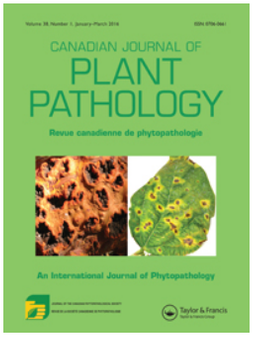

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Canadian Journal of Plant Pathology

ISSN: 0706-0661 (Print) 1715-2992 (Online) Journal homepage: http://www.tandfonline.com/loi/tcjp20

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To cite this article: Brent D. McCallum, Colin W. Hiebert, Sylvie Cloutier, Guus Bakkeren, Silvia B. Rosa, D. Gavin Humphreys, G. Francois Marais, Curt A. McCartney, Vinay Panwar, Christof Rampitsch, Barry J. Saville & Xiben Wang (2016) A review of wheat leaf rust research and the development of resistant cultivars in Canada, Canadian Journal of Plant Pathology, 38:1, 1-18, DOI: 10.1080/07060661.2016.1145598

To link to this article: http://dx.doi.org/10.1080/07060661.2016.1145598

Accepted author version posted online: 27 Jan 2016.
Published online: 02 Mar 2016.

Can. J. Plant Pathol., 2016
Vol. 38, No. 1, 1–18, http://dx.doi.org/10.1080/07060661.2016.1145598

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A review of wheat leaf rust research and the development of resistant cultivars in Canada

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(Accepted 18 January 2016)

Abstract: Wheat leaf rust, caused by *Puccinia triticina* Eriks., is of worldwide concern for wheat producers. The disease has been an annual problem for Canadian wheat producers since the early days of wheat cultivation in the 1800s, and research focused on combating this disease began in the early 1900s. Significant progress was made towards understanding the epidemiology of wheat leaf rust and developing genetic resistance in many countries worldwide. This review paper focuses exclusively on the research and development done in whole, or in part, in Canada. An integrated approach to controlling wheat leaf rust consisted of research in the following areas: the early research on wheat leaf rust in Canada, breeding and commercialization of high quality rust resistant wheat cultivars, discovery and genetic analysis of leaf rust resistance genes, the population biology and genetics of the *P. triticina*/wheat interaction. This review summarizes the research in each of these areas and the connections between the different aspects of the research. A multi-disciplinary team approach has been key to the advancements made within these diverse research fields in Canada since the early 1900s.

Keywords: breeding, brown rust, molecular biology, pathology, *Puccinia triticina*

Résumé: La rouille brune, causée par *Puccinia triticina* Eriks., préoccupe les producteurs de blé partout dans le monde. La maladie a été un problème annuel récurent pour les producteurs canadiens depuis les débuts de la culture du blé dans les années 1800, et des chercheurs se sont efforcées de trouver des moyens de lutter contre cette maladie dès les années 1900. Des progrès majeurs ont été faits dans de nombreux pays quant à la compréhension de l'épidémiologie de la rouille brune et au développement de la résistance à cette maladie. Cet article de synthèse met exclusivement l'accent sur les recherches et les progrès accomplis au Canada, en tout ou en partie. Une approche intégrée relative à la lutte contre la rouille brune a consisté en recherches dans les domaines suivants: les premières recherches sur la rouille brune au Canada; la sélection et la commercialisation de cultivars de blé de grande qualité, résistants à la rouille; la découverte et l'analyse génétique des gènes de résistance à la rouille; la biologie des populations et la génétique des interactions blé-*P. triticina*. Cet article présente un aperçu de la recherche qui a été faite dans chacun de ces domaines et les liens qui existent entre les différents aspects de cette recherche. Une équipe multidisciplinaire a été la clé des progrès réalisés dans ces diverses sphères de recherche au Canada depuis le début des années 1900.

Mots clés: biologie moléculaire, pathologie, *Puccinia triticina*, rouille brune, sélection

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Early research on wheat leaf rust in Canada

The initial focus of rust research on wheat in Canada was primarily on stem rust (caused by *Puccinia graminis* f. sp. *tritici* Eriks. & Henn.) as a consequence of the devastating losses in Western Canada during the early years of wheat production, such as the severe epidemics in 1902, 1904, 1916, 1923, 1935 and 1938 (Craigie 1945). The stem rust epidemic of 1916 was particularly disastrous, with the loss of approximately half of the wheat crop, estimated at over $102 million dollars at that time (Johnson 1961b). Leaf rust infection, while not as damaging as stem rust, was also severe during many of these early years of wheat production, including 1921, 1925, 1927, 1930, 1932 and 1935, and was moderately severe in 1923, 1924, 1931, 1934 and 1937 (Craigie 1939). Between 1921 and 1937, annual losses caused by leaf rust were estimated at 7% yield reduction in Manitoba (Craigie 1939). Losses due to the combined effects of leaf and stem rust in Manitoba and Saskatchewan were estimated at 16.8% yield loss between 1925 and 1935, representing an average annual loss of $31 million dollars at that time (Greaney 1936), with a current (2015 inflated) value of $532 million. The most widely grown wheat cultivars during 1910–1935 were 'Red Fife' (1870 until approximately 1910) and 'Marquis' (1911 until 1939) (McCallum & DePauw 2008), which were susceptible to stem and leaf rust (Dickinson 1976). In response to yield losses due to leaf and stem rust, the Dominion Rust Laboratory was established in Winnipeg in 1925 (Johnson 1961b). Research was initiated on yield loss estimates, chemical control, epidemiology, breeding for resistance, host/parasite interactions, and the fundamental biology of economically important rust fungi. Early chemical control of rust on wheat was mainly through the use of sulphur dusts (Bailey & Greaney 1928), while later research involved synthetic chemicals (Peturson et al. 1958). The production of genetically resistant cultivars, by utilizing stem rust (*Sr*) and leaf rust (*Lr*) resistance genes, was sought as an alternative to chemical control because of the cost savings, the difficulty in applying fungicides, and environmental concerns.

Breeding and commercialization of high quality rust resistant cultivars

Genetic resistance to stem rust was first introduced with the registration of the cultivar 'Thatcher' in 1935, and subsequently of cultivars 'Renown' (registered in 1937), 'Regent' (registered in 1939) and 'Redman' (registered in 1946) (Peturson 1958). 'Thatcher' was the most popular cultivar and dominated the market in Canada from 1939 (when it surpassed 'Marquis') until 1968, when it was overtaken by 'Manitou' (McCallum & DePauw 2008). While 'Thatcher' was resistant to stem rust, it was very susceptible to leaf rust. 'Renown' was the first leaf rust resistant cultivar to be widely grown in western Canada, having the leaf rust resistance gene *Lr14a* (Samborski 1985). This gene provided effective resistance for several years but its effectiveness was lost by 1945 due to changes in the *P. triticina* population (Johnson & Newton 1946). Leaf rust epidemics in the 1930s and 1940s were shown to substantially reduce yield, grade, and protein content of these stem rust resistant cultivars, particularly 'Thatcher' (Peturson & Newton 1939; Peturson et al. 1945, 1948). 'Thatcher' was nearly immune to stem rust from the 1930s to the 1950s; however, it was susceptible to race 15B that significantly increased in frequency in 1952 (Peturson 1958). Concurrent epidemics of leaf rust and stem rust race 15B during the 1950s caused severe losses on wheat (Peturson 1958). 'Selkirk' (registered in 1953) was resistant to stem rust race 15B, and had improved resistance to leaf rust. Its yield losses were less severe than 'Thatcher' under heavy leaf rust pressure (Peturson 1958; Samborski & Peturson 1960). 'Selkirk', containing leaf rust resistance genes *Lr10* and *Lr14a* and heterogeneous for *Lr16* (Samborski 1985; Martens & Dyck 1989), was taken up rapidly by producers and grown extensively in the rust prone area of Manitoba and eastern Saskatchewan (Fig. 1). However, 'Thatcher' remained more popular in the western prairies due to its higher yield potential in areas with lower rust epidemics (McCallum & DePauw 2008). Virulence in *P. triticini* to *Lr10* increased soon after the introduction of 'Selkirk', whereas virulence to *Lr16* started to increase 8 years after 'Selkirk' was widely grown (Samborski 1985).

