GENETIC STUDIES OF HOST - PATHOGEN INTERACTION BETWEEN *BRASSICA NAPUS* AND *LEPTOSPHAERIA MACULANS*.

A Thesis

Submitted to the Faculty of

Graduate Studies

The University of Manitoba

by

Mario Keri

In Partial Fulfillment of the

Requirement for the Degree

of

Ph.D.

Department of Plant Science

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FACULTY OF GRADUATE STUDIES

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ABSTRACT

Keri, M., Ph.D., The University of Manitoba, October, 1999. Genetic studies of host - pathogen interaction between *Brassica napus* and *Leptosphaeria maculans*.

Major Professor: Dr. S. R. Rimmer.

Leptosphaeria maculans (anamorph: *Phoma lingam*) causes blackleg disease of oilseed rape. Cultivars and lines of *Brassica* species were evaluated for reaction to isolates of *L. maculans* originating from different geographic regions. Based on interaction phenotypes (IP) expressed on cotyledons of the *Brassica napus* cultivars Westar, Glacier and Quinta, the isolates were divided into seven groups, consisting of aggressive and non-aggressive isolates; on the inclusion of five additional *B. napus* differentials, the aggressive isolates were further divided into 23 sub groups. The aggressive isolates originating from western Canada were mainly classed as pathogenicity group (PG) 2 while the isolates originating from eastern Canada were grouped mainly as PG 4. Further, based on reactions of all *B. napus* differentials, the PG 2 isolates were subdivided into 15 groups whereas the PG 3 and PG 4 isolates were further subdivided into eight groups.

Studies to characterize DNA from 18 aggressive isolates of *L. maculans* on the basis of RAPD were also undertaken, using 150 ten mer-oligonucleotide random primers. Twenty eight primers were informative and of these, 14 differentiated between and within the virulent isolates on the basis of DNA polymorphism.

Isolates belonging to PG 2 were highly monomorphic, whereas the PG 3 and PG 4 isolates were more variable. One isolate was identified by the primers as different from the sample PG 2 isolates used in the study and was designated PG 2-1. This isolate was later confirmed as different from PG 2 using the additional *B. napus* differential genotypes. Some PG 3 and PG 4 isolates formed subclusters. However this was not related to IP on the host genotypes.

The inheritance of resistance in the *B. napus* cultivars, Westar, Glacier and Quinta was investigated using four isolates of *L. maculans* belonging to four pathogenicity groups (PG). Quinta (resistant to PG 2-1, PG 2 and PG 3), Glacier (resistant to PG 2-1 and PG 2) and Westar (susceptible to all the four isolates) were reciprocally crossed to each other. The parents, F_1 and F_2 populations from the susceptible x resistant crosses, resistant x resistant crosses and their reciprocals were evaluated at the cotyledon stage, using the cotyledon inoculation method, with the isolates PL87-41 (PG 2-1), PL86-12 (PG 2), Lifolle 6 (PG 3) and Lifolle 5 (PG 4) chosen from different *L. maculans* pathogenicity groups. No differences were observed with direction of cross. Resistance to *L. maculans* was expressed as a dominant trait. A single dominant gene each in Glacier and Quinta determined resistance to the PG 2-1 isolates. Likewise, resistance to the PG 2 isolates of *L. maculans* was conferred by one dominant gene in Glacier. In Quinta however, two genes with duplicate recessive epistatic interaction controlled resistance to the PG 2 isolate of *L. maculans*. Resistance in Quinta to the PG 3 isolate of *L. maculans* was controlled by a single dominant gene, based on the F_2 populations derived from Glacier x Quinta crosses, thus confirming the results obtained from the Westar x

Quinta crosses. It was concluded that four genes determine resistance in the *B. napus* cultivars to the isolates used. All plants were susceptible to the PG 4 isolate.

The genetics of resistance in Quinta to four progeny isolates obtained from crosses of *L. maculans* was also investigated. Two resistant *B. napus* cultivars (Quinta) and the susceptible cultivar (Westar) were reciprocally crossed to each other. The parents, F_1 and F_2 populations from the susceptible x resistant crosses and their reciprocals were studied at the cotyledon stage, for resistance to two PG 2 isolates (1130 - B24 and 5174/11) and two PG 3 isolates (PL89 - 21 and 1130 - A18). Resistance to these isolates in Quinta was expressed as a qualitative trait and controlled by nuclear genes. A dominant gene determined resistance in Quinta to the PG 3 isolate 1130 - A18 and another dominant gene conferred resistance to the PG 3 isolate PL89 - 21. The genes conferring resistance to the two PG 3 isolates were not allelic but rather were linked (RF = 16.44%). Resistance in Quinta to the PG 2 isolate, 1130 - B24 was controlled by one dominant gene. The gene conferring resistance in Quinta to the PG 2 isolate, 1130 - B24 was linked to genes conferring resistance to the PG 3 isolates (1130 - A18 and PL89 - 21), with a RF of 9.49% and 6.96% respectively. Segregation of resistance in Quinta to the PG 2 isolate, 5174/11 did not fit a one gene model nor a two gene model.

FOREWORD

This thesis follows the manuscript style outlined by the Department of Plant Science, University of Manitoba. Manuscripts follow the style recommended by the Canadian Journal of Plant Pathology. Four manuscripts, each containing an abstract, an introduction, materials and methods, results and discussion are presented. The manuscripts are preceded by a general introduction and literature review, and followed by a general discussion, literature cited and appendices.

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CHAPTER 1

GENERAL INTRODUCTION

Leptosphaeria maculans (Desm. Ces & de Not.), anamorph: *Phoma lingam* (Tode:Fr.) Desm., the causal organism of blackleg disease of crucifers, is an important pathogen of oilseed rape in many oilseed rape production regions (Cook and Evans 1979). Based on pathogenicity studies numerous researchers have categorized isolates of *L. maculans* into at least two types (now designated as types A and B) and commonly referred to as aggressive or highly virulent types and non aggressive or weakly virulent respectively (Koch et al. 1991, Mengistu et al. 1991, Jensen 1994, Johnson & Lewis 1994, Plummer et al. 1994, Somda & Brun 1995). In the field both types cause disease on susceptible *Brassica* species, often producing similar symptoms. Serious economic losses due to seed yield reductions have been reported in various parts of the world (Rawlinson & Muthyalu 1979, Cargeeg & Thurling 1980a, Kharbanda 1989, Gugel & Petrie 1992, Salisbury et al. 1995, Sansford 1995, Fitt et al. 1997) and are usually attributed to the aggressive types of *L. maculans* (Martens et al. 1984, Petrie & Lewis 1985). The pathogen has been adequately controlled in many regions, using resistant cultivars and cultural methods (Rimmer and van den Berg 1992). In Australia, Canada and Europe, oilseed rape cultivars with moderate to high resistant reactions to the aggressive isolates of *L. maculans* are increasingly in use.

Genetic variation in populations of *L. maculans* in Canada has not been documented in any detail. Deficiency in this knowledge is partly due to the lack of

a suitable host differential set which can distinguish different pathotypes of *L. maculans*. For a breeding program to be effective, it is necessary to understand genetic variability both in the host and the pathogen. A number of genetic traits have been used to characterize isolates of *L. maculans* (Goodwin and Annis 1991, Koch et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995, Somda et al. 1996, Taylor et al. 1991b). However, their utility has been hampered by the unreliability of certain markers, e.g. pigment production, inadequacy of biochemical markers and electrophoretic karyotyping in identifying genetic variability within the aggressive populations of *L. maculans*.

Pathogenicity and virulence are two important genetic traits and are reliable and easy to score. In the *L. maculans* - *Brassica* pathosystem, virulence has been used as genetic marker for different isolates of *L. maculans* (Badawy et al. 1991, Ballinger et al. 1991, Koch et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995). The *B. napus* cultivars Westar, Glacier and Quinta have been used in many of the studies to differentiate isolates of *L. maculans* (Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995, Ansan-Melayah et al. 1995). Based on the cotyledon interaction phenotype (IP) of these cultivars, at least four pathogenicity groups (PG), often referred to as PG 1 (non-aggressive or B type), PG 2, PG 3 and PG 4 (aggressive types or A type) have been identified (Mengistu et al. 1991, Rimmer and van den Berg 1992). PG 1 isolates of *L. maculans* are avirulent on all 3 differential cultivars; PG 2 isolates are virulent only on Westar, PG 3 isolates are virulent on Westar and Glacier, and PG 4 isolates are virulent on Westar, Glacier

and Quinta. Initially PG 2 isolates were reported in western Canada (Mengistu et al. 1991, Kutcher et al. 1993). Subsequently, Ballinger et al. (1991), Kuswinanti et al. (1995), Kutcher et al. (1993), and Mengistu et al. (1991) characterized isolates of *L. maculans* into more pathogenicity groups, based on IP on differential cultivars and lines of different *Brassica* species. It is evident that the various results obtained in these studies may not be consistent or comparable, since different isolates of *L. maculans* and different *Brassica* species were used; moreover differential genotypes from a single *Brassica* species, for example *B. napus*, may possess genes which confer resistance to different genotypes of the pathogen and these may be influenced by different genetic backgrounds.

Researchers have sought to differentiate isolates of *L. maculans* on the basis of DNA polymorphism, since such genetic markers are selectively neutral. Differences between aggressive and non-aggressive isolates of *L. maculans* were identified on the basis of RAPD analysis (Goodwin and Annis 1991, Hassan et al. 1991, Schaffer & Wostemeyer 1992, Brun et al. 1997), RFLP (Johnson & Lewis 1990, Koch et al. 1991) and electrophoretic karyotypes (Taylor et al. 1991, Morales et al. 1993a, Plummer et al. 1994). Considering that genetic variation was demonstrated using host differential genotypes, such DNA based characterization of isolates of *L. maculans* would be more useful, were they able to identify further groupings, particularly within the aggressive isolates. However the above studies could only differentiate between the aggressive and non-aggressive isolates of *L. maculans*.

Limited genetic studies have been done in the *Brassica* - *L. maculans* pathosystem. Conflicting results were often obtained from such studies. This may be due to the different host material/genotypes and pathogen genotypes that were used. For example, Delwiche (1980) reported that cotyledon resistance to *L. maculans* in *B. napus* was controlled by dominant gene action. In contrast, Sawatsky (1989) reported that resistance of *B. napus* at the cotyledon stage is controlled by recessive gene action. Pang & Halloran (1996b) report that resistance in *B. napus* to *L. maculans* was determined as a quantitative trait. Ansan-Melayah et al. (1998) reported that resistance in *B. napus* cultivar Quinta to PG 3 isolates was controlled by one dominant gene. Also the inheritance of resistance in Glacier to PG 2 isolates was determined by a single dominant gene. Although Ansan-Melayah et al. (1998) concluded that resistance in Quinta to PG 2 isolates was controlled by two genes, their results for segregation for resistance was highly significant for either one or two genes. Pongam et al. (1998) studied the genetics of resistance in a doubled haploid line of *B. napus* cultivar 'Major' and found that one gene determined resistance to PG 2 isolates. There is consequently a need to better understand the genetics of host resistance and the inheritance of avirulence in *L. maculans* in this host pathosystem. The objective of this research is to better understand the genetics of host-pathogen interaction in the *Brassica* - *L. maculans* pathosystem.

CHAPTER 2

LITERATURE REVIEW

2.1.0 *Brassica* species

The Brassicaceae are composed of many genera and species which include both domesticated and wild species. Knowledge of both cultivated *Brassica* species and their wild relatives is important in order to establish long term breeding programs in oilseed rape (Wratten 1977, Roy 1978, Sacristan and Gerdemann 1986). Moreover, the wild relatives may also be of potential value as new crops. In studies of host-pathogen interactions, Badawy et al. (1991), Ballinger et al. (1991), Koch et al. (1991), Mengistu et al. (1991), Kutcher et al. (1993), Kuswinanti et al. (1995) used various genera and species of the Brassicaceae. However, these taxa differ in their genomic backgrounds and in terms of the disease resistance genes they may possess. Wild species of the Brassicaceae may possess desirable agronomic traits such as resistance to diseases, insects and nematodes that may be transferred to oilseed rape or to cultivated species (Mithen et al. 1987, Mithen and Lewis 1988, Sjödin and Glimelius 1988, Mithen and Herron 1991). For example, the hairiness of *Brassica incana* Ten. may confer resistance to certain pests, while the thickened pods of *Brassica macrocarpa* (Guss) Caruel and *Brassica hillarionis* Post may provide some resistance to pod shattering (Mithen and Herron 1991). Other desirable traits include male sterility, yellow seed and cold tolerance.

Brassica nigra (L.) Koch (n=8, BB genome), *Brassica oleracea* L. (n=9, CC genome) and the amphidiploids *Brassica carinata* Braun (n=17, BC genome), *Brassica juncea* Czern & Coss. (n=18, AB genome), Raphanobrassica L. (n=18, RC genome) are species related to *Brassica napus* L. (n=19, AC genome) and *Brassica rapa* L. (n=10, AA genome) (Morinaga 1934, U 1935, Kimber & McGregor 1995). The four cultivated species and their relationship to the other *Brassica* species have been described by U (1935).

2.1.1 Production of oilseed rape

In Canada, *B. napus*, *B. rapa*, *B. juncea*, *B. oleracea*, *B. nigra* and *Sinapis alba* L (n=12, D genome) (Kimber & McGregor 1995) are cultivated as vegetables, fodder, as a source of vegetable oil and for condiment (Love et al. 1990). Canola is the trade name for oilseed rape (*B. napus*, and *B. rapa*) cultivars with less than 2% erucic acid as a percent of the total fatty acids and less than 30 μMoles of aliphatic glucosinolates per gram oil-free meal (Downey & Rimmer 1993). Most oilseed rape cultivars are susceptible to blackleg. However recently *B. napus* cultivars resistant or moderately resistant to blackleg, e.g. Sentry and Quantum have been released. Cultivation of canola in western Canada has been increasing since the late 1960's (Petrie 1995). In Australia, epidemics due to blackleg drastically affected the rapeseed industry shortly after the introduction of the crop (Barbetti 1975, Roy and Reeves 1975, McGee and Emmett 1977, Rawlinson and Muthyalu 1979, Cargeeg and Thurling 1980a, Rimmer and Buchwaldt 1995)

2.2.0 The pathogen

Leptosphaeria maculans, [anamorph: *Phoma lingam* (Tode ex Fr.) Desm.], causal organism of blackleg in cruciferous plants, is an ascomycetous fungus (Puninthalingham & Holliday 1972, McGee & Emmett 1977). The disease is sometimes called canker (Martens et al. 1984, Newman & Bailey 1987) stem canker (Punithalingam & Holliday 1972, Newman 1984, Davies 1986), crown canker or dry rot (Punithalingam & Holliday, 1972, Kruger 1983). *L. maculans* is a hemibiotrophic parasite and both the perfect and imperfect stages are pathogenic. Two forms of this pathogen occur, either as a virulent or aggressive form or an avirulent or non-aggressive form. Variability in the rates of mycelial growth, pycnidial production and pigment production in liquid media have been correlated with aggressive (A type) and non-aggressive (B type) isolates of *L. maculans* (Petrie & Vanterpool 1966, Boerema 1976, McGee & Petrie 1978, Petrie 1978, Petrie 1979, Humpherson-Jones 1983a, Hanacziwskyj & Drysdale 1984, Hill et al. cited by Hill & Williams 1988, Koch et al. 1991). Severe disease may occur if aggressive isolates of *L. maculans* infect susceptible host genotypes at early growth stages (Martens et al. 1984). Yield losses in susceptible *B. napus* and *B. rapa* oilseed cultivars due to blackleg have been reported (McGee and Emmett 1977, Petrie 1995, Salisbury et al. 1995).

2.2.1 Occurrence

Leptosphaeria maculans occurs in most oilseed rape producing areas of the

world. In Canada, non-aggressive isolates of *L. maculans* were first detected in the 1960s (Vanterpool 1961, Vanterpool 1963). Since then both aggressive and non-aggressive isolates have been identified on the prairies (McGee & Petrie 1978, Petrie 1979 Petrie et al. 1985, Platford 1988, van den Berg et al. 1989), and Ontario (Peters & Hall 1989, Gugel & Petrie 1992, Rempel & Hall 1993). Likewise, both A and B types of *L. maculans* have been reported by researchers in Great Britain (Brown et al. 1976, Cook & Evans 1978, Gladders & Musa 1980, Humpherson-Jones 1983b, Newman 1984, Humpherson-Jones 1986, Gladders & Symonds 1995, Fitt et al. 1997), France (Lacoste et al. 1969, Somda and Brun 1995), Germany (Kruger 1983, Kuswinanti et al. 1995), Denmark (Jensen 1994), Australia (Bokor et al. 1975, McGee and Emmett 1977, Wood & Barbetti 1977, Cargeeg & Thurling 1980b, Plummer et al. 1994, Salisbury et al. 1995), and recently in North Dakota and Kentucky, U.S.A. (Lamey 1995). In Saskatchewan aggressive isolates of *L. maculans* were first detected in 1975 (McGee & Petrie 1978, Petrie 1979). Aggressive isolates have also been recorded in Alberta (Kharbanda 1989) and Ontario (Peters & Hall 1989).

There has been a progressive spread of *L. maculans* in Canada. With the introduction of cultivars with major gene resistance to *L. maculans* a high potential exists for the increase of the aggressive isolates of *L. maculans,* or for evolution of new virulent pathotypes of *L. maculans* (Petrie 1995, Mayerhofer et al. 1997). Moreover, sexual recombination between different genotypes of *L. maculans* has been demonstrated (Rimmer & van den Berg 1992).

Aggressive isolates of *L. maculans* have caused considerable yield losses in oilseed rape (Kharbanda 1989, Gugel & Petrie 1992, Petrie 1995, Salisbury et al. 1995, Sansford 1995). In Ontario, yield losses due to blackleg have been estimated to be over 25% in some fields (Gugel & Petrie 1992) whereas in western Canada, McGee & Petrie (1978), Petrie (1978), and Petrie et al. (1985b) reported losses of up to 50%. Losses as high as 50% have occurred in Germany (Gugel & Petrie 1992, Siedel et al. 1984 in Lamey 1995). In Logan county of Kentucky (USA), 75-90% losses have been reported (Lamey & Hershman 1993, Hershman 1992 in Lamey 1995). Similarly in the UK, up to 93% of crops were infected with blackleg in 1993 (Sansford 1995). This was the highest level of infection recorded in 10 years. In Australia, *L. maculans* eliminated the production of oilseed rape until resistant cultivars were developed (Bokor et al. 1975, Wood & Barbetti 1977, Cargeeg & Thurling 1980a, Cargeeg & Thurling 1980b, Salisbury et al. 1995).

2.3.0 The disease

All parts of the susceptible plant may be infected by the pathogen and susceptible genotypes of oilseed rape may become infected at any stage of development of the crop (Wratten 1977, Gabrielson 1983, Kruger 1983, Martens et al. 1984). When the disease is seed-borne, seedlings of the susceptible genotypes may be killed at emergence or shortly thereafter. Disease symptoms on such plants often resemble damping off. When infection occurs early in plant development (i.e. from emergence to six leaf stage) severe crown cankers may

develop (Wratten 1977, McGee and Petrie 1979, Martens et al. 1984) on susceptible genotypes. This can result in the complete girdling of the crown, lodging and/or the death of the plant. *Leptosphaeria maculans* may also colonize roots of host plants with no apparent symptoms, except darkening of the roots and root collar region (Gugel et al. 1990, Keri 1991).

2.3.1 Disease cycle

Leptosphaeria maculans overwinters as seed-borne mycelia, pycnidia on the seed coat, or as pycnidia or pseudothecia on infested crop residue. Ascospores are released from pseudothecia into the air and are wind-borne for distances up to 10 km (Bokor et al. 1975, McGee 1977, Petrie 1979, Gladders and Musa 1980). Discharge of ascospores follows a seasonal pattern in Australia, Canada and France (Alabouvette and Brunin 1970, McGee 1977, McGee and Petrie 1979). In Canada, ascospore discharge begins about July of the year after the canola crop has been harvested and continues throughout the summer and fall. However, in the second and subsequent years ascospore discharge begins early in the spring and continues until late autumn, decreasing quantitatively over the following years (McGee 1977).

Ascospores released into turbulent air may land on leaves where they germinate, infect and produce lesions on susceptible *Brassica* host genotypes. Once established, the fungus forms pycnidia which exude pycnidiospores (Martens et al. 1984, Hammond & Lewis 1986a). Pycnidiospores serve as secondary

inoculum to spread the pathogen locally, by water splash dispersal, to neighbouring plants. When conditions are conducive, severe disease epidemics may develop. Secondary inoculum, in the form of mycelia infested seed, may also serve to initiate or introduce the disease into new locations (Humpherson-Jones 1983a, Rimmer & Buchwaldt 1995). The fungus overwinters on crop debris and on other cruciferous weeds as mycelia, pycnidia and pseudothecia (Cunningham 1927, Gabrielson 1983, Hammond et al. 1985, Hammond & Lewis 1986a, Hammond & Lewis 1986b, McGee 1977, Hill & Williams 1988).

2.3.2 Control of disease

Seed-borne inoculum may not play an important role in the spread of blackleg disease in the susceptible crop (McGee 1977, Wood & Barbetti 1977), but it is important for introduction of the pathogen to new areas (Rimmer & Buchwaldt 1995). A seed source with a high percentage of infested seed had a high percentage of infected plants (Wood & Barbetti 1977). Up to 19% of plants in commercial fields developed blackleg when the seed source had 5.9% infected seed, but only 0.06% of plants were infected from seed with 0.5 % infection. Preventative measures against such introductions have been established in certain regions. Contaminated seed lots can be excluded by the use of seed testing methods that detect *L. maculans*, (Neergaard 1977) since even seed from healthy pods may be infested (Wood & Barbetti 1977).

Hot water treatment of seeds has been found to be effective (Walker 1923), but

this method may be impractical where large quantities of seeds are involved; moreover, the treatment may not completely eradicate the pathogen (Cunningham 1927, Gabrielson 1983). *Phoma lingam* was isolated from up to 18% of seed which was not treated with hot water. Williams (1967) treated cabbage seed from Europe once and Australia twice with hot -water treatment and reported that all treated seed from Europe and Australia was free of seed-borne blackleg. Chemical seed treatment has been used as an alternative in order to eradicate seed-borne pathogens. Treatments including a thiram seed coating or soaking (Maude et al. 1969), fenpropimorph (Maude et al. 1984), benomyl, iprodione, and thiabendazole (Maude et al. 1981, in Gabrielson 1983) have been investigated. However control of *L. maculans* seed-borne infection was variable. In growth chamber tests, Kharbanda (1989) found iprodione to be effective in the control of seed borne *L. maculans*.

Leptosphaeria maculans may persist in infected host tissue, hence the slower the breakdown of residue, the longer the persistence of the pathogen. Infested residue is the usual source of primary inoculum (i.e. pycnidiospores and ascospores). Decomposition of such residues depends on environmental conditions. In western Canada, infested residue may persist for 3 - 4 years. Consequently, a rotation of 3-5 years is recommended.

Ascospores are wind-borne. Therefore it may be necessary to implement measures to control the disease in adjacent fields (McGee and Emmet 1977) or even in distant fields. In Australia, delaying the seeding date so that the period of

heavy ascospore release does not coincide with the early and most susceptible stage of the crop has been used as an avoidance measure (McGee 1977). This is not practical in regions where ascospores are released throughout the year, or in regions such as Canada, where early frost in spring and fall may not permit such flexibility. In China, blackleg is not a problem. Likely reasons include flooding of the land for rice cultivation and harvesting rape stubble for fuel (Rimmer & Buchwaldt 1995).

Leptosphaeria maculans has been isolated from susceptible weed species such as *Sinapis arvense* L. (wild mustard) (Petrie & Vanterpool 1966, Petrie & Vanterpool 1968, Petrie 1978, Sjödin and Glimelius 1988). There is little evidence for specialization of this pathogen on the different *Brassica* species. Consequently the control of wild species and volunteer oilseed rape is desirable.

2.3.2.1 Chemical control

Brown et al. (1976), Morrall and Xi (1988), Xi and Morrall (1988) reported that applications of benomyl, triadimefon, flutriafol, iprodione, dimiconazole were all ineffective in the control of blackleg in oilseed rape cultivars. Only limited but uneconomical control was obtained by multiple foliar applications of prochloraz. In Australia, flutriafol was shown to be effective when applied at a rate of 75-100 g ai ha^{-1} (Ballinger et al. 1988a, Ballinger et al. 1988b). However, in Canada, Xi et al. (1988) and Xi et al. (1991a) observed only limited control of blackleg with flutriafol. Humpherson-Jones and Burchill (1982) reported that formation of pseudothecia in

the field or the in vitro release of ascospores may be restricted by chemical means. If found effective under field conditions, such chemical treatments may also interfere with sexual recombination between different genotypes of *L. maculans*.

2.3.2.2 Host resistance to control *L. maculans*

Host plant resistance may be defined as the ability of a given host plant to restrict and resist the growth of and colonization by a pathogen. The use of resistant cultivars of oilseed rape is an effective means to control blackleg of crucifers (Rimmer & Buchwaldt 1995). In Australia, Canada and Europe, a major objective of oilseed rape breeding is the development of resistant cultivars. Following serious yield losses in the late 70s in Australia, resistant cultivars were developed to ensure continued production of oilseed rape (Bokor et al. 1975, Rawlinson & Muthyalu 1979, Rimmer & Buchwaldt 1995, Salisbury et al. 1995). Similarly, *B. napus* cultivars in Canada were susceptible to *L. maculans* in the 1980's, but presently at least 20 cultivars are resistant or moderately resistant to *L. maculans*.

2.4.0 Sources of resistance

Various sources of resistance to *L. maculans* have been identified within the species *B. napus* (Wratten 1977, Roy 1978), *B. rapa* (Kutcher 1990, Ballinger et al. 1991) and *B. juncea* (Roy 1978, Roy 1984, Sacristan and Gerdemann 1986, Sjödin and Glimelius 1988, Ballinger et al. 1991, Keri 1991).

Seedling resistance was identified in a French winter type breeding line and in a North American spring type *B. napus* (Delwiche 1980, Sawatsky 1989); some winter rape cultivars were reported to have fair levels of resistance to *L. maculans* (Kutcher 1990, Rimmer and van den Berg 1992, Downey and Rimmer 1993). In Australia, the resistant lines Major and Ramses (Wratten 1977, Roy 1978) and in Europe, the cultivar Jet Neuf were used to develop new resistant cultivars (Rimmer, personal communication). Resistance to *L. maculans* in spring type *B. napus* cultivars, such as Maluka and Taparoo, was developed using the Japanese cultivars Chisaya, Chikuzen and Mutu natane as sources for resistance (Downey and Rimmer 1993).

Resistance to *L. maculans* has also been identified in *B. nigra* and *B. carinata* (Mithen et al. 1987, Brun et al. 1995). Similarly, resistance has been identified in *B. juncea* (Roy 1978, Ballinger et al. 1991, Keri 1991) and in other *Brassica* species (Kutcher 1990, Mithen & Herron 1991). The resistance in *B. juncea* was transferred to *B. napus* and *B. rapa* cultivars (Roy 1978, Roy 1984, Gugel et al. 1994). Mithen and Herron (1991) transferred resistance to *L. maculans* from wild *B. insularis*, *B. macrocarpa* and *B. atlantica* to *B. napus*, but the resulting synthetic lines of oilseed rape were all susceptible to *L. maculans* at the cotyledon stage. However foliar resistance was expressed as a reduction in lesion size but an increased amount of necrosis around the site of inoculation (Mithen and Herron 1991).

Resistance has been identified in the wild turnip *Erucastrum gallicum*. Similarly, *Arabidopsis thaliana*, *Diplotaxis muralis*, *Diplotaxis tenuifolia* and *Sisymbrina loeselii*

were shown to have cotyledons, leaves and stems that were more resistant to *L. maculans* than *B. napus* (Gugel et al. 1997). Segregation for resistance was observed in *Raphanus raphanistrum* at cotyledons and leaves, with the reactions ranging from highly susceptible to highly resistant; the stems were highly resistant (Chen and Séguin-Swartz 1997).

2.5.0 Morphological markers

Isolates of *L. maculans* have been discriminated on the basis of rate of mycelial growth and amount of mycelia produced (Cunningham 1927, Pound 1947, Petrie 1969, Petrie 1988). Differences between aggressive and non aggressive isolates in rate of growth in culture, pycnidia production and spore size have been reported. However, morphological markers are often limited in number. Further, morphological markers may not be accurate in determining genetic relationships in populations of *L. maculans* since their heritability may be low, and they may be subject to pleiotropic effects due to influences of the environment (Paterson et al. 1991).

2.5.1 Genetic markers

Differences between individuals of a given organism may be detected with the aid of markers. Biochemical markers (isozymes and toxins) and molecular markers [electrophoretic karyotypes, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic

DNA (RAPD)] have been used to discriminate isolates of *L. maculans* (Sacristan 1985, De March et al. 1986, Badawy & Hoppe 1989a, Badawy & Hoppe 1989b, Koch et al 1989, Goodwin and Annis 1991, Pedras & Séguin-Swartz 1992, Brun et al. 1997, Mahuku et al. 1996, Mahuku et al. 1997, Pongam et al. 1998). Direct markers such as pathogenicity and virulence have also been used to discriminate isolates of *L. maculans* (Ballinger et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995).

2.5.2 Physiological markers and biochemical markers

In liquid culture isolates of *L. maculans* produce pigments that range in colour from yellow to brown (McGee & Petrie 1978). Differences in the production of the brown pigment have been used to separate aggressive isolates of *L. maculans* from non-aggressive isolates (McGee & Petrie 1978, Delwiche 1980, Newman 1984, Humpherson-Jones 1986). Sippell & Hall (1995) reported that both aggressive and non aggressive isolates may produce pigment in culture. The amount of pigment produced, as defined by the intensity of colour, may vary considerably in single spore isolates of a culture or between different isolates of *L. maculans*. In other pathosystems where pigments have been used as genetic markers, similar variation in the intensity of pigments have been observed even among tissue or organs of a single isolate. Moreover, genotype x environment interactions may affect the quantity of pigment produced, hence making pigmentation an unreliable marker. When the genetic basis for pigment production is known, the pigment could be a

versatile genetic marker, since pigments can be scored visually, hence rapidly, easily and inexpensively.

Isozyme differences have been used to classify isolates of *L. maculans* (Hassan et al. 1991, Hanacziwskyj & Drysdale 1984, Hill et al. 1984, Sippell et al. 1988, Gall et al. 1995, Sippell & Hall 1995). Isozymes are a class of enzymes that share a common substrate but exhibit different electrophoretic mobilities. When different electromorphs are identified by genetic analysis as allelic, they are called allozymes. The frequent polymorphism of isozymes make them useful as genetic markers and their co-dominant nature permits the identification or estimation of heterozygosity. Isozymes have also been used to identify dominant, recessive or epistatic alleles in other systems. The technique is rapid and allows the different alleles or loci to be scored easily, and at low cost.

Differences between aggressive and non aggressive isolates of *L. maculans*, based on profiles of phytotoxins produced in liquid cultures, have been reported. Phytotoxins might also be useful as substitutes for the pathogen to screen host genotypes in breeding. Phytotoxins are secondary metabolites and a characteristic of the necrotic phase of *L. maculans* (Badawy & Hoppe 1989a, Badawy & Hoppe 1989b, Koch et al 1989, Pedras et al. 1990). At least eight phytotoxins are produced by aggressive isolates, including sirodesmin PL, deacylsirodesmin PL, sirodesmin H, sirodesmin J, sirodesmin K and phomalizarine (Badawy & Hoppe 1989a, Badawy & Hoppe 1989b, Koch et al 1989, Pedras et al. 1990, Pedras et al. 1992). Sirodesmin PL is the major phytotoxin produced by aggressive isolates of

L. maculans. Sjödin and Glimelius (1989) found resistant host genotypes that were insensitive to sirodesmin PL, indicating that sirodesmin PL is specific in action. However, Pedras et al. (1993) reported that the above mentioned phytotoxins are non specific in action. Most researchers suggest that the only differences between these phytotoxins on resistant and susceptible host genotypes is quantitative, rather than qualitative (De March et al. 1986, Badawy & Hoppe 1989a, Badawy & Hoppe 1989b, Koch et al 1989, Pedras et al. 1990). Recently, Pedras et al. (1993) reported a new phytotoxin (phomalide) which causes lesions on oilseed rape closely resembling the symptoms caused by the fungus on resistant and susceptible host genotypes. Phomalide was produced by germinating spores that were 24 - 60 h old, but not by older cultures; moreover, its activity was negated by sirodesmin PL (Pedras et al. 1993).

