+/ 20
1994	Thatcher-*Lr9*	13-2	PDD	2++	4	23/ 5
1994	AC Domain (*Lr10,12,16,34*)	41-3	TBB	4	4	23/ 10
1994	Biggar (*Lr13*)	114-1	TBG	4	4	23+/ 5
1994	Stoa	144-1	TDB	2	2	23/ 5-20
1994	Winter wheat	70-1	TDB	2n	4	0;/ TR
1994	Winter wheat	105-1	TDB	2	;12	; / TR
1994	Marshall	10-1	TDG	4	22+	23/ 5
1994	Little Club	36-1	TDG	4	2+3	;12/ 5
1994	Winter wheat	69-1	TDG	22+	12	0;/ TR
1994	Spring wheat	78-1	TDG	4	4	34/ 20-30
1994	Little Club	37-1	TFB	4	4	23/ 5
1994	Spring wheat	80-1	TFB	;12	12–	;1/ TR
1994	Marshall	10-3	TFG	4	4	23/ 20
1994	Kenya Farmer	32-1	TFM	22+	12	0;/ TR

[a] Where known, wheat cultivar and leaf rust resistance genes are listed. Spring wheat indicates collections obtained from farm fields where identity of cultivar was not known. Cultivars Little Club and Kenya Farmer are susceptible to leaf rust, as were winter wheats. All other have some effective leaf rust resistance.
[b] Virulence phenotypes are given in the three-letter code used in the *Prt* nomenclature (19).
[c] Infection types as described in the *Prt* nomenclature (19). Most common infection types listed first.
[d] Leaf rust severity (%) estimated using the Cobb scale (21). TR = trace level of uredinia.

that were collected before 1966. When known, the wheat line or cultivar from which the leaf rust isolates were collected was recorded, and the leaf rust resistance genotype was noted (Table 1). Collections from farm fields were recorded as spring wheat.

Each isolate was increased on primary leaves of Little Club wheat seedlings, in 10-cm-diameter pots, that had been treated at emergence with maleic hydrazide to prevent further leaf formation. Plants were inoculated after the first leaves had fully formed by atomizing a suspension of Dustrol (Ciba-Giegy Canada, Mississauga, ON) nonphytotoxic industrial oil (300 to 500 µl) mixed with 1 mg of urediniospores. The seedling plants were placed in a dew chamber at 15°C for at least 18 h, then removed to a greenhouse bench at 15 to 25°C with 8 h of supplemental fluorescent lighting. Clear plastic cylinders without tops were placed around each pot to reduce cross-contamination between isolates. After 10 to 14 days, urediniospores from each isolate were collected into size 00 gelatin capsules using a cyclone spore collector (24). The urediniospores were dried in a desiccator overnight, and then placed in glass tubes and stored at –80°C. Isolates were heat-shocked for 5 min at 40°C prior to inoculation on adult plants.

Host materials. Flag leaves of Thatcher near-isogenic lines RL 4031 (Tc*Lr13*), RL 6044 (Tc*Lr22a*), RL 6058 (Tc*Lr34*), RL 6082 (Tc*Lr35*), and RL 6114 (Tc*Lr13,34*) and Thatcher were inoculated at heading with all selected *P. recondita* f. sp. *tritici* isolates. Thatcher has the adult-plant resistance gene *Lr22b*. Almost all *P. recondita* f. sp. *tritici* isolates found in North America are virulent to this gene. Adult plants were inoculated in two separate tests. Lines Tc*Lr13,* Tc*Lr22a,* Tc*Lr34,* and Thatcher were inoculated with all leaf rust isolates in March 1995. All isolates were also tested on adult plants of Tc*Lr13,* Tc*Lr35,* Tc*Lr13,34,* and Thatcher in June 1995. The isolates were tested twice on Tc*Lr13* because they were polymorphic for virulence to this line. In both tests, four plants (one plant of each line) were grown together in 15-cm-diameter fiber pots in a sand–peat–soil mixture. The plants were grown in a greenhouse at 15 to 25°C, with 8 h of supplemental fluorescent lighting, and were fertilized with 20-20-20 NPK as needed. The plants were trimmed to two tillers each. Heads were removed from the plants after emergence to delay senescence of the flag leaves. Flag leaves in each pot were inoculated by atomizing 2 to 3 mg of urediniospores from a single *P. recondita* f. sp. *tritici* isolate in 500 µl of oil. After inoculation, the plants were placed in a dew chamber at 15°C for at least 18 h. After incubation, the plants were placed in greenhouses between 15 and 20°C, and the infection types (IT) on the flag leaves were evaluated 14 days later. Infection types