The subsequent leading cultivars in western Canada were 'Manitou' (1965), 'Neepawa' (1969) and 'Katepwa' (1981), all carrying *Lr13* (Samborski 1985; Campbell & Czarnecki 1987) and were each the most popular from 1968 to 1972, 1973 to 1985, and 1986 to 1995, respectively. Virulence for *Lr13* started to appear a few years after the introduction of 'Manitou' (Samborski 1985), and by 2002, nearly all isolates found in western Canada were virulent to this gene (McCallum & Seto-Goh 2005). Most of the popular cultivars developed since 'Katepwa' have a more diverse set of *Lr* genes, including the predominant cultivar from 1996–1997 'CDC Teal' (*Lr1, Lr13, Lr34*) (Hughes & Hucl 1993; Liu & Kolmer 1997b) and the leading cultivar from 1998–2005 'AC Barrie' (*Lr13, Lr16*) (McCaig et al. 1996; Kolmer 2001b). Other popular cultivars during this period were 'Columbus' (*Lr13, Lr16*) (Samborski & Dyck 1982),

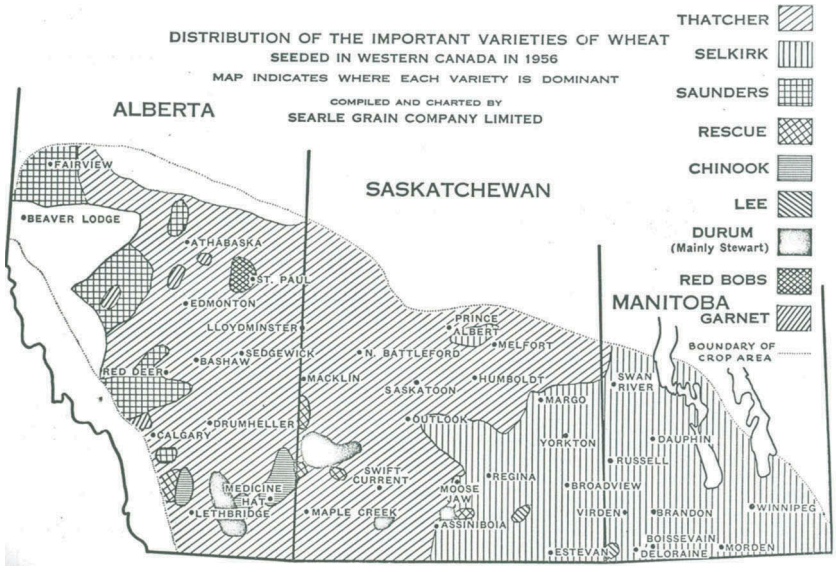

Fig. 1 Distribution of wheat varieties in Western Canada in 1956. Reprinted from Searle Grain Company Limited Annual Variety Survey Report 1956.

'Roblin' (*Lr1, Lr10, Lr13, Lr34*) (Dyck 1993a), 'Glenlea' (*Lr1, Lr13, Lr34*) (Dyck et al. 1985), 'AC Domain' (*Lr10, Lr12, Lr16*), 'AC Taber' (*Lr14a, Lr13, LrTb*) (Liu & Kolmer 1997a), 'AC Majestic' (*Lr13, Lr16*), 'AC Splendor' (*Lr13, Lr16*), 'AC Karma' (*Lr13, Lr16, LrTb*) (Kolmer & Liu 2002), 'Laura' (*Lr1, Lr10, Lr34*), 'Genesis' (*Lr13, Lr14a*) and 'Biggar' (*Lr13, Lr14a*) (Kolmer 1994).

Information on the resistance genes present in current and past cultivars can be used to develop a comprehensive picture of leaf rust resistance in Canadian wheat cultivars, facilitating future resistance breeding. The genes *Lr13, Lr14a, Lr16, Lr21* and *Lr34* have been most widely used in Canadian cultivars (Martens & Dyck 1989; Kolmer et al. 1991; Kolmer 1996; McCallum & DePauw 2008). While virulence to *Lr13, Lr14a*, and to some extent to *Lr16* has developed over time, *Lr34* still provides effective resistance after many years of utilization. In Canada, 'Glenlea' (*Lr1, Lr13* and *Lr34*) (Dyck et al. 1985) was the first major cultivar with *Lr34* released in 1972 and it has remained moderately resistant to leaf rust since then. Over the years, the unique effectiveness and the durability of *Lr34* were realized and it was subsequently incorporated into many cultivars. Most rust resistance genes are race-specific, conditioning resistance to some races of the pathogen but not others (Dyck & Kerber 1985). However, a few such as *Lr34* are not race-specific, controlling all races equally well and have not been overcome by mutations to virulence in the pathogen (McCallum et al. 2011a). The proportion of the CWRS (Canadian Western Red Spring wheat, the dominant bread wheat class) area seeded to cultivars with *Lr34* ranged from 20–40% from 1990 to 2009 (McCallum et al. 2011a). Dyck et al. (1985) recognized that *Lr34* conditioned resistance to stem rust as well as leaf rust in the cultivar 'Glenlea'. This was the first report of the pleiotropic nature of *Lr34*, which was subsequently reported to also reduce stripe rust, stem rust and other biotrophic diseases in numerous publications worldwide (McCallum et al. 2012).

Other resistance genes that have maintained their effectiveness over time include *Lr21* and *Lr22a*, isolated from *Aegilops tauschii* (Coss. syn. *Triticum tauschii*) and introgressed into bread wheat (Rowland & Kerber 1974). These genes have been deployed in cultivars such as 'AC Cora' (1994) (*Lr13, Lr21*), 'AC Minto' (1991) (*Lr11, Lr13, Lr22a*) (Townley-Smith et al. 1993a; Kolmer 1996), 'McKenzie' (1997) (*Lr10, Lr13, Lr16, Lr21*) (McCallum & Seto-Goh 2010), '5500HR' (2000) (*Lr22a+*), '5600HR' (1999) (*Lr22a+*) (Hiebert et al. 2007) and 'Snowstar' (*Lr21 +*) (Humphreys et al. 2013). Virulence to *Lr21* only recently (2011) appeared in the Canadian *P. triticina* population (McCallum et al. 2011c).

Combinations of resistance genes have often been found to confer more resistance than would be predicted from the effects of the genes in isolation. In the cultivar 'Selkirk', *Lr13* was found to act synergistically with *Lr16* and the gene combinations of *Lr13+Lr30+Lr11* and *Lr30 +Lr3ka* both resulted in more resistant seedling reactions than was expected if the genes were acting independently

(Samborski & Dyck 1982). Many cultivars in Canada and the USA have combinations of resistance genes with *Lr34* and/or *Lr13* (Kolmer 1996). The expression levels of many other *Lr* genes are significantly enhanced when paired with *Lr34* (German & Kolmer 1992) or *Lr13* (Kolmer 1992c). Cultivar 'Pasqua' (1991) (*Lr11, Lr13, Lr14b, Lr30, Lr34*) (Dyck 1993b; Townley-Smith et al. 1993b) represents a deliberate attempt to combine multiple resistance genes into a single cultivar. It has remained highly resistant since its release in 1991 up to the present, even though *Lr11, Lr13* and *Lr14b* became ineffective during that time and *Lr30* and *Lr34* only provide partial resistance when used individually (McCallum & Thomas 2011). The resistance gene *Lr34* was also shown to enhance stem rust resistance in cultivars derived from 'Thatcher' (Kerber & Aung 1999). Furthermore, it was thought that *Lr34* permitted the expression of stem rust resistance genes that were normally inhibited by a suppressor of stem rust resistance on chromosome 7D, i.e. *Lr34* acted as a 'nonsuppressor' (Kerber & Aung 1995). The 'nonsuppressing' effect of *Lr34* (or a closely linked gene) was also suggested by Dyck (1987). *Lr34* conferred stem rust and leaf rust resistance in 'Glenlea' (Dyck et al. 1985) and a number of other cultivars (Dyck & Samborski 1982). Navabi et al. (2005a) found a significant contribution to both leaf and stripe rust resistance from *Lr34/Yr18*, or genes closely linked to this locus, in a number of CIMMYT (International Maize and Wheat Improvement Center) wheat cultivars. Hiebert et al. (2011) showed that the resistance reaction to race Ug99 (TTKSK) of stem rust in the spring wheat variety 'Peace', associated with *SrCad*, was significantly lower in doubled haploid lines that also possessed *Lr34*.

