

Genetic Analysis of Blackleg and White Rust

Resistance in *Brassica rapa*

By

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BY

Patricia Anne Cuthbert

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree
of
Master of Science**

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Foreword

This thesis is written in a manuscript style as outlined by the Department of Plant Science, University of Manitoba. Two manuscripts are presented which follow the style of the Canadian Journal of Plant Science. Within each manuscript is an introduction, materials and methods, and results and discussion. A general abstract, a general introduction and a review of literature precede the manuscripts. Finally, a general discussion and conclusion, and literature cited follow the manuscripts.

Abstract

Cuthbert, Patricia Anne, M.Sc. The University of Manitoba, February 2000.

Genetic Analysis of Blackleg and White Rust Resistance in *Brassica rapa*

Major Professor: Dr. R. Scarth, Department of Plant Science

A major constraint to canola production in western Canada, particularly for *Brassica rapa* L., is susceptibility to diseases – specifically brown girdling root rot, blackleg and white rust. Blackleg, a fungal disease, is very serious in parts of Alberta, and in all regions of Saskatchewan and Manitoba. The fungus attacks the cotyledons, leaves, stems, and pods of its host plant. *Leptosphaeria maculans* (Desm.) Ces. and de. Not., an ascomycete, is the causal organism of blackleg of canola. White rust, caused by *Albugo candida* (Pers. ex Hook.) Kuntze., is a devastating disease of *B. rapa* throughout western Canada with yield losses up to 15 to 20%. White rust can affect any part of the plant, except the root, with white to cream-colored pustules from seedling stage onward. The most effective and economical long-term solution to control these diseases is the use of resistant cultivars, supplemented with use of proper management practices and chemical applications. To date no blackleg resistant, and only a few white rust resistant *B. rapa* cultivars are available for commercial production. However, to produce resistant cultivars, breeding programs need to understand the genetics of host disease resistance.

The first objective of this study was to determine the inheritance of resistance to blackleg caused by *L. maculans* in *B. rapa*. Intraspecific crosses were performed between four blackleg resistant populations and a blackleg susceptible population. Inheritance of resistance to the disease was determined by production of homozygous doubled haploid lines, from heterozygous blackleg resistant F₁ generations, using microspore culture and self-pollination to determine the doubled haploid line segregation of the population at the cotyledon and adult plant stages. Doubled haploid (DH) line (i.e., gametic) segregation for two (UMBL source) populations fit a 3:1 resistant to susceptible ratio, indicating there were two genes segregating in the inheritance of blackleg resistance at the cotyledon stage. The expression of resistance is dominant and the presence of either gene confers a highly resistant rating. Gametic segregation of the SYL ACB population fit a 1:1 resistant to susceptible ratio, indicating only one gene segregated in the inheritance of blackleg resistance at the cotyledon stage. Segregation ratios of adult *B. rapa* plants (UMBL and SYL ACB populations) screened in the field based blackleg nursery indicated only one gene segregates in the inheritance of blackleg resistance at the adult stage. Chi-square contingency tests indicated there was an association between blackleg resistance genes at the cotyledon and adult plant stages of the DH lines derived from [(UMBL-1 x SC25779 (F₃)) x UM971], [(UMBL-2 x SC25779 (F₃)) x UM971], and [(SYL ACB x SC25779 (F₃)) x UM971]. The number of DH lines derived for the SYL UM population was too low to evaluate for resistance and susceptibility.

The second objective of this study was to determine the inheritance of resistance to *A. candida* in *B. rapa*. Intraspecific crosses were performed between a pure-breeding white rust resistant population to four pure-breeding white rust susceptible populations. Inheritance of resistance to the disease was determined by production of homozygous doubled haploid lines, from heterozygous white rust resistant F₁ generations, using microspore culture and self-pollination to determine the doubled haploid line segregation of the population at the cotyledon stage. Doubled haploid line (i.e., gametic) segregation of the two (UMBL source) populations fit a 3:1 resistant to susceptible ratio, indicating there were two genes segregating in the inheritance of white rust resistance to race Ac7a at the cotyledon stage. These two genes may operate independently to confer white rust resistance in the dominant form. However, it is also possible that the genes for white rust resistance in these crosses interact in a dominant-recessive epistatic fashion, as originally proposed by Kalavacharla (1996). Assessment of the doubled haploid lines generated in this study does not distinguish between these two contrasting explanations. Segregation of the doubled haploid lines from the F₁'s of the SYL ACB population cross fit a 1:1 resistant to susceptible, indicating there was only one gene segregating in the inheritance of white rust resistance to race Ac7a. The number of DH lines derived for the SYL UM population was too low to evaluate for resistance and susceptibility.

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1.0

Introduction

1.0 Introduction

Brassica oilseed crops are one of the most important sources of edible oil in the world and are cultivated predominately in Canada, China, the Indian Subcontinent, and Western Europe (Downey and Röbbelen 1989). However, over the last decade as disease resistance and quality characteristics have been improved and incorporated, the major regions of production have grown to include Australia, United States, and South America.

In Canada, rapeseed oil is produced from two *Brassica* species, *B. napus* (Argentine rape or oilseed rape) and *B. rapa* (syn. *B. campestris*) (Polish rape or turnip rape). *B. rapa* is an important crop grown worldwide as a canola (low erucic, low glucosinolate rapeseed) crop due to its low levels of saturated fatty acids and seed chlorophyll in the seed oil. *B. rapa* cultivars are generally earlier maturing, but lower yielding than oilseed rape cultivars.

In 1993, *B. rapa* was the predominant canola species grown in western Canada at 58% of the total canola acreage (Canadian Grain Commission 1998). *B. rapa* acreage was the highest in Alberta at 75%, equivalent to *B. napus* in Saskatchewan, and was the lowest in Manitoba at approximately 25% (Canadian Grain Commission 1998). Since 1993, *B. rapa* production in western Canada has decreased to 18% of the total canola acreage (Canadian Grain Commission 1998). The major constraint to the production of *B. rapa* in

western Canada is susceptibility to diseases – principally brown girdling root rot, blackleg and white rust.

Blackleg, a fungal disease, is very serious in parts of Alberta, and in all regions of Saskatchewan and Manitoba. The fungus attacks the cotyledons, leaves, stems, and pods of its host plant. *Leptosphaeria maculans* (Desm.) Ces. and de. Not., an ascomycete, is the causal organism of blackleg of canola and exists in two forms: avirulent and virulent. The avirulent form of the fungus causes only superficial disease symptoms, while the virulent form causes severe crown cankers and economic yield losses. To control blackleg several management practices have been recommended: proper crop rotations, careful stubble and residue management, use of certified seed and control of volunteer canola and cruciferous weeds. Chemical seed treatments and applications of systemic fungicides help to reduce the amount of disease and inoculum present in a field.

Albugo candida (Pers. ex Hook.) Kuntze., the causal organism of white rust of canola, is a devastating disease throughout western Canada with yield losses up to 15 to 20% (Bernier 1974). White rust affects any part of the plant, except the root, with white to cream-colored pustules from seedling stage onward. This fungal disease has two types of infection: general and systemic, resulting in stunting of the entire plant and formation of pustules on all parts; or local with direct invasion of single leaves, stems, or flowers.

Stems have localized or extended swellings, sometimes sharp bends and proliferation from lateral buds giving a bushy growth. Various flower parts are deformed with pronounced distortion of flower pedicels referred to as stagheads. Several management practices have been recommended for control of this fungal disease including proper crop rotation, control of volunteer canola and cruciferous weeds and use of certified seed. Chemical control measures also help to reduce disease and inoculum present in a field.

Blackleg and white rust can be very serious diseases of *B. rapa* and with appropriate environmental conditions can cause total crop devastation. Therefore, the most economical and effective control measure for these diseases is the use of disease resistant cultivars, supplemented by the use of management and chemical control measures. However, the development of multiple disease resistant turnip rape cultivars requires the knowledge and understanding of the genetics of disease resistance.

The objectives of this study were to determine the inheritance of *L. maculans* and *A. candida* resistance in *Brassica rapa*. Inheritance of resistance to these diseases was determined by production of homozygous doubled haploid lines using microspore culture and self-pollination to determine the gametic segregation of the population. The knowledge gained from this inheritance study will be used in the development of *B. rapa* germplasm with resistance to blackleg and white rust diseases.

2.0 Literature Review

2.1 THE HOST

2.1.1 Oilseed *Brassicas*

The Cruciferae family, to which the genus *Brassica* belongs, contains many important crop plants and weeds. In domesticating the *Brassicas*, man has modified almost every plant part, including the root, stem, leaf, terminal and axillary buds, and seeds. The oilseed *Brassicas* are closely related to the cole vegetables; the condiment mustards and root crops, turnip, rutabaga, and radish; as well as weeds, such as black and wild mustard.

Brassica oilseeds are one of the most important sources of edible oil in the world and are cultivated predominately in Canada, China, the Indian Subcontinent, and Western Europe (Downey and Röbbelen 1989). However, over the last decade as disease resistance and quality characteristics have been improved and incorporated, the regions of major production have grown to include Australia, United States, and South America.

In Canada, rapeseed oil is produced from two *Brassica* species, *B. napus* (Argentine rape or oilseed rape) and *B. rapa* (syn. *B. campestris* L.) (Polish rape or turnip rape), while other *Brassica* species are used as vegetables or condiments (Liu 1987). The seeds of *Brassicas* normally contain over 40% oil,

while the meal contains 36 to 44% protein, which makes it a feedstock of high nutritive value (Kimber and McGregor 1995).

The advent of rapeseed breeding in Canada took place during World War II, when H.G. Neufeld made selections of *B. napus* from seed stocks introduced from Argentina, and the first commercial production of rapeseed oil in Canada occurred in 1943 (Appelqvist and Ohlson 1972).

The Canola Council of Canada has registered the term “canola” to describe cultivars of *B. napus* and *B. rapa* which meet specific requirements for erucic acid (less than 1% and taken as a percentage of the total fatty acids in the extracted oil) and glucosinolate content (less than 30 $\mu\text{mol/g}$ in the residual meal) (Eskin et al. 1996).

The first low erucic acid *B. napus* variety was Oro, registered in 1968, while the first low erucic acid *B. rapa* variety was Polar, registered in 1969. The first low erucic acid and low glucosinolate *B. napus* canola variety, Tower, was released in 1974 while the first ‘double low’ *B. rapa* canola variety, Candle, was released in 1977.

2.1.2 *Brassica rapa* Distribution & Economic Importance

Brassica rapa, a non-bulbing form of the true turnip, is believed to have the widest distribution of all oilseed *Brassicas*. It was found at least 2 000 years ago over an area extending from western Europe to eastern China and Korea, and from Norway to the Sahara and India (Hedge 1976). Burkill proposed that *Brassica rapa* originated somewhere in Europe as a biennial form and that the annual form evolved later (Prakash 1980). *B. rapa* was introduced to Canada in 1936 from Poland (Bell 1982).

Most cold-hardy cultivars of *Brassica* oilseeds during spring growth belong to *B. rapa* since this species has a relatively high growth rate under low temperatures. *B. rapa* is suited to production in the northern portions of the canola growing area in Canada due to its early maturity and high growth rate under low temperatures, reducing the importance of early seeding that is critical for the later maturing *B. napus*.

In 1993, *B. rapa* was the predominant canola species grown in western Canada at 58% of the total canola acres (Canadian Grain Commission 1998). *B. rapa* acreage was the highest in Alberta at 75%, equivalent to *B. napus* acreage in Saskatchewan and was lowest in Manitoba at approximately 25% (Canadian Grain Commission 1998). Since 1993, *B. rapa* production in western Canada has decreased to 18% of the total canola acreage (Canadian Grain Commission 1998). The major constraint to the production of *B. rapa* in

western Canada is susceptibility to diseases - principally brown girdling root rot, blackleg and white rust. Root rot is the major concern in the Peace River area of Alberta, where the disease can cause severe losses. Blackleg is a serious disease in *B. rapa* in parts of Alberta and in all regions of Saskatchewan and Manitoba. White rust can also be a serious disease of *B. rapa* throughout western Canada with yield losses of up to 15 to 20% (Bernier 1972). Other factors that have also contributed to the decrease in production of *B. rapa* canola cultivars in recent years include the availability of herbicide tolerant *B. napus* cultivars, which is not a production option in *B. rapa*; and earlier maturing *B. napus* canola varieties.

2.1.3 Genomic Relationship

Morinaga (1934 as cited by Olsson and Ellerstrom 1980) demonstrated the relationships among the cultivated *Brassica* species. *Brassica* species [*B. napus* L. (n=19), *B. juncea* (Czern & Coss (n=18) and *B. carinata* Braun (n=17)] were found to be amphidiploids through cytological evidence and had arisen through natural interspecific hybridization between diploid *Brassica* species with lower-chromosome numbers [*B. nigra* (L.) Koch. (n=8), *B. oleracea* L. (n=9) and *B. rapa* L. (n=10)]. This hypothesis was confirmed by artificially synthesizing *B. napus* through *B. rapa* and *B. oleracea* crosses (U 1935) (Figure 2.1). Later Downey *et al.* (1975) and Olsson and Ellerstrom (1980) obtained synthetic *B. juncea* and *B. carinata* from crosses of *B. nigra* and *B. rapa* or *B. oleracea*. Knowledge gained of inter-relationships within

the *Brassica* genus has helped researchers in the transfer of valuable characteristics such as early maturity, cytoplasmic male sterility, self-incompatibility, and disease resistance from *B. rapa* to *B. napus* (Liu 1985).

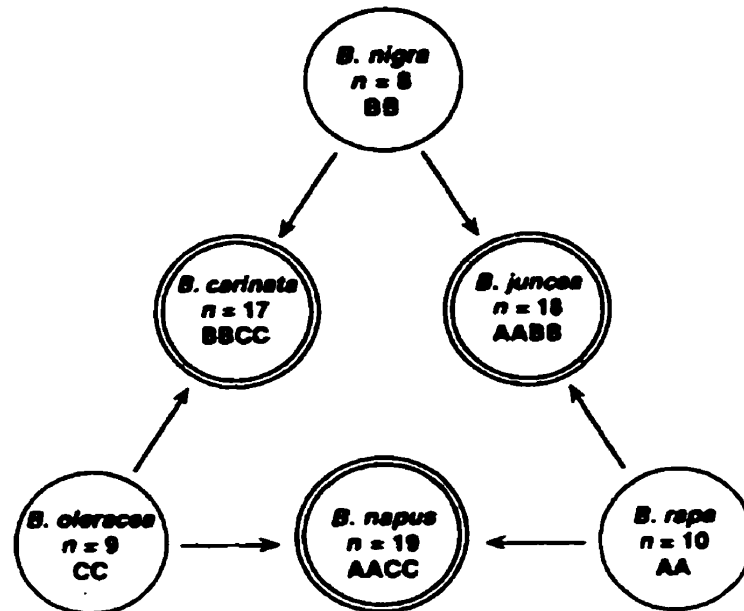


Figure 2.1. Genomic relationship of the *Brassica* species (U 1935)

2.1.4 Reproductive Biology of *B. rapa*

The inflorescence of *Brassica* plants is racemose and flowering is indeterminate beginning at the lowest bud on the main raceme. The flower is radial with four erect, prominent sepals, and four petals which alternate with the sepals in the form of a cross. There are six stamens, the two outer stamens being distinctly shorter than the inner four that surround the stigma. There are four nectaries spaced equidistant and between the two whorls of stamens. Two of the nectaries are at the base of the two outer stamens. The

stigma is receptive for pollination three days prior and three days after flowers open (Downey and Röbbelen 1989). The petal colour is normally pale yellow, but several shades of yellow have been identified and numerous genes have been reported to affect flower colour (Morice 1960; Alam and Aziz 1954).

B. rapa has a more compact bud arrangement than *B. napus* and unopened buds are sometimes found below opened flowers (Downey *et al.* 1980). The flowers in *B. rapa* are normally smaller and a darker yellow than *B. napus*. Three to five flowers open per day on the main raceme. The flowering period of *B. rapa* is approximately 3 to 4 weeks.

The diploid *B. rapa* relies on outcrossing for fertilization to occur, and has a self-incompatibility system that is controlled sporophytically by multi-allelic S-locus. Sporophytic self-incompatibility is an incompatibility reaction imparted to the pollen by the plant upon which it is borne. The number of S-alleles in *B. rapa* has been estimated to be approximately 100 (Nou *et al.* 1993). A population must have variability of these alleles in order for interpollination to occur. The main agents for pollination are wind and insects.

The S-locus, located in the pistil, is active in the papillar cells at the stigmatal surface and in the anthers sporophytically in the tapetal cells and gametophytically in microspores (Goring and Rothstein 1992). Pollen tube growth is inhibited at the stigmatal surface when self-pollination occurs

(Kandasamy *et al.* 1989). Two genes are involved in recognizing and enforcing this incompatibility reaction, the S-locus glycoprotein (SLG) and S-locus receptor kinase (SRK). When self-pollination occurs the SLG predominately accumulates in the papillar cell walls. The SLG is then modified and becomes competent to bind SRK (Nasrallah and Nasrallah 1993). The SRK becomes activated when self-pollen contacts the papillar cells at the stigmatal surface.

S-alleles, in the S-locus, function as a dominance series determined by the pollen parent which makes it important to have the correct combination of alleles in the stigma pollen grain for inhibition of self-pollination (Thompson and Taylor 1966). The expression of self-compatibility is a result of variants. Mutations in S-alleles have been found in *B. oleracea* and *B. rapa* that reduce the amount of SLG in the stigma (Nasrallah and Nasrallah 1993). These mutated S-alleles are sometimes referred to as self-fertile (S_f) alleles.

The stigma is unable to recognize self- versus cross-pollen until one day prior to anthesis or flower opening. Therefore this incompatibility reaction in *Brassicas* can be overcome through bud pollination, a time consuming and labour intensive method, before the stigma is biologically able to respond to self-pollen (Sun 1938). An alternative method that is less labour intensive involves increasing the levels of CO₂, which inhibits the SI rejection response by inhibiting the protein signal (O'Neill *et al.* 1984). NaCl spray treatments are

also effective at overcoming SI with the breakdown of proteins that accumulate at the stigmatal surface (Friesen and Scarth 1998; Fu *et al.* 1992).

2.1.5 Breeding Methods

Breeding procedures in an obligate cross-pollinated crop, such as *B. rapa*, are based largely on population improvement principles, i.e., increasing the frequency of genes in the population for the desired breeding objectives (Poehlman and Sleper 1995). Therefore, it is imperative that genes contributing to the enhancement of the desired objectives be present in the source population. Subjecting the source population and the selections from it, to various environmental stresses, according to the objectives of the breeding program will assist in identifying the superior genotypes and their progenies (Poehlman and Sleper 1995).

Breeding procedures in *B. rapa* include mass selection, recurrent selection, synthetic development, hybrid production and doubled haploid line production followed by population reconstruction.

Mass selection is a system of breeding in which individual plants are visually chosen for desirable traits, and the seed harvested and bulked to grow the next generation (Poehlman and Sleper 1995). This system of breeding is simple and easy. It is effective for improvement of characters with high

heritability that can be visibly identified. With a three-year cycle, new varieties can be developed quickly. A weakness of this system is the lack of control of the pollen source and the genes contributing to the progeny through the pollen gametes. Another weakness of mass selection is that selection for characters with low heritability is relatively ineffective, since plants superior due to phenotype cannot be distinguished from plants superior due to environmental influence.