were read according to the Stakman scale (26) and the scale used by Long and Kolmer (19): 0 = no visible sign of infection, ; = small hypersensitive flecks, 1 = small uredinia surrounded by necrosis, 2 = small uredinia surrounded by chlorosis, 3 = moderate uredinia without chlorosis, 4 = large uredinia without chlorosis, + = slightly larger uredinia than expected for the IT, – = slightly smaller uredinia than expected for the IT, c = more chlorosis than expected for the IT, and X = mesothetic mixture of uredinia and flecks. Isolates that produced infection type 0 to 2++ were considered avirulent, and those with IT 3 to 4 were considered virulent. The most common IT was listed first, followed by less frequent IT. The percentage of leaf rust severity on TcLr34, TcLr13,34, and Thatcher for each isolate was estimated using the modified Cobb scale (21). Infection type on each resistant Thatcher line was compared to the susceptible check Thatcher for each isolate.

Fifteen of the *P. recondita* f. sp. *tritici* isolates that had very low, intermediate, and high IT to adult plants of TcLr13 were inoculated to seedlings of lines TcLr13, RL 6000 (TcLr2a), RL 6123 (TcLr2a,13), RL 6048 (TcLr11), RL 6127 (TcLr11,13), RL6008 (TcLr17), RL 6129 (TcLr13,17), RL 6049 (TcLr30), RL 6134 (TcLr13,30), RL 6007 (TcLr3ka), RL 6126 (TcLr3ka,13), TcLr22a, TcLr34, TcLr13,34, TcLr35, RL 6081 (TcLr37), and Thatcher. Eight to 12 seeds of each line were planted in clumps in fiber flats filled with sand–peat–soil. Seedlings were grown in a greenhouse at 15 to 25°C with 8 h of supplemental fluorescent light. Plants were inoculated with 2 to 3 mg of urediniospores suspended in 500 µl of oil 8 days after planting, when the first leaves were fully expanded. Plants were incubated in a dew chamber at 15°C for at least 18 h, then removed to a greenhouse bench at 15 to 20°C. Infection types were evaluated 12 days after inoculation (19,26).

RESULTS

Adult-plant test. All isolates tested were avirulent on adult plants of TcLr22a and TcLr35, and had lower IT and rust severity on TcLr34 compared with Thatcher. All isolates had IT 2– on plants of TcLr22a, and IT ; on plants of TcLr35. On adult plants of TcLr34, all isolates had IT 23, with an estimated rust severity of 20 to 50%. On Thatcher, all isolates had IT 4, with severity of 90%, except isolate 59-2 (SBD), which was avirulent to gene Lr22b in Thatcher and had IT ; on Thatcher and all the Thatcher lines. The resistance response of TcLr34 could consistently be recognized by a direct comparison of the susceptible check Thatcher inoculated with the same isolates.

The tested isolates were polymorphic for virulence to adult plants with Lr13 (Table 1). In the March test, 13 isolates had IT ; to

;1, 25 isolates had IT 2– to 2++, and 29 isolates had IT 4. In the June test, 10 isolates had IT ; to ;12, 23 isolates had IT 12 to 2+3, and 34 isolates had IT 4. Most isolates produced the same or similar IT on TcLr13 between the two different tests. Of 67 isolates tested, only 11 had different avirulence–virulence ratings between the two tests. Three isolates had IT 4 in the March test and had IT 22+ to 2+3 in the June test. Eight isolates had IT ;12 to 2++ in March and IT 4 in June. Isolates with IT ; to ;1 had low IT in both tests.