A number of initially effective *Lr* genes deployed in Canada and throughout the world have become less effective because of the evolution of increased virulence in the *P. triticina* population. However, some *Lr* genes or gene combinations have proven durable over many years of wheat production. In order to understand what gene combinations conferred durable resistance, genetic analyses have been conducted on a number of durably resistant cultivars. One of these was the Australian cultivar 'Cook' that was found to have durable resistance to both leaf and stripe rusts because of the presence of *Lr34/Yr18* interacting with other *Lr* genes (Navabi et al. 2005b). Another was the South American cultivar 'Toropi', whose complementary genes (*LrTrp1* and *LrTrp2*) condition resistance to leaf and stripe rust (Barcellos Rosa 2012). Resistance in cultivars with multiple *Lr* genes (gene pyramids) has generally been durable. In Canada, the inheritance of leaf rust resistance has been investigated in many cultivars and breeding lines, including 'Kenyon' and 'Buck Manantial' (Dyck 1989), 'Chinese Spring' and 'Sturdy' (Dyck 1991), 'Westphal 12A' and 'BH1146′ (Kolmer & Liu 2001), and accessions 'V336′ and 'V618′ from the Watkins wheat collection (Dyck & Jedel 1989). As changes in the races of *P. triticina* have rendered many of the resistance genes ineffective, the ongoing discovery of new *Lr* genes is needed for incorporation into future cultivars.

Wheat lines with unique leaf rust resistance genes such as *Lr35* (Knox et al. 2000) have been released in Canada as germplasm lines for breeding. Resistance gene *Lr36* from *T. speltoides* (Dvorak & Knott 1990) has been deployed in the Canadian cultivar 'CDC Bounty' (P. Hucl, personal communication). Durum wheat has traditionally been very resistant to *P. triticina* in Canada. However, races that specifically attack durum wheat have been causing significant losses in Mexico and other durum growing countries, and these races generally have been found to be virulent on Canadian durum cultivars. Efforts are underway to understand and improve resistance to these durum-attacking races in Canadian germplasm (Singh et al. 2013).

Genetic resistance to leaf rust has provided significant protection against losses in Canadian wheat. The results of long-term resistance breeding efforts in Canada were clearly evident when a collection of 45 Canadian wheat cultivars, released from the early 1900s to the present, were grown in side-by-side field plots for comparison (Martens et al. 2014). Leaf rust resistance in wheat cultivars has improved steadily over time through the incorporation of newer effective genes and the development of gene pyramids. This was reflected in the lower flag leaf infection and lower yield losses due to leaf rust of recent cultivars compared with the older more susceptible cultivars (Fig. 2).

The development of 'Thatcher'-based single Lr *gene lines*

Genetics of rust resistance is often complicated by the presence of multiple resistance genes in the same line. Additionally, the expression of these genes is often influenced by the genetic background of the host line. Near-isogenic lines, that contain single *Lr* genes in a uniformly susceptible background, greatly facilitate research to understand the genetics of host/parasite interactions. The development of near-isogenic single *Lr* gene lines in the 'Thatcher' wheat background was initiated by Dr R. G. Anderson (Anderson 1966) and performed mainly by Dr P. Dyck, working at the Cereal Research Centre, Agriculture and Agri-Food Canada (CRC-AAFC) (Table 1). 'Thatcher' was very susceptible to nearly all *P. triticina* virulence phenotypes, making it a highly

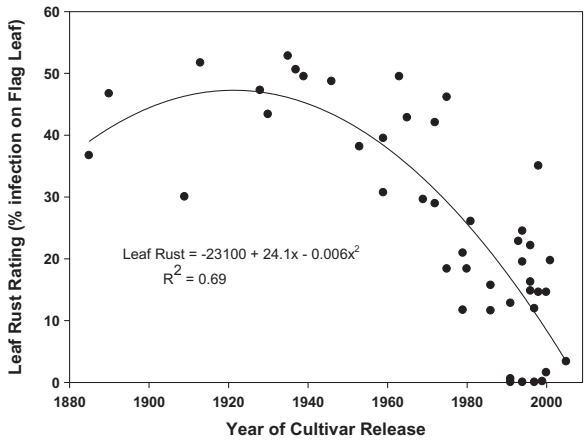

Fig. 2 Relationship between year of release and average leaf rust (% of flag leaf coverage) severity over the period of 2008–2010 at Carman, Manitoba for 45 Canadian wheat cultivars. From Martens et al. (2014).

suitable genetic background in which to incorporate these genes, although there are some very rare races avirulent on 'Thatcher'. Dr Dyck crossed lines with unique *Lr* genes to 'Thatcher' and then used 'Thatcher' as the recurrent parent in backcrosses (usually to the backcross 5 generation) to create near-isogenic lines for many of the known leaf rust resistance genes. These lines are used as the official reference stocks for most of the leaf rust resistance genes (McIntosh et al. 1995). Many of the 'Thatcher' near-isogenic lines have been adopted in various parts of the world as components of differential sets for virulence surveys and genetic analysis of this pathosystem – this represents one of Canada's most significant contributions to wheat leaf rust research globally. Because the 'Thatcher' near-isogenic lines simplified the genetics of differentials by placing them in a uniform background, they facilitate the careful investigation of the gene-for-gene relationship between the pathogen and the host. Furthermore, the widespread use of the same set of differential lines affords direct comparison of virulence in *P. triticina* populations across various laboratories. These lines have also been used extensively for tests of allelism to confirm the identity of hypothesized resistance genes in numerous cultivars (e.g. McCallum & Seto-Goh 2010).

Discovery and genetic analysis of leaf rust resistance genes

The early development of rust resistant wheat cultivars in Canada highlighted the need to identify new and broadly effective sources of genetic resistance to incorporate into future cultivars. Canadian researchers have been among the world leaders in the discovery and characterization of leaf rust resistance genes. There are currently 69 recognized leaf rust resistance genes/alleles, many of which were characterized in whole or in part in Canada (Table 2) (McCallum et al. 2012). These include *Lr2a, Lr2b* and *Lr2c* (Dyck & Samborski 1974), *Lr3a* (Dyck & Johnson 1983), *Lr3bg* and *Lr3ka* (Haggag & Dyck 1973),

Table 1. Near-isogenic lines of the wheat cultivar Thatcher with unique leaf rust resistance genes developed in Canada.

Gene	RL number	Pedigree
Lr1	RL6003	Thatcher*6/Centenario
Lr2a	RL6016	Thatcher*6/Webster
Lr2b	RL6019	Thatcher*6/Carina
Lr2c	RL6047	Thatcher*6/Brevit
Lr3a	RL6002	Thatcher*6/Democrat
Lr3bg	RL6042	Bage/8*Thatcher
Lr3ka	RL6007	Thatcher*6/Klein Anniversario
Lr9	RL6010	Transfer (*Aegilops umbellulata*)/6*Thatcher
Lr10	RL6004	Thatcher*6/Exchange
Lr11	RL6053	Thatcher*6//E-1/Hussar
Lr12	RL6011	Exchange/6*Thatcher
Lr13	RL6001	Prelude*6/Loros
Lr14a	RL6013	Selkirk/6*Thatcher
Lr14b	RL6006	Thatcher*6/Maria Escobar
Lr15	RL6052	Thatcher*6/W1483
Lr16	RL6005	Thatcher*6/Exchange
Lr17a	RL6008	Klein Lucero/6*Thatcher
Lr18	RL6009	Thatcher*7/Africa 43
Lr19	RL6040	Thatcher*7/Translocation 4 (*Lr19* derived from *Agropyron elongatum*)
Lr20	RL6092	Thatcher*6/Timmo
Lr21	RL6043	Thatcher*6/RL5406(Tetra Canthatch/*Aegilops squarrosa* var *meyeri*-RL5289)
Lr22a	RL6044	Thatcher*7/RL5404(Tetra Canthatch/*Aegilops squarrosa* var *strangulata*-RL5271)
Lr22b	Thatcher	Marquis/Iumillo Durum//Marquis/Kanred
Lr23	RL6012	Lee 310/6*Thatcher
Lr24	RL6064	Thatcher*6/3/Agent//2*Prelude/8*Marquis
Lr25	RL6084	Thatcher*7/Transec
Lr26	RL6078	Thatcher*6/St-1–25
Lr28	RL6079	Thatcher*6/C-77–1
Lr29	RL6080	Thatcher*6//CS7D/Ag#11
Lr30	RL6049	Thatcher*6/Terenzio
Lr32	RL6086	Thatcher*6/3/Thatcher/*Aegilops squarrosa*//Mq(K)
Lr33	RL6057	Thatcher*6/PI58548
Lr34	RL6058	Thatcher*6/PI58548
Lr35	RL5711	Marquis-K*8//RL5344/RL5346 (*Triticum monococcum*)
Lr37	RL6081	Thatcher*8/VPM
Lr38	RL6097	Thatcher*6/T7Kohn
Lr44	RL6147	Thatcher*6/*Triticum speltoides* 7831 85GN 438
Lr45	RL6144	Thatcher*6/St-1
Lr52	RL6107	Thatcher*6/V336
Lr60	RL6172	Thatcher*3/V860
Lr63	RL6137	Thatcher*6/TMR5-J14-12-24
Lr64	RL6149	Thatcher*6/8404
Lr67	RL6077	Thatcher*6/PI250413

Table 2. Leaf rust resistance genes discovered in whole or in part by Canadian researchers.