Recurrent selection is any breeding system designed to increase frequency of genes for particular quantitatively inherited characters by repeated cycles of selection based on progeny performance. A recurrent selection cycle involves identification of genotypes superior for the specific quantitative character being improved, and the subsequent intermating of the superior genotypes to obtain new gene combinations (Poehlman and Sleper 1995). Selection cycles may be repeated as long as superior genotypes are being generated. Progeny tests allow greater control over the genetic constitution of the population in the next generation in comparison to mass selection. There is a potential for loss of desirable characteristics if not included in the initial selection criteria.

Mass and pedigree selections are only useful in improving *B. rapa* if there is enough diversity within the selected populations to avoid inbreeding

depression. Both of these procedures require many cycles of selection to obtain a stable population with desirable characteristics.

Synthetic varieties are developed by crossing parents based on their performance and general combining ability as determined by the performance of progeny from different combinations (Becker 1988). A synthetic variety is an advanced generation of a seed mixture of strains, clones, inbreds, or hybrids among them, maintained for a number of generations by open-pollination (Poehlman and Sleper 1995). The component strains, clones, inbreds, or hybrids are maintained, and the synthetic is reconstituted at regular intervals.

The number of parents selected to create the synthetic must be large enough to minimize inbreeding depression and small enough to incorporate only individuals that will maintain the mean performance of the population (Becker 1988).

Synthetics of *B. rapa* are usually composed of two, or at most three, parental lines mixed in equal proportions and grown in isolation (Buzza 1995). *B. rapa* has an advantage in making synthetics as it is self-incompatible, which makes the outcome of mixing more predictable. The "Syn 1" seed from a two-component synthetic will be composed of 25% of the component parts and 50% of the F₁ hybrid from the cross between the two parents. This assumes

an equal propensity to cross between lines as within a line. The exploitation of heterosis using this system has been demonstrated by the first *B. rapa* synthetics (Hysyn 100 and Hysyn 110) which were registered in Canada in 1994 (Buzza 1995).

Heterosis (or hybrid vigour) is the increase in size, yield, growth, and or vigour of a hybrid resulting from a cross from genetically unlike homozygous parental lines (Poehlman and Sleper 1995). The first filial generation (F_1) is the hybrid and should result in the maximum expression of heterozygosity over any further generation produced by inter-crossing this population. Therefore, the principle of hybrid breeding is to find the parent genotypes that will combine to produce a superior yielding F_1 hybrid plant and to reproduce the F_1 genotype in every plant in the hybrid population faithfully (Poehlman and Sleper 1995).

A major obstacle in hybrid production is developing a method of reliable pollination control to minimize the amount of self-pollination occurring with parents, while maximizing cross-pollination between them. Since *B. rapa* is predominately cross-pollinated, and the number of different SI alleles present in its population is approximately 100 (Nou *et al.* 1993). There is an inherent concern for siblings of a particular *B. rapa* hybrid line to inter-pollinate one another within a row. A means of controlling pollen movement and/or production is necessary to ensure purity of hybrid seed and to be

economically viable. By promoting outcrossing between unrelated genotypes, a diverse assortment of favourable genes is available and heterosis can be exploited.

Five pollen control mechanisms are available to ensure purity of hybrid populations and these are cytoplasmic male sterility (CMS), genetic male sterility (GMS), self-incompatibility, manual emasculation, and gametocides.

Cytoplasmic male sterility (CMS) is the most popular system of producing hybrids. This system is composed of three lines, usually referred to as:

- **A-line** (male-sterile or female parent line);
- **B-line** (maintainer); and
- **R-line** (restorer or male parent line)

The genotypes of these lines are demonstrated in Figure 2.2. The A- and B-lines have the same nuclear genotype but different cytoplasms. The A-line has a cytoplasm conferring male sterility in the absence of nuclear genes, which can override this cytoplasmic trait. The B-line has a cytoplasm which allows normal pollen production. The R-line has a nuclear gene or genes, which overcome the effect of the “male-sterile” cytoplasm and restores normal male fertility.

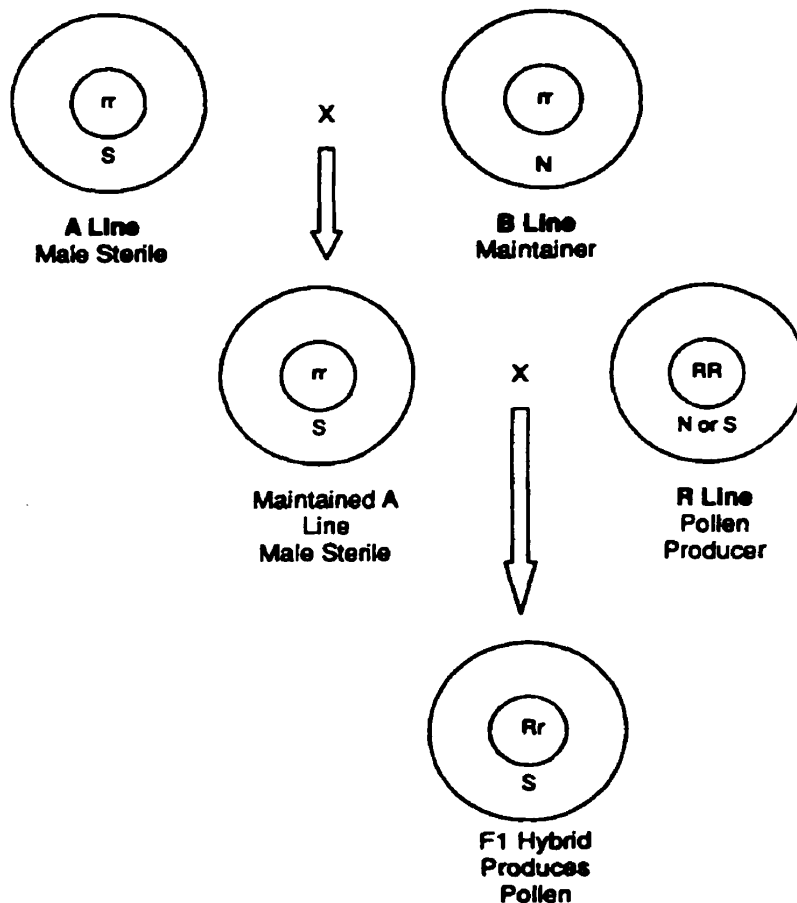


Figure 2.2. Cytoplasmic male sterility system (Poehlman and Sleper 1995)

Ideally, the above cross results in restoration of fertility and the expression of heterosis in the F_1 hybrid. The B-line is used to maintain the CMS A-line, which carries the maternally inherited S cytoplasm. This system has been developed in *B. napus* using several cytoplasmic systems including *nap*, *polima*, and *ogura*, all of which require improvements either in their genetic stability or stability under different environments (Downey and Röbbelen 1989). Any CMS system is difficult to maintain in self incompatible *B. rapa*, where inbreeding depression is strongly expressed (Buzza 1995).

Self-incompatibility is a pollination control system, which occurs naturally in *B. rapa*. The production of hybrids using the sporophytic self-incompatibility to ensure a high level of outcrossing has proven impractical. Inbred plants obtained through continuous bud pollination or doubled haploid derived inbreds from microspore/anther culture, exhibiting high levels of inbreeding depression and are very difficult to maintain by continuous selfing to ensure the presence of a homozygous S allele to allow stable SI expression. Thus the difficulties and costs associated with the production of parental seed stocks would severely limit the potential of this system for hybrid oilseed *B. rapa*, even if various double, triple, and four way cross schemes discussed by Thompson (1983) and Kott (1995) were employed. To avoid the potential problems of poor seed set in commercial hybrid fields, due to poor pollen movement among the self-incompatible plants, Kott (1995) proposed a four-way cross to overcome poor vigour. However, this procedure requires increased time for crossing and identification of compatible genotypes. This problem can be minimized by the identification of S alleles in *B. rapa* using restriction fragment markers of SLG PCR products. Nishio *et al.* (1996) identified DNA band patterns that could be used as markers for S alleles.

Traditional breeding procedures to establish complete homozygosity is a time consuming process that is confounded by the processes of segregation, recombination, and independent assortment, which leads to genetic variation. The production of doubled haploid lines is a valuable asset to a breeding

program since homozygosity is reached in one generation, versus five or six generations of inbreeding (Morrison and Evans 1988). The doubled haploid (DH) plants derived from the F_1 or offspring from an initial cross between desirable parents produce breeding lines that are completely homozygous at all loci, whereas some residual heterozygosity persists in lines obtained by even five or six generations of inbreeding. Finally, recessive genes that may be masked in diploid heterozygous breeding lines are uncovered and will be expressed in the phenotype of haploids or doubled haploids.

The DH lines represent a sample of the whole gametic array with distinct genetic contributions from the parents. Undesirable lines can be eliminated early in the selection process (Griffing 1975; Powell *et al.* 1986). Superior DH lines can be selected and bud-pollinated to create the next generation, with further selection for superior lines accomplished by testing phenotypic variation and environmental effects (Powell *et al.* 1990).

In *Brassicas*, DH lines are usually produced through the culture of male gametes or microspores (Ferrie and Keller 1995). Lichter (1982) first demonstrated microspore culture in *Brassicas*. The procedure used to produce DH lines in canola involves the selection of buds containing the late uninucleate to early binucleate microspores which have not undergone the first pollen mitosis (Keller *et al.* 1975; Pechan and Keller 1988). At this stage, microspores have the ability to switch from gametophytic to sporophytic

control by undergoing symmetric instead of asymmetric division which occurs *in vivo* to promote gametogenesis (Zaki and Dickinson 1990; 1991). Microspores can be characterized cytologically (Kott *et al.* 1988a) or using flow cytometry (Fuchs and Pauls 1992). Buds are macerated to release microspores, which are then cultured in a liquid medium to recover haploid embryos.

Haploid lines are then regenerated from these embryos and submersing the plant roots in a diluted solution containing a microtubule agent, such as colchicine doubles chromosomes. When applied prior to the first pollen mitosis colchicine promotes symmetric cell division. Application of colchicine at the single cell stage avoids chimeric expression of different ploidy levels and associated poor seed production (Mathias and Röbbelen 1991).

Microspores, haploid embryos, and haploid/doubled haploid plants have invaluable uses in plant breeding programs: mutant selection (Swanson *et al.* 1989); biochemical analysis of lipids (Taylor *et al.* 1993); biochemical mutagenesis and transformation studies (Ferrie and Keller 1995); genetic studies - RFLP mapping (Landry *et al.* 1991; Ferreira *et al.* 1994); and the physiological aspects of embryo maturation and plant regeneration (Taylor *et al.* 1993).

Limitations exist in the application of the DH line methodology in a breeding program. Some crops are recalcitrant to *in vitro* embryo production resulting in too few or weak DH plants being recovered, such as anther-derived albinos in cereals (Day and Ellis 1984). Recalcitrance may be genotype dependent (Ferrie *et al.* 1995) or due to conditions in DH line production procedures. Factors that influence DH line production include donor plant conditions, culture media, and culture environment.

Genetic manipulation of *B. rapa* is not as well developed as that for *B. napus*. The diploid *B. rapa* (AA) is more recalcitrant to tissue culture than the amphidiploid *B. napus* (AACC) (Baillie *et al.* 1992). It has been suggested that the A genome inhibits regeneration whereas the C genome contains shoot regeneration genes (Narasimhulu and Chopra 1988). Evolution of ethylene from *B. rapa* cells or tissue in culture has also been suggested to cause low levels of regeneration (Chi *et al.* 1991). It has been proposed that highly embryogenic genotypes have a high level of synchrony in the stages of microspore development (Kott *et al.* 1988b). Asynchronous cultures may inhibit embryogenesis of cells at the optimum stage of development due to endogenous toxins produced by the death of more mature cells (Pechan and Keller 1988).

Increasing the speed or frequency of regeneration of *B. rapa* and *B. napus* microspore derived embryos enhance the production of DH plants (Coventry

et al. 1988). This is accomplished by providing an *in vitro* culture environment for the haploid embryos, by inducing desiccation tolerance and dehydration of the embryos. To induce these conditions, abscisic acid (ABA), cold, and heat treatments along with desiccation have been implemented to increase the frequency of conversion from embryos to plants (Kott and Beversdorf 1990; Senaratna *et al.* 1991; Brown *et al.* 1993).

Self-pollination or inbreeding to produce homozygosity in obligate outcrossing crops leads to inbreeding depression, resulting in poor performance, due to the expression of deleterious homozygous recessive alleles. Inbreeding depression has been reported in DH lines of *B. rapa* expressed as poor germination rates, spindly branching, reduced height, late flowering, lack of pollen, low seed set, and poor seed quality (Dewan *et al.* 1995). DH line production does not avoid inbreeding by minimizing the repeated cycles of inbreeding. In order to exploit the benefits of DH development in cross-pollinating crops, there must be a recovery of performance to at least the level of the original heterozygous population. This can be accomplished by population reconstruction (Friesen and Scarth, preprint 2000).

2.2 THE PATHOGEN – *LEPTOSPHAERIA MACULANS*

2.2.1 Pathogen Description

Leptosphaeria maculans (Desm.) Ces. & de Not. [anamorph *Phoma lingam* (Tode ex Fr.) Desm.] is the causal organism of blackleg disease in canola. It is heterothallic, an ascomycete and a member of the order Pleosporales (Horst 1990).

“Pseudothecia of *L. maculans* are black, immersed becoming erumpent, globose with protruding ostioles, ranging in size from 300 to 500 µm in diameter and are normally found on woody plant tissue. Asci are cylindrical to clavate, sessile or short stipitate measuring 80 to 125 x 15 to 22 µm; the ascus wall is bitunicate (Punithalingam and Holliday 1972). Ascospores are hyaline and spindle shaped when young but yellow tan and five septate at maturity measuring 30 to 70 µm x 4 to 9 µm (Smith and Sutton 1964; Boerema 1976)” (Sawatsky 1989).

Two types of pycnidia of *P. lingam*, designated Group I and Group II, have been found on *Brassica* spp.. As a parasite, the pathogen produces Group I pycnidia with a pseudosclerenchymatous wall structure, which are initially closed developing a papillate opening (sometimes with a neck) (Boerema 1976). As a saprophyte, the pathogen produces Group II pycnidia with a pseudoparenchymatous wall structure (Boerema 1976). Group II pycnidia have dark walls, are often irregular in shape and may or may not have a

papilla. Hyaline, oval, single-celled pycnidiospores measuring 1-2 x 2.5-5 μm are exuded from the pycnidia (Boersma 1976).

2.2.2 Host Range and Distribution

Blackleg (*L. maculans*) is a worldwide fungal disease that attacks plants of the *Brassicaceae* family, including rutabaga, cole crops and several other cultivated crucifers. The pathogen has also been known to cause disease on several cruciferous weeds including *Thlaspi* spp., *Descurainia sophia* (L.) Webb, *Sisymbrium* spp., *Lepidium* spp. (Petrie and Vanterpool 1965) and *B. kaber* (DC.) L.C. Wheeler (syn. *Sinapis arvensis* L.) (Petrie 1979). The disease is found on all continents and was reported in 48 countries by 1978 (Commonwealth Mycological Institute 1978).

Blackleg has been a serious disease predominately in Australia, the prairie provinces of Canada, and Europe, especially France (Lamey 1995). Recently, the importance of the disease in canola/rapeseed has increased and has been associated with the increase in area of production, including Canada and Europe. Production of rapeseed in Australia began in the mid-1960s with varieties introduced from Canada (e.g., Target, Oro, Span) (Cutting 1975). During the early 1970s the production acreage in Australia increased rapidly; however, by 1972 the selected Canadian cultivars proved to be highly susceptible to blackleg causing a severe blackleg disease epidemic across Australia. This epidemic resulted in a significant decrease in

rapeseed acreage and did not increase substantially for a decade (Murray and Stovold 1970; Bokor *et al.* 1975; Wightman 1982).

There have also been yield losses as high as 50% reported in Germany (Seidel *et al.* 1984). As well, losses due to blackleg disease of up to 20% were recorded in Canada (Petrie 1978). In 1995, Pedras *et al.* indicated crop losses due to blackleg in Canada alone exceed thirty million dollars annually.

2.2.3 Disease Symptoms

Leptosphaeria maculans attack the cotyledons, leaves, stems and pods of its host plant. Symptoms appear first as water-soaked lesions on cotyledons, hypocotyls, and leaves. These lesions turn a white to grey colour, round to irregular in shape, and become dotted with numerous pin-head sized black fruiting bodies called pycnidia. When in a mature state and under moist conditions, the pycnidia exude spores in pink ooze.

Stem cankers are black to whitish-grey in colour and are of two types: at temperatures of 24/15°C cankers develop rapidly, and are dry with abundant pycnidia; while at lower temperatures 12/7°C they develop slowly, and are soft and slimy with no pycnidia (Barbetti 1975). These cankers may girdle the entire stem restricting pod fill, or causing lodging and death of the plant.

Invasion of the host follows a systemic pathway of infection (Hammond *et al.* 1985). The fungus colonizes on the cotyledons and leaves in the intercellular spaces of the lamina, and then follows the vascular strand into the petiole. The pathogen moves down the petiole and into the stem where it kills cells of the cortex causing cankers on upper portions of the stem and more commonly at the crown. Hammond and Lewis (1987a) and Mithen *et al.* (1987) found resistance to the pathogen was expressed in the leaves of *Brassica* species and therefore prevented systemic colonization. Hammond and Lewis (1987b) found resistance in the stem of oilseed rape plants after the leaves and petiole had been colonized.

Isolates of *L. maculans* can be separated into two groups namely virulent/aggressive and weakly virulent/non-aggressive (Rimmer and van den Berg 1992). The weakly virulent form of the fungus usually infects plants near maturity resulting in shallow stem lesions, and rarely forming extended cankers that girdle the stem. The highly virulent form of the fungus attacks the crop earlier and more seriously. If basal infection begins early, stem cankers appear from flowering onwards. As the season progresses, cankers penetrate, deepen and may girdle stem bases, often completely severing the plant. By mid July, plants may start to lodge. Less severely affected plants remain standing but have restricted moisture and nutrient flow, and ripen prematurely with shrivelled seed and pods (Alberta Agriculture, Food and Rural Development 1995).

Stem infection before the six-leaf stage is usually associated with serious yield loss (McGee and Petrie 1979). Pods and seeds may also be infected, with infected pods ripening and splitting prematurely resulting in seed loss. The seed beneath pod lesions may be sunken or shrivelled and pale grey in colour. The earlier pod infection occurs, the less likely that viable seed will be produced. Hard, black fruiting perithecia, slightly larger than pycnidia, form on the basal stem cankers late in the year or in the following year.

2.2.4 Disease Cycle

The disease cycle of *Leptosphaeria maculans* is illustrated in Figure 2.3. The pathogen survives from season to season either on infected seed or infected crop residue. Two types of spores are formed: asexual pycnidiospores which spread the disease a short distance within the crop canopy, and sexually produced ascospores which can be carried by the wind for several miles. During wet, windy weather the fruiting bodies discharge thousands of infectious spores (Canola Council of Canada, 1997). Ascospores have been found to travel 5 to 8 km in Australia (Bokor *et al.* 1975) and 1.5 km in Britain (Gladders and Musa 1980). In Canada, Petrie (1978) found severe infections of oilseed rape crops immediately adjacent to fields containing infested residue but only traces of infection in a crop 1 km from the nearest inoculum source.

Ascospore dispersal follows a seasonal pattern in France, Australia, and Canada (Alabouvette and Brunin 1970; McGee 1977; and Petrie 1979). The discharge in Canada begins in July of the year after establishment of the disease and continues throughout the summer and fall. In the second and subsequent years, discharge begins in early spring and continues into the fall. The amount of inoculum decreases significantly by the third year (McGee 1977).