2.5.3 Molecular markers

In the *L. maculans-Brassica* pathosystem, DNA markers have been used to differentiate or fingerprint aggressive and non-aggressive isolates of *L. maculans* (Johnson and Lewis 1990, Goodwin and Annis 1991, Hassan et al. 1991, Koch et al. 1991). Distinct differences have been reported between aggressive and non-aggressive isolates of *L. maculans* based on RFLP markers (Johnson and Lewis 1990). Similarly, based on RAPD markers, differences between aggressive and non-aggressive isolates have been reported within Canadian isolates of *L. maculans* (Goodwin and Annis 1991). Mahuku et al. (1996) and Brun et al. (1997)

distinguished the aggressive isolates of *L. maculans* from non-aggressive ones in infected tissue of *Brassica* species. Different isolates may vary in number and size of chromosomes. These differences have been used to distinguish the aggressive from non-aggressive *L. maculans* isolates (Taylor et al. 1991, Morales et al. 1993a, Plummer & Howlett 1993, Plummer et al. 1994). Electrophoretic karyotypes of selected aggressive and non-aggressive isolates indicated that, while non-aggressive isolates have 12 - 14 chromosomal bands, aggressive isolates have 6 - 8 distinct bands (Taylor et al. 1991, Morales et al. 1993a, Plummer & Howlett 1993, Plummer et al. 1994). Consequently, it has been suggested that aggressive and non-aggressive isolates of *L. maculans* belong to two different species (Goodwin and Annis 1991, Hassan et al. 1991, Meyer et al. 1992, Morales et al. 1993a, Morales et al. 1993b, Taylor & Borgman 1994).

2.5.4 Avirulence genes as markers

Pathogenicity and/or virulence are markers of practical importance for discriminating different isolates of *L. maculans*. In order to identify new genes conferring resistance to existing aggressive isolates of *L. maculans*, it is necessary to evaluate host plants for disease phenotypes. Different single genes (major genes) for resistance to *L. maculans* may be recognized when isolates of the pathogens representing different groups or races are used in such a screening procedure. This often can be time consuming and labour intensive, especially when the differential lines or genotypes used do not possess single genes for

resistance to the given pathogen. Pongam et al. (1999) evaluated 49 isolates of *L. maculans* from different regions using AFLP analysis. They reported that isolates from North Dakota, Georgia and western Canada were closely related to one another and that the isolates from Australia, Germany, France, UK and Ontario were a separate but closely related group. The PG 2 isolates found in North America however, formed several sub clusters. Pongam et al. (1999) used only 1 isolate from Canada.

2.5.5 Physiological specialization

In the *Brassica* - *L. maculans* pathosystem, aggressive isolates of *L. maculans* are characterized by pathogenicity tests on differential sets of plant genotypes. The host differential genotypes are composed of *B. napus* and various other *Brassica* species (Badawy et al. 1991, Ballinger et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995).

Mengistu et al. (1991) studied pathogenic variability in isolates of *L. maculans* from Australia, Europe and North America, using the *B. napus* cultivars Westar, Glacier and Quinta. Isolates of *L. maculans* were grouped into four pathogenicity groups (PG 1 to PG 4). The PG 1 isolates were avirulent on all the three cultivars and the PG 2 isolates were virulent (causing extensive necrotic response) on Westar. Koch et al. (1991) reported that PG 2 isolates also elicited an intermediate interaction phenotype (IP) on both Quinta and Glacier. The PG 3 isolates were virulent (causing extensive necrosis and sporulating) on both Westar and Glacier,

but they elicited an intermediate IP on Quinta (Koch et al. 1991, Mengistu et al. 1991). All three cultivars were susceptible to PG 4 isolates. The PG 2, PG 3 and PG 4 isolates also caused severe symptoms on some accessions of *B. rapa*, *B. oleracea* and *R. sativus*, but *B. nigra*, *B. carinata* and *B. juncea* were resistant to all the isolates (Mengistu et al. 1991).

Badawy et al. (1991) used *B. napus* cultivars (Glacier, Quinta, Jet Neuf and five other cultivars), *B. rapa* (five cultivars), *B. oleracea* (eight cultivars) and *B. juncea* (one cultivar) to differentiate isolates of *L. maculans* and classified the aggressive isolates as A-type and the weakly aggressive isolates as the B-type. Based on their results, A type isolates could be divided into three groups. They further characterized the isolates on the basis of lesion size and rate of sporulation into two additional sub-groups. One pathogenicity group (A2) caused disease on hypocotyls of the cultivar Jet Neuf (Badawy et al. 1991).

Ballinger et al. (1991) identified 14 distinct pathogenicity groups in populations of *L. maculans* from Australia. Twelve differential cultivars were used consisting of the spring *B. napus* cultivars Chisaya, Marnoo, Midas, Niklas, Wesbrook and RX3; winter type *B. napus* cultivars Jet Neuf and Rafal plus spring type *B. rapa* cultivars and the *B. juncea* cultivars Stoke and Zaria. All isolates tested were A types (Ballinger et al. 1991). Some differential cultivars were susceptible at the cotyledon stage but resistant at the adult stage. As a result, Ballinger et al. (1991) suggested that cotyledon resistance and adult plant resistance are two distinct traits. Isolates of *L. maculans* able to cause disease on *B. juncea* were identified (Ballinger et al.

1991, Keri 1991, Rimmer & van den Berg 1992). This suggests that the use of resistance genes originating from *B. juncea* to confer resistance in susceptible *B. napus* genotypes may not necessarily lead to a durable type of resistance to *L. maculans* in such new *B. napus* cultivars.

Kutcher et al. (1993) used *B. napus* (both spring and winter types), *B. rapa* and *B. juncea* accessions to differentiate isolates of *L. maculans*. Kutcher et al. (1993) found that Australian isolates of *L. maculans* were more pathogenic than Canadian isolates, and that Manitoba and Saskatchewan isolates were similar in their pathogenicity on the differential cultivars. Variation for pathogenicity was observed among isolates collected from a single field (Kutcher et al. 1993, Rempel & Hall 1993, Mahuku et al. 1997). The pathogen population was heterogeneous. Therefore, Kutcher et al. (1993) suggested that several genes for pathogenicity (sic) may occur in *L. maculans*.

Kuswinanti et al. (1995) differentiated isolates of *L. maculans* into six pathogenicity groups based on cotyledon reactions of the *B. napus* cultivars Lirabon, Quinta, Glacier and Jet Neuf. Isolates were further differentiated on 12 other *Brassica* genotypes including *B. napus*, *B. rapa*, *B. oleracea*, *B. juncea*, *B. nigra* and *B. carinata* cultivars and accessions. This resulted in the characterization of German isolates of *L. maculans* into 12 sub-groups, suggesting that populations of *L. maculans* in Germany are highly variable (Kuswinanti et al. 1995). However, little is known about the genetic basis of resistance in these differential cultivars to isolates of *L. maculans*.

2.5.6 Mating system

There is little information with regard to sexual reproduction in *L. maculans*. The few studies conducted to date are limited to the development of methods for crossing isolates of *L. maculans* (Petrie & Lewis 1985, Mengistu et al. 1991, Mengistu et al. 1993, Somda et al. 1997) with the intention to study and better understand the genetics of avirulence. The genetic control of mating type has been investigated by crossing A type isolates. Sexual reproduction in *L. maculans* is controlled by a single locus of a bipolar, heterothallic mating system (Venn 1979, Mengistu et al.1991, Gall et al. 1994, Ansan-Melayah et al. 1995, Somda et al. 1997). Recently successful matings between non-aggressive (B type) isolates of *L. maculans* have been achieved (Somda et al. 1997) but crosses between B-type and A type isolates have not been successful.

The inability of two isolates of a pathogenic fungus to mate may be due to a number of factors. For example *Tilletia caries* and *T. controversa* cultures that were repeatedly subcultured axenically, lost their ability to mate (Hoffman & Kendrick 1969, Trail 1985 cited by Mills & Churchill 1988). Crosses between isolates of *L. maculans* from cabbage and oilseed rape have been made but their fertility was reduced. The reduced fertility was thought to be due to the presence of heterogenic incompatibility factors (Hill & Williams 1988).

Parasexuality in *L. maculans* was demonstrated by Petrie (1969), using complementary biochemical mutants grown on minimal medium. Although

diploidization and haploidization of nuclei did not occur in these instances, somatic segregation for morphological markers was observed (Petrie 1969).

2.6.0 Genetics of avirulence

In the *L. maculans-Brassica* pathosystem, the study of genetics of avirulence of *L. maculans* has been hampered by a number of problems. It is only recently that, successful matings between aggressive isolates of *L. maculans* have been achieved in the laboratory (Mengistu et al. 1991, Mengistu et al. 1993, Gall et al. 1994, Ansan-Melayah et al. 1995, Somda et al. 1997). Similarly cultivars of *Brassica* sp. which can differentiate isolates of *L. maculans* were recently identified (Badawy et al. 1991, Ballinger et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995).

The relationship between genes for resistance in the differential cultivars Glacier and Quinta, and isolates of *L. maculans* belonging to different pathogenicity groups was studied by Rimmer and Mengistu (unpublished). Based on these results Rimmer and van den Berg (1992) postulated that two genes in the pathogen determine avirulence of PG 2 isolates, that Glacier has a single gene and Quinta two genes for resistance to PG 2 isolates, one of which is shared by Glacier. An earlier report by Keri & Rimmer (1998) supported this hypothesis. Similarly Ansan-Melayah et al. (1998) reported that avirulence in PG 3 isolates on Quinta was determined by a single gene. Ansan-Melayah et al. (1995) crossed a PG 3 isolate (a.2) to a PG 4 isolate (H5). The progeny (8 tetrads and 88 random spores) from

these crosses were tested on the differential cultivars Westar, Glacier and Quinta. Segregation for avirulence : virulence fitted a 1 : 1 ratio, indicative of a single locus in PG 3 isolates. Ansan-Melayah et al. (1998) also reported that at least one gene in PG 2 isolates determined avirulence on Glacier and Quinta. Segregation of progeny from PG 2 (PHW1245) X PG 4 (1.2.4) crosses and the test cross (PHW1245 X 1.2.4) X 7.9.06 reported by Ansan-Melayah et al. (1998), fitted a 1:1 ratio of avirulence : virulence on Glacier, indicating single gene control. Similarly, Ansan-Melayah et al. (1998) tested five out of eight tetrads from the above crosses on Quinta and reported that two genes determine avirulence in the PG 2 isolate (PHW1245). However, their data do not support this conclusion. Segregation of avirulence : virulence in most of the progeny isolates was significant for either a one or two gene model. Segregation in only one out of eight isolates of the tetrads fitted a 3 avirulent : 1 virulent ratio indicative of a two gene control. Pongam et al. (1998) crossed the same PG 2 isolate (PHW1245) to PG 4 (PHW1223) and studied segregation of avirulence : virulence on the *B. napus* cultivar Major. A single gene determined avirulence in the PG 2 (PHW1245) on *B. napus*. It is important to note that Ansan-Melayah et al. (1998) and Pongam et al. (1998) used different *B. napus* cultivars and different genotypes of *L. maculans*.

2.6.1 Genetics of resistance

In *B. napus*, cotyledon resistance to *L. maculans* may be controlled by either dominant or recessive genes (Delwiche 1980, Sawatsky 1989). Delwiche (1980)

reported that two dominant genes (Lm1 and Lm2) determined resistance in a French *B. napus* breeding line. These genes were thought to be linked. On the other hand, Sawatsky (1989) identified a single recessive gene controlling cotyledon resistance but two dominant genes determined adult plant resistance in the field. It has been postulated that a single gene for resistance occurs the *B. napus* cultivar Cresor (Dion et al. 1995) and single dominant genes occur in Maluka (Mayerhofer et al. 1997) and Major (Ferreira et al. 1995). Studies to determine the inheritance of resistance in the *B. napus* cultivars have used Glacier and Quinta in crosses with various susceptible *B. napus* cultivars; moreover the progeny from these crosses were inoculated using different isolates of *L. maculans* (Keri & Rimmer 1998, Ansan-Melayah et al. 1998). Keri & Rimmer (1998) crossed Quinta and Glacier to Westar and tested the parents, F_1 and F_2 with isolates of *L. maculans*. Ansan-Melayah et al. (1998) crossed Quinta and Glacier to Score and tested the parents, F_1 and F_2 with progeny isolates from crosses of *L. maculans*. Rimmer et al. (unpublished) tested the parents, F_1 and F_2 of Quinta by Westar crosses with progeny isolates from crosses of *L. maculans*. Resistance in Quinta to PG 3 isolates was controlled by one dominant gene (Rimmer et al. unpublished, Keri & Rimmer 1998, Ansan-Melayah et al. 1998). Similarly, Ansan-Melayah et al. (1998) also tested the parents (Glacier and Score), F_1 and F_2 with the PG 2 isolates (PHW1325, 14.3.01 or 14.1.01). The F_1 progeny were all resistant and the F_2 segregated for a single locus for resistance, suggesting that resistance in Glacier to PG 2 isolates was determined by a single gene. Similar results were

reported by Keri & Rimmer (1998). For the Quinta x Score crosses, Ansan-Melayah et al. (1998) tested the parents (Quinta and Score) with the PG 2 isolates PHW1325 or 14.3.01 but the F_1 and F_2 were tested with six tetrads from crosses of PG 2 (PHW1245) x PG 4 (1.2.4) and test cross (PHW1245 x 1.2.4) x 1.2.4. Although Ansan-Melayah et al. (1998) concluded that resistance in Quinta to PG 2 isolates is controlled by two genes, their segregation results for resistance in Quinta to PG 2 isolates was highly significant for either one or two gene model. Ferreira et al. (1995) studied the genetics of resistance in the double haploid of the *B. napus* cultivar 'Major' and found that one gene determined resistance to PG 2 isolates.

A better understanding of the mechanisms of resistance is required if breeders are to stay ahead of virulence changes in pathogen populations and to identify and use diverse sources of resistance. Studies involving both the host and pathogen may provide some additional clarification. Reliable DNA markers can assist in the selection of genotypes of *L. maculans*, for the purpose of isolate conservation, so that the widest possible genetic variation is included and unnecessary duplication avoided. Reliable DNA markers may also be used in lieu of differential host plants to characterize isolates of *L. maculans*. Molecular markers that are associated with different avirulence genes may facilitate the tagging of the avirulence genes in the pathogen population, such that their presence or absence in the pathogen may be inferred without having to score host plant genotypes for interaction phenotypes. In genetic studies, identity of recombinant individuals resulting from crosses using

different avirulence genotypes of *L. maculans* may be confirmed using DNA markers.

Often, the hope is that resistance genes incorporated into cultivars will provide long lasting protection against the pathogen. Information on specific interactions in *L. maculans-Brassica* pathosystem is available (Badawy et al. 1991, Ballinger et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995). However, different *Brassica* species have been used in these studies. Resistant or moderately resistant cultivars of *B. napus* are increasingly in use in oilseed producing areas. Moreover, in many cultivars of *B. napus*, the resistance to *L. maculans* is thought to have originated from a narrow genetic base (Rimmer & Buchwaldt 1995). It is possible that some of these resistance genes may alter the frequency of virulence alleles in pathogen populations (Petrie 1995, Mayerhofer et al. 1997). Few genetic studies have been undertaken to determine the inheritance of resistance in *B. napus*. Still fewer studies have been undertaken to elucidate the inheritance of resistance in both differential cultivars, Glacier and Quinta, to isolates of *L. maculans*. In order to address these shortcomings, this thesis investigates the differentiation of isolates of *L. maculans* using *B. napus* differential genotypes and characterizes the isolates based on DNA polymorphisms using the RAPD technique. It also investigates the basis of resistance in the *B. napus* differential cultivars Glacier and Quinta, using isolates from different pathogenicity groups and from crosses of isolates from different pathogenicity groups, in order to determine if the host pathogen interaction is a gene for gene based system.

CHAPTER 3

Characterization of isolates of *Leptosphaeria maculans* from different geographic regions based on cotyledon interaction phenotypes using an extended *Brassica napus* differential set.

3.1 Abstract

One hundred fifty-six isolates of *Leptosphaeria maculans* from different geographical regions were characterized into pathogenicity groups based on host-pathogen interaction using *Brassica* host differentials. Cotyledons of the *Brassica napus* cultivars Westar, Glacier and Quinta were inoculated with each isolate and interaction phenotypes classified as resistant or susceptible. The isolates of *L. maculans* were sub-divided into seven pathogenicity groups on the basis of the interaction phenotypes expressed on cotyledons of Westar, Glacier and Quinta. Further discrimination of the virulent isolates of *L. maculans* was achieved using five additional *B. napus* lines. The PG 2, PG 3 and PG 4 isolates were characterized into 23 sub groups using all the 11 host differential *Brassica* genotypes. Canadian PG 2 isolates could be grouped into 15 sub groups, whereas each of the PG 3 and PG 4 isolates of *L. maculans* from Europe and Australia had four sub groups. The additional differentials may be useful to study changes in virulence of *L. maculans* and in planning the deployment of resistance genes.

3.2 Introduction

Leptosphaeria maculans (Desm.) Ces & de Not. (Anamorph: *Phoma lingam* (Tode ex Fr.) Desm., the causal organism of blackleg of crucifers is a serious threat to the production of oilseed rape in many parts of the world (Gugel & Petrie 1992). Resistant cultivars are used to control this disease *L. maculans* where it is a problem. In Australia, the use of resistant cultivars provided sufficient control to allow the revival of this industry (Ballinger et al. 1991, Salisbury et al. 1995). It is possible that the control *L. maculans* by these resistant cultivars may be rendered ineffective due to build up in populations of new races (Petrie 1995, Mayerhofer et al. 1997).

In the *L. maculans-Brassica* pathosystem, a limited number of differential cultivars have been used to identify different isolates of the pathogen (Newman 1984, Badawy et al. 1991, Ballinger et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995). Isolates of *L. maculans* are often grouped as either non-aggressive or aggressive types, based primarily on cotyledon reactions of susceptible cultivars such as Westar. To date, four pathogenicity groups (PG) have been recognised using *Brassica napus* cultivars Westar, Glacier and Quinta (Koch et al. 1991, Mengistu et al. 1991). The PG 1 (B type) isolates of *L. maculans* are non-aggressive on Westar, Glacier and Quinta, and are quite distinct in many characteristics from the aggressive (A type) isolates (PG 2, PG 3 and PG 4).

Mengistu et al. (1991), Badawy et al. (1991), Ballinger et al. (1991), Kutcher et

al. (1993), Kuswinanti et al. (1995) used *B. napus* genotypes and other related *Brassica* species such as *B. oleracea*, *B. rapa*, *B. juncea*, *B. nigra*, *B. carinata* (Braun) and *R. sativus* to identify variability for virulence in aggressive (A type) isolates of *L. maculans.* Ballinger et al. (1991), characterized isolates of *L. maculans* into 14 races based on interaction phenotype (IP) on *B. napus* genotypes and other *Brassica* species. They did not find avirulent isolates, nor did they include Westar, Glacier and Quinta in their differential set. Similarly, Kuswinanti et al. (1995), used *B. napus* genotypes including Westar, Glacier and Quinta and other *Brassica* species to differentiate virulent isolates of *L. maculans* (PG 2, PG 3 and PG 4) into 18 sub groups. Non-aggressive (PG 1) isolates were also found but PG 2 isolates (A3, A4) were only present in collections from France and not from Germany. Differentiation of the aggressive isolates of *L. maculans* into different PG suggests that specific interactions may be involved in the *Brassica* - *L. maculans* pathosystem and that new virulence types may evolve if *B. napus* cultivars with resistance were widely cultivated. Cultivars with moderate to high levels of resistance to *L. maculans* were developed in Canada. Whether these cultivars will provide long lasting protection against the natural population of *L. maculans* is unknown. Little information is currently available on genetic variation in the populations of *L. maculans*. The objective of this study is to identify useful differential resistance genes particularly in *Brassica napus* and to investigate variability for virulence in *L. maculans* using isolates from western Canada and other regions.

3.3.0 Materials and methods

One hundred and fifty six isolates of *L. maculans* obtained from the major oilseed rape producing regions of the world (Australia, Canada, and Europe) were used in this study. However, only 70 isolates were tested on the complete set of nine differerentials. Most isolates of *L. maculans* were from the collection of the University of Manitoba. Other isolates were obtained from Drs G. Séguin-Swartz (Agriculture and Agri-food Canada, Saskatoon, J. P. Tewari (University of Alberta), P. Kharbanda (Alberta Agriculture) and P. Goodwin (University of Guelph). All aggressive isolates of *L. maculans* were passaged through Westar, at least twice, to maintain pathogenicity. Isolates of *L. maculans* used in the study were initially characterized based on their interaction phenotypes on cotyledons of Westar, Glacier and Quinta.

3.3.1 Inoculum preparation

Infected cotyledons of Westar were surface sterilized in a 0.6% sodium hypochlorite (household Javex) solution for 3-5 min and then rinsed with a second 0.6% sodium hypochlorite solution. Cotyledons were plated onto V8-Juice (Campbell Soup Company Ltd, Toronto, Canada) agar to which 1% streptomycin sulphate was added to restrict bacterial growth. The fungi were sub-cultured again, 5-7 days after the incubation of infested host tissue, to increase inoculum. All plating of cultures was done under a flow hood, and cultures were incubated under continuous cool white fluorescent lights (100 - 150 μ E S^{-1} m^{-2}) at 20°C. After 7-10

days, sporulating cultures were flooded with 10-15 mL of sterile distilled water and the surface of the plates scraped gently with a flamed glass rod to dislodge pycnidiospores. The mixture was filtered through sterile 'Miracloth' (Calbiochem, La Jolla, CA) into sterile 15 mL centrifuge tubes, and the spore suspension centrifuged at 4,500 rpm for 30 min. The supernatant was decanted, the spore pellet resuspended in 1 mL of sterile distilled water and the spore concentrate stored in 1 mL tubes at -20°C.

To prepare spores for host plant inoculation, the frozen concentrated spores were thawed at room temperature, and a few drops of the inoculum was resuspended in 10 mL of sterile distilled water. Quantification of the spore concentration was done with the aid of a haemocytometer and appropriate dilutions were made with sterile distilled water to obtain a final concentration of 1×10^7 spores mL^{-1}.

3.3.2 Host material

Nine selections of *B. napus* (Westar, Glacier, Quinta, Val-1, Dac-1, Dac-2, Quantum, Sprint and Sentry) and two accessions of *B. juncea* (UM3466 and UM3467) were used in this study (Table 3.1). Up to 20 seeds of each accession were planted in flats using a soil-less mix (Metromix ™, W. R. Grace and Co. Ltd., Ajax, Ontario). Glacier, Quinta, Val-1, Dac-1 and Dac-2 were selfed three times and the S_3 lines were used to determine the interaction phenotypes elicited by the test isolates. All plants were watered daily and kept at 21.5/14°C day/night

temperature and a 16 h photo period.

3.3.3 Cotyledon evaluation

Fully expanded cotyledons of the host differential genotypes were inoculated with the test isolates 6-7 days after seeding. Both cotyledons were wounded with a needle and a 10 μL droplet of inoculum placed onto each wound. The inoculum was allowed to dry overnight and the plants were returned to the growth chamber. Flats were not watered for one day and were fertilized twice using 20 - 20 - 20 (N - P - K), three and six days after inoculation respectively. True leaves from all plants were removed to ensure that the cotyledons continued to expand and remained green. Cotyledons were evaluated for interaction phenotypes (IP) 10-12 days after inoculation on a scale of 0 to 9 (Williams 1985).

3.4 Results and discussion

The respective *Brassica* differential cultivars/selections used in this study were either resistant or susceptible when tested with different isolates of *L. maculans* from the diverse pathogenicity groups. The mean IP elicited by PG 1 to PG 4 isolates on cotyledons of the cultivars Glacier, Quinta, Dac-1, Dac-2, Sprint, Sentry, Quantum and the *B. juncea* accessions (UM3466 and UM3467) ranged from 0 to 9. The PG 1 isolates caused resistant IP on most of the differential cultivars whereas the PG 4 isolates caused highly susceptible IP on most differential cultivars.

A summary of the IP of the differential accessions to isolates of *L. maculans* is presented in Appendix A, where R = resistant (IP 0 to 3.9), I = intermediate (IP 4 to 5.9) and S = susceptible (IP 6 to 9). Based on the reactions of Westar, Glacier and Quinta, the isolates of *L. maculans* from Australia, Canada and Europe could be classified either as PG 1, PG 2, PG 3 or PG 4, if the intermediate IP is grouped with the resistant class (Appendix A). Aggressive and non aggressive isolates of *L. maculans* were identified in all regions of Canada on the basis of IP on Westar, Glacier and Quinta. The aggressive isolates in western Canada were mainly PG 2. Only three isolates (PL88-16 from Manitoba, 831 from Saskatchewan and LMM89-9 from Alberta) were classed as PG 3. The Manitoba and Saskatchewan isolates were similar in virulence characteristics, suggesting that they have a common origin. Similar results were reported by Kutcher et al. (1993).

Based on IP observed on Westar, Glacier and Quinta nearly all of the isolates from eastern Canada were either PG 1 and PG 4, whereas isolates from Australia and Europe were mainly PG 3 or PG 4 . However, the latter finding could be due to the small number of isolates from these regions. In Canada, PG 4 isolates occurred only in Ontario suggesting that the origin of the isolates from western Canada and Ontario are different.

Since Glacier and Quinta are winter types, *B. napus* cultivars differential hosts with spring type growth habits would be desirable. Spring type *B. napus* cultivars would eliminate the need to vernalize the plants since they do not have cold dormancy. This would shorten the time to maturity of winter types *B. napus*

cultivars. Moreover, differential genotypes consisting of commercial cultivars *B. napus* may provide information about the evolution of new virulence genes in field populations of *L. maculans*, as well as being of a practical use to farmers and breeders.

When isolates were tested on all the differential cultivars including Sentry, Val-1, Quantum, Sprint and Dac-1 they could be further differentiated into 23 sub-groups, with the PG 2 isolates comprising 15 sub-groups while PG 3 and PG 4 could be classed into eight sub-groups (Table 3.2 and Table 3.3). Quantum was resistant to most (78%) of all isolates, while Val-1 was resistant to the fewest isolates (13%). Sprint, Sentry and Dac-1 were resistant to 37%, 33% and 28% of the PG 2 isolates respectively. This suggests that Quantum has one or more effective genes for resistance to PG 2 than the other differential accessions, whereas Dac-1 and Val-1 have the fewest resistance genes. However, based on IP, Sprint, Val-1 and Dac-1 were the most useful cultivars to characterize the isolates, since 85% of the PG 2 isolates used were differentiated by these cultivars. Sentry was used to characterize the seven isolates which were virulent on Sprint, Val-1 and Dac-1. The differential accessions Dac-2, UM3466 and UM3467 included in this study also were useful as they could differentiate Alberta isolates from Manitoba and Saskatchewan isolates; but they were not tested with the complete set of isolates used (Appendix A).

Isolates which were either virulent or avirulent on all additional differential genotypes were identified. Three isolates (PI89-15, PL89-20 and SK97-12) were

virulent on all five additional *B. napus* differentials and only one was avirulent. About 32% of the isolates were virulent on more than one differential genotype. This suggests that combined or broad virulence is prevalent within the *L. maculans* field isolates. Host differential genotypes may have more that one gene conferring resistance to some genotypes of *L. maculans*. Rare virulent types were also identified, albeit at very low frequencies. This is evident in that rare disease patterns were elicited on the differential genotypes (Appendix A). For example LMS89-54 was virulent on Glacier but avirulent on Westar and Quinta whereas 2367 was virulent on Westar and Quinta but avirulent on Glacier. Other examples include the isolates SK97-10 and SK97-19 which were virulent on both Quantum and Sprint. The potential for change in the relative frequencies of new virulent genotypes is real, and may depend on the selection pressure exerted by specific resistance genes in the host cultivars. Therefore, to understand the genetic structure of the pathogen and to identify genes in the host differentials which confer resistance to these isolates, isolates of *L. maculans* used in virulence studies should be from a wide spatial distribution and be representative of field populations. Of the PG 2 isolates from western Canada, 69% were avirulent on one to two of the additional *B. napus* differential cultivars and 22% of the PG 2 isolates were avirulent on three to four of the additional *B. napus* differentials.

Forty-six of 156 PG 2 isolates from western Canada were tested on all the *B. napus* differentials. The nomenclature of PG 2 isolates in western Canada has been based on sub-groupings of their IP on the Val-1 and Dac-1 differentials (Table

3.2) and on the percentage abundance of the respective sub-group. Ninety three percent of PG 2 isolates from western Canada were avirulent on at a least one of the differential cultivars. Isolates virulent on Val-1 but avirulent on Dac-1 were designated PG 2 and comprised 30% of the isolates. Isolates avirulent on Val-1 but virulent on Dac-1 comprised 13% of the population are referred to as PG 2-1. Fifty percent of the PG 2 isolates were virulent on both Val-1 and Dac-1. Isolates in the sub-group that were virulent on both Va-1 and Dac-1 are referred to as PG 2-2. Mengistu et al. (1991), Rimmer & van den Berg (1992) and Kutcher et al. (1993) identified all isolates of *L. maculans* in western Canada as PG 2. This study, however, demonstrates that the isolates of PG 2 comprise different virulence genotypes.

Host - pathogen specificity in the *Brassica* - *L. maculans* pathosystem, has been reported in Australia, Europe and Canada (Badawy et al. 1991, Ballinger et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995). In these studies, *Brassica* genotypes belonging to different species and various isolates of *L. maculans* were used. Consequently, only a limited and cautious comparison of the data is possible. Moreover, different measures for IP were used by the researchers. For instance, Kuswinanti et al. (1995) classified IP 1-2.9 rating as resistant, IP 3.0-5.9 as intermediate and IP 6-9 as susceptible. On the other hand, Ballinger et al. (1991) categorized IP ratings of 0-4 as resistant and IP 4.1-9 as susceptible. In the present study, the interaction phenotypes rated as 0 to 3.9 (R) were defined as resistant, IP 4 to 5.9 as intermediate (I) and IP 6 to 9 (S) as susceptible reactions.

This classification may be more useful for the characterization of resistance genes, as it would result in grouping the isolates into three classes of R, I and S. However, the R and S instead of R, I and S classification is preferred due to the ease of interpretation of the results.

On the basis of reactions of the differential hosts, Kuswinanti et al. (1995) identified 10 isolates of *L. maculans* in Germany, belonging to PG 1. Most isolates of *L. maculans* (62%) tested in Germany by Kuswinanti et al. (1995) were classed as PG 4, while 33% were PG 3 and only three isolates (5%) were PG 2. The latter three isolates originated in France with no PG 2 observed in German isolates (Kuswinanti et al. 1995). On the basis of IP on only the *B. napus* differentials, the 14 races of *L. maculans* identified by Ballinger et al. (1991) may be grouped into five pathogenicity groups; races 3, 6 and 11 were virulent on Chisaya and RX3 but avirulent on Rafal, races 4, 7 and 9 were virulent on Rafal but avirulent on Chisaya and RX3, races 5 and 8 were virulent on Rafal and RX3 but avirulent on Chisaya, race 10 was virulent on Chisaya and Rafal but avirulent on RX3 and races 1 and 2 were virulent on all 3 *B. napus* genotypes. No avirulent isolates were reported by Ballinger et al. (1991). However, Plummer et al. (1994) did report the presence of PG 1 isolates in Australia. It should be noted that Ballinger et al. (1991) did not include the cultivars Westar, Glacier and Quinta in their differential set.