Gene(s)	Common wheat or alien source of resistance gene	Chromosome location in common wheat	Thatcher isoline	Citation
Lr2a	Webster (*T. aestivum* L.)	2DS	RL6016	Dyck and Samborski (1968)
Lr2b	Carina (*T. aestivum* L.)	2DS	RL6019	Dyck and Samborski (1974)
Lr2c	Brevit (*T. aestivum* L.)	2DS	RL6047	Dyck and Samborski (1974)
Lr3a	Democrate (*T. aestivum* L.)	6BL	RL6002	Dyck and Samborski (1968)
Lr3bg	Bage (*T. aestivum* L.)	6BL	RL6042	Haggag and Dyck (1973)
Lr3ka	Klein Anniversario (*T. aestivum* L.)	6BL	RL6007	Haggag and Dyck (1973
Lr12	Opal (*T. aestivum* L.)	4BL	RL6011	Dyck et al. (1966)
Lr13	Frontana; Manitou (*T. aestivum* L.)	2BS	RL4031	Dyck et al. (1966)
Lr14a	Selkirk (*T. aestivum* L.)	7BL	RL6013	Dyck and Samborski (1970)
Lr14b	Rafaela; Maria Escobar (*T. aestivum* L.)	7BL	RL6006; RL6056	Dyck and Samborski (1970)
Lr16	Exchange; Selkirk (*T. aestivum* L.)	2BS	RL6005	Dyck and Samborski (1968)
Lr17a	Klein Lucero; Rafaela, EAP (*T. aestivum* L.)	2AS	RL6008; RL6054; RL6055	Dyck and Samborski (1968)
Lr18	Africa 43; Sabikei (*T. aestivum* L.)	5BL	RL6009	Dyck and Samborski (1968)
Lr19	*Thinopyrum ponticum* (*Podp.*) *Barkworth & D.R. Dewey*	7DL	RL6040; RL6085	Sharma and Knott (1966)
Lr21	*Ae. squarrosa* L. var *meyeri*	2DS	RL6043	Rowland and Kerber (1974)
Lr22a	*Ae. squarrosa* L. var *strangulata*	2DS	RL6044	Rowland and Kerber (1974)
Lr22b	Canthatch (*T. aestivum* L.)	2DS	Thatcher	Dyck (1979)
Lr23	Gaza (*T. turgidum* L. var. *durum*)	2BS	RL6012	McIntosh and Dyck (1975)
Lr30	Terenzio (*T. aestivum L.*)	4AL	RL6049	Dyck and Kerber (1981)
Lr32	*Ae. squarrosa* L.	3DS	RL6086	Kerber (1987), Kerber (1988)
Lr33	PI58548; PI268454; PI268316 (*T. aestivum L.)*	1BL	RL6057	Dyck et al. (1987)
Lr34	PI58548; Frontana; Chinese Spring, Terenzio, Bezostaja (*T. aestivum L.*)	7DS	RL6058; RL6091; RL6106; RL6159	Dyck (1987)
Lr35	*Ae. speltoides* Tausch.	2BS	RL6082	Kerber and Dyck (1990)
Lr36	*Ae. speltoides* Tausch.	6BS	N/A[1]	Dvorak and Knott (1990)
Lr44	*Triticum spelta* L.	1BL	RL6147	Dyck and Sykes (1994)
Lr52	V336; V618 (*T. aestivum* L.)	5BS	RL6107	Hiebert et al. (2005)
Lr53	*Triticum dicoccoides* L.	6BS	N/A	Marais et al. (2005)
Lr56	*Ae. sharonensis* Eig.	6AL	N/A	Marais et al. (2006)
Lr59	*Ae.peregrina* (*Hackel in J. Fraser*)	1AL	N/A	Marais et al. (2008)
Lr60	V860 (*T. aestivum* L.)	1DS	RL6172	Hiebert et al. (2008)
Lr62	*Ae. neglecta* Req. ex Bertol.	6AS	N/A	Marais et al. (2009)
Lr63	T. monococcum L.	3AS	RL6137	Kolmer et al. (2010)
Lr64	*T. turgidum* L.	6AL	RL6149	Dyck et al. (1994)
Lr67	PI250413 (*T. aestivum L.*)	4DL	RL6077	Hiebert et al. (2010b)
Lr70	KU3198 (*T. aestivum L.*)	5DS	N/A	Hiebert et al. (2014)

[1]Not available. A 'Thatcher' isoline was not developed for the *Lr* gene at the time of publication.

Lr12 and *Lr13* (Dyck et al. 1966), *Lr14a* and *Lr14b* (Dyck & Samborski 1970), *Lr16, Lr17a* and *Lr18* (Dyck & Samborski 1968), *Lr19* (Sharma & Knott 1966), *Lr21* and *Lr22a* (Rowland & Kerber 1974), *Lr22b* (Dyck 1979), *Lr23* (McIntosh & Dyck 1975), *Lr30* (Dyck & Kerber 1981), *Lr32* (Kerber 1987, 1988), *Lr33* (Dyck et al. 1987), *Lr34* (Dyck 1987), *Lr35* (Kerber & Dyck 1990), *Lr36* (Dvorak & Knott 1990), *Lr44* (Dyck & Sykes 1994), *Lr52* (Hiebert et al. 2005), *Lr53* (Marais et al. 2005), *Lr56* (Marais et al. 2006), *Lr59* (Marais et al. 2008), *Lr60* (Hiebert et al. 2008), *Lr62* (Marais et al. 2009), *Lr63* (Kolmer et al. 2010), *Lr64, Lr67* (Dyck et al. 1994; Hiebert et al. 2010b), *Lr70* (Hiebert et al. 2014) and a number of *Lr* genes with temporary designations (Innes & Kerber 1994; McIntosh et al. 1995). These sources were developed by screening large germplasm collections of bread wheat (*T. aestivum* L.) and related species for leaf rust resistance, discovering the genetic basis of the resistance using defined *P. triticina* race collections, mapping the resistance gene to a specific chromosome arm, determining allelism with other genes in the identified region, and in some cases transferring this resistance into high quality bread wheat germplasm and cultivars.

Most leaf rust resistance genes are effective from the early seedling stage throughout the life of the plant. Adult plant resistance genes, such as *Lr12, Lr13, Lr22a* and *Lr34*, are not normally expressed until the juvenile or

adult plant growth stages. The genetics of adult plant resistance was first investigated in the cultivars 'Exchange' and 'Frontana' (Dyck et al. 1966). Field screening was critical to identify adult plant resistance in germplasm collections. Several *T. aestivum* germplasm collections have been screened for both seedling and adult plant leaf rust resistance, including the Watkins wheat collection (Claude et al. 1986; Dyck 1994a), Ethiopian wheat (Dyck & Sykes 1995), Italian collections (Jedel et al. 1988) and other germplasm collections (Shang et al. 1986; Dakouri et al. 2013).

Broad-spectrum leaf rust resistance was also identified in related species, which was then transferred into *T. aestivum* through inter-specific hybridization with closely and more distantly related species. Resistance was identified in *Agropyron intermedium* chromosomal translocation lines (Dyck & Friebe 1993), *Triticum turgidum* spp. *dicoccoides* (Dyck 1994b), *Agropyron elongatum* (Sharma & Knott 1966), *Aegilops tauschii* (Rowland & Kerber 1974; Kerber 1987; Innes & Kerber 1994), *Ae. speltoides* (Knott & Dvorak 1976, 1981), an amphiploid of *Ae. speltoides* × *T. monoccocum* (Kerber & Dyck 1990), and *Thinopyrum intermedium* through partial amphiploids with durum (Zeng et al. 2013). Leaf rust resistance was also successfully transferred from the durum cultivars 'Medora' and 'Stewart' to bread wheat, but results were not clear for a transfer from an autotetraploid of *T. monococcum* to bread wheat (Dyck & Bartos 1994). Sometimes, the introduction of chromosomal segments from related species caused a reduction in yield and grain quality (Knott & Dvorak 1976). These effects were investigated for a number of inter-specific introgressions (Knott & Dvorak 1981; Dyck & Lukow 1988). Numerous genetic tools, such as synthetic tetraploids and the use of colchicine to double the chromosomal complement, were also developed in wheat during the process of these interspecies transfers of rust resistance (Knott & Dvorak 1976). Two of the resistance genes transferred from *Ae. tauschii* (*Lr21* and *Lr22a*) have been incorporated into many current Canadian cultivars and have provided effective resistance over time in a number of cultivars.