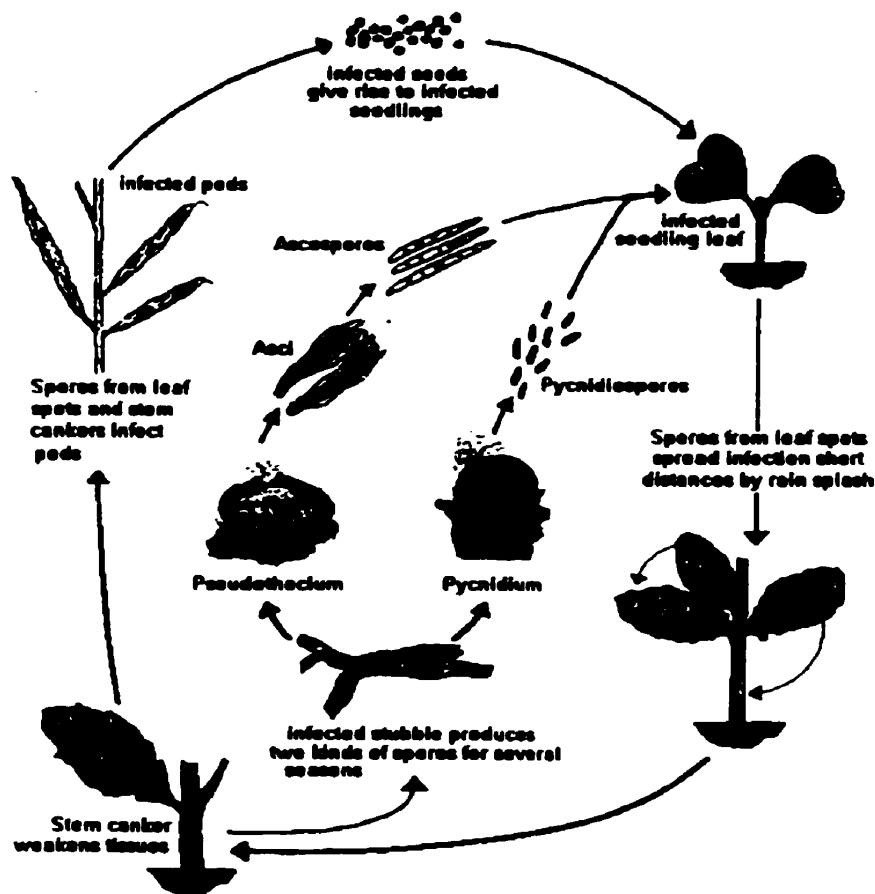


Figure 2.3. Blackleg disease cycle of *Brassica* oilseeds (Canola Council of Canada 1999)

2.2.5 Disease Control

2.2.5.1 Management Practices

Crop residue is an important source of inoculum, therefore a plan to reduce the risk of blackleg requires the following management practices: proper crop rotations, careful stubble and residue management, and control of volunteer canola and cruciferous weeds, especially wild mustard. (Canola Council of Canada 1999).

Infected residue is the primary source of inoculum and depending on the environmental conditions, it may take as long as 4 to 5 years to breakdown (Alabouvette and Brunin 1970). Therefore, producers should practice crop rotation and not grow canola on a field more frequently than once every four years. Infected residue should be buried as deeply as possible, and a shallow tillage or direct seeding used in the spring to avoid bringing infected canola residue back to the surface. Since the primary inoculum is also airborne, canola should not be seeded within one kilometer of infested land for three to four years (McGee and Emmett 1977).

The control of volunteer oilseed rape and susceptible cruciferous weeds is important to prevent the establishment of the pathogen in fields. Wild mustard (*Sinapis arvensis*) is highly susceptible to the virulent oilseed rape form of *L. maculans* (Petrie 1979).

Seeding of oilseed rape crops has been delayed in Australia as a means of avoiding periods of heavy ascospore release (McGee 1977). However, this is not a viable alternative in the prairie provinces of Canada due to environmental constraints, and the fact that ascospore production is heavy throughout the growing year and dependent on the environmental conditions.

2.2.5.2 Chemical Control

The control of *L. maculans* via management practices is only partially effective. The virulent form of the pathogen is seedborne; and therefore, can introduce the disease to areas that are not presently infected by the disease. As a preventative control seed testing methods which detect *L. maculans* have been developed (International Seed Testing Association 1965).

Hot water treatments have been used in the past to eradicate the pathogen from crucifer seeds. Walker (1923) found this treatment to be effective; however, other reported that it failed to eradicate the pathogen (Cunningham 1927; Gabrielson 1983). This type of treatment is impractical for large seed lots, but has been used extensively for vegetable *Brassicas* (e.g., cabbage) until the discovery of systemic fungicides.

Numerous seed treatments have been found to be effective in controlling the pathogen, for example thiram, fenpropimorph, benomyl, thiabendazole, and

iprodione. However, these treatments do not protect crops grown in infested fields.

A number of chemical control methods have been tested to control the disease in infested fields. Researchers were able to restrict pseudothecia formation and ascospore release from infected stubble *in vitro* by chemical means; however, on a commercial scale this would prove uneconomical (Humpherson-Jones and Burchill 1982).

Applications of systemic fungicides such as benomyl have generally given poor disease control unless excessively high rates were used (Brown *et al.* 1976). Xi and Morrall (1988a) showed that single foliar application of the fungicides: triadimefon, flutriafol, iprodione, and diniconazole were not effective in controlling blackleg disease. They concluded that at present there is limited potential to control blackleg disease in western Canada through the use of foliar fungicide applications.

In Australia, the systemic application of the fungicide flutriafol as a seed treatment was successful in reducing disease incidence (Xi and Morrall 1988b). Ballinger *et al.* (1988) found that Flutriafol applied as a fertilizer dressing on superphosphate granules, significantly reduced the levels of stem canker in areas where the disease was prevalent. However, in western Canada this fungicide had only limited effectiveness (Xi *et al.* 1989).

2.3 THE PATHOGEN – *ALBUGO CANDIDA*

2.3.1 Pathogen Description

Albugo candida (Pers. ex Hook.) Kuntze, an obligate parasite, is a diploid biotroph belonging to the family *Albuginaceae* of the order Peronosporales in the class Oomycetes (Horst 1990).

In *Albugo candida* (Figure 2.4) the mycelium is intercellular and feeds by haustoria, which penetrate the host cell walls through minute perforations, and expand on the inside of the cells into globose or knob-like structures. The mycelium grows and when maturity is reached, they produce short, club-shaped sporangiophores from the tips of a large number of hyphal branches in one locality. The sporangiophores are borne in close proximity to one another in solid layers immediately below the epidermis of the host. When the sporangiophores reach a certain growth stage, they begin to cut off a number of sporangia at their tips. Each sporangiophore gives rise to several sporangia produced in succession, therefore a chain of sporangia is formed with the oldest at the tip of the chain and the youngest at the base. As the sporangia mature, they become detached in the space between the sporangiophores and the epidermis of the host. Both the growth of the fungus and the production of numerous sporangia exert a pressure from below the host epidermis, causing it to bulge and eventually to burst over the growing sorus. Upon the bursting of the epidermis, the sporangia are released and form a white crust on the surface of the host. Individually the

The sporangia of *Albugo* germinate by zoospores or by germ tubes, depending on the temperature. When zoospores are produced, the sporangia extrude four to twelve zoospores in an advanced stage of differentiation, into sessile vesicle (Vanterpool 1959).

2.3.2 Host Range and Distribution

A. candida, the causal agent of white rust of crucifers, is an obligate parasite that attacks at least 29 genera of crucifers (*Brassicaceae*) including major agricultural crops, common weeds, and native species (Jacobson *et al.* 1998). Average yield losses due to white rust on turnip rape in western Canada have been between 1.2 and 9.0% in some years (Berkenkamp 1972; Harper and Pittman 1974; and Petrie 1973); however, in heavily infested fields, yield losses ranging from 30 to 60% have been reported (Bernier 1972).

The incorporation of resistance in predominately grown cultivars, e.g., Tobin and Reward (Scarth *et al.* 1992), has been used to control white rust. However, new pathotypes of *A. candida* virulent to the resistance utilized in Tobin can now be found in western Canada (Liu *et al.* 1996). Some recently turnip rape released cultivars (e.g., Colt, Eldorado, Horizon, and Klondike) are highly susceptible to the pathotypes of *A. candida* commonly found on *B. rapa* (Liu *et al.* 1996).

Ten physiologic "races" of *A. candida* have been identified and classified based on specificity to different crucifer species including race 2 on *B. juncea* (Pound and Williams 1963), race 7 on *B. rapa* (Petrie 1988), and race Accar on *B. carinata* (Liu and Rimmer 1992).

A. candida pathotypes are virulent on many genotypes of their homologous host species (Rimmer and Buchwaldt 1995). However, some may also cause disease on some genotypes of closely related species (Petrie 1988). Isolates from *B. rapa* and *B. juncea* in Canada are homothallic, whereas isolates from *B. oleracea* and *B. carinata* are heterothallic (Liu and Rimmer 1992).

2.3.3 Disease Symptoms

White to cream-colored masses, or pustules of "white rust", appear on the leaves from the seedling stage onward. Blisters may appear on any part of the plant except the root. They vary in size and shape and are often confluent in extended patches. There seem to be two types of infection: general and systemic, resulting in stunting of the entire plant and formation of pustules on all parts; or local, with direct invasion of single leaves, stems, or flowers. Upper surface of leaves often has yellow areas with white pustules on the underside. The latter are powdery when mature, and the epidermis is ruptured to free chains of sporangia that are carried by wind to moist surfaces. They germinate by 6 to 10 zoospores, swarmspores, which settle down, produce germ tubes, and enter the plants through stomata.

Stems have localized or extended swellings, sometimes sharp bends and proliferation from lateral buds giving a bushy growth. Various flower parts are deformed with pronounced distortion of flower pedicels. When these thickened parts die, oospores are formed to survive the winter in crop refuse.

Albugo is very sensitive to temperature changes. The spores germinate within the range of 1 to 18°C, but will do so much more quickly when the range is narrowed to 10 to 13°C. In order for the mycelium to penetrate the leaf, the temperature must rise to not less than 16°C and not more than 25°C, with an optimum of 20°C (University of Adelaide).

2.3.4 Disease Cycle

Primary inoculum of white rust, oospores, overwinters in residue, soil and as a contaminant in seed lots. Oospores, sexual state of the organism, are known to survive for long periods under dry conditions (Rimmer and Buchwaldt 1995). In the moist conditions of spring, the oospore germinates to produce a sessile vesicle or a short exit tube that terminates in a vesicle. Zoospores are released from the vesicle and swim to host tissue where they encyst and germinate, infecting the tissue. Secondary infection is by sporangia being produced in the leaf blisters and windborne to healthy tissue. Sporangia germinate by producing zoospores. Zoospores encyst and germinate to cause localized infections. Oospores eventually develop in swollen or hypertrophied tissue. Stagheads develop from infected flower

buds. At harvest, stagheads may be broken during threshing resulting in contamination of the seed with resting spores.

2.3.5 Disease Control

2.3.5.1 Management Practices

The precise life span of resting spores in the soil is not known, however, there is a definite deterioration over a period of several years, therefore it is imperative that sanitation practices are followed: proper crop rotation, control of volunteers and cruciferous weeds, and use of pathogen free seed (Canola Council of Canada 1999).

Producers should use crop rotations and not grow canola on a field more frequently than once every 3 to 4 years with non-cruciferous plants. Infected leaves and other plant parts should be buried as deeply as possible to reduce the amount of fungus present in a field.

The control of volunteer oilseed rape and susceptible cruciferous weeds is important to prevent the establishment of the pathogen in fields. Volunteer rapeseed and wild mustard plants are sources of inoculum and should be controlled early in the season.

2.3.5.2 Chemical Control

The development of acylalanine fungicides, e.g., metalaxyl (Ridomil; Subdue), has greatly improved the ability to control white rust in *B. rapa* with fungicide application (Ferreira and Boley 1991). Metalaxyl provides limited curative activity and some control of systemic infection (Ferreira and Boley 1991). This fungicide is rapidly taken up by the leaves, stems and roots, translocated acropetally, and is effective at low rates, but is not generally phytotoxic.

Applications should be made to the seed, soil and to the foliage. Frequency of application would vary according to the length of crop production and amount of rainfall experienced. In temperate environments a soil application and a minimum of 1 to 2 foliar applications during the crop cycle is recommended. To control white rust in turnip rape effectively, the fungicide must be available at the time of infection of cotyledons. Metalaxyl on seeds could act directly by inhibiting or killing zoospores arising from germinating oospores, which are most likely the primary source of inoculum for emerging cotyledons in the spring (Pound and Williams 1963; Verma and Petrie 1975; and Verma and Petrie 1980). Rimmer and Buchwaldt (1995) stated that oospore contamination may be controlled with metalaxyl though this chemical is not registered in Canada for use on *Brassica* oilseeds.

However, with the possibility of developing fungicide tolerant pathogen strains associated with metalaxyl, growers should consider using Ridomil MZ58

formulations with foliar fungicide applications. This formulation adds a second fungicide to the tank mix (Ferreira and Boley 1991).

Older, but less effective, fungicides for white rust control include Dithane Z-78, Blitox, wettable sulphur, fixed copper compounds, Bordeaux mixture, chlorothalonil, captan, dodine, mancozeb, metiram, maneb, and zineb (Ferreira and Boley 1991).

2.4 HOST PLANT DISEASE RESISTANCE

Cultural and chemical control help to decrease the disease risk of blackleg and white rust. However, alone they do not provide sufficient control in areas where turnip rape is grown. The most economical and effective control measure for these diseases is the use of resistant cultivars, supplemented by chemical/cultural control.

Resistance has been defined as a characteristic of the host plant, which enables it to resist growth and colonization, by a pathogenic organism (Crute 1985). Tolerance has been defined as the ability of a host cultivar to support invasion and colonization by the pathogen, similar to a second host cultivar, and yet to suffer less damage to its growth, development and reproduction (Crute 1985).

The development of resistant cultivars requires knowledge of the genetics of disease resistance inheritance, and an understanding of host-pathogen interactions. Multiple disease resistant turnip rape cultivars with good agronomic and quality characteristics would be beneficial and represent the most economical, effective and environmentally friendly means to ensure that canola production in western Canada is not limited by disease.

2.4.1 Inheritance of Blackleg Resistance in *Brassicas*

Resistance to *L. maculans* in turnip and oilseed rape has been a major breeding objective in Europe, Australia and recently Canada. “Blackleg disease of canola can cause devastating losses, however, it has been effectively been controlled in Europe since the introduction of the resistant cultivar Jet Neuf in 1977. Most resistant cultivars grown in Europe derive their resistance from this cultivar. So far there has been little evidence that this resistance has become any less effective and blackleg is not considered a major problem in that region today” (Rimmer and van den Berg 1992).

A number of researchers have attempted to transfer resistance from one species to another. For example, *Brassica* species with the B genome (*B. nigra*, *B. carinata*, and *B. juncea*) possess a hypersensitive type of resistance to blackleg that is effective throughout the life of the plant; therefore this resistance has been transferred to *B. rapa* and *B. napus* which have AA and AACC genomes, respectively (Rimmer and van den Berg 1992).

Although control via cultural and chemical means helps to reduce the amount of disease in a field, they do not provide control of disease. Therefore, most breeding programs have incorporated breeding for blackleg resistance into rapeseed lines with good agronomic and quality characteristics to prevent further yield losses due to disease.

2.4.1.1 *B. napus*

A number of researchers have studied the inheritance of blackleg resistance in *B. napus*. In 1980, Cargeeg and Thurling inoculated seedlings of different genotypes with Australian isolates of the pathogen and observed continuous variation in disease reaction, which is indicative of polygenic control of resistance (Rimmer and van den Berg 1992).

Delwiche (1980) determined cotyledon blackleg resistance of a French winter breeding line was controlled by single dominant gene (*Lm1*), while a second dominant gene (*Lm2*) conferred resistance in another line (Rimmer and van den Berg 1992). An independence test for these two genes indicated linkage. Another researcher, Sawatsky (1989), found that a single recessive gene controlled inheritance of cotyledon resistance in summer oilseed rape breeding lines.

Sawatsky (1989) screened the progeny of two summer rape lines crossed to susceptible cultivar Regent to determine adult plant resistance to blackleg. Results indicated that two dominant genes confer blackleg resistance in *B. napus*. The presence of dominant alleles at both loci conferred a high level of resistance, while a single dominant allele at either locus provided intermediate levels of resistance (Rimmer and van den Berg 1992).

2.4.1.2 *B. juncea*

There is considerable interest in western Canada to develop canola-quality *B. juncea*, because of its resistance to blackleg and its superior drought tolerance. *B. juncea* has been used for interspecific transfer of blackleg resistance to *B. napus* (Roy 1984). Resistance at the seedling stage of *B. juncea* or *B. carinata* is controlled by genes located on the B genome and is believed to be more effective than seedling resistance in *B. napus* (Roy 1984; Sacristan and Gerdemann 1986).

Keri *et al.* (1997) determined that the inheritance of genetic control of resistance to *L. maculans* in *B. juncea* is two nuclear genes with dominant recessive epistatic gene action.

2.4.1.3 *B. oleracea*

The inheritance of resistance to *L. maculans* in *B. oleracea* was studied in a cross between *B. alboglabra* and *B. insularis* in the *B. oleracea* group (Rimmer and van den Berg 1992). Results of this study conclude that two unlinked, duplicate genes determine hypersensitive resistance from *B. insularis* in *B. oleracea* (Mithen and Lewis 1988)

2.4.1.4 *B. rapa*

To date no information or studies have been released on the inheritance of *L. maculans* in *B. rapa*.

2.4.2 Inheritance of White Rust Resistance in *Brassicas*

White rust is prevalent on cultivated *Brassica* species and other crucifers in many parts of the world (Saharan and Verma 1992). In western Canada, *A. candida* races 2 and 7 are of economic importance, as they attack *B. juncea* and *B. rapa* crops, respectively (Petrie 1988). The development of white rust resistant cultivars is a primary goal of *B. juncea* and *B. rapa* breeding programs in western Canada.

2.4.2.1 Resistance to Race Ac7

The inheritance of resistance of some *Brassica* species to different races of *A. candida* has previously been studied and there is evidence of resistance being dominant with monogenic or digenic inheritance. Fan *et al.* (1983) studied the inheritance of resistance to *A. candida* race 7 (Ac7) in *B. napus* cultivar Regent and found that white rust resistance is conditioned by independent, dominant genes at three loci.

The inheritance of resistance to race 2 (Ac2) in a resistant cultivar of brown mustard (*B. juncea*) was studied by Tewari *et al.* (1988) who found that resistance was monogenic. Verma and Bhowmik (1989) studied the inheritance of resistance to a *B. juncea* pathotype of *A. candida* in the *B. napus* line BN-Sel and found that duplicate dominant genes governed resistance.

Kalavacharla (1996) reported the inheritance of resistance to races 7a and 7v of *A. candida* in *B. rapa* fit a dominant-recessive epistasis model. According to this model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs., assuming that these two genes are A and B determining the genetics of resistance and susceptibility. Therefore, resistance is conferred when the genotype of the plant is A_B_, A_bb, or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb.

“Applying this model for Ac7a, the two genes are designated as A^a and B^a while for Ac7v, they are designated as A^v and B^v. Since phenotypic linkage of reaction to Ac7a and Ac7v was seen, it was suggested that there may be present a total of four genes (A^a=A gene for Ac7a, B^a=B gene for Ac7a, A^v=A gene for Ac7v and B^v=B gene for Ac7v) of which A^a and A^v are tightly linked and which independently act as inhibitor genes to B^a and B^v. It is also possible that there are only three genes (A, B^a and B^v), with a common A gene for Ac7a and Ac7v, which acts as an inhibitor gene to B^a as well as B^v, with B^a and B^v being unlinked to each other” (Kalavacharla 1996).

