RESISTANCE OF BRASSICA JUNCEA CZERN & COSS TO BLACKLEG DISEASE CAUSED BY LEPTOSPHAERIA MACULANS (DESM.) CES. & DE NOT.

A Thesis

Submitted to the Faculty of

Graduate Studies

The University of Manitoba

by

Mario Keri

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

July, 1991.

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ABSTRACT

Keri, M., M.Sc., The University of Manitoba, July, 1991. Resistance of Brassica juncea to blackleg disease caused by Leptosphaeria maculans.
Major Professor: Dr. S. R. Rimmer.

Leptosphaeria maculans causes blackleg disease in many cultivated crucifers including Brassica juncea. To date only a limited number of plants from this species have been evaluated for resistance to this pathogen. Accessions of B. juncea were evaluated for reactions to 2 isolates of L. maculans (Plat2, Pl86-14) at the cotyledon stage (296 accessions) and to 1 isolate (Pl86-14) at the adult plant stage (258 accessions). Accessions were observed for consistency of interaction phenotype over 3 rating times, and for root infection. Most accessions of B. juncea were resistant at the cotyledon and adult plant stages but the roots were susceptible. Leptosphaeria maculans was recovered from a sample of infected roots. Most (76%) plants within accessions were resistant at the cotyledon and adult plant stages, but some plants resistant at the cotyledon stage were susceptible at the adult plant stage. Interaction phenotypes on most (77%) accessions were not consistent over the 3 ratings. Susceptible lines were subsequently selected from plants whose rating scores were $\geq$ 5.0 in any of the 3 ratings. A weak correlation ($r=0.28^{**}$) was found between cotyledon and adult tests.

The use of resistant host varieties is an effective method to control blackleg disease but little is known about the genetic control of resistance to L. maculans in Brassica species. The inheritance of resistance in B. juncea to L. maculans was investigated in greenhouse experiments. Three resistant parents (UM3021, UM3043, UM3323) were reciprocally crossed to the susceptible parent UM3132 and to each other. The parents, F_1 and F_2 plants of the crosses were tested for reactions to L. maculans at the cotyledon and adult plant stages. F_2 plants from the crosses involving the resistant

parents and F_3 plants obtained from susceptible F_2 plants from the cross UM3021 x UM3132 were not tested at the adult stage. Resistance in all 3 resistant lines was controlled by 2 nuclear genes with dominant recessive epistatic action. This is supported by the segregation for resistance (1:3) in some F_3 populations obtained from susceptible F_2 plants. No segregation occurred in F_2 progeny of resistant x resistant crosses.

The relationship between the levels of seed glucosinolates and resistance in B. juncea to L. maculans was investigated. The levels of seed glucosinolates in 3 resistant lines (UM3021, UM3043, UM3323) and 3 susceptible lines (UM3132, UM3466, UM3467) were determined. In addition, the levels of seed glucosinolates in F_1, F_2, and F_3 seeds of crosses between resistant and susceptible lines were determined as were the reactions of the plants to L. maculans. Resistance to L. maculans was controlled by nuclear genes but levels of seed glucosinolates was controlled by the genotype of the maternal plant. The predominant glucosinolate in seeds of the resistant lines UM3043, UM3323 was 2-propenyl glucosinolates and the predominant seed glucosinolate in the susceptible lines and the resistant line UM3021 was 3-butenyl glucosinolates. There were significant differences between the levels of the 2-propenyl glucosinolates in the resistant parents UM3323 & UM3043 and the parents UM3132, UM3466, UM3467 & UM3021. The levels of the 3-butenyl glucosinolates in the susceptible parents and resistant parent UM3021 were not significantly different. The 2-propenyl glucosinolate was dominant over 3-butenyl glucosinolate. No relationship between resistance in B. juncea to L. maculans and the major seed glucosinolates was observed.

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. Three 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.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Dr. S. R. Rimmer for his guidance, valuable advice and suggestions. As my mentor, he has been very understanding and patient; giving generously of his time. I am also thankful for his encouragements during the research and preparation of this thesis, for critically reviewing this thesis and for all assistance he accorded me.

Many thanks to Dr. Donald E. Harder and Dr. Peter B. E. McVetty for their advice and assistance as research Committee members, and for critically reviewing this manuscript.

I wish to extend my appreciation and thanks to Dr. Kees van den Berg for the time he accorded me and for his suggestions and advice. I am grateful to Dr. Rachael Scarth for her assistance in the course of the research and I thank Mr. Randy Kutcher for many a stimulating discussion.

I am grateful to Ms. Paula Parks for the technical assistance and for bearing with me especially when there was no more greenhouse space available. I am also thankful to the greenhouse staff Ian Brown, Linda Peran and Kelly Allen for their assistance and tolerance.

Special thanks are extended to Dr. Jim K. Daun and his family for their encouragement and care. I would also like to thank Mr. Robert Kellington, Mr. Arthur Quanbury, Mr Wayne Chase and their families for all their support.

Financial support from Garst Seed Company and the National Research Council of Canada (IRAP) program is gratefully acknowledged.

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GENERAL INTRODUCTION

Oilseed Brassica species are the third most important sources of edible vegetable oil in the world (Downey & Robbelen 1989). In Canada, oilseed rape (Brassica napus L. and Brassica rapa L. (syn. B. campestris L.) and mustard are significant economic crops (Martens et al. 1984, Downey & Robbelen 1989). The major cultivated species of mustard are Brassica juncea Czern & Coss (brown & oriental mustard) and Sinapis alba L. (yellow mustard).

Increased production of Brassica spp., especially of oilseed rape, has been threatened in many producing areas by the build up of diseases common to rape, mustard and the other crucifers. One such disease is blackleg, caused by Leptosphaeria maculans (Desm.) Ces. & de Not. and its asexual stage Phoma lingam (Tode ex Schw.) Desm. This pathogen occurs as aggressive and non-aggressive populations. Both the aggressive and non-aggressive populations of L. maculans have been reported in Canada (McGee & Petrie 1978, Petrie 1979, Martens et al. 1984) and in Britain (Humpherson - Jones 1983a). Aggressive populations cause more serious damage than non-aggressive ones, but disease severity fluctuates from year to year (McGee & Emmett 1977, Thurling & Venn 1977, McGee & Petrie 1978, Gladders & Musa 1980). The fluctuations in the aggressiveness of the pathogen suggest that L. maculans may respond to changes in host resistance (Cargeeg & Thurling 1980b, Delwiche 1980, Newman 1984) and/or environmental influences. Hence, there is need for new sources of more stable resistance.

Breeding for resistance to blackleg has been a major objective of oilseed rape improvement programs in many countries including Canada. This is due to the serious disease threat attributable to blackleg in Australia (Bokor et al. 1975, McGee 1977, McGee & Emmett 1977, Thurling & Venn 1977), France (Alabouvette & Brunin 1970), Kenya (Piening et al. 1975), England (Cook & Evans 1978) and more recently in Canada

(Petrie 1979). Resistant varieties, when available, have been able to increase and/or maintain yields economically. The use of host resistance for disease control is also environmentally desirable, and helps to ensure that yields remain stable and predictable over time.

Sources of resistance have been reported in many Brassica spp. including wild species. Resistance which can only protect the crops in the adult stage has been found in B. rapa (genome AA) and B. napus (genome AACC) (Roy & Reeves 1975). Brassica juncea, a related species with genome AABB, possesses complete resistance at both seedling and adult plant stages (Roy 1984). This resistance has also been said to be stable although little is known about this host-pathogen relationship or the genetic control of the resistance. Genetic control of resistance, to any given pathogen, must involve the association of two organisms - the host and the pathogen; and hence the interaction of their genotypes (Person & Mayo 1974). Such a relationship is expressed as the observable disease reaction or interaction phenotype (Ellingboe 1976). Evaluations of some Brassica spp. for reactions to L. maculans have been reported but no such formal evaluations of B. juncea have been made to date. In the evaluation of B. napus reactions to L. maculans, Williams (1985) used a cotyledon rating scale of 0-9, where 0 indicated highly resistant and 9 highly susceptible interaction phenotypes. While it is a satisfactory scale of measurement, it has been rather difficult, in some cases, to delineate a resistant reaction from a susceptible one.

The seedling and adult plant resistance in B. juncea to L. maculans has been transferred to B. napus (Roy 1984). However, such transfers were not very successful due to interspecific incompatibility and sterility of the F_1 progeny (Roy 1978). Little is known about how different genotypes in this host-parasite interaction affect the expression of blackleg disease (Cargeeg & Thurling 1980a), and about the host-parasite interaction in the Brassica spp. - L. maculans system. No study of host-parasite interactions has been done in the B. juncea - L. maculans pathosystem. Knowledge of

the causes of fluctuating compatibility (sensu Heath 1981) of host-parasite interactions, the use of methods that overcome interspecific incompatibility and sterility of the progeny, and cytogenetic studies may all contribute to a better understanding of resistance to L. maculans in canola.

Canola is a term used to describe low erucic acid, low glucosinolate cultivars that meet defined quality standards in Canada. As a result, low glucosinolate rapeseed meal has become an alternative to soybean meal as an animal feed supplement, and canola oil is regarded as a high quality oil for human consumption. Such improvement of oilseed rape is a desirable development. However, some reports have implied the involvement of glucosinolates in the resistance in Brassica spp. against foliar pathogens (Rawlinson 1979, Mithen et al. 1986). Brassica juncea is related to B. rapa and B. napus. It is resistant to blackleg disease, to which the latter two species are susceptible. Brassica juncea also has high levels of glucosinolates in the seed and leaves. Little is known about the relationship between seed glucosinolates and resistance to L. maculans in Brassica species.

This study was undertaken with the following objectives.

1. To screen and evaluate accessions of B. juncea for susceptibility and resistance to L. maculans.
2. To study the genetics of resistance in B. juncea to L. maculans.
3. To study the relationship of seed glucosinolates with the resistance of B. juncea to L. maculans.

LITERATURE REVIEW

2.1.0 The Host

2.1.1 Description

The Brassica oilseed crops, Brassica napus L. (Argentine type rape or summer rape), and Brassica rapa L. (Polish type rape or summer turnip rape), are species inter-related to Brassica juncea Czern & Coss and Brassica carinata Braun. They are commonly referred to as oilseed rape (oilseed type) or rapeseed and mustard (oilseed and condiments type) respectively (Downey & Rakow 1987). Hybridization of the diploid species Brassica nigra (L.) Koch (n=8), Brassica oleracea L. (n=9) and B. rapa (n=10) occurred naturally giving rise to the amphidiploid species, B. carinata (n=17), B. juncea (n=18) and B. napus (n=19) (Downey & Robbelen 1989).

The cultivation of oilseed rape is restricted to the temperate, warm-temperate zones (Kolte 1985) and to the sub-tropics (Downey & Rakow 1987). In Canada, primarily summer forms of B. napus and B. rapa are grown (Canola Growers Manual 1989). Canada is a major producer of oilseed rape (Downey & Robbelen 1989) and a major supplier of mustard to the world market (Martens et al. 1984). The major cultivated species are B. juncea (brown & oriental mustard) and Sinapis alba L. (syn. B. hirta - yellow mustard). The latter, a distant relative of the oilseed crops is grown in greater quantities in Manitoba than the former.

2.1.2 History of Host

Rapeseed production began in Canada in 1942 with the Argentine type rapeseed (Stefansson 1983), but commercial production started in 1943 to supply lubrication oil for marine engines during the second world war (Boulter 1983). In 1956-57, edible oil

was first processed (Boulter 1983) and since then, continuous efforts by Canadian breeders have resulted in the release of varieties of oilseed rape low in erucic acid and glucosinolates (Downey & Rakow 1987). These quality attributes are desirable in products for human consumption and animal feed (Fenwick et al. 1983). Such varieties are referred to as double low and well known as canola. A recent release of a canola cultivar low in linolenic acid has shortened hydrogenation time and increased oil stability (Scarth et al. 1988). Improvements in oil and meal quality, and in the agronomic aspects of oilseed rape, resulted in increased desirability of oilseed products at home and in the world market, making oilseed rape the second most valuable grown crop (McVetty 1988, Canola Growers Manual 1989) and the fastest growing seed crop in Canada (Boulter 1983).

Brassica juncea (mustard), an amphidiploid of B. rapa and B. nigra is considered a plant of Asiatic origin (Kolte 1985). In Canada, commercial production of mustard started in 1936 with about 40 hectares (Statistics Canada 1976). It is currently grown for condiment in all the prairie provinces and has potential as an oilseed crop in western Canada (Pawlowski 1970, Love 1988, Woods et al. 1991), especially in the southern drier parts of the prairies, where temperatures are rather high for B. napus. Mustard has better tolerance to high salinity and high temperature conditions than oilseed rape (Pawlowski 1970, Singh 1987). In India, it is grown as a winter crop when weather conditions are mild and favourable, either as a pure crop or intercropped with wheat, barley and chick peas (Singh 1987).

There are two forms of the cultivated mustard, an early maturing dwarf type with less foliage and poor yields, and a late maturing, profusely branched and high yielding type (Singh 1987). The cultivars grown in Canada are high yielding and have a maturity period intermediate between B. napus and B. rapa (Pawlowski 1970, Woods et al. 1991); the cultivars 'Domo' and 'Cutlass' out yielded Westar by up to 20% in Canada (Love 1988, Woods et al. 1991).

Brassica juncea competes more readily with weeds and is more resistant to shattering and lodging than B. napus and B. rapa (Pawlowski 1970, Woods et al. 1991). It is also considerably more resistant to blackleg disease than B. napus and B. rapa.

Brassica juncea (oriental mustard) has high contents of both oil and protein (Woods et al. 1991). The oil content of B. juncea is similar to that of B. rapa cultivar 'Echo', but it has a higher protein content than the latter; moreover in Echo, the relationship between oil and protein contents is inverse (Pawlowski 1970). The use of low glucosinolate cultivars in this crop would enhance its promotion as an oilseed crop and its increased production in the prairies (Love 1988).

Brassica juncea is presently used in Asia as a vegetable crop, as an oilseed crop, spice plants and for condiments. In Japan, the meal is used as a nitrogen fertilizer, while in Canada, it is mainly produced for use in the preparation of hot table mustard.

2.2.0 The Pathogen

Leptosphaeria maculans (Desm.) Ces. & de Not. (Phoma lingam (Tode ex Fr.) Desm. is the causal organism of blackleg of crucifers (Smith & Sutton 1964, Punithalingam & Holliday 1972, McGee & Emmett 1977) and one of the most severe diseases attacking B. napus and B. rapa (Downey & Rakow 1987); however, most lines of B. juncea are resistant to the fungus (Roy 1984, Downey & Rakow 1987). In the literature, blackleg disease of crucifers is known by various names; such as stem canker (Punithalingam & Holliday 1972, Davies 1986, Newman 1984), canker (Martens et al. 1984, Newman & Bailey 1987), crown canker or dry rot (Punithalingam & Holliday 1972, Kruger 1983).

2.2.1 Description

Leptosphaeria maculans is a hemibiotrophic parasite of Brassicaceae (Boerema 1976). It is a heterothallic, bipolar ascomycete of the order Sphaeriales (Smith & Sutton 1964, Cargeeg & Thurling 1980b). The mycelia of this fungus are septate, branched and are hyaline when young, but become pigmented or dark walled with age (Boerema et al. 1981). The fungus may produce pseudosclerenchymatous perithecia (pseudothecia) of the perfect state, as well as pseudoparenchymatous and pseudosclerenchymatous pycnidia of its imperfect state (Boerema 1976) on dead host material. Ascospores are spindle shaped, multinucleate and haploid; they are hyaline when young becoming yellow tan at maturity (Smith & Sutton 1964, Boerema 1976)

Phoma lingam (Tode ex Fr.) Desm. is the imperfect state of L. maculans. Pycnidia of P. lingam are large and initially closed but later develop papillate openings (porus) sometimes as a neck (Boerema et al. 1981). Pycnidia vary widely in size and shape both between and within strains (sensu Boerema 1976). Pycnidiospores are hyaline, guttulate and cylindrical in shape (Punithalingam & Holliday 1972).

In culture, L. maculans is highly variable in terms of growth rate, pycnidia production and pigment production (Boerema 1976) characteristics which are often associated with differences in pathogenicity (Petrie & Vanterpool 1966). Non-aggressive isolates are fast growing, producing few pycnidia on V-8 or prune lactose yeast agar, while the aggressive isolates grow more slowly and produce abundant pycnidia on the same media (McGee & Petrie 1978). Non-aggressive isolates of the fungus produce a red/brown pigment in Czapek-Dox medium while the aggressive isolates do not (McGee & Petrie 1978, Humpherson-Jones 1983a). Aggressive isolates of L. maculans also produce abundant Sirodesmin PL toxin compared to the non-aggressive isolates. On starch gels, non-aggressive isolates produce a fast migrating band for malate

dehydrogenase (EC11137) compared to aggressive isolates (Hill et al. 1984 cited by Hill & Williams 1988).

2.2.2 Host Range

The pathogen attacks virtually all members of the Cruciferae including the economically important species B. oleracea var. capitata, B. rapa (syn. B. campestris, or turnip), Brassica napobrassica L., Raphanus sativus L., S. alba, B. napus, B. carinata, B. juncea (Williams 1974, Punithalingam & Holliday 1972, Commonwealth Mycological Institute 1978, Gabrielson 1983).

Its wild host range includes Raphanus raphanistrum L. in Australia, Brassica kaber L. (syn. Brassica arvense, S. arvense) in Canada (Petrie & Vanterpool 1968, Petrie 1978); Thlaspi arvense L. (Petrie & Vanterpool 1965, McGee & Petrie 1978), Descurania sophia (L.) Webb. (Petrie & Vanterpool 1965), Mathiola incana L., Lepidium spp. and Sisymbrium spp. (Petrie & Vanterpool 1966, Punithalingam & Holliday 1972, Commonwealth Mycological Institute 1978).

2.3.0 The Disease

Leptosphaeria maculans can attack all parts of the plants causing damping-off of seedlings (Van Bakel 1968 cited by Gabrielson 1983), cotyledon infections (Delwiche 1980, Kruger 1983, Davies 1986), leaf spots, stem cankers, crown cankers and root infections (Punithalingam & Holliday 1972, Piening et al. 1975, Kruger 1983, Van den Berg et al. 1989). It can also infect seed stalks, siliques and seeds of susceptible plants (Boerema 1976, Kruger 1983, Martens et al. 1984). However, the most important phase of the disease resulting in yield loss is the stem canker phase, which arises from early infections of plants and usually forms at the base of the stem (Martens et al. 1984, Davies

1986). Early infection of susceptible plants results in premature ripening and production of shrivelled seeds, if any at all. Such infected plants tend to shatter before healthy ones are ripened. Severely infected plants frequently lodge by breaking at the root-collar where the basal portion of the stem is infected and may be completely girdled.

2.3.1 Disease symptoms

Necrotic spots develop on cotyledons and/or leaves. Such lesions are usually dirty-white in colour and irregularly shaped. The infection then spreads into the stem, causing grey-brown to dirty-white discolourations often beginning near the base of the scar remaining from fallen infected leaves. Infected tissue may be dotted with numerous easily visible pycnidia (Kruger 1983, Martens et al. 1984). Another lesion type forms on stems (Kruger 1983) above the fifth node and has been shown to require pollen or fallen floral parts (Hammond & Lewis 1986a) for infection to occur.

2.3.2 The disease cycle

Leptosphaeria maculans overwinters on infested crop residue (McGee & Emmett 1977, Petrie 1978, Martens et al. 1984). In the absence of host tissue, the organism does not persist for long in the soil. When conditions are conducive, ascospores and/or pycnidiospores are produced. The ascospores become airborne, infecting plants for long distances around the site of spore liberation.

Oilseed rape can be infected by either ascospores or pycnidiospores as early as the time of seedling emergence, since spores may either be present on seed or brought by wind (Wood & Barbetti 1977, Kruger 1983, Davies 1986). Within the crop, new infections produce pycnidia from which pycnidiospores ooze in the presence of free moisture. These usually are the cause of localized infections or spread of disease.

Infection may also be initiated by seed-borne mycelia or pycnidia on seed (Kruger 1983). Infected seed may be important in the spread of the disease to new areas (Wood & Barbetti 1977, Kruger 1983, Martens et al. 1984, Davies 1986) but the pycnidia and perithecia on host residue are more important in the epidemiology of the disease (Boerema 1976, McGee & Emmett 1977).

2.3.3 Disease Control

Infested crop residues have been associated with severe crop infections (McGee & Emmett 1977, Petrie 1978). In Australia, crop failures occurred when crops were sown close to, or on infested crop residue. The volume of crop residues can be reduced in one year by 90% when crop rotation is practised (McGee 1977), and the incidence of disease can be decreased by increasing distances from infested trash, or by use of shelter belts. In Canada, severe blackleg infections occurred where infested rape residue was present either in the field, or in an adjacent field; the inoculum on rapeseed thrash being reduced in 2 years (Petrie 1978). Consequently a 3 - 5 year rotation is recommended as a control practice.

Late seeding, in order to avoid critical ascospore release periods is used as a control measure in Australia (Bokor et al. 1975, McGee & Emmett 1977), Germany and the Netherlands (Kruger 1983). However this practice may result in reduced yields.

The use of disease free seed can prevent introductions of the disease to new areas. Seed may be treated with fungicides such as iprodione, thiram, fenpropimorph and benomyl. The latter fungicide has particularly been shown to increase yields and reduce the incidence of blackleg. However the reports regarding the effectiveness of these chemicals are conflicting (Brown et al. 1976, Thurling & Venn 1977, Kruger 1983).

Aggressive isolates of L. maculans have been found on weed hosts in Canada (Petrie & Vanterpool 1965, McGee & Petrie 1978, Petrie 1979) and Australia (McGee

1977). Consequently weed control is an important measure for the control of blackleg disease.

The use of resistant varieties is often the most economical means of controlling plant diseases. In Canada a few cultivars of oilseed rape possess fair resistance to L. maculans (Anon 1991); and in Europe and Australia, the disease is controlled by the use of a few cultivars possessing adult plant resistance (Roy 1978, Cargeeg & Thurling 1980b, Newman & Bailey 1987).

2.4.0 History of disease

2.4.1 Occurrence in Canada

Blackleg was first reported in Canadian oilseed rape fields in 1961 (Vanterpool 1961). Severe infections of fields in North Battleford and Annaheim (Vanterpool 1963) were further indications of the prevalence of blackleg and its serious potential.

Petrie (1973a) included blackleg as part of a foot rot disease complex, even though 11% of the plates had L. maculans cultures. The high frequency of occurrence of blackleg in damaged or injured plants was demonstrated when the fungus was isolated from both cortical and hypertrophied inner tissues; 91% of the plants had blackleg in conjunction with herbicide injury and approximately 65% of the stems had blackleg (Petrie 1973b). Undamaged plants were rarely infected, indicating that injury is required for successful colonization.

In 1975, aggressive isolates of L. maculans were obtained from Saskatchewan fields (McGee & Petrie 1978, Petrie 1979). A severe localized outbreak of the aggressive populations later occurred with high incidence in the fields examined, when precipitation was very high (Petrie et al. 1985). The aggressive populations of L. maculans now predominate and contribute to serious crop losses in Saskatchewan. Since then

aggressive isolates of L. maculans have spread to the neighbouring provinces of Manitoba and Alberta. Aggressive populations of L. maculans have also recently been reported in Ontario (Peters & Hall 1989).

2.4.2 Economic Importance

Oilseed rape was introduced to Australia in 1968 as an alternative crop to wheat. Increased cultivation of oilseed rape was followed by the wide spread occurrence of blackleg in 1971 (Bokor et al. 1975, Wood & Barbetti 1977, Cargeeg & Thurling 1980b). Losses due to blackleg were the major limiting factor in the establishment of this industry in Australia.

In Great Britain the area sown to oilseed rape increased from 24.5 x 10^3 ha in 1974 to 55 x 10^3 ha in 1977. The infection levels remained low up to 1976 but became high in 1977 - 1978 (Cook & Evans 1978, Gladders & Musa 1980). Similarly in France the expansion of winter rape production in 1964 - 1965 was followed by an epidemic of blackleg in 1966 (Brown et al. 1976).

In Canada (Saskatchewan), the acreage sown to oilseed rape increased from 731 x 10^3 in 1966 to 2,737 x 10^3 ha in 1971 (Statistics Canada 1976). Two to 3 years following the 1970 - 1973 production peak the aggressive pathogen was detected (Petrie 1975, McGee & Petrie 1978). Fortunately disease severity fluctuates from year to year although the causes of such fluctuations have not yet been determined (McGee & Emmett 1977, Thurling & Venn 1977, McGee & Petrie 1978, Gladders & Musa 1980). For example a serious outbreak of blackleg occurred in 1982 and the average yield losses for the province of Saskatchewan was 6%, with losses of up to 50% observed in the 17 fields surveyed (Petrie 1985b). In 1984, yield losses were much higher - reported at 7.2% with mean losses in the infected fields at 25.2%; however, in 1985, the yield losses estimated were lower - reported at 12.2% (Petrie 1986). Blackleg was the most important

of all the diseases surveyed. In Manitoba, the production of oilseed rape increased from 79.4 x 10^3 acres in 1969 to 547 x 10^3 in 1980 (Statistics Canada 1984). No blackleg was observed for the period between 1978 - 1980 (Rimmer & Platford 1982). In 1987, blackleg was detected in the southwest and the northwest regions of the province, causing 62% average infected fields with 30% infection level in the southwest, and 31% fields infected with 24% infection level in the northwest (Platford 1988). Blackleg was detected in the central region only in one field and was not found in the eastern region of the province (Platford 1988); however it was detected even in the eastern region in 1989 (Van den Berg et al. 1989). In Manitoba, blackleg is progressively intensifying; moreover the steady increase in the production of oilseed rape in Canada from 1,401 x 10^3 ha in 1981 to 3,652 x 10^3 in 1988 (Statistics Canada 1988) may be matched by increased severity of the disease and probably disease epidemics, unless resistant cultivars are introduced quickly.

2.5.0 Host - Parasite interaction

The use of resistant cultivars to control plant diseases may be followed by the recurrence of disease on these cultivars due to changes in pathogen virulence. Proper understanding of the interactions in a host-parasite system entails knowledge of the genes involved in both organisms (Lawrence 1988). Flor (1942) realized this factor and consequently studied the genetics of the interaction of resistance genes in the host (Linum usitatissimum L.) and the virulence genes of the pathogen (Melampsora lini L.) in the flax-flax rust pathosystem (Flor 1946, Flor & Comstock 1971); he established, that in most cases, host resistance is dominant and that the pathogen's virulence is recessive.

2.5.1 The gene - for - gene model

Pathogenicity (virulence) on flax varieties possessing 1 gene for resistance to the avirulent pathogen was conditioned by a single gene in the parasite. Similarly, virulence on flax varieties possessing 2, 3, 4 or 5 genes for resistance was conditioned by corresponding 2, 3, 4 or 5 genes (respectively) in the fungus. These studies culminated in the important inference that: For each gene conferring resistance in the host, there is in the pathogen, a correspondingly related and specific gene conferring virulence (Flor 1955, Flor & Comstock 1971, Lawrence 1988).

Where resistance is dominant, a diploid pathogen will be virulent on a host plant if the host plant is homozygous recessive for resistance at all loci (with both the virulent and avirulent isolates) or when the host's resistance genes are all matched by the pathogen's homozygous recessive genotype at the corresponding loci (Zadoks & Schein 1979). Where the host's resistance genes occur at more than 1 locus, a single gene for resistance will confer resistance to the recessive genotype of the pathogen (Zadoks & Schein 1979, Lawrence 1988). Such a gene-for-gene relationship enables the use of interaction phenotypes in the identification of either genotypes of the interacting organisms (Flor & Comstock 1971) and the use of single resistance genes to identify specific interactions in host-parasite systems (Flor 1955).

2.5.2 Epistasis and resistance

When more than 1 resistance gene is effective against a pathogen and the genes act independently, the interaction phenotype expressed, e.g. rating=1 or 3 for 2 genes, is usually the lower one i.e. rating=1 (Roelfs 1988). However interactions between resistance genes may occur (Vanderplank 1984, Roelfs 1988). During epistatic interactions, the presence or absence of resistance alleles at one locus affects the

expression of alleles at another locus, both in the host and the pathogen (Vanderplank 1984). Commonly reported epistatic relationships in a 2 gene system are the 9:7 and 15:1 ratios. Rarely, 13:3 ratios of resistant:susceptible plants are reported. Virulence for either of 2 resistance genes separated may occur in an epistatic relationship, but virulence for the 2 genes combined in a cultivar is rare in the wheat-stem rust system (Vanderplank 1984).