Molecular markers linked to many of the *Lr* genes provide opportunities for marker-assisted selection of leaf rust resistance. Molecular markers were developed in Canada for *Lr1* (Cloutier et al. 2007), *Lr13* (Hiebert et al. 2015), *Lr16* (McCartney et al. 2005), *Lr21* (J. Thomas unpublished), *Lr22a* (Hiebert et al. 2007), *Lr25* and *Lr29* (Procunier et al. 1995), *Lr32* (Thomas et al. 2010), *Lr34* (Dakouri et al. 2010), *Lr35* (Gold et al. 1999), *Lr52* (Hiebert et al. 2005), *Lr60* (Hiebert et al. 2008), *Lr67* (Hiebert et al. 2010b) and *Lr70* (Hiebert et al. 2014). The various alleles at the *Lr34* locus were analysed for their frequency and inheritance in Canadian (McCallum et al. 2011a) and international wheat germplasm (Dakouri et al. 2014). A potential evolutionary path for the diversity found at the *Lr34* locus was also proposed by Dakouri et al. (2014).

Two of the *Lr* genes that are common in Canadian germplasm, *Lr13* and *Lr16*, have been the focus of recent studies. *Lr13* is a race-specific adult plant resistance gene that can provide effective resistance in combination with other *Lr* genes. Genetic analysis and mutation studies have shown that *Lr13* and *Ne2* appear to be a single pleiotropic gene (Hiebert et al. 2015). *Ne2* causes progressive necrosis in hybrids that also carry *Ne1*, a phenotype also referred to as hybrid necrosis. *Lr16* can also confer effective resistance, particularly when combined with *Lr34* (Hiebert et al. 2010a). Fine mapping of *Lr16* has produced SNP markers based on resistance gene analogues (RGAs) that co-segregate with *Lr16* in large populations (Kassa et al. 2014). These markers are useful for marker-assisted selection and map-based cloning of *Lr16*.

The leaf rust resistance gene *Lr1* was cloned and sequenced by a research team led by Canadian researchers (Cloutier et al. 2007). It was found to belong to the coiled-coil nucleotide-binding site and leucine-rich repeat domain-containing class of resistance genes and is a member of a large gene family. However, the sequence of *Lr1* was not related to either *Lr10* or *Lr21*, the only other cloned seedling *Lr* genes to date. The cloned copy of *Lr1* behaved in a dose-dependent manner in transgenic lines, expressing partial resistance in hemizygous lines and full resistance in homozygous lines. This correlates well with the reaction of non-transformed plants that are either heterozygous or homozygous for *Lr1* in segregating populations (B. McCallum, unpublished data).

Understanding how these *Lr* genes interact with *P. triticina* avirulence genes to condition resistance requires a good understanding of the population biology of *P. triticina*, and detailed analysis of the host/parasite interaction using physiological and molecular genetic approaches.

Population biology and genetic analysis of *Puccinia triticina*

Population biology of Puccinia triticina

An initial step in understanding leaf rust epidemiology in wheat was to monitor epidemics of leaf rust and characterize the *P. triticina* population for its changing virulence profile. Knowledge about population diversity, race

structure, and genetic lineages are extremely important for predicting new introductions and/or mutants that can overcome an effective host resistance gene. Since 1931, annual virulence surveys have been conducted in Canada through extensive field surveys during the growing season to estimate the severity and distribution of the disease. This collaborative effort involved researchers across the country. Infected leaf samples were collected annually during these surveys to obtain live rust isolates representative of the pathogen population. Purified isolates were then analysed for their virulence spectra by inoculating the above-mentioned differential wheat lines and observing the disease reactions. Detailed virulence information was used to track the evolution of new and potentially damaging races and to determine the effectiveness of the resistance genes in wheat cultivars in production as well as those that could be incorporated into future cultivars. When virulence surveys for *P. triticina* were initiated in Canada in 1931, virulence phenotypes or races were originally given numbers based on their reaction to the eight existing standard differentials (Newton & Johnson 1941), which carried resistance genes *Lr1, Lr2a, Lr2c, Lr3* and *Lr11*. Modified race designation systems (UN or Unified Numeration and International Standard) evolved over time using *Lr1, Lr2a, Lr2c* and *Lr3* to differentiate virulence phenotypes (Kolmer 1989). The use of various wheat cultivars or lines as differentials confounded the interaction between the resistance and avirulence genes due to the presence of multiple resistance genes in some lines and the effect of various genetic backgrounds. The development of the 'Thatcher' based near-isogenic lines simplified the host/parasite genetics of this system, since each line had a single unique resistance gene. The currently used North American race designation system uses a four letter code system to define the reaction on 16 of these 'Thatcher' single *Lr* gene near-isogenic wheat lines (Set 1: *Lr1, Lr2a, Lr2c, Lr3*; Set 2: *Lr9, Lr16, Lr24, Lr26*; Set 3: *Lr3ka, Lr11, Lr17, Lr30*; (Long & Kolmer 1989), Set 4 [subsequently added]: *LrB, Lr10, Lr14a* and *Lr18*).

As a result of ongoing annual surveys, Canada has developed a continuous historical record of the *P. triticina* population from 1931 to the present day. These annual surveys were published in the Canada Department of Agriculture reports (1931–1960), the Canadian Plant Disease Survey (1960–1979) and the *Canadian Journal of Plant Pathology* (1980–present), and were summarized for the periods of 1931–1955 (Johnson 1956), 1956–1987 (Kolmer 1989) and 1987–1997 (Kolmer 1999). The annual virulence surveys since 1997 (Kolmer 2001b; McCallum & Seto-Goh 2002, 2003, 2005, 2006a, 2006b, 2008, 2009; McCallum et al. 2004, 2010, 2011b, 2013) have not been summarized to date. During the period 1931–1955, distinct distributions of virulence phenotypes were found in each of the geographic regions of Canada. The regions were defined as Eastern Canada (all provinces east of Manitoba), the Prairies (Manitoba, Saskatchewan and Alberta) and British Columbia (Johnson 1956). Over 90% of the wheat-growing area in Canada is in the three Prairie provinces (McCallum & DePauw 2008). The wheat cultivars grown in the Prairies from 1931–1955 appeared to have had a strong selective effect on the virulence phenotypes in the region over time (Johnson 1956). A similar effect was noticed for the period 1956–1987 (Kolmer 1989). Selection was the strongest in this region because there was an emphasis on using genetic resistance to leaf rust in the cultivars grown in the prairies of Canada and the USA.

Cereal rust pathogens are often able to change genetically, through mutation and sexual or asexual recombination, to become virulent to resistance genes present in the host. This process was termed 'Man-guided evolution' (Johnson 1961a) because resistance genes deployed in prevalent wheat cultivars led to the appearance and selection of phenotypes with virulence to these resistance genes. *Puccinia triticina* populations in Canada have evolved virulence over time to many of the resistance genes used in Canadian wheat cultivars, such as *Lr1, Lr10, Lr12, Lr13* and *Lr14a. Lr14a* was effective when initially deployed in 'Renown' (1937), but cultivars with this gene were susceptible by 1945 (Johnson & Newton 1946). When 'Selkirk', with *Lr10, Lr14a*, and heterogeneous for *Lr16* (Samborski 1985; Martens & Dyck 1989), became widely grown in 1955 (McCallum & DePauw 2008), there was selection for virulence detected for *Lr10* and *Lr16*. The frequency of isolates virulent to *Lr10* increased rapidly from 0% in 1952 to 80% of the isolates in 1958 (Anderson 1961). However, isolates virulent to *Lr16* were not detected until 1962, reaching a high of 56% in 1967. Virulence to *Lr16* subsequently declined to near 0% (Samborski 1985) after 'Selkirk' was replaced in 1968 with 'Manitou' followed by 'Neepawa' and 'Katepwa' (all with *Lr13*). Virulence to *Lr13* soon increased (Kolmer 1989) with the introduction of these cultivars. Virulence to *Lr16* disappeared entirely in Manitoba and Saskatchewan from 1989–1994 but reappeared after 1995, reaching a high of 74% in 2001 when 'AC Barrie' (*Lr13, Lr16*) was the predominant cultivar (McCallum & Seto-Goh 2005; McCallum & DePauw 2008). After 2001, virulence to *Lr16* declined again to low levels; however, 'AC Barrie' and single gene lines with *Lr16* remained susceptible in the field, indicating that most virulence phenotypes were likely heterozygous for *Lr16* virulence (McCallum & Seto-Goh 2005) which

resulted in an intermediate infection type. Heterozygosity for virulence was found to be common for many resistance genes in the Canadian *P. triticina* population (Kolmer 1992d).