Isolates 56-1 (BBB), 64-1 (CHB), and 59-1 (MBB) were collected before the release of wheat cultivars with Lr13 in western Canada. These isolates had IT ; on adult plants of TcLr13 (Table 1). Six of the isolates collected in 1989 had low IT on adult plants of TcLr13 in both tests, and one isolate had IT ;12 in March and IT 4 in the June test. None of the isolates from 1989 were virulent to TcLr13 in both tests. Twenty-one of the isolates collected in 1994 had low IT on TcLr13 in both tests, and 25 had high IT. Variation for virulence on TcLr13 occurred within virulence phenotypes as defined by IT on seedling differentials. Of the nine MBR isolates tested, two had low IT to Lr13 in both tests, one had a low IT in March and a high IT in June, and seven had high IT in both tests. Of the eight MFM isolates tested, five had low IT to TcLr13 in both tests, two had low IT in March and high IT in June, and one had high IT in both tests. Of the four TDG isolates tested, one had low IT on TcLr13 in both tests, two had high IT in March and low IT in June, and one had high IT on TcLr13 in both tests. A 2 × 2 contingency test (27) (hosts: lines with Lr13 versus those without; *P. recondita* f. sp. *tritici*: isolates virulent to Lr13 versus those avirulent) showed that isolates collected in 1994 with high IT to TcLr13 in March and June were significantly associated with hosts with Lr13 (X^2 = 5.08, 1 df). However, six isolates that had IT 2 to 2+ to TcLr13 in both tests were collected from cultivars or lines with Lr13.

Nineteen isolates collected in 1994, which had IT 23 on adult plants of TcLr34 and IT 4 on plants with TcLr13, had IT 23 and varied for rust severity on adult plants of TcLr13,34 (Table 1). Sixteen isolates had low IT on plants of TcLr13 in both tests and had very low IT and low rust severity on adult plants of TcLr13,34. However, seven isolates from 1994 had IT 4 on plants of TcLr13, yet had IT 0; to 12 and low rust severity on adult plants of TcLr13,34 (Table 1). Four other isolates had low IT on TcLr13 in both tests and IT 23 on plants of TcLr13,34. Eight isolates that varied for virulence to TcLr13 in the two tests had low IT on plants with TcLr13,34, and two other isolates that had different readings on TcLr13 had IT 23 to plants of TcLr13,34.

Seedling test. Most isolates tested had IT 3+ on plants with TcLr13 in the seedling test (Table 2). Two isolates, which were avirulent to TcLr13 in the adult-plant test, produced moderate-large uredinia surrounded by chlorosis (IT 3c to 3+c) on seedling plants of TcLr13. Enhancement of seedling resistance genes by Lr13 was most strongly and frequently expressed to isolates 118-1 (NBB), 89-2 (MBB), and 56-1 (BBB), which had IT ; on adult plants of TcLr13. These isolates also had intermediate IT on lines with genes Lr11, Lr17, Lr30, and Lr3ka. Thatcher lines with gene pairs Lr11, Lr13; Lr17, Lr13; Lr30, Lr13; and Lr3ka, Lr13 expressed enhanced resistance relative to the single gene lines to these three isolates. Thatcher lines with gene pairs Lr11, 13; Lr30, 13; and Lr3ka, 13 also had enhanced resistance relative to the single gene lines to isolate 80-1 (TFB), which had IT 12 on adult plants of TcLr13. Enhancement of seedling resistance by Lr13 was observed less frequently to the isolates that had intermediate or high IT on adult plants of TcLr13 (Table 2). For these isolates in most cases, IT on the lines with the paired resistance genes were the same as on the single gene lines.