3.0

Genetic Analysis of Blackleg Resistance in

Brassica rapa

3.1 Introduction

Blackleg, caused by *Leptosphaeria maculans* (Desm.) Ces. and de. Not., is one of the most important diseases of *Brassica* species. The pathogen is a constant threat to crop production, especially to oilseed *Brassica* species (*B. napus* L. and *B. rapa* L. (syn. *B. campestris*)) in parts of Alberta and in all regions of Saskatchewan and Manitoba.

Blackleg symptoms can occur on the cotyledons, leaves, stems, and pods of the host plant. The fungus exists in two forms: avirulent and virulent. The avirulent form of the fungus causes only superficial disease symptoms, while the virulent form causes severe crown cankers and economic yield losses. To control blackleg several management practices have been recommended: proper crop rotations, careful stubble and residue management, use of certified seed and control of volunteer canola and cruciferous weeds. Chemical seed treatments and applications of systemic fungicides help to reduce the amount of disease and inoculum present in a field.

Although these management practices and chemical applications aid to reduce the amount of disease and inoculum present in the field, they are not effective enough for economical long-term control of the disease. The most effective method to ensure canola production, especially *B. rapa*, in western Canada is not limited by this disease in the future is the use of resistant cultivars, supplemented with proper management practices and chemical

applications. To date no *B. rapa* cultivars resistant to blackleg have been released. However, the incorporation of blackleg resistance into canola lines with desirable agronomic qualities has become a major objective of breeding programs in countries where the disease has seriously reduced yields. In order to breed for resistance, more knowledge concerning the genetics of resistance would be valuable for effective breeding in oilseed *Brassicas*.

The genetics of resistance to *L. maculans* in *Brassica* species (*B. napus*, *B. juncea*, and *B. oleracea*) has been reviewed (Rimmer and van den Berg 1992), but despite the importance of the disease, information on the number of genes involved in resistance and their effects and allelic relationships in *B. rapa* is rather limited.

No information is available on the inheritance of blackleg resistance in *B. rapa*. Therefore the objective of this study was to determine the inheritance of *L. maculans* resistance in *B. rapa* derived from two different sources. Inheritance of the disease was determined by production of homozygous blackleg resistant doubled haploid lines using microspore culture and self-pollination of heterozygous genotypes to determine the doubled haploid line (i.e., gametic) segregation of the population. The knowledge gained from this inheritance study can be used in the development of *B. rapa* germplasm with resistance to blackleg.

3.2 Materials and Methods

3.2.1 Blackleg Resistant Populations

Four *B. rapa* populations with blackleg resistance were produced: UMBL-1, UMBL-2, Syl ACB, and Syl UM. The two populations of UMBL were developed at the University of Manitoba through interspecific crosses between *B. napus* (Maluka, DH 88-676, and DH 88-756) and *B. rapa* (RRW83-5370, and S83-5009) breeding lines. The two Syl populations were produced by Dr. Don Woods at Beaverlodge, Alberta through crosses between adapted *B. rapa* cultivars (AC Sunshine) and *B. rapa* spp. *sylvestris*. These populations were selected and re-selected by the originators for blackleg resistance using the cotyledon and petiole inoculation method and evaluated in the University of Manitoba Blackleg Nursery.

Due to sporophytic self-incompatibility in *B. rapa*, the four blackleg resistant populations were crossed in January 1996, prior to commencement of this project, with a self compatible *B. rapa* source (UMBL-1 x SC25779 (F₃)), (UMBL-2 x SC25779 (F₃)), (Syl ACB x SC25779 (F₃)), (Syl UM x SC25779 (F₃)) and re-selected for blackleg resistance to allow further crossing and the production of double haploid lines through microspore culture procedures.

3.2.2 Greenhouse Crosses

A reselection (UM971) of the *B. rapa* cultivar, Reward, registered by the University of Manitoba, has superior white rust resistance and good

agronomic and quality characteristics, however, it is susceptible to blackleg. Intraspecific crosses between four blackleg resistant populations and one blackleg susceptible *B. rapa* crosses were completed at the commencement of this project in May 1997: [(UMBL-1 x SC25779 (F₃)) x UM971]; [(UMBL-2 x SC25779 (F₃)) x UM971]; [(Syl ACB x SC25779 (F₃)) x UM971]; and [(Syl UM x SC25779 (F₃)) x UM971]. Forty seeds of UM971 were planted at the same time as the blackleg resistant crosses, but were not inoculated, because several plants of UM971 were required to ensure adequate pollen production to complete the crosses.

After seed set had occurred, watering of the plants was gradually reduced and they became mature and dry. Seed was harvested from each dry plant and packaged in individual envelopes. During the fall of 1997, the F₁ seed from these crosses were grown out to the bud stage and microspore culture was performed to produce homozygous doubled haploid lines.

3.2.3 Microspore Culture

Heterozygous blackleg resistant populations (F₁) obtained from the intraspecific greenhouse crosses were seeded: September 12 1997, January 22 1998, and July 7, 1998; inoculated September 19 1997, January 29 1998, and July 14 1998; and rated: September 29 1997, February 11 1998, and July 24 1998, respectively to perform microspore culture. Microspore culture

protocol for *Brassica rapa* (Ferrie and Keller 1995) was followed to produce doubled haploid lines from November 1997 to December 1998.

3.2.4 Cotyledon Inoculation

Five seeds of each homozygous doubled haploid line were planted with five seeds of a susceptible check in Metromix® a soilless potting mix (W.R. Grace and Co. Ltd., Ajax, Ontario) in "jiffy" pots within flats. Flats were placed in an environmentally controlled growth room, with a 16-hour photoperiod, 1500 $\mu\text{mol}/\text{m}^2$ PAR at bench surface, and 20/15 °C day/night temperature growing conditions. The flats were watered daily with tap water and fertilized with a solution of 20-20-20 (nitrogen-phosphorous-potassium) fertilizer on the 5th and 10th day after seeding. At seven days after seeding when the cotyledons had fully expanded, each cotyledon was wounded with a sterile needle and a 10 μl drop of spore suspension (*L. maculans* isolate P182-12) containing 10^6 pycnidiospores per ml placed over the wounded area. Seedlings were rated eleven days after inoculation to allow symptoms to appear using a 0 to 9 rating scale (Table 3.1 and Figure 3.1). Plants scoring 0, 1, or 3 were resistant, and plants rating 7, 9 were susceptible. Intermediate scores of 5 were rarely observed (Appendix B).

Plants with a response of 0 or 1 (resistant) were planted into 15 cm plastic pots and placed in the greenhouse. Plants were self-pollinated using a 3% salt spray to overcome self-incompatibility. Watering of the plants was

gradually reduced after seed set to allow the plants to mature and dry. Seed was harvested from each plant and packaged in individual envelopes.

Cotyledon inoculations were replicated 4 times on each of the homozygous lines to attain the gametic segregation of each blackleg resistant doubled haploid line.

3.2.5 Adult Plant Resistance to Blackleg

During the summer of 1999, a field trial was conducted in the Blackleg Nursery at AgQuest Independent Research Farm, Minto, Manitoba. One-hundred and twenty five blackleg resistant doubled haploid lines (produced in 1997/98 by microspore culture), seven resistant *B. rapa* populations (UMBL-1, UMBL-2, SYL ACB, SYL UM, 96-103-154, 96-103-188, 96-103-290), a moderately susceptible *B. rapa* cultivar (Tobin), two susceptible *B. rapa* cultivars (Reward, UM971), and a susceptible *B. napus* cultivar (Westar) were planted using a small-plot cone seeder on June 11. The field was inoculated with the same isolate of *L. maculans* (P182-12) used to test for cotyledon resistance. The field trial was established to evaluate resistance levels for the homozygous lines at the adult plant stage.

Single nursery row plots of each entry were sown in a randomized complete block with 4 replications. A susceptible check (Westar and Reward) was sown every 10th row as a spreader row, and a check for comparison

purposes. Each row was 3 m long and contained 100 seeds. The nursery rows were spaced 60 cm apart.

Disease development was observed on adult plants and scored using a rating scale 0 to 5, as shown in Table 3.2. Twenty-five plants were rated per row (Appendix C). Plants scoring 0,1 or 2 are considered resistant and plants scoring 4 or 5 are considered susceptible. Intermediate scores of 3 are rarely observed, but counted as resistant.

3.2.6 Statistical Analysis

Data for this experiment was analyzed using Chi-square goodness of fit tests and Chi-square contingency tests. Chi-square goodness of fit test measures the size of discrepancy between the observed and expected results. Chi-square contingency test allows the comparison of one set of observations taken under particular conditions to those of a similar nature taken under different conditions. There are no expected values for the Chi-square contingency test; this test is simply to determine whether the results are dependent (contingent upon) or independent of the conditions in which they were observed. The significance level for testing hypotheses for Chi-square goodness of fit and Chi-square contingency tests is five percent. Degree of freedom is equal to one for the Chi-square tests conducted in this research.

Table 3.1. Blackleg disease reaction of cotyledons of <i>Brassica</i> lines inoculated with <i>Leptosphaeria maculans</i> isolates (Delwiche 1980)	
DISEASE REACTION	DESCRIPTION
0	No darkening of tissue around wound. Typical response of non-inoculated cotyledon.
1	Limited blackening around wound; lesion diameter 0.5 to 1.5 mm. Faint chlorotic halo may be present. Sporulation absent.
3	Dark necrotic lesion 1.5 to 3.0 mm. Chlorotic halo may be present. Sporulation absent.
5	Non-sporulating, 3 to 6 mm lesion, sharply delimited by darkened necrotic tissue. May show greyish-green tissue collapse characteristic of susceptible reactions (7 & 9), or dark necrosis throughout.
7	Greyish-green tissue collapse. Lesion 3 to 5 mm, with sharply delimited non-darkened margins.
9	Rapid tissue collapse at about 10 days accompanied by profuse sporulation in large lesions (> then 5 mm) with diffuse, non-darkened margins.

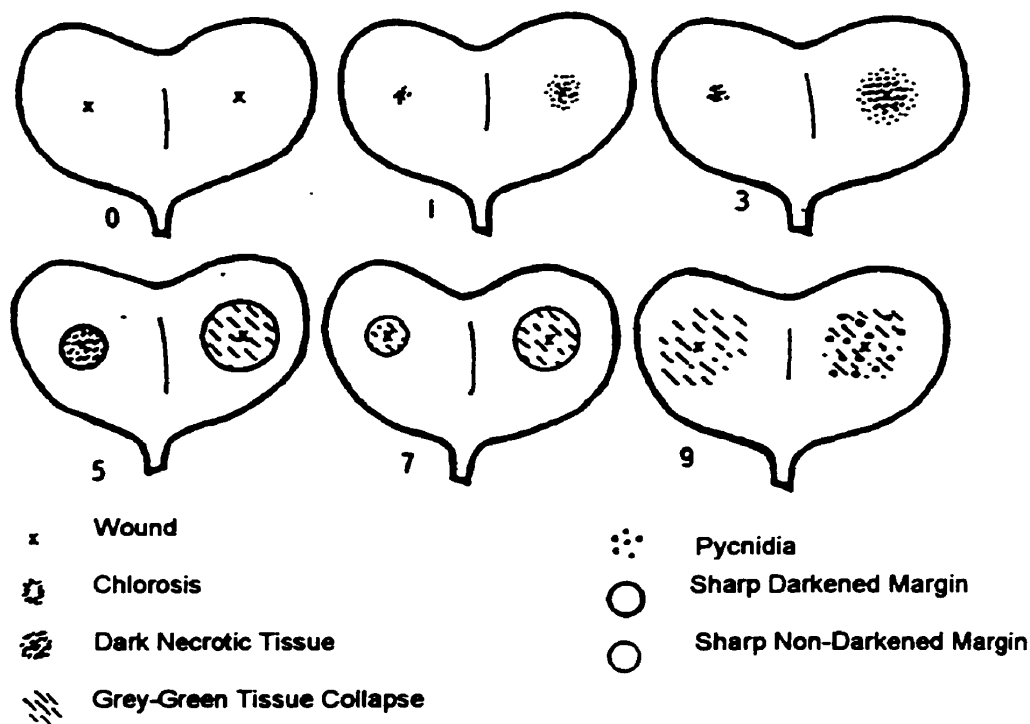


Figure 3.1. Blackleg disease reaction of cotyledons of *Brassica* lines inoculated with *Leptosphaeria maculans* isolates (Delwiche 1980)

Table 3.2. Blackleg rating scale for crown canker due to *Leptosphaeria maculans* based on length and circumference measurements

Score	Lesion Length on Stem	Circumference of Stem Girdled by Lesion
0	No infection	No infection
1	< 10 mm	< 25 %
2	10 to 19 mm	25 to 50 %
3	20 to 29 mm	50 to 75 %
4	30 mm and greater	75 to 100 %
5	Plant dead	Plant dead

3.3 Results and Discussion

3.3.1 Parents and F₁'s of Greenhouse Crosses

Intraspecific crosses between four blackleg resistant populations and one blackleg susceptible population were completed [(UMBL-1 x SC25779 (F₃)) x UM971]; [(UMBL-2 x SC25779 (F₃)) x UM971]; [(SYL ACB x SC25779 (F₃)) x UM971]; and [(SYL UM x SC25779 (F₃)) x UM971], resulting in the production of heterozygous blackleg resistant F₁ generations (Table 3.3).

Table 3.3. Reaction of parents and F₁'s of intraspecific crosses to produce heterozygous blackleg resistant F₁ generations to develop homozygous doubled haploid lines, (R) resistance and (S) susceptibility to blackleg isolate P182-12

Parents & F ₁ Generation	Reaction to blackleg isolate P182-12
(UMBL-1 x SC25779 (F ₃))	R
(UMBL-2 x SC25779 (F ₃))	R
(SYL ACB x SC25779 (F ₃))	R
(SYL UM x SC25779 (F ₃))	R
UM971	S
[(UMBL-1 x SC25779 (F ₃)) x UM971] F ₁	R
[(UMBL-2 x SC25779 (F ₃)) x UM971] F ₁	R
[(SYL ACB x SC25779 (F ₃)) x UM971] F ₁	R
[(SYL UM x SC25779 (F ₃)) x UM971] F ₁	R

3.3.2 Microspore Culture

Microspore culture was performed from November 1997 to December 1998 on heterozygous blackleg resistant populations (F_1) obtained from intraspecific greenhouse crosses to produce homozygous doubled haploid lines. While performing microspore culture, several problems were encountered which reduced the number of doubled haploid lines obtained. These problems included contamination; production of albino and dead embryos; recalcitrant, non-responsive *B. rapa* genotypes; and poor seed set on doubled haploid plants. Table 3.4 summarizes the number of doubled haploid lines produced from F_1 's of each cross.

Table 3.4. Doubled haploid line production

Cross F_1 's	Number of DH Lines	Comments Re: microspore culture
[(UMBL-1 x SC25779 (F_3)) x UM971]	72	Highly responsive
[(UMBL-2 x SC25779 (F_3)) x UM971]	74	Highly responsive
[(SYL ACB x SC25779 (F_3)) x UM971]	26	Responsive
[(SYL UM x SC25779 (F_3)) x UM971]	2	Non-responsive

In this study, F_1 's derived from crosses of the UMBL populations were highly responsive to microspore culture, while F_1 's from the crosses of the SYL populations were responsive to non-responsive. There are several possible explanations. Narashimhulu and Chopra (1988) suggest that the A genome inhibits regeneration. Evolution of ethylene from *B. rapa* cells or tissue in culture has also been suggested to cause low levels of regeneration (Chi et

al. 1991). It has also been proposed that highly embryogenic genotypes have a high level of synchrony in the stages of microspore development (Kott *et al.* 1988b). Asynchronous cultures may inhibit embryogenesis of cells at the optimum stage of development due to endogenous toxins produced by the death of more mature cells (Pechan and Keller 1988).

Based on the observations formulated while performing microspore culture, all of these events contributed in some way to the non-responsive nature of the SYL populations. Further study and varying techniques used in microspore culture may lead to the production of a greater number of doubled haploid lines for the SYL cross derived F_1 's.

3.3.3 Assessment of doubled haploid line gametic segregation ratios

3.3.3.1 Cotyledon reactions

UMBL populations fitted a 3:1 resistant to susceptible segregation ratio, indicating there were two genes segregating in the inheritance of blackleg resistance at the cotyledon stage, for example A,a and B,b (Table 3.5.). The expression of resistance is dominant and the presence of either gene confers a highly resistant rating, for example AAbb, aaBB, AABB are resistant and aabb is susceptible.

Gametic segregation ratio of the SYL ACB population fit a 1:1 resistant to susceptible, indicating there was only one gene segregating in the inheritance of blackleg resistance at the cotyledon stage (Table 3.5.).

3.3.3.2 Adult plant reactions

UMBL populations fit a 1:1 resistant to susceptible segregation ratio, indicating there was only one gene segregating in the inheritance of blackleg resistance at the adult stage (Table 3.6.).

SYL ACB population fit a 1:1 resistant to susceptible ratio, indicating there was only one gene segregating in the inheritance of blackleg resistance at the adult stage (Tables 3.6.).

In the SYL ACB population the same nine plants were resistant at the cotyledon stage and the adult plant stage, therefore this strongly suggests that the cotyledon blackleg resistance gene is the same gene for adult blackleg resistance.

Due to the nature of the population, the number of doubled haploid lines for the SYL ACB cross was low, therefore the results should be interpreted with caution and need to be confirmed with further study. The number of doubled haploid lines produced for the SYL UM cross was extremely low, therefore they were not analyzed in the results section.

Table 3.5. Observed segregation ratios of doubled haploid lines derived from cross F₁'s for resistance (R) and susceptibility (S) at the cotyledon stage to blackleg isolate P182-12

Doubled haploid lines from Cross F ₁ 's	DH lines Observed R:S	Segregation Ratio R:S	X ²	P-value
[(UMBL-1 x SC25779 (F ₃)) x UM971]	50 : 18	3 : 1	0.0784	0.9 – 0.5
[(UMBL-2 x SC25779 (F ₃)) x UM971]	24 : 13	3 : 1	2.027	0.5 – 0.10
[(SYL ACB x SC25779 (F ₃)) x UM971]	9 : 9	1 : 1	0.0000	0.995

Table 3.6. Observed segregation ratios of doubled haploid lines derived from cross F₁'s for resistance (R) and susceptibility (S) at the adult stage to blackleg isolate P182-12/natural field inoculum

Doubled haploid lines from Cross F ₁ 's	DH lines Observed R:S	Segregation Ratio R:S	X ²	P-value
[(UMBL-1 x SC25779 (F ₃)) x UM971]	36 : 32	1 : 1	0.2352	0.9 – 0.5
[(UMBL-2 x SC25779 (F ₃)) x UM971]	19 : 18	1 : 1	0.0270	0.9 - 0.5
[(SYL ACB x SC25779 (F ₃)) x UM971]	9 : 9	1 : 1	0.0000	0.995

3.3.4 Linkage Studies for Blackleg Genes Effective at Cotyledon and Adult Plant Stages

Chi-square contingency tests were calculated between reaction of cotyledons to *L. maculans* isolate P182-12 and adult plant reactions to *L. maculans* isolate P182-12/natural field inoculum, Table 3.7, 3.8, and 3.9. The Chi-square value for the UMBL and SYL ACB populations were significant at the 0.05 level and provides evidence that there is an association between the gene(s) for cotyledon and adult plant resistance to *L. maculans* isolate P182-12. This indicates that one or two dominant genes, depending on the source, conferring *L. maculans* resistance at the cotyledon and adult plant stages in *B. rapa* are found in clusters in regions of chromosomes.