The *P. triticina* populations in western and eastern Canada are fairly distinct both for virulence (Kolmer 1991a, 1992a) and molecular markers (Kolmer et al. 1995; Kolmer 2001a). The western population was found to diverge significantly from the eastern population after 1937 as a consequence of the selective effect of resistant cultivars commonly grown in the Prairies, which also reduced the diversity of the prairie population (Kolmer 1991b). The *P. triticina* populations in Canada were characterized by distinct clusters of isolates that had similar virulence and molecular phenotypes within clusters (Kolmer et al. 1995), and demonstrated strong linkage disequilibrium between loci because of the selection for virulence and the asexual nature of the population. Linkage disequilibrium was higher in the more selected western population than in the eastern population. The eastern population was subject to less intense selection for virulence, and may have undergone some level of sexual recombination (Kolmer 1992b), which would have significantly reduced linkage disequilibrium (Liu & Kolmer 1998). Kolmer (1992a) found that there were significant differences in the frequency of virulence phenotypes between Ontario and Quebec collections of *P. triticina* from 1990, and those from winter and spring wheat in eastern Canada. Manitoba and Saskatchewan collections were not significantly different from each other, but were different from those from eastern Canada. Some common virulence and molecular phenotypes are still found annually in both western and eastern Canada, which could be due to a common origin in the USA (McCallum & Seto-Goh 2006a, 2006b). Wang et al. (2010a) analysed the *P. triticina* populations in Canada from 1997 to 2007 and found that the Quebec and Ontario population was distinct from that in Manitoba and Saskatchewan and the isolates formed six genetically similar clusters similar to those found in the USA.

Selection for virulence was investigated using a mixture of virulence phenotypes cultured for eight asexual uredinial generations on host lines with different combinations of resistance genes (Kolmer 1990). Changes in the populations observed on some lines over time were thought to be due to differences in urediniospore production between the virulence phenotypes (fitness), rather than selection for virulence *per se*. However, selection for virulence reduced the diversity of a randomly produced sexual population in response to resistance genes in the host cultivar (Kolmer 1993). Selection of a sexual population on multi-lines with various combinations of resistance genes demonstrated a predominance of virulence phenotypes heterozygous for virulence, and that unnecessary genes for virulence, in which the corresponding resistance genes were not present, were not deleterious to fitness (Kolmer 1995). Selection for virulence to *Lr16* on wheat lines carrying this gene was demonstrated in a greenhouse study with a mixture of virulence phenotypes (Kolmer 1990). Because *P. triticina* inoculum is blown northward into Canada from the USA, the distribution of virulence phenotypes in Canada is also influenced by the *Lr* genes in wheat cultivars grown in the USA. Virulence to *Lr24, Lr17* and other genes has appeared at relatively high levels in Canadian *P. triticina* populations even though these genes have not been deployed in Canadian wheat cultivars (Kolmer 1989, 1999; McCallum & Seto-Goh 2006b). This was hypothesized to be a consequence of the selective effect of wheat cultivars carrying those genes in the USA (Fig. 3). Virulence for *Lr9* and *Lr21* has only evolved recently in Canada (in 2006 [McCallum & Seto-Goh 2009] and 2011 [McCallum et al. 2011c], respectively), and has remained relatively low to date, whereas virulence to *Lr24, Lr17* and *Lr2a* fluctuates over time but is relatively high in the Canadian *P. triticina* population (Fig. 3). The Canadian population of *P. triticina* was also tested annually for virulence to adult plant resistance genes *Lr12, Lr13, Lr22a, Lr34, Lr35* and *Lr37*. The frequency of virulence to *Lr12, Lr13* and *Lr37* was very high, whereas it was normally less than 10% on *Lr35*, and no virulent isolates were found for *Lr22a* and *Lr34* (McCallum & Seto-Goh 2005, 2006a, 2006b, 2008, 2009; McCallum et al. 2010, 2011b, 2013; B. McCallum, unpublished data).

Puccinia triticina in Canada evolved new combinations of virulence relatively rapidly, resulting in a highly variable pathogen population. For example, 22 to 72 different *P. triticina* virulence phenotypes were identified annually in Canadian surveys from 2000 to 2013 (sample sizes varied from 97 to 420 isolates) (McCallum & Seto-Goh 2002, 2003, 2004, 2005, 2006a, 2006b, 2008, 2009; McCallum et al. 2010, 2011b, 2013; B. McCallum, unpublished). During this time, 305 unique virulence phenotypes were identified in Canada, based on the standard set of 16 'Thatcher' near-isogenic differential wheat lines. Even more variation was found when these virulence phenotypes were tested on race-specific adult plant resistance genes for the 2000–2013 period listed above, and during earlier years (Kolmer 1997). Moreover, the predominant virulence phenotypes changed regularly, although there was some continuity from year to year (Table 3). This variability could have arisen from a variety of sources, including mutation, host selection, and sexual, asexual or parasexual recombination. The potential for sexual

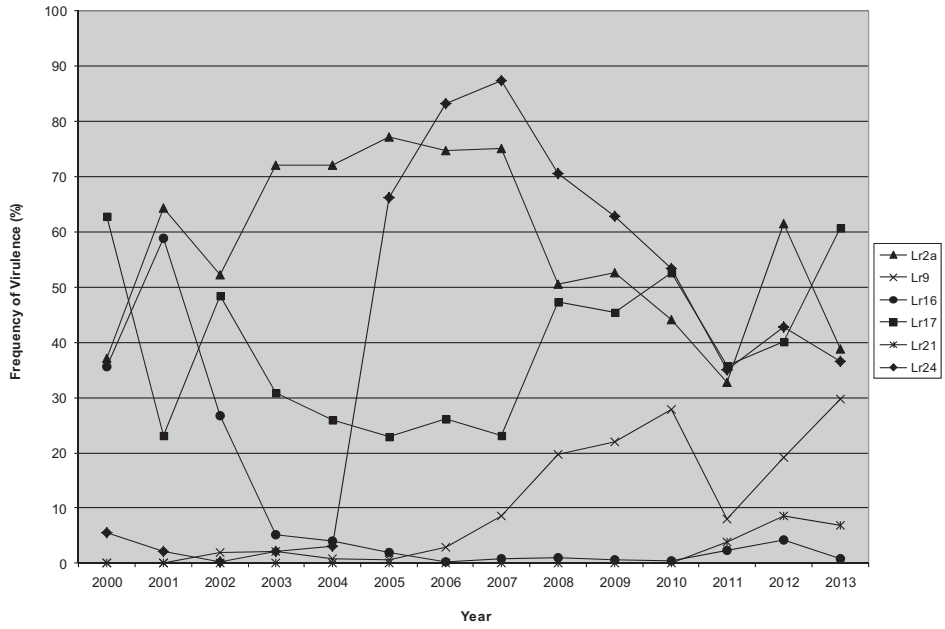

Fig. 3 Virulence frequency to leaf rust resistance genes *Lr2a, Lr9, Lr16, Lr17, Lr21* and *Lr24* in the Canadian *Puccinia triticina* population 2000–2013. Data from McCallum & Seto-Goh 2003, 2004, 2005, 2006a, 2006b, 2008, 2009; McCallum et al. 2010, 2011b, 2013, and B. McCallum (unpublished data).

Table 3. Frequency (%) of predominant *P. triticina* virulence phenotypes in Canada from 2000 to 2013.