All isolates tested had high IT on seedling plants of TcLr22a, IT 23 on seedling plants of TcLr34, and IT X on seedling plants of TcLr37 (Table 2). Most isolates had IT 33+ with varying amounts of chlorosis to seedling plants of TcLr35. Isolate 178-2 (PBR) had IT ;22+ on plants with TcLr35. Interaction for enhanced resistance was not observed in seedling plants of TcLr13,34, as compared with TcLr34.

DISCUSSION

All of the tested isolates were avirulent to the adult-plant resistance genes Lr22a and Lr35, and produced intermediate IT to adult plants with Lr34. The Thatcher lines with Lr22a and Lr35 are highly resistant in rust nursery tests (J. A. Kolmer, *unpublished*). The lack of isolates with virulence to these genes and the high levels of field resistance that they confer indicate that genes Lr22a and Lr35 would provide effective resistance in a breeding program. The isolates were highly polymorphic for virulence to Lr13. With the exception of isolate 59-2 (SBD), all isolates were virulent to Lr22b in Thatcher.

Gene Lr22a was derived from *Triticum taushii* (5) and currently is present only in the Canadian cultivar AC Minto (J. A. Kolmer, *unpublished*). Since this cultivar has been grown in a limited amount, the *P. recondita* f. sp. *tritici* population in Canada has not been widely exposed to Lr22a. AC Minto (Lr11, Lr13, Lr22a) and TcLr22a are highly resistant in the field to leaf rust (J. A. Kolmer, *unpublished*). Resistance conditioned by Lr22a was not evident in the seedling test. Virulence to Lr22a should be evaluated in juvenile or adult plants.

Gene *Lr34* has been widely used in North American spring wheats over the last 30 years (16). All isolates had intermediate IT 23 and lower rust severity to Tc*Lr34* compared with the susceptible check Thatcher. It is remarkable that isolates with equal virulence to Tc*Lr34* and Thatcher have not yet been found. Gene *Lr34* was initially described as a modifier of *Lr13* in the cultivar Frontana (8) and only later isolated, characterized singly, and mapped to chromosome 7D (2). In the field, lines with only *Lr34* can have variable amounts of leaf rust severity, with a mixture of small and large uredinia. At times, lines with only *Lr34* can appear to be susceptible. Cultivars and lines with *Lr34* combined with other genes that condition effective resistance have been highly resistant in the field (3,4,9). Resistance conditioned by *Lr34* could be distinguished in the seedling test, although in other tests seedlings with *Lr34* were indistinguishable from the susceptible check Thatcher (3,4,9). Dyck and Samborski (7) originally isolated *Lr34* in seedling plants that were tested in cooler temperatures and reduced light intensities.

Gene *Lr35* was derived from *Aegilops speltoides* (10) and is not present in any commercially grown wheat cultivar. Although resistance conditioned by Tc*Lr35* is optimally evaluated on flag leaves, primary leaves of seedling plants had variable amounts of chlorosis surrounding the uredinia. Kerber and Dyck (10) showed that resistance was expressed starting at the second leaf stage. In a Marquis cultivar background, male gametes with *Lr35* had a lower transmission rate, which resulted in distorted segregation ratios. Transmission of male gametes with *Lr35* was improved in a Thatcher background (10). Gene *Lr35* is linked with *Sr39* and is associated with higher protein content in a Marquis background (E. R. Kerber, *personal communication*).