Several researchers have found disease resistance genes and similar DNA sequences frequently clustered together in regions of chromosomes (Aarts 1998; Okubara 1997; Chong *et al.* 1994; O'Donoughue *et al.* 1996). In *B. napus* genes for resistance to blackleg at the cotyledon and adult plant stages are different, however linked (10 centimorgans apart) and clustered together on linkage group N7. (Rimmer, personal communication). Since UMBL blackleg resistant populations were derived from interspecific crosses between *B. napus* and *B. rapa* we expected to see similar results in this study, i.e., there is linkage associated between the cotyledon and adult blackleg resistance genes and they are found in gene clusters.

Due to the low number of doubled haploid lines produced, the results of the SYL ACB population should be interpreted cautiously and further study performed to confirm these results.

3.3.5 Summary

Blackleg is a serious disease that limits the production of *B. rapa* in western Canada. Disease resistant cultivars with good agronomic and quality characteristics would be the most economical, effective, and environmentally friendly means to ensure that *B. rapa* production in western Canada is not limited by disease.

Development of disease resistant cultivars requires knowledge of the genetics of disease resistance inheritance. Information gained from this study indicated there were one or two dominant genes for blackleg resistance, depending on the source of resistance, at the cotyledon stages. One of the dominant genes also confers blackleg resistance at the adult plant stage, which is contingent upon the cotyledon resistance gene(s) for UMBL-1 and UMBL-2 populations. This relatively simple inheritance for blackleg resistance can be quickly and easily incorporated into *B. rapa* cultivars.

Table 3.7. Chi-square contingency test of UMBL-1 population doubled haploid lines derived from [(UMBL-1 x SC25779 (F₃) x UM971] cross F₁'s for association of cotyledon and adult plant reactions to blackleg isolate P182-12/natural field inoculum

	Blackleg Cotyledon		TOTAL	
	Resistant	Susceptible		
Blackleg Adult	Resistant	36	0	36
	Susceptible	14	18	32
TOTAL		50	18	68
<hr/>				
X ² value				24.7258

Table 3.8. Chi-square contingency test of UMBL-2 population doubled haploid lines derived from [(UMBL-2 x SC25779 (F₃) x UM971] cross F₁'s for association of cotyledon and adult plant reactions to blackleg isolate P182-12/natural field inoculum

	Blackleg Cotyledon		TOTAL	
	Resistant	Susceptible		
Blackleg Adult	Resistant	19	0	19
	Susceptible	5	13	18
TOTAL	24	13	37	
X ² value				18.1048

Table 3.9. Chi-square contingency test of SYL ACB population doubled haploid lines derived from [(SYL ACB x SC25779 (F₃) x UM971] cross F₁'s for association of cotyledon and adult plant reactions to blackleg isolate P182-12/natural field inoculum

		Blackleg Cotyledon		TOTAL
		Resistant	Susceptible	
Blackleg Adult	Resistant	9	0	9
	Susceptible	0	9	9
TOTAL		9	9	18
X ² value				2592.0000

4.0

Genetic Analysis of White Rust Resistance in *Brassica rapa*

4.1 Introduction

Albugo candida (Pers. ex Hook.) Kuntze., the causal organism of white rust on *Brassicas*, is an obligate parasite that attacks at least 29 genera of crucifers including major agricultural crops, common weeds, and native species (Jacobson *et al.* 1998). In western Canada, average yield losses due to white rust on turnip rape have been between 1.2 and 9.0% in some years (Berkenkamp 1972; Harper and Pittman 1974; and Petrie 1973). In heavily infested fields, yield losses ranging from 30 to 60% have been reported (Bernier 1972).

To date, at least 10 physiologic "races" of *A. candida* have been identified and classified on specificity to different *Brassica* species including race 2 on *B. juncea*, and race 7 on *B. rapa*, and race Accar on *B. carinata* (Hill *et al.* 1988; Pound and Williams 1963; Liu and Rimmer 1992).

White rust affects any part of the plant, except the root, with white to cream-colored masses from seedling stage onward. This fungal disease has two types of infection: general and systemic, resulting in stunting of the entire plant and formation of pustules on all parts; or local with direct invasion of single leaves, stems, or flowers. Stems have localized or extended swellings, sometimes sharp bends and proliferation from lateral buds giving a bushy growth. Various flower parts are deformed with pronounced distortion of flower pedicels referred to as stagheads. Several management practices

have been recommended for control of this fungal disease including proper crop rotation, control of volunteer canola and cruciferous weeds and use of certified seed. Chemical control measures also help to reduce inoculum and disease present in a field.

Cultural and chemical control helps to decrease the risk of white rust, however, alone they do not provide sufficient control in areas where *B. rapa* is grown. The most economical and effective long-term control measure is the use of resistant cultivars, supplemented by cultural and chemical control.

In western Canada, *A. candida* races 2 and 7 are of economic importance, as they attack *B. juncea* and *B. rapa* crops, respectively (Petrie 1988). Therefore, development of white rust resistant cultivars is a primary goal of *B. juncea* and *B. rapa* breeding programs in western Canada. The development of resistant cultivars requires knowledge of the genetics of disease resistance inheritance.

In 1996, Kalavacharla reported the inheritance of resistance to races 7a and 7v of *A. candida* in *B. rapa* fitted a dominant-recessive (13:3) epistasis model. According to this model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs. Assuming that these two genes are A and B determining the genetics of resistance and susceptibility, resistance is conferred when the genotype of the plant is A_B_, A_bb, or

aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb. To date, no other studies have been undertaken to determine the inheritance of white rust in *B. rapa*.

The objective of this study was to confirm the inheritance of white rust resistance to race 7a in *B. rapa* by the production of doubled haploid lines using microspore culture and self-pollination to determine the gametic segregation of the population. The knowledge gained from this inheritance study can be used in the development of *B. rapa* germplasm with resistance to white rust.

4.2 Materials and Methods

4.2.1 White Rust Resistant Population

A reselection of the *B. rapa* cultivar, Reward, registered by the University of Manitoba, was used in the intraspecific greenhouse crosses because of its superior resistance to white rust and good agronomic and quality characteristics.

4.2.2 White Rust Susceptible Populations

Four white rust susceptible *B. rapa* populations with good blackleg resistance were produced: UMBL-1, UMBL-2, SYL ACB, and SYL UM. UMBL populations were developed at the University of Manitoba through interspecific crosses between *B. napus* (Maluka, DH 88-676, and DH 88-756) and *B. rapa* (RRW83-5370, and S83-5009). SYL populations were produced by Dr. Don Woods at Beaverlodge, Alberta through crosses between adapted *B. rapa* cultivar (AC Sunshine) and *B. rapa* spp. *sylvestris*.

Due to sporophytic self-incompatibility in *B. rapa*, the four white rust susceptible populations were crossed in January 1996, prior to commencement of this project, with a self compatible *B. rapa* source (UMBL-1 x SC25779 (F₃)), (UMBL-2 x SC25779 (F₃)), (SYL ACB x SC25779 (F₃)), (SYL UM x SC25779 (F₃)) to allow further crossing and production of double haploid lines through microspore culture procedures.

4.2.3 Greenhouse Crosses

Intraspecific crosses between one white rust resistant population (UM971) and four white rust susceptible populations were completed at the commencement of this project in May 1997: [(UMBL-1 x SC25779 (F₃)) x UM971]; [(UMBL-2 x SC25779 (F₃)) x UM971]; [(Syl ACB x SC25779 (F₃)) x UM971]; and [(Syl UM x SC25779 (F₃)) x UM971].

After seed set had occurred, watering of the plants was gradually reduced and they became mature and dry. Seed was harvested from each dry plant and packaged in individual envelopes. During the fall of 1997, the F₁ seed from these crosses were grown out to the bud stage and microspore culture was performed to produce homozygous doubled haploid lines.

4.2.4 Microspore Culture

Heterozygous white rust populations (F₁ generations) obtained from the intraspecific greenhouse crosses were seeded: September 12 1997, January 22 1998, and July 7, 1998; inoculated September 19 1997, January 29 1998, and July 14 1998; and rated: September 29 1997, February 11 1998, and July 24 1998, respectively to perform microspore culture.

Microspore culture protocol for *Brassica rapa* (Ferrie and Keller 1995) was followed to produce homozygous doubled haploid lines from November 1997 to December 1998.

4.2.5 White Rust Cotyledon Inoculations

Five seeds of each white rust homozygous doubled haploid lines were planted with five seeds of a susceptible check, Torch, in Metromix[®] a soilless potting mix (W.R. Grace and Co. Ltd., Ajax, Ontario) in "jiffy" pots within flats. Flats were placed in an environmentally controlled growth room, with 16-hour photoperiod, 1500 $\mu\text{mol}/\text{m}^2$ PAR at bench surface, and 20/15 °C day/night temperature. The flats were watered daily with tap water and fertilized with a solution of 20-20-20 (nitrogen-phosphorous-potassium) fertilizer on the 5th and 10th day after seeding. At seven days after seeding when the cotyledons had fully expanded, plants were tested for white rust resistance by inoculating cotyledons with a zoospore suspension (10^4 zoospores per ml). Inoculum preparation, inoculation methods, and incubation conditions were as described by Williams (1985). Briefly, zoosporangia were germinated in a small volume of double distilled water at 12°C, zoospore concentration was adjusted, inoculum (10 μl) was applied to the adaxial surface of the cotyledons using an Eppendorf repeater pipette, and inoculated seedlings were incubated in the a misting chamber for 48 hours. After incubation, seedlings were returned to the growth room.

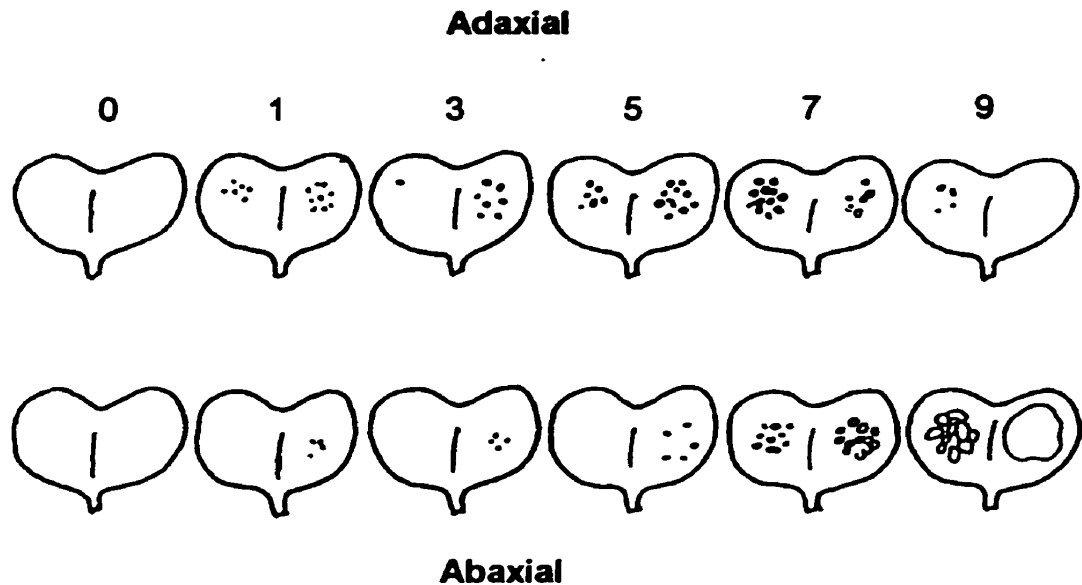
Seven days after inoculation, disease reactions were observed and scored using a rating scale 0 to 9, as shown in Figure 4.1 (Williams 1985). Cotyledons that showed no symptoms or small necrotic flecks on the adaxial surface without sporulation were scored 0 or 1 and were considered resistant,

whereas those showing scattered or coalescing pustules on the abaxial or adaxial surface were scored as 7 or 9 and were considered susceptible. Intermediate scores (3 and 5) rarely were observed.

Cotyledon inoculations were replicated 4 times on each of the homozygous doubled haploid lines to attain the gametic segregation for white rust resistance.

4.2.6 Statistical Analysis

Data for this experiment was analyzed using Chi-square goodness of fit tests. Chi-square goodness of fit test measures the size of discrepancy between the observed and expected results. The significance level for testing hypotheses for Chi-square goodness of fit tests is five percent. Degree of freedom is equal to one for the Chi-square tests conducted in this research.



Symptoms and signs for white rust ratings on the adaxial/abaxial cotyledon surfaces are:

- 0 - no symptoms on either cotyledon surface,
- 1 - necrotic flecks/none to few necrotic flecks,
- 3 - few, minute pustules/none to very few pustules,
- 5 - few to many small pustules/ few small pustules,
- 7 - many to few small pustules/many large pustules,
- 9 - very few to no pustules/large coalescing pustules.

Figure 4.1. White rust disease reaction of cotyledons of *Brassica* lines inoculated with *Albugo candida* races (Williams 1985)

4.3 Results and Discussion

4.3.1 Parents and F₁'s of Greenhouse Crosses

Intraspecific crosses between a white rust resistant population and four white rust susceptible populations were completed [(UMBL-1 x SC25779 (F₃)) x UM971]; [(UMBL-2 x SC25779 (F₃)) x UM971]; [(Syl ACB x SC25779 (F₃)) x UM971]; and [(Syl UM x SC25779 (F₃)) x UM971], resulting in the production of heterozygous white rust resistant F₁ generations (Table 4.1).

Table 4.1. Reaction of parents and F₁'s of intraspecific crosses to produce heterozygous white rust resistant F₁ generations to develop homozygous doubled haploid lines, (R) resistance and (S) susceptibility to white rust race Ac7a

Parents & F ₁ Generation	Reaction to race Ac7a
UM971	R
(UMBL-1 x SC25779 (F ₃))	S
(UMBL-2 x SC25779 (F ₃))	S
(SYL ACB x SC25779 (F ₃))	S
(SYL UM x SC25779 (F ₃))	S
[(UMBL-1 x SC25779 (F ₃)) x UM971] F ₁	R
[(UMBL-2 x SC25779 (F ₃)) x UM971] F ₁	R
[(SYL ACB x SC25779 (F ₃)) x UM971] F ₁	R
[(SYL UM x SC25779 (F ₃)) x UM971] F ₁	R

4.3.2 Microspore Culture

Microspore culture was performed from November 1997 to December 1998 on the heterozygous white rust resistant populations (F_1) obtained from four intraspecific crosses to produce homozygous doubled haploid lines. While performing microspore culture, several problems were encountered which reduced the number of doubled haploid lines obtained. These problems included contamination; production of albino and dead embryos; recalcitrant, non-responsive *B. rapa* genotypes; and poor seed set on doubled haploid plants. Table 4.2 summarizes the number of doubled haploid lines produced from F_1 's of each cross.

Table 4.2. Doubled haploid line production

Cross F_1 's	Number of DH Lines	Comments Re: microspore culture
[(UMBL-1 x SC25779 (F_3)) x UM971]	72	Highly responsive
[(UMBL-2 x SC25779 (F_3)) x UM971]	74	Highly responsive
[(SYL ACB x SC25779 (F_3)) x UM971]	26	Responsive
[(SYL UM x SC25779 (F_3)) x UM971]	2	Non-responsive

In this study, F_1 's derived from crosses of the UMBL populations were highly responsive to microspore culture, while F_1 's from the crosses of the SYL populations were responsive to non-responsive. There are several explanations. Narashimhulu and Chopra (1988) suggest that the A genome inhibits regeneration. Evolution of ethylene from *B. rapa* cells or tissue in culture has also been suggested to cause low levels of regeneration (Chi *et*

al. 1991). It has also been proposed that highly embryogenic genotypes have a high level of synchrony in the stages of microspore development (Kott *et al.* 1988b). Asynchronous cultures may inhibit embryogenesis of cells at the optimum stage of development due to endogenous toxins produced by the death of more mature cells (Pechan and Keller 1988).

Based on the observations formulated while performing microspore culture, all of these events contributed in some way to the non-responsive nature of the SYL populations. Further study and varying techniques used in microspore culture may lead to the production of a greater number of doubled haploid lines for the SYL cross derived F₁'s.

4.3.3 Assessment of Doubled Haploid Line Gametic Segregation Ratios

4.3.3.1 Cotyledon Plant Reactions

The doubled haploid lines from F₁'s of the UMBL population crosses fit a 3:1 resistant to susceptible ratio, indicating there were two genes segregating in the inheritance of white rust resistance at the cotyledon stage, Table 4.3. These two genes may operate independently to confer white rust resistance in the dominant form. However, it is also possible that the genes for white rust resistance interact in a dominant-recessive fashion, as originally proposed by Kalavacharla (1996). Assessment of the doubled haploid lines generated in this study does not distinguish between two dominant genes and

two epistatic genes operating to control white rust resistance, therefore further research is required to confirm Kalavacharla's hypothesis.

The doubled haploid lines from F_1 's of the SYL ACB population cross fit a 1:1 resistant to susceptible ratio, indicating there is only one gene segregating in the inheritance of white rust resistance in *B. rapa* to race Ac7a (Table 4.3). Although the same white rust resistant parent (UM971) was used for each intraspecific cross, the number of resistance genes varies between the UMBL populations and the SYL ACB population. Perhaps one of the genes is being suppressed in the SYL ACB population.

Due to the nature of the population, the number of doubled haploid lines for the SYL ACB cross was low, therefore the results should be interpreted with caution and need to be confirmed with further study. The number of doubled haploid lines produced for the SYL UM cross was extremely low, therefore they were not analyzed in the results section.

Kalavacharla (1996) studied the inheritance of white rust resistance in *B. rapa* to races Ac7a and Ac7v. Homogeneous *B. rapa* populations were obtained through sib-matings. The inheritance of white rust resistance in *B. rapa* to Ac7a and Ac7v fit a two-gene model of dominant-recessive epistasis (Kalavacharla 1996).

Table 4.3. Observed segregation ratios of doubled haploid lines derived from cross F₁'s for resistance (R) and susceptibility (S) at the cotyledon stage to white rust race Ac7a

Doubled haploid lines from Cross F ₁ 's	DH lines Observed R:S	Segregation Ratio R:S	χ^2	P-value
[(UMBL-1 x SC25779 (F ₃)) x UM971]	46 : 22	3 : 1	1.9608	0.5 – 0.1
[(UMBL-2 x SC25779 (F ₃)) x UM971]	23 : 14	3 : 1	3.2522	0.1 – 0.05
[(SYL ACB x SC25779 (F ₃)) x UM971]	5 : 4	1 : 1	0.0000	0.995

4.3.3 Summary

White rust is a serious disease that limits the production of canola in western Canada. Disease resistant canola cultivars with good agronomic and quality characteristics would be the most economical, effective, and environmentally friendly means to ensure canola, particularly *B. rapa*, production in western Canada is not limited by disease.

Development of resistant cultivars requires knowledge of the genetics of disease resistance inheritance. Gametic segregation of the UMBL populations fits a 3:1 resistant to susceptible, indicating there were two genes segregating in the inheritance of white rust resistance to race Ac7a at the cotyledon stage. These two genes may operate independently to confer white rust resistance in the dominant form. However, it is also possible that the genes for white rust resistance interact in a dominant-recessive epistatic fashion, as originally

proposed by Kalavacharla (1996). Assessment of the doubled haploid lines generated in this study does not distinguish between these two contrasting explanations, therefore further research is required to confirm Kalavacharla's hypothesis. The results obtained for the SYL ACB population should be interpreted cautiously and further studies should be done to confirm that only one gene is involved in the inheritance of white rust resistance to race Ac7a. This relatively simple inheritance for white rust resistance can be quickly and easily incorporated into *B. rapa* cultivars.