2.6.0 Brassica - L. maculans pathosystem

The resistance of Brassica species to L. maculans is frequently referred to as two separate types: Seedling resistance and adult plant resistance (Thurling & Venn 1977, Cargeeg & Thurling 1980b, Delwiche 1980, Roy 1984, Sjodin & Glimelius 1988). Seedling resistance often conditions resistance to specific races of the pathogen (Delwiche 1980), while resistance at the adult stage is often against all prevalent races (Kruger 1983, Kolte 1985). However little is known about the interactions in the Brassica - L. maculans pathosystem (Hill & Williams 1988). Few studies have been done on the resistance of the host and almost none on either the pathogen or both host and pathogen.

2.6.1 Virulence studies

Recently, isolates of L. maculans from widely separated regions were tested for differential virulence on cotyledons of B. napus cultivars Westar, Quinta and Glacier. The isolates were then grouped into four pathogenicity groups (PG1 - PG4). PG1 was avirulent on Westar, PG2 was virulent on Westar only, PG3 was virulent on Westar and Glacier and PG4 was virulent on all 3 cultivars (Mengistu et al. 1989, Koch et al. 1991). Crosses between a PG2 isolate and a PG4 isolate of L. maculans have been made. The virulence of the parents and of their progenies were determined on the same cultivars of

B. napus (Rimmer 1989, personal communication). The virulence of L. maculans is determined by 2 major genes. (Rimmer 1989, personal communication).

2.6.2 Aspects of host resistance

Breeding for host resistance can be an economic and effective approach to controlling diseases. It starts with the search for resistance. Both searching and breeding for resistance are major objectives of breeding programs, aiming at incorporating resistance to blackleg in oilseed rape in Australia (Cargeeg & Thurling 1980a), France (Brown et al. 1976), Germany (Kruger 1983), England (Cook & Evans 1978, Newman 1984) and Canada. Some success has been achieved in these respects.

Resistance has been found in several Brassica spp. either at the adult stage or at both the seedling and adult plant stages. Adult plant resistance has been found in the B. napus cultivars 'Novoski', 'Ceska' and 'Zollerngold' (Cargeeg & Thurling 1980b), and a high level of resistance has been reported in the French cultivar 'Ramses' (Wratten 1977, Roy 1978). However, complete resistance (i.e. seedling & adult plant resistance) has not been found in B. napus.

Brassica juncea has been reported to be more resistant to blackleg than B. rapa, B. napus and B. carinata. These reports are based either on field observation of the disease and/or results of interspecific crosses. In an interspecific cross of B. napus with a resistant B. juncea, Roy (1978) recovered resistant adult plants of B. napus type and suggested that the genes for adult plant resistance are located on the A-genome. Because some B. carinata showed resistance at both the seedling and adult stages, the genes for seedling resistance are thought to be located on the B-genome, common to both B. carinata and B. juncea but absent in Brassica napus (Roy 1984, Sacristan & Gerdemann 1986). However in their investigations of B. nigra, B. juncea, B. carinata and B. napus,

Sjodin & Glimelius (1988) reported cultivars with the B-genome that were also susceptible at the seedling stage.

The search for resistance to blackleg has also been carried out using new in vitro selection techniques. Sacristan & Gerdemann (1986) obtained resistant progeny from interspecific crosses of B. juncea, B. carinata and B. napus. The resistance from B. carinata was lost after one generation of backcrossing unlike that from B. juncea; and a higher degree of resistance than that of Jet Neuf was obtained as a result of such backcrossings and single plant selections. Consequently some genes for resistance are thought to occur on either the A-genome, or that recombination between the B and C-genomes occured more readily than between A and B-genomes (Sacristan & Gerdemann 1986). These investigations however, centered on the transfer of resistance interspecifically but provided little knowledge on the nature of the resistance.

2.6.3.0 Genetics of host resistance

Knowledge regarding the genetics of resistance in the Brassicaceae to L. maculans is scanty (Hill & Williams 1989). Genetic studies are important steps towards effective breeding for disease resistance. Few studies have been done on either seedling or adult plant resistance in oilseed rape to L. maculans, and little is known about the mechanism of disease resistance.

2.6.3.1 Seedling resistance

The few studies to date are limited to the nature of host resistance. Thurling & Venn (1977) observed that in B. napus seedling resistance to blackleg was continuous. However, significant interactions occurred between the isolates of L. maculans and B. napus; being particularly significant for latent period, duration of infection and partial

field resistance. Cargeeg & Thurling (1980a) suggested that the resistance of B. napus to L. maculans is polygenic in nature. Similarly the rate of growth of the fungus on calli of B. napus cultivars, Jet Neuf and Lesira, also varied quantitatively (Sacristan 1982).

As a result of genetic studies, Delwiche (1980) suggested that resistance in B. napus to L. maculans was oligogenic rather than polygenic in nature. She reported that resistance to L. maculans in two B. napus cultivars was controlled by two dominant linked genes. Differential interaction between isolates of the fungus and cultivars of B. napus occurred (Delwiche 1980). This study was only done in the greenhouse and was limited to seedling plants.

Contradictory results were obtained by Sawatsky (1989). She conducted studies on resistance of B. napus breeding lines 'R8314, R8317' (resistant) and the cultivar 'Regent' (susceptible) to isolate 'Pl85-10' of L. maculans in the greenhouse. Sawatsky (1989) reported that a single recessive gene detemined the seedling resistance of both resistant parents in the greenhouse.

2.6.3.2 Adult plant resistance

Adult plant resistance is being used to control blackleg disease in Europe and Australia. Partial adult plant resistance was found in B. napus derived from cell cultures (Sacristan 1982).

Mithen and Lewis (1988) studied the inheritance of resistance to L. maculans in crosses between B. oleracea and Brassica insularis Moss. They reported that two dominant and independent genes controlled the inheritance of resistance to L. maculans in the hybrid. Sawatsky (1989) also studied resistance in adult plants of the crosses 'R8314 x Regent', 'R8317 x Regent', resistant x susceptible cultivars respectively, both in the field and greenhouse. Resistance of the adult F_1 plants in the field was intermediate between that of the crossed parents. The F_2 population from the cross

'R8314 x Regent' fitted a 9:7 ratio of resistant to susceptible, when the intermediate class was grouped with the susceptibles, for 4 of the 6 families; the other two families had reversed ratios (Sawatsky 1989). Crosses involving R8317 and Regent had only two families, one fitting a 3:1 and the other had excess resistant plants (Sawatsky 1989). In greenhouse studies, resistance was dominant in the F_1 population, and the F_2 populations could fit a 9:7, 15:1 and a 9:6:1 ratios when the phenotypes are grouped into resistant : (intermediate + susceptible), (resistant + intermediate) : susceptible, and resistant : intermediate : susceptible respectively (Sawatsky 1989). Field results were not grouped to match the greenhouse groupings except for one, i.e. resistant : (intermediate + susceptible). The F_2 population from the cross 'R8317 x Regent' fitted a 3:1 ratio similar to one family from the same cross tested in the field. Also both tests fitted a 9:7 ratio when the intermediates are grouped with the susceptible. Sawatsky (1989) concluded by reporting that two dominant genes conferred the resistance in B. napus to L. maculans, and that a single gene conferred the intermediate reaction. She attributed the variations to genetic background and environmental differences. These studies suggest that the resistance in oilseed rape to L. maculans is oligogenically controlled.

2.6.4 Host specialization

Evidence for specific interactions between the host and the pathogen have been reported (Thurling & Venn 1977, Cargeeg & Thurling 1980a, Delwiche 1980, Newman 1984, Hammond & Lewis 1987a, Hammond & Lewis 1987c). Such differential interactions as reported can be grouped into three classes: Those expressed at the cotyledon stage (Delwiche 1980), on stems (Thurling & Venn 1977, Hammond & Lewis 1987b) and on the leaves (Wratten and Murray 1977, Hammond & Lewis 1987c, Mithen et al. 1987).

Thurling & Venn (1977) tested 53 cultivars of B. napus and B. rapa at the adult stem stage with three populations of L. maculans and reported significant interactions between them. Thirty six of the cultivars were classified as susceptible to all the three populations, while some were resistant to only one population, but either susceptible or moderately resistant to the other two populations. Only one cultivar (Zollerngold) was resistant to all the three populations. All cultivars were susceptible at the cotyledon stage. Hammond & Lewis (1987b) observed that only the aggressive isolate Lm1 invaded the pith of B. napus cv. Rapora, whereas the weakly aggressive isolate Lm3 was restricted to the stem cortex. They also reported that , when challenged with the three isolates of L. maculans, the leaves of B. napus cv. Rapora reacted differentially. The resistant reaction was expressed in the young leaves but lost with leaf age, and at higher temperatures the incidence of infection was lower (Hammond & Lewis 1987c).

Such reports of interactions between rapeseed and L. maculans, while suggesting that several isolates of the pathogen may occur where rapeseed is widely grown, caution one about the dangers of moving infected plant material between wide geographical areas e.g. Australia and Canada.

2.6.5 Pathogenicity

Leptosphaeria maculans occurs both as aggressive and non-aggressive populations on Brassica spp. in Canada (McGee & Petrie 1978, Petrie 1979) and in Great Britain (Humpherson - Jones 1983b). The aggressive populations attack crops in their early growth causing serious damage while the non-aggressive populations attack crops later in their growth causing very little damage (Martens et al. 1984). Aggressive populations may cause very little damage to the plants if infection occurs in the advanced stage of growth (Hammond & Lewis 1986b). Severe outbreaks of the disease caused by aggressive populations occurred in Australia on oilseed rape of Canadian origin and in

the United States on cabbage (Gabrielson 1974). It was suggested that the relationship between periods of inoculum availability and crop susceptibility were more favourable for disease development in Australia than in Canada, where the pathogen was less aggressive (McGee & Petrie 1978). In 1975, an aggressive isolate similar to that occurring in Australia and the U.S.A. was reported in Canada (McGee & Petrie 1978, Petrie 1979).

2.7.0 Mechanisms of resistance

Disease resistance mechanisms may involve factors related to the environment of the interacting genotypes, other than nutrients. These mechanisms may be expressed during the pre-penetration period or after penetration (Goodman et al. 1986), resulting in sub-optimal associations between the host-parasite genotypes (Bailey 1983). Sound breeding strategies will depend on identification of the pathogen's races and on some knowledge of the genetic systems involved in host-parasite interaction. Resistance mechanisms often involve structural and biochemical processes (Parry 1990). Little is known about the morphological, physiological and biochemical aspects of the resistance of oilseed rape to L. maculans. Some knowledge regarding the host's resistance mechanisms may allow breeders to develop/incorporate new or novel types of resistance.

2.7.1 Morphological mechanisms

Field resistance has been attributed to a number of factors. The rapid loss of cotyledons followed by a quick development of leaves, lignification and vessel blockage in resistant rapeseed, and early differentiation of the xylem and woody tissues (Hammond & Lewis 1987a) have all been thought to inhibit the development of the fungus in oilseed rape - L. maculans pathosystem. Recent reports show that host

colonization is systemic and ends with the necrotic stem canker phase (Hammond et al. 1985, Hammond & Lewis 1986b). In the foliar necrotic phase, the fungus is restricted to the intercellular spaces of the parenchyma and the adjacent cells; the fungus does not kill the cells in advance and the necrotic cells are several millimeters behind the hyphal front; an indication of biotrophic invasion. These findings imply that breeding for leaf and stem resistance could prevent systemic infections (Hammond & Lewis 1986b) and consequently, canker development. In a breeding program at Wagga, Australia, no complete resistance to leaf infections was found, but the production of pycnidia on some infected leaves was either reduced or prevented (Wratten & Murray 1977). This may reduce the initial inoculum and secondary plant infections.

2.7.2 Other Defense Mechanisms

In general, penetration of resistant plants often occurs as easily as in susceptible ones, but the active accumulation of anti-microbial (fungitoxic) compounds may interfere with host colonization (Macer 1960, Bailey 1983, Goodman et al. 1986). When invaded, a resistant plant may respond to microbial organisms actively (Goodman et al. 1986) by accumulating fungitoxic compounds such as phytoalexins.

Compounds with antifungal activity that meet the criteria of classification as phytoalexins have recently been reported in B. rapa, R sativus, B. napus and B. juncea (Dahiya & Rimmer 1988, Rouxel et al. 1989). Methoxybrassinin and cyclobrassinin have been shown to accumulate in leaf and stem tissues of B. napus treated with $AgNO_3$ solution or L. maculans suspension (Dahiya & Rimmer 1988). The two compounds differ in the speed and duration of accumulation at the infection site, and their effectiveness; methoxybrassinin being faster and effective than cyclobrassinin (Dahiya & Rimmer 1988, Rouxel et al. 1989).

Rouxel et al. (1989) treated leaves of both B. napus (susceptible) and B. juncea (resistant) with both $AgNO_3$ and $CuCl_2$ solutions, and L. maculans suspension. They reported another phytoalexin, brassilexin, that accumulated much faster in the resistant species than in the susceptible one. Consequently Rouxel et al. (1989) suggested that susceptibility could either be due to the levels of the phytoalexins that the pathogen can tolerate, or that Sirodesmin PL actively inhibits the synthesis of the phytoalexin.

Sirodesmin PL is one of the toxins produced by L. maculans which can induce necrosis and symptoms on seedling plants similar to those induced by the pathogen; it is a codeterminant of pathogenicity (Sacristan 1982) and has been used to screen protoplasts and embyo cultures, in the hope that resistance to the toxin may also be expressed as resistance to the pathogen. Inherited resistance in Brassica spp. to L. maculans has not been found using the toxin as a selector (Hill & Williams 1988), but some progress has been made. Sjodin & Glimelius (1989) reported that, regardless of the resistance of the intact plants, protoplasts of test plants were sensitive to the toxin. Insensitivity to the toxin occurred only in the more differentiated tissues such as cell aggregates, leaves or roots of resistant plant material, whereas material susceptible to L. maculans was sensitive to Sirodesmin PL (Sjodin & Glimelius 1989).

Mechanisms of host resistance to either the toxin or the pathogen may differ. The probable occurrence of tolerance to Sirodesmin PL or lack of expression by necrosis, as in biotrophic invasion, or when the pathogen is unable to produce toxins in certain host genotypes will only complicate the matter i.e. resistance or tolerance against the toxin does not imply resistance or tolerance to the pathogen.

2.7.3 Glucosinolates

Increased interest in rapeseed products, both in the domestic and world markets has led plant breeders to select for higher oil and lower glucosinolate contents in varieties

suitable for western Canada (Wetter & Craig 1959). Such improvements while desirable (Heaney & Fenwick 1980a, Fenwick et al. 1983) have been thought to result in increased susceptibility of Brassica spp. to certain diseases (Greenhalgh & Mitchell 1976, Rawlinson 1979).

Intact glucosinolates are non-toxic but when tissues are damaged, the enzyme myrosinase is able to hydrolyse glucosinolates to yield glucose, sulphate ion, isothiocyanates, nitrites and thiocyanates (Fenwick et al. 1983, Underhill 1980). The latter 3 compounds are responsible for the characteristic flavour and pungent taste of the mustards, radishes and horse radishes (Heaney & Fenwick. 1980b, Robbelen & Thies 1980, Underhill 1980, Sang et al. 1984). The hydrolysis products of glucosinolates have been reported to be toxic to insects, bacteria and many foliar pathogens (Nayar & Thorsteinson 1963, Greenhalgh & Mitchell 1976, Fenwick et al. 1983) including L. maculans (Mithen et al. 1986). The hydrolysis products of the indole glucosinolates inhibited the growth of L. maculans in culture (Mithen et al. 1986). Cultivars of B. napus with low incidence of blackleg had high levels of glucosinolates and erucic acid (Hanacziwskyj & Drysdale 1984). The resistance of leaves of the Brassicaceae to L. maculans has been associated with lesion size and the levels of glucosinolates. Plants with small localized lesions had higher levels of the alkenyl glucosinolates than those plants with either large lesions or systemic infections (Mithen et al. 1987). The ability of the host plant to produce allyl isothiocyanates in large amounts contributes to the growth restriction of the fungus (Greenhalgh & Mitchell 1976, Hammond et al. 1985, Mithen et al. 1986). The content of glucosinolates in the leaves of Brassicaceae resistant to L. maculans (small localized lesions) was higher than in the leaves of the susceptible plants; with the alkenyl glucosinolates forming the largest proportion of the total foliar glucosinolates (Greenhalgh & Mitchell 1976, Mithen et al. 1987).

The high levels of flavour volatiles in resistant plants may not be the actual cause of the resistant reactions (Greenhalgh & Mitchell 1976, Fenwick et al. 1983, Holley &

Jones 1985). In the wild species studied, hydroxy butenyls formed the largest proportion of the total glucosinolates, indicating that a different mechanism, other than alkenyl glucosinolates, may be involved in the expression of resistance in these plants. Holley & Jones (1985) used B. juncea species that did not contain inhibitory levels of isothiocyanates but which were not infected with the yeast organism (Nematospora spp.). Also no relationship occurred between concentrations of allyl isothiocyanates and resistance to Plasmodiophora brassicae Woronin (Link & Walker 1943 cited by Holley & Jones 1985). It is probable that breeding for low glucosinolates in the seed of the Brassicae and the level of selections may result in the loss of resistance genes due to genetic linkage between these two characteristics, or that the resistance genes themselves are pleiotrophic. The intensity of selections may also result in the decrease of resistance genes which were originally present (Wood 1986), if such selections are made in the absence of the pathogen. Consequently the resistance genes to L. maculans may have been eliminated with the commercially undesirable traits.

Evaluation of Brassica juncea Czern & Coss accessions for reaction to Leptosphaeria maculans (Desm.) Ces. & de Not.

3.1 Abstract

Two hundred and ninety six accessions of Brassica juncea were evaluated at the cotyledon stage for reactions to two isolates of Leptosphaeria maculans (Plat2, Pl86-14). The consistency of interaction phenotype over time was examined. In addition 258 of the accessions were evaluated at the adult stage for reactions to the isolate Pl86-14, and at harvest time for root infection. Most accessions of B. juncea were resistant at both the cotyledon and adult stages but susceptible to root infection. One accession of B. juncea was susceptible to L. maculans at all stages. The aggressive isolate of L. maculans was re-isolated from roots of infected plants regardless of cotyledon or stem reactions. Interaction phenotypes on cotyledons of 77% of accessions were not consistent over time. Eight lines of B. juncea susceptible to L. maculans at all stages of growth were susbsequently selected from plants whose interaction phenotypes over time were $\geq$ 5.0. Most plants within accessions were resistant at cotyledon and adult stages; however some plants were resistant at the cotyledon stage but susceptible at the adult stage. Further testing of the latter group at only the cotyledon stage, without detaching the cotyledons resulted in infection of the non-inoculated stems. A weak correlation ($r=0.28^{**}$) was found between cotyledon and adult stem tests.

3.2 Introduction

Blackleg disease caused by Leptosphaeria maculans (Desm.) Ces. & de Not. is a considerable threat to the production of oilseed rape in many parts of the world including Canada. Knowledge regarding sources of resistance to and specificity of L. maculans is important for programs involved in breeding for resistance to this disease. Different cultivars of Brassica napus L. and B. rapa L. (syn. B. campestris) have been studied as sources for resistance to L. maculans (Thurling & Venn 1977, Roy 1978). This has resulted in the identification in B. napus, of adult plant resistance to L. maculans (Alabouvette et al. 1974, Lammerink 1979). All B. napus cultivars tested were susceptible at the seedling stage, both in the field and the greenhouse (Helms & Cruickshank 1979). No complete resistance, i.e. at both the seedling and adult plant stages, has been found (Thurling & Venn 1977, Roy 1978, Helms & Cruickshank 1979, Newman & Bailey 1987).

Brassica juncea Czern & Coss (mustard), a species related to B. napus and B. rapa, possesses both seedling and adult plant resistance (Roy 1984, Sacristan & Gerdemann 1986). Resistance to L. maculans has also been identified in Brassica insularis Moss., a wild species from Sardinia (Mithen & Lewis 1988), Brassica carinata Braun (Sacristan & Gerdemann 1986) and in representatives of other Brassicaceae (Sjodin & Glimelius 1988). High levels of resistance have been transferred by interspecific crosses from B. juncea to B. napus (Roy 1984, Sacristan & Gerdemann 1986) and from B. carinata to B. napus (Sacristan & Gerdemann 1986). Only the resistance from B. juncea persisted after the first back cross.

Brassica juncea is an attractive alternative to B. napus and B. rapa. It performs particularly well under warm drier conditions. Its potential as an oilseed crop in Canada is very high (Woods et al. 1991), especially if and when canola quality B. juncea become available (Love 1988). Increased production of canola is threatened by the potential

damage that blackleg can cause. Reports concerning the resistance of B. juncea to L. maculans have generally been based on either observations of the disease reaction in the field, or results of crosses between selected resistant B. juncea plants with other Brassica spp. (Roy 1984, Sacristan & Gerdemann 1986). No formal evaluations of B. juncea have been made to date. Hence, this study was initiated to:

1. evaluate accessions of B. juncea from the collection of the University of Manitoba, for reactions to L. maculans,
2. select susceptible plants for a study of the genetics of resistance of B. juncea to L. maculans and
3. find optimum time for disease measurement at the cotyledon and adult stages.

3.3.0 Materials and methods

3.3.1 Inoculum

Two aggressive isolates (Plat2 & P186-14) of L. maculans from widely different regions in Manitoba were used. The isolates were recognised as aggressive on the basis of their interaction phenotype on, and were maintained by repeated passage through 'Westar', a susceptible B. napus cultivar.

3.3.2 Inoculum preparation

Infested host material was surface sterilized in 5% sodium hypochlorite (household javex) solution for 2-3 minutes, then rinsed thoroughly 2-3 times in sterile distilled water and transferred onto V8-juice agar plates to which 1% streptomycin sulphate and rose bengal (40 mg/L) were added. All plating was done under a laminar

flow hood, and plates were kept under near UV light or cool fluorescent light at room temperature. After 5-6 days, abundantly sporulating cultures were flooded with 5-10 mL of sterile distilled water and their surface gently scraped with a flamed glass slide. The mixture was filtered through 4 layers of sterile cheese cloth into centrifuge tubes and centrifuged for 30 minutes at 4500 rpm. The supernatant was decanted and the pelleted spores stored frozen. When desired, the spore concentrate was thawed, and a few drops resuspended in 10 mL of sterile distilled water. Quantification of spores was done using a haemocytometer and appropriate dilutions made to obtain a final concentration of 1 x 10^7 spore mL^{-1}.

3.3.3 Host material

Two hundred and ninety six accessions of B. juncea randomly selected from the University of Manitoba seed collection were evaluated in this study. Two hundred fifty eight of these accessions were evaluated at both the cotyledon and adult stages. Seeds were planted in flats (5 x 10 jiffy pots) using a soilless mix (Metromix™, W. R. Grace & Co. Ltd., Ajax, Ontario) as the planting medium. Up to 10 seeds from each accession were planted. All flats were watered daily and kept in the growth chamber at 24/20°C day/night temperatures and a 16h photoperiod.

3.3.4 Cotyledon evaluations

Cotyledons were wounded when fully expanded, 6-7 days after planting, and simultaneously tested with both isolates of L. maculans, one to either cotyledon. The 2 isolates were differentiated by marking one cotyledon.

A drop of inoculum (10 uL) was introduced to the wound and the inoculum allowed to dry onto the wounds. Flats were not watered until the following day.

Cotyledons were evaluated for interaction phenotype (IP), 10 days after inoculation using the cotyledon evaluation method (Table 3.1). To determine the optimum time for rating, up to 3 ratings were done for 261 to 296 accessions - on the 10^{th}, 12^{th} and 15^{th} day after inoculation.

3.3.5 Adult plant evaluations

All cotyledons were detached from seedlings to halt disease progress. Up to 10 seedlings per accession were potted (1 seedling per pot) in the soilless mix. Adult plants were inoculated at growth stage (GS) 3 - 3.2 (Harper & Berkenhamp 1975) using the isolate Pl86-14. The inoculum was prepared in similar manner and concentration as for cotyledon evaluation. Fully elongated stem (2^{nd} - 3^{rd}) node intervals were pierced, and the inoculum (10 uL) delivered into the stem via the wound.

Plants were rated for stem lesions weekly for 5 weeks, beginning 10 days after inoculation. The key of Newman (1984) was slightly modified and used for stem evaluations (Table 3.2). Notes were also taken for lesion colour, presence of pycnidia, purpling of stems, wilting of plants and internal darkening of stems. All adult plants were inoculated, rated and kept till harvest, either in a greenhouse or a growth chamber. Growth chambers were maintained at 24/20^{o}C day/night temperature and 16h photoperiod, but the greenhouse temperature varied depending on the season. The greenhouse temperatures for the months of May, June, and July were not as strictly controlled as those for the winter months.

3.3.6 Root evaluations

After harvest, pots were watered thoroughly to facilitate removal of roots. Roots were then rated for presence of infection expressed as darkening of roots (Table 3.3). A

sample of roots was plated on V8-juice agar medium. The medium was prepared as described earlier. Fungal growth was observed for mycelial colour and presence of pycnidia.

3.4 Results

Interaction phenotypes (IP) on cotyledons of B. juncea were observed 8 - 10 days after inoculation with both aggressive isolates of L. maculans. The IP in B. juncea - L. maculans system was slow to develop compared to Westar. Frequently, yellowing and purpling of cotyledons of resistant plants were observed 1 - 10 days after inoculation, but lesions on the susceptible B. juncea were more restricted compared to lesions on B. napus cv. Westar. At the adult stage, greyish-white lesions with abundant pycnidia were observed on susceptible lines. Purple streaks (usually along the length of colonization) and/or limited necrosis (with purple borders) were commonly observed on resistant lines, but no pycnidia formed in the lesions. Rapid development of stem lesions occurred at 4 to 5 weeks after inoculation. Infection of the roots often was preceded by darkening of the pith and resulted in wilting of some inoculated plants. Internal tissues of infected roots were often brown, becoming black at plant maturity. The aggressive isolate of L. maculans was re-isolated from the darkened roots.

The results for all accessions tested against the 2 isolates of L. maculans are presented in Appendix 3.1 (cotyledon reactions) and Appendix 3.2 (cotyledon & adult reactions). One accession of B. juncea (UM3115) was highly susceptible to both isolates at the cotyledon stage (IP=9), on day 10 and to Pl86-14 at the adult stage (IP=8) four weeks after inoculation. All UM3115 plants died. Based on mean IP for plants within accessions, all B. juncea accessions tested were resistant to L. maculans at both the cotyledon and adult stages, when IP 0 = most resistant and IP 9 = most susceptible for

cotyledon reactions, and at the adult stage where IP 0 = most resistant and IP 8 = most susceptible.

The mean IP of the accessions are grouped into classes (0-9). The number of accessions of B. juncea within each class (cotyledon & adult reactions) are summarised in Table 3.4. Three accessions (UM3115, UM3403 & UM3064) had a mean IP greater than 3.0 on first cotyledon rating; but on the third cotyledon rating 72 accessions had a mean IP greater than 3.0 when Pl86-14 was used as inoculum (Table 3.4). Most accessions had low mean IP ratings. At day 10 of cotyledon ratings, 99% of the accessions had mean IP within the range 0-3. When Plat2 was used as inoculum, 96% and 83% of the accessions were rated within the lower (0 - 3) range of the evaluation scale at day 12 and day 15 respectively. When Pl86-14 was used as inoculum, 90% and 72% of the accessions were rated within the range (0 - 3) at day 12 and day 15 respectively. Similarly, at the adult stage, 1 accession had a mean IP rating greater than 3.0, ten days after stem inoculation, compared to 38 accessions with same mean IP rating at week 4 (Table 3.4). The mean IP of most accessions was within the 0 - 5 range in week 1 to week 4, with 85% of the accessions having a low rating (IP 0 - 3) at four weeks.

Based on IP ratings, accessions are grouped by the highest observed IP of plants within accessions (Tables 3.5 and 3.6). Most (288) accessions of B. juncea were rated within the lower range (IP 0 - 3) of the cotyledon evaluation scale at day 10, when Plat2 was used as inoculum, while 280 accessions were rated within the same range (IP 0 - 3) of the scale using Pl86-14 as inoculum (Table 3.5). At the adult stage (week 1), 257 accessions were rated within the lower range (IP 0-3) of the adult stage evaluation (Tables 3.6); with the number of accessions in the lower range of cotyledon and adult stage scale decreasing over time.