Twenty eight percent of the isolates used in the present study were PG 1. These isolates could be subdivided into 10 groups based on IP on all the differential genotypes (Appendix A). So far, differences among the PG 1 isolates

of *L. maculans* have only been identified using RAPD (Goodwin and Annis 1991). Based on IP on Westar, Glacier and Quinta, 46% of the isolates were PG 2 isolates; only 10% of the isolates were PG 3 and 15% were PG 4 isolates. The majority of the isolates used in the present study originated in Canada.

To facilitate the interpretation of results, IP 0-5.9 was defined as resistant and IP 6-9 as susceptible, since sporulation of the pathogen is present only on IP 6-9. The inclusion of more *B. napus* differential genotypes may result in the further differentiation of the isolates of *L. maculans*. On the basis of IP on all the *B. napus* differential genotypes used, the aggressive isolates of *L. maculans* can be sub divided into 23 subgroups comprising 15 subgroups of PG 2 isolates, 4 subgroups of PG 3 isolates and 4 subgroups of PG 4 isolates (Table 3.2, Appendix A). It is conceivable that more subgroups may be identified among the PG 3 and PG 4 if more isolates originating from Australia, Europe and Ontario, were included in the study. Indeed, Kuswinanti et al. (1995) reported 16 subdivisions among the PG 3 and PG 4 isolates from Germany. However, if only the *B. napus* differential genotypes are used to characterize the isolates and intermediate IP are grouped with resistant IP the isolates of *L. maculans* used by Kuswinanti et al. (1995) fall into seven subgroups composing five subgroups of PG 4 and 2 subgroups of PG 3. Similarly considering only the four *B. napus* differential genotypes used by Badawy et al. (1991), the 124 isolates of *L. maculans* they tested can be classed into three PG.

Efficient discrimination of isolates of *L. maculans* on the basis of differential host

resistance genes is necessary, since this may permit detection of new virulent pathotypes which may threaten widely grown cultivars and facilitate an appropriate response by breeders. This information would enable the efficient use and deployment of genes providing resistance to *L. maculans* (Mayerhofer et al. 1997); moreover it may facilitate the maintenance of selected and/or rare pathotypes of *L. maculans* isolates useful in genetic studies for resistance and virulence. If isolate collections are made over long periods of time, the study of variation for virulence in populations of *L. maculans* will be possible and may reveal changes in the frequency of virulence phenotypes. The identification of variation within the population of the pathogen will depend on how well, and unambiguously, the IP can be categorized, the efficacy of host differential cultivars, the number of genes for resistance in each differential cultivar and the environmental test conditions. In the present study, *Brassica* differentials providing effective resistance against PG 2, PG 3 and their sub-groupings were identified. These cultivars/accessions are likely sources of resistance genes for anticipatory breeding for resistance (sensu McIntosh and Brown 1997).

Table 3.1. Name or strain, year of release or acquisition, species, and pedigree or origin of *Brassica* accessions used as host differentials to characterize virulence phenotypes of isolates of *Leptosphaeria maculans*.

Host name	year	Species	Pedigree and/or Origin
Westar	1982	*B. napus*	/Boronowski x Tower/Zephyr/568 - 2895 /Midas/Tower, Canada.
Glacier	1987	*B. napus*	Ledos/Rapol x Hector/, USA.
Quinta	1976	*B. napus*	ORO x Rapol * 3. Germany.
Sentry	1996	*B. napus*	Jet Neuf / Regent * 2 //Karat // Legend, Canada.
Quantum	1995	*B. napus*	DH from (Maluka x line 88 - 53473), Canada.
Sprint	1996	*B. napus*	Tested as D481, Canada.
Dac -1	1996	*B. napus*	China.
Dac - 2	1996	*B. napus*	China.
Val (1)	1996	*B. napus*	Africa.
UM 3466	----	*B. juncea*	Shekhar, India.
UM 3467	----	*B. juncea*	India.

Table 3.2. Differentiation of pathogenicity group (PG 2) isolates based on interaction phenotype (R=resistant, S=susceptible), on eight *Brassica napus* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculation.

		Differential host genotypes							
Group	No.*	West	Glac	Quin	Quan	Sent	Spri	Val-1	Dac-1
PG 2	1	S	R	R	R	R	R	R	R
	2	S	R	R	R	R	R	R	S
	2	S	R	R	R	R	R	S	R
	1	S	R	R	R	R	R	S	S
	2	S	R	R	R	R	S	S	R
	7	S	R	R	R	R	S	S	S
	1	S	R	R	R	S	S	R	S
	3	S	R	R	R	S	R	S	R
	6	S	R	R	R	S	R	S	S
	4	S	R	R	R	S	S	S	R
	7	S	R	R	R	S	S	S	S
	2	S	R	R	S	S	S	R	S
	2	S	R	R	S	S	R	S	S
	3	S	R	R	S	S	S	S	R
	3	S	R	R	S	S	S	S	S
Total	46	0	46	46	36	15	17	6	13

* No. = number of isolates in each sub-group. Isolates for which a rating was not obtained for one or more differential hosts were omitted.

Table 3.3. Differentiation of pathogenicity group (PG 3 and PG 4) isolates based on interaction phenotype (R=resistant, S=susceptible), on eight *Brassica napus* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculation.

		Differential host genotype							
Group	No.*	West	Glac	Quin	Quan	Sent	Spri	Val-1	Dac-1
PG 3	2	S	S	R	S	R	S	S	S
	1	S	S	R	R	S	S	S	S
	1	S	S	R	S	S	R	S	S
	8	S	S	R	S	S	S	S	S
PG 4	1	S	S	S	R	R	S	R	S
	1	S	S	S	R	R	S	S	R
	1	S	S	S	R	R	S	S	S
	9	S	S	S	S	S	S	S	S
Total	24	0	0	12	4	5	1	1	1

* No. = number of isolates in each sub-group. Isolates for which a rating was not obtained for one or more differential hosts were omitted.

CHAPTER 4

Differentiation of isolates of *Leptosphaeria maculans* from different geographic regions based on Random Amplified Polymorphic DNA (RAPD) analysis.

4.1 Abstract

Leptosphaeria maculans, the causal organism of blackleg disease of crucifers occurs as aggressive and non-aggressive forms. One hundred fifty-six isolates of *L. maculans* were characterized on the basis of host-pathogen interaction on *Brassica* host differential accessions. Random amplified DNA polymorphism (RAPD) analysis was then used to characterize 18 of the 156 isolates, selected on the basis of their virulence, mating type and geographic origin. DNA was extracted from the isolates and analysed for polymorphism using 150 ten mer, oligonucleotide arbitrary primers. A total of 28 primers were informative. Fourteen primers differentiated isolates of *L. maculans* into two distinct groups consistent with the pathogenicity grouping and geographic origin. Isolates of *L. maculans* from western Canada formed one distinct cluster and isolates from Australia and Europe formed a second cluster. The DNA polymorphism of PG 3 and PG 4 isolates exhibited more variability than isolates of PG 2. Two isolates of *L. maculans* originating from the same colony were identified by the primers as a subset of PG 2. These isolates were later confirmed as different from PG 2 using host differentials other than Westar, Glacier and Quinta and were designated as

PG 2-1. In this study, variability of *L. maculans* isolates appeared to depend on the geographic origin. Further, the characterizations of the isolates of *L. maculans* based on the DNA polymorphism using the RAPD technique, was consistent with the pathogenicity groupings of the isolates based on IP of the host differentials cultivars.

4.2 Introduction

Blackleg caused by *Leptosphaeria maculans* (Desm.) Ces & de Not., anamorph: *Phoma lingam* (Tode:Fr.) Desm., is an important disease in many oilseed rape producing areas of the world (Gabrielson 1983, Gugel & Petrie 1992, Rimmer & Buchwaldt 1995). Isolates of *L. maculans* can infect all parts of susceptible hosts, including severe damage at the crown which often results in premature ripening or death of the plant (Gabrielson 1983, Martens et al. 1984) and serious yield loss (Rawlinson & Muthyalu 1979, Cargeeg & Thurling 1980a, Gugel & Petrie 1992, Salisbury et al. 1995, Sansford 1995, Fitt et al. 1997). Pathogen isolates are highly variable in many characteristics (McGee and Petrie 1978, Delwiche 1980, Humpherson-Jones 1983a, Petrie 1988) including virulence (Koch et al. 1989, Pedras et al. 1990, Pedras & Séguin-Swartz 1992, Kutcher et al. 1993, Kuswinanti et al. 1995).

Aggressive (A type) and non-aggressive (B type) isolates of *L. maculans* (Johnson & Lewis 1990) were characterized into pathogenicity groups (PG) based on the interaction phenotypes (IP) on the *Brassica napus* cultivars Westar, Glacier and Quinta. Four PG of *L. maculans* were recognized by Koch et al. (1991) and Mengistu et al. (1991). Non-aggressive or B type (PG 1) isolates of *L. maculans* are quite distinct from the aggressive or A type (PG 2, PG 3 and PG 4) isolates (Koch et al. 1991, Mengistu et al. 1991). Furthermore, different subgroups were characterized among PG 2 isolates in western Canada based on IP on Val-1 and Dac-1 differential accessions (Chapter 3, this thesis). The PG 2 isolates are

virulent on Val-1 but avirulent on Dac-1 and the PG 2-1 isolates are avirulent on Val-1 but virulent on Dac-1. The PG 2-2 isolates are virulent on both Val-1 and Dac-1. Differentiation of isolates of *L. maculans* on the basis of host plant reaction is a useful method but is time consuming and labourious. Characterization of isolates of *L. maculans* on the basis of molecular markers may be as reliable and quicker.

Isolates of *L. maculans* have been compared on the basis of their electrophoretic karyotypes (Taylor et al. 1991b, Plummer et al. 1994). Electrophoretic karyotypes of selected A and B type isolates indicated that while the B type isolates of *L. maculans* have 12-14 chromosomal bands, the A type isolates had only 6 - 8 distinct bands (Taylor et al. 1991). On the basis of restriction fragment length polymorphism (RFLP), Johnson and Lewis (1990), Hassan et al. (1991), Koch et al. (1991) found distinct differences between the A type and B type isolates of *L. maculans*. Similarly, genetic differences between the aggressive and non-aggressive isolates of *L. maculans* were reported, using the random amplified polymorphic DNA (RAPD) technique (Goodwin and Annis 1991, Schaffer & Wostemeyer 1992, Schaffer & Wostemeyer 1994, Mahuku et al. 1996, Mahuku et al. 1997). Goodwin and Annis (1991) identified differences among the B type isolates of *L. maculans* from either Ontario or western Canada. No differences were found between the A type isolates from western Canada and Ontario (Goodwin and Annis 1991). These and other studies have led to the conclusions that there are major genetic differences between the aggressive or A

type and non-aggressive or B type isolates of *L. maculans* (Badawy et al. 1991, Goodwin and Annis 1991, Hassan et al. 1991, Hill et al. 1984, Koch et al. 1989, Taylor 1993, Taylor & Borgmann 1994). Information from these studies with regard to genetic variability and the genetic structure among aggressive isolates of *L. maculans* is however, limited. Little is known about genetic variability in populations of *L. maculans*. The objective of this study was to examine the relationships among aggressive isolates of *L. maculans* using RAPD.

4.3.0 Materials and methods

4.3.1 Origin of fungal isolates

Eighteen isolates of *L. maculans* were used in this study. These isolates were chosen based on geographical region of origin, pathogenicity group, mating type and growth characteristics (Table 4.1). Isolates from Canada, Australia, Europe and USA were included in the study. All the isolates of *L. maculans* used in this study were from the collection of the University of Manitoba.

4.3.2 Fungal cultures

Seventy-five mL of Czapek-Dox (DIFCO Laboratories, Detroit, MI, USA) medium in 125 mL conical flasks were inoculated with 50 μL of concentrated spore suspension of *L. maculans*. The medium was supplemented with 1% streptomycin sulphate to retard bacterial growth. The 18 isolates were analysed in three

batches of six, with each isolate replicated three times and with each batch having two non-inoculated flasks as controls. Isolates were incubated for 10 - 14 days in a water bath maintained at 25°C.

Following incubation, approximately 10 mL of media from each flask containing the liquid cultures were each plated onto three petri plates containing V-8 juice agar and incubated at 20°C and 16 h photo period in incubators. The cultures were examined after 4-5 days both visually and using a light microscopy to determine the purity of the isolates. Samples that were contaminated with bacteria and/or other fungi were discarded, and new cultures prepared.

The mycelia from all liquid cultures were filtered using sterile filter paper and rinsed with sterile distilled water. Mycelia from each test isolate were then placed each into 50 mL centrifuge tubes and the tops sealed with punctured wax paper. All tubes were placed in a lyophilizer and the mycelia dried for at least 48 h. Leaves of two lentil genotypes were also included as controls.

4.3.3 DNA extraction

Mycelia of test isolates of *L. maculans* and the leaves of the two lentil genotypes were frozen with liquid nitrogen and ground into a fine powder in centrifuge tubes, using sterile glass pestles. The extraction buffer (100 mM Tris-HCl (pH 8.0), 50 mM Na_2EDTA, 500 mM NaCl and 1.25% SDS) was added to the mycelial powder and the mixture vortexed to mix thoroughly the contents of the tubes. The tubes were incubated at 65°C for 30 min, and ammonium acetate (CH_3

$CooNH_4$) added to a final concentration of 2.5 M. The contents of the tubes were gently mixed and placed on ice for 20 min, followed by centrifugation at 8000 rpm for 15 min. The supernatant was transferred to new tubes and nucleic acids precipitated using 0.6 volumes of isopropanol alcohol and centrifuged again at 8000 rpm for 10 min to pellet the DNA. The supernatant was discarded carefully and the pelleted DNA re-dissolved in 300 μL TE, and 10 μL of 2.8 M NaCl. Twenty μL of proteinase K (10 mg/mL) were added and the contents of the tubes incubated again at 65°C for 20 min. Two hundred μL of a 5% CTAB in 0.7 M NaCl solution were added and the contents of the tubes incubated for another 20 min.

The solutions were extracted twice with equal volumes of chloroform/isoamyl-alcohol (25:1). The supernatant was removed and 5 μL of RNAse (10 mg/mL) were added and the mixture incubated for 1 hr at 37°C. The RNAse was extracted twice using phenol/chloroform (1:1). DNA was precipitated with 1 volume isopropanol, washed twice with 70% ethyl alcohol and dissolved in 200 μL sterile HPLC grade water. DNA concentration and purity was determined using UV-spectrophotometry.

4.3.4 DNA amplification

One hundred and fifty arbitrary primers obtained from University of British Columbia (UBC primers) were used to screen DNA from 18 isolates of *L. maculans* representing four pathogenicity groups (Table 4.1) to determine which primers

yielded informative amplification products. DNA from two lentil genotypes was included as control.

DNA amplification reactions were performed in 25 μL reaction mixtures each containing 25 ng / μL template genomic DNA from each *L. maculans* isolate sample, 1 unit Taq DNA polymerase (Promega, Madison, WI), Promega 100 x buffer (50 mM KCl, 10 mM Tris HCl (pH 8.0), 0.1% Triton x-100, 2.0 mM $MgCl_2$, 200 nM oligonucleotide (10 mer) primer and 100 μM of each dNTP (Promega, Madison, WI).

All amplification reactions were performed using a thermocycler (MJ 100, MJ Research, Inc. Watertown, MA, USA). The thermocycler was programmed for an initial cycle of 94°C for 2 min (denaturation step) followed by 45 cycles at 94°C for 1 min, 35°C for 1 min (annealing step) and 72°C for 2 min (elongation step). The fastest possible transition between temperatures was used. A final elongation step was included at 72°C for 10 min, which was immediately followed by cooling to 4°C and holding at this temperature until samples were removed. To check reproducibility of amplicons, the amplification of all sample DNA templates was repeated twice.

Twenty μL of each amplification product, to which 3 μL stop buffer (6 x bromophenol blue in sucrose) was added as a tracking dye, were resolved by electrophoresis in 1.4% agarose gels (1% seakem + 1% Nusieve, Fisher Chem.) at 70-80 V/cm for 3 hr. The gels were stained with ethidium bromide (10 mg/mL)

and the resolved bands visualized by exposure to UV-fluorescent light. A DNA size marker was included to determine the size of the fragments resolved.

4.3.5 RAPD analysis

On the basis of the position of the DNA bands resolved on the agarose gel, the assumption was made that bands of the same size were homologous. Reproducible amplified DNA fragments from all the isolates of *L. maculans* were scored as present (1) or absent (0). Only bright bands were used in the analysis. To determine the relationship between the different isolates of *L. maculans*, the formula $S = 2N_{xy} / (N_x + N_y)$ was used, where S = the similarity coefficient, N_{xy} = the number of shared bands by only two isolates, N_x and N_y = the number of amplified fragments from isolate "x", and isolate "y", respectively (Nei and Li 1979). The similarity coefficients were converted to genetic distance using the equation $D = (1/S) - 1$, where D = genetic distance and S = similarity coefficient (Swofford and Olsen 1990). A distance matrix for the isolates was generated by averaging distance across all primers/bands and then a dendogram was constructed using the average linkage cluster analysis method of the software package SAS (SAS Institute Inc. 1989).

4.4 Results and discussion

Up to 10 DNA fragments were amplified for each primer. The size of amplicons ranged from 0.10 Kb to 2.7 Kb with most bands lying between 0.5 Kb and 2.3 Kb

(Figures 4.1 - 4.5). Approximately 80% of the primers produced amplicons which were non informative. Amplicons produced by 12% of the arbitrary primers were faint. Only two primers produced no amplification products. Thirty primers (20%) produced polymorphic bands. Of these primers 14 were informative, based on the DNA polymorphism identified from the 18 isolates of *L. maculans* (Table 4.2). An average of two to three polymorphic bands were produced by the primers with one to two polymorphic markers observed in each pathogenicity group. Examples of some of the informative markers are presented in Figures 4.1 to 4.6. The primers UBC 357 and UBC 382 characterized PG 2 isolates as different from the other isolates based on two distinct fragment sizes. Bands produced by the primer UBC 357 ranged from 0.65 Kb to 1.48 Kb (Fig. 4.1). Fragment size 1.48 Kb amplified by UBC 357 differentiated the PG 2 isolates from other groups (Fig. 4.1). Primer UBC 382 distinguished only the PG 2 isolates from other isolates of *L. maculans* based on the 0.73 Kb fragment (Fig. 4.2). A total of eight amplicons, ranging from 0.66 Kb, were produced by the primer UBC 740. The 0.66 Kb fragment could differentiate the PG 2-1 and PG 2 from PG 3 and PG 4 isolates (Fig. 4.3). Similarly, based on marker UBC 337, the PG 2-1 and PG 2 isolates were different from most PG 3 and PG 4 isolates, based on the 1.47 Kb fragment size (Fig. 4.4). The primer UBC 710 identified differences between isolates from Australia (PG 3) and Germany (PG 4) from other isolates of *L. maculans* (Fig. 4.5). Ansan-Melayah et al. (1995) reported nine primers which were informative in progeny from crosses of PG 3 and PG 4 isolates of *L. maculans.* Similarly, Nicholson et al. (1993) using

20 RAPD primers also identified differences among three races of *Bipolaris maydis*. Race O and race C were characterized as different from race T, based on RAPD profiles of 16 primers on seven isolates. Only four primers produced amplicons which differed for all the three races (Nicholson et al. 1993).

The 18 isolates of *L. maculans* used in this study, were characterized using 13 easily scored RAPD bands, eight of which were polymorphic (Table 4.3). Based on average linkage cluster analysis, the isolates were separated into distinct clusters (Figure 4.6). The clusters were consistent with the pathogenicity grouping of the isolates and their region of origin. The North American (PG 2-1 and PG 2) isolates formed a separate cluster with 79% similarity between them. The Australian and European, consisting of PG 3 and PG 4 isolates, formed another cluster, with 83% similarity between them. The only isolate from Ontario was clustered together with one isolate from Europe (France) with 55% similarity between them. There was a large distance ($d > 1$) at which the North American cluster of the isolates of *L. maculans* merged with the cluster of Australian and European isolates.

Mills et al. (1992), using 40 RAPD primers to study molecular variability in isolates of *Colletotrichum gloeosporiodes*, were able to classify the isolates based on their pathogenicity on different host species and their geographic origins. Similarly, Vaillancourt & Hanau (1992) reported the clustering of isolates of *C. graminicola* on the basis of geographic origin. On the other hand, Crowhurst et al. (1991) characterized isolates of *Fusarium solani* f. sp. *cucurbitae* into two distinct

groups corresponding with the two mating types. In the present study no such correspondence with mating types was apparent (Fig. 4.6).

The PG 2 isolates of *L. maculans* were monomorphic and were closely related to the PG 2-1 isolates used. That the PG 2 isolates in this study shared identical fingerprints suggests that they probably originated from few initial genotypes. The PG 3 isolates were more closely clustered with the PG 4 isolates. These isolates of *L. maculans* were mainly from Australia and Europe, suggesting that they may have had a common progenitor. Based on the polymorphic RAPD markers, variability within the PG 3 and the PG 4 isolates was high. The isolates from Australia and Europe had approximately a 75% similarity. Further, the Australian isolates formed two sub clusters with a 60% similarity. Vaillancourt & Hanau (1992) found that isolates of *C. graminicola* from different locations were highly polymorphic and only the isolates from one geographic region (Peru) were monomorphic. Likewise, Grajal-Martin et al. (1993), using RAPD analysis, found high variability in races of *Fusarium oxysporum* f. sp. *pisi*; only race 2 isolates were monomorphic.

RAPD fingerprints of the isolates of *L. maculans* were consistent with the virulence - based PG classification. The utility of these RAPD markers is clearly demonstrated by the close correspondence between the pathogenicity grouping and their respective RAPD markers, the identification of close similarities among PG 2 isolates, as well as variability among Australian and European isolates. However the usefulness of RAPD markers has been questioned, primarily due to

the uncertain homology of the migrating bands and for other reasons as well. Therefore, to ensure that the RAPD markers are dependable and reproducible, it is necessary to convert them into specific markers and undertake a genetic study to determine the inheritance of the markers. Such specific markers would then be desirable for fingerprinting isolates from the field which could be convenient for monitoring changes in frequencies of virulence genes in various populations of *L. maculans*. The sample size (N=18) used in this study suggests that RAPD analysis can successfully and adequately fingerprint *L. maculans* of isolates. However accurate classification of genotypes of *L. maculans* will be dependent on the availability of suitable differentials to characterize field isolates. For example, the subset of PG 2, PG 2-1, was identified by RAPD prior to its characterization by host differentials. The RAPD technique identified additional subgroups within the PG 3 and PG 4 isolates but this has not yet been corroborated on the basis of host pathogen interaction. Kuswinanti et al. (1995) identified at least 16 sub groups within the PG 3 and PG 4 isolates of *L. maculans* using *Brassica* host genotypes to differentiate the isolates. The RAPD technique coupled with the use of specific markers may enable the monitoring of local pathogen populations. The information gathered from broad geographic areas will be useful in designing effective strategies for the deployment of new resistant cultivars (McIntosh and Brown 1997) and may lead to the better understanding of the evolution of races in the pathogen.

Table 4.1. Isolate identity, mycelial colour, pathogenicity group (PG), mating type [MAT1 (-) and MAT2 (+)] and origin of isolates of *Leptosphaeria maculans* studied using RAPD markers

Isolate	Mycelial colour	PG	Mating type	Origin
PI87-1	Yellow green	4	-	Canada (Ontario)
PI85-9	Yellow green	2	-	Canada (Manitoba)
PI86-12 (2)	White	2	+	Canada (Manitoba)
PI89-19	White	3	+	Australia
PI89-21	Yellow green	3	-	Australia
PI89-28	Yellow green	3	+	Australia
PI87-41 (1)	White	2	+	United States (USA)
PI87-41	Yellow green	2	+	United States (USA)
NGermany	Yellow green	3	-	North Germany
Stockoy3	White	3	-	Belgium
Stockoy1	White	3	-	Belgium
Bamberg1	White	4	-	Germany
Bamberg3 (1)	White	4	-	Germany
Bamberg4	White	4	-	Germany
Lifolle5	White	4	+	France
SA10G	Yellow green	2	-	Canada (Saskatchewan)
PI87-5	Yellow green	2	-	Canada (Manitoba)
PI86-4	Yellow green	2	-	Canada (Manitoba)

Table 4.2. The primer number, sequence, differentiated pathogenicity (PG), percentage (%) GC and number of bands amplified by 14 informative primers identified in this study.

Primer number	Sequence	Pathogenicity group	% GC	Number of bands
UBC 301	5' CGGTGGCGAA 3'	PG 3/PG 4	70	9
UBC 308	5' AGCGGCTAGG 3'	PG 2/PG 4	70	8
UBC 315	5' GGTCTCCTAG 3'	PG 2-1	60	5
UBC 337	5' TCCCGAACCG 3'	PG 2/PG 4	70	8
UBC 350	5' TGACGCGCTC 3'	PG 2-1/PG 2	70	8
UBC 356	5' GCGGCCCTCT 3'	PG 2/PG 3	80	9
UBC 357	5' AGGCCAAATG 3'	PG 2	50	7
UBC 358	5' GGTCAGGCCC 3'	PG 2-1/PG 2	80	7
UBC 382	5' ATACACCAGC 3'	PG 2	50	8
UBC 710	5' GGTGGTGGGG 3'	PG 3/PG 4*	80	6
UBC 731	5' CCCACACCAC 3'		70	6
UBC 734	5' GGAGAGGGAG 3'		70	7
UBC 736	5' GAGGGAGGAG 3'		70	6
UBC 740	5' GGAGGGAGGA 3'	PG 2-1/PG 2	70	8

* PG 3 are from Australia and PG 4 are from Germany.

Figure 4.1. RAPD electrophenograms of 18 isolates of *L. maculans* from different regions. The primer UBC 357 was used to amplify complementary sequences from genomic DNA of the isolates. The bands were separated on a 1.4% agarose gel, stained with ethidium bromide and visualized under UV - transluminescence. Lane 1 = Size marker (lambda DNA/HIND III & pUC18/Hinf 1), lane 2 = Ontario isolate, lanes 3 & 4 = Manitoba isolates, lanes 5 to 7 = Australian isolate, lanes 8 & 9 = Manitoba isolates, lane 10 = German isolate, lanes 11 & 12 = German, Belgian isolates, lanes 13, 14 and 16 = French isolates, lane 17 = Saskatchewan isolate, lanes 18 & 19 = Manitoba isolates, lane 20= negative control (no DNA), lanes 21 - 22 = Lentil genotypes. Numbers at top of figure indicate pathogenicity group.

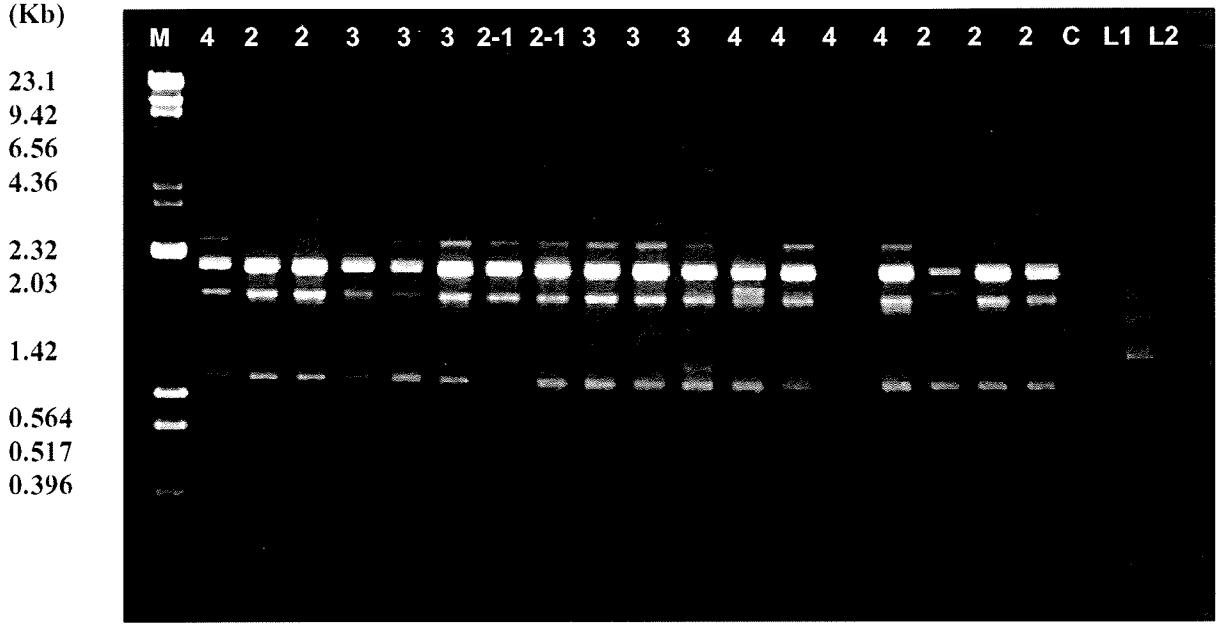
(Kb)
23.1
9.42
6.56
4.36
2.32
2.03
1.42
0.564
0.517
0.396
M 4 2 2 3 3 3 2-1 2-1 3 3 3 4 4 4 4 2 2 2 C L1 L2

Figure 4.2. RAPD electrophenograms of 18 isolates of *L. maculans* from different regions. The primer UBC 382 was used to amplify complementary sequences from genomic DNA of the isolates. The bands were separated on a 1.4% agarose gel, stained with ethidium bromide and visualized under UV - transluminescence. Lane 1 = Size marker (lambda DNA/HIND III & pUC18/Hinf 1), lane 2 = Ontario isolate, lanes 3 & 4 = Manitoba isolates, lanes 5 to 7 = Australian isolates, lanes 8 & 9 = Manitoba isolates, lane 10 = German isolate, lanes 11 & 12 = German, Belgian isolates, lanes 13 - 16 = French isolates, lane 17 = Saskatchewan isolate, lanes 18 & 19 = Manitoba isolates, lane 20 = negative control (no DNA), lanes 21 - 22 = Lentil genotypes. Numbers at top of figure indicate pathogenicity group.

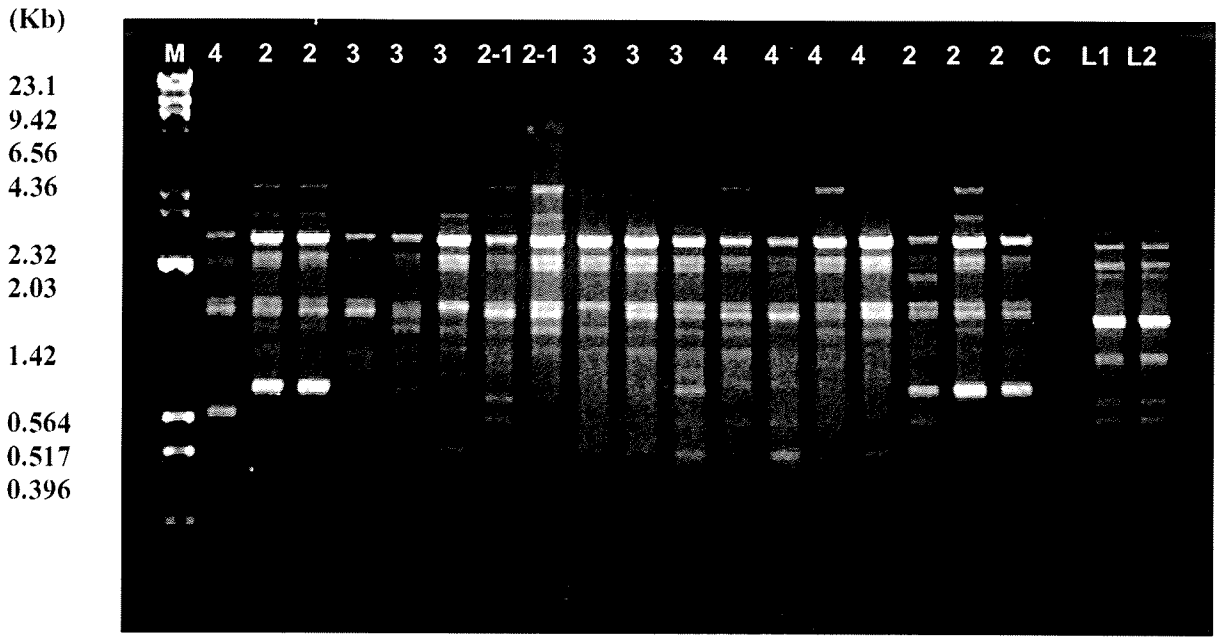
(Kb)
23.1
9.42
6.56
4.36
2.32
2.03
1.42
0.564
0.517
0.396
M 4 2 2 3 3 3 2-1 2-1 3 3 3 4 4 4 4 2 2 2 C L1 L2

Figure 4.3. RAPD electrophenograms of 18 isolates of *L. maculans* from different regions. The primer UBC 740 was used to amplify complementary sequences from genomic DNA of the isolates. The bands were separated on a 1.4% agarose gel, stained with ethidium bromide and visualized under UV - transluminescence. Lane 1 = Size marker (lambda DNA/HIND III & pUC18/Hinf 1), lane 2 = Ontario isolate, lanes 3 & 4 = Manitoba isolates, lanes 5 to 7 = Australian isolates, lanes 8 & 9 = Manitoba isolates, lane 10 = German isolate, lanes 11 & 12 = German, Belgian isolates, lanes 13 - 16 = French isolates, lane 17 = Saskatchewan isolate, lanes 18 & 19 = Manitoba isolates, lane 20 = negative control (no DNA), lanes 21 - 22 = Lentil genotypes. Numbers at top of figure indicate pathogenicity group.