5.0

General Discussion

5.0 General Discussion and Conclusions

Inheritance of resistance of *Brassica* species to various isolates and races of *L. maculans* and *A. candida*, respectively, has been previously studied by researchers, with resistance being dominant and under the influence of one or two genes (Rimmer and van den Berg 1992; Kalavacharla 1996). To date, limited information has been published on the inheritance of resistance to blackleg (isolate P182-12) and white rust (race Ac7a) in *B. rapa*.

The objectives of this study were to determine the inheritance of blackleg and white rust resistance in *B. rapa*. Genetic studies of *B. rapa* are difficult to do because the self-incompatibility of the crop makes it tedious to produce a homogeneous population to obtain accurate results. By using doubled haploid lines for this study, the results were achieved easily and efficiently.

5.1 Inheritance of blackleg resistance in *B. rapa* to isolate P182-12

Intraspecific crosses between four blackleg resistant populations (UMBL-1, UMBL-2, SYL ACB, and SYL UM) and a blackleg susceptible population (UM971) were performed. Inheritance of the disease was determined by production of doubled haploid lines, from the heterozygous blackleg resistant F₁ generations of the four intraspecific crosses, using microspore culture and self-pollination to determine the gametic segregation of the population at the cotyledon and adult stage for blackleg resistance and susceptibility.

5.1.1 Cotyledon Reactions

Doubled haploid line (i.e., gametic) segregation ratios for the UMBL populations fit a 3:1 resistant to susceptible, indicating there were two genes segregating in the inheritance of blackleg resistance at the cotyledon stage, for example A,a and B,b. The expression of resistance is dominant and the presence of either gene confers a highly resistant rating, for example AAbb, aaBB, AABB are resistant, while aabb is susceptible.

Gametic segregation ratios of the SYL ACB population fit a 1:1 resistant to susceptible, indicating there was only one gene segregating in the inheritance of blackleg resistance at the cotyledon stage.

5.1.2 Adult Plant Reactions

Segregation ratios of adult *B. rapa* plants (UMBL and SYL ACB populations) screened in the field based blackleg nursery indicated only one gene segregates in the inheritance of blackleg resistance at the adult stage.

Due to the nature of the population, the number of doubled haploid lines for the SYL ACB cross was low, therefore the results should be interpreted with caution and need to be confirmed with further study. The number of doubled haploid lines produced for the SYL UM cross was extremely low, therefore they were not analyzed in the results section.

5.2 Inheritance of white rust resistance in *B. rapa* to race Ac7a

Intraspecific crosses between a white rust resistant population (UM971) and four white rust susceptible populations (UMBL-1, UMBL-2, SYL ACB, and SYL UM) were performed. Inheritance of the disease was determined by production of doubled haploid lines, from the heterozygous white rust resistant F_1 generation of the four intraspecific crosses, using microspore culture and self-pollination to determine the gametic segregation of the population at the cotyledon and adult stage for blackleg resistance and susceptibility.

5.2.1 Cotyledon Reactions

White rust resistance results obtained in this study agreed with the results and interpretation reported by Kalavacharla (1996). Gametic segregation of the UMBL populations fit a 3:1 resistant to susceptible, indicating there were two genes segregating in the inheritance of white rust resistance to race Ac7a at the cotyledon stage. These two genes may operate independently to confer white rust resistance in the dominant form. However, it is also possible that the genes for white rust resistance interact in a dominant-recessive epistatic fashion, as originally proposed by Kalavacharla (1996). Assessment of the doubled haploid lines generated in this study does not distinguish between these two contrasting explanations, therefore further research is required to confirm Kalavacharla's hypothesis.

Segregation of the doubled haploid lines from the F₁'s of the SYL ACB population cross fitted a 1:1 resistant to susceptible, indicating there was only one gene segregating in the inheritance of white rust resistance to race Ac7a. Although the same white rust resistant parent (UM971) was used for each intraspecific cross, the number of resistance genes varies between the UMBL populations and the SYL ACB population. Perhaps one of the genes is being suppressed in the SYL ACB population.

Due to the nature of the population, the number of doubled haploid lines for the SYL ACB cross was low, therefore the results should be interpreted with caution and need to be confirmed with further study. The number of doubled haploid lines produced for the SYL UM cross was extremely low, therefore they were not analyzed in the results section.

5.3 Linkage Analysis of Blackleg Resistance Genes

Chi-square contingency tests of UMBL and SYL ACB populations indicated there was an association between blackleg resistance genes at the cotyledon and adult plant stages, Table 3.7, 3.8 and 3.9. There are two dominant genes found in gene clusters in regions of chromosomes conferring blackleg resistance at the cotyledon and adult plant stages in *B. rapa* (based on sources studied in this research). Similar results have been found in *B. napus*. Blackleg resistance gene(s) at the cotyledon and adult plant stages were proven to be clustered together on linkage group N7 (10 centimorgans

apart) (Rimmer, personal communication). Since UMBL blackleg resistant populations were derived from interspecific crosses between *B. napus* and *B. rapa* we expected to see similar results in this study, i.e., there is linkage associated between the cotyledon and adult blackleg resistance genes.

Due to the low number of doubled haploid lines produced, the results of the SYL ACB population should be interpreted cautiously and further study performed to confirm these results.

5.4 Linkage Analysis of Blackleg and White Rust Resistance

The same four crosses were used in these studies to investigate the inheritance of blackleg resistance at the cotyledon and adult plant stages and white rust resistance. Doubled haploid lines produced in all crosses were assessed for the three traits. Chi-square contingency tests were used, for the first ever in *B. rapa*, to determine if the genes for blackleg resistance are linked to the genes for white rust resistance. Tables 5.1 to 5.4 provide the results of these Chi-square tests. In all cases, the genes for blackleg resistance assort independently of the genes for white rust resistance. This indicates that breeding for combined blackleg and white rust resistance will be fairly simple and efficient.

5.5 Summary

Blackleg and white rust are serious diseases that limit production of *B. rapa*, in western Canada. Disease resistant cultivars with good agronomic and quality characteristics, supplemented with proper management practices and chemical applications would be the most economical, and effective long-term solution to ensure *B. rapa* production is not limited by disease in the future. The knowledge gained from this inheritance study can be used in the development of *B. rapa* germplasm with resistance to blackleg and white rust diseases.

5.6 Future Research

The development of more doubled haploid lines from the SYL parent crosses; and verification of doubled haploid line segregation ratios to confirm that a single dominant gene confers blackleg and/or white rust resistance in these crosses.

The development of *B. rapa* cultivars with long-lasting blackleg and white rust resistance via gene pyramiding requires further research to determine if there is any association of genes between resistant sources. This can be accomplished by intercrossing the doubled haploid F_1 's from all four intraspecific crosses.

Verification of Kalavacharla's hypothesis for white rust resistance in *B. rapa* can be confirmed by intercrossing the heterozygous white rust resistant F_1 's within each intraspecific cross to determine the F_2 segregation ratio of each population.

Table 5.1. Chi-square contingency test of UMBL-1 population doubled haploid lines derived from [(UMBL-1 x SC25779 (F₃) x UM971] cross F₁'s for association of cotyledon reactions to blackleg isolate P182-12 and cotyledon reactions to white rust race Ac7a

		Blackleg Cotyledon		TOTAL
		Resistant	Susceptible	
White Rust Cotyledon	Resistant	34	12	46
	Susceptible	16	6	22
TOTAL		50	18	68
X ² value				0.0361

Table 5.2. Chi-square contingency test of UMBL-2 population doubled haploid lines derived from [(UMBL-2 x SC25779 (F₃) x UM971] cross F₁'s for association of cotyledon reactions to blackleg isolate P182-12 and cotyledon reactions to white rust race Ac7a

		Blackleg Cotyledon		TOTAL
		Resistant	Susceptible	
White Rust Cotyledon	Resistant	16	7	23
	Susceptible	8	6	14
TOTAL		24	13	37
X ² value				0.1702

Table 5.3. Chi-square contingency test of UMBL-1 population doubled haploid lines derived from [(UMBL-1 x SC25779 (F₃) x UM971] cross F₁'s for association of adult plant reactions to blackleg isolate P182-12/natural field inoculum and cotyledon plant reactions to white rust race Ac7a

		White Rust Cotyledon		TOTAL
		Resistant	Susceptible	
Blackleg Adult	Resistant	25	11	36
	Susceptible	21	11	32
TOTAL		46	22	68
X ² value				0.0058

Table 5.4. Chi-square contingency test of UMBL-2 population doubled haploid lines derived from [(UMBL-2 x SC25779 (F₃) x UM971] cross F₁'s for association of adult plant reactions to blackleg isolate P182-12/natural field inoculum and cotyledon plant reactions to white rust race Ac7a

		White Rust Cotyledon		TOTAL
		Resistant	Susceptible	
Blackleg Adult	Resistant	14	5	19
	Susceptible	9	9	18
TOTAL		24	13	37
X ² value				0.0217

6.0

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6.0 Literature Cited

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Appendix A

Appendix A: Summary of Doubled Haploid Lines Resistant (R) and Susceptible (S) Ratings

Blackleg - Cotyledon

Blackleg - Adult

White Rust - Cotyledon

DH Lines	R	MR	MS	S	R	MR	MS	S	R	MR	MS	S
UMBL-1 1A												
1				*				*	*			
2	*				*				*			
3	*							*	*			
4	*				*				*			*
5	*				*				*			
6	*				*				*			
7	*							*	*			
8	*							*	*			
9	*							*	*			
10	*				*				*			
11	*				*				*			
12				*				*	*			
13				*				*	*			
14				*				*	*			
15				*				*	*			
16	*				*				*			
17	*				*				*			
18	*				*				*			
19	*				*				*			
20	*				*				*			
UMBL-1 6F												
1				*				*	*			*
2	*				*				*			
3				*				*	*			
4				*				*	*			
5	*				*				*			*
6	*				*				*			*
7	*				*				*			
8				*				*	*			
9	*				*				*			
10	*				*				*			
11	*				*				*			
12	*				*				*			
13				*				*	*			
14	*				*				*			
15	*							*	*			
16	*				*				*			
17	*				*				*			
UMBL-1 6F												
1	*				*			*	*			*
2	*							*	*			*
3	*				*				*			*
4	*				*				*			*
5	*				*				*			*
6	*				*				*			*
7	*				*			*	*			*
8	*				*			*	*			*
9	*				*			*	*			*
10				*				*	*			*
11	*				*				*			*
12	*				*			*	*			*
13				*				*	*			*
14	*				*				*			*
15	*				*				*			*
16	*				*				*			*
17				*				*	*			*
18	*				*			*	*			*
19				*				*	*			*
20	*							*	*		*	
21	*							*	*			
22	*				*				*			
23				*				*	*			
24	*				*			*	*			
25	*				*				*			
26	*				*			*	*			
27	*				*			*	*			*
28	*							*	*			*
29				*				*	*		*	
30				*				*	*			*
UMBL-1 7C												
1				*				*	*			*
TOTAL	50	0	0	18	36	0	0	32	46	0	2	22

R = Resistant
MR = Moderately Resistant
MS = Moderately Susceptible
S = Susceptible

Appendix A: Summary of Doubled Haploid Lines Resistant (R) and Susceptible (S) Ratings

Blackleg - Cotyledon					Blackleg - Adult					White Rust - Cotyledon				
DH Lines	R	MR	MS	S	R	MR	MS	S		R	MR	MS	S	
UNABL-2 2F														
1	*				*					*				
2				*				*		*				
3	*							*		*				
4	*				*					*				
5	*				*					*				
6				*				*		*				
7	*				*								*	
8	*				*								*	
9	*				*						*			
10	*				*						*			
11	*				*						*			
12				*				*					*	
13				*				*					*	
14	*				*						*			
15				*				*		*				
16				*				*			*			
17	*				*								*	
18	*				*						*			
19	*				*						*			
20	*							*		*				
21				*				*		*				
22	*				*					*				
23				*				*		*				
24	*				*					*				
25				*				*					*	
26	*				*								*	
27	*				*					*				
28				*				*					*	
29				*				*					*	
30	*				*					*				
31				*				*		*				
UNABL-2 4H														
1	*				*					*				
UNABL-2 8B														
1				*				*					*	
2	*				*								*	
3	*							*					*	
4	*							*					*	
5	*							*					*	
TOTAL	24	0	0	13	19	0	0	18		16	7	0	14	

Blackleg - Cotyledon					Blackleg - Adult					White Rust - Cotyledon				
DH Lines	R	MR	MS	S	R	MR	MS	S		R	MR	MS	S	
STL ACB 2H														
1				*				*		*				
2	*				*									
3				*				*						
4	*				*								*	
5	*				*									
6	*				*					*				
7	*				*					*				
8	*				*					*				
9	*				*									
10	*				*									
11				*				*						
12				*				*						
13				*				*						
14				*				*						
15	*				*								*	
16				*				*		*				
17				*				*					*	
18				*				*					*	
TOTAL	9	0	0	9	9	0	0	9		5	0	0	4	
STL UM 3A														
1				*				*						
2	*				*									
TOTAL	1	0	0	1	1	0	0	1		0	0	0	0	

Appendix B

Appendix B: Summary of Blackleg Doubled Haploid Line Cotyledon Inoculation Scores

UMBL-1 1A										UMBL-1 4F										UMBL-1 4E										UMBL-1 4D										UMBL-1 4C										UMBL-1 4B										UMBL-1 4A										UMBL-1 40										UMBL-1 39										UMBL-1 38										UMBL-1 37										UMBL-1 36										UMBL-1 35										UMBL-1 34										UMBL-1 33										UMBL-1 32										UMBL-1 31										UMBL-1 30										UMBL-1 29										UMBL-1 28										UMBL-1 27										UMBL-1 26										UMBL-1 25										UMBL-1 24										UMBL-1 23										UMBL-1 22										UMBL-1 21										UMBL-1 20										UMBL-1 19										UMBL-1 18										UMBL-1 17										UMBL-1 16										UMBL-1 15										UMBL-1 14										UMBL-1 13										UMBL-1 12										UMBL-1 11										UMBL-1 10										UMBL-1 9										UMBL-1 8										UMBL-1 7										UMBL-1 6										UMBL-1 5										UMBL-1 4										UMBL-1 3										UMBL-1 2										UMBL-1 1										UMBL-1 0										UMBL-1 -1										UMBL-1 -2										UMBL-1 -3										UMBL-1 -4										UMBL-1 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Appendix B: Summary of Blackleg Doubled Haploid Line Cotyledon Inoculation Scores

[illegible]

Appendix B: Summary of Blackleg Doubled Haploid Line Cotyledon Inoculation Scores

[illegible]

Appendix C

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
785	1	REWARD	0	1	0	0	1	1	1	1	0	0	2	2	1	1	0	0	0	0	1	0	0	0	0	0	0	0.44	
879	1		0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0.20	
983	1		0	0	0	0	0	0	1	0	0	2	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0.24	
1113	1		0	2	0	1	4	3	2	2	2	2	0	0	0	1	0	0	4	3	2	0	1	1	2	0	1	1.32	
S			2	1	1	0	0	1	2	0	3	4	3	3	1	3	0	0	1	0	0	0	3	0	1	0	1	1.20	
S			3	2	4	0	2	0	0	2	1	4	1	0	0	2	0	2	2	1	1	2	1	2	0	0	2	1.36	
S			1	0	5	0	0	0	1	1	1	1	0	0	0	1	0	0	0	2	1	0	1	1	2	1	2	0.84	
S			0	0	0	0	0	1	1	0	1	0	0	0	0	2	0	0	0	1	0	1	2	0	1	0	0	0.40	S
706	111	SYL ACB 2H1																											
891	111		0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0.24	
1001	111		2	0	4	1	3	0	0	0	0	0	3	0	0	1	0	2	0	4	1	0	0	0	4	2	4	1.24	
1156	111		4	0	2	0	0	0	1	2	0	0	0	0	1	1	0	0	0	0	1	0	0	2	0	3	2	0.76	S
733	120	SYL ACB 2H10																											
947	120		0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	0	0	0.26	
1018	120		0	0	0	3	0	0	2	0	0	2	0	2	0	2	0	1	0	0	2	0	0	1	0	3	1	0.76	
1196	120		0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1					0.55	R
722	121	SYL ACB 2H11																											
945	121		1	0	1	0	1	0	0	0	0	1	0	1	0	1	2	0	1	0	0	0	0	0	0	0	0	0.36	
1046	121		4	1	0	2	1	0	2	1	0	0	0	0	0	0	0	0										0.69	
1215	121		0	0	0	0	0	2	2	0	0	0	0	3	0	0	0	0	0	1	1	3	1	0	0	0	0	0.52	S
706	122	SYL ACB 2H12																											
897	122		0	0	0	0	0	0	1	0	0	0	3	1	0	0	0	0	0	0	0	1	2	0	0	2	2	0.46	
1099	122		0	2	0	0	0	1	0	0	2	0	0	0	4	1	2	2	1	0	0	1	3	1	4	2	0	1.04	
1122	122		1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0							0.32	S
732	123	SYL ACB 2H13	1	1	0	2	0	4	0	4	1	0	0	0	1	0												1.00	
883	123		0	0	0	0	0	0	0	2	2	0	1	0	2	0	2	0	4	1	1	1	2	0	0	0	0	0.72	
1004	123		1	0	0	1	3	1	5	1	1	1	2	1	0	0	3	3	2	2	0	2	1	0	2	0	2	1.36	
1212	123		0	0	0	0	2	0	0	0	1	0	0	1	1	1	1	2	3	1	1	2	3	0	1	0	0	0.60	S
706	124	SYL ACB 2H14																											
903	124		0	1	0	0	0	0	0	0	0	2	3	0	0	0	1	1	0	0	0	0	2	1	0	0	1	0.46	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Ranking
1008	124		1	1	1	1	0	0	0	2	0	0	4	3	0	2	3	4	3	2	0	0	2	4	2	2	0	1.48	
1148	124		0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0									0.31	8	
704	125	SYL ACB 2H15	0	1	0	2	2	0	3	2	0	1	0	0	0	1	0	0	0	0	0	3	0	0	1	0	0	0.64	
846	125		0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	2	1							0.39		
1039	125		0	1	1	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0.44		
1133	125		1	0	0	0	1	1	0	0	1	0	1	1	0	0	3	1	0	0	1	0	0	1	0	1	0	0.52	R
787	126	SYL ACB 2H16	0	0	0	0	0	1	0	0	0	1	0	0	0	0	2	0	0	2							0.33		
899	126		0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	2	0	0	1	0	0	0	0.24	
1065	126		3	2	1	2	2	0	0	1	0	2	4	1	2	2	0	2	0	0	0	0	3	0	0	1	2	1.20	
1185	126		0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0.24	8
830	127	SYL ACB 2H17	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.08	
917	127		0	0	2	0	0	0	0	0	0	0	4	0	2	0	1	0	0	0	0	1	0	1			0.50		
1026	127		4	2	1	0	0	0	0	0	0	0	0	1	2	1	3	2	1	2	4	3	4	4	3	2	3	1.68	
1233	127		1	1	0	0	0	0	0	1	1	0	0	0	0	0	4	0	0	0	0	1					0.45	8	
721	128	SYL ACB 2H18	0	1	1	0	0	1	0	2	3	0	3	1	0	0	0	4	2	0	2	1	0	4	1	0	2	1.12	
862	128		1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0.24	
1069	128		0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0.24	
1137	128		0	0	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	1	2	0	0	1	0	0.44	8
781	112	SYL ACB 2H2																											
950	112		0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0.24	
1071	112		1	1	3	2	2	3	2	2	1	3	0	0	2	0	2	1	0	0	0	0	0	0	0	0	0	1.00	
1177	112		0	1	0	0	1	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0.32	R
705	113	SYL ACB 2H3																											
860	113		2	2	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0	2	0	1	1	0	0	0	0.52	
1029	113		1	0	0	0	1	2	4	0	0	0	1	2	0	0	2	0	0	1	2	1	3	0	0	1	0	0.84	
1180	113		0	1	0	1	0	0	1	0	1	0	1	0	0	0	1	3	2	1	0						0.63	8	
774	114	SYL ACB 2H4																											
931	114		1	0	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	2	0	0.40	
1110	114		0	2	0	0	0	0	2	0	0																0.44		
1221	114		0	0	0	0	0	0	1	0	0	1	0	2	2	1	0	0	0	1	1	1	0	0	0	1	0	0.44	R