Although all accessions of B. juncea were resistant to L. maculans at both the cotyledon and adult stages, the roots of most plants within accessions were infected

regardless of the ratings for cotyledon and stem reactions. Eighty eight percent of the accessions tested in the greenhouse had darkened roots. The aggressive isolate of L. maculans was isolated from darkened roots of 50 accessions of B. juncea sampled from the greenhouse tests.

Since all accessions of B. juncea were resistant to L. maculans, further evaluations for susceptibility were made on the basis of single plants within accessions. The reactions of B. juncea plants at the cotyledon stage over the 3 ratings are summarised in Table 3.7. When Plat2 was used as inoculum, 99% of the plants were rated in the 0 - 3 range at day 10; most of these plants were rated in IP class 0 and 1. At day fifteen, 86% of the plants were rated in the 0 - 3 range; fewer plants were rated in IP class 0 and 1, and more plants were rated in IP class greater than 3.0 compared to day 10. Similar results were obtained using Pl86-14; with fewer plants occurring in IP 0 class compared to Plat2 (Table 3.7). At the adult stage (weeks 1 - 3), most plants were rated within the lower range (IP 0 - 3) of the adult evaluation scale compared to weeks 4 and 5 (Table 3.8). Plants from which cotyledon IP ratings greater than 5.0 were observed after 12 or 15 days were sampled and re-tested with the 2 isolates of L. maculans at the cotyledon stage and with Pl86-14 at the adult stage. Seven lines (plants) UM3001, UM3132, UM3366, UM3403, UM3460, UM3466 & UM3467, were susceptible at both the cotyledon (IP=9) and adult stage (IP greater than 7). The susceptible plant (UM3001) was selected from repeated selfing of a field selected UM3001 with dark roots. A benomyl drench (0.25 g 500 mL^{-1}) was used frequently and/or when desired, to rescue any wilting/dying plants.

A summary of individual plant reactions to L. maculans at both the cotyledon and adult stages indicated that 76% of the plants were resistant at both the cotyledon and adult stages. Some (22%) plants were resistant to L. maculans at the cotyledon stage but susceptible at the adult stage. A sample of these plants was re-tested at the cotyledon stage. The cotyledons were not detached after rating, and stems were not inoculated. Cotyledons were symptomless (IP=0) but infection of stems occurred. Three plants

(0.16%) were susceptible at the cotyledon stage and resistant at the adult stage. However these could be a result of experimental error. A poor correlation was found between cotyledon (day 10) and adult (week 4) reactions (r=0.28, P=0.001).

The IP of 77% of the accessions changed from a lower rating at day 10 to a higher one at day 15 after inoculation. The IP of 59 out of 258 accessions was consistent over the 3 cotyledon ratings. Analysis of variance was performed on the data using plants within accessions as replicates to determine the best time of resistance selection in B. juncea. The IPs at day 15 after cotyledon inoculation had the widest range (IP 0 - 7.0) and the lowest error mean square compared to days 12 (IP 0 - 5.8 $\pm$ 0.53) and 10 (IP 0 - 4.6 $\pm$ 0.50). Likewise IP at week 5 had the widest range (IP 0 - 8.0) and the lowest error mean square compared to week 4 (IP 0 - 7.8 $\pm$ 0.13) week 3 (IP 0 - 7.3 $\pm$ 0.40) and week 2 (IP 0 - 6.7 $\pm$ 0.68). However the occurrence of saprophytic growth at day 15 and week 5 cannot be ruled out. Consequently day 12 and week 4 would be the best time to select for disease resistance.

3.5 Discussion

This study indicates that the resistance of B. juncea to L. maculans is not 'absolute and complete' (sensu Roy 1984, Sacristan & Gerdemann 1986) but that variability for expression of resistance occurs both among plants and at different plant parts (i.e. cotyledon, stem & root). Based on the average IP of plants within accessions, all tested accessions of B. juncea were resistant to L. maculans at the cotyledon and adult stages. Most B. juncea accessions were resistant to L. maculans at both the cotyledon and adult stages, but the roots were susceptible. Wittern et al. (1985) cited by Newman & Bailey (1987) reported darkening of internal crown tissue of oilseed rape, inoculated with L. maculans, regardless of the external symptoms of plants. Roots' susceptibility to L. maculans may prove more damaging than the stem canker phase, especially in wet

fields or in wet years. Hence plants should also be screened for root infection. Aggressive isolates of L. maculans were isolated from roots of B. juncea regardless of their IP ratings. Field root samples were often contaminated with other organisms. Van den Berg et al. (1989) also isolated L. maculans from roots of oilseed rape obtained from the field. In practice it is more likely that early root infections by L. maculans would be confused with other soil borne diseases, e.g. root rots. Since many B. juncea plants which were resistant to L. maculans at the cotyledon and adult stages subsequently showed root infections, the resistance may promote development of virulent pathotypes. Sexual recombination in the woody root tissue (Boerema 1976) may give rise to new pathotypes. It would be desirable to transfer the resistance in B. juncea to L. maculans to susceptible B. napus and B. rapa. However only plants resistant at the cotyledon, stem and root stages should be used in crosses to transfer the resistance genes.

Most plants within accessions resistant to L. maculans at the cotyledon stage were also resistant at the adult stage. Roy (1978) and Sjodin & Glimelius (1988) reported resistance in B. juncea at both the cotyledon and adult stages. In this study, one accession and 8 lines were susceptible at the cotyledons, stems, and roots. Most (7) susceptible B. juncea lines were re-selected from plants with IP equal to or greater than 5.0, twelve to fifteen days after inoculation. The line UM3001 was selected from a field plant in which the roots were susceptible. This suggests incomplete resistance due to heterozygosity of the genotypes. Lesions and sporulation were often restricted in susceptible B. juncea lines compared to B. napus cv. Westar. Restriction of lesion size and sporulation on the susceptible B. juncea lines (e.g. UM3132, UM3001) may imply the involvement of resistance mechanisms other than major genes. Moreover susceptible plants may carry genes for resistance against other isolates of the pathogen. Hence plants should be screened with a range of isolates, or in the field.

A few plants within accessions were resistant to L. maculans at the cotyledon stage (i.e. no necrosis occurred, IP=0) but susceptible at the adult stage. Inoculating only

the cotyledons, but not the stems resulted in stem lesions. This suggests biotrophic infections of the cotyledons. Hammond et al. (1985) reported biotrophic invasion of B. napus leaves in the B. napus - L. maculans system. It is impossible to distinguish a genuine resistant reaction (IP=0) from a biotrophic reaction (IP=0) by eye. Consequently, selection of resistant genotypes should be based on the occurrence of the hypersensitive response (IP=1-3). A hypersensitive response would deprive the pathogen of living cells (Parry 1990) required for the initial biotrophic stage (Hammond et al. 1985).

A longer exposure time is required for adequate separation of resistant and susceptible B. juncea lines. Interaction phenotypes on cotyledons of B. juncea changed over time in most (199/258) accessions tested; tending towards more susceptibility. Susceptible B. juncea lines were subsequently selected from lines derived from some of these accessions, suggesting heterozygosity of these genotypes. This implies incomplete action of the resistance genes or the occurrence of modifying factors. Differences in IP due to homozygosity and heterozygosity of host material have important implications when breeding for disease resistance (Roelfs 1988). If the action of resistance genes is incomplete, or modifying factors are involved, the process of transferring resistance genes to susceptible B. juncea plants or the susceptible B. napus and B. rapa, will be more complicated and lengthy. More information about the genetic control of resistance in B. juncea to L. maculans is required.

At the adult stage, rapid development of lesions occurred 4-5 weeks after stem inoculation, suggesting the involvement of active or physiological resistance factors. The phytoalexins methoxybrassinin, cyclobrassinin and brassilexin have been shown to build up faster in resistant Brassica spp. than in susceptible ones (Dahiya & Rimmer 1988, Rouxel et al. 1989). This may increase time for disease to be manifested depending on the duration, effectiveness and concentration of the phytoalexins. Further investigation into the components of resistance in B. juncea to L. maculans is required.

Table 3.1. Interaction phenotype classes on cotyledons of Brassica spp. after inoculation with isolates of Leptosphaeria maculans (Delwiche, 1980).

Class	Description
0	No darkening around wound. Typical response of non-inoculated cotyledons.
1	Limited blackening around wound, lesion diameter is 0.5 - 1.5 mm. Sporulation absent but chlorotic halo may be present.
3	Dark necrotic lesions, 1.5 - 3.0 mm, chlorotic halo may be present but sporulation absent.
5	Lesion diameter is 3 - 6 mm with sharply delineated dark necrotic margins. May show grey-green tissue collapse as in 7 and 9 or may have dark necrosis throughout. There is no sporulation.
7	Grey-green tissue collapse 3 - 5 mm diameter, sharply delimited with non-darkened margin.
9	Rapid tissue collapse about 10 days followed by profuse sporulation in large (greater than 5 mm) lesions that have diffuse non-darkened margins.

Table 3.2. Interaction phenotype classes on adult stems of Brassica species inoculated with Leptosphaeria maculans. Rating scores are based on length and circumference of stem lesions obtained by addition of scale (C+L) (Newman, 1984).

Score (S)	Lesion circumference or stem girdling (C)	Lesion length on stem (L)
0	no infection	no infection
1	less than 25% girdling	less than 10 mm
2	25 - 49% girdling	10 - 19 mm
3	50 - 74% girdling	20 - 29 mm
4	75 - 100% girdling	greater than 30 mm
5	plant dead	plant dead

Table 3.3. Interaction phenotype classes on roots of Brassica juncea after inoculation at cotyledon and adult stage with isolates of Leptosphaeria maculans. Ratings are based on percentage root darkening after harvest.

Score (S)	Percent root darkening (circumference)
0	no darkening (no disease)
1	less than 10% darkening
3	10% - 24% darkening
5	25% - 49% darkening
7	50% - 74% darkening
9	75% - 100% darkening

Table 3.4. Number of Brassica juncea accessions in each mean interaction phenotype group, when challenged at the cotyledon and adult stages with two isolates of Leptosphaeria maculans.

		Interaction phenotype groupings									
Isolate	Time	0-1	1.1-2	2.1-3	3.1-4	4.1-5	5.1-6	6.1-7	7.1-8	8.1-9	Total
	Day				(Cotyledon)						
Plat2	10	259	28	7	1	0	0	0	0	1	296
	12	202	49	34	7	3	0	0	0	1	296
	15	137	39	39	23	15	2	3	0	1	259
Pl86-14	10	236	42	15	2	0	0	0	0	1	296
	12	153	63	51	18	7	3	0	0	1	296
	15	66	55	66	28	31	9	3	0	1	259
Pl86-14	Week				(Adult stage)						
	1	257	0	0	0	0	1	0	0	0	258
	2	212	34	1	0	0	1	0	0	0	258
	3	160	60	13	22	2	0	0	1	0	258
	4	72	115	33	14	11	12	0	1	0	258

Table 3.5. Reactions of Brassica juncea accessions at the cotyledon stage to two isolates of Leptosphaeria maculans. Values are number of accessions grouped by highest occurring interaction phenotype taken over time.

		Interaction phenotype						
Isolate	Time(days)	0	1	3	5	7	9	Total
Plat2	10	77	178	33	3	3	2	296
	12	52	145	59	27	6	7	296
	15	39	96	53	44	20	9	261
Pl86-14	10	70	156	54	9	3	4	296
	12	40	108	74	49	16	9	296
	15	33	29	76	70	39	14	261

Table 3.6. Reactions of Brassica juncea accessions at the adult stage to one isolate of Leptosphaeria maculans (Pl86-14). Values are number of accessions grouped by highest occurring interaction phenotype taken over five weeks.

	Interaction phenotype									
Week	0	1	2	3	4	5	6	7	8	Total
1	195	61	1	0	0	0	0	0	1	258
2	66	117	34	22	6	6	0	0	7	258
3	28	83	49	26	23	17	18	4	10	258
4	27	14	60	54	26	18	9	34	16	258
5	0	0	5	11	4	6	2	48	36	112

Table 3.7. Reactions of Brassica juncea plants at the cotyledon stage to two isolates of Leptosphaeria maculans. Values are percentage plants within each interaction phenotype class taken over time.

Isolate	Time	Interaction phenotype						Total
		0	1	3	5	7	9	
Plat2	10	41.6	52.6	4.54	0.5	0.2	0.6	100
	12	26.8	55.9	13.02	3.3	0.5	0.9	100
	15	21.9	43.1	20.95	10.3	2.6	1.1	100
Pl86-14	10	34.9	54.8	8.7	0.8	0.2	0.7	100
	12	21.2	51.6	17.3	7.6	1.3	1.1	100
	15	18.0	30.7	28.4	16.2	5.4	1.3	100

Table 3.8. Reactions of Brassica juncea plants at the adult stage to one isolate (Pl86-14) of Leptosphaeria maculans. Values are percentage plants within each interaction phenotype taken over five weeks.

Week	Interaction phenotype									Total
	0	1	2	3	4	5	6	7	8	
1	93.67	5.5	0.2	0.4	0.0	0.0	0.0	0.1	0.2	100
2	62.7	28.9	3.6	2.9	0.5	0.7	0.0	0.0	0.7	100
3	38.8	42.2	7.6	4.1	2.4	1.8	1.5	0.5	1.2	100
4	24.9	38.8	16.8	7.9	3.4	2.3	1.1	3.1	1.9	100
5	3.5	26.1	18.8	10.2	4.2	2.6	6.1	21.0	7.7	100

The inheritance of resistance in Brassica juncea Czern & Coss to Leptosphaeria maculans (Desm.) Ces. & de Not.

4.1 Abstract

The inheritance of resistance to two isolates of Leptosphaeria maculans (Plat2, PI86-14) was examined in Brassica juncea. Three resistant parents (UM3021, UM3043, UM3323) were each reciprocally crossed to the susceptible parent (UM3132) and to each other. The parents and F_1 from all the crosses and F_2 from crosses involving the resistant parents and the susceptible parent (UM3132) were evaluated at the cotyledon and adult stages. Similar results were obtained for cotyledon and adult stem reactions. F_3 families of susceptible F_2 plants from the cross involving one resistant parent (UM3021) were also evaluated. Resistance of B. juncea to L. maculans was controlled by two nuclear genes with dominant recessive epistatic action in all three resistant lines. This conclusion is supported by the fact that some F_3 families derived from susceptible F_2 plants segregated for resistance in a ratio of 1 resistant : 3 susceptible. No segregation occurred in the progeny of crosses between resistant parents.

4.2 Introduction

The stem canker phase of blackleg disease caused by Leptosphaeria maculans (Desm.) Ces. & de Not. is important in many oilseed rape producing areas of the world, as far as yield reduction is concerned (McGee & Emmett 1977, Rawlinson & Muthyalu 1979, Newman & Bailey 1987). Most oilseed rape cultivars in Canada are susceptible to blackleg. In Europe a few cultivars provide adult plant resistance. Knowledge regarding the genetics of resistance in Brassica spp. to L. maculans would be an important step towards effective breeding for disease resistance in oilseed rape.

The resistance of Brassica napus L. to L. maculans was suggested to be polygenically determined (Wratten & Murray 1977, Cargeeg & Thurling 1980a) based on the observed disease development in the field. However, Delwiche (1980) carried out genetic studies in the greenhouse using two cultivars of winter oilseed rape (B. napus) and reported that one dominant gene conferred cotyledon resistance to L. maculans. More recently, Sawatsky (1989) suggested that one recessive gene controlled seedling resistance in summer rape lines to L. maculans, and that two dominant genes conferred adult plant resistance. Mithen & Lewis (1988) reported two dominant genes that control resistance to L. maculans in hybrids of Brassica oleracea L. and Brassica insularis Moss.

Brassica juncea Czern & Coss is a potential oilseed crop in western Canada (Woods et al. 1991) and is more resistant to blackleg at both the seedling and adult plant stages than either B. rapa or B. napus (Roy 1984, Sacristan & Gerdemann 1986). Resistance to L. maculans has been transferred from B. juncea to other Brassica spp. by interspecific hybridization (Roy 1984), but knowledge regarding the inheritance of resistance in B. juncea to L. maculans is not available. This study was undertaken to determine the genetic control of resistance in B. juncea to L. maculans.

4.3.0 Materials and methods

4.3.1 Host material

Three accessions of B. juncea (UM3021, UM3323 & UM3043) were used as resistant parents and the accession UM3132 was used as the susceptible parent. All parents were single plant selections selected for resistance or susceptibility based on the interaction phenotype (IP) at both cotyledon and adult stages, growth stages (GS) 1, and GS 3 - 3.2 (Harper & Berkenkamp 1975) respectively. Resistant and susceptible plants used in the genetic study were S_2 plants. The B. napus cultivar 'Westar' was included in all tests as a susceptible check.

4.3.2 Crossing procedure

Reciprocal crosses were made between resistant and susceptible, and between resistant and resistant plants. In addition, each resistant and susceptible plant was selfed to obtain S_3 progeny. Selfed and crossed flowers were isolated in glycine bags to avoid pollen cross contamination. Fifty F_1 plants from each reciprocal cross were tested at the cotyledon stage (GS 1); and from each reciprocal cross 1 to 7 F_1 plants were randomly selected. These F_1 plants (1 - 7) were tested at the adult stage (GS 3 - 3.2), grown to maturity, and selfed to obtain F_2 progeny of each cross. F_2 plants (26-136) from each F_1 plant were also selfed to obtain F_3 seeds. The parents (S_2 & S_3), the F_1 and F_2 plants were all tested with the 2 isolates of L. maculans (Plat2 & Pl86-14) at the cotyledon stage and with Pl86-14 at the adult stage. F_2 plants of resistant x resistant crosses were not tested at adult stage. F_3 lines derived from susceptible F_2 plants of the cross (UM3021 x UM3132) were also tested only at the cotyledon stage.

Reciprocal crosses between different parent plants are numbered using arabic numerals (1 to 7); where more than one F_1 plant has been selected from a given parental cross, the F_2 families derived therefrom are designated by identical arabic numerals but different alphabet (e.g. 1A, 1B to 1G). F_2 families derived from different F_1 plants of different parental crosses are designated by different arabic numerals and may have identical alphabet letters (e.g. 1A, 2A to 7A).

4.3.3 Inoculum

Two aggressive isolates of L. maculans (Plat2 & Pl86-14) selected from the collection of the University of Manitoba were used in this study. The isolates were from widely separated regions of Manitoba and were maintained by repeated passage through Westar.

4.3.4 Inoculum preparation

Infected host material was surface sterilized in 5% sodium hypochlorite (household javex) solution for 2 - 3 minutes, then rinsed 2 - 3 times in sterile distilled water and transferred onto V8-juice agar plates to which 1% streptomycin sulphate and rose bengal (40 mg/L) were added. All plating was done under a laminar flow hood, and plates were kept under near UV light and/or cool fluorescent light at room temperature. After 5 - 7 days, sporulating cultures were flooded with 5 -10 mL of sterilized distilled water and their surface gently scraped with a flamed glass slide to release the pycnidiospores. The mixture was then filtered through 4 layers of sterilized cheese cloth into centrifuge tubes and centrifuged for 30 minutes at 4500 rpm. The supernatant was decanted and the peleted spores stored at -10°C to -15°C. When desired, the spore concentrate was thawed and a few drops re-suspended in 10 mL of sterile distilled water.

Quantification of spores was done using a haemocytometer and appropriate dilutions made to obtain a final concentration of $1x10^7$ spore mL^{-1}.

4.3.5 Cotyledon evaluations

Cotyledons were wounded when fully expanded, 6-7 days after planting and simultaneously inoculated with both isolates of L. maculans, one to either cotyledon. A drop of inoculum (10 uL) was introduced to the wounds and the inoculum allowed to dry onto the wounds. Plants were not watered until the following day. Ten days after inoculation, cotyledons were rated using a scale of 0 to 9, where 0 = no disease and 9 = very susceptible (Williams 1985). Resistant and susceptible parents were each re-tested for range and uniformity of reactions using both isolates of L. maculans. All cotyledon evaluations were done in growth chambers, maintained at 24/20°C day/night temperatures, and 16 hour photoperiod.

4.3.6 Adult plant evaluations

Adult plants were inoculated at GS 3 - 3.2 using pycnidiospores of the isolate Pl86-14. The inoculum (10 uL) was injected into the lower portion of the stem, (2nd - 3rd) node above the crown using a hypodermic syringe. Plants were first rated 10 days after inoculation and followed by weekly ratings over four weeks. The key of Newman (1984) was slightly modified (p. 31) and used for stem evaluations. Adult plants were inoculated, rated and kept till harvest either in the growth chamber or the greenhouse. Growth chambers were maintained at 24/20°C day/night temperature but the greenhouse temperatures varied depending on the season. The greenhouse temperatures for the months of May, June and July were not as strictly controlled as those for winter months.

4.3.7 Statistical analysis

The Chi-square test for goodness of fit was used to analyse the F_2 and F_3 segregation data. F_2 families of the same cross were tested for homogeneity using the Chi-square test.

4.4.0 Results and discussion

4.4.1 Parental tests

The mean IPs' of the 3 resistant parents following cotyledon inoculation with isolates of L. maculans were less than 0.7 $\pm$ 0.14 (Table 4.1, S_2 lines). The mean IP for susceptible parents was greater than 8.3 $\pm$ 0.18. Similar results were obtained in tests of the selfed parental material (Table 4.1, S_3 lines). The mean IPs' of the selfed resistant parents were equal to or less than 0.3 $\pm$ 0.05. Susceptible parents had a mean IP greater than 8.6 $\pm$ 0.07 (Table 4.1). Interaction phenotypes (IP) were consistent even after 10 days from first rating. At the adult stage (four weeks after inoculation), the adult IP (AIP) for resistant and susceptible parents were greater than 0.6 $\pm$ 0.4 and 7.0 $\pm$ 0.32 respectively. The expression of a resistant or susceptible IP at the cotyledon stage in the B. juncea - L. maculans pathosystem occurs within a 0 - 3 score for resistant and 5 - 9 for susceptible genotypes respectively (Table 4.1). At the adult stage, AIP for resistant parents occurred within the 0 - 3 range and for susceptible parents within the 6 - 8 range, based on the data from the parents. No segregation for resistance or for susceptibility occurred in the (S_2 & S_3) progeny of either the susceptible or resistant parents.

4.4.2 F_1 (susceptible x resistant crosses)

The mean IP of the F_1 populations from the susceptible x resistant crosses and their reciprocals were between 0.02 and 1.28 (Table 4.2). Most scores for F_1 reactions were within the two lowest ranges of the rating scale (IP 0-1) compared to the parents (IP 0-3). All F_1 progeny from the UM3323 & UM3043 crosses with susceptible parent (UM3132) were uniformly resistant (IP 0 - 1). However segregation for susceptibility occurred in the F_1 from the cross UM3021 x UM3132 indicating heterozygosity in UM3021 (Table 4.2). When tested at the adult stage, AIP 4 - 5 were often accompanied by purple streaks and in some cases sporulation occurred with plants four weeks after inoculation. Consequently AIP 4 and 5 are also considered as susceptible AIP. No differences occurred between reciprocal crosses of resistant and susceptible plants. Based on the F_1, the resistance in B. juncea (test lines) to L. maculans is controlled by dominant nuclear genes. This conforms with the dominant gene control in other Brassica - L. maculans pathosystems (Delwiche 1980, Mithen & Lewis 1988) but is in contrast to the recessive gene control in the B. napus - L. maculans system (Sawatsky 1989). Resistant and susceptible IP for the parents and F_1 were consistent at both cotyledon and adult stage, hence only one set of data is presented.

4.4.3 Resistant x resistant crosses

Data for F_1 and F_2 populations of crosses between all the resistant parents and their reciprocals are presented in Tables 4.3 and 4.4 respectively. F_2 populations of the crosses between the resistant parents (UM3323 x UM3021) and (UM3323 x UM3043) and their reciprocals were all resistant and similar to the responses of the F_1 and their respective parents. This indicates that the resistance genes in UM3323 are allelic to those in UM3021 and UM3043. Crosses between UM3021 and UM3043 and their reciprocals

were predominantly resistant (Table 4.4). One or two plants with IP 7 were observed in some crosses, but these could be due to epistatic effects and/or some unknown environmental effects, associative and dissociative effects of the genes, or even an experimental error. Nevertheless based on the fact that these IPs in the F_2 did not cover the range of IP defined earlier as susceptible, no segregation for susceptibility was inferred in crosses between resistant parents. These results indicate that the resistant genes in the three resistant parents (UM3323, UM3043, UM3021) are allelic.

4.4.4 Pooled F_2 (susceptible x resistant crosses)

Chi-square tests for homogeneity of variances gave either significant values and/or non-significant values for 13:3 and/or 3:1 ratios depending on individual parents of a cross. Pooled F_2 segregation ratios for the crosses UM3323 x UM3132 (Table 4.5), UM3021 x UM3132 (Table 4.6) and UM3132 x UM3043 (Table 4.7) fit a 13:3 ratio of resistant : susceptible plants. However, in the reciprocal crosses, the pooled F_2 segregation ratios for some crosses of UM3132 x UM3323 (Table 4.8) and UM3132 x UM3021 (Table 4.9) fit either 3:1 and/or 13:3 ratios. The hypothesis that two genes with dominant recessive epistatic interaction may be involved was tested by screening for resistance in the progeny of susceptible F_2 families. Segregation for resistance occurred in F_3 families of susceptible F_2 plants of the cross UM3132 x UM3021, fitting 1:3 or 0:1 ratios of resistant : susceptible plants (Table 4.10). These results indicate that resistance to L. maculans in B. juncea lines UM3323, UM3043, UM3021 is controlled by two nuclear genes with dominant recessive epistasis. The two gene control of resistance of B. juncea to L. maculans is consistent with the two gene system reported for other Brassica species by Delwiche (1980), Mithen & Lewis (1988) and Sawatsky (1989). Delwiche (1980) reported two linked genes that determine the resistance of B. napus to L.

maculans, but Sawatsky (1989) reported two epistatic dominant genes controlling adult resistance in B. napus to L. maculans. No F_3 segregations were studied in either case.

4.4.5 F_2 populations (susceptible x resistant crosses)

The F_2 segregation of 13 resistant : 3 susceptible can be explained by a two resistance gene system in which one gene (A) when homozygous recessive is epistatic to the other gene (B) when dominant. The genotype (aa) in the presence of a dominant gene (B) will confer susceptibility; the homozygous gene (BB) may be more susceptible than the heterozygous gene (Bb); and the homozygous recessive gene (bb) will confer resistance when associated with the genotype (aa). The genotypes of both susceptible and resistant parents of particular crosses will certainly influence the resulting segregation ratios. In this study, F_2 segregation ratios for the cross UM3323 x UM3132 fit a 13:3 ratio, indicating that UM3323 is uniform for same parental genotype (Table 4.5). They were allocated the genotypes (AAbb x aaBB) respectively. The reciprocal cross UM3132 x UM3323 fit both 13:3 and 3:1 ratios (Table 4.8). Crosses 1-3 which fit the same ratio (13:3) were allocated the genotypes (aaBB x AAbb). Crosses 4 and 5 fit 13:3 and 3:1 ratios (Table 4.8). Based on no segregation in parents and the F_1, the genotypes postulated for susceptible and resistant parents are (aaBB x AABb) respectively.

Data for the cross UM3132 x UM3043 are presented in Table 4.7. F_2 ratios for crosses 3 and 5 fit a 13:3 ratio. The genotypes of the respective parents are aaBB x AAbb. Crosses (1, 2, 4, 6 and 7) fitting both 13:3 and 3:1 were allocated the genotypes (aaBB x AABb) for susceptible and resistant parents respectively.

No segregation was observed in parents UM3132 and UM3021; however a few susceptible plants occurred in some F_1 populations of the cross UM3021 x UM3132 and their reciprocals. UM3021 x UM3132 F_2 crosses 1 to 5 fit 13:3 ratio (Table 4.6); hence

the resistant and susceptible parents were allocated the genotype AAbb x aaBB respectively. However, the cross UM3132 x UM3021 fit either 13:3, 3:1 or 1:3 ratios (Table 4.9). Segregation for resistance occurred in the F_3 families of susceptible F_2 plants (including samples from the population fitting a 1:3 ratio), hence providing support for the hypothesis of dominant recessive epistasis (Table 4.10). However, some F_3 families had reversed segregation ratios. This may have been caused either by unknown environmental conditions, differences in the genetic background or other unknown host-parasite interaction factors. The genotypes of parents for the cross UM3132 x UM3021 are summarized in Table 4.11.