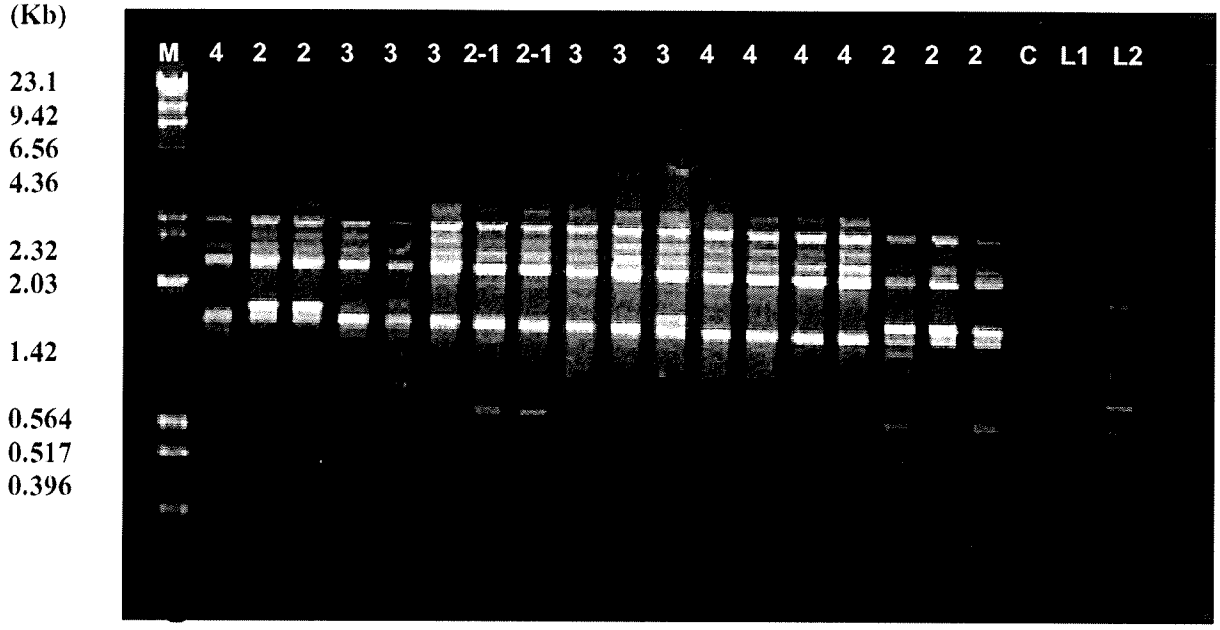
(Kb)
23.1
9.42
6.56
4.36
2.32
2.03
1.42
0.564
0.517
0.396
M 4 2 2 3 3 3 2-1 2-1 3 3 3 4 4 4 4 2 2 2 C L1 L2

Figure 4.4. RAPD electrophenograms of 18 isolates of *L. maculans* from different regions. The primer UBC 337 was used to amplify complementary sequences from genomic DNA of the isolates. The bands were separated on a 1.4% agarose gel, stained with ethidium bromide and visualized under UV - transluminescence. Lane 1 = Size marker (lambda DNA/HIND III & pUC18/Hinf 1), lane 2 = Ontario isolate, lanes 3 & 4 = Manitoba isolates, lanes 5 to 7 = Australian isolates, lanes 8 & 9 = Manitoba isolates, lane 10 = German isolate, lanes 11 & 12 = German, Belgian isolates, lanes 13 - 16 = French isolates, lane 17 = Saskatchewan isolate, lanes 18 & 19 = Manitoba isolates, lanes 20 - 21 = Lentil genotypes, lane 22 = negative control (no DNA). Numbers at top of figure indicate pathogenicity group.

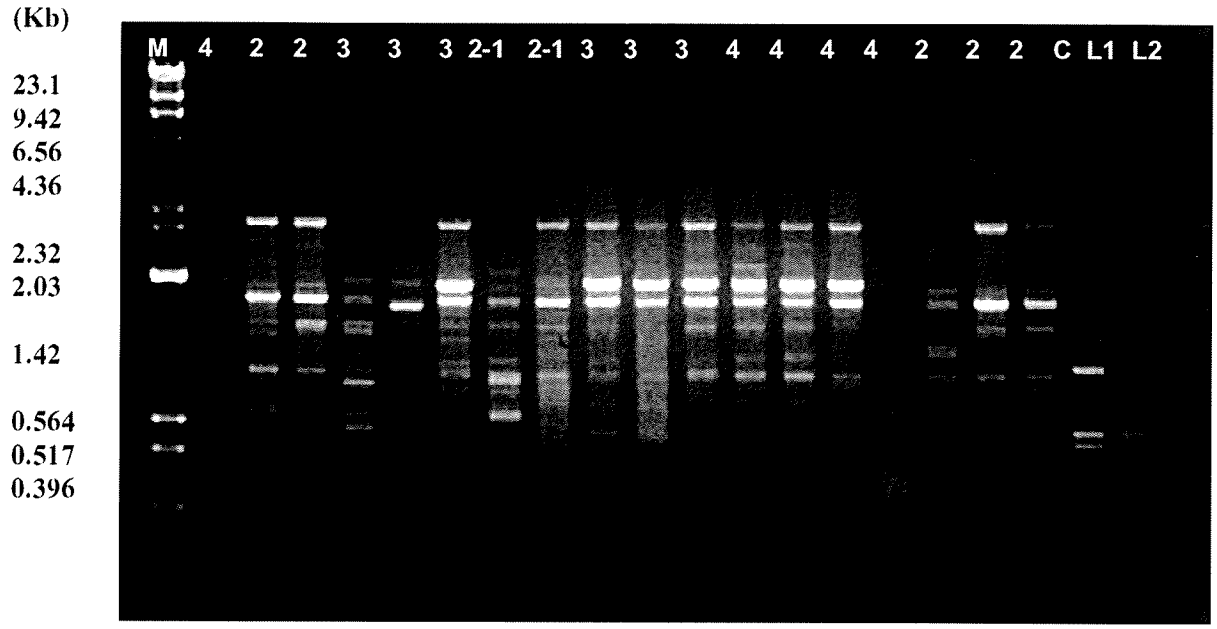
(Kb)
23.1
9.42
6.56
4.36
2.32
2.03
1.42
0.564
0.517
0.396
M 4 2 2 3 3 3 2-1 2-1 3 3 3 4 4 4 4 2 2 2 C L1 L2

Figure 4.5. RAPD electrophenograms of 18 isolates of *L. maculans* from different regions. The primer UBC 710 was used to amplify complementary sequences from genomic DNA of the isolates. The bands were separated on a 1.4% agarose gel, stained with ethidium bromide and visualized under UV - transluminescence. Lane 1 = Size marker (lambda DNA/HIND III & pUC18/Hinf 1), lane 2 = Ontario isolate, lanes 3 & 4 = Manitoba isolates, lanes 5 to 7 = Australian isolates, lanes 8 & 9 = Manitoba isolates, lane 10 = German isolate, lanes 11 & 12 = German, Belgian isolates, lanes 13 - 16 = French isolates, lane 17 = Saskatchewan isolate, lanes 18 & 19 = Manitoba isolates, lanes 20 - 21 = Lentil genotypes, lane 22 = negative control (no DNA). Numbers at top of figure indicate pathogenicity group.

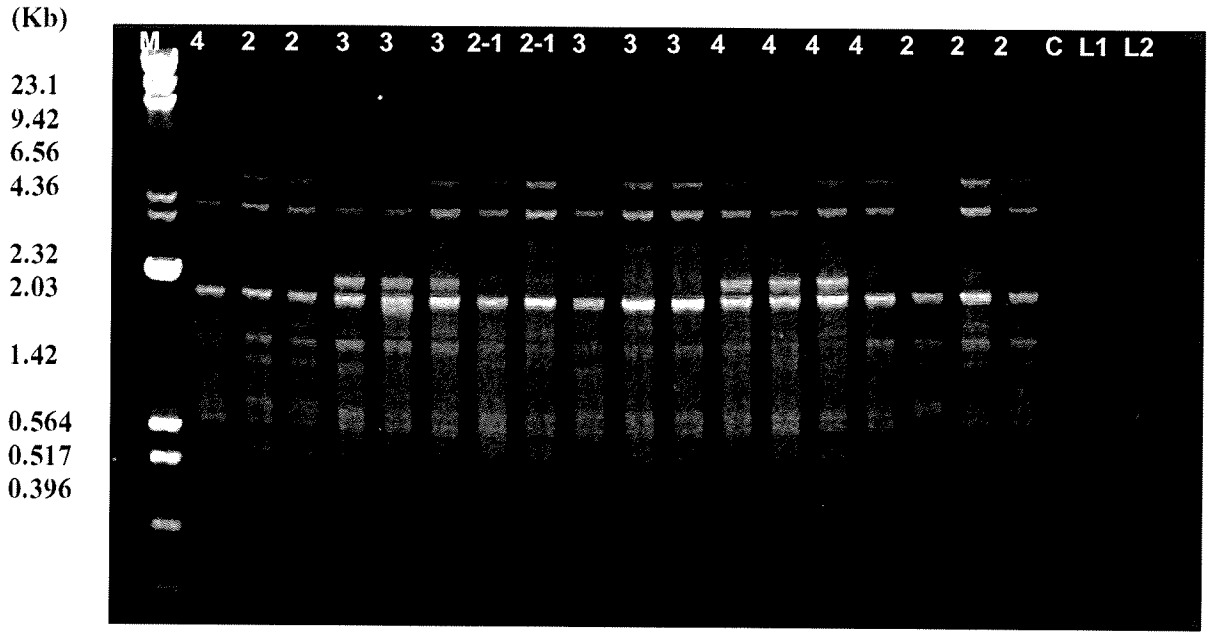
(Kb)
23.1
9.42
6.56
4.36
2.32
2.03
1.42
0.564
0.517
0.396
M 4 2 2 3 3 3 2-1 2-1 3 3 3 4 4 4 4 2 2 2 C L1 L2

Figure 4.6. Phenogram depicting the relationship between 18 isolates of *L. maculans* from different regions following average linkage cluster analysis. A total of 8 polymorphic bands were used. The pathogenicity group (PG), origin, mating types and name (No.) of each isolate is shown adjacent to the clusters. The average distance between clusters is indicated above the phenogram.

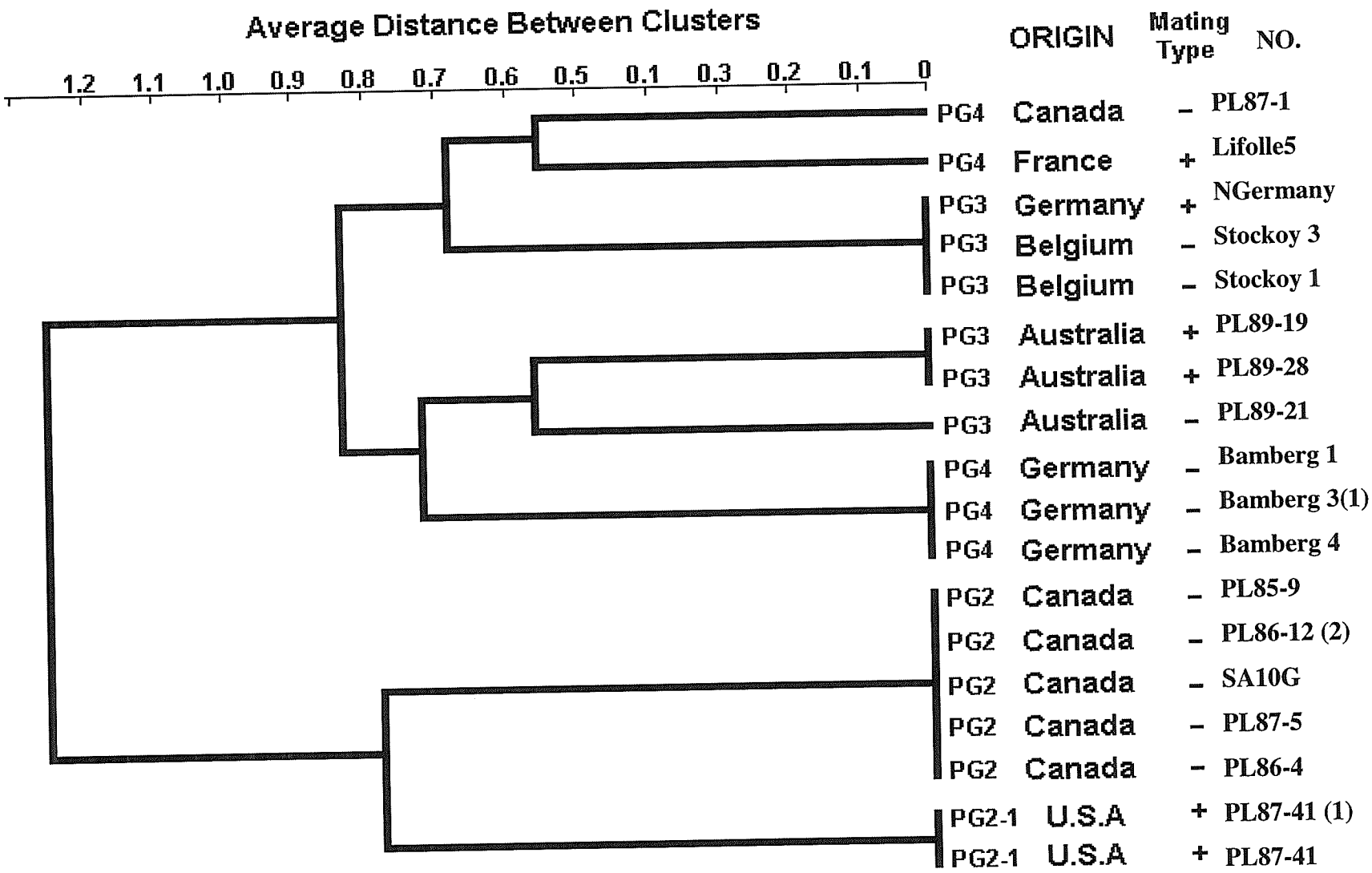
Average Distance Between Clusters
1.2 1.1 1.0 0.9 0.8 0.7 0.6 0.5 0.1 0.3 0.2 0.1 0
ORIGIN
Mating Type
NO.
PG4 Canada – PL87-1
PG4 France + Lifolle5
PG3 Germany + NGermany
PG3 Belgium – Stockoy 3
PG3 Belgium – Stockoy 1
PG3 Australia + PL89-19
PG3 Australia + PL89-28
PG3 Australia – PL89-21
PG4 Germany – Bamberg 1
PG4 Germany – Bamberg 3(1)
PG4 Germany – Bamberg 4
PG2 Canada – PL85-9
PG2 Canada – PL86-12 (2)
PG2 Canada – SA10G
PG2 Canada – PL87-5
PG2 Canada – PL86-4
PG2-1 U.S.A + PL87-41 (1)
PG2-1 U.S.A + PL87-41

Table 4.3. Data matrix for eighteen isolates of *Leptosphaeria maculans* from which clear and easily distinguishable RAPD bands (B1 to B13) were obtained.

Isolate	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
Pl86-4	0	0	0	0	0	1	0	1	0	0	0	0	0
PL87-5	0	0	0	0	0	1	0	1	0	0	0	0	0
SA10G	0	0	0	0	0	1	0	1	0	0	0	0	0
Lifolle5	0	1	0	0	0	0	1	0	0	0	0	0	1
Bamberg4	0	1	0	0	0	0	1	0	0	1	0	0	1
Bamberg3(1)	0	1	0	0	0	0	1	0	0	1	0	0	1
Bamberg1	0	1	0	0	0	0	1	0	0	1	0	0	1
Stokoy1	0	0	0	0	0	0	1	0	0	0	0	0	1
Stokoy3	0	0	0	0	0	0	1	0	0	0	0	0	1
Ngermany	0	0	0	0	0	0	1	0	0	0	0	0	1
PL87-41	0	0	1	0	0	1	0	0	0	0	0	0	0
PL87-14(1)	0	0	1	0	0	1	0	0	0	0	0	0	0
PL89-28	0	1	0	0	0	0	0	0	0	1	0	0	1
PL89-21	0	0	0	0	0	0	0	0	0	1	0	0	1
PL89-19	0	0	0	0	0	0	0	0	0	1	0	0	1
PL86-12(2)	0	0	0	0	0	1	0	1	0	0	0	0	0
PL85-9	0	0	0	0	0	1	0	1	0	0	0	0	0
PL87-1	0	1	0	0	0	0	1	0	1	0	0	0	1

CHAPTER 5

The inheritance of resistance in *Brassica napus* differential cultivars to four isolates of *Leptosphaeria maculans*.

5.1 Abstract

Two resistant *B. napus* cultivars (Glacier and Quinta) and one susceptible *B. napus* cultivar (Westar) were selected for genetic analysis on the basis of their reactions to isolates of *L. maculans* that belonged to four different pathogenicity groups. The susceptible and resistant parents were reciprocally crossed to each other. The parents, F_1 and F_2 populations from the susceptible x resistant crosses, resistant x resistant crosses and their reciprocals were evaluated at the cotyledon stage with four isolates, each representing a different pathogenicity group (PG 2-1, PG 2, PG 3 and PG 4). Cotyledon resistance in *B. napus* to *L. maculans* was determined to be a qualitative trait controlled by nuclear genes. In both Glacier and Quinta resistance to the PG 2-1 isolate was controlled by a single dominant gene. Cotyledon resistance to the PG 2 isolate, however, was controlled by one dominant gene in Glacier, but by two genes with duplicate recessive epistatic interaction in Quinta. Resistance to the PG 3 isolate of *L. maculans* in Quinta was controlled by a single dominant gene.

Few or no segregants were observed in F_2 populations derived from the Glacier x Quinta crosses when inoculated with PG 2-1 or PG 2 isolates suggesting that the genes conferring resistance in Glacier and Quinta to these two PGs may be allelic,

the same gene, or that the genes are tightly linked. Cotyledon resistance to the PG 3 isolate in the Quinta was controlled by a single dominant gene. These results support those obtained from the susceptible by resistant crosses. Thus, at least four genes collectively determine resistance in Glacier and Quinta to the isolates of *L. maculans* used in this study. The parents, F_1 and F_2 populations derived from Glacier and Westar crosses were all susceptible to the PG 3 isolate. All parents and progeny of crosses involving Westar and Glacier or Quinta were susceptible to the PG 4 isolate.

5.2 Introduction

Blackleg caused by *Leptosphaeria maculans* (Desm.) Ces and de Not. is a serious disease of oilseed rape in many countries where oilseed rape is grown. Most cultivars of oilseed rape grown in Canada between 1980 and 1990, were susceptible to *L. maculans*. Breeding for resistance to *L. maculans* is a major objective of oilseed rape improvement in Canada, since the use of resistant oilseed rape cultivars may ensure that crop yields are maintained at desirable and predictable levels. Currently, in Canada, there is an increasing number of recommended cultivars which provide moderate to high level of resistance to the prevalent forms of *L. maculans*. This improvement is particularly important, since growing susceptible cultivars can lead to serious yield losses (Kharbanda 1989, Gugel & Petrie 1992, Salisbury et al. 1995, Sansford 1995).

Genetic studies of both host and pathogen can provide pertinent information for an effective breeding program. Such studies would indicate the ease with which resistance genes may be transferred from one genotype to another, and the speed with which selection of desirable genotypes may be achieved. Resistance to *L. maculans* in different *Brassica* species may be determined by various genetic mechanisms (Delwiche 1980, Mithen and Lewis 1988, Sawatsky 1989, Pang & Halloran 1996a, Pang & Halloran 1996b, Keri et al. 1997, Pongam et al. 1998, Ansan-Melayah et al. 1998). Resistance in different *Brassica* species or cultivars has been described as polygenic (Cargeeg and Thurling 1980a, Pang & Halloran 1996b), dominant (Delwiche 1980, Mithen and Lewis 1988, Keri & Rimmer 1998),

recessive (Sawatsky 1989) and dominant recessive epistasis (Pang & Halloran 1996c, Keri et al. 1997). In *B. napus*, cotyledon resistance to *L. maculans* is controlled by two genes (Delwiche 1980, Rimmer and van den Berg 1992); in a cross involving *B. oleracea* L. and *B. insularis* Moss (Mithen and Lewis 1988) and in crosses between resistant and susceptible *B. juncea* Coss & Czern. cultivars (Pang & Halloran 1996c, Keri et al. 1997), at least two genes conferring resistance to *L. maculans* isolates were identified respectively.

Isolates of *L. maculans* can be differentiated into pathogenicity groups based on the *B. napus* cultivars Westar, Glacier and Quinta (Koch et al. 1991, Mengistu et al. 1991). Based on mapping studies Ferreira et al. (1995) identified a single locus which determines resistance to PG 2 isolates in the *B. napus* cultivar Major. Subsequently, Pongam et al. (1998) inferred that a single gene determined resistance in Major based on segregation of avirulence in progeny isolates from crosses of PG 2 and PG 4 isolates of *L. maculans*. Similarly, Ansan-Melayah et al. (1995) and Ansan-Melayah et al. (1998) crossed Quinta and Glacier to Score and reported that resistance to PG 3 isolates in *B. napus* cv. Quinta is controlled by a single dominant gene as is resistance to PG 2 isolates in Glacier. Resistance to PG 2 isolates in Quinta appears to be more complex, with Keri & Rimmer (1998) and Ansan-Melayah et al. (1998) reporting that resistance to PG 2 isolates in Quinta is determined by two genes. In order to investigate the basis of the resistance genes in the differential cultivars Glacier and Quinta, and to examine whether this host pathogen interaction is a gene - for - gene based system this

study was undertaken to determine the inheritance of resistance in the *B. napus* differential cultivars (Quinta and Glacier) to differential isolates of *L. maculans.*

5.3.0 Materials and methods

5.3.1 Pathogen isolates

Four isolates of *L. maculans* (PL87-41, PL86-12, Lifolle 6 and Lifolle 5) representing four pathogenicity groups (PG 2-1, PG 2, PG 3 and PG 4 respectively) were used for all tests in this study. The isolates were characterized as aggressive and belonging to different pathogenicity groups based on the interaction phenotypes elicited on cotyledons of the *B. napus* differential cultivars Westar, Glacier, Quinta, Val-1 and Dac-1. Westar was susceptible to the PG 2-1, PG 2, PG 3 and PG 4 isolates used. Glacier was susceptible to the PG 3 and PG 4 but resistant to PG 2-1 and PG 2 isolate. The PG 2-1 and PG 2 isolates were differentiated on Val-1 and Dac-1. Val-1 was susceptible to the PG 2 but resistant to the PG 2-1 isolate, while Dac-1 was susceptible to the PG 2-1 but resistant to the PG 2 isolate. Quinta was susceptible only to the PG 4 isolate. The isolates used in this study were selected randomly from a sample of 156 isolates with known pathogenicity groups. All isolates were passaged at least twice through Westar (PG 2 and PG 2-1), Glacier (PG 3) or Quinta (PG 4), before their usage in the genetic study.

5.3.2 Inoculum preparation

Diseased cotyledons from inoculated differential cultivars were surface sterilized in a 0.6% sodium hypochlorite (household Javex) solution for 2-3 minutes. The cotyledons were rinsed in a second 0.6% solution of sodium hypochlorite and plated onto V8-Juice (Campbell Soup Company Ltd, Toronto, Canada) agar dishes with 1% streptomycin sulphate in order to inhibit bacterial growth. A second sub-culture to increase inoculum from the colonized plates was done after 5-7 days after incubating the infested host tissue. All handling and plating of inoculum was done under a laminar flow hood. The cultures were incubated under cool white fluorescent light at 20°C. After 7-10 days, sporulating cultures were flooded with 10-15 mL of sterile distilled water and their surface gently scraped with a flamed glass rod or slide to dislodge or release spores from pycnidia. The mixture was filtered through sterile Miracloth (Calbiochem, La Jolla, CA, USA) into sterile 15 mL centrifuge tubes and the spore suspension was centrifuged at 4,500 rpm for 30 minutes. The supernatant was decanted, the spore pellet re-suspended in approximately 1 mL of sterile distilled water and stored frozen as a concentrate at -20°C. To prepare spores for inoculation, the spore concentrate was left at room temperature to thaw and a few drops resuspended in 10 mL of sterile distilled water. The spores were quantified using a haemocytometer and adjusted by dilutions using sterile distilled water to a final concentration of 10^7 spores mL^{-1}.

5.3.3 Host material and crosses

Three differential cultivars of *B. napus*, Westar (spring type), Glacier and Quinta (winter types) were used in this study. Up to 50 seeds of each differential cultivar were planted in flats using a soilless mix (Metromix ™, W.R. Grace and Co. Ltd. Ajax, Ontario). Each cultivar was initially tested with a representative PG 1, PG 2, PG 3 and PG 4 isolate and plants with a resistant (IP 0-3) or susceptible (IP 9) reaction to the respective pathogenicity group were selected for use in the genetic study. Up to 14 single plants were selected for each cultivar for use as parental plants. The winter type cultivars Quinta and Glacier, were vernalized for six weeks to break dormancy and then returned to the greenhouse for crossing. The Quinta and Glacier parents were reciprocally crossed to each other and to Westar. The racemes used in the crosses were each isolated in glycine bags to avoid pollen cross contamination. Selected racemes from each parent used in the crosses were also isolated in glycine bags to self the plant and to avoid cross contamination by foreign pollen. The Quinta, Glacier and Westar parents and the F_1 plants from all the crosses were then tested with the four isolates representing each pathogenicity group. Up to six F_1 plants from each cross were randomly selected to generate F_2 populations. The parents, F_1 and F_2 plants from each cross were again tested as a batch with all the four isolates used in the study. Quinta, Glacier and Westar were included in every test as control. All plants were maintained at 21.5/14°C day/night temperature with a 16 h photo period and watered daily using tap water.

5.3.4 Cotyledon evaluation

Fully expanded cotyledons of the cultivars were inoculated with the isolates approximately 7 - 8 days after seeding. One lobe of one cotyledon was marked with two pin marks to identify one isolate and the diagonally opposite lobe was marked with one pin mark to identify another isolate. The other isolates were identified based on their position on the respective cotyledon lobe relative to either the 2-pin mark, or the single pin mark.

The two marked lobes of the cotyledons were then wounded and a 10 μL droplet of inoculum from each of two isolates was placed onto the wounds. Inoculum was allowed to dry for approximately 2 h and the two unmarked lobes of cotyledons were also wounded and inoculated with 10 μL inoculum of the remaining 2 isolates of *L. maculans*. The inoculum was allowed to dry onto the wounds and the plants were not watered for 1 day.

All plants were fertilized twice with a 20 - 20 - 20 (N - P - K) water soluble fertilizer, amended with micronutrients, three and six days after inoculation. True leaves from all plants were clipped back to ensure that the cotyledons expanded and remained green.

Cotyledon reactions were evaluated 10 days after inoculation using the cotyledon evaluation method (Williams 1985). Cotyledons were observed for an additional 2-3 days to note any changes in cotyledon reactions. The interaction phenotypes (IP) 0-4 were defined as resistant and interaction phenotypes 5-9 as susceptible.

5.4.0 Results

5.4.1 Parental tests

All Glacier and Quinta parental plants were resistant to isolates of PG 2-1 and PG 2 and all the Quinta parental plants were also resistant to the PG 3 isolate (Table 5.1). The parent Glacier was susceptible to the PG 3 isolate and both Quinta and Glacier parental plants were also susceptible to the PG 4 isolate. The cultivar Westar, was susceptible to all the isolates.

5.4.2 F_1, Quinta X Westar crosses

The F_1 from the Quinta x Westar crosses and their reciprocals were resistant to the PG 2-1. Most of the F_1 from the Quinta x Westar cross were resistant to the PG 2 isolate (Table 5.2). However, most F_1 populations segregated for IP to the PG 2 isolate (Appendix B. 4). When Quinta was used as the male parent most of the progeny were susceptible or gave segregation ratios close to 1:1. Alternatively when Quinta was used as the female parent, the F_1 families were either mostly resistant or gave a segregation ratio close to a 1:1. On the basis of mean IP, the F_1 from the Quinta x Westar crosses were also resistant to the PG 3 isolate (Table 5.2). Similar results were observed with the reciprocal crosses. Segregation for susceptibility occurred in the F_1 of some resistant x susceptible crosses when inoculated with the PG 3 isolates (Appendix B. 6). All the F_1 plants from the Quinta

x Westar crosses and their reciprocals were susceptible to the PG 4 isolate (Appendix B. 8).

5.4.3 F_1, Glacier x Westar crosses

The F_1 from most of the Glacier x Westar crosses and reciprocals were resistant to both PG 2-1 and PG 2 isolates of *L. maculans* (Table 5.3). Only the F_1 from one particular cross (W6G6), and their reciprocal crosses were susceptible to the PG 2 isolate (Table 5.3, Appendix B. 12). Individual F_1 plant reactions to PG 2-1 or PG 2 isolates indicated that segregation for susceptibility occurred in some F_1 populations (Appendix B. 10 & Appendix B. 12). All F_1 plants from Glacier x Westar crosses and the reciprocals were susceptible to PG 3 and PG 4 isolates.

5.4.4 F_1, Glacier x Quinta crosses

The F_1 populations from the Glacier x Quinta crosses and their reciprocals were resistant to PG 2-1, PG 2 and PG 3 (Table 5.4, Appendix B. 19, Appendix B. 20 & Appendix B. 21). Segregation for susceptibility to the PG 2 and the PG 3 isolates was observed in some F_1 populations of the Glacier x Quinta crosses and the reciprocals (Appendix B. 20 & Appendix B. 21). Most F_1 plants from Glacier x Quinta crosses and the reciprocals were susceptible to PG 4 (Appendix B. 22). However, in the cross G2QB and their reciprocals, the F_1 were mostly resistant to the PG 4 isolate.

5.4.5 F_2, Quinta x Westar crosses

Segregation data for F_2 populations from the Quinta x Westar crosses, their reciprocals and the combined Quinta x Westar crosses are presented in Table 5.5 - 5.7. The F_2 populations from the Quinta x Westar crosses fit a 3 resistant : 1 susceptible segregation ratio when inoculated with the PG 2-1 isolate (Table 5.5). When inoculated with the PG 2 isolate, the segregation ratios fit an expected segregation ratio of 9 resistant : 7 susceptible (Table 5.6). In a few of these crosses however, different F_2 populations fit either a segregation ratio of 3 resistant:1 susceptible or a 1 resistant: 3 susceptible ratio (Appendix B. 5). The combined data for all F_2 populations from the Quinta x Westar crosses and their reciprocals fit a 9 resistant:7 susceptible segregation ratio (Table 5.6). The F_2 populations of Quinta x Westar crosses and their reciprocals fit a 3:1 segregation ratio, when inoculated with the PG 3 isolate (Table 5.7). All the F_2 populations from these crosses were susceptible to the PG 4 isolate.