Gene *Lr13* was first used in Canada in the cultivar Manitou, which was released in 1966. Manitou was highly resistant when first released. By 1974, however, leaf rust ratings on Manitou had increased to 60% (18). The widespread use of cultivars with *Lr13* selected *P. recondita* f. sp. *tritici* phenotypes with virulence to this gene. Since Manitou, every cultivar released by the Cereal Research Centre in Winnipeg, with the exception of AC Domain, has had *Lr13*. Cultivars with only *Lr13*, however, still have some effective resistance. Leaf rust severities on Neepawa and Katepwa in rust nursery tests in recent years have generally been 30 to 50%, with moderately resistant to moderately susceptible responses (J. A. Kolmer, *unpublished*). Despite the widespread use of cultivars with *Lr13*, isolates avirulent to this gene have been maintained in the *P. recondita* f. sp. *tritici* population in Manitoba and Saskatchewan. Isolates avirulent to *Lr13* may originate from susceptible winter wheats in the southern U.S. plains. There was an association between isolates with virulence to *Lr13* and host cultivars known to have *Lr13*. However, isolates that had intermediate IT 2 to 2+ to Tc*Lr13* were also collected from hosts with this gene. Isolates with intermediate IT to *Lr13* are still capable of infection and reproduction on cultivars with this gene. This may help explain why isolates avirulent to *Lr13* can still be found.

In this study, the frequency of virulence to *Lr13* was highest in isolates collected in 1994. As isolates virulent to *Lr13* increase in frequency, the effectiveness of *Lr13* resistance will be eroded. If virulence to *Lr13* continues to increase, cultivars with combinations of *Lr13* and other genes (e.g., Roblin *Lr1, Lr10, Lr13, Lr34*; Columbus *Lr13, Lr16*; AC Minto *Lr11, Lr13, Lr22a*; AC Cora *Lr13, Lr21*) may start to have higher leaf rust severities and response readings in rust nurseries and farm fields.

All of the isolates that varied for avirulence–virulence to *Lr13* in the two tests had intermediate IT in one of the tests. Isolates with intermediate IT are probably heterozygous for virulence to *Lr13* and may be more sensitive to temperature and light conditions than are homozygous avirulent isolates. Virulence to *Lr13* was determined to be conditioned by a single gene in *P. recondita* f. sp. *tritici* (P. L. Dyck, *personal communication*).

Most isolates that produced high IT on adult plants of Tc*Lr13* in both tests and IT 23 on adult plants of Tc*Lr34* produced IT 23 with variable rust severity on plants of Tc*Lr13,34*. However, some isolates that were completely virulent to Tc*Lr13* in both tests produced very low IT on plants of Tc*Lr13,34*. The isolates were tested on the Tc*Lr34* and Tc*Lr13,34* lines at different times, which could have affected the results since expression of *Lr34* can be influenced by temperature (22). Selected *P. recondita* f. sp. *tritici* isolates from the 1995 virulence survey in Canada were tested on adult plants of Tc*Lr13*, Tc*Lr34*, and Tc*Lr13,34* simultaneously (J. A. Kolmer, *unpublished*). A number of isolates from the 1995 survey also produced IT 3+ on Tc*Lr13*, IT 23 on Tc*Lr34*, and IT 0; on Tc*Lr13,34*. The IT expressed by adult plants with *Lr34* generally has been stable in the ambient greenhouse conditions in Winnipeg in recent virulence surveys and in previous work (9).

Genes *Lr13* and *Lr34* apparently can interact for enhanced resistance even to *P. recondita* f. sp. *tritici* isolates that produce a moderate to high IT on plants with both genes singly. Previously, German and

Table 2. Infection types[a] of *Puccinia recondita* f. sp. *tritici* isolates with low, intermediate, and high infection types to adult plants of Thatcher near-isogenic lines with *Lr13* to seedlings of near-isogenic Thatcher lines with single and two genes for leaf rust resistance