In most cases, in this study the B. juncea cotyledon reactions, IP 0-3 and IP 5-9 were grouped as resistant and susceptible IP respectively. At the adult stage the occurrence of extensive purple streaks make determination of AIP more difficult. In this study AIP 0-3 were grouped as resistant and AIP 4-8 as susceptible. The grouping of IP may influence the postulation of gene action (Sawatsky 1989). The heterozygosity of parents and of the pathogen, and/or differences in the genetic background will also affect the nature of the host-parasite interaction (Ellingboe 1981). High tempteratures may sometimes enhance epistatic interactions (Vanderplank 1984); this may make it difficult to obtain consistent gene action i.e. at cotyledon and adult stages.

The genes for resistance in UM3323 are allelic to those in UM3021 and UM3043. Although some segregation for susceptibility occurred in the F_2 of crosses involving UM3021 and UM3043, the expected 13:3 F_2 segregation ratio was not observed due to excess resistant IP. Possible explainations include, the occurrence of biotrophic infections, epistatic and/or environmental effects, dissociative effects of the resistance genes, differences in the genetic background and probability of a third non-allelic gene.

The two genes identified in this study may both have to be transferred to any susceptible lines if B. juncea type resistance is to be maintained. The dominant gene has major effects on the expression of IP, and the recessive gene has modifying effects on the

expression of the dominant allele. Hence, both dominant and recessive control of resistance are possible depending on the genotype of the original parent. The identification of lines with AABB and aabb genotypes would facilitate future studies into the mechanisms of action of the genes, the associative and dissociative effects of different gene combinations and/or environment on host-parasite interaction. The genotype aabb can easily be obtained in F_3 families of susceptible F_2 plants and the genotype AABB may be identified by a testcross. All the F_1 will be resistant and all the F_2 will fit a 13:3 ratio. However it will be necessary to select many F_1 families per cross in order to differentiate between the genotype AABB and AABb. Furthermore, separation of the two genes into single gene lines may facilitate the development of differential series.

Table 4.1. Plant number, interaction phenotype, disease severity and frequency of phenotype for Brassica juncea lines challenged at the cotyledon stage with Leptosphaeria maculans*.

Parent number	Reaction[1] R	S	Total	Means ± SE[2]	Frequency of phenotype 0	1	3	5	7	9
S_2 lines										
UM3021	47	0	47	0.45 ± 0.139	36	6	5	0	0	0
UM3043	46	0	46	0.63 ± 0.140	27	14	5	0	0	0
UM3323	54	0	54	0.61 ± 0.114	29	21	4	0	0	0
UM3132	0	50	50	8.48 ± 0.179	0	0	0	5	3	42
UM3466	0	43	43	8.54 ± 0.174	0	0	0	3	4	36
UM3467	0	48	48	8.38 ± 0.180	0	0	0	4	7	37
S_3 lines										
UM3021	198	0	198	0.25 ± 0.056	178	7	14	0	0	0
UM3043	196	0	196	0.25 ± 0.050	168	18	10	0	0	0
UM3323	200	0	200	0.30 ± 0.052	172	28	0	0	0	0
UM3132	0	200	200	8.72 ± 0.071	0	0	0	13	2	185
UM3466	0	206	206	8.79 ± 0.058	0	0	0	8	6	192
UM3467	0	188	188	8.66 ± 0.070	0	0	0	11	10	167

* Isolates used = Plat2 and P186-14, both from Manitoba.
1 Reaction (R=resistant, S=susceptible)
2 SE=Standard error.

Table 4.2. Plant number, interaction phenotype, disease severity and frequency of phenotype for F_1 populations from susceptible x resistant cross and their reciprocals, challenged at the cotyledon stage with Leptosphaeria maculans*.

Cross	Reaction[1] R	S	Total	Means ± SE[2]	Frequency of phenotype 0	1	3	5	7	9
3132x3323	50	0	50	0.18 ± 0.055	41	9	0	0	0	0
3323x3132	50	0	50	0.02 ± 0.020	49	1	0	0	0	0
3132x3021	499	41	540	0.68 ± 0.060	427	24	48	41	0	0
3021x3132	159	33	192	1.28 ± 0.140	109	39	11	29	4	0
3132x3043	50	0	50	0.26 ± 0.063	37	13	0	0	0	0
3043x3132	50	0	50	0.38 ± 0.069	31	19	0	0	0	0

* Isolates used = Plat2 and P186-14, both from Manitoba.
1 Reaction (R=resistant, S=susceptible)
2 SE=Standard error.

Table 4.3. Plant number, interaction phenotype, disease severity and frequency of phenotype for F_1 populations from resistant x resistant crosses of Brassica juncea and their reciprocals, challenged at the cotyledon stage with Leptosphaeria maculans*.

Cross	Reaction[1] R	S	Total	Means ± SE[2]	Frequency of phenotype 0	1	3	5	7	9
3323x3021	48	0	48	0.29 ± 0.07	34	14	0	0	0	0
3021x3323	50	0	50	0.22 ± 0.06	39	11	0	0	0	0
3323x3043	46	0	46	0.22 ± 0.06	36	10	0	0	0	0
3043x3323	48	0	48	0.38 ± 0.07	30	18	0	0	0	0
3021x3043	46	0	46	0.43 ± 0.07	26	20	0	0	0	0
3043x3021	50	0	50	0.40 ± 0.07	30	20	0	0	0	0

* Isolates used = Plat2 and P186-14, both from Manitoba.
1 Reaction (R=resistant, S=susceptible)
2 SE=Standard error.

Table 4.4. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (resistant x resistant) challenged with Leptosphaeria maculans.

	Cross	Reaction R	S	Total	Model
1.	3323x3021	651	0	651	1:0
	3021x3323	618	0	618	1:0
2.	3323x3043	585	0	585	1:0
	3043x3323	721	0	721	1:0
3.	3021x3043	128	2	130	1:0
		123	1	124	1:0
		131	1	132	1:0
		126	1	127	1:0
		129	2	131	1:0
		62	1	63	1:0
		132	0	132	1:0
		136	0	136	1:0
	Total	967	8	975	1:0
	Pooled				1:0
4.	3043x3021	133	0	133	1:0
		42	0	42	1:0
		114	1	115	1:0
		130	1	131	1:0
		129	1	130	1:0
	Total	548	3	551	1:0
	Pooled				1:0

NB: Crosses between resistant parents were not tested at adult.

Table 4.5. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (UM3323 x UM3132 crosses) challenged with Leptosphaeria maculans .

Cross	Reaction			X^2			
	R	S	Total	3:1	P	13:3	P
1.	28	4	32	2.667	0.10-0.20	0.821	0.30-0.50
2.	25	4	29	1.943	0.10-0.20	0.446	0.30-0.50
3.	25	5	30	1.111	0.20-0.30	0.079	0.70-0.90
4.	27	2	29	5.069*	0.01-0.05	2.631	0.10-0.20
5.	20	6	26	0.051	0.70-0.90	0.304	0.50-0.70
6.	27	2	29	5.069*	0.01-0.05	2.631	0.10-0.20
Total	152	23	175	15.909		6.912	
Pooled				13.122**	< 0.001	3.603	0.05-0.10
Homogeneity df=5				2.788	0.70-0.90	3.309	0.50-0.70

Table 4.6. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (UM3021 x UM3132 crosses) challenged with Leptosphaeria maculans.

Cross	Reaction			X^2			
	R	S	Total	3:1	P	13:3	P
1A	101	23	124	2.753	0.05-0.10	0.003	>0.95
1B	93	25	118	0.915	0.30-0.50	0.461	0.30-0.50
1C	94	30	124	0.043	0.70-0.90	2.412	0.10-0.20
1D	83	15	98	5.184*	0.01-0.05	0.763	0.30-0.50
1E	79	19	98	1.646	0.10-0.20	0.026	0.70-0.90
1F	104	15	119	9.751**	0.001-0.01	2.951	0.05-0.10
1G	88	27	115	0.142	0.50-0.70	1.688	0.10-0.20
Total	642	154	796	20.434		8.304	
Pooled				18.004**	< 0.001	0.190	0.70-0.90
Homogeneity df=6				6.866	0.30-0.50	8.114	0.20-0.30
2A	58	7	65	7.021**	0.001-0.01	2.718	0.05-0.10
2B	81	18	99	2.455	0.10-0.20	0.021	0.70-0.90
2C	64	16	80	1.067	0.20-0.30	0.082	0.70-0.90
2D	73	19	92	0.928	0.30-0.50	0.219	0.50-0.70
2E	45	10	55	1.364	0.20-0.30	0.012	0.90-0.95
Total	321	70	391	12.833		3.050	
Pooled				10.406**	0.10-0.20	0.183	0.50-0.70
Homogeneity df=4				2.427	0.50-0.70	2.867	0.50-0.70
3A	79	19	98	1.646	0.10-0.20	0.026	0.70-0.90
3B	91	24	115	1.046	0.20-0.30	0.339	0.50-0.70
3C	104	20	124	5.204*	0.01-0.05	0.559	0.30-0.50
3D	104	15	119	9.751**	0.001-0.01	2.951	0.05-0.10
3E	91	20	111	2.886	0.05-0.10	0.038	0.70-0.90
Total	469	98	567	20.533		3.912	
Pooled				18.004**	< 0.001	0.798	0.30-0.50
Homogeneity df=4				2.529	0.50-0.70	3.114	0.50-0.70

Table 4.6. (continued)

4A	94	18	112	4.762*	0.01-0.05	0.528	0.30-0.50
4B	36	11	47	0.064	0.70-0.90	0.668	0.30-0.50
4C	45	5	50	6.000*	0.01-0.05	2.513	0.10-0.20
4D	21	5	26	0.057	0.70-0.90	0.004	0.95
4E	73	9	82	8.602**	0.001-0.01	3.253	0.05-0.10
Total	269	48	317	19.484		6.966	
Pooled				16.430**	< 0.001	2.692	0.10-0.20
Homogeneity df=4				3.054	0.50-0.70	4.274	0.30-0.50
5A	97	14	111	9.084**	0.001-0.01	2.745	0.05-0.10
5B	91	20	111	2.906	0.05-0.10	0.039	0.70-0.90
5C	87	18	105	3.457	0.05-0.10	0.178	0.50-0.70
5D	80	11	91	8.092**	0.001-0.01	2.651	0.10-0.20
Total	355	63	418	23.539		5.613	
Pooled				21.975**	< 0.001	3.715	0.05-0.10
Homogeneity df=3				1.564	0.50-0.70	1.898	0.50-0.70

Table 4.7. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (UM3132 x UM3043 crosses) challenged with Leptosphaeria maculans at the cotyledon stage.

Cross	Reaction			X^2			
	R	S	Total	3:1	P	13:3	P
1.	67	15	82	1.972	0.10-0.20	0.011	0.90-0.95
2.	88	18	106	3.635	0.05-0.10	0.218	0.50-0.70
3.	91	16	107	5.760*	0.01-0.05	1.013	0.30-0.50
4.	89	19	108	3.161	0.05-0.10	0.095	0.70-0.90
5.	89	14	103	7.149**	0.001-0.01	1.799	0.10-0.20
6.	79	22	101	0.558	0.30-0.50	0.610	0.30-0.50
7.	76	21	97	0.581	0.30-0.50	0.535	0.30-0.50
Total	579	125	704	22.816		4.281	
Pooled				19.705**	< 0.001	0.456	0.50-0.70
Homogeneity df=6				3.111	0.70-0.90	3.823	0.70-0.90

NB: No reciprocal Crosses for this cross were tested.

Table 4.8. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (UM3132 x UM3323 crosses) challenged with Leptosphaeria maculans.

	Cross	Reaction R	Reaction S	Total	X^2 3:1	P	13:3	P
1.	A	72	14	86	3.488	0.05-0.10	0.345	0.50-0.70
	1B	52	18	70	0.019	0.70-0.90	2.229	0.10-0.20
	Total	124	32	156	3.508		2.573	
	Pooled				1.675	0.10-0.20	0.330	0.50-0.70
	Homogeneity df=1				1.832	0.10-0.20	2.244	0.10-0.20
2.	A	57	12	69	2.130	0.10-0.20	0.084	0.70-0.90
	2B	74	19	93	1.036	0.20-0.30	0.172	0.50-0.70
	2C	71	18	89	1.082	0.20-0.30	0.127	0.70-0.90
	2D	58	15	73	0.772	0.30-0.50	0.155	0.50-0.70
	2E	42	15	57	0.053	0.70-0.90	2.142	0.10-0.20
	Total	302	79	381	5.073		2.680	
	Pooled				3.696	0.05-0.10	0.996	0.30-0.50
	Homogeneity df=4				1.377	0.70-0.90	1.684	0.70-0.90
3.	A	35	14	49	0.333	0.50-0.70	3.083	0.05-0.10
	3B	37	10	47	0.348	0.50-0.70	0.202	0.50-0.70
	3C	36	11	47	0.058	0.70-0.90	0.677	0.30-0.50
	3D	39	10	49	0.551	0.30-0.50	0.086	0.70-0.90
	3E	37	12	49	0.007	0.90-0.95	1.049	0.30-0.50
	Total	184	57	241	1.297		5.097	
	Pooled				0.234	0.50-0.70	3.792	0.05-0.10
	Homogeneity df=4				1.063	0.70-0.90	1.305	0.70-0.90
4.	A	37	12	49	0.007	0.90-0.95	1.049	0.30-0.50
	4B	46	11	57	0.988	0.30-0.50	0.010	0.90-0.95
	4C	39	19	58	1.862	0.10-0.20	7.412**	<0.01
	4D	34	14	48	0.444	0.50-0.70	3.419	0.05-0.10
	4E	38	10	48	0.444	0.50-0.70	0.137	0.70-0.90
	Total	196	66	260	3.745		12.027	
	Pooled				0.020	0.50-0.70	7.513**	< 0.01
	Homogeneity df=4				3.725	0.30-0.50	4.514	0.30-0.50
5.	A	39	10	49	0.551	0.30-0.50	0.086	0.70-0.90
	5B	35	15	50	0.667	0.30-0.50	4.109*	0.01-0.05
	5C	32	19	51	4.085*	0.01-0.05	11.339**	<0.01
	5D	34	16	50	1.307	0.20-0.30	5.507*	0.01-0.05
	5E	41	10	51	0.791	0.30-0.50	0.021	0.70-0.90
	Total	181	70	251	7.400		21.062	
	Pooled				1.117	0.20-0.30	13.706**	< 0.001
	Homogeneity df=4				6.283	0.10-0.20	7.356	0.10-0.20

Table 4.9. Cross, observed segregation ratios and Chi-square tests for F_2 reactions of Brassica juncea (UM3132 x UM3021 crosses) challenged with Leptosphaeria maculans.

Cross	Reaction			X^2			
	R	S	Total	3:1	P	13:3	P
1.	103	29	132	0.646	0.30-0.50	0.898	0.30-0.50
2.	93	32	125	0.024	0.70-0.90	3.887*	0.01-0.05
3.	91	34	125	0.323	0.50-0.70	5.908*	0.01-0.05
4.	37	85	122	128.847**	< 0.001	207.314**	< 0.001
5.	97	22	119	2.692	0.10-0.20	0.005	0.90-0.95
6.	103	28	131	0.919	0.30-0.50	0.592	0.30-0.50
7.	98	30	128	0.167	0.50-0.70	1.846	0.10-0.20
8.	32	9	41	0.203	0.50-0.70	0.270	0.50-0.70
Total	654	269	923	134.820		220.720	
Pooled				8.454**	< 0.001	65.394**	< 0.001
Homogeneity df=7				126.366**	< 0.001	155.326**	< 0.001
Total#	617	184	801	4.974		13.406	
Pooled				1.758	< 0.10-0.20	9.361*	< 0.001-0.01
Homogeneity df=6				3.216	< 0.70-0.90	4.045	< 0.20-0.30

Cross 4. is excluded from pooled data.

Table 4.10. Family, observed segregation for resistance in Brassica juncea and Chi-square tests for F_3 reaction of susceptible F_2 families to Leptosphaeria maculans.

Family F_2 number	Reaction R	S	Total	Model	X^2	P
3. 3132x3021	11	41	52	1:3	0.231	0.50-0.70
	10	35	45	1:3	0.067	0.70-0.90
	0	44	44	0:1		
	29	15	44	3:1		
	21	23	44	1:3	2.735	0.05-0.10
	11	34	45	1:3	0.007	0.90-0.95
4. 3132x3021	1	44	45	0:1		
	11	31	42	1:3	0.000	> 0.95
	16	32	48	1:3	1.361	0.20-0.30
	14	26	40	1:3	1.633	0.20-0.30
	13	33	46	1:3	0.116	0.70-0.90
	10	32	42	1:3	0.000	> 0.95
7. 3132x3021	19	22	41	1:1		
	10	35	45	1:3	0.067	0.90-0.95
	41	0	41	1:0		
	13	32	45	1:3	0.189	0.50-0.70
	42	3	45	1:0		
	29	16	45	3:1	2.141	0.10-0.20
8. 3132x3021	0	47	47	0:1		
	30	16	46	3:1	1.85	0.10-0.20
	12	34	46	1:3	0.000	> 0.95
	11	29	40	1:3	0.033	0.70-0.90
	16	31	47	1:3	1.596	0.20-0.30
	0	53	53	0:1		

Table 4.11. Observed F_1 interaction phenotype and Chi-square models for F_2 and F_3 reactions of Brassica juncea (UM3132 x UM3021 cross) challenged with Leptosphaeria maculans.

Cross	Reaction F_1	Reaction F_2		F_3*	Proposed genotype of parent plants
1.	Res#	13:3	3:1	++	aaBB x AABb
2.	Seg@		3:1	--	aaBB x AaBB
3.	Seg		3:1	++	aaBB x AaBB
4.	Seg		1:3	++	aaBB x Aabb
5.	Seg	13:3	3:1	--	aaBB x AaBB
6.	Res	13:3	3:1	--	aaBB x AABb
7.	Res	13:3	3:1	++	aaBB x AABb
8.	Res	13:3	3:1	++	aaBB x AABb

#. Res=resistant.
@. Seg=segregating.
*. tested in F_3 = ++; Not tested in F_3 = --.
NB: F_3 families were not tested for adult reactions.

The relationship of the major seed glucosinolates in seed of Brassica juncea Czern & Coss with the resistance to Leptosphaeria maculans (Desm.) Ces. & de Not.

5.0 Abstract

The relationship between levels of seed glucosinolates in Brassica juncea and the resistance to Leptosphaeria maculans was investigated. Levels of glucosinolates in seed of three resistant lines (UM3021, UM3043, UM3323) and three susceptible lines (UM3132, UM3466, UM3467) were determined. Crosses were made between resistant parents and UM3132 and the reactions of the progeny to L. maculans evaluated. Also the levels of glucosinolates in the F_1, F_2 and F_3 seeds were determined. Resistance in B. juncea to L. maculans is controlled by nuclear genes but the levels of the major seed glucosinolates (2-propenyl & 3-butenyl glucosinolates) are controlled by the genotype the maternal plant. Levels of 2-propenyl glucosinolates in seed of resistant lines UM3043 and UM3323 were significantly different from the levels in the susceptible lines and the resistant line UM3021. However levels of 3-butenyl glucosinolates in seeds of the susceptible lines and the resistant parent UM3021 were not significantly different from each other. The 2-propenyl glucosinolates were the dominant seed glucosinolates. There was no relationship between the levels of seed glucosinolates in B. juncea and resistance to L. maculans.

5.1 Introduction

Most Brassica napus L. and Brassica rapa L. (syn. Brassica campestris) cultivars are susceptible at all growth stages to Leptosphaeria maculans (Desm.) Ces. & de Not., the causal organism of blackleg in crucifers. Resistance to L. maculans in oilseed rape may be expressed at the cotyledon (Williams & Delwiche 1980), foliar (Wratten 1977, Hammond & Lewis 1987c, Mithen et al. 1987), or adult (Thurling & Venn 1977, Cargeeg & Thurling 1980b, Hammond & Lewis 1987a) stages. Wratten (1977) and Mithen et al. (1987) attributed the (foliar) resistance in some Brassica spp. to the high levels of glucosinolates in resistant cultivars compared to the susceptible ones. Brassica napus and B. rapa cultivars have a high percentage of 3-butenyl glucosinolate as component of the total glucosinolates. The resistance in B. juncea to L. maculans is reportedly due to high levels of 2-propenyl glucosinolates (Mithen et al. 1987). Most B. juncea cultivars in Canada have a high percentage of the total glucosinolates as 2-propenyl glucosinolates. Lower levels of 2-propenyl glucosinolates in B. oleracea L. cultivars contributed to susceptibility to Peronospora parasitica (Pers. ex Fr.) Fr., the causal organism of downey mildew of cabbage (Holley & Jones 1985). High levels of 2-propenyl glucosinolates in germinating seeds of Brassica spp. protected seedlings from infection by fungi (Holley & Jones 1985).

Development of canola quality B. juncea may result in the expansion of the canola industry in Canada. Reducing the levels of seed glucosinolates genetically may alter crop host resistance to foliar pathogens (Greenhalgh & Mitchell 1976, Rawlinson 1979). Such low glucosinolate cultivars may be threatened by blackleg disease. In this study, the relationship between levels of seed glucosinolates in B. juncea with resistance to L. maculans is investigated.

5.2.0 Materials and methods

5.2.1 Inoculum

Two aggressive isolates (Plat2 & P186-14) of L. maculans from Manitoba were used. The isolates were recognised as aggressive on the basis of their interaction phenotypes on 'Westar', a susceptible B. napus cultivar. The inoculum was prepared in the same manner as described in section 3.

5.2.2 Host material

Three resistant lines (UM3021, UM3043 & UM3323) and 3 susceptible lines (UM3132, UM3466 & UM3467) were used to determine the relationship of resistance to L. maculans in B. juncea with levels of seed glucosinolates. Brassica juncea lines were selected for their reactions to L. maculans at the cotyledon and adult stages using the method of Williams (1985) and Newman (1984) respectively. Parent plants representing resistant and susceptible lines were each self-pollinated and reciprocally cross-pollinated. All pollinations were controlled, and cross-pollinations preceded by emasculations. Progeny of the self-pollinated parents and the F_1 seeds were tested for their reactions to L. maculans at the cotyledon and adult stages. F_1 and F_2 plants were self-pollinated to obtain F_2 and F_3 seeds respectively.

Seeds were planted in flats (5 x 10 jiffy pots) and seedlings in pots (1 per pot) using a soilless mix (Metromix™, W. R. Grace & Co. Ltd., Ajax, Ontario) as the planting medium. All flats and pots were watered daily and kept in a growth chamber at 24/20°C day/night temperatures and a 16h photoperiod.

5.2.3 Analysis of seed glucosinolates

Glucosinolate composition is affected by pod position (Kondra & Downey 1970). Consequently a bulk sample per plant was used for the analysis. All the seed from each plant was threshed and a 1 g sample taken. Seeds from the self-pollinated parents (S_2) and the F_1, F_2 and F_3 seeds, were analysed for glucosinolate levels. The analysis was performed using the method of Daun & McGregor (1981). Benzyl glucosinolate was used as the internal standard.

5.3 Results and Discussion

The most important component glucosinolates in seeds of resistant and susceptible B. juncea test lines are the 2-propenyl and 3-butenyl glucosinolates (Table 5.1). The 2-propenyl glucosinolates accounted for 66% and 99% of the total glucosinolates in the seeds of the resistant lines UM3043 and UM3323 respectively. In seeds of susceptible lines UM3132, UM3466, UM3467 and resistant line UM3021, the 2-propenyl glucosinolates accounted for 20% to 30% and the 3-butenyl glucosinolates for 70% to 77% of the total glucosinolates. The mean levels of 2-propenyl glucosinolates in the seeds of the resistant lines UM3043 and UM3323 were significantly different from those of the susceptible parents and resistant line UM3021 (Table 5.1). Similarly, mean levels of the 3-butenyl glucosinolate in seed of susceptible lines were significantly different from mean 3-butenyl glucosinolate levels in seed of the resistant lines UM3043 and UM3323, but mean levels of 3-butenyl glucosinolate in seed of susceptible parents were not significantly different from mean 3-butenyl glucosinolate levels in seeds of resistant line UM3021 (Table 5.1). This suggests that mechanisms other than levels of seed glucosinolates are involved in the resistance of B. juncea to L. maculans, and that the role of the major glucosinolates in seed of B. juncea with regard to resistance to L.

maculans may be a minor one if any. Greenhalgh & Mitchell (1976) reported that high levels of allyl isothiocyanates were formed by both resistant and susceptible Brassica oleracea in response to infection by P. parasitica. Similarly Walker (1943) cited by Greenhalgh & Mitchell (1976) observed no relationship between levels of 2-propenyl glucosinolates and the resistance of B. oleracea to club root.

In the F_1 of crosses between susceptible and resistant B. juncea lines, no difference in IP occurred with direction of cross; when resistance = IP 0-3 and susceptibility = IP 5-9. All F_1 plants were resistant to L. maculans, indicating that resistance in B. juncea to L. maculans is controlled by nuclear genes. Sufficient seed for analysis was available from F_1 plants of the cross UM3132 x UM3323. The mean levels of the 2-propenyl and/or 3-butenyl differed significantly with direction of cross and depended on the levels in the female parent (Table 5.2). This indicates that the levels of 2-propenyl and 3-butenyl glucosinolates in the seeds of resistant and susceptible B. juncea lines are controlled by the maternal genotype rather than the genotype of the embryo. This also suggests that the levels of seed glucosinolates and resistance to L. maculans in B. juncea are not controlled by the same gene. The control of seed glucosinolate levels by the maternal genotype has been reported in other Brassica spp. (Kondra & Stefansson 1970, Love et al. 1990). The levels of 2-propenyl glucosinolates in F_2 seeds of the crosses between resistant and susceptible lines (UM3132 x UM3323) and their reciprocals, accounted for 63% - 67% of the total glucosinolates (Table 5.2). This further reflects the dominance of inheritance of the 2-propenyl glucosinolates over 3-butenyl glucosinolates. Similar results were obtained by Love (1988).

Data obtained from sample F_3 seeds of resistant and susceptible F_2 plants of the cross UM3021 x UM3132 are presented in Table 5.3. Average levels of 2-propenyl glucosinolates in F_3 seeds of resistant and susceptible F_2 plants accounted for 48% - 85% and 38% - 59% of the total seed glucosinolates respectively. The average levels of 3-butenyl glucosinolates accounted for 15% - 51% and 40% - 62% of the total seed

glucosinolates in F_3 seeds of resistant and susceptible F_2 (UM3021 x UM3132) plants respectively. The levels of 2-propenyl glucosinolates in seed of resistant and susceptible F_2 plants are plotted against those of 3-butenyl glucosinolates in Figure 5.1. An inverse relationship is indicated for seed levels of 2-propenyl glucosinolates verses 3-butenyl glucosinolates. The scatter of resistant and susceptible IP through out the figure further supports the conclusion that there is no relationship between resistance in B. juncea to L. maculans with the levels of the major glucosinolates in the seed of B. juncea. Hence the levels of glucosinolates in seed of B. juncea may be reduced without affecting their resistance to L. maculans.

Table 5.1. Glucosinolate content in seeds of resistant (R) and susceptible (S) Brassica juncea lines as determined by the method of the Canadian Grain Commission[1]. Values are means* ± SE.

Line Number	IP	N	Glucosinolates (umol g^{-1} meal) Allyl[2]	But[3]	Pent[4]	Hobut[5]	Total
UM3043	R	4	120.0±6.2b	61.6±7.6d	0.3±0.1cd	0.5±0.0a	182.4a
UM3323	R	8	151.0±6.4a	1.9±0.6c	0.1±0.0d	0.1± .b	153.1bc
UM3021	R	5	49.3±1.2c	114.0±2.0a	0.8±0.1bc	0.2±0.0ab	164.3b
UM3132	S	6	39.2±2.1cd	121.0±6.0a	1.1±0.1b	0.4±0.1ab	161.7bc
UM3466	S	5	33.6±2.7d	122.0±4.1a	1.7±0.3a	0.4±0.1ab	157.7bc
UM3467	S	7	41.6±2.2cd	102.0±4.6b	1.1±0.1b	0.4±0.1ab	145.1c

*Means under each column followed by the same letter are not significantly different for each glucosinolate, L.S.D at 5% level.
1 Daun & McGregor (1981).
2 Allyl (2-Propenyl).
3 But (3-Butenyl).
4 Pent (4-Pentyl).
5 Hobut (2 Hydroxy-3-Butenyl).

Table 5.2. Glucosinolate content in seeds of selfed and cross pollinated Brassica juncea lines as determined by the method of the Canadian Grain Commission[1]. Values are means* ± SE.