5.4.6 F_2, Glacier x Westar crosses

The F_2 populations of the Glacier x Westar crosses and their reciprocals fit a 3 resistant:1 susceptible segregation, when inoculated with either the PG 2-1 (Table 5.8) or PG 2 isolates (Table 5.9). The Chi - square tests for pooled and/or homogeneity on all combined data for the Glacier x Westar crosses were significant for a 3:1 segregation ratio (Table 5.8, Table 5.9). In only one case involving cross 6, did the segregation ratios for the F_2 families fit a 1 resistant : 3

susceptible genetic model when plants were inoculated with the PG 2 isolates (Appendix B. 13). All the F_2 populations were susceptible to the PG 3 and PG 4 isolates.

5.4.7 F_2, Glacier x Quinta crosses

The F_2 populations from the Glacier x Quinta crosses and their reciprocals were predominantly resistant to the PG 2-1 and the PG 2 isolates of *L. maculans* (Appendix B. 23 & Appendix B. 24). Pooled data from these crosses were significant for a 3:1 segregation ratio. However, the homogeneity Chi - square was not significant (Table 5.10). The F_2 populations from these crosses predominantly fit a 3 resistant: 1 susceptible segregation ratio when tested with the PG 3 isolate (Appendix B. 25). Most F_2 progeny of crosses involving Glacier or Quinta were susceptible to the PG 4 isolate. However, the F_2 from the cross G2QB and their reciprocals, were mostly resistant to the PG 4 isolate.

5.5 Discussion

The *B. napus* cultivars Westar, Glacier and Quinta have been used by various researchers (Badawy et al. 1991, Koch et al. 1991, Mengistu et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995, Pongam et al. 1999) as differential hosts for isolates of *L. maculans*. However, information on the genetics of resistance was derived using different genotypes of both *B. napus* and *L. maculans*. Following map based studies, Ferreira et al. (1995) reported that a single gene conferred

resistance in the cultivar Major to PG 2 isolates. Subsequently Pongam et al. (1998) identified a complementary gene for avirulence in PG 2 isolates based on reactions of the cultivar Major to progeny isolates from crosses of *L. maculans*. The present study reports on the inheritance of resistance using the three *B. napus* cultivars Westar, Glacier and Quinta originally used as differentials by Koch et al. (1991). The F_1 and F_2 generations were each inoculated simultaneously with a PG 2-1, PG 2, PG 3 and PG 4 isolate. Based on the reactions of F_1 plants, segregation for susceptibility occurred in most of the reciprocal crosses between Quinta, Glacier and Westar, when tested with the PG 2-1, PG 2 and PG 3 isolates. Most F_1 populations segregated for susceptibility to the PG 2 isolate with the segregation being close to a 1:1 ratio. This would be expected if the interaction between the host and pathogen is determined by at least 2 genes. For instance, when the resistance genes in the host are heterozygous at either locus (i.e. R_1 r_1 or r_2R_2) and the genotype of the PG 2 isolate was A_1 a_2) a 1:1 segregation may be obtained. Moreover, the F_1 from W6G6 cross and their reciprocals were all susceptible to the PG 2 isolate. This suggests that the Quinta and Glacier parents used in the study were heterozygous and heterogeneous.

Resistance to the PG 2-1 in both Quinta and Glacier was controlled by a single dominant gene in each cultivar (Table 5.5 and Table 5.8). Excessive numbers of resistant plants in some populations of the Quinta x Westar and the Glacier x Westar crosses resulted in significant Chi-square values (Appendix B. 3 & Appendix B. 11). Also segregation for susceptibility was observed in some families

of the Quinta x Glacier crosses when tested with the PG 2-1 isolate. This is likely due to linkage of the genes conferring resistance to PG 2-1 and other resistance genes in both cultivars (data not included). Moreover, genetic background or environment, may influence the expression of IP.

Based on results of a genetic study of the progeny from crosses between isolates of *L. maculans* tested on Westar, Glacier and Quinta, Rimmer and van den Berg (1992) postulated that Glacier and Quinta had one and two genes respectively for resistance to PG 2 isolates, and that one of the genes was common in both cultivars. The results of the present study, using the same cultivars, generally support the hypothesis of Rimmer and van den Berg (1992).

Resistance in Quinta to PI86-12 (PG 2) isolate of *L. maculans* was controlled by two nuclear genes. However, Rimmer and van den Berg (1992) were unable to postulate the genetic model for the two resistance genes possibly because the parental PG 2 and PG 4 isolates and the progeny from their crosses were not tested on the F_2 from crosses of resistant x susceptible plants. Ansan-Melayah et al. (1998) studied the inheritance of resistance in F_2 populations from Quinta x Score crosses using progeny isolates from the *L. maculans* crosses, PHW1245 x 1.2.4 and (PHW1245 x 1.2.4) x 1.2.4. Data presented by Ansan-Melayah et al. (1998) did not fit a one or two gene model. The data in the present study suggested that the resistance in Quinta to the PG 2 isolate of *L. maculans* may be explained by a 9:7 genetic model of resistant : susceptible plants. With this model, two independent dominant genes determine resistance to *L. maculans*; but either

gene pair when homozygous recessive is epistatic to the other dominant resistance gene. Hence the genotypes R_1R_1 r_2r_2, R_1r_1 r_2r_2, r_1r_1 R_2R_2, r_1 r_1 R_2r_2 and r_1r_1 r_2r_2 result in a susceptible interaction phenotype when host plants are challenged with PG 2 isolates. All other genotypes (R_1- R_2-, R_1R_1 R_2- and R_1- R_2R_2) exhibit a resistant reaction to the PG 2 isolates.

The F_2 populations from the Quinta x Westar crosses number 1, 5, 6 and their reciprocals fit a 9:7 model of resistant: susceptible plants when tested with PG 2 isolates (Appendix B. 5). The genotype of the Quinta parents in these crosses may be either R_1R_1 R_2R_2 or R_1r_1 R_2r_2; the latter genotype (R_1r_1 R_2r_2) is however more likely, since the parents were selfed only once. Some of the F_2 populations from Quinta x Westar crosses indicated that the Quinta parent plants numbers 2 and 4 segregated for only one locus of resistance to the PG 2 isolate (Appendix B. 5). In the crosses QBW2 and QDW4, the data fit a 3 resistant : I susceptible segregation ratio, indicating that a single dominant gene determined resistance to the PG 2 isolate. However, in the reciprocal cross (W2QB), the F_2 populations fit a segregation of 9 resistant : 7 susceptible ratio, suggesting that two genes conferred resistance to the PG 2 isolate (Appendix B. 5). In the cross QBW2, the segregation ratio of the progeny in this reciprocal cross fitted a 3 resistant : 1 susceptible ratio rather than a 9 resistant : 7 susceptible genetic model, suggesting that only a single dominant gene was expressed. The excess of resistant progeny plants in the former as compared to the latter model may be due to environmental effects on the gene action, effects due to genetic background in Quinta, and/or the

interaction effects of the environment and Quinta genetic background. Alternatively the genes conferring resistance in Quinta to *L. maculans* could be tightly linked. In the reciprocal cross (W4QD), the data fit a 1 resistant: 3 susceptible segregation ratio, suggesting that a single recessive resistance gene. The anomalies in gene action in the above crosses may be attributed to environmental effects on one or both the genes which confer resistance to the PG 2 isolate in Quinta, linkage of different resistance genes and/or differences in pollen viability. Excess of plants with susceptible IP occurred in cross W4QD; the data fit a 1: 3 segregation ratio, indicating that a single gene determined resistance to the PG 2 isolate. It is important to note that some F_1 plants were susceptible while others were resistant to PG 2 isolates. Consequently if a susceptible F_1 plant was selfed to derive F_2 populations, it is conceivable that a 1 resistant: 3 susceptible segregation ratio could be obtained. The contradiction in gene action in this cross could be due to either the effect of environment on certain genotypes or the heterozygosity and heterogeneity of the parents.

The duplicate recessive epistatic model can be confirmed by two methods. The intercrossing of susceptible genotypes will result in F_1 progeny which are all resistant if the susceptible genotype (R_1R_1 r_2r_2) is crossed to the susceptible genotype (r_1r_1 R_2R_2). All the F_1 and F_2 plants will be susceptible if the susceptible genotypes (R_1- r_2r_2 , r_1r_1 R_2-) are crossed to the susceptible genotype (r_1 r_1 r_2r_2). A 1 resistant : 1 susceptible segregation ratio would be obtained in F_1 generation if the genotype of susceptible parent (R_1R_2 r_2r_2) and susceptible parent (r_1r_1 R_2r_2)

are crossed. Two kinds of F_2 populations will occur; one F_2 population will fit a 9:7 genetic model and the other F_2 population will all be susceptible to the PG 2 isolate.

F_2 populations from the Glacier x Westar crosses and their reciprocals fit a 3 resistant:1 susceptible segregation ratio for reactions to the PG 2 isolate. This indicates that a single dominant gene determines resistance in Glacier to the PG 2 isolate. Similar results were obtained by Ansan-Melayah et al. (1998) when F_2 populations from Glacier x Score crosses were tested with the PG 2 isolates (PHW1325, 14.3.01 or 14.1.01). The results of this study are in agreement with the postulate of Rimmer and van den Berg (1992), that a single gene in Glacier confers resistance to PG 2 isolates. However, combined data for the F_2 populations from the Glacier x Westar crosses were significant for a 3:1 segregation ratio. The data in one cross (G6W6) also indicated that a recessive gene confers resistance to the PG 2 isolate used; thus, depending on the original parent population, either a dominant or a recessive gene action may be observed. This further supports the inference that the Quinta and Glacier original seed lot or seed source were composed of heterogeneous and heterozygous genotypes. Similar results with regard to conflicting gene action in the *Brassica* - *L. maculans* pathosystem were reported by other researchers (Delwiche 1980, Sawatsky 1989). Delwiche (1980), using *B. napus* cultivars, identified two dominant linked genes which controlled resistance to an isolate of *L. maculans* at the cotyledon stage; in

contrast, Sawatsky (1989) reported only a single recessive gene conferring resistance in *B. napus* cultivars to a PG 2 isolate at the cotyledon stage.

The F_2 populations from the Quinta x Westar crosses and their reciprocals fit a 3:1 genetic model of resistant: susceptible plants, when tested with the PG 3 isolate, indicating that a single dominant gene conferred resistance to the PG 3 isolate in the differential cultivar Quinta. Ansan-Melayah et al. (1998) also identified a single gene for resistance in F_2 populations from Quinta x Score using the PG 3 isolates a.2 or 11.26.11. However they did not determine the genetics of resistance to the PG 3 isolates in F_2 populations from Quinta x Glacier crosses. In this study F_2 progeny of the Glacier x Quinta crosses and their reciprocals fit a 3:1 segregation model of resistant: susceptible plants, confirming that one dominant gene determined resistance in Quinta to PG 3 isolates (Appendix B. 25).

In the Glacier x Quinta crosses, their reciprocals and combined data, the F_2 populations were homogeneous for a 3:1 segregation ratio. However, the Chi-square values for the pooled data were either significant or highly significant for a 3:1 segregation ratio, suggesting that the Glacier and Quinta parents are heterozygous at the locus for resistance to PG 3 isolates of *L. maculans.* Alternatively, the genes conferring resistance to the PG 3 isolate may be linked to other resistance genes. Data for linkage analysis has not been included herein.

The F_1 and F_2 plants from the G2QB crosses were mostly resistant to the PG 4 isolate used. It is possible that resistant progenies may be obtained from reciprocal crosses of Glacier and Quinta. However, at the time of testing this

particular cross, the growth chambers broke down or malfunctioned. Therefore, that most of the F_1 and F_2 from the G2QB crosses were resistant was attributed to the break down of the growth chambers and the resultant high temperatures. The parents, F_1 and F_2 populations from each cross were tested as a batch. In summary, this study identified at least four different genes for resistance to *L. maculans* residing in the *B. napus* cultivars Quinta and Glacier. Quinta has a gene for resistance to the PG 2-1, another gene for resistance to the PG 3 and two genes for resistance to the PG 2, while Glacier has at least one gene for resistance to PG 2-1 and another gene for resistance to the PG 2 isolates. The gene conferring resistance to the PG 2 in Glacier is same with one of the two genes conferring resistance in Quinta to PG 2 isolate.

Table 5.1. Mean interaction phenotype of parental plants of *Brassica napus* cultivars Westar (W), Quinta (Q) and Glacier (G) following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

Parent Number	Isolate Number and Pathogenicity group (PG) PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
	Interaction phenotype rating [1]				
W1	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	21
W2	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	18
W3	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	18
W4	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	24
W5	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	18
W6	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	14
W7	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	20
QA	3.1 ± 0.12	2.6 ± 0.12	3.3 ± 0.10	8.4 ± 0.25	33
QB	2.6 ± 0.13	2.8 ± 0.15	3.4 ± 0.11	8.9 ± 0.11	32
QC	3.2 ± 0.06	2.8 ± 0.15	4.0 ± 0.09	9.0 ± 0.00	28
QD	3.2 ± 0.11	3.5 ± 0.12	4.1 ± 0.09	8.9 ± 0.00	26
QE	3.2 ± 0.08	1.9 ± 0.14	3.6 ± 0.09	8.6 ± 0.15	24
QF	3.0 ± 0.08	2.5 ± 0.14	3.4 ± 0.08	8.3 ± 0.17	17
G1	1.7 ± 0.17	1.6 ± 0.16	9.0 ± 0.00	9.0 ± 0.00	34
G2	2.1 ± 0.20	1.8 ± 0.19	9.0 ± 0.00	3.8 ± 0.27	28
G3	1.4 ± 0.14	1.5 ± 0.16	9.0 ± 0.00	9.0 ± 0.00	38
G4	1.0 ± 0.00	1.2 ± 0.20	9.0 ± 0.00	9.0 ± 0.00	22
G5	1.1 ± 0.06	1.2 ± 0.09	7.9 ± 0.17	8.8 ± 0.09	33
G6	2.0 ± 0.19	3.1 ± 0.21	9.0 ± 0.00	9.0 ± 0.00	30

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Table 5.2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

Cross	Isolate Number and Pathogenicity group (PG)				Number of plants
	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	
	Interaction phenotype rating [1]				
W1QA	2.8 ± 0.15	5.5 ± 0.51	3.3 ± 0.16	6.8 ± 0.67	38
W2QB	3.1 ± 0.11	4.9 ± 0.29	3.6 ± 0.13	8.1 ± 0.24	54
W3QC	2.8 ± 0.13	7.6 ± 0.22	4.3 ± 0.08	9.0 ± 0.00	51
W4QD	3.4 ± 0.07	7.2 ± 0.18	4.3 ± 0.09	8.8 ± 0.05	52
W5QE	3.1 ± 0.18	5.4 ± 0.35	4.3 ± 0.14	9.0 ± 0.00	49
W6QF	3.0 ± 0.34	5.2 ± 0.41	6.3 ± 0.33	8.7 ± 0.05	36
QAW1	3.1 ± 0.09	4.1 ± 0.23	4.3 ± 0.13	9.0 ± 0.00	53
QBW2	3.4 ± 0.18	4.2 ± 0.30	4.6 ± 0.23	8.9 ± 0.02	47
QCW3	2.6 ± 0.14	4.1 ± 0.31	4.2 ± 0.14	8.8 ± 0.02	56
QDW4	2.3 ± 0.15	4.3 ± 0.32	3.7 ± 0.12	8.9 ± 0.07	58
QEW5	1.9 ± 0.16	4.4 ± 0.38	3.6 ± 0.17	9.0 ± 0.00	53
QFW6	2.1 ± 0.14	5.4 ± 0.44	3.1 ± 0.22	8.9 ± 0.04	57

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Table 5.3. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

Cross	Isolate Number and Pathogenicity group (PG)				Number of plants
	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	
	Interaction phenotype rating [1]				
W1G1	3.1 ± 0.23	2.7 ± 0.39	9.0 ± 0.00	9.0 ± 0.00	46
W2G2	2.6 ± 0.13	1.3 ± 0.19	9.0 ± 0.00	9.0 ± 0.00	46
W3G3	1.5 ± 0.19	2.8 ± 0.14	9.0 ± 0.00	9.0 ± 0.00	25
W4G4	3.0 ± 0.00	3.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	42
W6G6	2.5 ± 0.22	9.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	16
W7G7	3.0 ± 0.00	3.0 ± 0.00	9.0 ± 0.00	9.0 ± 0.00	16
G1W1	3.3 ± 0.23	3.1 ± 0.20	8.0 ± 0.00	8.0 ± 0.00	56
G2W2	2.4 ± 0.16	2.7 ± 0.18	8.0 ± 0.00	8.0 ± 0.00	60
G3W3	4.0 ± 0.23	4.2 ± 0.25	8.1 ± 0.04	7.9 ± 0.04	58
G4W4	2.3 ± 0.15	3.1 ± 0.21	8.1 ± 0.04	8.0 ± 0.04	59
G6W6	2.8 ± 0.08	7.2 ± 0.15	8.0 ± 0.09	8.1 ± 0.08	64
G7W7	3.3 ± 0.07	3.5 ± 0.10	8.8 ± 0.05	8.8 ± 0.04	63

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Table 5.4. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

Cross	Isolate Number and Pathogenicity group (PG)				Number of plants
	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	
	Interaction phenotype rating [1]				
G1QA	2.2 ± 0.17	3.1 ± 0.11	4.2 ± 0.14	8.5 ± 0.09	46
G2QB	1.9 ± 0.17	3.4 ± 0.17	4.1 ± 0.14	2.6 ± 0.00	37
G3QC	1.7 ± 0.15	3.1 ± 0.07	4.1 ± 0.17	9.0 ± 0.00	59
G4QD	1.0 ± 0.00	3.1 ± 0.05	3.4 ± 0.18	9.0 ± 0.00	54
G5QE	1.2 ± 0.10	3.0 ± 0.06	3.5 ± 0.22	9.0 ± 0.00	47
G6QF	1.3 ± 0.10	3.2 ± 0.11	2.3 ± 0.16	8.1 ± 0.17	47
QAG1	3.6 ± 0.20	3.4 ± 0.20	3.7 ± 0.18	8.9 ± 0.20	44
QBG2	2.6 ± 0.14	3.5 ± 0.18	4.1 ± 0.18	3.4 ± 0.24	33
QCG3	3.9 ± 0.25	4.4 ± 0.34	4.4 ± 0.15	8.2 ± 0.25	45
QDG4	2.3 ± 0.19	3.4 ± 0.15	3.9 ± 0.15	8.6 ± 0.19	51
QEG5	1.6 ± 0.14	3.3 ± 0.15	4.1 ± 0.17	8.5 ± 0.14	42
QFG6	1.7 ± 0.16	3.4 ± 0.16	4.1 ± 0.22	8.1 ± 0.16	38

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Table 5.5. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta and their reciprocal crosses, following cotyledon inoculation with the PG 2 - 1 isolate PL87-41 of *Leptosphaeria maculans*.

Cross	Observed IP R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	587	196	783	3:1	5.336	
Pooled				3:1	0.000	> 0.95
Homogeneity (df=5)				3:1	5.336	0.30-0.50
Quinta x Westar						
Total plants	390	125	515	3:1	13.001	
Pooled #				3:1	0.146	0.70-0.90
Homogeneity (df=3)				3:1	12.855*	0.01-0.05
Westar x Quinta Combined						
Total plants	1211	347	1558	3:1	52.407	
Pooled				3:1	6.183*	0.01-0.05
Homogeneity (df=11)				3:1	46.224**	< 0.001

Cross QAW1 and QBW2 were excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 5.6. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta and their reciprocal crosses, following cotyledon inoculation with the PG 2 isolate PL86-12 of *Leptopshaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	306	232	538	9:7	5.447	
Pooled #				9:7	0.086	0.30-0.50
Homogeneity (df=3)				9:7	5.361	0.10-0.20
Quinta x Westar						
Total plants	290	190	480	9:7	7.429	
Pooled #				9:7	3.386*	0.01-0.05
Homogeneity (df=3)				9:7	4.430	0.30-0.50
Westar x Quinta Combined						
Total plants	875	694	1569	9:7	18.631	
Pooled				9:7	0.146	0.50-0.70
Homogeneity (df=11)				9:7	18.483	0.05-0.10

All crosses testing significant for a 9:7 genetic model were excluded from pooled data.

* = significant at the 0.05 probability level.

Data for combined crosses include the crosses testing significant for a 9:7 ratio.

Table 5.7. Crosses, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta and their reciprocal crosses following cotyledon inoculation with the PG 3 isolate Lifolle 6 of *Leptosphaeria maculans*.

Cross	Observed IP R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	510	199	709	3:1	4.823	
Pooled				3:1	3.559	0.05-0.10
Homogeneity (df=5)				3:1	1.266	0.90-0.95
Quinta x Westar						
Total plants	440	136	576	3:1	8.002	
Pooled #				3:1	0.593	0.30-0.50
Homogeneity (df=4)				3:1	7.409	0.30-0.50
Westar x Quinta Combined						
Total plants	1028	375	1403	3:1	17.809	
Pooled				3:1	2.236	0.10-0.20
Homogeneity (df=9)				3:1	15.573	0.05-0.10

Cross QEW5 was excluded from pooled data.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 5.8. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar x Glacier and their reciprocal crosses following cotyledon inoculation with the PG 2-1 isolate PL87-41 of *Leptospaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Glacier						
Total plants	558	186	744	3:1	6.099	
Pooled #				3:1	0.000	> 0.95
Homogeneity (df=4)				3:1	6.099	0.10-0.20
Glacier x Westar						
Total plants	524	146	670	3:1	7.284	
Pooled #				3:1	3.680	0.05-0.10
Homogeneity (df=4)				3:1	3.604	0.30-0.50
Westar x Glacier Combined						
Total plants	1235	341	1576	3:1	46.049	
Pooled				3:1	9.506**	0.001-0.01
Homogeneity (df=11)				3:1	36.544**	< 0.001

Cross G2W2 was excluded from pooled data.
** = significant at the 0.01 probability level.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 5.9. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar x Glacier and their reciprocal crosses following cotyledon inoculation with the PG 2 isolate PL86-12 of *Leptosphaeria maculans.*

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Glacier						
Total plants	301	123	424	3:1	3.873	
Pooled #				3:1	3.635	0.05-0.10
Homogeneity (df=2)				3:1	0.238	0.70-0.90
Glacier x Westar						
Total plants	387	147	534	3:1	8.083	
Pooled #				3:1	1.820	0.10-0.20
Homogeneity (df=3)				3:1	6.263	0.05-0.10
Westar x Glacier Combined						
Total plants	1064	511	1575	3:1	55.214	
Pooled				3:1	46.214**	< 0.001
Homogeneity (df=11)				3:1	9.000	0.50-0.70

Cross W2G2, W6G6 and reciprocals were excluded from pooled data.
** = significant at the 0.01 probability level.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 5.10. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier x Quinta and their reciprocal crosses following cotyledon inoculation with the PG 3 isolate Lifolle 6 of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Glacier x Quinta						
Total plants	443	176	619	3:1	6.708	
Pooled #				3:1	3.891*	0.01-0.05
Homogeneity (df=3)				3:1	2.817	0.30-0.50
Quinta x Glacier						
Total plants	541	241	782	3:1	16.974	
Pooled $				3:1	14.119**	< 0.001
Homogeneity (df=4)				3:1	2.855	0.50-0.70
Glacier x Quinta Combined						
Total plants	984	417	1401	3:1	23.682	
Pooled				3:1	16.962**	< 0.001
Homogeneity (df=8)				3:1	6.720	0.50-0.70

Crosses G1QA and G5QE were excluded from pooled data.
$ Cross QEG5 was also excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

CHAPTER 6

The genetics of resistance in the *Brassica napus* differential cultivar Quinta to progeny isolates from crosses of *Leptosphaeria maculans*.

6.1 Abstract

Evidence suggests that variation for virulence exists among isolates of *Leptosphaeria maculans* and that interactions between certain genotypes of *Brassica napus* and isolates of *L. maculans* may be specific. A resistant *B. napus* differential cultivar (Quinta) was crossed to the susceptible *B. napus* cultivar Westar. The parents, F_1 and F_2 populations from the crosses and their reciprocals were evaluated at the cotyledon stage with two PG 2 isolates (5174/11 and 1130 - B24) and two PG 3 isolates (PL89 - 21 and 1130 - A18). The results indicated that cotyledon resistance in *B. napus* to *L. maculans* is a qualitative trait and is controlled by nuclear genes. Resistance in Quinta to the PG 3 isolate (1130 - A18) was controlled by a dominant gene. Similarly, resistance to the PG 3 isolate (PL89 - 21) was controlled by one dominant gene. Resistance in Quinta to the PG 2 isolate (1130 - B24) was also controlled by one dominant gene. However, segregation of resistance in Quinta to the PG 2 isolate (5174/11) neither fit a one gene model nor a two - gene model. The genes conferring resistance to the two PG 3 isolates of *L. maculans* are not allelic but rather are linked (RF = 16.44%). The gene conferring resistance in Quinta to the PG 2 isolate (1130 - B24) is linked to genes conferring resistance to the PG 3 isolates (1130 - A18 and PL89 - 21),

with RFs = 9.49% and 6.96% respectively. The reasons for the distorted segregation of resistance in Quinta to the PG 2 isolate (5174/11) are not clear.

6.2 Introduction

Leptosphaeria maculans (Desm.) Ces and de Not., the causal organism of blackleg disease of oilseed rape, is a heterothallic fungus. Little is known about the nature of the interaction between the resistance genes in *Brassica* species and specific isolates of *L. maculans*. Since Koch et al. (1991) identified Westar, Glacier and Quinta as suitable cultivars for the differentiation of isolates of *L. maculans*, some progress in the elucidation of physiological specialization has been achieved (Badawy et al. 1991, Ballinger et al. 1991, Kutcher et al. 1993, Kuswinanti et al. 1995). It is important therefore to undertake genetic studies of both the host and pathogen, since they can provide pertinent information that is necessary for an effective breeding program and facilitate pursuits for a better understanding of host-pathogen interaction.

In a review of host resistance to *L. maculans*, Rimmer and van den Berg (1992) alluded to the involvement of a gene-for-gene interaction in this host-pathosystem and presented a hypothesis of a resistance gene model for Glacier and Quinta based on the segregation pattern of the avirulence gene in the pathogen. The gene-for-gene hypothesis stipulates that each gene conferring resistance in the host plant to a given pathogen, is matched by a corresponding and complementary gene in the pathogen, which confers avirulence to the host plant (Flor 1971, Crute 1985). Both resistance in the host plant and avirulence in the pathogen were expressed as dominant phenotypes in most host pathogen interactions reported. There is however limited information with regard to the genetics of

resistance in *B. napus* to *L. maculans* or on the genetics of avirulence in *L. maculans*. Rimmer and van den Berg (1992) also postulated that PG 2 isolates comprise two genotypes, A_1A_2 and A_1a_2. The genotype a_1A_2 would represent PG 3 and the a_1a_2 genotype would represent PG 4 isolates. If this hypothesis is correct, one would expect all F_1 plants from crosses of resistant x susceptible plants to be resistant and the F_2 populations from crosses of resistant x susceptible plants to fit a 3 resistant: 1 susceptible segregation ratio if the genotype of the isolate is A_1A_2 and a two gene model is assumed. On the other hand, a 1 resistant: 1 susceptible segregation ratio would be expected in the F_1 from crosses of resistant x susceptible plants if the genotype of the PG 2 test isolate is A_1a_2. The segregation of the F_2 populations would, however, depend on whether the parents are homozygous at the two loci and on the genotype of the particular plant which generated the F_2 population. The objective of this study was to test the above mentioned hypothesis and also determine the genetics of host-pathogen interaction using progeny isolates from crosses between different genotypes of *L. maculans* and the differential cultivar Quinta.

6.3.0 Materials and methods

6.3.1 Pathogen isolates

Four isolates of *L. maculans*, two of pathogenicity group (PG) 2 and two isolates of PG 3 were used in this study. The parent isolates 5174/11 (PG 2) and

PL89-21 (PG 3), and two progeny from their cross 1130-B24 (PG 2) and 1130-A18 (PG 3) were selected, based on their IP on Westar, Glacier and Quinta, from among 120 progeny isolates of *L. maculans*. All isolates were passaged through Westar before use in the genetic study.

6.3.2 Inoculum

Diseased cotyledons from the inoculated differential cultivars were surface sterilized in a 0.6% sodium hypochlorite (household Javex) solution for 2-3 minutes. The cotyledons were rinsed in a second 0.6% solution of the sodium hypochlorite and plated onto V8-Juice (Campbell Soup Company Ltd, Toronto, Canada) agar with 1% streptomycin sulphate in order to inhibit bacterial growth. A second sub-culture for inoculum production from the colonized plates was done 5-7 days after incubating the infested host tissue. All handling and plating of inoculum was done under the flow hood. Culture plates were incubated under cool white fluorescent light at 20°C. After 7-10 days, sporulating cultures were flooded with 10-15 mL of sterile distilled water and their surface gently scraped with a flamed glass rod or slide to dislodge or release spores from pycnidia. The mixture was filtered through sterile Miracloth (Calbiochem, La Jolla, CA) into sterile 15 mL centrifuge tubes and the spore suspension was centrifuged at 4,500 rpm for 30 minutes. The supernatant was decanted, the spore pellet re-suspended in approximately 1 mL of sterile distilled water and stored frozen as a concentrate at -20°C.

To prepare spores for inoculation, the spore concentrate was thawed at room temperature and a few drops resuspended in 10 mL of sterile distilled water. The spore concentration was quantified using a haemocytometer and adjusted with sterile distilled water to a final concentration of 10^7 spores mL^{-1}.

6.3.3 Host material

Three differential cultivars of *B. napus*, Westar (spring type) and Quinta (winter types) were used in this study. Seeds of the differential cultivars were planted in flats using a soilless mix (Metromix ™, W.R. Grace and Co. Ltd. Ajax, Ontario). The cultivars were evaluated only at the cotyledon stage for their reactions to the four isolates representing the two pathogenicity groups. Each cultivar was initially tested with a representative PG 1, PG 2, PG 3 and PG 4 isolate, and plants with resistant (IP 0 - 3) or susceptible (IP 9) reaction to the respective pathogenicity group were selected for use in the genetic study. Up to 8 single plants were selected for each cultivar for use as parents. The winter type cultivars Quinta was vernalized for six weeks to break dormancy and then returned to the greenhouse for crossing. The Quinta parent was reciprocally crossed to Westar and the crossed racemes isolated in glycine bags to avoid pollen cross contamination. Selected racemes from each parental plant were also isolated in glycine bags to obtain inbred seed and to avoid cross contamination by foreign pollen. Quinta and Westar parentals and the F_1 plants from all the crosses were then tested with the four isolates representing the two pathogenicity groups. Up to six F_1 plants from

each cross were randomly selected to generate F_2 populations. The parents, F_1 and F_2 plants from each cross were again tested as a batch with all the four isolates used in the study. Quinta, Glacier and Westar were included in every test as control. All plants were kept at 21.5/14°C day/night temperature and a 16 h photo period and watered daily using tap water.

6.3.4 Cotyledon evaluation

Fully expanded cotyledons of the cultivars were inoculated with the four isolates approximately seven to eight days after seeding. One lobe of one cotyledon was marked with two close pin marks to identify one isolate and the diagonally opposite lobe was marked with only one pin mark to identify another isolate. The marked lobes of the cotyledons were then wounded and a 10 μL droplet of inoculum from each of two isolates was placed onto the wounds. The inoculum was allowed to dry for approximately 2 h and the two unmarked lobes of cotyledons were then wounded and inoculated with 10 μL inoculum of the remaining two isolates of *L. maculans*. These two isolates were identified based on their position on the respective cotyledon lobe relative to either the two pin marked lobe, or the single pin - marked lobe. The inoculum was allowed to dry onto the wounds and the flats were not watered for one day.