Virulence	Year													
Phenotype[a]	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
MBDS	54.1[b]	18.5	30.2	13.4	10.0	4.0	1.4	1.3	0.5	0.3	0	1.4	6.4	12.1
MBTN	0	0	0	0	0	0	0	0	1.0	0	2.8	4.2	4.3	8.6
MFDS	0	0	0	0	0	0.2	5.1	0.8	5.7	1.0	0.5	0.7	0	0.8
MFPS	0	0	0	0	0	5.7	4.8	3.9	4.9	4.5	3.5	6.0	3.0	6.3
MLDS	0	0	0.7	0	0	0	1.4	8.4	18.9	20.6	26.1	7.4	2.6	7.8
TBBG	0	0	0	0	14.8	10.2	1.7	0	0.2	0	4.5	8.5	19.2	11.7
TBBJ	0.6	1.7	9.9	57.0	41.2	7.1	3.4	0	0.2	0.3	1.3	0.7	0.9	0.8
TDBG	0	0	0	0	0.3	44.5	50.1	54.3	22.9	15.8	11.8	9.5	2.6	2.0
TDBJ	0.3	0	0	0	0.6	8.6	16.7	16.5	23.1	28.1	22.8	13.0	6.4	7.0
TGBJ	21.6	39.5	22.0	3.1	3.3	0.7	0	0	0	0	0	0	0	0
THBJ	5.7	14.6	1.4	0	0	0	0	0	0	0	0	0	0	0
TNBG	0	0	0	0	0	0	0.3	0	0	0	1.0	2.1	14.5	10.5
Others	17.7	25.6	35.8	26.5	29.7	19.0	15.0	14.7	22.6	29.4	25.7	47.9	46.4	44.5
Number of isolates	366	362	295	97	330	420	353	381	104	310	399	284	234	265
Number of virulence phenotypes	23	41	40	23	46	39	31	22	48	39	41	72	54	37

[a]The first three letters of the virulence phenotype determined according to Long & Kolmer (1989), Set 4 [subsequently added]: *LrB, Lr10, Lr14a* and *Lr18*.
[b]Data from McCallum & Seto-Goh 2003, 2004, 2005, 2006a, 2006b, 2008; McCallum et al. 2010, 2011b, 2013; and B. McCallum (unpublished data).

recombination in *P. triticina* was first investigated in Canada by Brown and Johnson (1949). They determined the relative abilities of various species of *Thalictrum*, native to North America, to act as alternate or sexual hosts for *P. triticina* and made initial selfing studies for the predominant races of the time. These authors thought that while sexual reproduction of *P. triticina* on *Thalictrum* species in North America was not common, it could occasionally occur and lead to diversity in the *P. triticina* population. They discovered that many of the races were heterozygous for avirulence, since the selfed progeny populations segregated for virulence to many of the differentials. Canadian populations were compared with other worldwide collections of *P. triticina*, and some of the

Canadian race groups clustered with similar groups of races from other regions (Kolmer & Liu 2000).

Results from more than 75 years of annual virulence surveys of *P. triticina* in Canada demonstrated the diverse and rapidly changing nature of these populations. Various factors have contributed to this dynamic population structure, but the primary drivers are mutation and host selection. Selection for virulence on host resistance genes has been observed in the virulence surveys over time and demonstrated in greenhouse simulations. The fact that *P. triticina* populations have evolved to overcome many of the resistance genes used in Canadian cultivars demonstrates that there is an ongoing need to identify and incorporate new sources of genetic resistance.

Genetic analysis of Puccinia triticina

The genetic basis of avirulence in *P. triticina* was investigated at great length in the 1960s and 1970s by Drs Samborski and Dyck (CRC-AAFC). *Puccinia triticina* genetically is a dikaryotic fungus, which has been termed a functional diploid. Populations of the pathogen that segregate for virulence were generated through a series of selfing crosses (since many virulence/avirulence loci are heterozygous in most isolates) and genetic crosses between different virulence phenotypes on *Thalictrum*. The parents and progeny were then screened for virulence/avirulence on host lines with single genes for resistance in the 'Thatcher' background. Populations from self-fertilized isolates of four different virulence phenotypes were evaluated for virulence on the eight standard wheat differential lines used in the 1960s (Samborski & Dyck 1968). Avirulence to most of these lines was conditioned by single dominant genes, although there were some cases of avirulence being recessive and where additional genes affected the disease response. A second study involving these eight differential wheat lines that used cross-fertilized and self-fertilized *P. triticina* populations (Haggag et al. 1973) also supported the hypothesis of avirulence being primarily conditioned by single dominant genes. Since some of these differential lines contained more than one resistance gene, the interpretation was confounded by more than one specific gene-for-gene interaction. These results were confirmed with hybrid *P. triticina* populations using near-isogenic backcross lines in the 'Thatcher' background with single resistance genes (Samborski & Dyck 1976). Avirulence to the adult plant resistance gene *Lr22b*, found in 'Thatcher', was also shown to be conditioned by a single dominant gene (Bartos et al. 1969).

Development of a genetic linkage map for *P. triticina* using avirulence and molecular markers (McCallum et al. 2004) should facilitate studies on the fundamental nature of avirulence genes. The cloning of defined avirulence genes in *P. triticina* should provide insight into the nature of the resistance responses and identification of the resistance genes in *T. aestivum* that interact with these avirulence genes. Using Simple Sequence Repeat (SSR) markers (Wang et al. 2006, 2010b) and Amplified Fragment Length Polymorphism (AFLP) markers, initial linkage groups were established in the *P. triticina* mapping population produced by Samborski and Dyck (1968). Recent next-generation DNA sequencing methods have generated the complete genome sequences of 57 progeny of *P. triticina* cross Race 1 × Race 9 (Samborski & Dyck 1968) and over 20,000 Single Nucleotide Polymorphisms (SNPs) have been revealed, creating numerous markers, some of which are linked to potential avirulence genes (Bakkeren, McCallum, Joly, Mulock, unpublished data). In addition to asexual and sexual reproduction, *P. triticina* can reproduce through parasexual or somatic recombination where somatic hyphae from two different isolates can fuse and combine nuclei, ultimately producing new genotypes different from either parent (Wang & McCallum 2009).

Improved DNA and RNA sequencing technologies have now made possible the detailed genetic analysis of *P. triticina* and other cereal rusts. Initially, a large library of expressed sequence tags (ESTs) from *P. triticina* was developed and sequenced to reveal the expressed genes and their relative abundance in the various spore stages and in isolated haustoria of the fungus (Xu et al. 2011). Deep sequencing of the genome and transcriptome of a number of *P. triticina* isolates has allowed comparative genomics with related fungal species and the identification of genes critical to the infection process (Bakkeren et al. 2012; Bruce et al. 2014).

The *Puccinia triticina*/wheat interaction

The interaction between *P. triticina* and wheat is influenced by a number of factors, including temperature, genetic background of the host, genetics of avirulence in *P. triticina*, and the interaction between resistance and avirulence genes. Johnson and Newton (1937) demonstrated that very high temperatures inhibited pustule development of *P. triticina*. Newton and Johnson (1941) determined that moderate temperature rises differentially affected the reactions of resistant lines, with some becoming more resistant and others becoming more susceptible. Dyck and Johnson (1983) used single resistance gene lines in the 'Thatcher'

background to determine the temperature sensitivity, in the 10–20°C range, of the resistance genes known at that time. Not only did temperature sensitivity vary between genes, but as the temperature increased, most genes became less effective while some were unaffected and others became more effective.

The expression of host resistance can also be influenced by the growth stage of the plant. Newton and Johnson (1943) were the first Canadian researchers to identify adult plant resistance to leaf rust in a number of cultivars, including 'Thatcher' and 'Marquis'. This resistance was not expressed at the seedling stage but was expressed as the plant matured to the jointing and flag leaf stages. Resistance in the cultivars 'Exchange' and 'Selkirk' was found to increase from the seedling to the adult plant stage but declined as the plant matured (Samborski & Ostapyk 1959). The adult plant resistance in 'Thatcher' and 'Marquis' was later determined to be a single recessive gene (Bartos et al. 1969). This gene was subsequently found to be allelic to *Lr22* that was transferred from *Ae. tauschii*, and was designated *Lr22b* while the *Ae. tauschii* allele received the *Lr22a* designation (Dyck 1979). Adult plant resistance was also genetically characterized in 'Exchange' (*Lr12*) and 'Frontana' (*Lr13*) (Dyck et al. 1966), and PI250 413 (*Lr67*) (Dyck & Samborski 1979; Hiebert et al. 2010b). The most important of the adult plant resistance genes, *Lr34*, was isolated from the cultivars 'Terenzio', 'Lageadinho' and 'PI58548' (Dyck 1987). It has subsequently been found to be widely distributed in Canadian germplasm and is one of the most common resistance genes worldwide (McCallum et al. 2011a; Dakouri et al. 2013), conferring durable leaf rust resistance. Analysis of adult plant resistance genes is time and space consuming, because of the need to grow plants to maturity to determine rust reaction phenotypes. Recently, a technique was developed to inoculate separate tillers on a single plant with different races, improving the efficiency of adult plant resistance gene phenotyping by permitting the same plant to be used for testing multiple races (Rosa et al. 2014).