Parent or Cross	IP	N	Glucosinolates (umol g^{-1} meal) Allyl[2]	But[3]	Pent[4]	Hobut[5]	Total
$P_1$3132	S	6	39.2±2.1d	121.0±6.0a	1.1±0.1a	0.4±0.1b	161.0ab
$F_1P_1xP_2$	R	6	41.1±2.6d	114.0±3.0b	1.1±0.1a	0.8±0.1a	156.7ab
$F_2P_1xP_2$	R	19	102.0±1.4c	59.1±0.8c	0.2±0.0b	0.2±0.0bc	161.5ab
$F_2P_2xP_1$	R	21	112.0±2.4c	54.3±1.5c	0.2±0.0b	0.3±0.0cd	166.6a
$F_1P_2xP_1$	R	6	135.0±3.8b	1.6±0.3d	0.1±0.0c	0.1± .d	136.1c
$P_2$3323	R	8	151.0±6.4a	1.9±0.6d	0.1±0.0bc	0.1± .cd	153.0b

*Means under each column followed by the same letter are not significantly different for each glucosinolate, L.S.D at 5% level.
1 Daun & McGregor (1981).
2 Allyl (2-Propenyl).
3 But (3-Butenyl).
4 Pent (4-Pentyl).
5 Hobut (2 Hydroxy-3-Butenyl).

Table 5.3. Glucosinolate contents in F_3 seeds from a sample of resistant and susceptible F_2 plants of the cross (3021x3132) as determined by the method of Canadian Grain Commission[1]. Values are means ± SE of replicates.

Plant or Cross	IP	N	Glucosinolates (umol g^{-1} meal) Allyl[2]	But[3]	Pent[4]	Hobut[5]	Total
1	R	8	155.0±17.80	42.2±12.30	0.3±0.03	0.6±0.14	198.09
	S	6	105.0±17.10	72.3±14.80	0.5±0.15	0.8±0.24	178.58
2	R	13	147.0± 9.62	40.0± 8.29	0.2±0.01	0.4±0.07	187.53
	S	6	87.2±16.50	83.6± 9.90	0.4±0.07	0.5±0.07	171.62
3	R	16	155.0± 9.29	27.9± 7.48	0.2±0.02	0.2±0.05	183.26
	S	4	69.5± 8.51	109.0±10.80	0.6±0.13	0.7±0.13	179.75
4	R	16	101.0±13.20	71.9±12.30	0.5±0.09	0.5±0.10	173.84
	S	8	66.0± 3.65	96.1± 5.00	0.6±0.05	0.6±0.09	163.29
5	R	8	84.0±15.30	89.8±14.30	0.5±0.12	0.4±0.05	174.69
	S	11	80.1±12.00	87.2±12.10	0.6±0.11	0.5±0.08	164.37
6	R	14	138.0±11.60	41.4±10.90	0.3±0.03	0.4±0.10	180.01
	S	15	91.5±12.70	83.9±11.40	0.5±0.06	0.5±0.07	175.58
7	R	16	97.8± 9.00	57.0± 8.43	0.4±0.05	0.2±0.03	155.34
	S	7	59.4± 6.96	97.2± 7.59	0.7±0.12	0.3±0.03	157.57

1 Daun & McGregor (1981).
2 Allyl (2-Propenyl).
3 But (3-Butenyl).
4 Pent (4-Pentyl).
5 Hobut (2 Hydroxy-3-Butenyl).

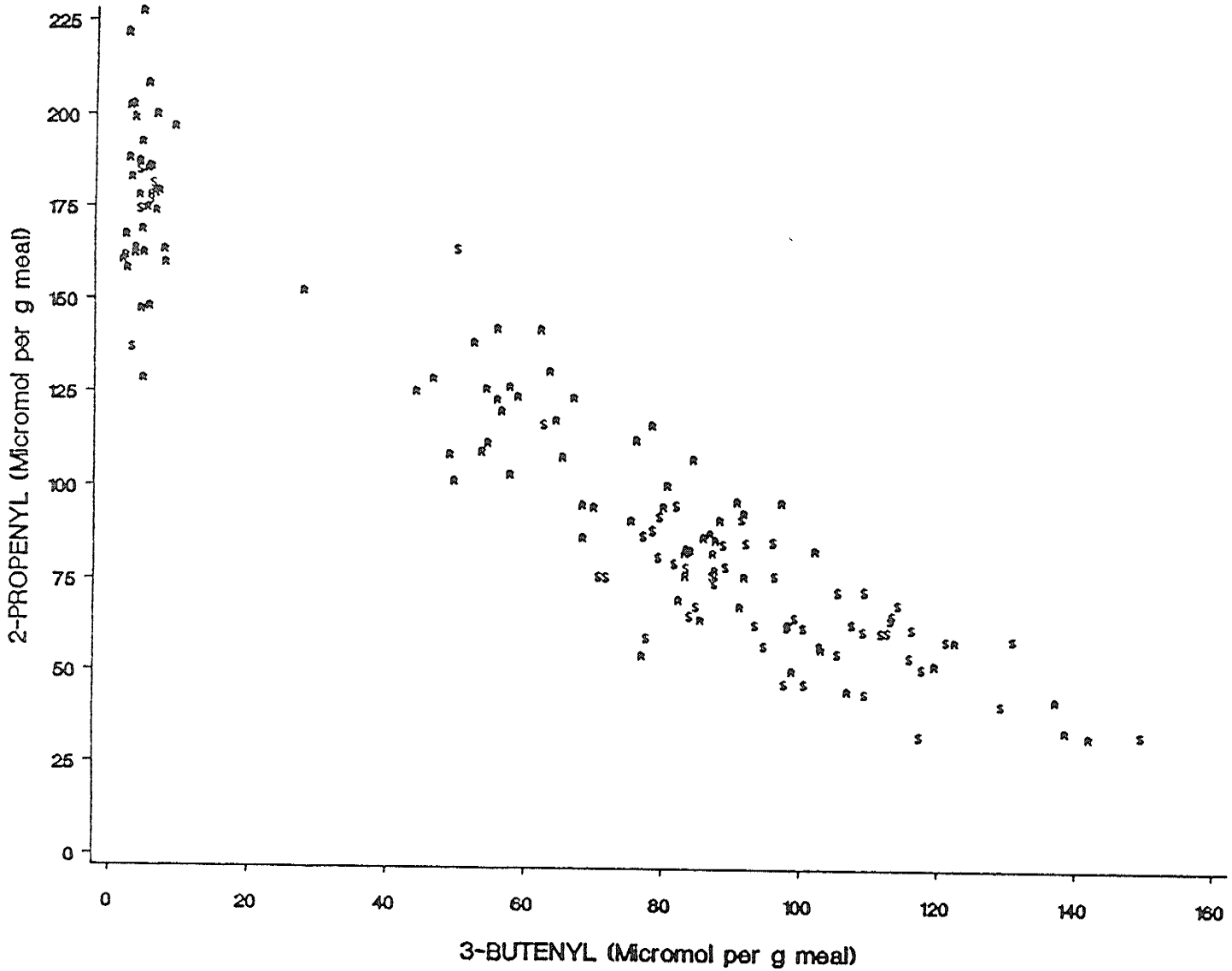

Figure 5.1. The relationship between the major seed glucosinolates with reaction to Leptosphaeria maculans in resistant (R) and susceptible (S) Brassica juncea challenged at cotyledon stage with 2 isolates of (P186-14 & Plat2) and at adult stem with isolate P186-14.

GENERAL DISCUSSION

Resistance in B. juncea to L. maculans is expressed at both the cotyledon and adult plant stages. Based on observation of interaction phenotypes (IP), using a limited number of plants, this resistance is often referred to as 'complete and stable' (Roy 1984) or as 'absolute and complete' (Sacristan & Gerdemann 1986). Evaluation of accessions of B. juncea at the University of Manitoba indicated that there is variability for reactions to L. maculans at the cotyledon and adult stages on aerial parts of the plant and also in the roots in the adult stage. Most accessions expressed resistance in both the cotyledon and stems but were susceptible to root infection. It is possible that root infections in B. napus and B. rapa are not recognized or perhaps overlooked because of the current importance of stem and crown canker. It is also conceivable that susceptibility of roots and subsequent development of the sexual stage on root residues may provide for sexual recombinations and/or development of virulent pathotypes. Consequently, screening for resistance to root infections in B. juncea should be considered in breeding programs.

Interaction phenotypes on most accessions were not consistent over time, often tending towards higher IPs (i.e. increasing susceptibility) with duration of infection. Hence, a longer exposure to infection may be required to adequately separate resistant genotypes from susceptible ones. This may be due to the incomplete action of the resistance genes. That this may be the result of heterozygosity of host material is supported by the fact that susceptible lines were selected from selfed lines whose IP were equal to/or greater than 5.0 in any of the 3 ratings. There was often less damage by the pathogen on such heterozygous genotypes compared to the susceptible ones; nevertheless, the fungus was able to colonize the host, albeit restrictedly. Again, the widespread use of cultivars with such genotypes may allow for the development of new pathotypes and/or increase of inoculum.

Most plants within accessions were resistant to L. maculans at both the cotyledon and adult stages; however some plants with a resistant IP at the cotyledon stage were later infected at the adult stage even when the stems had not been inoculated. This suggests that biotrophic or latent infections may occur as early as the cotyledon stage and results in subsequent disease development. Cytological studies are needed to verify this. For breeding purposes, selections at the cotyledon and adult stages should be based on the occurrence of the hypersensitive response (IP = 1-3). Better still, inoculation of the cotyledons, without detachment, and subsequent selection of plants at the adult stage may be used in preliminary breeding tests to select for cotyledon and/or adult resistance. This latter method would be preferable over inoculation of both cotyledons and stems because it follows the natural development of the disease in the field more closely.

The inheritance of resistance in B. juncea to L. maculans was studied under controlled environmental conditions using two isolates at the cotyledon stage and with one isolate at the adult stage. It is difficult to decide whether or not intermediate IPs (e.g. IP=5) should be assigned to either the resistant or susceptible phenotype. As a result, IPs' on Brassica species in genetic studies have often been categorized arbitrarily. In this study no segregation for resistance occurred when plants with IP = 5 -9 were selfed and the progeny were challenged with isolates of L. maculans at the cotyledon stage, nor did segregation for susceptibility occur when plants with IP = 0 - 3 were selfed and tested with the same isolates of L. maculans. This suggests that for B. juncea cotyledon reactions, IP 0-3 = resistant and IP 5-9 = susceptible.

Resistance in B. juncea to L. maculans is controlled by two nuclear genes with dominant recessive epistatic gene action. Consequently the inheritanceof resistance may be perceived as quantitative (Strickberger 1985). The resistance of B. juncea has been described as 'complete and stable' (Roy 1984) or 'absolute and complete'(Sacristan & Gerdemann 1986). Consequently both the major and modifier (sensu Strickberger 1985) genes may have to be transferred to susceptible cultivars or other susceptible and

compatible species if B. juncea type resistance to L. maculans is to be maintained. However it is unlikely that 'stable' resistance can easily be transferred to a susceptible cultivar or to another species unless special care is taken to also select for the recessive gene. The differential nature of resistance to L. maculans has been demonstrated in B. napus. It is possible that the resistance conferred by the two genes in B. juncea when separated singly into monogenic lines will be differential in nature. Also, in view of the gene-for-gene hypothesis, different host-pathogen genotype combinations may result in different genotypic ratios and possibly manifesting different IPs'. Hence knowledge of the resultant IP of particular heterozygous genes may be important for inference of effective gene combinations. There is need to test the stability of both the dominant and recessive genes, singly and in different combinations. These genes may be used in the development of differential series.

Resistance genes in the parent UM3323 are allelic to those in parents UM3021 and UM3043. A few susceptible plants were observed in the F_2 of crosses involving UM3021 and UM3043 but the segregation ratio did not fit the expected 13:3 ratio due to excess resistant IP. It is probable that factors such as epistatic and/or environmental effects on certain genotypes, or the occurrence of biotrophic/latent infections may be involved. Also the presence of a third resistance gene or of dissociative gene effects could result in some segregation for susceptibility.

Epistatic genes are greatly influenced by the environment and the stability of the genes may depend on whether combined genes are associated or dissociated (Vanderplank 1984). Blackleg disease has been known to fluctuate from year to year though the cause of such fluctuations have not been known (Cargeeg & Thurling 1980b). That epistasis may be the cause is a plausible postulation and further studies into the effect of environmental factors such as temperature, moisture and humidity may provide an insight. The identification of lines with AABB and aabb genotypes would facilitate future studies into the mechanisms of action of the genes, the associative and dissociative

effects of different gene combinations, and/or environmental effects on host-parasite interaction.

Studies on the relationship of the major seed glucosinolates in B. juncea with resistance to L. maculans indicated that either of the major seed glucosinolates (2-propenyl or 3-butenyl) may occur predominantly in B. juncea irrespective of the reactions to L. maculans. There were no significant differences between the major seed glucosinolates (2-propenyl & 3-butenyl) in the resistant line UM3021 and the susceptible lines UM3132, UM3466 & UM3467, but the major seed glucosinolate levels of the aforementioned lines were significantly different from those of the resistant lines UM3043 and UM3323. In addition, the resistance in B. juncea to L. maculans was controlled by nuclear genes but the levels of the major glucosinolates were controlled by the genotype of the maternal plant. That there is no relationship between resistance in B. juncea with the levels of major seed glucosinolates was further indicated by the results obtained from resistant and susceptible F_2. This suggests that reactions to L. maculans and the levels of the major seed glucosinolates are not controlled by the same gene. This is significant in that glucosinolate levels in seed of B. juncea may be reduced genetically possibly without adversely affecting resistance to L. maculans. Nevertheless, more studies on the inter-relationship of seed glucosinolate and foliar glucosinolate levels with the resistance to L. maculans are needed.

LITERATURE CITED

Alabouvette, C., and B. Brunin. 1970. Recherches sur la maladie du colza due a Leptosphaeria maculans (Desm.) Ces. et de Not. I. Role des restes de culture dans la conservation et la dissemination du parasite. Ann. Phytopathol. 2(3): 463-475.

Alabouvette, C. B., B. Brunin, and J. Louvet. 1974. Recherches sur la maladie du colza due a Leptosphaeria maculans (Desm.) Ces. et de Not. IV. Pouvoir infectieux des pycnospores et sensibilite varietale. Ann. Phytopathol. 6: 265-275.

Anon. 1991. Field Crop Variety Recommendation for Manitoba. Manitoba Agriculture.

Bailey, J. A. 1983. Biological perspecitve of host-parasite interactions. In: The dynamics of host defence. eds. J. A. Bailey and B. J. Deverall. Academic Press. Australia.

Barbetti, M. J. 1975. Effects of temperature on development and progression in rape of crown canker caused by Leptosphaeria maculans. Aust. J. Exp. Agric. and Anim. Husb. 15: 705-708.

Boerema, G. H. 1976. The Phoma species studied in culture by Dr. R. W. G. Dennis. Trans. Br. Mycol. Soc. 67(2): 289-319.

Boerema, G. H., H. A. Van Kesteren, and W. M. Loerakker. 1981. Notes on Phoma. Trans. Br. Mycol. Soc. 77(1): 61-74.

Bokor, A., M. J. Barbetti, A. G. P. Brown, G. C. MacNish, and P.M. Wood. 1975. Blackleg of rapeseed. J. Agric. Western Aust. 16:7-10.

Bonman, J. M., R. L. Gabrielson, P. H. Williams, and P. A. Delwiche. 1981. Virulence of Phoma lingam to cabbage. Plant Disease 65(11): 865-867.

Brown, A. G. P., M. J. Barbetti, and P. M. Wood. 1976. Effect of benomyl on blackleg disease of rape in Western Australia. Aust. J. Exp. Agric. Anim. Husb. 16: 276-279.

Boulter, G. S. 1983. The history and marketing of rapeseed oil in Canada. In: High and low erucic acid rapeseed oils. Eds J.K. G. Kramer, F. D. Sauer & W. J. Pigden. pp. 61-89. Academic Press, Toronto.

Canola Growers Manual. 1989. Canola Council, Winnipeg, Manitoba, Canada.

Cargeeg, L. A., and N. Thurling. 1980a. Contribution of host - pathogen interactions to the expression of the blackleg disease of spring rape (Brassica napus L.) caused by Leptosphaeria maculans (Desm.) Ces. et de Not. Euphytica 29: 465-476.

Cargeeg, L. A., and N. Thurling. 1980b. Seedling and adult plant resistance to blackleg (Leptosphaeria maculans (Desm.) Ces. et de Not.) in spring rape (Brassica napus L.). Aust. J. Agric. Res. 31: 37-46.

Commonwealth Mycological Institute. 1978. Distribution maps of plant diseases. Map No. 73. Edition 4. Commonwealth Agricultural Bureaux.

Cook, R. J., and E. J. Evans. 1978. Build up of diseases with intensification of oilseed rape in England. In: Proceedings of the 5th international rapeseed conference. Vol. 1. June 12-16. pp 333-337. Malmo, Sweden.

Dahiya, J. S., and S. R. Rimmer. 1988. Phytoalexin accumulation in tissues of Brassica napus inoculated with Leptosphaeria maculans. Phytochemistry 27(10): 3105-3107.

Davies, J. M. L. 1986. Diseases of oilseed rape. In: Oilseed rape. eds. Scarisbrick, D. H. and Daniels, R. W. 309 pp. Collins professional technical books. London.

Daun, J. K., and D. I. McGregor. 1981. Glucosinolate analysis of rapeseed (Canola). Method of the Canadian grain commission grain research laboratory. Canadian grain commission, Winnipeg, Man. 25 pp.

De March, G., G. Seguin-Swartz, and G. A. Petrie. 1986. Virulence and culture filrate phytotoxicity in Leptosphaeria maculans: Perspectives for in vitro selection. Can. J. Plant Pathol. 8: 422-428.

Delwiche, P. A. 1980. Genetic aspects of blackleg (Leptosphaeria maculans) resistance in rapeseed (Brassica napus). Ph. D. Thesis. University of Wisconsin Madison W.I. 144 pp.

Dennis, R. W. G. (1946). Notes on some British fungi ascribed to Phoma and related genera. Trans. Br. Mycol. Soc. 29: 11-42.

Downey, R. K., and G. F. W. Rakow. 1987. Rapeseed and mustard. In: 'Principles of cultivar development. Vol. 2 Crop Species'. eds. W. R. Fehr MacMillan Pub. Co. NY. pp. 437-486.

Downey, R. K., and G. Robbelen. 1989. Brassica Species. In: Oil crops of the world: Their breeding and utilization. eds. G. Robbelen, R. K. Downey and A. Ashri. pp. 339-362. McGraw Hill Publishing Company.

Ebba,T. A., and C. Person. 1975. Genetics of fungal pathogens. Genet. Supp. 79: 397-408.

Ellingboe, A. H. 1976. Genetics of host-parasite interactions. Encycl. Plant Physiology New Ser. 4: 761-778.

Ellingboe, A. H. 1981. Changing concepts in host-pathogen genetics. Ann. Rev. Phytopathol. 19: 125-143.

Fenwick, G. R., R. K. Heaney, and W. J. Mullin. 1983. Glucosinolates and their break down products in food and food plants. CRC Critical Reviews in Food Science and Nutrition 18: 123-201.

Flor, H. H. 1942. Inheritance of pathogenicity in Melamsora lini. Phytopathology 32 (8): 653-669.

Flor, H. H. 1946. Genetics of Pathogenicity in Melampsora lini. Journal of Agricultural research. 73(11,12): 335-357.

Flor, H. H. 1955. Host-parasite interaction in flax rust- its genetics and other implications. Phytopathology. 45: 680-685.

Flor, H. H., and V. E. Comstock. 1971. Flax cultivars with multiple rust conditioning genes. Crop Sci. 11: 64-66.

Gabrielson, R. L. 1974. Washington's all-out attack on blackleg. American Vegetable Grower. pp. 21 and 25.

Gabrielson, R. L. 1983. Blackleg disease of crucifers caused by Leptosphaeria maculans (Phoma lingam) and its control. Seed Sci. and Technol. II. pp. 749-780.

Gladders, P., and T. M. Musa. 1980. Observations on the epidemiology of Leptosphaeria maculans stem canker in winter oilseed rape. Plant Pathol. 29: 28-37.

Goodman, R. N., Z. Kiraly, and K. R. Wood. 1986. The Biochemistry and physiology of plant disease. 433 pp. University of Missouri Press.

Greenhalgh, J. R., and N. D. Mitchell. 1976. The involvement of flavour volatiles in the resistance to downey mildew of wild and cultivated forms of Brassica oleracea. New Phytol. 77: 391-398.

Hammond, K. E., and B. G. Lewis. 1986a. Superficial stem lesions on oilseed rape caused by Leptosphaeria maculans in the presence of anther components. Trans. Br. Mycol. Soc. 86(1): 175-178.

Hammond, K. E., and B. G. Lewis. 1986b. The timing and sequence of events leading to stem canker disease in populations of Brassica napus var. oleifera in the field. Plant Pathol. 35: 551-564.

Hammond, K. E., and B. G. Lewis. 1987a. The establishment of systemic infection in leaves of oilseed rape by Leptosphaeria maculans. Plant Pathol. 36:135-147.

Hammond, K. E., and B. G. Lewis 1987b. Variation in stem infections caused by aggressive and non-aggresive isolates of Leptosphaeria maculans on Brassica napus var. oleifera. Plant Pathol. 36: 53-65.

Hammond, K. E., and B. G. Lewis. 1987c. Differential responses of oilseed rape leaves to Leptosphaeria maculans. Trans. Br. Mycol. Soc. 88(3): 329-333.

Hammond, K. E., B. G. Lewis, and T. M. Musa. 1985. A systemic pathway in the infection of oilseed rape plants by Leptosphaeria maculans. Plant Pathol. 34: 557-565.

Hanacziwskyj, P., and R. B. Drysdale. 1984. Variation in pathogenicity of Leptosphaeria maculans to oilseed rape and other brassicas. Aspects Appl. Biol. 6: 343-353.

Harper, F. R., and B. Berkenkamp. 1975. Revised growth-stage key for Brassica campestres and B. napus. Can. J. Plant Sci. 55: 657-658.

Heaney, R. K., and G. R. Fenwick. 1980a. Glucosinolates in Brassica vegetables. Analysis of 22 varieties of brussel sprouts (Brassica oleracea var gemmifera). J. Sci. Food Agric. 31: 785-793.

Heaney, R. K., and G. R. Fenwick. 1980b. The glucosinolate content of Brassica vegetables. A chemotaxonomic approach to cultivar identification. J. Sci Food Agric. 31: 794-801.

Heath, M. C. 1981. A generalized concept of host-parasite specificity. Phytopathology 71(11): 1121-1123.

Helms, K., and I. A. M. Cruickshank. 1979. Germination-inoculation technique for creening cultivars of oilseed rape and mustard for resistance to Leptosphaeria maculans. Phtopathol Z. 95: 77-86.

Hill, C. B., and P. H. Williams. 1988. Leptosphaeria maculans cause of blackleg of crucifers. Advances in Plant Pathol. 6: 169-174.

Holley, R. A., and J. D. Jones. 1985. The role of myrosinase in the development of toxicity toward Nematospora in mustard seed. Can. J. Bot. 63: 521-526.

Humpherson-Jones, F. M. 1983a. Pathogenicity studies on isolates of Leptosphaeria maculans from Brassica seed production crops in south-east England. Ann. Appl. Biol. 103: 37-44.

Humpherson-Jones, F. M. 1983b. The Occurrence of Alternaria brassicicola, Alternaria brassicae and Leptosphaeria maculans in Brassica seed crops in south-east England between 1976 and 1980. Plant Pathol. 32: 33-39.

Koch, E., K. Song, T. C. Osborn, and P. H. Williams. 1991. Relationship between pathogenicity and phylogeny based on restriction fragment length polymorphism in Leptosphaeria maculans. Mol. Plant-Microbe Interac. 4(4): 341-349.

Kolte, S. J. 1985. Diseases of annual edible oilseed crops. Volume 11: Rapeseed - mustard and sesame diseases. pp 9-82.

Kondra, Z. P., and R. K. Downey. 1970. Glucosinolate content of rapeseed (Brassica napus L. and Brassica campestris L.) meal as influenced by pod position on the plant. Crop Science 10: 54-56.

Kondra, Z. P., and B. R. Stefansson. 1970. Inheritance of the major glucosinolates of rapeseed (Brassica napus) meal. Can. J. Plant Sci. 50: 643-647.

Kruger, W. 1983. Oilseed rape: Pests and diseases. Semmundo. Saatzucht GmbH, Billstr. 139, D-2000 Hamburg 28. Printed by Schuthedruck, D-2100 Hamburg 90.

Lammerink, J. 1979. Blackleg (Leptosphaeria maculans) in New Zealand oilseed rape. Cruciferae Newsletter 4:25.

Lawrence, G. J. 1988. Melampsora lini, rust of flax and linseed. In: Advances in plant pathology. Genetics of plant pathogenic fungi. Vol. 6: 314-331. ed. G. S. Sidhu. Academic Press.

Love, H. K. 1988. The inheritance of seed aliphatic glucosinolates in oilseed Brassica species. Ph.D. Thesis, University of Saskatchewan. 226 pp.

Love, H. K., R. K. Rakow, J. P. Raney, and R. K. Downey. 1990. Genetic control of 2-propenyl and 3-butenyl glucosinolate synthesis in mustard. Can. J. Plant Sci. 70: 425-429.

Macer, R. C. F. 1960. Nature and exploitation of crop plant resistance to disease. Nature 4728: 857-859.

Martens, J. W., W. L. Seaman, and T. G. Atkinson. 1984. Diseases of field crops in Canada. 160 pp. The Canadian Phytopathological Society.

McGee, D. C. 1977. Blackleg (Leptosphaeria maculans (Desm.) Ces. et de Not.) of rapeseed in Victoria: Sources of infection and relationships between inoculum, environmental factors and disease severity. Aust. J. Agric. Res. 28: 53-62.

McGee, D. C., and R. W. Emmett. 1977. Blackleg (Leptosphaeria maculans (Desm.) Ces. et de Not.) of rapeseed in Victoria: Crop losses and factors which affect disease severity. Aust. J. Agri. Res. 28: 47-51.

McGee, D. C., and G. A. Petrie. 1978. Variability of Leptosphaeria maculans in relation to blackleg of oilseed rape. Phytopathology 68: 626-630.

McVetty, P. B. E. 1988. Hybrid Canola: Development and field performance. In: Technical and scientific papers presented at Manitoba Agri-Form. Dec. 13 & 14 1988.

Mengistu, A., S. R. Rimmer, E. Koch, and P. H. Williams. 1989. Pathogenicity grouping of Leptosphaeria maculans isolates based on three cultivers of Brassica napus. Phytopathology 79(10): 1207 (Abst).

Mithen, R. F., B. G. Lewis, and G. R. Fenwick. 1986. In vitro activity of glucosinolates and their products against Leptosphaeria maculans. Trans. Br. Mycol. Soc. 87(3): 433-440.

Mithen, R. F., B. G. Lewis, R. K. Heaney, and G. R. Fenwick. 1987. Resistance of leaves of Brassica species to Leptosphaeria maculans. Trans. Br. Mycol. Soc. 88(4): 525-531.

Mithen, R. F., and B. G. Lewis. 1988. Resistance to Leptosphaeria maculans in hybrids of Brassica oleracea and Brassica insularis. J. Phytopathology 123: 253-258.

Nayar, J. K., and A. J. Thorsteinson. 1963. Further investigations into the chemical basis of insect-host plant relationships in an oligophagous insect, Plutella maculipennis (Curtis) (Lepidoptera: Plutellidae). Can. J. Zool. 41: 923-929.

Newman, P. L. 1984. Differential host-parasite interaction between oilseed rape and Leptosphaeria maculans, the causal fungus of stem canker. Plant Pathol. 33: 205-210.

Newman, P. L., and D. J. Bailey. 1987. Screening for resistance to canker (Leptosphaeria maculans) in winter oilseed rape (Brassica napus ssp. oleifera). Plant Pathol. 36: 346-354.

Parry, D. W. 1990. Plant pathology in agriculture. Cambridge University Press, Cambridge. 385pp.

Pawlowski, S. H. 1970. Commercial potential of interspecific crosses among several Brassica species. In: Proceedings of the international conference on the science, technology and marketing of rapeseed and rapeseed products. September 20-23, 1970. Published by the Rapeseed Association of Canada in co-operation with the Department of Industry, Trade and Commerce. Ottawa.

Person, C., and G. M. E. Mayo. 1974. Genetic limitations and models of specific interactions between a host and its parasite. Can. J. Bot. 52:1339-1347.