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
737	115	SYL ACB 2H5																											
912	115		0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0.12	
1047	115		1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10	
1121	115		1	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0.24	R
725	116	SYL ACB 2H6																											
905	116		0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0.20	
987	116		0	1	0	0	2	0	0	0	0	0	1	0	2	0	1	0	0	0	0	0	0	2	1	0	1	0.44	
1153	116		0	0	1	1	2	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	0	0	1	0.44	R
783	117	SYL ACB 2H7	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0.12	
872	117		1	0	0	0	1	0	1	1	0	0	2	0	1	0	0	0	0	0	1	0	0	1	0	0	2	0.44	
1072	117		0	1	2	0	0	0	2	0	0	0	2	0	2	0	0	0	0	0	3	1	1	0	3	1	0	0.72	
1151	117		0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0.20	R
816	118	SYL ACB 2H8																											
930	118		0	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0.26	
1022	118		1	0	0	1	0	3	0	0	0	0	0	3	0	0	1	2	0	0	0	0						0.55	
1191	118		1	0	1	0	0	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	0	1	1	1	0	0.52	R
783	119	SYL ACB 2H9																											
958	119		0	0	1	2	1	0	0	0	0	1	0	1	1	0	2	0	2	2	0	0	0	0	0	0	0	0.52	
1027	119		0	1	0	0	0	2	0	2	0	0	0	0	0	2	0	2	0	0	0	2	1	0	0	3	3	0.72	
1243	119		0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0	1							0.26	R
787	109	SYL UM 2A1	0	0	0	2	0	2	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0.40	
857	109		1	5	0	0	0	0	0	0	0	0	0	1	3	4	0	3	0	2	0	2	0	0	1	2	0	0.90	
975	109		1	0	0	0	3	0	0	0	2	0	1	1	0	0	1	2	0	3	3	3	0	1	1	0	0	0.90	
1149	109		1	1	1	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0.52	S
706	110	SYL UM 2A2	2	0	0	0	0	1	2	0	1	1	0	0	0	0	0	1	1	1	1	1	1	0				0.57	
933	110		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	2	1	0.32	
1010	110		0	0	0	2	1	0	2	2	1	1	0	1	1	2	1	0	1	2	1	0	0	0	2	1	0	0.84	
1169	110		2	0	0	1	0	2	1	0	0	0	0	1	0	0	0	2	0	1	0	1	0	1	0	0	1	0.52	R
758	2	TOBIN	1	1	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.32	
845	2		0	0	2	0	2	1	0	0	0	0	0	3	0	0	0	0	1	0	0	0	2	0	0	1	0	0.48	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
1006	2		0	0	0	0	0	0	2	0	2	2	1	0	0	3	0	0	0	2								0.67	
1175	2		0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0.20	8
811	3	UM971	0	0	0	0	0	0	0	0	1	0	1	2	1	1	0	0	4	1	2	2	0	0	0	0	0	0.60	
864	3		0	0	1	0	0	1	0	0	1	1	0	2	0	0	0	2	0	0	0	2	0	0	0	0	1	0.44	
992	3		1	1	2	0	3	2	0	0	0	0	0	3	0	0	0	0	0	0	3	3	2	2	0	3	0	1.00	
1229	3		0	0	0	0	0	4	1	0	2	0	0	0	1	1	0	0	1	0	0	0	0	2	0	0	3	0.60	8
834	4	UMBL-11A1	0																									0.00	
954	4		0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	4	1	1	0	1	0	2	0.52	
995	4		0	1	0	0	2	0	0	1	0	2	0	1	0	0	0	0	2	0								0.50	
1152	4		0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	1	0			0.23	8	
802	13	UMBL-11A10	2	1	1	2	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0				0.36	
939	13		0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	3						0.55	
1076	13		0	0	2	1	0	0	0	0	0	2	0	1	0	0	0	1	2	0	0	0	0	0	1	0	0	0.40	
1173	13		1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	0	0.26	R
766	14	UMBL-11A11	1	0	2	2	2	1	1	2	2	2																1.50	
913	14		0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	2	1	0.26	
1056	14		0	0	1	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0.24	
1155	14		0	1	1	0	1	2	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0.36	R
749	15	UMBL-11A12																											
844	15		0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	4	0	0	0.26	
1023	15		2	0	0	0	2	1	1	2	0	0	0	1	1	0	0	3	0	0	0	0						0.65	
1214	15		0	1	1	1	1	4	2	0	3	1	0	1	1	0	0	1	1	1	1	0	0	3	1	0	0	0.66	8
797	16	UMBL-11A13	0	0	1	0	0	1	2																			0.97	
851	16		0	0	0	1	1	4	0	0	0	0	0	5	4	1	0	2	1	2	0	2	0	0	0	2	0	1.00	
1094	16		0	0	2	0	0	0	0	0	0	0	2	0	0													0.31	
1129	16		0	0	0	2	1	0	2	1	1	0	1	0	0	1	0	0	1	1	0	1	2	1	0	0	1	0.64	8
752	17	UMBL-11A14	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	
858	17		0	1	1	0	0	0	0	0	0	2	2	4	2	4	5	5	0	0	2	0	1	2	1	0	2	1.36	
1073	17		1	2	0	0	1	0	1	1	0	1	1	3	1	2	0	2	0	1	0	2	0	0	0	0	1	0.80	
1209	17		1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	1	1	1	0	2	0.46	8

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
720	18	UMBL-1 1A15	2	3	3	2	1	4	3	4	1																	2.56	
938	18		1	0	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	4	3	2	1	0	2	0.72	
1096	18		2	1	1	0	2	1	0	0	1	0	0	2														0.83	
1241	18		2	2	1	0	1	1	0	2	0	0	0	0	0	1												0.71	S
789	19	UMBL-1 1A16	2	0	0	0	0	0	0																			0.29	
908	19		0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	3	1	0	0.36	
1058	19		1	0	0	0	1	2	0	0	0	1	0	0	0	0	1	1	0	1	1	0	1	1	0	0	0	0.44	
1202	19		0	0	0	1	0	1	0	1	0	1	1	0	0	0	2	1	0	0	0	0	1	0	0	0	0	0.38	R
963	20	UMBL-1 1A17	1	0	1	0	1	0	0	0	0	3	4	0	0	0	1	0	1	1	1	0	1	0	0	1	0	0.84	
1086	20		0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	4	1	2	0	0	2	0	0	0.58	
1248	20		1	1	1	0	0	0	0	1	0	0	0	0	0	1	0	2	0	0	0	0						0.35	
762	20		0	1	0	1	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0.38	R
823	21	UMBL-1 1A18	0	0	0	1	0																					0.20	
946	21		0	0	0	1	1	0	0	1	1	0	1	1	0	0	1	1	0	0	0	0	0	3	2	1	0	0.56	
988	21		0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	0.28	
1136	21		1	0	0	0	0	0	0	1	0	0	1	0	0	0	2	0	2	0	1	0	0	0	1	0	0	0.38	R
792	22	UMBL-1 1A19	1	2	0	0	0	0	0	1	0	0	0	0	0													0.31	
973	22		0	0	0	0	0	3	1	2	0	0	0	0	2	2	2	2	0	0	1	0	0	0	1	0	0	0.64	
1057	22		0	2	2	0	0	0	0	0	0	0	0	0	1	1	1	1	2	0	1	0	0	0	0	0	1	0.48	
1217	22		0	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	2	1	1	1	1	0	0	0	1	0.48	R
819	5	UMBL-1 1A2	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1	3	3	0	1	0	0	0	2	3	0.68	
972	5		0	0	0	0	1	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	0	0	1	0.38	
1028	5		0	0	0	1	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0.20	
1139	5		0	0	0	1	0	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	1	0	2	1	0.40	R
818	23	UMBL-1 1A20	0	1	1	1	0	2	1	0	0	0	0	0	0	0												0.43	
987	23		1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	3	0	0	0	0	0	1	0.32	
986	23		0	0	0	1	0	2	0	0	0	0	0	3	0	0	0	1	0	1	3	0	0	0	0	0	0	0.44	
1116	23		1	1	0	0	1	1	2	1	0	0	0	2	0	0	0	1	1	0	1	2	2	2	0	1	1	0.80	R
731	6	UMBL-1 1A3	0	1	0	3	1	5	0	2	0	0	0	1	0	2	1	0	0	0	0	0	0	0	4	0	0	0.80	
876	6		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0							0.18	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating	
1052	6		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0.16		
1242	6		1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	8	
822	7	UNBL-1 1A4	1	0																								0.50		
916	7		0	0	0	0	0	1	0	1	1	0	0	1	0	0	1	1	0	1	1	0	1	1	0	1	1	0.46		
1100	7		0	0	1	1	3	1	1	1	2	3	0	2	1	0	0	2	1	0	2	0						1.05		
1161	7		0	0	0	1	0	0	3	3	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0.52	R	
779	8	UNBL-1 1A5	0	2	0	0	2	0	0	0	3	2	2	0	1	1	1	2	0	1	2	1	1	2	2	0	0	1.00		
961	8		0	1	1	1	1	0	1	0																		0.63		
966	8		2	2	0	0	0	1	2	0	0	0	1	0	2	0	0	0	1	0	1	0	0	0	0	1	0	0.52		
1219	8		0	0	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0.36	R	
750	9	UNBL-1 1A6	0	0	0	1	0	0	2	1	2	0	0															0.55		
861	9		1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0.20		
1031	9		0	0	0	1	1	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0.32		
1147	9		0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	2				0.32	R	
769	10	UNBL-1 1A7	0	3	0	0	0	0	0	1	2	0	0	3	0	0	0	0	0	0	0	0	1	0	3	0	4	0	0.66	
943	10		0	1	0	0	0	0	0	1	0	0	0	1	0	2	1	0	0	2	0	0	0	1	1	0	0	0.40		
960	10		2	0	2	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	2	0	0	1	0	0.52		
1203	10		0	0	0	0	0	0	1	2	1	0	0	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0.40	8	
734	11	UNBL-1 1A8	0	2	2	2	4	2	3	0	4	1	1	0	0	4	0	0	0	0	0	0	2	2	0	1	0	1.20		
936	11		3	1	2	1	0	0	0	0	2	1	0	0	0	1	0	0	1	0	1	1	4	3	2	4	4	1.24		
1067	11		0	0	0	0	0	1	2	0	4	0	0	4	2	0	0	0	0	1	1	0	0	1	1	0	1	0.72		
1134	11		0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	1	0	0				0.32	8	
837	12	UNBL-1 1A9	1	1	2	1	2	2	1	1	0	2	0	0	0	4	0	0	2	1	1	1	0	0	0	0	2	0.66		
866	12		0	0	1	1	0	0	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0			0.35		
1109	12		2	0	2	3	3	1	1	0	2	2	0	1	0	0	1	0	0	0	0	0	0	0	2	0	1	0.64		
1131	12		0	0	0	1	0	0	0	0	0	0	2	1	0	0	2	1	1	0	2	0	1	1	1	2	0	0.60	8	
776	24	UNBL-1 4F1	1	0	4	3	1	3	2	5	3	2	2	3	2	0	0	0	0	3	2	2	0	1	0	0	1	1.67		
877	24		0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0.16		
1019	24		1	3	0	0	0	2	1	1	2	2	1	0	0	1	0	1	0	1	0	0	2	1	0	3	0	0	0.84	
1160	24		0	0	0	0	0	2	2	2	0	1	0	0	2	0	2	0	0	1	0	0	0	0	0	1	2	0.60	8	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating	
801	33	UMBL-1 4F10	1	1	0	1	0	0	1	0	2	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0.40	
959	33		0	1	1	1	0	2	1	1	3	0	0	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0.56		
1081	33		0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0.24		
1117	33		1	0	0	0	1	0	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.24	R	
755	34	UMBL-1 4F11	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.20		
929	34		0	1	0	2	1	0	1	0	0	0	1	1	2	0	1	0	2	0								0.67		
1108	34		2	0	2	0	2	0	0	0	0	2	0	1	1	0	1	0	0	2	0	3	0	1				0.77		
1216	34		1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1				0.23	R	
708	35	UMBL-1 4F12	1	1	0	0	0	0	0	2	0	0	0	2	0	1	0	0	0	1	0	0	0	0	0	1	1	0.40		
983	35		0	0	0	0	1	1	1	0	0	0	1	0	0	1	0	0	2	0	0	0	0	0	0	1	0	0.32		
977	35		1	0	1	0	0	0	0	0	1	0	2	2	2	1	2	2	2	2	0	1	2	1	0	2	2	1.04		
1213	35		0	0	1	0	1	1	1	0	0	0	0	1	1	1	1	0	1	1	0	0	1	0	0	0	0	0.44	R	
777	36	UMBL-1 4F13	0	2	0	0	0	1	0	2	0	3	1	0	4	0	4	0	4	2	4	0	1	0	1	0	0	1.16		
854	36		0	0	0	0	0	0	0	0	1	0	2	2	2	0	0	0	0	0	0	4	3	2	0	2	2	0.90		
1081	36		0	0	0	1	0	1	0	0	1	0	0	2	0	2	2	0	2	1	3	2	0	0	4	4	0	1.00		
1187	36		0	0	0	0	1	0	1	2	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0.28	S	
831	37	UMBL-1 4F14	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0.20		
839	37		0	1	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.24		
1046	37		0	0	2	0	0	0	0	0	0	0	0															0.18		
1237	37		0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	3	2	0	1	0	0.44	R	
711	38	UMBL-1 4F15	0	0	0	0	0	0	2	0	0	0	0	0	1	0	2	2	0	4	0	0						0.55		
889	38		0	0	1	0	2	2	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0.32		
1043	38		1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0.18		
1146	38		4	1	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				0.55	S	
705	39	UMBL-1 4F16	1	0	3	2	2	3	0	0	1	0	1	1	3	2	1	0	1	0	2	0	0	0	0	0	0	0	0.92	
923	39		0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.20		
994	39		0	0	0	0	0	0	2	0	0	1	0	1	0	1	2	2	2	0	2	2	0	2	1	1	2	0.84		
1184	39		1	2	0	0	2	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	1	0.48	R	
832	40	UMBL-1 4F17	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0			0.22		
915	40		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0.08		

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
979	40		0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0.20	
1218	40		1	1	0	0	0	0	0	0	0	1	1	0	0	0											0.29	R	
814	25	UMBL-1 4F2	2	2	0	0																					1.00		
843	25		0	0	1	1	0	1	1	0	1	0	0	1	0	1	0	3	0	2	2	1	0	0	1	0	0	0.64	
1040	25		2	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	2	2	0	0	2	0	1		0.54	
1228	25		1	0	0	0	0	1	1	1	1	1	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0.48	R
719	26	UMBL-1 4F3	4	0	4	0	1	0	1	2	0	0	2	0	0	0	2	0	1	2	1	1	5	2	0	1	5	1.36	
861	26		0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	3	0	1	0	0	0	1	0	0.32	
1098	26		0	1	1	0	2	2	1	0	2	0	0	0	2	0	0	0	0	0	0	2	4	1	0	0	2	0.80	
1238	26		1	0	0	0	0	2	1	0	1	0	1	1	0	1	0	2	2	0	2	2	0	0	0	0	0	0.72	S
742	27	UMBL-1 4F4	3	2	0	1	4	2	2	1	2	3	0	1	3	0	1	0	3	0	2	0	2	3	1	3	4	1.72	
960	27		0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0.39	
998	27		0	0	2	1	1	1	2	0	0	1	5	0	3	0	2	1	2	1							1.22		
1208	27		0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	4	0	1	0	0	1	0	0.44	S
748	28	UMBL-1 4F5	2	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	2	0				0.39	
863	28		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	1	0	1	0.24	
1032	28		0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	0	0	0	0.24	
1235	28		3	0	0	0	0	0	1	3	3	2	1	3	3	2	0	0	2	0	1	0	1					1.19	R
770	29	UMBL-1 4F6	0	0	0	0	0	2	0	0	1	2	1	1	1	1	0	1	0									0.59	
832	29		0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0.12	
1044	29		0	0	2	0	0	2	1	0	0	1	1	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0.44	
1230	29		0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0					0.19	R
833	30	UMBL-1 4F7	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.16	
962	30		1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	2	1	1	0	2	3	2	2	0.72	
1005	30		0	2	2	0	0	1	0	0	0	0	0	2	2	0	0	0	1	2	2	0	0	0	2	3	0	0.78	
1228	30		2	0	0	0	0	0	1	2	1	2	1	1	1	0	0	1	1	0	0	2	1					0.78	R
803	31	UMBL-1 4F8	0	2	1	0	2	0	1	0	0	1	2	0	1	2	1	1	0	0	0	0	0	0	0	0	2	0.64	
965	31		0	1	1	0	1	0	0	4	5	3	0	0	1	0	0	0	1	0	0	0	0	0	2	2	0	0.64	
1013	31		0	0	0	0	0	2	1	2	0	2	0	0	2	0	1	0	0	2	0	0	0	0	0	1	0	0.52	
1193	31		0	0	1	0	1	0	0	1	2	1	0	0	1	0	1	0	0	1	0	2	1	0	0	1	0	0.52	S

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Ranking	
758	32	UNBL-1 4F9	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0.28		
842	32		0	0	0	0	1	0	1	0	0	0	0	0	3	0	1	1	0	0	1	2	1	0	0	1	0	0.48		
1007	32		2	2	2	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.44		
1197	32		1	0	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	0	0	0	1	0	1	0	1	0.80	R	
809	41	UNBL-1 6F1																												
848	41		0	0	1	0	0	2	0	2	1	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	0.44		
1074	41		0	0	2	2	2	1	1	1	0	0	0	1	0	0	0	2	2	0	1	1	2	0	3	2	1	0.88		
1130	41		0	1	0	0	0	0	0	0	1	0																0.20	R	
728	50	UNBL-1 6F10																												
852	50		0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	4	5	5	0	0.80		
982	50		0	0	0	1	1	1	0	0	2	0	1	0	0	1	0	0	0	2	1	2	0	0	2	3	2	0.78		
1182	50		0	0	1	1	1	0	3	1	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0.52	S	
817	51	UNBL-1 6F11	2	2	0	0	2	0	0	0	0	1	0	0	0	1	0	2	2	0	0	0	0	0	1	0	0	0.52		
911	51		2	0	1	0	0	0	0	1	0	1	0	0	0	0	2	2	1	0	2	0	0	0	0	1	0	0.52		
1059	51		0	1	0	1	0	0	0	0	1	2	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0.38		
1244	51		0	1	0	0	1	0	1	3	3	0	1	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0.80	R	
812	52	UNBL-1 6F12																												
908	52		1	0	1	0	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.32		
980	52		0	0	0	2	1	0	0	1	0	0	0	0	0	1	2	1	0	0	1	2	0	0	0	1	0	0.48		
1141	52		1	0	0	0	0	1	1	0	1	0	1	1	1	1	0	1	1	1	0	0	0	0	0	1	0	0.48	R	
773	53	UNBL-1 6F13																												
920	53		0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	2	1	0	0	0	1	0	0.32		
1089	53		3	4	2	2	0	1	2	1	4	0	1	0	2	0	1	0	2	0	0	1	0	1	1	2	0	1.20		
1114	53		0	0	0	1	0	0	0	0	1	0	0	1	1	0	3	1	4	1	0	1	0	2	1	2	2	0.84	S	
738	54	UNBL-1 6F14	1	1																								1.00		
841	54		0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0.20		
1054	54		0	0	0	0	0	0	0	3	1	0	0	1	0	0	1	0	3	0	0	0	1	0	2	0	0	0.48		
1159	54		1	1	2	1	0	0	1	1	1	2	1	1	0	1	0	3	0	0	0	0	1	1	0	0	0	0.72	R	
813	55	UNBL-1 6F15	2	0	0	2	3	0	0	0	2	3	2	1	0	0	0	1	0	0	0	0	0	0	0	1	2	0	0.78	
927	55		0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	2	2	1	0	0	2	2	0	0.44		