All the plants were fertilized twice with a 20 - 20 - 20 (N - P - K) water soluble fertilizer, amended with micronutrients, three days after inoculation and 6 days

after inoculations. True leaves from all plants were clipped back to ensure that the cotyledons expanded and remained green.

Cotyledon reactions were evaluated 10 days after inoculation using the cotyledon evaluation method (Williams 1985). Cotyledons were observed for an additional 2-3 days in order to record any changes in the cotyledon reactions. The interaction phenotypes 0-4.9 were defined as resistant and interaction phenotypes 5-9 as susceptible.

6.4.0 Results

6.4.1 Parental tests

All the Quinta plants used as parents in this genetic study were resistant to both PG 2 isolates (1130 - B24 and 5174/11) and to both PG 3 isolates (PL89-21 and 1130 - A18). The cultivar Westar was susceptible to all four isolates used in this study.

6.4.2 F_1, Quinta X Westar crosses

The F_1 from the Quinta x Westar crosses and their reciprocals were resistant to isolate 1130 - B24 (Table 6.1, Appendix C. 1). Majority of the F_1 plants from the Quinta x Westar crosses were resistant to isolate 5174/11 based on their mean IP (Table 6.1, Table 6.2), although segregation in some F_1 families was observed. Segregation for susceptibility in F_1 families when inoculated with the isolate

5174/11, was close to a 1 resistant:1 susceptible ratio (Table 6.2). No differences were observed with direction of cross, although populations were small. Similarly, based on mean IP, the F_1 from the Quinta x Westar crosses were resistant to the PG 3 isolates (1130 - A18 and PL89-21) (Table 6.1, Appendix C. 3 & Appendix C. 4). No differences were observed with the direction of cross. Segregation for IP was observed in some F_1 families of all crosses when inoculated with the isolates 1130 - B24, 5174/11, 1130 - A18 and PL89-21.

6.4.3 F_2, Quinta x Westar crosses

The F_2 data of the Quinta x Westar crosses gave a good fit to a 3 resistant : 1 susceptible segregation ratio (Table 6.3, Appendix C. 5) when F_2 populations were inoculated with isolate 1130 - B24. When inoculated with the other PG 2 isolate (5174/11), the F_2 populations from Quinta x Westar crosses and their reciprocals did not fit a one or two gene model (Table 6.4). In a few families however, F_2 populations fit either a 1:3 or a 5:11 segregation ratio of resistant : susceptible plants (Appendix C. 6). The pooled data was neither homogeneous for a 1 resistant : 3 susceptible, nor a 5 resistant : 11 susceptible segregation ratio. The F_2 data fit a 3 resistant : 1 susceptible ratio when the families were inoculated with the isolates 1130-A18 or PL89 - 21 (Table 6.5, Table 6.6 respectively, Appendix C. 7 & Appendix C. 8).

6.5 Discussion

Since Koch et al. (1991) first identified the cultivars Westar, Glacier and Quinta as suitable differential host genotypes for isolates of *L. maculans*, Rimmer and Mengistu (in Rimmer and van den Berg 1992) initiated a study of the genetics of avirulence in *L. maculans* and suggested that a gene-for-gene system is likely involved in the *Brassica* - *L. maculans* pathosystem. They postulated that, one gene determines resistance to PG 2 isolates in Glacier; that two genes confer resistance to PG 2 isolates in Quinta and that Glacier had one gene for resistance to PG 2 isolates in common with Quinta. Evidence supporting this hypothesis is presented in a separate paper (see Chapter 5). In their study, Rimmer and Mengistu (in Rimmer and van den Berg (1992) further postulated that at least two avirulence genotypes (A_1A_2 and A_1a_2) occur among PG 2 isolates and PG 3 would then be a_1A_2. This genetic model was tested by challenging each F_2 plant simultaneously with the two PG 2 genotypes of *L. maculans* in question. The crosses between Quinta and Westar were selected instead of Glacier x Westar crosses because Quinta was thought to have at least two genes conferring resistance to PG 2 isolates of *L. maculans* (Rimmer and van den Berg 1992) and consistently gave reliable results (Koch et al. 1991).

No significant differences occurred with the direction of cross indicating that resistance in Quinta to the isolates of *L. maculans* was determined by nuclear genes. The segregation ratios of F_2 populations from the Quinta x Westar crosses and their reciprocals, for reactions to the isolates 1130 - B24, 1130 - A18 and

PL89 - 21, were consistent with a 3:1 genetic model, indicating that a single dominant gene conferred resistance to each of these genotypes of *L. maculans*. The data in this study suggest that the isolate 1130-B24 is one of the key PG 2 genotypes postulated by Rimmer and van den Berg (1992). One piece of evidence is that segregation for resistance in F_2 populations from the Quinta x Westar crosses and their reciprocals to the isolate 1130-B24 fit a 3:1 ratio. This isolate obviously behaves differently from the PG 2 isolate PL86 - 12 which resulted in a 9:7 segregation ratio (see Chapter 5). This suggests that PG 2 isolates comprise various genotypes. These may be further differentiated by new host differential genes.

If one assumes that the reactions in Quinta to isolate 1130 - B24 (PG 2) is represented by the alleles R_1 / r_1, isolate 1130 - A18 (PG 3) by R_2 / r_2, and isolate PL89 - 21 (PG 3) by R_3 / r_3, and assuming the independent assortment of the resistance genes, the F_2 populations from the Quinta x Westar crosses may be analyzed for segregation of the resistance genes by taking a pair of genes at a time. Contrary to what would be expected if two unlinked dominant genes determined resistance to *L. maculans* in Quinta, segregation of the F_2 populations from the Quinta x Westar cross was highly significant ($p < 0.0001$) for a 9:3:3:1 ratio. Distorted segregation ratios can, however, result from either the departure of phenotypes, from a 3:1 segregation ratio due to the R/r alleles, or the departure from independent assortment due to linkage of the resistance genes. Chi-square tests for segregation at each resistance gene were non-significant [resistance (R_1

/ r_1) to PG 2 (1130 - B24), $X^2 = 0.0360$; (R_2 / r_2) to PG 3 (1130 - A18), $X^2 = 0.899$; (R_3 / r_3) to PG 3 (PL89 - 21), $X^2 = 0.182$] for a 3:1 segregation ratio. This indicated that the departure of the Quinta x Westar F_2 populations from a 9:3:3:1 genetic model was not due to the relative scarcity of certain phenotypes as determined by the respective resistance alleles. Further, the independence tests for linkage were highly significant ([(R_1) to PG 2 (1130 - B24) / (R_2) to PG 3 (1130 - A18), X^2 = 1,564.00; (R_1) to PG 2 (1130 - B24) / (R_3) to PG 3 (PL89 - 21), X^2 = 1,171.58, and (R_2) to PG 3 (1130 - A18) / (R_3) to PG 3 (PL89 - 21), X^2 = 1,304.96, thus confirming that the departure of the Quinta x Westar F_2 populations from a 9:3:3:1 segregation ratio was due to linkage. A test for linkage intensity indicated that resistance gene (R_1) to the PG 2 isolate (1130 B24) was tightly linked to the resistance genes (R_2 and R_3) conferring resistance to the PG 3 isolates (1130 - A18 and PL89 - 21), with recombination frequencies of 6.96% and 9.49% respectively. Surprisingly, the resistance genes (R_2 and R_3) to the PG 3 isolates (1130 - A18 and PL89 - 21) are two separate genes which are tightly linked and have a recombination frequency of 16.44%. The order of these genes is (R_2) - (R_1) - (R_3). This implies that different genes conferring resistance to isolates of *L. maculans* of the same PG may be uncovered when individual plants of segregating populations are inoculated with different isolates of the same PG.

Ansan-Melayah et al. (1998) reported that two genes determine resistance in Quinta which are matched by two avirulence genes in PG 2 isolates. Their data using PG 2 isolates did not support the conclusion that two genes conferred

avirulence of these isolates on Quinta. Ansan-Melayah et al. (1998) tested generations from the Score x Quinta crosses with six PG 2 progeny isolates from crosses of the PG 2 (PHW1245) x the PG 4 (1.2.4). Segregation for resistance to the PG 2 isolates in Quinta was significant for a one or two gene model. They suggested that the distorted segregation ratio was due to linkage. The significant Chi-square values could also be due to pooling of the data from the six different PG 2 progeny isolates. It appears that Ansan-Melayah et al. (1998) did not consistently test more than one isolate on each plant of each generation. Results from the present study (Chapter 3) indicated that the PG 2 isolates are comprised of various genotypes. Segregation of F_2 populations from crosses of Quinta x Westar to the PG 2 (5174/11) isolate neither fit a one gene model nor a two gene model. The distorted segregation ratios were possibly due to the linkage of genes conferring resistance to PG 2 and PG 3 isolates. Moreover, the interactions between the PG 2 (5174/11) isolate and F_2 populations of Quinta x Westar crosses suggest that the gene which determines resistance to the PG 2 (5174/11) isolate is not the same gene as that which confers resistance to the PG 2 (1130 - B24) isolate of *L. maculans*. Linkage analysis suggested that these two genes are independent. It is also worth noting that reactions to the PG 2 isolate (1130 - B24) are controlled by a dominant gene whereas the reactions to the PG 2 isolate (5174/11) are controlled by one or two recessive genes. Plummer & Howlett (1993) reported variability in karyotypes of *L. maculans* for both field isolates and

isolates from crosses. Chromosome polymorphism may cause some of the discrepancies observed among PG 2 isolates 1130 - B24 and 5174/11

At least three genes for resistance to *L. maculans* were identified in the *B. napus* differential cultivar Quinta. Two independent loci confer resistance in Quinta to PG 2 isolates of *L. maculans* which would be matched by two independent avirulence loci in the pathogen. The present study also showed that two genes determine resistance in Quinta to two PG 3 isolates of *L. maculans* which would be matched by two avirulence genes in the pathogen. This would therefore require at least a three gene model to explain the gene-for-gene interaction in this pathosystem and hence supports the second hypothesis of Rimmer and van den Berg (1992). For the sake of simplicity, the first hypothesis of Rimmer and van den Berg (1992) is preferred.

If the genotypes R_1 R_1 , R_2 R_2 and R_3 R_3 represent the resistance genes in Quinta, the resistance genes in Glacier would be represented by either R_1 R_1 or R_2 R_2. The matching avirulence genotypes in *L. maculans* would be A_1, A_2 and A_3. Quinta and Glacier are likely to have one gene in common which could either be R_1 R_1 or R_2 R_2. A different gene determines resistance to the PG 2 (1130 - B24) isolate and at least another different gene determines resistance to the PG 2 (5174/11) isolate. However the nature of interactions between the PG 2 (5174/11) isolate and Quinta is not clear. Evidence that the genotype of 5174/11 is A_1a_2 a_3 may be derived from the fact that the F_1. segregation was close to a 1:1 ratio.

Single genes determine resistance to each of PG 3 (1130 - A18) and PG 3 (PL89 - 21) in Quinta. That these two resistance genes are tightly linked to each other and to the gene conferring resistance to the PG 2 (1130 - B24) isolate, has been demonstrated. Ansan-Melayah et al. (1998) reported that a single gene determined resistance to PG 3 in Quinta and this was matched by a single avirulence gene in PG 3 isolates. They tested Score and Quinta parents and the F_1 from Score x Quinta crosses with the PG 3 (a.2 and 11.26.11) but the F_2 generation was tested with only the isolate (11.26.11).

To better answer the questions raised in this paper, it is necessary to first study the genetics of resistance to isolate 5174/11 in Glacier and also to identify the allelic relationships between the resistance genes in Quinta and Glacier using the progeny of *L. maculans* isolates above. Precise determination of the map distance between the identified resistance genes may be obtained if genotypes, homozygous for one gene but heterozygous for the other, are identified from the F_2 populations and selfed to obtain F_3 populations. These may then be inoculated with various isolates and scored to determine the genotype of each F_2 plant to be used in the mapping of the resistance genes.

Table 6.1. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with four progeny isolates from crosses of *Leptosphaeria maculans*.

Cross	Isolate Number and Pathogenicity group (PG)				Number of plants
	1130 - B24 PG 2	5174/11 PG 2	1130 - A18 PG 3	PL89 - 21 PG 3	
	Interaction phenotype rating [1]				
W1QA	2.4 ± 0.25	6.1 ± 0.70	1.4 ± 0.25	2.3 ± 0.00	9
W2QB	3.1 ± 0.10	4.0 ± 1.19	2.7 ± 0.30	2.7 ± 0.18	10
W3QC	2.3 ± 0.38	5.7 ± 0.96	3.1 ± 0.31	3.3 ± 0.17	9
W4QD	2.6 ± 0.23	3.3 ± 1.47	2.5 ± 0.30	3.0 ± 0.21	11
W5QE	3.3 ± 0.25	3.0 ± 0.58	3.0 ± 0.25	3.3 ± 0.33	4
QAW1	3.3 ± 0.33	3.6 ± 1.00	2.0 ± 0.45	2.9 ± 1.00	9
QBW2	2.9 ± 0.39	2.3 ± 0.63	3.0 ± 0.35	3.3 ± 0.45	13
QCW3	2.4 ± 0.30	4.3 ± 0.64	2.4 ± 0.29	3.3 ± 0.17	17
QDW4	2.8 ± 0.30	3.6 ± 0.60	1.9 ± 0.29	2.7 ± 0.21	14
QEW5	3.3 ± 0.18	2.5 ± 0.72	1.9 ± 0.34	3.2 ± 0.22	16

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Table 6.2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar x Quinta crosses and their reciprocals following cotyledon inoculation with the PG 2 isolate 5174/11 of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									Total
		0	1	3	4	5	6	7	8	9	Plants
W1QA	6.1 ± 0.70	2			1			1	1	4	9
W2QB	4.0 ± 1.19	3	1	1	1		1		1	2	10
W3QC	5.7 ± 0.96	1		2	1		1		1	3	9
W4QD	3.3 ± 1.47	4	3						3	1	11
W5QE	3.0 ± 0.58	1		1	1	1					4
QAW1	3.6 ± 1.00	5				1				3	9
QBW2	2.3 ± 0.63	8			1		3		1		13
QCW3	4.3 ± 0.64	4	2		3	3	3		2	2	19
QDW4	3.6 ± 0.60	4	1		3		5		1		14
QEW5	2.5 ± 0.72	8	1	1			6				20

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Table 6.3. Cross, interaction phenotype, genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with the PG 2 isolate 1130B - 24 of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	898	300	1198	3:1	4.559	
Pooled				3:1	0.000	> 0.95
Homogeneity (df=4)				3:1	4.559	0.30-0.50
Quinta x Westar						
Total plants	907	290	1197	3:1	7.293	
Pooled				3:1	0.381	0.50-0.70
Homogeneity (df=4)				3:1	6.912	0.10-0.20
Westar x Quinta Combined						
Total plants	1805	590	2395	3:1	11.852	
Pooled				3:1	0.171	0.50-0.70
Homogeneity (df=9)				3:1	11.681	0.20-0.30

Table 6.4. Cross interaction phenotype, genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with the PG 2 isolate 5174/11 of *Leptosphaeria maculans*

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	412	785	1197	5:11	10.257	
Pooled				5:11	5.597**	0.01-0.05
Homogeneity (df=4)				5:11	4.660	0.30-0.50
Quinta x Westar						
Total plants	426	771	1197	5:11	33.756	
Pooled				5:11	11.037**	< 0.001
Homogeneity (df=4)				5:11	22.719**	< 0.001
Westar x Quinta Combined						
Total plants	838	1556	2394	5:11	44.013	
Pooled				5:11	15.705**	< 0.001
Homogeneity (df=9)				5:11	28.308**	< 0.001

** = significant at the 0.01 probability level.

Table 6.5. Cross interaction phenotype, genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with the PG 3 isolate 1130A - 18 of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	899	297	1196	3:1	8.822	
Pooled				3:1	0.018	0.70-0.90
Homogeneity (df=4)				3:1	8.804	0.05-0.10
Quinta x Westar						
Total plants	902	297	1199	3:1	13.990	
Pooled				3:1	0.034	0.70-0.90
Homogeneity (df=4)				3:1	13.956**	< 0.001
Total plants	751	219	970	3:1	3.962	
Pooled #				3:1	3.036	0.05-0.10
Homogeneity (df=3)				3:1	0.926	0.70-0.90
Westar xQuinta Combined						
Total plants	1801	594	2395	3:1	22.812	
Pooled				3:1	0.050	0.70-0.90
Homogeneity (df=9)				3:1	22.762*	0.001-0.01

Cross QDW4 was excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 6.6. Cross, interaction phenotype, genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with the PG 3 isolate PL89 - 21 of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	866	332	1198	3:1	6.757	
Pooled				3:1	4.702*	0.01-0.05
Homogeneity (df=4)				3:1	2.055	0.70-0.90
Quinta x Westar						
Total plants	901	292	1193	3:1	9.177	
Pooled				3:1	0.175	0.50-0.70
Homogeneity (df=4)				3:1	9.161	0.05-0.10
Westar x Quinta Combined						
Total plants	1767	624	2391	3:1	15.934	
Pooled				3:1	1.537	0.50-0.70
Homogeneity (df=9)				3:1	14.397	0.05-0.10

* = significant at the 0.05 probability level.

CHAPTER 7

GENERAL DISCUSSION

In Canada, breeding for resistance to *L. maculans* in *B. napus* has resulted in the release of cultivars with moderate to high levels of resistance. If widely grown these resistant cultivars may provide an opportunity for the selection of new specific virulence types in *L. maculans* populations (Mayerhofer et al. 1997, Petrie 1995). To control blackleg effectively, it is important to understand the structure of genetic variability for virulence in the pathogen population as well as the genetic mechanisms of resistance in host cultivars.

An important aspect in the determination of virulence composition of a pathogen on a given crop species is obviously the presence of resistance genes in the commercial crop cultivars or species. Badawy et al. (1991), Ballinger et al. (1991), Kuswinanti et al. (1995), Kutcher et al. (1993) tested different *Brassica* genotypes belonging to different species with different *L. maculans* genotypes. In this study, emphasis is placed on *B. napus* (nine cultivars or accessions) to differentiate isolates of *L. maculans* originating from different geographic regions (Australia, Canada, and Europe). At least seven PGs of *L. maculans* were identified using *B. napus* accessions Westar, Glacier and Quinta. Isolates of *L. maculans* in Canada are predominantly PG 1, PG 2 or PG 4 based on the IP elicited on Westar, Glacier and Quinta. Isolates of *L. maculans* from Ontario were PG 1 or PG 4, whereas isolates of *L. maculans* from western Canada were predominantly PG 1 or PG 2. Nearly all the virulent isolates of *L. maculans* in western Canada, were PG 2.

Similar results were reported by Mengistu et al. (1991), Rimmer and van den Berg (1992), Kutcher et al. (1993). Most isolates of *L. maculans* from Manitoba and Saskatchewan elicited a similar pattern of virulence on the differential host genotypes suggesting that both Manitoba and Saskatchewan isolates of *L. maculans* have a common origin. The pathogen's introduction into these regions is likely via infested seed; consequently it is necessary to monitor seed introduced from Ontario into western Canada for isolates of *L. maculans* endemic to Ontario.

Isolates of *L. maculans* from Ontario were predominantly PG 4 whereas isolates from Australia and Europe were predominantly PG 3 and PG 4. Since no PG 4 isolates of *L. maculans* were identified in western Canada, the origin of the disease initiating isolates of *L. maculans* in western Canada and Ontario must be different. Virulent types (PG 3 and PG 4) were common to Australia, Europe and Ontario, (Canada). Australia and Europe are separated from Canada by a large body of water, hence the likely source of inoculum between these regions is via imported seed or infested crop residue. It is likely that Ontario isolates of *L. maculans* originated from Europe, considering that the source of some canola seed in Ontario is European. In Canada, identification of aggressive isolates of *L. maculans* was first reported in 1975 in Saskatchewan and in 1980 in Manitoba. Resistance genes in *B. napus* cultivars were introduced much earlier in Australia and Europe as compared to their introduction in *B. napus* cultivars in Canada. This could have resulted in the observed variation on the pathogen over time in Australia and Europe.

Certain virulent genotypes occurred at very low frequencies, indicating that some virulence genes and/or gene combinations may be present at very low frequencies in western Canada. The small sample size from each region and the limited area of sampling within the various geographic regions, may be one reason for this observation. It may be necessary to undertake detailed studies in using representative sample isolates from these each geographic region, if a thorough understanding of the pathogens' genetic structure is to be made. Isolates of *L. maculans* which were virulent on more than one host differential genotype were also identified. Similar findings are discernable in the reports of Ballinger et al. (1991), Kutcher et al. (1993) and Kuswinanti et al. (1995). The ability of some isolates of *L. maculans* for virulence on more than one differential host genotype indicates that combined virulence occurs. Based on the reactions of all the differential genotypes employed more than one gene, conferring resistance to genotypes of *L. maculans*, occurs. Also, the present study suggests that different virulence genes and/or combinations of virulence genes exist in *L. maculans* populations of isolates. Moreover, sexual recombination within *L. maculans* has been demonstrated (Rimmer and van den Berg 1992). Therefore sexual recombination in isolates may alter the frequencies of certain virulence genes in the different populations of *L. maculans*.

Various researchers, studying host-pathogen specificity in the *Brassica* - *L. maculans* pathosystem, have used different cutoff points to define resistance and susceptibility as well as different genotypes of *L. maculans* and diverse *Brassica*

species and genotypes. Only a limited and cautious comparison of the data from the present study is posssible with the data of Ballinger et al. (1991) and Kuswinanti et al. (1995). Based on IP of the *B. napus* differential accessions, PG 2 isolates of *L. maculans* could further be differentiated into 15 sub-groups. Ballinger et al. (1991), Kutcher et al. (1993) and Kuswinanti et al. (1995) also identified more subgroups among PG 3 and PG 4 isolates, when *B. juncea* genotypes and other *Brassica* sp. were included in the differential sets. Kuswinanti et al. (1995) categorized aggressive isolates of *L. maculans* into four PGs using *B. napus* genotypes. They identified 10 isolates of *L. maculans* belonging to PG 1. Only three isolates were found to be PG 2 and these consisted of isolates from France. No PG 2 isolates were identified among isolates from Germany. The remaining isolates were either PG 3 or PG 4. Kuswinanti et al. (1995) further differentiated PG 3 and PG 4 isolates of *L. maculans* into 16 sub groups. On the other hand, Ballinger et al. (1991), also using *B. napus* genotypes (Chisaya, Rafal and RX3), identified 14 races of *L. maculans*. These subgroups fall into five major groups. Ballinger et al. (1991) did not identify any non aggressive isolates. However, they did not include the cultivars Westar, Glacier and Quinta in their differential set, thus no comparison of their results with those of other researchers can be made.

This study suggests that the number of PG identified in *L. maculans* will depend on many factors, including the number of resistance genes in host plants that are differential to various genotypes of *L. maculans,* the gene action of the

resistance genes involved, as well as the linkage and epistatic relationships of the resistance genes. Different genes may confer resistance to the same genotype of *L. maculans.* Moreover, it is desirable to diversify resistance genes incorporated into new commercial cultivars. It is desirable to develop single gene differential lines in order to characterize individual resistance genes. This will also facilitate a better understanding of the virulence composition of *L. maculans* populations and the use of various disease control strategies. One strategy is to combine three or more resistance genes in a cultivar so as to reduce the chances of the pathogen evolving virulence to a single specific resistance gene, and to increase the range of resistance within the host genotype to different isolates. Another control strategy is to deploy mixtures of cultivars possessing different resistance genes to check the development of highly virulent genotypes. The findings reported here will enable breeders to keep ahead of new races of the pathogen which may develop as a result of the release and widespread use of resistant genes in commercial cultivars.

Characterization of isolates of *L. maculans,* on the basis of DNA polymorphism, will complement the knowledge obtained from host-pathogen interaction studies. The RAPD data herein suggests that RAPD analysis can satisfactorily differentiate and identify local differences in virulence genotypes among isolates of *L. maculans.* In present study, 14 of 28 primers were useful in differentiating isolates of *L. maculans.* Ansan-Melayah et al. (1995) also characterized PG 3 and PG 4 isolates of *L. maculans* based on their RAPD profiles. Further isolates of *L.*

maculans belonging to different PGs were discriminated into two distinct groups based on DNA polymorphism. PG 2 isolates of were homogeneous, and hence may reflect an isolated genetic pool. One isolate was identified as a subset of PG 2. This isolate was later identified as different from PG 2 on the basis of host pathogen interaction and was designated PG 2-1. The PG 3 and PG 4 isolates of *L. maculans* were more heterogeneous, hence genetic recombination between these different genotypes may result in the pathogen overcoming the present resistant cultivars (Petrie 1995, Mayerhofer et al. 1997).

Taylor (1993) reported a simple method to detect aggressive isolates of *L. maculans* in contaminated seed of oilseed rape. Considering that the homology of migrating DNA bands can not be ascertained, the development of specific primers from RAPD markers would be useful for a more accurate characterization of this pathogen, and hence improve the precision for the identification of the aggressive isolates of *L. maculans* in infested seed.

The differentiation of field isolates of *L. maculans* on the basis of host - pathogen interaction requires that isolates be axenically cultured from infested host tissue. This can be time consuming and laborious, requiring controlled environments. Moreover only a limited number of field isolates can be handled this way. In contrast RAPD analysis is simple to use, cost effective and can handle hundreds of samples per day. However, RAPD analysis is prone to errors. Identical temperature profile settings in different PCR machines may not yield the

same amplicons. Also, changes in the concentration of magnesium ions can result in unreliable fingerprints.

Research on the genetics of resistance in *B. napus* cultivars suggests that cotyledon resistance to *L. maculans* is controlled by a single recessive gene (Sawatsky 1989) or by two dominant linked genes (Delwiche 1980). Cargeeg & Thurling (1980a) and Pang & Halloran, (1996b) suggested that resistance to *L. maculans* was polygenic. Limited research has been done on the genetics of resistance in the *B. napus* differential cultivars Westar, Glacier and Quinta. In this study a single dominant gene determines resistance in Glacier to a PG 2 isolate while resistance to the same PG 2 isolate in Quinta is controlled by two nuclear genes, compatible with a 9:7 genetic model of resistant to susceptible plants. The data also suggests that, depending on the original parent population, resistance to the PG 2 isolate of *L. maculans* may be determined by one or two genes. Since some F_2 populations of Quinta x Westar crosses segregated for only one locus of resistance to the PG 2 isolate, the original seed lot or seed source was probably heterogeneous.

A single dominant gene conferred resistance to the PG 3 isolate of *L. maculans* in the differential cultivar Quinta. Similar results were obtained when progeny from Quinta x Glacier were challenged with PG 3 isolates, confirming that one dominant gene determined resistance to PG 3 isolates in Quinta. In all, at least four genes for resistance to *L. maculans* were identified in the *B.napus* differential cultivars Quinta and Glacier. The *B. napus* cultivar Quinta has at least four resistance

genes, while Glacier has at least two genes for resistance to isolates of *L. maculans*.

Rimmer and van den Berg (1992), using the cultivars Westar, Glacier and Quinta as differential host genotypes to study isolates of *L. maculans*, suggested that a gene-for-gene system is likely involved in the *Brassica* - *L. maculans* pathosystem and that one gene determined resistance to PG 2 isolates in Glacier; that two genes conferred resistance to PG 2 isolates in Quinta and that Glacier had one gene for resistance to PG 2 isolates in common with Quinta. The present study, using the same differential host cultivars, supports the hypotheses of Rimmer and van den Berg (1992). Rimmer and van den Berg (1992) further postulated that at least two avirulence genotypes occur among PG 2 isolates. A single nuclear gene conferred resistance in Quinta to the PG 2 isolate (1130-B24). However, the resistance in Quinta to the PG 2 isolate (5174/11) neither fit a one gene model nor a two gene model. Moreover, PG 2 isolate (5174/11) behaves differently from PG 2 isolates (1130-B24 and (PL86-12). The data suggest that the gene determining resistance to the PG 2 (5174/11) isolate is not allelic to the gene conferring resistance to the PG 2 (1130 - B24) isolate and that the PG 2 isolates may comprise various genotypes. Further tests are required in order to determine the relationship of these genes.

A single dominant nuclear gene determines resistance in Quinta to the PG 3 (1130 - A18) and another dominant nuclear gene confers resistance to the PG 3 isolate (PL89 - 21). Linkage analysis indicates that the resistance to the two PG 3

isolates (1130 - A18 and PL89 - 21) is determined by two tightly linked genes. To confirm this, there is need to study the genetics of resistance in Glacier to the PG 3 isolates (1130 - A18 and PL89 - 21), in order to determine the relationships of the resistance genes using the four progeny isolates above.

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Appendix A. Isolates, interaction phenotypes (R=resistant, I= intermediate, S=susceptible), on eight *Brassica* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculations with isolates of *Leptosphaeria maculans* from different geographical regions.

	Brassica Differential Host Genotypes										
Isolate	West	Glac	Quin	Sent	Val-1	Quan	Spri	Dac-1	Dac-2	3466	3467
	Interaction phenotypes										
PL84-9	R	R	R	.	.	.		.	.	.	.
PL87-31A	R	R	R	.	.	.	.	.	.	.	.
BR84-FC64	R	R	R	.	.	.	.	.	.	.	.
OS84-49A	R	R	R	.	.	.	.	.	.	R	R
PL84-11	R	R	R	R	R	R	R	.	.	.	.
PL84-8	R	R	R	.	S	R	R	S	.	.	.
PL84-10	R	R	R	S	R	R	S	S	.	.	.
Unity	R	R	R	S	S	R	R	R	R	R	R
PL87-57	R	R	R	S	S	S	S	.	R	.	.
2379	R	R	R	S	.	S	S	S	I	.	.
2382	R	R	R	R	R	R	I	R	R	.	.
LM6	R	R	R	R	R	R	R	R	.	.	.
AB 97-16	R	R	R	R	R	R	R	R	R	.	.
AB 97-14	.	.	.	.	R	.	R	R	.	.	.
88-28A	R	R	R	.	.	.	.	.	.	I	I
88-23	R	R	R	.	.	.	.	.	.	I	I
LM5	R	R	S	R	R	R	R	.	R	.	.
LMS 89-54	R	S	I	R	R	R	R	.	R	.	.
PL84-13	R	I	I	R	R	R	R	.	.	.	.
PL87-54	R	I	I	R	R	R	R	R	.	.	.
OS84-53B	R	I	I	.	.	.	.	.	.	I	.
PL87-50	R	R	I	.	.	.	.	.	.	.	.
PL87-52	R	R	I	.	.	.	.	.	.	.	.
PL87-55	R	R	I	.	.	.	.	.	.	.	.
92-14-3	R	R	I	.	.	.	R	.	R	.	.
OS84-47	R	R	I	.	.	.	.	.	.	R	
BL4	R	R	I	.	.	.	.	.	.	I	I
PL87-27	R	R	I	.	.	.	.	.	.	.	.
LMS 89-12	R	R	I	R	R	R	R	S	R	.	.
OS84-53A	R	R	I	R	R	I	R	R	.	I	I
92-30-1	R	R	I	I	R	R	I	R	.	R	I
92-22-1	R	R	I	I	R	I	R	R	.	R	I

NB: duplicated Isolates are different single isolates originating from same culture.

Appendix A (cont.). Isolates, interaction phenotypes (R=resistant, I=intermediate, S=susceptible, .=not tested), on eight *Brassica* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculations with isolates of *Leptosphaeria maculans* from different geographical regions.