Peters, P., and R. Hall. 1989 Differentiation of strains of Leptosphaeria maculans from rapeseed in Ontario. Can. J. Plant Path. 11(2): 197. (Abstract).

Petrie, G.A. 1973a. Diseases of Brassica species in Saskatchewan, 1970-1972. II. Stem, pod & leaf spots. Can. Plant Dis. Surv. 53(2): 83-87.

Petrie, G.A. 1973b. Herbicide damage and infection of rape by the blackleg fungus, Leptosphaeria maculans. Can. Plant Dis. Surv. 53(1): 26-28.

Petrie, G.A. 1978. Occurrence of a highly virulent strain of blackleg (Leptosphaeria maculans) on rape in Saskatchewan (1975-1977). Can. Plant Dis. Surv. 58(2): 21-25.

Petrie, G.A. 1979. Prevalence of a highly virulent strain of blackleg (Leptosphaeria maculans) in seed samples of rape and turnip rape produced in western Canada in 1976 and 1977. Can. J. Plant Sci. 59: 899-901.

Petrie, G. A. 1985a. Saskatchewan rapeseed/canola disease survey. Can. Plant Dis. Surv. 65(2): 47-49.

Petrie, G. A. 1985b. Yield losses in Saskatchewan rapeseed/canola crops from basal stem cankers of blackleg (Leptosphaeria maculans) in 1982, with notes on other diseases. Can. Plant Dis. Surv. 65(2): 43-46.

Petrie, G. A. 1986. Blackleg and other diseases of canola in Saskatchewan in 1984 and 1985. Can. Plant Dis. Surv. 66(2): 51-53.

Petrie, G. A. 1988. The rapid differentiation of virulent and weakly virulent strains of Leptosphaeria maculans (blackleg or stem canker) and related pycnidial fungi from Brassica seeds and stems. Can. J. Plant Pathol. 10: 188-190.

Petrie, G. A., and P. A. Lewis. 1985. Sexual compatibility of isolates of the rapeseed blackleg fungus Leptosphaeria maculans from Canada, Australia, and England. Can. J. Plant Pathol. 7: 253-255.

Petrie, G. A., K. Mortensen, and J. Dueck. 1985. Blackleg and other diseases of rapeseed in Saskatchewan, 1978 to 1981. Can. Plant Dis. Surv. 65(2): 35-41.

Petrie, G. A., and T. C. Vanterpool. 1965. Diseases of rape and cruciferous weeds in Saskatchewan in 1965. Can. Plant Dis. Surv. 44: 111-112.

Petrie, G. A., and T. C. Vanterpool. 1966. Diseases of rape, mustard and cruciferous weeds in the prairie provinces. Can. Plant Dis. Surv. 46(4): 117-120.

Petrie, G. A., and T.C. Vanterpool. 1968. Diseases of crucifers in Saskatchewan in 1967. Can. Plant Dis. Surv. 48(1): 25-27.

Piening, L., E. Okolo, and D. Harder. 1975. Blackleg of rapeseed in Kenya. E. Afr. agric. For. J. 41(2): 110-113.

Platford, G. 1988. Manitoba canola survey. Technical and scientific papers presented at Manitoba Agri-Forum. Dec. 13-14, 1988.

Punithalingam, E., and P. Holliday. 1972. Lephosphaeria maculans. Commonwealth Mycological Institute (CMI) descriptions of pathogenic fungi and bacteria. No. 331.

Rakow, G., and D. L. Woods. 1987. Outcrossing in rape and mustard under Saskatchewan prairie conditions. Can. J. Plant Sci. 67: 147-151.

Rawlinson, C. J. 1979. Light spot of oilseed rape: an appraisal with comments on strategies for control. Proceedings Crop Protection Conference - Pests and Diseases.

Rawlinson, C. J., and G. Muthyalu. 1979. Diseases of Winter oilseed rape - occurrence, effects and control. Journal of Agric. Sci. Cambridge 93: 593-606.

Rimmer, S.R., and R. G. Platford. 1982. Manitoba rapeseed disease survey 1978-1980. Can. Plant Dis. Surv. 62(2): 45-49.

Robbelen, G., and W. Thies. 1980. Variation in rapeseed glucosinolates and breeding for improved meal quality. In: 'Brassica crops and wild allies. Biology and Breeding.' eds. S. Tsunoda, K. Hinata and C. Gomez-Campo. Japan Scientific Societies press, Tokyo. pp. 285-299.

Roelfs, A. P. 1988. Genetic control of phenotypes in wheat stem rust. Ann. Rev. Phytopathol. 26: 351-67.

Rouxel, T., Y. Chupeau, R. Fritz, A. Kollman, and J. F. Bousquet. 1988. Biological effects of Sirodesmin PL, a phytoalexin produced by Leptosphaeria maculans. Plant Science 57: 45-53.

Rouxel, T., A. Sargniguet, A. Kollman, and J. F. Bousquet. 1989. Accumulation of a phytoalexin in Brassica spp in relation to a hypersensitive reaction to Leptosphaeria maculans. Physiological and Molecular Plant Pathology 34: 507-517.

Roy, N. N. 1978. A study on disease variation in the populations of an interspecific cross of Brassica juncea L. x Brassica napus L. Euphytica 27: 145-149.

Roy, N. N. 1984. Interspecific transfer of Brassica juncea-type blackleg resistance to Brassica napus. Euphytica 33: 295-303.

Roy, N. N., and J. Reeves. 1975. Breeding better rape and linseed for western Australia. J. Agric. Western Aust. 16: 93-97.

Sacristan, M. D. 1982. Resistance responses to Phoma lingam of plants regenerated from selected cell and embryogenic cultures of haploid Brassica napus. Theor. Appl. Genet. 61: 193-200.

Sacristan, M. D., and M. Gerdemann. 1986. Different behavior of Brassica juncea and Brassica carinata as sources of Phoma lingam resistance in experiments of interspecific transfer to B. napus. Z. Pflanzenzuecht 97: 304-314.

Sang, J. P., I. R. Minchinton, P. K. Johnstone, and R. J. W. Truscott. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can. J. Plant Sci. 64: 77-93.

Sawatsky, W. M. 1989. Evaluation of screening techniques for resistance to Leptosphaeria maculans and genetic studies of resistance to the disease in Brassica napus. MSc. Thesis. University of Manitoba. 85 pp.

Scarth, R., P. B. E. McVetty, S. R. Rimmer, and B. R. Stefansson. 1988. Stellar, a low linolenic - high linoleic acid summer rape. Can. J. Plant Sci. 68: 509-511.

Singh, H. 1987. Present status of production, constrains and research achievements of Indian mustard in India. In 'Oil crops: Niger and Rapeseed/mustard'. Proceedings of the third oil crops network workshop held in Addis Ababa, Ethiopia, 6-10 October 1986. International Development Research Centre Manuscript Report.

Sjodin, C., and K. Glimelius. 1988. Screening for resistance to blackleg Phoma lingam (Tode ex Fr.) Desm. within Brassicaceae. J. Phytopathology. 123: 322-332.

Sjodin, C., and K. Glimelius. 1989. Diffferences in response to the toxin sirodesmin PL produced by phoma lingam (Tode ex Fr.) Desm. on protoplasts, cell aggregates and intact plants of resistance and susceptible Brassica species. Theor. Appl. Genet. 77: 76-80.

Smith, H. C., and B. C. Sutton. 1964. Leptosphaeria maculans the ascogenous state of Phoma lingam. Trans. Br. Mycol. Soc. 47(2): 159-165.

Statistics Canada. 1976. Selected agricultural statistics for Canada 1935-1975.

Statistics Canada. 1984. Handbook of selected agricultural statistics 1976-83.

Statistics Canada 1988. Handbook of selected agricultural statistics 1981-1988.

Stefansson, B. R. 1983. The development of improved rapeseed cultivars. In: High and low erucic acid rapeseed oils. Eds J.K. G. Kramer, F. D. Sauer & W. J. Pigden. pp. 144-159. Academic Press, Toronto.

Strickberger, M. W. 1985. Genetics. Third edition. 842 pp. MacMillan Publishing Company. New York.

Thurling, N., and L. A. Venn. 1977. Variation in the responses of rapeseed (Brassica napus and Brassica campestris) cultivars to blackleg (Leptosphaeria maculans) infection. Aust. J. Exp. Agric. and Anim. Husb. 17: 445-451.

Underhill, E. W. 1980. Glucosinolates. In: Secondary plant products. eds. E. A. Bell and B. V. Charlwood. pp. 493-511. Springer-Verlag Berlin, Heidelberg, New York.

Van den Berg, C. G. J., R. G. Platford, R. Kutcher, and S. R. Rimmer. 1989. Blackleg and other diseases in canola. In: Technical and scientific papers presented at a conference for Agricultural professionals. Manitoba Agri-Forum. December 12 & 13, 1989.

Vanderplank, J. E. 1982. Host-pathogen interactions in plant disease. 207 pp. Academic Press Inc. New York.

Vanderplank, J. E. 1984. Disease resistance in plants. Second edition. 194 pp. Academic Press, Inc. New York.

Vanterpool, T.C. 1961. Rape diseases in Saskatchewan in 1961. Can. plant Dis. Surv. 44(5): 372-373.

Vanterpool, T.C. 1963. Blackleg (Phoma lingam). Can. Plant Dis. Surv. 48(4): 214.

Wetter, L. R., and B. M. Craig. 1959. Varietal and environmental effects on rapeseed. I. Isothiocyanate and thiooxazolidine content. Can. J. Plant Sci. 39: 395-399.

Williams, P. H. 1974. Blackleg and black rot - continuing threat to cabbage production? American Vegetable Grower. pp. 20 and 22.

Williams, P. H. 1985. Crucifer Genetics Cooperative (CrGC) Resource Book. 1985. Department of Plant Pathology, University of Wisconsin Madison WI.

Woods, D. L., J. J. Capcara, and R.K. Downey. 1991. The potential of mustard (Brassica juncea (L.) Coss) as an edible oil crop on the Canadian prairies. Can. J. Plant Sci. 71: 195-198.

Wood, P. McR., and M. J. Barbetti. 1977. The role of seed infection in the spread of blackleg of rape in western Australia. Aust. J. Exp. Agric. and Anim. Husb. 17: 1040-1044.

Wood, R. K. S. 1986. Introductory comments on host-parasite interaction. In: Biology and molecular biology of plant-pathogen interactions. ed. J. A. Bailey. Springer-Verlag Berlin Hiedelberg.

Wratten, N. 1977. Breeding for resistance to blackleg (Leptosphaeria maculans (Desm.) Ces. et de Not) in rape (Brassica campestris L. and Brasica napus L.) In: 3rd Int. Cong. Soc. Adv. Breed. Res. Asia & Oceania (SOBRAO). 3d(vi): 46-23-46-25.

Wratten, N., and G. M. Murray. 1977. A population improvement approach for developing resistance to blackleg in rapeseed. In: 3rd Int. Cong. Soc. Adv. Breed. Res. Asia & Oceania (SOBRAO).

Zadoks, J. C., and R. D. Schein. 1979. Epidemiology and plant disease management. Oxford Univeristy Press.

Appendix 3.1. Mean disease severity ratings for Brassica juncea accessions challenged at cotyledon stage with 2 isolates of Leptosphaeria maculans (Pl86-14 & Plat2). Values are means of 5 to 10 plants (Upper values) ± SE (Lower values) 10, 12 and 15 days after inoculations in descending disease severity at day 10. *University of Manitoba accession number.

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3115	9.00	9.00	9.00	3115	9.00	9.00	9.00
	0.00	0.00	0.00		0.00	0.00	0.00
3403	4.60	5.80	6.20	3403	3.80	4.60	6.20
	0.50	0.53	0.53		0.80	0.88	0.53
3064	3.80	5.80	.	3132	2.79	2.38	3.25
	0.80	1.31	.		0.68	0.51	0.51
3116	3.00	5.00	5.00	3370	2.20	3.40	6.20
	0.00	0.42	0.42		0.33	0.27	0.33
3132	2.89	3.00	3.50	3479	2.20	3.00	5.60
	0.66	0.55	0.59		0.33	0.42	0.31
3379	2.60	4.60	7.00	3396	2.20	3.40	5.40
	0.50	0.78	0.00		0.53	0.78	0.27
3370	2.60	5.40	6.20	3488	2.20	2.60	3.80
	0.27	0.27	0.33		0.33	0.50	0.68
3396	2.60	4.20	5.40	3373	2.20	3.00	3.80
	0.50	0.53	0.27		0.33	0.00	0.33
3488	2.60	3.00	5.40	3480	2.20	2.20	3.60
	0.27	0.42	0.50		0.33	0.33	0.60
3118	2.60	3.40	3.80	3314	2.00	2.40	2.80
	0.27	0.50	0.33		0.84	1.11	1.08
3479	2.20	3.40	5.60	3385	1.80	2.60	3.80
	0.33	0.50	0.31		0.53	0.50	0.53
3113	2.20	4.20	5.00	3122	1.80	2.60	3.80
	0.33	0.53	0.42		0.33	0.50	0.33
3363	2.20	3.80	5.00	3111	1.80	2.60	3.40
	0.33	0.68	0.42		0.33	0.50	0.27
3373	2.20	3.40	4.60	3116	1.80	3.00	3.00
	0.33	0.27	0.27		0.33	0.42	0.42

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3122	2.20 0.33	3.40 0.65	4.60 0.50	3118	1.80 0.33	3.00 0.42	3.00 0.42
3460	2.20 1.14	2.60 1.07	3.80 0.90	3064	1.80 0.33	4.20 1.31	. .
3097	2.20 0.33	3.00 0.42	3.00 0.42	3010	1.67 1.05	1.67 1.05	2.33 1.48
3354	2.20 0.33	2.60 0.27	3.00 0.42	3312	1.60 0.31	3.40 0.50	4.60 0.65
3314	2.00 0.84	2.40 1.11	2.80 1.08	3056	1.60 0.91	2.60 1.13	. .
3056	2.00 1.17	2.20 1.14	. .	3379	1.40 0.27	4.60 0.78	6.20 0.53
3415	1.80 0.33	2.60 0.27	4.20 0.53	3406	1.40 0.27	1.80 0.53	4.60 0.50
3417	1.80 0.33	2.20 0.33	3.80 0.33	3491	1.40 0.27	1.80 0.33	4.60 0.27
3480	1.80 0.33	1.80 0.33	4.60 0.65	3363	1.40 0.27	2.20 0.33	4.20 0.53
3111	1.80 0.33	2.60 0.58	3.20 0.47	3472	1.40 0.27	1.80 0.33	4.20 0.33
3357	1.80 0.33	2.20 0.33	3.80 0.33	3482	1.40 0.27	1.80 0.33	3.80 0.68
3467	1.80 0.53	3.40 0.98	4.60 0.88	3368	1.40 0.27	3.80 0.33	3.80 0.33
3126	1.80 0.33	3.40 0.50	4.20 0.53	3483	1.40 0.27	1.80 0.33	3.80 0.68
3143	1.80 0.33	3.00 0.60	3.80 0.33	3417	1.40 0.27	1.40 0.27	3.80 0.33
3366	1.80 0.53	2.60 0.65	3.40 0.78	3481	1.40 0.27	2.20 0.33	3.60 0.60

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3400	1.80	3.00	3.80	3467	1.40	1.80	2.60
	0.97	1.10	1.02		0.27	0.53	1.07
3342	1.75	4.50	6.00	3466	1.40	2.20	2.60
	1.11	1.26	1.00		0.27	0.80	0.78
3337	1.67	2.00	3.00	3354	1.40	1.40	1.80
	1.67	1.53	1.15		0.27	0.27	0.33
3103	1.60	2.00	3.00	3382	1.33	1.33	1.67
	0.40	0.42	0.42		0.88	0.88	0.67
3365	1.50	2.00	2.50	3348	1.33	1.33	1.33
	1.19	1.00	1.50		0.88	0.88	0.88
3376	1.40	4.20	4.60	3357	1.20	2.20	3.40
	0.27	0.61	0.58		0.33	0.33	0.27
3406	1.40	2.60	4.20	3145	1.20	2.20	3.40
	0.27	0.50	0.68		0.33	0.33	0.27
3491	1.40	1.80	4.20	3126	1.20	1.60	2.60
	0.27	0.33	0.33		0.33	0.40	0.50
3472	1.40	2.20	4.60	3325	1.00	3.00	5.00
	0.27	0.33	0.27		0.00	0.42	0.00
3085	1.40	2.60	3.80	3376	1.00	3.40	4.60
	0.27	0.27	0.68		0.00	0.27	0.50
3368	1.40	3.80	4.40	3399	1.00	2.60	4.60
	0.27	0.33	0.31		0.00	0.50	0.27
3482	1.40	1.80	5.40	3113	1.00	2.60	4.20
	0.27	0.33	0.27		0.37	0.65	0.68
3483	1.40	1.80	3.80	3387	1.00	1.40	4.20
	0.27	0.33	0.80		0.00	0.27	0.33
3099	1.40	4.60	5.80	3360	1.00	1.40	4.20
	0.27	0.27	0.53		0.00	0.27	0.53
3390	1.40	3.00	4.20	3415	1.00	1.00	4.20
	0.27	0.42	0.33		0.00	0.00	0.53

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3413	1.40 0.27	2.20 0.53	3.40 0.27	3390	1.00 0.00	1.80 0.33	3.40 0.27
3095	1.40 0.27	2.60 0.50	3.80 0.33	3413	1.00 0.00	1.80 0.33	3.40 0.27
3466	1.40 0.27	2.20 0.80	3.80 0.90	3309	1.00 0.00	3.00 0.00	3.40 0.27
3393	1.40 0.27	2.20 0.80	3.00 0.73	3470	1.00 0.00	1.00 0.00	3.00 0.60
3109	1.40 0.27	3.80 0.33	4.60 0.50	3460	1.00 0.37	1.40 0.27	3.00 1.03
3089	1.40 0.27	2.60 0.50	3.00 0.42	3469	1.00 0.00	1.80 0.33	2.80 0.63
3487	1.40 0.27	1.40 0.27	1.80 0.53	3152	1.00 0.00	2.60 0.27	2.80 0.36
3453	1.40 0.27	1.40 0.27	1.40 0.27	3101	1.00 0.00	3.00 0.42	2.60 0.50
3375	1.40 0.68	3.00 0.63	3.00 0.63	3143	1.00 0.00	1.00 0.00	2.60 0.27
3348	1.33 0.88	3.33 1.67	3.33 1.67	3485	1.00 0.00	1.00 0.00	2.60 0.65
3312	1.20 0.20	2.20 0.33	3.00 0.60	3420	1.00 0.00	1.00 0.00	2.60 0.27
3145	1.20 0.33	2.20 0.33	3.40 0.27	3422	1.00 0.00	1.00 0.00	2.60 0.27
3463	1.20 0.33	1.80 0.33	3.00 0.60	3476	1.00 0.00	1.00 0.00	2.60 0.27
3114	1.20 0.33	3.40 0.65	4.60 0.65	3498	1.00 0.00	1.00 0.00	2.60 0.27
3439	1.20 0.33	1.40 0.27	1.80 0.53	3093	1.00 0.00	1.80 0.33	2.20 0.33

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3450	1.20 0.33	1.40 0.27	2.60 0.27	3500	1.00 0.00	1.00 0.00	2.20 0.33
3325	1.00 0.00	3.80 0.33	5.00 0.00	3502	1.00 0.00	1.00 0.00	2.20 0.33
3461	1.00 0.00	2.60 1.07	4.60 0.88	3468	1.00 0.00	1.00 0.00	2.20 0.33
3399	1.00 0.00	2.20 0.33	4.60 0.27	3459	1.00 0.00	0.80 0.13	1.80 0.33
3387	1.00 0.00	2.20 0.33	4.20 0.33	3434	1.00 0.00	1.00 0.00	1.80 0.33
3360	1.00 0.00	1.80 0.53	4.20 0.53	3473	1.00 0.00	1.40 0.27	1.80 0.53
3385	1.00 0.00	3.80 0.68	4.60 0.50	3079	1.00 0.00	1.00 0.00	1.40 0.27
3481	1.00 0.00	1.80 0.33	4.00 0.54	3464	1.00 0.00	1.00 0.00	1.40 0.27
3309	1.00 0.00	1.80 0.33	1.80 0.33	3069	1.00 0.00	1.40 0.27	1.40 0.27
3083	1.00 0.00	2.80 0.55	5.80 0.80	3458	1.00 0.00	1.00 0.00	1.40 0.27
3470	1.00 0.00	2.20 0.53	5.40 0.27	3455	1.00 0.00	1.00 0.00	1.40 0.27
3091	1.00 0.00	2.60 0.78	4.20 0.53	3489	1.00 0.00	1.00 0.00	1.40 0.27
3152	1.00 0.00	2.20 0.33	2.40 0.43	3313	1.00 0.00	1.40 0.27	1.40 0.27
3469	1.00 0.00	1.80 0.33	4.80 0.55	3432	1.00 0.00	1.00 0.00	1.00 0.00
3101	1.00 0.00	3.00 0.42	3.80 0.33	3452	1.00 0.00	1.00 0.00	1.00 0.00

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3454	1.00	3.00	3.80	3503	1.00	1.00	1.00
	0.00	0.60	0.53		0.00	0.00	0.00
3485	1.00	1.40	3.00	3445	1.00	1.00	1.00
	0.00	0.27	0.60		0.00	0.00	0.00
3420	1.00	1.00	2.60	3429	1.00	1.00	1.00
	0.00	0.00	0.27		0.00	0.00	0.00
3422	1.00	1.00	2.60	3431	1.00	1.00	1.00
	0.00	0.00	0.27		0.00	0.00	0.00
3476	1.00	1.00	2.60	3075	1.00	1.00	1.00
	0.00	0.00	0.27		0.00	0.00	0.00
3498	1.00	1.00	2.60	3442	1.00	1.00	1.00
	0.00	0.00	0.27		0.00	0.00	0.00
3093	1.00	2.20	3.00	3495	1.00	1.00	1.00
	0.00	0.33	0.42		0.00	0.00	0.00
3500	1.00	1.00	3.00	3497	1.00	1.00	1.00
	0.00	0.00	0.00		0.00	0.00	0.00
3502	1.00	1.00	3.00	3499	1.00	1.00	1.00
	0.00	0.00	0.00		0.00	0.00	0.00
3468	1.00	1.00	2.20	3501	1.00	1.00	1.00
	0.00	0.00	0.33		0.00	0.00	0.00
3071	1.00	2.60	3.00	3041	1.00	3.40	.
	0.00	0.50	0.60		0.00	0.98	.
3473	1.00	1.00	2.20	3045	1.00	2.60	.
	0.00	0.00	0.33		0.00	0.78	.
3434	1.00	1.00	3.00	3029	1.00	1.80	.
	0.00	0.00	0.42		0.00	0.33	.
3459	1.00	1.00	3.40	3019	1.00	1.40	.
	0.00	0.00	0.50		0.00	0.27	.
3106	1.00	3.00	3.80	3035	1.00	1.40	.
	0.00	0.60	0.33		0.00	0.27	.

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3381	1.00	4.00	5.00	3047	1.00	1.40	.
	0.71	1.00	1.41		0.00	0.27	.
3069	1.00	2.20	2.20	3049	1.00	1.00	.
	0.00	0.53	0.53		0.00	0.00	.
3313	1.00	1.00	1.00	3051	1.00	1.00	.
	0.00	0.00	0.00		0.00	0.00	.
3079	1.00	2.20	4.20	3054	1.00	1.00	.
	0.00	0.33	0.33		0.00	0.00	.
3464	1.00	1.00	3.00	3461	0.80	3.00	5.00
	0.00	0.00	0.00		0.13	1.03	0.84
3458	1.00	1.00	2.20	3077	0.80	2.20	4.20
	0.00	0.00	0.33		0.13	0.53	0.33
3455	1.00	1.00	1.80	3083	0.80	2.80	3.40
	0.00	0.00	0.33		0.13	0.55	0.65
3489	1.00	1.00	1.80	3151	0.80	2.60	3.40
	0.00	0.00	0.33		0.13	0.27	0.50
3443	1.00	1.00	2.20	3091	0.80	1.00	3.00
	0.00	0.00	0.33		0.13	0.00	0.00
3478	1.00	1.00	2.60	3095	0.80	1.20	3.00
	0.00	0.00	0.27		0.13	0.33	0.00
3432	1.00	1.00	2.60	3308	0.80	3.00	3.00
	0.00	0.00	0.27		0.13	0.00	0.00
3452	1.00	1.00	2.20	3311	0.80	2.00	2.60
	0.00	0.00	0.33		0.13	0.42	0.27
3503	1.00	1.00	2.20	3324	0.80	1.40	2.60
	0.00	0.00	0.33		0.13	0.27	0.50
3445	1.00	1.00	1.80	3150	0.80	1.20	2.60
	0.00	0.00	0.33		0.13	0.33	0.65
3429	1.00	1.00	1.60	3463	0.80	1.00	2.20
	0.00	0.00	0.31		0.13	0.00	0.33

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3431	1.00 0.00	1.00 0.00	1.40 0.27	3494	0.80 0.13	1.40 0.27	2.20 0.53
3075	1.00 0.00	1.00 0.00	1.00 0.00	3071	0.80 0.13	2.00 0.60	2.00 0.60
3442	1.00 0.00	1.00 0.00	1.00 0.00	3443	0.80 0.13	1.00 0.00	1.40 0.27
3495	1.00 0.00	1.00 0.00	1.00 0.00	3439	0.80 0.39	1.40 0.27	1.40 0.27
3497	1.00 0.00	1.00 0.00	1.00 0.00	3147	0.80 0.13	1.40 0.27	1.40 0.27
3499	1.00 0.00	1.00 0.00	1.00 0.00	3400	0.80 0.20	1.00 0.00	1.00 0.00
3501	1.00 0.00	1.00 0.00	1.00 0.00	3471	0.80 0.13	1.00 0.00	1.00 0.00
3471	1.00 0.00	1.00 0.00	3.00 0.42	3465	0.80 0.13	1.00 0.00	1.00 0.00
3440	1.00 0.00	1.00 0.00	1.40 0.27	3487	0.80 0.13	1.00 0.00	1.00 0.00
3474	1.00 0.00	1.00 0.00	1.40 0.27	3081	0.80 0.13	0.80 0.13	1.00 0.00
3437	1.00 0.00	1.00 0.00	1.00 0.00	3453	0.80 0.13	1.00 0.00	1.00 0.00
3457	1.00 0.00	1.00 0.00	3.40 0.27	3440	0.80 0.13	1.00 0.00	1.00 0.00
3484	1.00 0.33	1.25 0.25	2.50 0.50	3474	0.80 0.13	1.00 0.00	1.00 0.00
3346	1.00 0.55	1.40 0.68	1.80 0.97	3437	0.80 0.13	1.00 0.00	1.00 0.00
3425	1.00 0.55	1.80 0.49	2.20 0.49	3323	0.80 0.13	1.00 0.00	1.00 0.00

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3338	1.00	1.20	2.60	3436	0.80	0.80	0.80
	1.00	0.97	0.75		0.13	0.13	0.13
3041	1.00	3.40	.	3025	0.80	1.80	.
	0.00	0.98	.		0.13	0.49	.
3045	1.00	2.60	.	3022	0.80	1.20	.
	0.00	0.78	.		0.13	0.33	.
3029	1.00	1.80	.	3037	0.80	1.00	.
	0.00	0.33	.		0.13	0.00	.
3019	1.00	1.40	.	3039	0.80	0.80	.
	0.00	0.27	.		0.13	0.13	.
3035	1.00	1.40	.	3007	0.80	0.80	.
	0.00	0.27	.		0.13	0.13	.
3047	1.00	1.40	.	3033	0.80	0.80	.
	0.00	0.27	.		0.13	0.13	.
3049	1.00	1.00	.	3009	0.80	0.80	.
	0.00	0.00	.		0.13	0.13	.
3051	1.00	1.00	.	3355	0.75	0.75	1.00
	0.00	0.00	.		0.75	0.75	0.71
3054	1.00	1.00	.	3048	0.75	1.00	.
	0.00	0.00	.		0.16	0.00	.
3037	1.00	1.40	.	3002	0.67	0.67	0.67
	0.00	0.27	.		0.21	0.21	0.21
3048	1.00	1.00	.	3085	0.60	1.80	4.20
	0.00	0.00	.		0.16	0.33	0.53
3039	1.00	1.00	.	3065	0.60	2.20	3.80
	0.00	0.00	.		0.16	0.77	0.53
3077	0.80	3.00	5.00	3441	0.60	1.00	3.80
	0.13	0.42	0.60		0.16	0.00	0.33
3151	0.80	2.60	2.20	3099	0.60	3.00	3.40
	0.13	0.27	0.33		0.16	0.42	0.50