Thatcher line	Isolates														
	NBB 118-1	MBB 89-2	BBB 56-1	TFB 80-1	PBR 178-2	MDR 84-1	KBG 89-1	MBR 45-1	MDB 106-1	MFM 73-1	MCR 19-1	MBR 17-1	PBL 152-1	TFB 37-1	MFM 94-1
Lr13-Adult	;	;	;	12	12	2	2	22+	22+	2+	4	4	4	4	4
Lr13-Seedling	3+c	33+	3c	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3c
Lr2a	;2	0	0	3+	2+	0;	3+	0;	0;	0;	0;	0;	;2–	3+	0
Lr2a,13	;	0	0	3+	22+c	0;	3+	0;	0	0;	0;	0;	;2–	3+	0
Lr11	12–	;22–	;2–	;2–	3+	3+	3+	3+	;22–	;2–	3+	3+	2–	2–	;2–
Lr11,13	;1=	;12–c	;1+	;1–c	3+	3+	3+	3+	;12–	;2–	3+	3+	2–	2–	;2–
Lr17	;1	;1	;1–	;1	;1–	;11+	;1–	;1–	;1	;1	;1	;1+	;1–	;11+	;1–
Lr17,13	;1=	;1–c	;	;1	;1–	;1	;1–	;1–	;1–	;1	;1	;1=	;1–	;1–	;1–
Lr30	;22–	;2–	;2–	;22–	3+	3+	;2–	3+	;2–	33+	3+	3+	23c	22–	33+
Lr30,13	;1=	;1+c	;1–	;11+	3+	3+	;2–	3+	;2–	33+	3+	3+	2+c	22–	3
Lr3ka	;2–	;12=	;1=	22–	3+	3+	;12–	3+	;2–c	33+	3+	3+	3+	2–	3
Lr3ka,13	;1=	;1+c	;1–	;22–c	3+	3+	;2–	3+	;2–c	33+	3+	3+	3+	22+	23–
Lr22a	3+	3+	33+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	33+
Lr34	2+3	2+3	2+3	23	23	23	23	2–3	23	23	23	23	23	2+3	23–
Lr35	3+c	33+	33+	33+c	;22+	33+c	33+c	33+c	33+c	33+c	33+	33+	33+c	33+c	2–3
Lr37	X	X	;12–	X	X	;23	;12	X	X	X	;23	X	X	X	;12–
Lr13,34	2+3	23	23	23	23	23	23	23	23	23	2+3	22+c	23	2+3	23
Thatcher	4	3+	3+	3+	3+	3+	3+	3+	4	3+	3+	3+	3+	3+	3+

[a] Infection types as described in Long and Kolmer (19).

Kolmer (9) found that *Lr34* enhanced resistance in seedling and adult plants only with other resistance genes that conditioned some degree of effective resistance when present singly. In rust nursery tests and farm fields, wheats with *Lr13,34* can have variable levels of leaf rust severity, usually ranging from trace levels to 30%, depending on the severity of the leaf rust epidemic. Interaction between *Lr13* and *Lr34,* even to isolates that are completely virulent to *Lr13,* may help explain why wheats with this gene combination have had durable leaf rust resistance (3,4,8,23). Interaction between *Lr13* and *Lr34* for enhanced resistance was not evident in the seedling test.

In the seedling test, lines with *Lr13* paired with genes *Lr2a, Lr11, Lr17, Lr3ka,* and *Lr30* best expressed resistance to isolates that had very low IT on adult plants of Tc*Lr13,* and had an intermediate IT on plants with the seedling resistance genes. Gene interaction was not as frequently observed with isolates that produced intermediate or high IT to adult plants of Tc*Lr13,* even though many of these isolates also produced intermediate IT on plants with some of the seedling resistance genes. Virulence of the isolates to adult plants with *Lr13* affected the degree of interaction for enhanced resistance between resistance genes in seedling tests.

All isolates tested produced an IT X to plants with *Lr37* in the seedling test. Tc*Lr37* (6) is highly resistant in field tests (J. A. Kolmer, *unpublished*). *Lr37* along with genes *Lr22a* and *Lr35* offers additional sources of highly effective leaf rust resistance. These genes can be backcrossed into adapted germ plasm by screening progeny lines at the appropriate growth stage using *P. recondita* f. sp. *tritici* isolates virulent to the recurrent parent.

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