	Brassica Differential Host Genotypes										
Isolate	West	Glac	Quin	Sent	Val-1	Quan	Spri	Dac-1	Dac-2	3466	3467
	Interaction phenotypes										
WV151	R	I	R	I	R	R	R	I	.	R	I
2383	.	.	.	.	.	R	.	.	I	.	.
LMS 89-26	R	I	I	.	.	.	S	S	.	.	.
88-28B	R	I	I	.	.	.	.	.	.	.	.
PL87-29	R	I	I	.	.	.	.	.	.	.	.
92-06-3	R	I	I	.	R	R	R	.	.	I	S
PL87-24	R	I	I	R	R	.	R	R	.	.	.
LMA 89-79	R	I	I	R	R	R	R	S	.	.	.
LMA 89-79	R	I	I	R	R	R	R	S	.	.	.
LMS 89-24	R	I	I	R	R	R	S	.	R	.	.
LMM 89-11	R	I	I	R	I	R	S	S	R	.	.
PL87-7	S	R	R	.	.	.	.	.	.	.	.
PL87-17	S	R	R	.	.	.	.	.	.	.	.
PL89-12	S	R	R	.	.	.	.	.	.	.	.
PL89-13	S	R	R	.	.	.	.	.	.	.	.
PL89-29	S	R	R	.		.	.	.	.	.	.
PL86-6	S	R	R	R	R	R	R	.	.	.	.
PL85-2	S	R	R	R	R	R	R	R	.		
2358	S	R	R	R	R	R	I	S	R	.	
89-23	S	R	R	R	S	R	S		.	R	I
PL89-28	S	R	R	S	S	R	R	R	R	.	.
PL87-10	S	R	R	I	.	R	R	.	.	.	.
PL86-27	S	R	R	I	R	R	R	S	R	.	.
PL85-9	S	R	R	I	S	R	R	R	.	.	.
PL86-14	S	R	R	I	S	R	R	I	R	.	.
PL85-10	S	R	R	I	S	R	I	S	S	.	.
PL86-19	S	R	R	I	S	R	S	.	.	.	.
PL87-5	S	R	R	I	.	I	R	.	.	.	.
PL87-41	S	R	R	S	R	S	S	S	R	.	.
PL89-3	S	R	R	S	S	R	I	.	.	.	.
PL86-4	S	R	R	S	S	R	R	.	.	.	.
PL89-16	S	R	R	S	S	R	R	.	.	.	.

NB: duplicated Isolates are different single isolates originating from same culture.

Appendix A (cont.). Isolates, interaction phenotypes (R=resistant, I=intermediate, S=susceptible, .=not tested), on eight *Brassica* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculations with isolates of *Leptosphaeria maculans* from different geographical regions.

	Brassica Differential Host Genotypes										
Isolate	West	Glac	Quin	Sent	Val-1	Quan	Spri	Dac-1	Dac-2	3466	3467
	Interaction phenotypes										
PL86-12	S	R	R	S	S	R	R	R	R	.	.
PL89-22	S	R	R	R	S	R	R	.	R	.	.
PL89-23	S	R	R	R	S	R	R	.	R	.	.
PL89-24	S	R	R	.	.	R	.	.	.	.	.
SA10G	S	R	R	S	S	R	R	S	.	.	.
PL88-5	S	R	R	S	S	R	I	S	R	.	.
PL89-1	S	R	R	S	S	R	I	.	.	.	
767	S	R	R	S	S	R	S	I	.	R	S
PL89-2	S	R	R	S	S	I	S	S	.	.	.
PL89-15	S	R	R	S	S	S	S	S	.	.	.
PL89-20	S	R	R	S	S	S	S	S	.	.	.
LMA 89-64	S	R	I	.	.	.	.	.	.	.	.
PL85-7	S	R	I	.	.	.	R	.	.	.	.
LMA 89-81	S	R	I	.	.	.	.	S	.	.	.
LMA 89-81	S	R	I	I	S	R	S	S	.	.	.
562B	S	R	I	S	.	R	R	.	.	R	S
PL87-15	S	R	I	S	S	S	S	R	.	.	.
672	S	R	I	R	S	R	S	S	S	R	S
683	S	R	I	R	S	I	S	R	S	R	I
567	S	R	I	I	S	R	S	.	S	.	.
661	S	R	I	I	S	R	S	S	S	R	S
677	S	R	I	I	S	R	S	S	I	R	S
785A	S	R	I	S	S	R	S	S	S	R	S
785B	S	R	I	S	S	R	R	S	.	R	S
821	S	R	I	S	S	R	R	S	.	R	S
751	S	R	I	S	S	R	S	S	S	I	S
SK 97-7	S	R	I	S	S	R	S	R	R	.	.
SK 97-8	S	R	I	S	S	R	S	R	R	.	.
AB 97-13	S	R	R	S	S	S	I	S	R	.	.
AB 97-15	S	R	R	S	S	S	R	S	.	.	.
AB 97-19	S	R	R	S	S	S	S	R	.	.	.
AB 97-17	S	R	R	S	S	.	.	.	.	.	.

NB: duplicated Isolates are different single isolates originating from same culture.

Appendix A (cont.). Isolates, interaction phenotypes (R=resistant, I=intermediate, S=susceptible, .=not tested), on eight *Brassica* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculations with isolates of *Leptosphaeria maculans* from different geographical regions.

	Brassica Differential Host Genotypes										
Isolate	West	Glac	Quin	Sent	Val-1	Quan	Spri	Dac-1	Dac-2	3466	3467
	Interaction phenotypes										
SK 97-9	S	R	I	S	S	R	R	S	R	.	.
840	S	R	I	S	S	R	S	S	.	.	.
SK 97-10	S	R	I	S	S	S	S	R	R	.	.
SK 97-12	S	R	I	S	S	S	S	S	I	.	.
2367	S	R	S	S	R	S	S	S	.	.	.
656	.	.	.	.	.	.	R	S	R	.	.
S562A	S	I	R	.	.	.	.	.	.	R	.
659	S	I	R	R	S	R	S	S	S	R	S
766	S	I	R	I	S	I	S	S	S	R	.
602	S	I	R	S	S	R	S	R	S	R	S
PL86-21	S	I	R	S	S	R	S	S	.	.	.
PL86-9	S	I	I	.	.	.	.	.	.	.	.
LMS 89-53	S	I	I	.	.	.	.	.	.	.	.
676B	S	I	I	.	.	.	.	.	.	R	R
LMS 89-19	S	I	I	R	R	R	R	.	R	.	.
778	S	I	I	S	R	R	S	S	I	R	S
UNITY(x2)	S	I	I	S	S	R	R	.	R	.	.
742	S	I	I	I	S	R	S	S	S	R	S
676A	S	I	I	S	S	R	S	S	.	I	S
LMS 89-60	S	I	I	S	S	S	R	.	.	.	.
2379(x2)	S	.	.	S	.	S	S	.	.	.	.
PL89-18	S	S	R	I	S	S	S	S	S	.	.
WA32	S	S	R	I	S	S	S	S	S	.	.
PL89-19	S	S	R	S	.	R	S	S	.	.	.
PL88-16	S	S	R	S	S	S	S	.	.	.	.
Ngerm	S	S	R	S	S	S	S	S	S	.	.
Rennes3	S	S	R	S	S	S	S	S	S	.	.
Rennes4	S	S	R	S	S	S	S	S	S	.	.
Stockoy1	S	S	R	S	S	S	S	S	S	.	.
Stockoy2	S	S	R	S	S	S	S	S	S	.	.
Stockoy3	S	S	R	S	S	S	S	S	S	.	.
Lifolle6	S	S	R	S	S	S	S	S	.	.	.

NB: duplicated Isolates are different single isolates originating from same culture.

Appendix A (cont.). Isolates, interaction phenotypes (R=resistant, I=intermediate, S=susceptible, .=not tested), on eight *Brassica* differential genotypes (West=Westar, Glac=Glacier, Quin=Quinta, Sent=Sentry, Val-1, Quan=Quantum, Spri=Sprint, and Dac-1) following cotyledon inoculations with isolates of *Leptosphaeria maculans* from different geographical regions.

	Brassica Differential Host Genotypes										
Isolate	West	Glac	Quin	Sent	Val-1	Quan	Spri	Dac-1	Dac-2	3466	3467
	Interaction phenotypes										
PL89-19	S	S	R	S	.	S	S	S	S	.	.
PL89-21	S	S	R	S	S	S	S	S	I	.	.
LMM 89-9	S	S	I	.	.	.	.	.	.	.	.
831	S	S	I	S	S	S	I	S	S	R	S
2353	S	S	S	S	.	.	.	.	.	.	.
2355	S	S	S	.	.	.	.	.	.	.	.
2357	S	S	S	.	.	.	.	.	.	.	.
2361	S	S	S	.	.	.		.	.	.	.
2362	S	S	S	.	.	.	.	.	.	.	.
2368	S	S	S	.	.	.	.	.	.	.	.
2372	S	S	S	.	.	.	.	.	.	.	.
2363	S	S	S	S	R	S	S	S	.	.	.
PL87-1	S	S	S	S	R	S	S	S	.	.	.
2365	S	S	S	S	R	S	S	S	.	.	.
WA74	S	S	S	.	S	.	R	S	.	.	.
WA51	S	S	S	S	S	S	S	S	.	.	.
2354	S	S	S	S	S	S	S	S	.	.	.
Bamberg1	S	S	S	S	S	S	S	S	.	.	.
Bamberg2	S	S	S	S	S	S	S	S	.	.	.
Bamberg3	S	S	S	S	S	S	S	S	.	.	.
Bamberg4	S	S	S	S	S	S	S	S	R	.	.
Lifolle1	S	S	S	S	S	S	S	S	R	.	.
Lifolle3	S	S	S	S	S	S	S	S	R	.	.
Lifolle2	S	S	S	S	S	S	S	S	S	.	.
Lifolle5	S	S	S	S	S	S	S	S	S	.	.
LMS 89-21	S	S	S	I	R	R	S	S	S	.	.
LMA 89-10	S	S	S	I	S	R	S	R	S	.	.
PL89-27	S	S	S	I	S	R	S	S	R	.	.

NB: duplicated Isolates are different single isolates originating from same culture.

Appendix B. 1. Mean interaction phenotype of parental plants of *Brassica napus* cultivars Westar (W), Quinta (Q) and Glacier (G) following cotyledon inoculation with four isolates of *Leptosphaeria maculans* from the four pathogenicity groups.

	Isolate Number and Pathogenicity group (PG)				
Parent Number	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
	Interaction phenotype rating [1]				
W1	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	21
W2	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W3	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W4	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	24
W5	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W6	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	14
W7	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	20
QA	3.1±0.12	2.6±0.12	3.3±0.10	8.4±0.25	33
QB	2.6±0.13	2.8±0.15	3.4±0.11	8.9±0.11	32
QC	3.2±0.06	2.8±0.15	4.0±0.09	9.0±0.00	28
QD	3.2±0.11	3.5±0.12	3.9±0.09	8.9±0.20	26
QE	3.2±0.08	1.9±0.14	3.6±0.09	8.6±0.15	24
QF	3.0±0.08	2.5±0.14	3.4±0.08	8.3±0.17	17
G1	1.7±0.17	1.6±0.16	9.0±0.00	9.0±0.00	34
G2	2.1±0.20	1.8±0.19	9.0±0.00	3.8±0.27	28
G3	1.4±0.14	1.5±0.16	9.0±0.00	9.0±0.00	38
G4	1.0±0.00	1.2±0.20	9.0±0.00	9.0±0.00	22
G5	1.1±0.06	1.1±0.09	7.9±0.17	8.8±0.09	33
G6	2.0±0.19	3.1±0.21	9.0±0.00	9.0±0.00	30

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Appendix B. 2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with the PG 2-1 isolate (PL87-41) of *Leptosphaeria maculans*.

		IP Frequency distribution [1]							
Cross	Mean IP rating [2]	0	1	3	4	5	6	8	Total plants
W1QA	2.8 ± 0.15		3	20	2				25
W2QB	3.1 ± 0.11		5	33	14				52
W3QC	2.8 ± 0.13		8	33	8				49
W4QD	3.4 ± 0.07	1		26	25				52
W5QE	3.1 ± 0.18	1	7	11	22				41
W6QF	3.0 ± 0.34		10	3	14	1		1	30
QAW1	3.1 ± 0.09		3	38	12				53
QBW2	3.4 ± 0.18		3	29	8		6		46
QCW3	2.6 ± 0.14		15	32	9				56
QDW4	2.3 ± 0.15		26	28	2	1			57
QEW5	1.9 ± 0.16		27	17	3				47
QFW6	2.1 ± 0.14		30	25	1				56

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 3. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with PL87-47 (PG 2-1) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	87	28	115	3:1	0.026	0.70-0.90
W2QB	85	27	112	3:1	0.048	0.70-0.90
W3QC	96	25	121	3:1	1.215	0.20-0.30
W4QD	85	39	124	3:1	2.727	0.05-0.10
W5QE	123	34	157	3:1	0.766	0.30-0.50
W6QF	111	43	154	3:1	0.554	0.30-0.50
Total plants	587	196	783		5.336	
Pooled				3:1	0.000	> 0.95
Hom (df = 5)				3:1	5.336	0.30-0.50
QAW1	103	18	121	3:1	6.614*	0.01-0.05
QBW2	131	8	139	3:1	27.456**	< 0.001
QCW3	136	37	173	3:1	1.019	0.30-0.50
QDW4	136	29	165	3:1	4.462*	0.01-0.05
QEW5	42	17	59	3:1	0.458	0.30-0.50
QFW6	76	42	118	3:1	7.062*	0.001-0.01
Total plants	624	151	775		47.072	
Pooled				3:1	12.577**	< 0.0001
Hom (df = 5)				3:1	34.495**	< 0.0001

*, ** = significant at the 0.05 and 0.01 probability levels respectively.

Appendix B. 4. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with PL86-12 (PG 2) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total plants
W1QA	5.5 ± 0.51		3	16	1		1	2	1	14	38
W2QB	4.9 ± 0.29		2	16	14	1		14	6	1	54
W3QC	7.6 ± 0.22			3				11	21	15	50
W4QD	7.2 ± 0.18				5	1		24	18	4	52
W5QE	5.4 ± 0.35		1	10	9	1	8	1	6	5	41
W6QF	5.2 ± 0.41		4	4	8	4	5	3	3	5	36
QAW1	4.1 ± 0.23		2	22	22		1	1	4	1	53
QBW2	4.2 ± 0.30		4	18	12	6	5	2	3	2	47
QCW3	4.1 ± 0.31		10	22	7	2	4	4	6	1	56
QDW4	4.3 ± 0.32		10	17	4	1	5	3	7	3	58
QEW5	4.4 ± 0.38		13	12	9		3	3	9	4	53
QFW6	5.4 ± 0.44		14	12	1			3	11	16	57

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 5. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with PL86-12 (PG 2) of *Leptopshaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	73	42	115	9:7	2.747	0.05-0.10
W2QB	55	57	112	9:7	2.322	0.10-0.20
W3QC	37	86	123	1:3	1.694	0.10-0.20
W4QD	27	96	123	1:3	0.611	0.30-0.50
W5QE	92	64	156	9:7	0.366	0.30-0.50
W6QF	86	69	155	9:7	0.010	0.70-0.90
#Total plants	306	232	538		5.447	
Pooled				9:7	0.090	0.30-0.50
Hom (df = 3)				9:7	5.361	0.10-0.20
QAW1	82	43	125	9:7	3.770	0.05-0.10
QBW2	98	44	142	3:1	2.714	0.05-0.10
QCW3	112	66	178	9:7	2.954	0.10-0.20
QDW4	117	46	163	3:1	0.738	0.05-0.10
QEW5	62	56	118	9:7	0.659	0.30-0.50
QFW6	34	25	59	9:7	0.050	0.70-0.90
#Total plants	290	190	480		7.429	
Pooled				9:7	3.386	0.01-0.05
Hom (df = 3)				9:7	4.430	0.30-0.50

All crosses testing significant for a 9:7 segregation ratio were excluded in pooled data.

Appendix B. 6. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocals crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total plants
W1QA	3.3 ± 0.16		3	20	4	5					32
W2QB	3.6 ± 0.13		2	22	24	4		1			53
W3QC	4.3 ± 0.08			2	30	19					51
W4QD	4.3 ± 0.09			3	33	15					51
W5QE	4.3 ± 0.14			8	17	14	1	1			41
W6QF	6.3 ± 0.33	1		2	4	8	3	6	3	9	36
QAW1	4.3 ± 0.13			6	34	10		2	1		53
QBW2	4.6 ± 0.23			12	14	16		1	1	3	47
QCW3	4.2 ± 0.14		2	8	24	20	1		1		56
QDW4	3.7 ± 0.12		2	19	30	5	2				58
QEW5	3.6 ± 0.17		4	20	23	5				1	53
QFW6	3.1 ± 0.22	1	15	19	10	10	1			1	57

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.

[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 7. Crosses, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

	Observed IP					
Crosses	R	S	Total plants	Genetic Model	X^2	P
W1QA	80	35	115	3:1	1.812	0.10-0.20
W2QB	84	28	112	3:1	0.000	> 0.95
W3QC	90	33	123	3:1	0.165	0.50-0.70
W4QD	86	37	123	3:1	1.694	0.10-0.20
W5QE	85	34	119	3:1	0.811	0.30-0.50
W6QF	85	32	117	3:1	0.345	0.50-0.70
Total plants	510	199	709		4.824	
Pooled				3:1	3.559	0.05-0.10
Hom (df = 5)				3:1	1.266	0.90-0.95
QAW1	92	33	125	3:1	0.131	0.70-0.90
QBW2	112	26	138	3:1	2.792	0.05-0.10
QCW3	94	33	127	3:1	0.066	0.70-0.90
QDW4	103	24	127	3:1	2.522	0.10-0.20
QEW5	78	40	118	3:1	4.983*	0.01-0.05
QFW6	39	20	59	3:1	2.492	0.10-0.20
Total # plants	440	136	576		8.002	
Pooled				3:1	0.593	0.30-0.50
Hom (df = 5)				3:1	7.409	0.30-0.50

Cross QEW5 excluded in pooled data.
* = significant at the 0.05 probability level.

Appendix B. 8. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									Total plants
		0	1	3	4	5	6	7	8	9	
W1QA	6.8 ± 0.67	2	4	1					1	17	24
W2QB	8.1 ± 0.24	2	2		1			1	4	42	52
W3QC	9.0 ± 0.00									49	49
W4QD	8.8 ± 0.05	1								51	52
W5QE	9.0 ± 0.00									49	49
W6QF	8.7 ± 0.05	1						1	1	43	46
QAW1	9.0 ± 0.00									53	53
QBW2	8.9 ± 0.02								1	46	47
QCW3	8.9 ± 0.02								2	54	56
QDW4	8.9 ± 0.07							1		28	29
QEW5	9.0 ± 0.00									47	47
QFW6	8.9 ± 0.04		1							56	57

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.

[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 9. Cross, interaction phenotype(IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans.*

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	48	67	115	0:1		
W2QB	6	106	112	0:1		
W3QC	3	125	128	0:1		
W4QD	2	123	125	0:1		
W5QE	6	113	119	0:1		
W6QF	9	106	115	0:1		
Total plants	74	640	714			
Pooled				0:1		
Hom (df = 5)				0:1		
QAW1	3	118	121	0:1		
QBW2	4	135	139	0:1		
QCW3	3	119	122	0:1		
QDW4	3	122	125	0:1		
QEW5	6	125	131	0:1		
QFW6	1	58	59	0:1		
Total plants	20	677	684	0:1		
Pooled				0:1		
Hom (df = 5)				0:1		

Appendix B. 10. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with PL87-41 (PG 2-1) isolate of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									
		0	1	3	4	5	6	7	8	9	Total plants
W1G1	3.1 ± 0.23		6	36	1				2	1	46
W2G2	2.6 ± 0.13		10	33	3						46
W3G3	1.5 ± 0.19		19	5	1						25
W4G4	3.0 ± 0.00			42							42
W6G6	2.5 ± 0.22		4	12							16
W7G7	3.0 ± 0.00			16							16
G1W1	3.3 ± 0.23		10	32	5	2	4		3		59
G2W2	2.4 ± 0.16		23	29	7	1					60
G3W3	4.0 ± 0.23			39	5		8		5	1	58
G4W4	2.3 ± 0.15		24	29	6						59
G6W6	2.8 ± 0.08		7	55	2						64
G7W7	3.3 ± 0.07			44	14	5					60

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.

[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 11. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with PL87-41 (PG 2-1) isolate of *Leptospaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1G1	106	46	152	3:1	2.246	0.10-0.20
W3G3	131	34	165	3:1	1.699	0.10-0.20
W4G4	77	28	105	3:1	1.838	0.10-0.20
W6G6	120	36	156	3:1	0.308	0.30-0.50
W7G7	124	42	166	3:1	0.010	0.90-0.95
Total # plants	558	186	744		6.099	
Pooled				3:1	0.000	> 0.95
Hom (df = 4)				3:1	6.099	0.10-0.20
G1W1	107	23	130	3:1	3.703	0.05-0.10
G2W2	153	9	162	3:1	32.67**	< 0.0001
G3W3	88	31	119	3:1	0.070	0.70-0.90
G4W4	96	31	127	3:1	0.024	0.70-0.90
G6W6	104	31	135	3:1	0.299	0.50-0.70
G7W7	129	30	159	3:1	3.189	0.05-0.10
Total plants	677	155	832		39.951	
Pooled				3:1	18.006**	< 0.001
Hom (df = 5)				3:1	21.944**	< 0.001
Total # plants	524	146	670		7.284	
Pooled				3:1	3.681	0.05-0.10
Hom (df = 4)				3:1	3.604	0.30-0.50

Cross G2W2 excluded in pooled data
** = significant at the 0.01 probability level.

Appendix B. 12. Mean interaction phenotype (IP) on F_1 plants of the Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with PL86-12 (PG 2) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total plants
W1G1	2.7 ± 0.39		25	13			1		2	4	45
W2G2	1.3 ± 0.19		42	3						1	46
W3G3	2.8 ± 0.14		3	21	1						25
W4G4	3.0 ± 0.00			42							42
W6G6	9.0 ± 0.00									16	16
W7G7	3.0 ± 0.00			16							16
G1W1	3.1 ± 0.20		10	34	6	1	2		2		54
G2W2	2.7 ± 0.18		20	25	9	5	1				60
G3W3	4.2 ± 0.25		1	30	12		7		7	1	58
G4W4	3.1 ± 0.21		16	21	16	5			1		59
G6W6	7.2 ± 0.15			2			16	10	33	3	64
G7W7	3.5 ± 0.10			39	16	3	1	1			60

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.

[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 13. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with PL86-12 (PG 2) isolate of *Leptosphaeria maculans.*

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1G1	109	44	153	3:1	1.153	0.20-0.30
W2G2	148	18	166	3:1	17.743**	< 0.0001
W4G4	72	32	104	3:1	1.838	0.30-0.50
W6G6	39	116	155	1:3	0.002	> 0.95
W7G7	120	47	167	3:1	0.880	0.30-0.50
Total plants #	301	123	424		3.873	
Pooled				3:1	3.635	0.05-0.10
Hom (df =3)				3:1	6.263	0.05-0.10
G1W1	102	28	130	3:1	0.831	0.30-0.50
G2W2	147	14	161	3:1	22.830**	< 0.0001
G3W3	83	36	119	3:1	1.751	0.10-0.20
G4W4	84	43	127	3:1	5.493*	0.01-0.05
G6W6	42	93	135	1:3	2.689	0.10-0.20
G7W7	118	40	158	3:1	0.008	0.90-0.95
Total plants	576	254	830		33.598	
Pooled				3:1	13.894**	< 0.0001
Hom (df = 5)				3:1	19.704**	< 0.0001
Total plants #	387	147	534		8.083	
Pooled				3:1	1.820	0.10-0.20
Hom (df = 3)				3:1	6.263	0.05-0.10

Cross W2 x G2, W6 x G6 and reciprocals were excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.

Appendix B. 14. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									
		0	1	3	4	5	6	7	8	9	Total plants
W1G1	9.0 ± 0.00									44	44
W2G2	9.0 ± 0.00									45	45
W3G3	9.0 ± 0.00									25	25
W4G4	9.0 ± 0.00									42	42
W6G6	9.0 ± 0.00									16	16
W7G7	9.0 ± 0.00									16	16
G1W1	8.0 ± 0.00								56		54
G2W2	8.0 ± 0.00								60		60
G3W3	8.1 ± 0.04								53	5	58
G4W4	8.1 ± 0.04								54	7	59
G6W6	8.0 ± 0.09						4	4	43	13	64
G7W7	8.8 ± 0.05								11	48	59

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 15. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1G1	0	143	143	0:1		
W2G2	0	166	166	0:1		
W3G3	1	165	166	0:1		
W4G4	0	105	105	0:1		
W6G6	0	156	156	0:1		
W7G7	0	167	167	0:1		
Total plants	1	902	903	0:1		
Pooled				0:1		
Hom (df = 5)				0:1		
G1W1	0	130	130	0:1		
G2W2	0	162	162	0:1		
G3W3	0	119	119	0:1		
G4W4	0	127	127	0:1		
G6W6	0	135	135	0:1		
G7W7	0	160	160	0:1		
Total plants	0	833	833	0:1		
Pooled				0:1		
Hom (df = 5)				0:1		

Appendix B. 16. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									
		0	1	3	4	5	6	7	8	9	Total plants
W1G1	9.0 ± 0.00									45	45
W2G2	9.0 ± 0.00									45	45
W3G3	9.0 ± 0.00									25	25
W4G4	9.0 ± 0.00									42	42
W6G6	9.0 ± 0.00									16	16
W7G7	9.0 ± 0.00									16	16
G1W1	8.0 ± 0.00								55		55
G2W2	8.0 ± 0.00								60		60
G3W3	7.9 ± 0.04	1							52	5	58
G4W4	8.0 ± 0.04	1							51	8	60
G6W6	8.1 ± 0.08						4	3	48	9	64
G7W7	8.8 ± 0.04							1	9	49	59

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 17. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1G1	0	150	150	0:1		
W2G2	0	165	165	0:1		
W3G3	7	158	165	0:1		
W4G4	1	104	105	0:1		
W6G6	13	151	164	0:1		
W7G7	0	167	167	0:1		
Total plants	21	895	916			
Pooled				0:1		
Hom (df = 5)				0:1		
G1W1	0	130	130	0:1		
G2W2	8	154	162	0:1		
G3W3	0	119	119	0:1		
G4W4	3	124	127	0:1		
G6W6	0	135	135	0:1		
G7W7	2	157	159	0:1		
Total plants	13	819	832	0:1		
Pooled				0:1		
Hom (df = 5)				0:1		

Appendix B. 18. Mean interaction phenotype on the Glacier parental plants of *Brassica napus* Glacier (G) x Quinta (Q) differential cultivars following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

	Isolate Number and pathogenicity group (PG)				
Parent Number	PI87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
	Interaction phenotype rating [1]				
G1	1.7±0.17	1.6±0.16	9.0±0.00	9.0±0.00	34
G2	2.1±0.20	1.8±0.19	9.0±0.00	3.8±0.27	28
G3	1.4±0.14	1.5±0.16	9.0±0.00	9.0±0.00	38
G4	1.0±0.00	1.2±0.20	9.0±0.00	9.0±0.00	22
G5	1.1±0.06	1.12±0.09	7.9±0.17	8.8±0.09	33
G6	2.0±0.19	3.1±0.21	9.0±0.00	9.0±0.00	30
QA	2.5±0.27	1.4±0.16	2.8±0.26	8.9±0.07	22
QB	2.6±0.18	2.5±0.19	4.2±0.14	4.4±0.19	26
QC	1.4±0.16	1.2±0.13	3.9±0.19	9.0±0.00	25
QD	1.0±0.00	1.0±0.00	3.1±0.26	9.0±0.00	31
QE	2.1±0.28	1.2±0.13	3.9±0.25	9.0±0.00	21
QF	1.4±0.18	1.0±0.00	2.0±0.23	9.0±0.00	22

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985. Numbers are mean interaction phenotype ± S.E.

Appendix B. 19. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with PL87-41 (PG 2-1) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
G1QA	2.2 ± 0.17		21	21	3	1					46
G2QB	1.9 ± 0.17		21	16							37
G3QC	1.7 ± 0.15		39	12	6						57
G4QD	1.0 ± 0.00		52								52
G5QE	1.2 ± 0.10		41	3	1						45
G6QF	1.3 ± 0.10		42	6							48
QAG1	3.6 ± 0.20		5	18	16	2	1			2	44
QBG2	2.6 ± 0.14		7	26							33
QCG3	3.9 ± 0.25		9	11	20	1	9	1	1	1	53
QDG4	2.3 ± 0.19		19	29	2	1					51
QEG5	1.6 ± 0.14		30	12							42
QFG6	1.7 ± 0.16		25	13							38

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 20. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with PL86-12 (PG 2) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
G1QA	3.1 ± 0.11			38	7						45
G2QB	3.4 ± 0.17			21	16						37
G3QC	3.1 ± 0.07			53	5						58
G4QD	3.1 ± 0.05			52	2						54
G5QE	3.0 ± 0.06			43	2						45
G6QF	3.2 ± 0.11			39	8						47
QAG1	3.4 ± 0.20		4	20	16		1	2	1		44
QBG2	3.5 ± 0.18			18	14	1					33
QCG3	4.4 ± 0.34			20	15	4	4	3	5		53
QDG4	3.4 ± 0.15			33	16	1	1				51
QEG5	3.3 ± 0.15			28	14						42
QFG6	3.4 ± 0.16			23	15						38

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 21. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
G1QA	4.2 ± 0.14			11	19	11	3	1			45
G2QB	4.1 ± 0.14		1	9	17	10					37
G3QC	4.1 ± 0.17		5	14	17	17	5				58
G4QD	3.4 ± 0.18	1	9	11	21	11					53
G5QE	3.5 ± 0.22		10	6	17	11	1				45
G6QF	2.3 ± 0.16	2	14	26	3						45
QAG1	3.7 ± 0.18		4	16	20	2	1		1		44
QBG2	4.1 ± 0.18		1	9	14	8		1			33
QCG3	4.4 ± 0.15		1	3	31	13	2	2	1		53
QDG4	3.9 ± 0.15		1	13	28	8				1	51
QEG5	4.1 ± 0.17		2	8	14	11	3				42
QFG6	4.1 ± 0.22		1	11	16	6	2	1		1	38

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 22. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
G1QA	8.5 ± 0.17	3								42	45
G2QB	2.6 ± 0.17		9	25	3						37
G3QC	9.0 ± 0.00									59	59
G4QD	9.0 ± 0.00									52	52
G5QE	9.0 ± 0.10									47	47
G6QF	8.1 ± 0.07	6								41	47
QAG1	8.9 ± 0.20								4	40	44
QBG2	3.4 ± 0.14		1	22	6		2	1			33
QCG3	8.2 ± 0.25	2							19	24	53
QDG4	8.6 ± 0.19	2							4	45	51
QEG5	8.5 ± 0.14	2							7	33	42
QFG6	8.1 ± 0.16	4							3	31	38

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix B. 23. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with PL87-41 (PG 2-1) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
G1QA	150	1	151	1:0		
G2QB	146	0	146	1:0		
G3QC	146	0	146	1:0		
G4QD	170	1	171	1:0		
G5QE	149	0	149	1:0		
G6QF	157	0	157	1:0		
Total plants	918	2	920			
Pooled				1:0		
Hom (df = 5)				1:0		
QAG1	98	66	164	1:0		
QBG2	140	2	142	1:0		
QCG3	100	57	157	1:0		
QDG4	135	19	154	1:0		
QEG5	151	6	157	1:0		
QFG6	167	0	167	1:0		
Total plants	791	150	941			
Pooled				1:0		
Hom (df = 5)				1:0		

Appendix B. 24. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with PL86-12 (PG 2) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
G1QA	151	0	151	1:0		
G2QB	146	0	146	1:0		
G3QC	146	0	146	1:0		
G4QD	170	0	170	1:0		
G5QE	149	0	149	1:0		
G6QF	156	0	156	1:0		
Total plants	918	0	918			
Pooled				1:0		
Hom (df = 5)				1:0		
QAG1	157	4	161	1:0		
QBG2	142	0	142	1:0		
QCG3	67	90	157	1:0		
QDG4	150	1	151	1:0		
QEG5	156	1	157	1:0		
QFG6	168	0	168	1:0		
Total plants	840	96	936			
Pooled				1:0		
Hom (df = 5)				1:0		

Appendix B. 25. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier (G) x Quinta (Q) crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic model	X^2	P
G1QA	97	56	153	9:7	2.893	0.05-0.10
G2QB	98	48	146	3:1	4.831*	0.01-0.05
G3QC	111	35	146	3:1	0.037	0.50-0.70
G4QD	121	50	171	3:1	1.421	0.20-0.30
G5QE	73	77	150	9:7	3.208	0.05-0.10
G6QF	113	43	156	3:1	0.419	0.50-0.70
Total plants $	443	176	619		6.708	
Pooled				3:1	3.891*	0.01-0.05
Hom (df =3)				3:1	2.817	0.30-0.50
QAG1	110	52	162	3:1	3.984*	0.05-0.10
QBG2	108	35	143	3:1	0.000	> 0.95
QCG3	104	53	157	3:1	5.964*	0.01-0.05
QDG4	101	50	151	3:1	4.876*	0.01-0.05
QEG5	97	60	157	9:7	1.735	0.10-0.20
QFG6	118	51	169	3:1	2.148	0.10-0.20
Total plants #	541	241	782		16.974	
Pooled				3:1	14.119**	< 0.001
Hom (df =4)				3:1	2.855	0.50-0.70

$ Cross G1QA and G5QE excluded in pooled data.
# Cross QEG5 excluded in pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.