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3308	0.80 0.13	1.00 0.00	2.20 0.53	3454	0.60 0.16	1.80 0.33	2.60 0.65
3311	0.80 0.13	2.00 0.42	2.60 0.27	3393	0.60 0.16	1.00 0.00	2.60 0.78
3324	0.80 0.13	1.20 0.33	2.40 0.58	3109	0.60 0.16	1.60 0.58	2.20 0.53
3150	0.80 0.13	1.60 0.40	1.80 0.33	3366	0.60 0.16	0.80 0.13	2.00 0.42
3149	0.80 0.39	1.60 0.40	2.20 0.53	3097	0.60 0.16	1.20 0.33	2.00 0.60
3494	0.80 0.13	1.40 0.27	1.80 0.33	3106	0.60 0.16	0.60 0.16	1.80 0.33
3456	0.80 0.13	1.00 0.00	2.20 0.53	3456	0.60 0.16	1.40 0.27	1.80 0.33
3147	0.80 0.13	1.20 0.33	1.20 0.33	3154	0.60 0.16	1.40 0.27	1.80 0.33
3465	0.80 0.13	1.00 0.00	3.00 0.60	3448	0.60 0.16	1.00 0.00	1.80 0.33
3323	0.80 0.13	1.00 0.00	1.00 0.00	3103	0.60 0.16	1.00 0.37	1.60 0.40
3492	0.80 0.13	1.00 0.00	2.20 0.53	3457	0.60 0.16	1.00 0.00	1.00 0.00
3462	0.80 0.13	1.00 0.00	1.80 0.33	3450	0.60 0.16	1.00 0.00	1.00 0.00
3435	0.80 0.13	1.00 0.00	1.00 0.00	3380	0.60 0.24	0.80 0.20	1.00 0.00
3081	0.80 0.13	1.00 0.00	1.80 0.33	3492	0.60 0.16	1.00 0.00	1.00 0.00
3067	0.80 0.13	1.20 0.33	1.60 0.40	3416	0.60 0.24	0.80 0.20	1.00 0.00

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3436	0.80	0.80	0.80	3462	0.60	1.00	1.00
	0.13	0.13	0.13		0.16	0.00	0.00
3391	0.80	2.00	1.60	3490	0.60	1.00	1.00
	0.20	0.63	0.60		0.16	0.00	0.00
3353	0.80	1.80	3.40	3405	0.60	0.60	0.80
	0.58	0.97	0.75		0.24	0.24	0.20
3404	0.80	1.80	1.80	3391	0.60	0.40	0.80
	0.58	0.97	0.97		0.24	0.24	0.20
3025	0.80	1.80	.	3401	0.60	0.60	0.80
	0.13	0.49	.		0.24	0.24	0.20
3022	0.80	1.20	.	3343	0.60	0.60	0.60
	0.13	0.33	.		0.24	0.24	0.24
3007	0.80	0.80	.	3346	0.60	0.60	0.60
	0.13	0.13	.		0.24	0.24	0.24
3033	0.80	0.80	.	3011	0.60	0.60	.
	0.13	0.13	.		0.16	0.16	.
3493	0.75	1.00	2.50	3043	0.60	0.60	.
	0.16	0.00	0.33		0.16	0.16	.
3477	0.75	1.00	3.00	3451	0.56	1.22	2.56
	0.16	0.00	0.93		0.18	0.36	0.78
3438	0.75	1.00	1.00	3381	0.50	0.75	1.50
	0.16	0.00	0.00		0.29	0.25	0.50
3355	0.75	1.25	1.25	3371	0.50	0.50	1.50
	0.75	1.25	1.25		0.29	0.29	0.50
3335	0.75	1.25	2.00	3477	0.50	1.00	1.00
	0.75	1.25	1.68		0.19	0.00	0.00
3002	0.67	0.67	0.67	3484	0.50	1.00	1.00
	0.21	0.21	0.21		0.19	0.00	0.00
3383	0.67	1.00	1.67	3365	0.50	0.75	1.00
	0.33	0.00	0.67		0.29	0.25	0.00

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3065	0.60	3.40	4.60	3438	0.50	1.00	1.00
	0.16	0.50	0.65		0.19	0.00	0.00
3441	0.60	1.40	3.80	3342	0.50	0.75	0.75
	0.16	0.27	0.33		0.29	0.25	0.25
3486	0.60	1.00	2.60	3349	0.50	0.75	0.75
	0.16	0.00	0.27		0.29	0.25	0.25
3154	0.60	1.40	1.40	3073	0.40	2.20	3.80
	0.16	0.27	0.27		0.16	0.53	0.33
3448	0.60	1.00	1.40	3149	0.40	1.20	2.40
	0.16	0.00	0.27		0.16	0.33	0.58
3430	0.60	1.00	2.20	3486	0.40	1.00	2.20
	0.16	0.00	0.33		0.16	0.00	0.33
3490	0.60	1.00	1.00	3328	0.40	1.00	2.20
	0.16	0.00	0.00		0.16	0.00	0.33
3446	0.60	1.00	2.20	3430	0.40	0.40	1.80
	0.16	0.00	0.53		0.16	0.16	0.33
3424	0.60	1.00	2.00	3433	0.40	1.40	1.80
	0.16	0.00	0.33		0.16	0.27	0.53
3380	0.60	1.20	2.60	3478	0.40	1.00	1.40
	0.24	0.49	0.98		0.16	0.00	0.27
3401	0.60	1.40	1.40	3388	0.40	0.80	1.40
	0.24	0.40	0.40		0.24	0.20	0.40
3343	0.60	1.80	2.00	3153	0.40	0.80	1.40
	0.24	0.97	0.89		0.16	0.13	0.27
3009	0.60	0.80	.	3148	0.40	1.00	1.40
	0.16	0.13	.		0.16	0.00	0.27
3011	0.60	0.60	.	3446	0.40	1.00	1.00
	0.16	0.16	.		0.16	0.00	0.00
3043	0.60	0.60	.	3424	0.40	1.00	1.00
	0.16	0.16	.		0.16	0.00	0.00

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3451	0.56	0.78	1.67	3418	0.40	0.60	1.00
	0.18	0.15	0.65		0.24	0.24	0.00
3371	0.50	1.25	3.00	3435	0.40	1.00	1.00
	0.29	0.63	0.82		0.16	0.00	0.00
3449	0.50	1.00	2.50	3414	0.40	0.40	1.00
	0.19	0.00	0.33		0.24	0.24	0.00
3475	0.50	0.88	2.00	3408	0.40	0.40	0.80
	0.19	0.13	0.38		0.24	0.24	0.20
3349	0.50	3.50	4.50	3425	0.40	0.60	0.60
	0.29	0.96	1.26		0.24	0.24	0.24
3362	0.50	2.75	4.50	3404	0.40	0.40	0.60
	0.29	1.31	2.06		0.24	0.24	0.24
3073	0.40	3.00	5.40	3340	0.40	0.40	0.60
	0.16	0.60	0.65		0.24	0.24	0.24
3328	0.40	1.40	2.20	3378	0.40	0.60	0.60
	0.16	0.27	0.33		0.24	0.24	0.24
3433	0.40	1.00	1.00	3411	0.40	0.60	0.60
	0.16	0.00	0.00		0.24	0.24	0.24
3148	0.40	1.00	1.00	3375	0.40	0.40	0.40
	0.16	0.00	0.00		0.24	0.24	0.24
3364	0.40	1.40	2.60	3027	0.40	2.20	.
	0.24	0.40	0.75		0.16	0.33	.
3496	0.40	1.00	1.80	3005	0.40	0.80	.
	0.16	0.00	0.33		0.16	0.13	.
3416	0.40	2.20	2.20	3015	0.40	0.80	.
	0.24	0.49	0.49		0.16	0.13	.
3427	0.40	0.80	2.60	3383	0.33	0.67	0.67
	0.16	0.13	0.27		0.33	0.33	0.33
3414	0.40	0.60	1.00	3018	0.33	0.33	0.33
	0.24	0.24	0.00		0.21	0.21	0.21

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3405	0.40	1.60	2.20	3344	0.25	2.50	2.50
	0.24	0.60	0.80		0.25	1.44	1.44
3408	0.40	0.80	1.20	3493	0.25	1.00	1.50
	0.24	0.20	0.49		0.16	0.00	0.33
3347	0.40	1.20	2.80	3334	0.25	1.00	1.25
	0.24	0.97	1.62		0.25	0.71	0.63
3341	0.40	2.20	2.80	3449	0.25	1.00	1.00
	0.24	1.16	1.20		0.16	0.00	0.00
3378	0.40	0.80	1.00	3475	0.25	0.88	1.00
	0.24	0.20	0.00		0.16	0.13	0.00
3340	0.40	1.00	1.20	3362	0.25	0.50	0.75
	0.24	0.55	0.49		0.25	0.29	0.25
3352	0.40	1.20	1.80	3377	0.25	0.25	0.75
	0.24	0.49	0.49		0.25	0.25	0.25
3027	0.40	2.20	.	3089	0.20	1.80	1.80
	0.16	0.33	.		0.13	0.53	0.53
3005	0.40	0.80	.	3114	0.20	1.20	1.60
	0.16	0.13	.		0.13	0.33	0.40
3015	0.40	0.80	.	3444	0.20	0.80	1.60
	0.16	0.13	.		0.13	0.13	0.40
3053	0.40	0.60	.	3421	0.20	0.40	1.40
	0.16	0.16	.		0.20	0.24	0.40
3062	0.40	0.40	.	3364	0.20	1.00	1.20
	0.16	0.16	.		0.20	0.55	0.49
3382	0.33	1.33	1.67	3427	0.20	0.80	1.00
	0.33	0.88	0.67		0.13	0.13	0.00
3018	0.33	0.33	0.33	3067	0.20	0.60	1.00
	0.21	0.21	0.21		0.13	0.16	0.37
3344	0.25	3.50	3.50	3428	0.20	0.60	1.00
	0.25	0.96	0.96		0.20	0.24	0.00

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3334	0.25	1.25	4.00	3353	0.20	0.40	0.80
	0.25	0.63	0.58		0.20	0.24	0.20
3377	0.25	0.75	1.50	3347	0.20	0.20	0.80
	0.25	0.25	0.50		0.20	0.20	0.20
3367	0.25	0.50	0.75	3341	0.20	0.00	0.80
	0.25	0.29	0.25		0.20	0.00	0.20
3444	0.20	0.80	2.00	3392	0.20	0.60	0.80
	0.13	0.13	0.60		0.20	0.24	0.20
3388	0.20	1.00	1.40	3407	0.20	0.60	0.60
	0.20	0.00	0.40		0.20	0.24	0.24
3153	0.20	0.80	1.40	3352	0.20	0.20	0.40
	0.13	0.13	0.27		0.20	0.20	0.24
3421	0.20	2.20	2.60	3394	0.20	0.40	0.40
	0.20	0.49	0.75		0.20	0.24	0.24
3418	0.20	1.40	1.40	3398	0.20	0.20	0.20
	0.20	0.40	0.40		0.20	0.20	0.20
3428	0.20	1.40	1.40	3031	0.20	1.20	.
	0.20	0.40	0.40		0.13	0.33	.
3411	0.20	0.60	0.60	3013	0.20	0.20	.
	0.20	0.24	0.24		0.13	0.13	.
3407	0.20	1.60	2.00	3329	0.00	1.20	2.20
	0.20	0.87	1.26		0.00	0.33	0.33
3369	0.20	1.20	1.20	3419	0.00	1.00	1.00
	0.20	0.49	0.49		0.00	0.00	0.00
3394	0.20	1.00	1.40	3423	0.00	0.80	1.00
	0.20	0.00	0.40		0.00	0.20	0.00
3398	0.20	1.00	1.60	3496	0.00	1.00	1.00
	0.20	0.55	0.60		0.00	0.00	0.00
3031	0.20	1.20	.	3326	0.00	0.60	1.00
	0.13	0.33	.		0.00	0.16	0.00

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3017	0.20	0.40	.	3361	0.00	0.00	0.80
	0.13	0.16	.		0.00	0.00	0.20
3013	0.20	0.20	.	3374	0.00	0.40	0.80
	0.13	0.13	.		0.00	0.24	0.20
3010	0.00	0.00	0.00	3412	0.00	0.50	0.75
	0.00	0.00	0.00		0.00	0.29	0.25
3329	0.00	2.00	3.80	3332	0.00	0.00	0.60
	0.00	0.42	0.53		0.00	0.00	0.24
3419	0.00	2.20	2.60	3338	0.00	0.00	0.60
	0.00	0.49	0.40		0.00	0.00	0.24
3423	0.00	2.20	2.60	3369	0.00	0.40	0.60
	0.00	0.49	0.75		0.00	0.24	0.24
3326	0.00	0.60	1.00	3410	0.00	0.60	0.60
	0.00	0.16	0.00		0.00	0.24	0.24
3392	0.00	0.60	0.80	3359	0.00	0.25	0.50
	0.00	0.24	0.20		0.00	0.25	0.29
3374	0.00	0.80	1.40	3367	0.00	0.25	0.50
	0.00	0.20	0.40		0.00	0.25	0.29
3361	0.00	0.20	1.80	3351	0.00	0.20	0.40
	0.00	0.20	0.80		0.00	0.20	0.24
3412	0.00	1.50	3.00	3397	0.00	0.20	0.40
	0.00	0.50	1.41		0.00	0.20	0.24
3410	0.00	0.60	0.60	3389	0.00	0.20	0.40
	0.00	0.24	0.24		0.00	0.20	0.24
3332	0.00	0.00	3.60	3409	0.00	0.40	0.40
	0.00	0.00	1.83		0.00	0.24	0.24
3359	0.00	1.25	2.00	3372	0.00	0.00	0.33
	0.00	0.63	0.58		0.00	0.00	0.33
3409	0.00	0.80	1.20	3358	0.00	0.33	0.33
	0.00	0.20	0.49		0.00	0.33	0.33

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3351	0.00	0.60	1.80	3339	0.00	0.00	0.25
	0.00	0.24	0.73		0.00	0.00	0.25
3397	0.00	1.00	1.60	3336	0.00	0.00	0.25
	0.00	0.55	0.60		0.00	0.00	0.25
3389	0.00	0.80	1.60	3426	0.00	0.50	0.25
	0.00	0.20	0.60		0.00	0.29	0.25
3358	0.00	0.33	0.33	3395	0.00	0.25	0.25
	0.00	0.33	0.33		0.00	0.25	0.25
3372	0.00	1.00	1.33	3402	0.00	0.20	0.20
	0.00	1.00	0.88		0.00	0.20	0.20
3426	0.00	0.50	1.00	3333	0.00	0.00	0.20
	0.00	0.29	0.71		0.00	0.00	0.20
3395	0.00	0.75	0.75	3386	0.00	0.40	0.20
	0.00	0.75	0.75		0.00	0.24	0.20
3339	0.00	1.50	2.50	3356	0.00	0.50	0.00
	0.00	1.19	0.96		0.00	0.29	0.00
3336	0.00	0.50	1.50	3337	0.00	0.00	0.00
	0.00	0.29	0.87		0.00	0.00	0.00
3386	0.00	0.60	0.20	3335	0.00	0.00	0.00
	0.00	0.24	0.20		0.00	0.00	0.00
3402	0.00	0.40	1.00	3384	0.00	0.00	0.00
	0.00	0.24	0.55		0.00	0.00	0.00
3333	0.00	0.20	0.80	3350	0.00	0.00	0.00
	0.00	0.20	0.58		0.00	0.00	0.00
3356	0.00	0.75	3.00	3510	0.00	0.00	0.00
	0.00	0.25	0.00		0.00	0.00	0.00
3384	0.00	0.40	1.20	3345	0.00	0.00	0.00
	0.00	0.24	0.49		0.00	0.00	0.00
3350	0.00	0.33	0.67	3004	0.00	0.00	0.00
	0.00	0.33	0.33		0.00	0.00	0.00

Appendix 3.1 (continued).

	PI86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3510	0.00	0.00	0.40	3006	0.00	0.00	0.00
	0.00	0.00	0.16		0.00	0.00	0.00
3345	0.00	0.20	0.20	3008	0.00	0.00	0.00
	0.00	0.20	0.20		0.00	0.00	0.00
3004	0.00	0.00	0.00	3012	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3006	0.00	0.00	0.00	3014	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3008	0.00	0.00	0.00	3016	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3012	0.00	0.00	0.00	3024	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3014	0.00	0.00	0.00	3026	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3016	0.00	0.00	0.00	3028	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3024	0.00	0.00	0.00	3030	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3026	0.00	0.00	0.00	3032	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3028	0.00	0.00	0.00	3034	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3030	0.00	0.00	0.00	3036	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3032	0.00	0.00	0.00	3038	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3034	0.00	0.00	0.00	3040	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3036	0.00	0.00	0.00	3042	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3038	0.00	0.00	0.00	3044	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3040	0.00	0.00	0.00	3046	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3042	0.00	0.00	0.00	3050	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3044	0.00	0.00	0.00	3052	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3046	0.00	0.00	0.00	3055	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3050	0.00	0.00	0.00	3057	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3052	0.00	0.00	0.00	3059	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3055	0.00	0.00	0.00	3066	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3057	0.00	0.00	0.00	3068	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3059	0.00	0.00	0.00	3070	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3066	0.00	0.00	0.00	3072	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3068	0.00	0.00	0.00	3074	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3070	0.00	0.00	0.00	3076	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3072	0.00	0.00	0.00	3078	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3074	0.00	0.00	0.00	3080	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00

Appendix 3.1 (continued).

	Pl86-14				Plat2		
UMNO*	10	12	15	UMNO*	10	12	15
3076	0.00	0.00	0.00	3082	0.00	0.00	0.00
	0.00	0.00	0.00		0.00	0.00	0.00
3078	0.00	0.00	0.00	3060	0.00	4.00	.
	0.00	0.00	0.00		0.00	0.99	.
3080	0.00	0.00	0.00	3061	0.00	0.80	.
	0.00	0.00	0.00		0.00	0.39	.
3082	0.00	0.00	0.00	3017	0.00	0.40	.
	0.00	0.00	0.00		0.00	0.16	.
3060	0.00	2.00	.	3003	0.00	0.40	.
	0.00	0.84	.		0.00	0.16	.
3061	0.00	0.40	.	3053	0.00	0.20	.
	0.00	0.16	.		0.00	0.13	.
3003	0.00	0.40	.	3062	0.00	0.00	.
	0.00	0.16	.		0.00	0.00	.
3001	0.00	0.00	.	3001	0.00	0.00	.
	0.00	0.00	.		0.00	0.00	.
3021	0.00	0.00	.	3021	0.00	0.00	.
	0.00	0.00	.		0.00	0.00	.
3023	0.00	0.00	.	3023	0.00	0.00	.
	0.00	0.00	.		0.00	0.00	.
3058	0.00	0.00	.	3058	0.00	0.00	.
	0.00	0.00	.		0.00	0.00	.
3063	0.00	0.00	.	3063	0.00	0.00	.
	0.00	0.00	.		0.00	0.00	.
Maluka	0.00	1.70	.	Maluka	0.00	1.20	.
	0.00	0.45	.		0.00	0.42	.
Westar	9.00	9.00	.	Westar	9.00	9.00	.
	0.00	0.00	.		0.00	0.00	.

Appendix 3.2. Mean disease severity ratings for Brassica juncea accessions challenged at cotyledon stage with 2 isolates of Leptosphaeria maculans (Pl86-14 & Plat2) and at adult stem stage with Pl86-14. Values are means of 5 to 10 plants (Upper values) ± SE (Lower values) 10 days (B2, A2), after inoculations. Mean disease severity ratings are arranged in descending disease severity at week 4.
*University of Manitoba accession number.

	Cotyledon Tests		Adult stem tests					
UMNO*	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3115	9.00	9.00	5.10	6.70	7.30	7.80	8.00	7.00
	0.00	0.00	0.86	0.68	0.40	0.13	0.00	0.00
3342	5.00	1.00	1.00	2.00	4.00	6.00	.	.
	.	.	.	.	.	.	.	.
3358	0.00	0.00	0.50	2.25	4.00	5.75	.	.
	0.00	0.00	0.29	0.25	0.58	1.03	.	.
3404	0.80	0.40	0.40	3.00	4.00	5.60	.	.
	0.58	0.24	0.24	0.32	0.45	0.81	.	.
3405	0.40	0.60	0.00	3.40	4.60	5.60	.	.
	0.24	0.24	0.00	0.75	0.75	0.75	.	.
3411	0.20	0.40	0.40	2.00	4.00	5.60	.	.
	0.20	0.24	0.24	0.55	1.14	1.40	.	.
3407	0.20	0.20	0.00	2.60	4.00	5.40	.	.
	0.20	0.20	0.00	0.40	0.55	0.93	.	.
3359	0.00	0.00	0.00	2.00	3.40	5.40	.	.
	0.00	0.00	0.00	0.45	0.51	0.93	.	.
3339	0.00	0.00	0.67	1.67	4.00	5.33	.	.
	0.00	0.00	0.33	0.67	1.73	1.76	.	.
3347	0.25	0.25	0.50	2.50	4.00	5.25	.	.
	0.25	0.25	0.50	0.50	0.71	0.85	.	.
3388	0.25	0.50	0.75	2.50	4.50	5.25	.	.
	0.25	0.29	0.25	1.04	1.50	1.75		
3369	0.20	0.00	0.20	1.60	3.40	5.20	.	.
	0.20	0.00	0.20	0.51	1.03	1.36	.	.
3374	0.00	0.00	0.80	2.60	3.80	5.20	.	.
	0.00	0.00	0.20	0.75	1.07	1.36	.	.
3380	0.60	0.60	0.40	2.40	4.00	5.00	.	.
	0.24	0.24	0.24	0.60	0.55	0.55	.	.

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3408	0.40	0.40	0.00	3.00	4.00	5.00	.	.
	0.24	0.24	0.00	0.95	1.14	1.38	.	.
3362	0.00	0.00	0.00	1.50	3.50	5.00	.	.
	0.00	0.00	0.00	0.50	0.50	0.00	.	.
3372	0.00	0.00	0.00	2.00	3.75	5.00	.	.
	0.00	0.00	0.00	0.71	0.85	0.91	.	.
3346	1.00	0.60	0.00	1.40	2.80	4.80	.	.
	0.55	0.24	0.00	0.24	0.49	0.86	.	.
3410	0.00	0.00	0.40	2.80	3.80	4.80	.	.
	0.00	0.00	0.24	0.66	0.66	0.66	.	.
3382	0.25	1.00	0.50	2.50	3.50	4.75	.	.
	0.25	0.71	0.29	0.65	0.87	0.95	.	.
3355	0.60	0.60	0.00	1.80	3.20	4.60	.	.
	0.60	0.60	0.00	0.49	0.66	0.81	.	.
3341	0.25	0.00	0.25	1.00	2.50	4.50	.	.
	0.25	0.00	0.25	0.00	0.65	0.87	.	.
3350	0.00	0.00	0.50	2.00	3.00	4.50	.	.
	0.00	0.00	0.29	0.41	0.82	1.04	.	.
3375	1.40	0.40	0.40	2.00	3.20	4.40	.	.
	0.68	0.24	0.24	0.45	0.73	0.81	.	.
3364	0.40	0.20	0.20	1.40	3.20	4.00	.	.
	0.24	0.20	0.20	0.40	1.16	1.30	.	.
3344	0.25	0.25	0.25	1.50	2.50	4.00	.	.
	0.25	0.25	0.25	0.50	0.96	1.47	.	.
3333	0.00	0.00	0.00	1.67	3.33	4.00	.	.
	0.00	0.00	0.00	0.67	1.33	1.53	.	.
3345	0.00	0.00	0.40	1.80	2.80	4.00	.	.
	0.00	0.00	0.24	0.37	0.66	1.00	.	.
3402	0.00	0.00	0.40	1.80	3.00	4.00	.	.
	0.00	0.00	0.24	0.97	1.38	1.70	.	.

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3361	0.00	0.00	0.00	2.00	3.00	3.80	.	.
	0.00	0.00	0.00	0.45	0.95	1.07	.	.
3386	0.00	0.00	0.20	1.40	3.20	3.80	.	.
	0.00	0.00	0.20	0.24	0.86	1.07	.	.
3409	0.00	0.00	0.40	1.80	3.20	3.80	.	.
	0.00	0.00	0.24	0.80	1.36	1.59	.	.
3351	0.00	0.00	0.25	1.75	2.75	3.75	.	.
	0.00	0.00	0.25	0.75	1.31	1.65	.	.
3371	0.50	0.50	0.00	2.00	2.75	3.50	.	.
	0.29	0.29	0.00	1.22	1.60	2.02	.	.
3334	0.25	0.25	0.00	1.25	2.25	3.50	.	.
	0.25	0.25	0.00	0.63	1.11	1.44	.	.
3336	0.00	0.00	0.25	1.25	2.25	3.50	.	.
	0.00	0.00	0.25	0.25	0.75	1.32	.	.
3476	1.00	1.00	0.80	1.40	2.40	3.40	.	.
	0.00	0.00	0.20	0.51	0.51	0.68	.	.
3337	1.67	0.00	0.33	1.33	2.00	3.33	.	.
	1.67	0.00	0.33	0.33	1.00	1.86	.	.
3474	1.00	0.80	0.00	0.00	1.20	3.00	.	.
	0.00	0.20	0.00	0.00	0.37	0.84	.	.
3335	0.75	0.00	0.00	1.25	2.00	3.00	.	.
	0.75	0.00	0.00	0.25	0.71	1.08	.	.
3365	1.50	0.50	0.00	1.25	2.00	2.75	.	.
	1.19	0.29	0.00	0.63	0.91	1.11	.	.
3475	0.50	0.25	0.25	0.25	1.25	2.75	.	.
	0.29	0.25	0.25	0.25	0.25	0.85	.	.
3377	0.25	0.25	0.25	1.50	2.00	2.75	.	.
	0.25	0.25	0.25	0.65	0.91	1.11	.	.
3118	2.60	1.80	0.00	1.10	2.10	2.60	5.80	3.00
	0.27	0.33	0.00	0.78	0.84	0.83	1.05	0.97

Appendix 3.2 (continued).