In the wheat–*P. triticina* pathosystem, most *Lr* genes are single dominant genes, and most avirulence genes are inherited as single dominant genes (Samborski 1985). However, both avirulence and resistance for a number of gene pair combinations demonstrated incomplete dominance. *Puccinia triticina* isolates heterozygous for virulence to a particular resistance gene often produce intermediate reactions on host plants carrying this gene (Samborski 1963; Kolmer & Dyck 1994). Similarly, many resistance genes confer an intermediate level of resistance when the host line is heterozygous (Kolmer & Dyck 1994).

Physiology of the Puccinia triticina*/wheat interaction*

Physiology of the *P. triticina*/wheat interaction was first investigated by using detached wheat leaves, inoculated with *P. triticina* and floated on solutions containing benzimidizole (Samborski et al. 1958) with various additives (Samborski & Forsyth 1960). Some enzyme inhibitors and metabolites prevented the development of rust pustules. A subsequent study analysing host metabolism determined that most fungus inhibiting compounds were found to also affect the host (Samborski et al. 1961). Significant changes in the physiology of leaf rust-infected wheat leaves, compared with uninfected leaves, included an increase in ribonuclease activity (Rohringer et al. 1961), which was also higher in the susceptible or compatible interaction than in the resistant or incompatible interaction.

The interactions of the 'Thatcher' near-isogenic lines for *Lr2a, Lr3, Lr9* and *LrB* with various races of *P. triticina* were analysed microscopically to determine the timing and extent of resistance responses, such as lignin and callose deposition and host cell death (Wang et al. 2013). These observations were correlated to the development and growth of the fungus within the host tissues to understand how these resistance genes slow down and sometimes stop the growth of the rust fungus within the host leaves. Future studies should determine how these are coordinated to condition resistance which will hopefully lead to more durable forms of genetic resistance.

Gene expression and silencing in the Puccinia triticina*/wheat interaction*

Gene expression in the *P. triticina*/wheat interaction was analysed using *Lr1* interacting with *Avr1* (Fofana et al. 2007). Significant differences were found between compatible and incompatible interactions using microarrays with 7728 ESTs hybridized with RNA from compatible or incompatible interactions involving host lines with *Lr1* and *P. triticina* isolates with or without *Avr1*, sampled at different time points after inoculation. A similar but more comprehensive analysis conducted using the Affymetrix GeneChip® wheat genome array comprising more than 60,000 probe sets revealed the central role of COI1, the primary component of jasmonic acid signalling, and the shift towards carbon conservation 24 hours after infection (Kumar et al. 2014).

Another approach to better understand the *P. triticina*/wheat interaction has focused on the biology and genetics of the pathogen. To obtain a gene catalogue, an EST library was developed from various spore stages of *P. triticina* and from the *P. triticina*/wheat interaction

including isolated haustoria of the fungus (Hu et al. 2007a; Xu et al. 2011). These ESTs have proven to be a rich source from which SSR markers can be derived (Wang et al. 2010b). Since ESTs are derived from expressed genes, a correlation with gene expression was tentatively made and dramatic differences in the transcriptomes of the various spore and infection stages were found. Several candidate pathogenicity genes were identified based on comparative analyses with other model pathogens (Hu et al. 2007b).

Since genetic manipulations such as gene deletion and genetic transformation are as yet not feasible in *P. triticina*, performing functional gene studies is challenging. To address this challenge, a novel Host Induced Gene Silencing (HIGS) approach was developed (Panwar et al. 2013a, 2013b), where pathogen gene sequences are expressed in the host but post-transcriptional gene silencing produces a suppressed fungal gene phenotype. Disease severity has been significantly reduced by silencing sequences targeted to predicted *P. triticina* pathogenicity genes. When these genes were effectively silenced, disease symptoms were reduced after leaf, stem or stripe rust fungal inoculations. This confirmed the role of these fungal genes in pathogenicity but also showed that this approach could possibly lead to alternative ways for crop protection (Bakkeren et al. 2012; Panwar et al. 2013a). Through collaboration with Canadian researchers, the complete draft genome sequence of *P. triticina* race 1 (BBBD) was publicly released in 2010 (http://www.broadinstitute.org). This resource has supported the *P. triticina* genetic mapping and has allowed comprehensive analyses of pathogenicity and virulence factors of this fungus (Fellers et al. 2013; Bruce et al. 2014).

Proteomics of the Puccinia triticina*/wheat interaction*

A relatively new tool for analysing pathosystems at the molecular level is proteomics. Unlike genomics methods, proteomics measures proteins directly, along with changes in their abundance and post-translational modification status both in space and time. This is a tall order, and most progress has been made in model-organisms fueled by advances in mass spectrometry and bioinformatics. However, the wheat-rust pathosystem has no suitable model for comparison to facilitate progress. The first forays into rust proteomics, although the term had not yet been coined, were made at the Cereal Research Centre by Kim et al. (1982) and Howes et al. (1982). They compared the proteomes of resting and germinated urediospores, respectively, of *P. graminis* f. sp. *tritici* by 2-D gel electrophoresis, and demonstrated that there were race-specific differences in the protein patterns on the gels. However, at that time they were unable to identify any of the proteins, and the significance of their discovery remains unknown.

The first recent publication of the proteome of an incompatible interaction between *P. triticina* and the wheat cultivar 'Thatcher' highlighted more of the difficulties still being faced (Rampitsch et al. 2006). At the time, *P. triticina* Race 1 had not yet been sequenced, necessitating protein identification through cross-species database searching, and manual *de novo* sequencing of peptides for which no credible match could be found. In addition, using 2-D gels of whole unenriched tissue resulted in superficial proteome coverage. All of the important biochemical events occur at levels far below the sensitivity of the approach available at the time. This prompted research into enriched haustoria (Song et al. 2011), which revealed many more proteins. It was clear that more work would be needed to provide details on the biochemistry of this pathosystem. This work led to the production of monoclonal antibodies to enable purification of Race 1 (BBBD) haustoria to near-homogeneity (Rampitsch et al. 2015). The isolation of the haustorial proteome of this race can now be used in conjunction with the sequenced genome to identify proteins and genes which could be involved in pathogenesis. These antibodies were used to compare the proteomes of haustoria from different races of *P. triticina*, which could be the key to identifying the proteins associated with the virulence differences between the races (Rampitsch et al. 2013).

Summary

Research on wheat leaf rust in Canada has been conducted for more than a century, generating tremendous knowledge and resources. Progress has been made on controlling wheat leaf rust through an integrated approach with the development of leaf rust resistant cultivars, identification of novel resistance sources, understanding virulence dynamics of the *P. triticina* population, and analysing the host/parasite interaction. Resistant cultivars were developed for Canadian farmers through the use of available effective genes, the incorporation of genes from related species into adapted wheat backgrounds (e.g. 'Thatcher'), and gene pyramiding. Monitoring virulence in the *P. triticina* population in Canada annually has been crucial for the early detection of virulence to resistance genes used in cultivars, and has demonstrated the selective effect of resistance genes used in Canada and the USA on the pathogen population. A multidisciplinary team of researchers has determined the underlying genetic basis for many resistance and avirulence genes, and the

complex interaction between these genes. The body of knowledge generated by pioneering Canadian rust scientists has been capitalized upon by successive generations of dedicated scientists in Canada and around the world, to further understand the *P. triticina*/wheat system towards even more effective and durable genetic control of leaf rust in the future.

Acknowledgements

We thank Pat Seto-Goh, Barbara Mulock, Elsa Reimer and Winnie McNabb for excellent technical assistance on the recent wheat leaf rust pathology portions of this research. Thanks also to Mira Popovic, Jadwiga Budzinski, Sasanda Nilmalgoda and Ghassan Mardli, Eva Beimcik, Leslie Bezte, Rob Linning, Abdulsalam Dakouri and David Joly for their contributions to this research.

Funding

We gratefully acknowledge financial assistance for much of the research cited from Agriculture and Agri-food Canada, the Western Grains Research Foundation, the Ontario Research Fund – Research Excellence.

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