Appendix B. 25B. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 6 (PG 3) isolate of *Leptosphaeria maculans*.

	Observed IP					
Crosses	R	S	Total plants	Genetic Model	X^2	P
G1QA	97	56	153	11:5	2.039	0.10-0.20
G2QB	98	48	146	11:5	0.179	0.30-0.50
G3QC	111	35	146	11:5	3.599	0.05-0.10
G4QD	121	50	171	11:5	0.322	0.50-0.70
G5QE	73	77	150	9:7	3.208	0.05-0.10
G6QF	113	43	156	11:5	0.987	0.30-0.50
Total plants	613	309	922		10.330	
Pooled				11:5	2.200	0.10-0.20
Hom (df =5)				11:5	8.134	0.10-0.20
QAG1	110	52	162	11:5	0.054	0.70-0.90
QBG2	108	35	143	11:5	3.055	0.05-0.10
QCG3	104	53	157	11:5	0.460	0.30-0.50
QDG4	101	50	151	11:5	0.244	0.50-0.70
QEG5	97	60	157	9:7	1.735	0.10-0.20
QFG6	118	51	169	11:5	0.091	0.70-0.90
Total plants	638	301	939		5.638	
Pooled				11:5	0.284	0.50-0.70
Hom (df = 5)				11:5	5.354	0.30-0.50

Appendix B. 26. Cross, interaction phenotype (IP), genetic model (resistant (R): susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with Lifolle 5 (PG 4) isolate of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
G1QA	1	150	151	0:1		
G2QB	141	5	146	0:1		
G3QC	0	144	144	0:1		
G4QD	1	166	167	0:1		
G5QE	5	145	150	0:1		
G6QF	9	148	157	0:1		
Total plants	157	758	915			
Pooled				0:1		
Hom (df = 5)				0:1		
QAG1	7	156	163	0:1		
QBG2	138	5	143	0:1		
QCG3	12	145	157	0:1		
QDG4	5	148	153	0:1		
QEG5	7	151	158	0:1		
QFG6	8	158	166	0:1		
Total plants	177	763	940			
Pooled				0:1		
Hom (df = 5)				0:1		

Appendix C. 1. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar x Quinta crosses and their reciprocals following cotyledon inoculation with isolate 1130 - B24 (PG 2) of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
W1QA	2.4 ± 0.25	1	1	7							9
W2QB	3.1 ± 0.10			9	1						10
W3QC	2.6 ± 0.38	1	2	5	1						9
W4QD	2.9 ± 0.23	1	1	8	1						11
W5QE	3.3 ± 0.25			3	1						4
QAW1	3.3 ± 0.33		1	3	5						9
QBW2	3.4 ± 0.39	1	2	6	3		1				13
QCW3	2.9 ± 0.30	3	4	3	7						17
QDW4	3.0 ± 0.30	1	2	8	2	1					14
QEW5	3.3 ± 0.18		1	12	6	1					20

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix C. 2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar x Quinta crosses and their reciprocals following cotyledon inoculation with PG 2 isolate 5174/11 (PG 2) of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
W1QA	6.1 ± 0.70	2			1			1	1	4	9
W2QB	4.0 ± 1.19	3	1	1	1		1		1	2	10
W3QC	5.7 ± 0.96	1		2	1		1		1	3	9
W4QD	3.3 ± 1.47	4	3						3	1	11
W5QE	3.0 ± 0.58	1		1	1	1					4
QAW1	3.6 ± 1.00	5				1				3	9
QBW2	2.3 ± 0.63	8			1		3		1		13
QCW3	4.3 ± 0.64	4	2		3	3	3		2	2	17
QDW4	3.6 ± 0.60	4	1		3		5		1		14
QEW5	2.5 ± 0.72	8	1	1			6				16

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix C. 3. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar x Quinta crosses and their reciprocals following cotyledon inoculation with isolate 1130 - A18 (PG 3) of *Leptosphaeria maculans*.

Cross	Mean IP rating [2]	IP Frequency distribution [1]									
		0	1	3	4	5	6	7	8	9	Total Plants
W1QA	1.4 ± 0.25	5		3	1						9
W2QB	2.7 ± 0.30		2	7	1						10
W3QC	3.1 ± 0.31		1	5	3						9
W4QD	2.7 ± 0.30	1	2	7	1						11
W5QE	3.0 ± 0.25		1	1	2						4
QAW1	2.0 ± 0.45	3	1	3	2						9
QBW2	3.0 ± 0.35	1	2	4	5	1					13
QCW3	2.4 ± 0.29	4	3	6	6						19
QDW4	1.9 ± 0.29	7		3	3	1					14
QEW5	1.9 ± 0.34	7	3	7	2	1					20

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix C. 4. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar x Quinta crosses and their reciprocals following cotyledon inoculation with isolate PL89 - 21 (PG 3) of *Leptosphaeria maculans*.

		IP Frequency distribution [1]									
Cross	Mean IP rating [2]	0	1	3	4	5	6	7	8	9	Total Plants
W1QA	2.3 ± 0.00	2		7							9
W2QB	2.7 ± 0.18	2		5	3						10
W3QC	3.3 ± 0.17			6	3						9
W4QD	3.0 ± 0.21	1		4	5	1					11
W5QE	3.3 ± 0.33	1			2	1					4
QAW1	2.9 ± 1.00	2	1	1	3	2					9
QBW2	3.3 ± 0.45	2	1	2	3	3	1				12
QCW3	3.3 ± 0.17	3		4	9	3					19
QDW4	2.7 ± 0.21	3		7	3	1					14
QEW5	3.2 ± 0.22	2	1	7	8	2					20

[1] Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.
[2] Numbers are mean interaction phenotype ± S.E.

Appendix C. 5. Cross, interaction phenotype (IP), genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with isolate 1130B - 24 (PG 2) of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	187	75	262	3:1	1.837	0.10-0.20
W2QB	149	58	207	3:1	1.006	0.30-0.50
W3QC	160	47	207	3:1	0.581	0.30-0.50
W4QD	193	57	250	3:1	0.645	0.30-0.50
W5QE	209	63	272	3:1	0.490	0.30-0.50
Total plants	898	300	1198	3:1	4.559	
Pooled				3:1	0.000	> 0.95
Homogeneity (df=4)				3:1	4.559	0.30-0.50
QAW1	169	49	218	3:1	0.740	0.30-0.50
QBW2	218	70	288	3:1	0.074	0.70-0.90
QCW3	175	48	223	3:1	1.437	0.20-0.30
QDW4	158	70	228	3:1	3.953*	0.01-0.05
QEW5	187	53	240	3:1	1.089	0.20-0.30
Total plants	907	290	1197	3:1	7.293	
Pooled				3:1	0.381	0.50-0.70
Homogeneity (df=4)				3:1	6.912	0.10-0.20

* = significant at the 0.05 probability level.

Appendix C. 6. Cross interaction phenotype (IP), genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with isolate 5174/11 (PG 2) of *Leptosphaeria maculans*

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	82	180	262	5:11	0.000	> 0.95
W2QB	66	142	208	5:11	0.022	0.70-0.90
W3QC	74	134	208	5:11	1.813	0.10-0.20
W4QD	84	163	247	5:11	0.875	0.30-0.50
W5QE	106	166	272	5:11	7.547**	0.01-0.001
Total plants	412	785	1197	5:11	10.257	
Pooled				5:11	5.597**	0.01-0.05
Homogeneity (df=4)				5:11	4.660	0.30-0.50
QAW1	101	117	218	5:11	23.076**	< 0.001
QBW2	104	183	287	5:11	3.322	0.05-0.10
QCW3	84	139	223	5:11	4.276*	0.01-0.05
QDW4	74	154	228	5:11	0.154	0.50-0.70
QEW5	63	178	241	5:11	2.928	0.05-0.10
Total plants	426	767	1197	5:11	33.756	
Pooled				5:11	11.037**	< 0.001
Homogeneity (df=4)				5:11	22.719**	< 0.001

*, ** = significant at the 0.05 and 0.01 probability levels respectively.

Appendix C. 7. Cross interaction phenotype (IP), genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with isolate 1130A - 18 (PG 3) of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	181	81	262	3:1	4.891*	0.01-0.05
W2QB	159	49	208	3:1	0.231	0.50-0.70
W3QC	152	54	206	3:1	0.162	0.50-0.70
W4QD	197	50	247	3:1	2.981	0.05-0.10
W5QE	210	63	273	3:1	0.539	0.30-0.50
Total plants	899	297	1196	3:1	8.822	
Pooled				3:1	0.018	0.70-0.90
Homogeneity (df=4)				3:1	8.804	0.05-0.10
QAW1	170	47	217	3:1	1.292	0.20-0.30
QBW2	226	63	289	3:1	1.579	0.20-0.30
QCW3	174	49	223	3:1	1.090	0.20-0.30
QDW4	151	78	229	3:1	10.028**	0.001-0.01
QEW5	181	60	241	3:1	0.001	> 0.95
Total plants	902	297	1199	3:1	13.990	
Pooled				3:1	0.034	0.70-0.90
Homogeneity (df=4)				3:1	13.956**	< 0.001
Total plants	751	219	970	3:1	3.962	
Pooled #				3:1	3.036	0.05-0.10
Homogeneity (df=3)				3:1	0.926	0.70-0.90

Cross QDW4 was excluded from pooled data.

*, ** = significant at the 0.05 and 0.01 probability levels respectively.

Appendix C. 8. Cross, interaction phenotype (IP), genetic model (resistant (R):susceptible (S), Chi-square values (X^2) and probabilities for goodness of fit for F_2 plants of *Brassica napus* Westar x Quinta crosses following cotyledon inoculation with isolate PI89 - 21 (PG 3) of *Leptosphaeria maculans*.

	Observed IP					
Cross	R	S	Total plants	Genetic Model	X^2	P
W1QA	182	79	261	3:1	3.863*	0.01-0.05
W2QB	149	59	208	3:1	1.256	0.20-0.30
W3QC	153	54	207	3:1	0.130	0.70-0.90
W4QD	186	63	249	3:1	0.012	0.90-0.95
W5QE	196	77	273	3:1	1.496	0.20-0.30
Total plants	866	332	1198	3:1	6.757	
Pooled				3:1	4.702*	0.01-0.05
Homogeneity (df=4)				3:1	2.055	0.70-0.90
QAW1	173	45	218	3:1	2.208	0.10-0.20
QBW2	228	58	286	3:1	3.399	0.05-0.10
QCW3	163	60	223	3:1	0.432	0.50-0.70
QDW4	158	69	227	3:1	3.526	0.05-0.10
QEW5	179	60	239	3:1	0.001	> 0.95
Total plants	901	292	1193	3:1	9.177	
Pooled				3:1	0.175	0.50-0.70
Homogeneity (df=4)				3:1	9.161	0.05-0.10

*= significant at the 0.05 probability level.

Appendix D. A hypothetical gene - for- gene model based on the reactions (R = resistant and S = Susceptible) of six *B. napus* differential genotypes following inoculations with genotypes of *Leptosphaeria maculans* (A = avirulent and a = virulent) belonging to different pathogenicity groups (PG).

	Pathogen/ Genotype				
Host	**PG 2 -2**	**PG 2 -1**	**PG 2**	**PG 3**	**PG 4**
Genotype	A1 a2 a3 a4	a1 a2 A3 A4	a1 A2 a3 A4	A1 a2 a3 A4	a1 a2 a3 a4
Quinta					
R1-R2-R3-R4R4	R	R	R	R	S
Glacier					
r1r1R2-R3-r4r4	R	R	R	S	S
Val-1					
r1r1-r2r2R3-r4r4	S	R	S	S	S
Dac-1					
r1r1-R2-r3r3r4r4	S	S	R	S	S
Dac-2					
R1-r2r2r3r3R4r4	?	S	S	R	S
Westar					
r1r1r2r2r3r3r4r4	S	S	S	S	S

Table A. Number of isolates of *Leptosphaeria maculans* used in the study, by source of origin and pathogenicity grouping (PG).

	Pathogenicity Group					
Source	X	PG 1	PG 2	PG 3	PG 4	TOTAL
Canada	2	43	52	2	10	109
Europe				7	8	15
Australia			1	4	3	8
TOTAL	2	43	53	13	21	132

Table 5.1. Mean interaction phenotype of parental plants of *Brassica napus* cultivars Westar (W), Quinta (Q) and Glacier (G) following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

	Isolate Number and Pathogenicity group (PG)				
Parent	PL87-41	PL86-12	Lifolle 6	Lifolle 5	Number
Number	PG 2-1	PG 2	PG 3	PG 4	of plants
W1	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	21
W2	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W3	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W4	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	24
W5	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	18
W6	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	14
W7	9.0±0.00	9.0±0.00	9.0±0.00	9.0±0.00	20
QA	3.1±0.12	2.6±0.12	3.3±0.10	8.4±0.25	33
QB	2.6±0.13	2.8±0.15	3.4±0.11	8.9±0.11	32
QC	3.2±0.06	2.8±0.15	4.0±0.09	9.0±0.00	28
QD	3.2±0.11	3.5±0.12	4.1±0.09	8.9±0.00	26
QE	3.2±0.08	1.9±0.14	3.6±0.09	8.6±0.15	24
QF	3.0±0.08	2.5±0.14	3.4±0.08	8.3±0.17	17
G1	1.7±0.17	1.6±0.16	9.0±0.00	9.0±0.00	34
G2	2.1±0.20	1.8±0.19	9.0±0.00	3.8±0.27	28
G3	1.4±0.14	1.5±0.16	9.0±0.00	9.0±0.00	38
G4	1.0±0.00	1.2±0.20	9.0±0.00	9.0±0.00	22
G5	1.1±0.06	1.2±0.09	7.9±0.17	8.8±0.09	33
G6	2.0±0.19	3.1±0.21	9.0±0.00	9.0±0.00	30

Table 5.2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

	Isolate Number and Pathogenicity group (PG)				
Parent Number	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
W1QA	2.8 ± 0.15	5.5 ± 0.51	3.3 ± 0.16	9.0±0.00	38
W2QB	3.1 ± 0.11	4.9 ± 0.29	3.6 ± 0.13	9.0±0.00	54
W3QC	2.8 ± 0.13	7.6 ± 0.22	4.3 ± 0.08	9.0±0.00	51
W4QD	3.5 ± 0.07	7.2 ± 0.18	4.3 ± 0.09	9.0±0.00	52
W5QE	3.2 ± 0.18	5.4 ± 0.35	4.3 ± 0.14	9.0±0.00	41
W6QF	3.2 ± 0.34	5.2 ± 0.41	6.5 ± 0.33	9.0±0.00	36
QAW1	3.1 ± 0.09	4.1 ± 0.23	4.3 ± 0.13	9.0±0.00	53
QBW2	3.4 ± 0.18	4.2 ± 0.30	4.6 ± 0.23	8.3±0.27	47
QCW3	2.6 ± 0.14	4.1 ± 0.31	4.2 ± 0.14	9.0±0.00	56
QDW4	2.2 ± 0.15	4.3 ± 0.32	3.7 ± 0.12	9.0±0.00	58
QEW5	1.9 ± 0.16	4.4 ± 0.38	3.6 ± 0.17	8.8±0.09	53
QFW6	2.1 ± 0.14	5.4 ± 0.44	3.2 ± 0.22	9.0±0.00	57

Table 5.3. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

	Isolate Number and Pathogenicity group (PG)				
Parent Number	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
W1G1	3.1 ± 0.23	2.7 ± 0.39	9.0±0.00	9.0±0.00	46
W2G2	2.6 ± 0.13	1.3 ± 0.19	9.0±0.00	9.0±0.00	46
W3G3	1.5 ± 0.19	2.8 ± 0.14	9.0±0.00	9.0±0.00	25
W4G4	3.0 ± 0.00	3.0 ± 0.00	9.0±0.00	9.0±0.00	42
W6G6	2.5 ± 0.22	9.0 ± 0.00	9.0±0.00	9.0±0.00	16
W7G7	3.0 ± 0.00	3.0 ± 0.00	9.0±0.00	9.0±0.00	16
G1W1	3.3 ± 0.23	3.1 ± 0.20	9.0±0.00	9.0±0.00	54
G2W2	2.4 ± 0.16	2.7 ± 0.18	9.0±0.00	8.3±0.27	60
G3W3	4.0 ± 0.23	4.2 ± 0.25	9.0±0.00	9.0±0.00	58
G4W4	2.3 ± 0.15	3.1 ± 0.21	9.0±0.00	9.0±0.00	59
G6W6	2.8 ± 0.08	7.2 ± 0.15	9.0±0.00	8.8±0.09	64
W7G7	3.3 ± 0.07	3.5 ± 0.10	9.0±0.00	9.0±0.00	60

Table 5.3. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses, following cotyledon inoculation with four isolates of *Leptosphaeria maculans*.

	Isolate Number and Pathogenicity group (PG)				
Parent Number	PL87-41 PG 2-1	PL86-12 PG 2	Lifolle 6 PG 3	Lifolle 5 PG 4	Number of plants
G1QA	3.1 ± 0.23	2.7 ± 0.39	9.0±0.00	9.0±0.00	46
G2QB	2.6 ± 0.13	1.3 ± 0.19	9.0±0.00	9.0±0.00	46
G3QC	1.5 ± 0.19	2.8 ± 0.14	9.0±0.00	9.0±0.00	25
G4QD	3.0 ± 0.00	3.0 ± 0.00	9.0±0.00	9.0±0.00	42
G5QE	2.5 ± 0.22	9.0 ± 0.00	9.0±0.00	9.0±0.00	16
G6QF	3.0 ± 0.00	3.0 ± 0.00	9.0±0.00	9.0±0.00	16
QAG1	3.3 ± 0.23	3.1 ± 0.20	3.7 ± 0.18	9.0±0.00	54
QBG2	2.4 ± 0.16	2.7 ± 0.18	4.1 ± 0.18	8.3±0.27	60
QCG3	4.0 ± 0.23	4.2 ± 0.25	4.4 ± 0.15	9.0±0.00	58
QDG4	2.3 ± 0.15	3.1 ± 0.21	3.9 ± 0.15	9.0±0.00	59
QEG5	2.8 ± 0.08	7.2 ± 0.15	4.1 ± 0.17	8.8±0.09	64
QFG6	3.3 ± 0.07	3.5 ± 0.10	4.1 ± 0.22	9.0±0.00	60

Table 5.4. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with isolate Lifolle 6 (PG 3) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution									Total plants
		0	1	3	4	5	6	7	8	9	
G1QA	4.2 ± 0.14			11	19	11	3	1			45
G2QB	4.1 ± 0.14		1	9	17	10					37
G3QC	4.1 ± 0.17		5	14	17	17	5				58
G4QD	3.5 ± 0.18	1	9		11	21	11				53
G5QE	3.5 ± 0.22		10	6	17	11	1				45
G6QF	2.4 ± 0.16	2	14	26	3						45
QAG1	3.7 ± 0.18		4	16	20	2	1		1		44
QBG2	4.1 ± 0.18		1	9	14	8		1			33
QCG3	4.4 ± 0.15		1	3	31	13	2	2	1		53
QDG4	3.9 ± 0.15		1	13	28	8				1	51
QEG5	4.1 ± 0.17		2	8	14	11	3				42
QFG6	4.1 ± 0.22		1	11	16	6	2	1		1	38

Table 5.2. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with isolate PL87-41 (PG 2-1) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution							
		0	1	3	4	5	6	8	Total plants
W1QA	2.8 ± 0.15		3	20	2				25
W2QB	3.1 ± 0.11		5	33	14				52
W3QC	2.8 ± 0.13		8	33	8				49
W4QD	3.5 ± 0.07	1		26	25				52
W5QE	3.2 ± 0.18	1	7	11	22				41
W6QF	3.2 ± 0.34		10	3	14	1		1	30
QAW1	3.1 ± 0.09		3	38	12				53
QBW2	3.4 ± 0.18		3	29	8		6		46
QCW3	2.6 ± 0.14		15	32	9				56
QDW4	2.2 ± 0.15		26	28	2	1			57
QEW5	1.9 ± 0.16		27	17	3				47
QFW6	2.1 ± 0.14		30	25	1				56

Cotyledon Rating Scale of Delwiche &Williams, see Williams 1985.

Table 5.3. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocal crosses following cotyledon inoculation with isolate PL86-12 (PG 2) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution									
		0	1	3	4	5	6	7	8	9	Total plants
W1QA	5.5 ± 0.51		3	16	1		1	2	1	14	38
W2QB	4.9 ± 0.29		2	16	14	1		14	6	1	54
W3QC	7.6 ± 0.22			3				11	21	15	50
W4QD	7.2 ± 0.18				5	1		24	18	4	52
W5QE	5.4 ± 0.35		1	10	9	1	8	1	6	5	41
W6QF	5.2 ± 0.41		4	4	8	4	5	3	3	5	36
QAW1	4.1 ± 0.23		2	22	22		1	1	4	1	53
QBW2	4.2 ± 0.30		4	18	12	6	5	2	3	2	47
QCW3	4.1 ± 0.31		10	22	7	2	4	4	6	1	56
QDW4	4.3 ± 0.32		10	17	4	1	5	3	7	3	58
QEW5	4.4 ± 0.38		13	12	9		3	3	9	4	53
QFW6	5.4 ± 0.44		14	12	1			3	11	16	57

Table 5.4. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Quinta (Q) and their reciprocals crosses following cotyledon inoculation with isolate Lifolle 6 (PG 3) of *Leptosphaeria maculans*.

Crosses	Mean Interaction Phenotype	IP Frequency distribution									
		0	1	3	4	5	6	7	8	9	Total plants
W1QA	3.3 ± 0.16		3	20	4	5					32
W2QB	3.6 ± 0.13		2	22	24	4		1			53
W3QC	4.3 ± 0.08			2	30	19					51
W4QD	4.3 ± 0.09			3	33	15					51
W5QE	4.3 ± 0.14			8	17	14	1	1			41
W6QF	6.5 ± 0.33	1		2	4	8	3	6	3	9	36
QAW1	4.3 ± 0.13			6	34	10		2	1		53
QBW2	4.6 ± 0.23			12	14	16		1	1	3	47
QCW3	4.2 ± 0.14		2	8	24	20	1		1		56
QDW4	3.7 ± 0.12		2	19	30	5	2				58
QEW5	3.6 ± 0.17		4	20	23	5				1	53
QFW6	3.2 ± 0.22	1	15	19	10	10	1			1	57

Table 5.5. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with isolate PL87-41 (PG 2-1) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution									Total plants
		0	1	3	4	5	6	7	8	9	
W1G1	3.1 ± 0.23		6	36	1				2	1	46
W2G2	2.6 ± 0.13		10	33	3						46
W3G3	1.5 ± 0.19		19	5	1						25
W4G4	3.0 ± 0.00			42							42
W6G6	2.5 ± 0.22		4	12							16
W7G7	3.0 ± 0.00			16							16
G1W1	3.3 ± 0.23		10	32	5	2	4		3		59
G2W2	2.4 ± 0.16		23	29	7	1					60
G3W3	4.0 ± 0.23			39	5		8		5	1	58
G4W4	2.3 ± 0.15		24	29	6						59
G6W6	2.8 ± 0.08		7	55	2						64
G7W7	3.3 ± 0.07			44	14	5					60

Table 5.6. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Westar (W) x Glacier (G) and their reciprocal crosses following cotyledon inoculation with isolate PL86-12 (PG 2) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution									
		0	1	3	4	5	6	7	8	9	Total plants
W1G1	2.7 ± 0.39		25	13			1		2	4	45
W2G2	1.3 ± 0.19		42	3						1	46
W3G3	2.8 ± 0.14		3	21	1						25
W4G4	3.0 ± 0.00			42							42
W6G6	9.0 ± 0.00									16	16
W7G7	3.0 ± 0.00			16							16
G1W1	3.1 ± 0.20		10	34	6	1	2		2		54
G2W2	2.7 ± 0.18		20	25	9	5	1				60
G3W3	4.2 ± 0.25		1	30	12		7		7	1	58
G4W4	3.1 ± 0.21		16	21	16	5			1		59
G6W6	7.2 ± 0.15			2			16	10	33	3	64
G7W7	3.5 ± 0.10			39	16	3	1	1			60

Table 5.7. Mean interaction phenotype (IP) on F_1 plants of *Brassica napus* Glacier (G) x Quinta (Q) and their reciprocal crosses, following cotyledon inoculation with isolate Lifolle 6 (PG 3) of *Leptosphaeria maculans*.

Cross	Mean Interaction Phenotype	IP Frequency distribution									Total plants
		0	1	3	4	5	6	7	8	9	
G1QA	4.2 ± 0.14			11	19	11	3	1			45
G2QB	4.1 ± 0.14		1	9	17	10					37
G3QC	4.1 ± 0.17		5	14	17	17	5				58
G4QD	3.5 ± 0.18	1	9		11	21	11				53
G5QE	3.5 ± 0.22		10	6	17	11	1				45
G6QF	2.4 ± 0.16	2	14	26	3						45
QAG1	3.7 ± 0.18		4	16	20	2	1		1		44
QBG2	4.1 ± 0.18		1	9	14	8		1			33
QCG3	4.4 ± 0.15		1	3	31	13	2	2	1		53
QDG4	3.9 ± 0.15		1	13	28	8				1	51
QEG5	4.1 ± 0.17		2	8	14	11	3				42
QFG6	4.1 ± 0.22		1	11	16	6	2	1		1	38

Table 5.8. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta and their reciprocal crosses, following cotyledon inoculation with isolate PL87-41 (PG 2-1) of *Leptosphaeria maculans*.

Cross	Interaction Phenotype R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	587	196	783	3:1	5.336	
Pooled				3:1	0.000	> 0.95
Homogeneity (df=5)				3:1	5.336	0.30-0.50
Quinta x Westar						
Total plants	390	125	515	3:1	13.001	
Pooled #				3:1	0.146	0.70-0.90
Homogeneity (df=3)				3:1	12.855*	0.01-0.05
Westar x Quinta Combined						
Total plants	1211	347	1558	3:1	52.407	
Pooled				3:1	6.183*	0.01-0.05
Homogeneity (df=11)				3:1	46.224**	< 0.001

Cross QAW1 and QBW2 were excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.
Data for combined crosses include the crosses testing significant for a 3:1 ratio.

Table 5.9. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta and their reciprocal crosses, following cotyledon inoculation with isolate PL86-12 (PG 2) of *Leptopshaeria maculans*.

Cross	Interaction Phenotype R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	306	232	538	9:7	5.447	
Pooled #				9:7	0.086	0.30-0.50
Homogeneity (df=3)				9:7	5.361	0.10-0.20
Quinta x Westar						
Total plants	290	190	480	9:7	7.429	
Pooled #				9:7	3.386*	0.01-0.05
Homogeneity (df=3)				9:7	4.430	0.30-0.50
Westar x Quinta Combined						
Total plants	875	694	1569	9:7	18.631	
Pooled				9:7	0.146	0.50-0.70
Homogeneity (df=11)				9:7	18.483	0.05-0.10

All crosses testing significant for a 9:7 genetic model were excluded from pooled data.
* = significant at the 0.05 probability level.

Table 5.10. Crosses, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Westar x Quinta and their reciprocal crosses following cotyledon inoculation with isolate Lifolle 6 (PG 3) of *Leptosphaeria maculans*.

	Interaction Phenotype					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Quinta						
Total plants	510	199	709	3:1	4.823	
Pooled				3:1	3.559	0.05-0.10
Homogeneity (df=5)				3:1	1.266	0.90-0.95
Quinta x Westar						
Total plants	440	136	576	3:1	8.002	
Pooled #				3:1	0.593	0.30-0.50
Homogeneity (df=4)				3:1	7.409	0.30-0.50
Westar x Quinta Combined						
Total plants	1028	375	1403	3:1	17.809	
Pooled				3:1	2.236	0.10-0.20
Homogeneity (df=9)				3:1	15.573	0.05-0.10

Cross QEW5 was excluded from pooled data.

Table 5.11. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Westar x Glacier and their reciprocal crosses following cotyledon inoculation with isolate PL87-41 (PG 2-1) of *Leptospaeria maculans*.

Cross	Interaction Phenotype R	S	Total plants	Genetic Model	X^2	P
Westar x Glacier						
Total plants	558	186	744	3:1	6.099	
Pooled #				3:1	0.000	> 0.95
Homogeneity (df=4)				3:1	6.099	0.10-0.20
Glacier x Westar						
Total plants	524	146	670	3:1	7.284	
Pooled #				3:1	3.680	0.05-0.10
Homogeneity (df=4)				3:1	3.604	0.30-0.50
Westar x Glacier Combined						
Total plants	1235	341	1576	3:1	46.049	
Pooled				3:1	9.506**	0.001-0.01
Homogeneity (df=11)				3:1	36.544**	< 0.001

Cross G2W2 was excluded from pooled data.
** = significant at the 0.01 probability level.

Table 5.12. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Westar x Glacier and their reciprocal crosses following cotyledon inoculation with isolate PL86-12 (PG 2) of *Leptosphaeria maculans*.

	Interaction Phenotype					
Cross	R	S	Total plants	Genetic Model	X^2	P
Westar x Glacier						
Total plants	301	123	424	3:1	3.873	
Pooled #				3:1	3.635	0.05-0.10
Homogeneity (df=2)				3:1	0.238	0.70-0.90
Glacier x Westar						
Total plants	387	147	534	3:1	8.083	
Pooled #				3:1	1.820	0.10-0.20
Homogeneity (df=3)				3:1	6.263	0.05-0.10
Westar x Glacier Combined						
Total plants	1064	511	1575	3:1	55.214	
Pooled				3:1	46.214**	< 0.001
Homogeneity (df=11)				3:1	9.000	0.50-0.70

Cross W2G2, W6G6 and reciprocals were excluded from pooled data.
** = significant at the 0.01 probability level.

Table 5.13. Cross, interaction phenotype (IP), genetic model resistant (R):susceptible (S), Chi-square values (X^2) and probabilities (P) for goodness of fit for F_2 plants from *Brassica napus* Glacier x Quinta crosses following cotyledon inoculation with isolate Lifolle 6 (PG 3) of *Leptosphaeria maculans*.

	Interaction Phenotype					
Cross	R	S	Total plants	Genetic Model	X^2	P
Glacier x Quinta						
Total plants	443	176	619	3:1	6.708	
Pooled #				3:1	3.891*	0.01-0.05
Homogeneity (df=3)				3:1	2.817	0.30-0.50
Quinta x Glacier						
Total plants	541	241	782	3:1	16.974	
Pooled $				3:1	14.119**	< 0.001
Homogeneity (df=4)				3:1	2.855	0.50-0.70
Glacier x Quinta Combined						
Total plants	984	417	1401	3:1	23.682	
Pooled				3:1	16.962**	< 0.001
Homogeneity (df=8)				3:1	6.720	0.50-0.70

Crosses G1QA and G5QE were excluded from pooled data.
$ Cross QEG5 was also excluded from pooled data.
*, ** = significant at the 0.05 and 0.01 probability levels respectively.