	Cotyledon Tests		Adult stem tests					
UMNO*	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3439	1.20	0.80	0.40	0.60	1.20	2.60	.	.
	0.49	0.58	0.24	0.24	0.20	0.40	.	.
3471	1.00	0.80	0.40	0.80	1.40	2.60	.	.
	0.00	0.20	0.24	0.58	0.51	0.75	.	.
3340	0.40	0.40	0.40	0.80	1.40	2.60	.	.
	0.24	0.24	0.24	0.49	0.87	1.60	.	.
3367	0.20	0.00	0.20	0.60	1.80	2.60	.	.
	0.20	0.00	0.20	0.24	0.92	1.47	.	.
3312	1.20	1.60	0.00	0.80	1.70	2.50	3.30	2.00
	0.20	0.31	0.00	0.13	0.21	0.34	0.37	0.97
3132	2.56	2.44	0.00	0.17	2.00	2.44	2.94	1.13
	0.60	0.62	0.00	0.09	0.67	0.63	0.62	0.56
3071	1.00	0.86	0.00	0.57	0.57	2.43	6.00	6.00
	0.00	0.14	0.00	0.20	0.20	0.48	0.87	1.00
3487	1.40	0.80	0.00	0.00	1.20	2.40	.	.
	0.40	0.20	0.00	0.00	0.37	0.60	.	.
3338	1.00	0.00	0.20	0.80	1.40	2.40	.	.
	1.00	0.00	0.20	0.37	0.75	1.29	.	.
3431	1.00	1.00	0.00	0.00	1.00	2.40	.	.
	0.00	0.00	0.00	0.00	0.00	0.24	.	.
3489	1.00	1.00	0.20	0.20	0.80	2.40	.	.
	0.00	0.00	0.20	0.20	0.20	0.75	.	.
3492	0.80	0.60	0.40	0.40	1.20	2.40	.	.
	0.20	0.24	0.24	0.24	0.20	0.60	.	.
3414	0.40	0.40	0.20	0.20	1.80	2.40	.	.
	0.24	0.24	0.20	0.20	0.37	0.75	.	.
3143	1.80	1.00	0.00	0.80	1.80	2.30	4.80	3.40
	0.33	0.00	0.00	0.20	0.47	0.40	0.84	0.50
3323	0.80	0.80	0.00	0.40	1.30	2.30	3.60	3.00
	0.13	0.13	0.00	0.16	0.42	0.50	0.93	1.16

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3060	0.00	0.00	0.00	0.50	1.00	2.30	3.80	3.80
	0.00	0.00	0.00	0.17	0.00	0.33	0.77	1.06
3021	0.00	0.00	0.00	0.71	1.29	2.29	3.71	0.71
	0.00	0.00	0.00	0.29	0.47	0.52	0.97	0.18
3378	0.50	0.50	0.00	1.00	1.50	2.25	.	.
	0.29	0.29	0.00	0.58	0.87	1.31	.	.
3412	0.00	0.00	0.00	1.00	1.75	2.25	.	.
	0.00	0.00	0.00	0.71	1.03	1.31	.	.
3037	1.00	0.78	0.00	1.67	1.78	2.22	3.13	0.33
	0.00	0.15	0.00	0.80	0.80	0.80	0.87	0.33
3314	2.00	2.00	0.20	0.70	1.50	2.20	2.30	1.80
	0.84	0.84	0.13	0.33	0.40	0.47	0.45	0.92
3383	0.80	0.40	0.00	1.00	1.60	2.20	.	.
	0.20	0.24	0.00	0.45	0.68	0.92	.	.
3343	0.60	0.60	0.20	1.20	1.60	2.20	.	.
	0.24	0.24	0.20	0.20	0.60	1.20	.	.
3111	2.00	2.00	0.00	0.75	1.00	2.13	5.63	4.88
	0.38	0.38	0.00	0.16	0.00	0.30	1.03	0.85
3013	0.25	0.25	0.00	0.50	1.25	2.13	2.88	2.38
	0.16	0.16	0.00	0.19	0.45	0.67	1.01	1.18
3017	0.25	0.00	0.00	0.38	1.88	2.13	2.50	0.00
	0.16	0.00	0.00	0.18	0.91	0.90	0.91	0.00
3043	0.56	0.56	0.00	1.67	1.89	2.11	4.00	0.00
	0.18	0.18	0.00	0.80	0.77	0.75	1.01	0.00
3403	4.60	3.80	0.00	1.00	1.30	2.00	6.90	5.44
	0.50	0.80	0.00	0.79	0.78	0.70	0.23	1.03
3488	2.60	2.20	0.00	0.00	1.20	2.00	.	.
	0.40	0.49	0.00	0.00	0.20	0.63	.	.
3406	1.44	1.44	0.00	0.89	1.44	2.00	5.67	3.67
	0.29	0.29	0.00	0.20	0.38	0.37	0.80	1.18

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3390	1.40	1.00	0.00	0.40	0.70	2.00	6.30	5.10
	0.27	0.00	0.00	0.16	0.15	0.26	0.42	0.97
3348	1.33	1.33	0.00	1.33	1.67	2.00	.	.
	0.88	0.88	0.00	0.33	0.67	1.00	.	.
3085	1.25	0.63	0.00	0.75	1.88	2.00	5.88	2.88
	0.25	0.18	0.00	0.25	0.81	0.80	0.64	1.04
3048	1.00	0.75	0.00	1.38	1.63	2.00	6.00	0.00
	0.00	0.16	0.00	0.96	0.94	0.89	0.78	0.00
3455	1.00	1.00	0.00	0.00	0.80	2.00	.	.
	0.00	0.00	0.00	0.00	0.37	0.84	.	.
3457	1.00	0.60	0.00	0.00	0.80	2.00	.	.
	0.00	0.24	0.00	0.00	0.37	0.95	.	.
3308	0.80	0.80	0.00	0.70	1.30	2.00	2.40	1.67
	0.13	0.13	0.00	0.15	0.21	0.45	0.48	1.01
3025	0.78	0.78	0.00	0.78	2.00	2.00	3.22	1.00
	0.15	0.15	0.00	0.22	0.58	0.58	0.80	0.50
3477	0.75	0.50	0.50	0.50	1.00	2.00	.	.
	0.25	0.29	0.29	0.29	0.00	0.41	.	.
3352	0.40	0.20	0.00	0.80	1.20	2.00	.	.
	0.24	0.20	0.00	0.37	0.58	0.95	.	.
3356	0.00	0.00	0.00	1.00	1.25	2.00	.	.
	0.00	0.00	0.00	0.00	0.25	0.41	.	.
3029	1.00	1.00	0.00	0.40	1.90	1.90	5.00	0.78
	0.00	0.00	0.00	0.16	0.59	0.59	0.91	0.78
3051	1.00	1.00	0.00	0.90	1.40	1.90	6.00	1.33
	0.00	0.00	0.00	0.80	0.78	0.71	0.71	0.55
3049	1.00	1.00	0.00	0.67	1.22	1.89	4.00	2.33
	0.00	0.00	0.00	0.17	0.15	0.35	0.88	1.17
3363	2.20	1.40	0.00	0.30	0.70	1.80	4.40	2.30
	0.33	0.27	0.00	0.15	0.15	0.25	0.87	1.03

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3440	1.00	0.80	0.20	0.40	1.00	1.80	.	.
	0.00	0.20	0.20	0.40	0.32	0.49	.	.
3486	0.60	0.40	0.00	0.00	0.80	1.80	.	.
	0.24	0.24	0.00	0.00	0.37	0.73	.	.
3005	0.40	0.40	0.00	0.50	1.10	1.80	3.40	2.30
	0.16	0.16	0.00	0.17	0.10	0.33	0.83	1.03
3384	0.00	0.00	0.00	0.60	1.40	1.80	.	.
	0.00	0.00	0.00	0.24	0.75	0.92	.	.
3423	0.00	0.00	0.60	0.60	1.00	1.80	.	.
	0.00	0.00	0.24	0.24	0.32	0.66	.	.
3379	2.78	1.44	0.00	0.67	0.67	1.78	5.78	4.78
	0.52	0.29	0.00	0.17	0.17	0.32	0.72	0.95
3009	0.56	0.78	0.00	0.67	1.56	1.78	3.56	2.78
	0.18	0.15	0.00	0.24	0.41	0.36	0.85	1.10
3435	0.75	0.50	0.25	0.50	1.00	1.75	.	.
	0.25	0.29	0.25	0.29	0.00	0.48	.	.
3449	0.50	0.25	0.00	0.00	0.75	1.75	.	.
	0.29	0.25	0.00	0.00	0.25	0.63	.	.
3354	2.14	1.29	0.00	0.14	0.71	1.71	4.14	1.86
	0.40	0.29	0.00	0.14	0.18	0.36	0.99	0.70
3417	1.80	1.40	0.00	0.10	0.50	1.70	3.20	2.80
	0.33	0.27	0.00	0.10	0.31	0.37	0.87	1.14
3099	1.40	0.60	0.00	0.60	1.30	1.70	3.10	2.90
	0.27	0.16	0.00	0.16	0.47	0.40	0.74	1.12
3047	1.00	1.00	0.00	0.70	1.50	1.70	4.50	1.00
	0.00	0.00	0.00	0.21	0.27	0.26	0.73	0.73
3079	1.00	1.00	0.10	0.40	1.70	1.70	3.50	3.67
	0.00	0.00	0.10	0.16	0.21	0.21	0.54	0.58
3106	1.00	0.60	0.00	0.50	0.90	1.70	7.80	5.60
	0.00	0.16	0.00	0.17	0.35	0.21	0.13	0.52

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3309	1.00	1.00	0.00	0.30	0.70	1.70	2.10	0.80
	0.00	0.00	0.00	0.15	0.15	0.30	0.31	0.39
3420	1.00	1.00	0.00	0.44	1.00	1.67	2.78	2.89
	0.00	0.00	0.00	0.18	0.29	0.33	0.86	1.16
3113	2.20	1.00	0.00	0.50	1.00	1.60	4.70	6.40
	0.33	0.37	0.00	0.17	0.21	0.22	0.91	0.31
3357	1.80	1.20	0.00	0.20	0.80	1.60	2.40	4.10
	0.33	0.33	0.00	0.13	0.29	0.31	0.76	1.07
3480	1.80	2.20	0.20	0.20	0.80	1.60	.	.
	0.49	0.49	0.20	0.20	0.20	0.40	.	.
3483	1.40	1.40	0.20	0.20	0.80	1.60	.	.
	0.40	0.40	0.20	0.20	0.20	0.68	.	.
3484	1.20	0.60	0.00	0.20	1.00	1.60	.	.
	0.49	0.24	0.00	0.20	0.77	1.17	.	.
3437	1.00	0.80	0.20	0.20	1.00	1.60	.	.
	0.00	0.20	0.20	0.20	0.00	0.24	.	.
3501	1.00	1.00	0.00	0.40	0.80	1.60	1.80	0.25
	0.00	0.00	0.00	0.16	0.13	0.22	0.13	0.16
3154	0.60	0.60	0.00	0.20	0.90	1.60	2.70	0.78
	0.16	0.16	0.00	0.13	0.31	0.34	0.45	0.78
3027	0.40	0.40	0.00	0.90	1.40	1.60	4.30	1.80
	0.16	0.16	0.00	0.18	0.27	0.34	0.94	0.87
3370	2.56	2.33	0.00	0.33	1.11	1.56	4.78	3.89
	0.29	0.33	0.00	0.17	0.51	0.44	0.78	1.01
3075	1.00	1.00	0.00	0.78	1.22	1.56	4.00	2.11
	0.00	0.00	0.00	0.15	0.15	0.24	0.88	1.07
3387	1.00	1.00	0.00	0.33	0.67	1.56	6.33	4.11
	0.00	0.00	0.00	0.17	0.24	0.24	0.55	0.99
3022	0.89	0.89	0.00	0.33	1.56	1.56	5.00	0.14
	0.11	0.11	0.00	0.17	0.82	0.82	1.12	0.14

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3067	0.78	0.22	0.00	0.56	0.89	1.56	3.22	2.00
	0.15	0.15	0.00	0.24	0.42	0.58	0.91	0.96
3116	3.00	1.80	0.00	0.80	1.10	1.50	4.90	2.60
	0.00	0.33	0.00	0.13	0.23	0.22	0.90	0.91
3460	2.20	1.00	0.00	0.00	0.75	1.50	.	.
	1.71	0.55	0.00	0.00	0.25	0.65	.	.
3077	0.80	0.80	0.00	0.80	1.00	1.50	4.00	6.60
	0.13	0.13	0.00	0.13	0.15	0.17	0.86	0.27
3311	0.80	0.80	0.00	0.20	1.00	1.50	2.20	1.43
	0.13	0.13	0.00	0.13	0.15	0.22	0.25	0.72
3493	0.75	0.25	0.25	0.25	0.75	1.50	.	.
	0.25	0.25	0.25	0.25	0.25	0.65	.	.
3073	0.40	0.40	0.00	0.40	0.60	1.50	4.00	4.80
	0.16	0.16	0.00	0.16	0.16	0.22	0.71	0.87
3058	0.00	0.00	0.00	0.40	1.30	1.50	4.20	0.10
	0.00	0.00	0.00	0.16	0.15	0.27	0.99	0.10
3063	0.00	0.00	0.00	0.00	0.50	1.50	4.40	1.20
	0.00	0.00	0.00	0.00	0.17	0.31	0.81	0.81
3396	2.56	2.33	0.00	0.22	0.56	1.44	5.00	3.00
	0.56	0.58	0.00	0.15	0.18	0.18	0.93	1.13
3039	1.00	0.78	0.00	0.67	1.22	1.44	2.00	2.00
	0.00	0.15	0.00	0.17	0.28	0.34	0.55	0.88
3041	1.00	1.00	0.00	0.56	1.00	1.44	2.33	0.56
	0.00	0.00	0.00	0.18	0.00	0.18	0.62	0.34
3062	0.33	0.00	0.00	0.33	1.00	1.44	4.11	1.33
	0.17	0.00	0.00	0.17	0.33	0.29	0.92	0.90
3366	2.14	0.57	0.00	0.43	1.00	1.43	4.00	3.14
	0.74	0.20	0.00	0.20	0.00	0.20	1.09	1.37
3095	1.40	0.80	0.00	0.20	0.80	1.40	2.20	2.40
	0.27	0.13	0.00	0.13	0.13	0.22	0.73	1.05

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3453	1.40	0.80	0.00	0.00	0.60	1.40	.	.
	0.40	0.20	0.00	0.00	0.24	0.60	.	.
3045	1.00	1.00	0.00	0.40	0.90	1.40	3.20	1.90
	0.00	0.00	0.00	0.16	0.23	0.16	0.85	0.85
3091	1.00	0.80	0.00	0.40	1.00	1.40	4.20	3.90
	0.00	0.13	0.00	0.22	0.58	0.52	0.93	0.98
3360	1.00	1.00	0.00	0.40	0.80	1.40	4.30	2.70
	0.00	0.00	0.00	0.16	0.39	0.31	0.86	0.97
3452	1.00	1.00	0.00	0.00	0.60	1.40	.	.
	0.00	0.00	0.00	0.00	0.24	0.60	.	.
3464	1.00	1.00	0.00	0.00	0.40	1.40	.	.
	0.00	0.00	0.00	0.00	0.24	0.87	.	.
3481	1.00	1.40	0.20	0.40	0.80	1.40	.	.
	0.00	0.40	0.20	0.40	0.80	1.40	.	.
3147	0.80	0.80	0.00	0.30	0.90	1.40	1.90	0.00
	0.13	0.13	0.00	0.15	0.18	0.16	0.18	0.00
3150	0.80	0.80	0.00	0.40	0.70	1.40	1.90	0.13
	0.13	0.13	0.00	0.16	0.15	0.27	0.23	0.13
3462	0.80	0.60	0.00	0.00	0.80	1.40	.	.
	0.20	0.24	0.00	0.00	0.20	0.51	.	.
3401	0.60	0.60	0.00	0.00	0.80	1.40	.	.
	0.24	0.24	0.00	0.00	0.20	0.51	.	.
3418	0.20	0.40	0.00	0.00	0.80	1.40	.	.
	0.20	0.24	0.00	0.00	0.20	0.40	.	.
3068	0.00	0.00	0.00	0.00	1.20	1.40	.	.
	0.00	0.00	0.00	0.00	0.80	0.93	.	.
3415	1.75	1.00	0.00	0.25	0.63	1.38	5.38	2.25
	0.37	0.00	0.00	0.16	0.38	0.26	0.94	0.96
3033	0.88	0.88	0.00	0.38	0.75	1.38	2.00	1.57
	0.12	0.12	0.00	0.00	0.16	0.37	0.42	0.90

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3015	0.38	0.38	0.00	0.50	0.88	1.38	2.50	1.25
	0.18	0.18	0.00	0.19	0.23	0.37	0.94	0.90
3109	1.44	0.56	0.00	0.67	0.67	1.33	5.78	4.44
	0.29	0.18	0.00	0.17	0.17	0.17	0.86	0.93
3393	1.44	0.67	0.00	0.22	0.89	1.33	6.33	4.78
	0.29	0.17	0.00	0.15	0.20	0.17	0.67	1.01
3368	1.22	1.22	0.00	0.00	0.33	1.33	4.56	3.89
	0.22	0.22	0.00	0.00	0.17	0.24	0.99	1.23
3007	0.78	0.78	0.00	0.78	1.22	1.33	1.78	0.22
	0.15	0.15	0.00	0.15	0.28	0.24	0.36	0.15
3081	0.78	0.78	0.00	0.67	1.22	1.33	4.00	2.89
	0.15	0.15	0.00	0.17	0.22	0.17	0.82	1.02
3065	0.56	0.56	0.00	0.33	0.78	1.33	3.22	3.56
	0.18	0.18	0.00	0.17	0.22	0.24	0.88	1.03
3031	0.22	0.22	0.00	0.33	0.44	1.33	3.11	0.11
	0.15	0.15	0.00	0.17	0.24	0.17	0.70	0.11
3353	0.00	0.00	0.00	0.67	1.00	1.33	.	.
	0.00	0.00	0.00	0.67	1.00	1.33	.	.
3064	3.80	1.80	0.00	0.60	0.80	1.30	2.30	0.20
	0.80	0.33	0.00	0.16	0.13	0.15	0.58	0.13
3373	2.20	2.20	0.00	0.20	0.60	1.30	3.20	1.80
	0.33	0.33	0.00	0.13	0.22	0.15	0.93	0.88
3152	1.00	1.00	0.00	0.20	0.90	1.30	1.60	0.00
	0.00	0.00	0.00	0.13	0.10	0.15	0.16	0.00
3151	0.80	0.80	0.00	0.70	1.00	1.30	1.70	0.00
	0.13	0.13	0.00	0.15	0.21	0.21	0.26	0.00
3001	0.00	0.00	0.00	0.57	1.29	1.29	1.86	0.57
	0.00	0.00	0.00	0.20	0.29	0.29	0.34	0.43
3097	2.50	0.75	0.00	0.75	1.25	1.25	2.50	4.88
	0.33	0.16	0.00	0.25	0.56	0.56	0.80	0.85

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3056	1.38	1.13	0.00	0.63	1.25	1.25	3.88	0.75
	1.10	0.85	0.00	0.18	0.25	0.25	0.97	0.49
3126	1.89	1.00	0.00	0.22	0.56	1.22	3.22	0.67
	0.35	0.29	0.00	0.15	0.24	0.15	0.97	0.44
3093	1.00	1.00	0.00	0.33	0.78	1.22	3.67	3.33
	0.00	0.00	0.00	0.24	0.32	0.22	1.05	0.97
3114	1.20	0.20	0.00	0.40	0.50	1.20	3.20	2.80
	0.33	0.13	0.00	0.16	0.17	0.20	0.88	0.70
3145	1.20	1.20	0.00	0.20	0.60	1.20	1.50	1.44
	0.33	0.33	0.00	0.13	0.16	0.13	0.27	0.75
3035	1.00	1.00	0.00	0.50	0.90	1.20	2.90	0.00
	0.00	0.00	0.00	0.17	0.28	0.25	0.53	0.00
3083	1.00	0.80	0.00	0.70	1.00	1.20	3.40	4.60
	0.00	0.13	0.00	0.15	0.15	0.13	0.79	0.78
3443	1.00	0.80	0.00	0.00	0.80	1.20	.	.
	0.00	0.20	0.00	0.00	0.20	0.37	.	.
3498	1.00	1.00	0.40	0.60	0.80	1.20	.	.
	0.00	0.00	0.24	0.40	0.37	0.49	.	.
3499	1.00	1.00	0.00	0.00	0.40	1.20	1.60	0.00
	0.00	0.00	0.00	0.00	0.16	0.13	0.16	0.00
3438	0.80	0.60	0.20	0.20	0.80	1.20	.	.
	0.20	0.24	0.20	0.20	0.20	0.37	.	.
3011	0.60	0.60	0.00	0.40	0.80	1.20	2.80	1.20
	0.16	0.16	0.00	0.16	0.20	0.25	0.66	0.81
3416	0.40	0.60	0.00	0.00	0.80	1.20	.	.
	0.24	0.24	0.00	0.00	0.20	0.37	.	.
3332	0.00	0.00	0.00	0.60	0.80	1.20	.	.
	0.00	0.00	0.00	0.24	0.37	0.58	.	.
3392	0.00	0.20	0.20	0.20	0.40	1.20	.	.
	0.00	0.20	0.20	0.20	0.24	0.73	.	.

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3419	0.00	0.00	0.00	0.00	1.00	1.20	.	.
	0.00	0.00	0.00	0.00	0.00	0.20	.	.
3103	1.60	0.60	0.00	0.10	0.20	1.10	6.10	3.20
	0.40	0.16	0.00	0.10	0.13	0.10	0.72	0.94
3101	1.00	1.00	0.00	0.40	0.60	1.10	4.00	4.80
	0.00	0.00	0.00	0.16	0.27	0.18	0.80	0.81
3497	1.00	1.00	0.00	0.10	0.40	1.10	2.10	0.78
	0.00	0.00	0.00	0.10	0.16	0.18	0.23	0.78
3053	0.40	0.00	0.00	0.40	0.80	1.10	3.90	1.10
	0.16	0.00	0.00	0.16	0.25	0.28	0.90	0.66
3153	0.20	0.40	0.00	0.20	0.60	1.10	1.60	0.14
	0.13	0.16	0.00	0.13	0.16	0.23	0.22	0.14
3061	0.00	0.00	0.00	0.40	1.00	1.10	2.40	1.40
	0.00	0.00	0.00	0.16	0.15	0.10	0.50	0.67
3089	1.44	0.22	0.00	0.56	0.67	1.00	3.11	3.22
	0.29	0.15	0.00	0.18	0.17	0.00	0.81	0.95
3376	1.40	1.00	0.00	0.20	0.40	1.00	3.90	3.10
	0.27	0.00	0.00	0.13	0.16	0.15	0.81	1.06
3482	1.40	1.40	0.40	0.40	0.60	1.00	.	.
	0.40	0.40	0.24	0.24	0.24	0.45	.	.
3385	1.00	1.80	0.00	0.30	0.40	1.00	4.70	3.60
	0.00	0.53	0.00	0.15	0.16	0.15	0.98	1.06
3422	1.00	1.00	0.00	0.30	0.30	1.00	4.60	4.60
	0.00	0.00	0.00	0.15	0.15	0.21	0.92	0.87
3425	1.00	0.40	0.60	0.60	0.80	1.00	.	.
	0.55	0.24	0.24	0.24	0.20	0.32	.	.
3429	1.00	1.00	0.20	0.40	1.00	1.00	.	.
	0.00	0.00	0.20	0.40	0.32	0.32	.	.
3432	1.00	1.00	0.00	0.00	0.80	1.00	.	.
	0.00	0.00	0.00	0.00	0.20	0.32	.	.

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3478	1.00	0.40	0.60	0.80	0.80	1.00	.	.
	0.00	0.24	0.24	0.37	0.37	0.45	.	.
3485	1.00	1.00	0.00	0.00	0.75	1.00	.	.
	0.00	0.00	0.00	0.00	0.25	0.41	.	.
3495	1.00	1.00	0.00	0.00	0.50	1.00	1.63	0.00
	0.00	0.00	0.00	0.00	0.19	0.00	0.26	0.00
3391	0.80	0.60	0.00	0.20	0.60	1.00	.	.
	0.20	0.24	0.00	0.20	0.24	0.45	.	.
3446	0.60	0.40	0.20	0.20	0.60	1.00	.	.
	0.24	0.24	0.20	0.20	0.24	0.45	.	.
3490	0.60	0.60	0.40	0.40	0.80	1.00	.	.
	0.24	0.24	0.24	0.24	0.37	0.55	.	.
3349	0.50	0.50	0.00	0.75	1.00	1.00	.	.
	0.29	0.29	0.00	0.25	0.00	0.00	.	.
3148	0.40	0.40	0.00	0.30	0.50	1.00	1.50	0.00
	0.16	0.16	0.00	0.15	0.17	0.26	0.43	0.00
3421	0.25	0.25	0.00	0.00	0.75	1.00	.	.
	0.25	0.25	0.00	0.00	0.25	0.41	.	.
3398	0.20	0.20	0.20	0.20	0.80	1.00	.	.
	0.20	0.20	0.20	0.20	0.20	0.32	.	.
3428	0.20	0.20	0.00	0.00	0.60	1.00	.	.
	0.20	0.20	0.00	0.00	0.24	0.45	.	.
3003	0.00	0.00	0.00	0.57	0.71	1.00	1.86	0.29
	0.00	0.00	0.00	0.20	0.29	0.31	0.91	0.18
3069	1.00	1.00	0.00	0.30	0.60	0.90	4.40	5.90
	0.00	0.00	0.00	0.15	0.27	0.23	0.81	0.71
3149	0.80	0.40	0.00	0.50	0.60	0.90	1.30	0.00
	0.39	0.16	0.00	0.17	0.22	0.18	0.26	0.00
3399	1.00	1.00	0.00	0.11	0.11	0.89	3.89	2.89
	0.00	0.00	0.00	0.11	0.11	0.11	1.10	1.16

Appendix 3.2 (continued).

UMNO*	Cotyledon Tests		Adult stem tests					
	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3019	1.00	1.00	0.00	0.25	0.75	0.88	1.38	1.75
	0.00	0.00	0.00	0.16	0.31	0.30	0.46	0.96
3054	1.00	1.00	0.00	0.63	0.88	0.88	3.88	1.13
	0.00	0.00	0.00	0.18	0.12	0.12	0.87	0.85
3122	2.20	1.80	0.00	0.60	0.70	0.80	3.70	1.40
	0.33	0.33	0.00	0.16	0.15	0.13	0.94	0.67
3479	2.20	2.20	0.20	0.20	0.60	0.80	.	.
	0.49	0.49	0.20	0.20	0.40	0.58	.	.
3434	1.00	1.00	0.00	0.00	0.60	0.80	.	.
	0.00	0.00	0.00	0.00	0.24	0.37	.	.
3496	0.40	0.00	0.00	0.00	0.60	0.80	.	.
	0.24	0.00	0.00	0.00	0.24	0.37	.	.
3400	1.50	0.75	0.00	0.00	0.50	0.75	.	.
	1.19	0.25	0.00	0.00	0.29	0.48	.	.
3426	0.00	0.00	0.00	0.00	0.50	0.75	.	.
	0.00	0.00	0.00	0.00	0.29	0.48	.	.
3413	1.40	1.00	0.00	0.20	0.70	0.70	5.10	4.70
	0.27	0.00	0.00	0.13	0.21	0.21	0.98	1.04
3313	1.00	1.00	0.00	0.00	0.30	0.70	1.30	0.00
	0.00	0.00	0.00	0.00	0.15	0.15	0.21	0.00
3324	0.80	0.80	0.00	0.00	0.60	0.70	0.90	0.00
	0.13	0.13	0.00	0.00	0.16	0.15	0.23	0.00
3395	0.00	0.00	0.00	0.00	0.33	0.67	.	.
	0.00	0.00	0.00	0.00	0.33	0.67	.	.
3450	1.20	0.60	0.00	0.00	0.40	0.60	.	.
	0.49	0.24	0.00	0.00	0.24	0.40	.	.
3442	1.00	1.00	0.20	0.40	0.60	0.60	.	.
	0.00	0.00	0.20	0.40	0.40	0.40	.	.
3458	1.00	1.00	0.20	0.40	0.60	0.60	.	.
	0.00	0.00	0.20	0.24	0.24	0.24	.	.

Appendix 3.2 (continued).

	Cotyledon Tests		Adult stem tests					
UMNO*	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3076	0.00	0.00	0.00	0.00	0.00	0.50	.	.
	0.00	0.00	0.00	0.00	0.00	0.50	.	.
3394	0.20	0.20	0.00	0.00	0.20	0.40	.	.
	0.20	0.20	0.00	0.00	0.20	0.40	.	.
3445	1.00	1.00	0.00	0.00	0.20	0.20	.	.
	0.00	0.00	0.00	0.00	0.20	0.20	.	.
3024	0.00	0.00	0.20	0.20	0.20	0.20	.	.
	0.00	0.00	0.13	0.13	0.13	0.13	.	.
3389	0.00	0.00	0.00	0.00	0.20	0.20	.	.
	0.00	0.00	0.00	0.00	0.20	0.20	.	.
3397	0.00	0.00	0.00	0.20	0.20	0.20	.	.
	0.00	0.00	0.00	0.20	0.20	0.20	.	.
3078	0.00	0.00	0.10	0.10	0.10	0.10	.	.
	0.00	0.00	0.10	0.10	0.10	0.10	.	.
3381	1.00	0.50	0.00	0.00	0.00	0.00	.	.
	0.71	0.29	0.00	0.00	0.00	0.00	.	.
3018	0.40	0.40	0.00	0.00	0.00	0.00	.	.
	0.24	0.24	0.00	0.00	0.00	0.00	.	.
3014	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3016	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3023	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3026	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3028	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3030	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.

Appendix 3.2 (continued).

	Cotyledon Tests		Adult stem tests					
UMNO*	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3032	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3034	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3036	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3038	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3040	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3042	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3044	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3046	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3050	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3052	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3055	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3057	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3059	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3066	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3070	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.

Appendix 3.2 (continued).

	Cotyledon Tests		Adult stem tests					
UMNO*	B2	A2	Week1	Week2	Week3	Week4	Week5	Root
3072	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3074	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3080	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
3082	0.00	0.00	0.00	0.00	0.00	0.00	.	.
	0.00	0.00	0.00	0.00	0.00	0.00	.	.
Maluka	1.20	1.70	0.10	0.40	0.80	1.40	1.60	0.00
	0.42	0.45	0.10	0.16	0.25	0.22	0.27	0.00
Westar	9.00	9.00	3.88	5.55	6.66	7.26	7.65	7.00
	0.00	0.00	0.18	0.16	0.12	0.09	0.08	0.00