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
1104	55		0	0	0	0	1	2	2	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	2	0	2	0.60	
1246	55		0	0	1	0	0	1	0	0	0	0	0	0	3	0	0	0	0	2	1						0.42	R	
709	56	UMBL-1 6F16	1	0	0	1	1	0	0	2	0	2	1	0	0	0	0	3	1	0	0	0	1	1	0			0.61	
901	56		0	1	1	2	0	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0	0	1	1	0	0.56	
1041	56		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2							0.22		
1132	56		1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	3	0	1	0	0	0	0.44	R
735	57	UMBL-1 6F17	2	4	0	2	2	0	0	0	0	0	2	0	0	1	2	0	3	3	0	0	1	2	1	0	0	1.00	
999	57		0	1	0	0	0	0	2	2	0	2	2	3	0	0	0	0	1	1	0	0	0	1	0	0	0	0.60	
1064	57		0	1	0	0	0	0	2	0	1	1	1	0	0	1	0	1	0	0	0	0	1	2			0.50		
1166	57		2	4	0	0	0	2	4	4	1	0	0	4	2	2	1	1	1	0	1	0	0	1	4	4	1	1.56	S
747	58	UMBL-1 6F18	3	0	0	0	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	3		0.52	
935	58		0	0	0	0	0	0	0	0	3	2	2	1	1	2	0	1	0	0	0	2	1	0	0	0	1	0.64	
1079	58		0	0	0	0	1	0	1	0	0	0	1	1	0	1	1	1	0	0	0	0					0.35		
1183	58		1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	R
815	59	UMBL-1 6F19	0	1	0	0	1	2	0	0	1	1	0	1	1	2	0	1	0	1	0	2	4	4	0	2	1	1.00	
974	59		0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0	0.32	
1037	59		1	0	2	0	1	3	2	0	1	0	0	0	0	3	1	4	4	2	0	1	0	0	3	1	0	1.16	
1223	59		0	1	0	0	1	1	0	0	1	0	1	2	1	1	0	0	0	0	0	0	1	0	0	2	1	0.62	S
714	42	UMBL-1 6F2																											
836	42		0	0	1	1	1	1	4	1	0	0	0	0	0	1	0	0	0	1	2	0	1	4	0	0	1	0.76	
1055	42		2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2				0.24		
1162	42		0	2	1	0	1	1	1	0	0	0	0	0	2	2	1	0	1	0	0	1	0	0	0	1	1	0.60	S
717	60	UMBL-1 6F20	1	0	1	0	0	1	2	1	0	0	1	1	0	1	2	2	1	1	0	1	1	0	0	1		0.75	
875	60		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0							0.06		
1003	60		1	1	4	4	3	4	3	2	2	2	4	1	2	1	4	2	3	2	1	1	2	0	0	1	0	2.00	
1245	60		0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0		0.36	S
764	61	UMBL-1 6F21	0	2	0	1	0	1	0	1	2	0	0	0	0	0	0	0	1	0	0	1	2	1	1	2	4	0.79	
902	61		0	0	0	0	0	0	4	1	1	1	1	0	0	1	2	0	0	0	0	1	1	2	1	1	1	0.72	
961	61		1	0	0	0	0	0	0	0	0	1	0	0	0	0	4	1	0	3	1	0	0	1	0	1	1	0.56	
1207	61		0	0	2	1	1	0	0	1	0	0	0	0	3	0	0	0	0	2	1	2	1	2	1	0	0	0.66	S

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating	
765	62	UMBL-1 6F22	0	0	1	0	0	1	1	0	1	0	0	0	2	1	0	0	1	1	2	2	0	0	0	1	0	0.56		
859	62		0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	2	2	1	0	0	2	0	1	0	0.48		
1042	62		1	0	0	0	0	0	2	1	0	1	0	0	0	0	1	0	0	0	1	0					0.35			
1236	62		0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1									0.31	R		
754	63	UMBL-1 6F23	1	1	2	2	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.44		
971	63		0	1	0	0	0	0	0	0	0	4	2	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0.44		
1064	63		0	2	4	0	0	0	0	2	1	1	1	2	0	3	1	1	0	2	3	2	4	3	0	0	4	1.44		
1188	63		0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1						0.32	S		
804	64	UMBL-1 6F24	1	0	0	0	0	0	0	2	0	0	0	1	0	3	2	0	2	0	0	0	0	0	0	0	1	0.48		
919	64		0	5	0	1	2	2	0	0	1	2	0	0	0	3	0	0	0	3	2	0	0	1	0	0	0	0.86		
1035	64		0	0	1	0	1	1	0	0	4	5	1	0	0	0	0	1	0	1	1						0.84			
1126	64		0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	4	0.36	S	
713	65	UMBL-1 6F25	0	2	0	0	0	0	1	1	2	0	2	0	0	2	0	0	0	0	0	0	2	0	2	1	1	0	0.64	
949	65		0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0.24		
1108	65		1	2	2	1	1	0	1	2	1	0	0	0	1	0	0	0	2	0	2	0	0	0				0.73		
1123	65		0	0	2	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	2	0	1	1	1	0	0.40	R	
828	66	UMBL-1 6F26	1	1	0	1	1	0	0	0	0	0	5	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0.48		
914	66		0	0	0	0	2	0	0	0	1	0	0	1	0	2	0	0	0	0	0	0	1	0	0	0	0.29			
1016	66		0	0	0	2	0	1	4	0	1	2	1	0	0	1	1	1	0	0	0	0	0	0	2	1	0	0.86		
1112	66		0	0	0	1	1	0	0	1	0	0	0	1	0												0.31	S		
766	67	UMBL-1 6F27	0	3	2	1	0	0	0	0	0	0	2	0	2	0	0	1	3	0	0	0	1	0	0			0.65		
966	67		0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0.20		
1103	67		1	1	0	0	1	0	1	0	2	0	0	0	1	2	0	0	1	0	0	0	0	1	0	3	1	0.60		
1239	67		0	0	0	0	0	2	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	0	0	2	0.40	R	
743	68	UMBL-1 6F28	0	0	0	2	2	0	0	2	2	3	4	4	4	0	0	2	2	3	0	1	4	3	4	4	0	1.84		
937	68		2	0	0	1	0	1	1	0	0	0	1	0	1	1	0	0	0	0	0	2	1	0	1	2	2	0.64		
991	68		1	1	2	0	1	1	1	1	0	1	2	3	1	1	3	1	0	2	0	1	0	0	1	0	0	0.86		
1222	68		0	0	0	0	1	1	2	0	0	0	0	0	1	1	0										0.40	S		
759	69	UMBL-1 6F29	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0.16		
862	69		0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	2	0	2	0	1	0	1	0	1	0	0.44		

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
1067	60		0	3	1	0	0	0	1	1	0	1	3	2	1	3	2	0	1	0	4	4	4	2	1	2	0	1.44	
1179	60		0	1	0	0	1	1	1	0	1	0	0	1	0	0	1	1	1	0	0	0	2	1	2	1	1	0.64	S
739	43	UMBL-1 6F3																											
866	43		1	0	0	2	0	2	0	0	1	0	2	0	0	4	0	0	0	4	0	0	0	2	1	1	0	0.80	
1062	43		0	0	0	0	0	0	0	0	0	2	0	1	0	2	0	0	0	1								0.39	
1204	43		0	0	1	0	1	1	0	0	0	0	0	0	4	3	2	0	0	2	2	1	0	0	1	0	0	0.72	S
829	70	UMBL-1 6F30	1	1	1	0	0	0	0	0	1	0	0	1	0	2	0	0										0.44	
853	70		0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	4	0	0	0	0.64	
1009	70		1	1	0	0	1	0	0	0	1	2	0	1	0	1	2	0	0	0	2	2	3	2	0	1	3	0.62	
1247	70		0	1	0	0	1	0	0	0	0	1	0	0	0	1	0											0.27	S
764	44	UMBL-1 6F4	1	2	1	0	0	0	1	2	0	1	0	0	0													0.62	
866	44		0	0	1	0	2	2	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	1	0	1	0.44	
1033	44		0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	1	0	0	0	2	1	1	0	0	3	0.56	
1167	44		0	0	0	1	0	0	0	1	0	0	1	1	2	3	0	0	1	0	0	0	0	0	1	0	0	0.44	R
712	45	UMBL-1 6F5	1	3	0	0																						1.00	
856	45		0	0	0	0	0	0	1	1	0	1	0	1	1	0	2	0	0	2	1	2	2	2	2	2	1	0.84	
1068	45		2	0	0	0	1	1	0	0	1	0	1	0	2	1	0	2	1	0	1	1	1	1	2	0	0	0.72	
1194	45		0	1	3	2	1	0	1	1	1	1	0	0	0	1	0											0.60	R
800	46	UMBL-1 6F6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0							0.05	
924	46		0	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0								0.26	
1063	46		0	1	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	1	2	0.36	
1231	46		1	0	0	0	0	1	0	0	0	1	1	0	1	1	1	1	0	0	1	0	1	0	0	0	0	0.36	R
736	47	UMBL-1 6F7																											
907	47		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	0	0.20	
976	47		1	2	0	0	0	1	1	0	0	1	0	0	1	4	1	2	0	0	2	1	1	0	2	4	1	1.00	
1176	47		1	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.24	S
825	48	UMBL-1 6F8	0	0	0	0	0	0	0	0	0	0	0															0.00	
909	48		0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	2	0	0	0	0.24	
1049	48		0	0	1	2	0	2	0	1	2	1	0	0	0	2	0	0	2	0	0	0	0	0	2	0	1	0.64	
1172	48		1	0	0	0	1	1	0	0	1	1	0	1														0.60	R

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
746	49	UMBL-1 6F9	1	0	0	0	0	1	0	1	0	0	2	0	0	0	0	0	4	2	2	2	2	0	1	2	1	0.76	
876	49		1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	4	2	0	2	0	0	1	0.56	
999	49		0	0	0	0	0	0	0	0	0	0	2	1	1	4	1	0	2	2	2	4	1	1	0	1	0	0.86	
1199	49		0	0	1	1	0	0	0	1	1	1	0	1	1	0	0	1	1	0	0	0	1	1	0	0	0	0.44	8
740	71	UMBL-1 7C1																											
934	71		0	0	0	1	1	0	0	0	0	0	1	1	0	0	4	1	1	2	1	0	0	4	2	1	0	0.80	
1051	71		1	0	0	0	0	2	0	2	1	0	0	0	0	0	0	0	4	0	0	0	0	1	0	1	2	0.56	
1157	71		0	0	1	0	0	1	1	1	0	0	0	0	1	0	4	1	1	0	1	0	0	0	4	1	1	0.72	8
710	72	UMBL-2 2F1																											
847	72		3	0	0	0	0	1	0	2	0	3	0	2	2	2	2	2	1	0								1.11	
1087	72		0	0	0	0	0	0	0	0	0	0															0.00		
1144	72		0	1	0	1	1	0	0	0	0	0	1	0	1												0.36	R	
741	81	UMBL-2 2F10																											
874	81		0	1	0	0	1	0	2	1	0	2	0	2	0	0	2	0	2	2	0							0.79	
1036	81		1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	0	1	0	0	0	2	0	0.44	
1136	81		0	0	0	1	1	2	1	0	1	2	0	0	2													0.77	R
791	82	UMBL-2 2F11	2	1	2																							1.67	
922	82		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0								0.17	
1034	82		1	2	2	0	1	2	1	0	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0.64	
1142	82		3	2	2	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0					0.57	R
771	83	UMBL-2 2F12	0	0	0	0	0	0	4	0	0	1	0	1	0	1	2	0	1	2	0	1						0.65	
871	83		0	1	0	0	0	0	1	0	0	0	1	1	0	4	4	2	0	4	3	3	3	1	4	0	0	1.28	
993	83		3	4	3	0	0	1	0	0	2	2	0	0	0	1	2	0	0	2	0	1	0	0	1	0	0	0.86	
1189	83		0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0.36	8
703	84	UMBL-2 2F13	1	2	1	3	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.29	
864	84		1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0.28	
1088	84		1	1	0	2	4	0	2	4	4	1	1	1	2	0	3	0	2	0	0	0	1	0	1	2	3	1.40	
1227	84		3	2	1	1	0	0	1	0	0	0	1	0	3	2	1	0	0	2	3	1	0	0	2	2	1	1.04	8
799	85	UMBL-2 2F14	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0.16	
963	85		0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	2	0.26	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Range
1080	85		0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0.16	
1170	85		0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2	3	2	1	0	3	2	1	0.66	R
744	86	UNBL-2 2F15	0	0	0	0	2	0	0	0	4	0	2	1	0	0	0	3	0	1	0	0	0	0	0	0	0	0.52	
856	86		2	0	0	0	0	0	1	0	0	4	0	1	4	0	0	0	1	0	0	1	0	0	0	0	0	0.56	
997	86		0	0	1	1	0	1	0	0	0	2	0	4	0	1	3	0	2	1	2	0	0	1	0	0	0	0.78	
1208	86		1	0	0	1	0	2	0	0	4	0	1	0	1	2	0	0	1	3	1	3	1	4	0	1	4	1.20	S
772	87	UNBL-2 2F16	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0									0.24	
994	87		0	0	0	0	0	3	1	0	1	0	1	0	1	0	2	0	1	2	0	1	0	0	0	0	0	0.52	
984	87		3	1	0	0	0	0	0	2	1	2	2	2	0	3	2	3	4	4	2	0	2	0	1	0	0	1.36	
1176	87		0	2	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0.32	S
827	88	UNBL-2 2F17	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0.20	
873	88		0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0				0.27	
1107	88		0	2	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	2	0	0	0	0.36	
1125	88		0	0	1	2	2	2	1	0	0	1	0	0	1	1	0	0	0	2	2	0	0	1	0	1	0	0.66	R
781	89	UNBL-2 2F18	0	0	1	2	0	0	1	0	0	0	0	0	0	0	3	0	0	2	3	0	0	0	2	2		0.67	
948	89		0	0	0	1	0	0	0	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0.24	
1102	89		0	0	1	0	0	1	1	2	0	0	0	0	1	0	2	0	0	0	0	2	1	3				0.64	
1174	89		0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	3	1	0	1	1	0.52	R
806	90	UNBL-2 2F19	2	2	0	0	1	0	1	0	1	0	1	0	3	0	0	0	1	0	0	0	1	0	1	3	0	0.66	
855	90		2	2	0	0	0	0	1	3	0	0	0	0	1	1	0	0	1	3	0	1	0	0	0	0	0	0.60	
1091	90		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					0.00		
1234	90		1	1	0	1	1	1	0	0	1	1	1	0	3	0	0	0	1	0	2	2	2	0	3	2	0	0.92	R
807	73	UNBL-2 2F2	0	1	0	3	1	1	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0.46	
869	73		0	0	0	1	0	4	3	0	3	0	1	0	0	0	1	1	3	0	1	0	0	0	0	0	0	0.72	
1017	73		0	3	0	1	0	4	0	0	0	4	2	1	4	0	0	2	0	0	0	0	1	2	3	0	0	1.06	
1220	73		0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	2	1	0	0	0	0	0.36	S
723	91	UNBL-2 2F20	0	2	2	0	2	2	2	0	5	0	1	0	2	1	0	1	3	5								1.56	
964	91		0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1	1	0.32	
1011	91		1	1	1	1	1	2	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.46	
1184	91		1	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	0	1	0	0	1					0.36	S

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Ranking
782	92	UMBL-2.2F21	1	1	2	0	0	0	1	2	0	0	0	4	0	3	0	0	0	1								0.83	
870	92		0	0	1	0	1	0	0	1	0	0	1	0	2	0	5	1	2	0	0	1	0	0	0	3	0	0.72	
1077	92		1	1	0	0	1	1	1	0	0	0	1	0	1	1	2	0	0	0	3	2	2	2	2	0	0	0.64	
1163	92		0	0	2	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0.28	8
784	93	UMBL-2.2F22	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0										0.25	
941	93		1	1	0	0	1	3	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	2	1	1	0.56	
1038	93		1	2	0	0	0	0	2	1	0	0	2	0	1	0	0	1	1	1	1	0	0	0	3	0	2	0.68	
1166	93		1	1	2	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	2	1	0	0	0	1	0	0.44	R
780	94	UMBL-2.2F23	0	0	2	0	0	0	0	0	1	1	0	0	1	0	1	4	0	2	2	1	0	1				0.73	
840	94		0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0.20	
1070	94		0	2	1	4	0	4	2	0	0	2	2	2	2	1	1	0	0	1	1	0	2	2	0	1	2	1.26	
1168	94		0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0							0.26	8
751	95	UMBL-2.2F24	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	0	0	0	0.24	
806	95		1	0	2	2	3	0	0	0	0	0	0	0	0	2	0	0	0	1	0	1	1	1	2	1	0	0.66	
1101	95		0	0	0	1	0	2	0	0	2	0	2	1	0	0	0	0	0	0	0	0	1	2	0	1	0	0.46	
1182	95		1	1	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0					0.24	R
808	96	UMBL-2.2F25	1	2	0	0	2	2	0	0	0	1	0	1	0	0	0	0	2	0	0	1	1	3	0	1	0	0.66	
900	96		0	0	0	0	3	1	3	2	3	2	1	1	4	2	4	0	1	1	1	1	1	2	2	2	4	1.64	
1050	96		0	0	0	1	0	0	0	0	0	0	2	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0.28	
1200	96		0	1	0	0	0	1	0	0	0	1	0	1	1	0	1	0	1	1	0							0.42	8
729	97	UMBL-2.2F26	3	0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	3	1	0	0	3	0	0	2	0	0.66	
802	97		2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	2	0	1	0	0	0.32	
1021	97		1	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	2	0	0.44	
1158	97		1	0	1	0	0	0	0	0	0	0	0	0	0	2	1	1	2	0	0	2	0	0	0	1	1	0.46	R
716	98	UMBL-2.2F27	3	2	2	1	2	0	1	0	3	1	2	1	0	0	1	1	1	1	3	0	1	2	2	0	0	1.28	
944	98		0	0	0	0	0	0	0	0	2	0	1	0	0	0	1	0	0	2	1	1	0	1	0	1	1	0.44	
1083	98		1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.16	
1145	98		0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0.28	R
757	98	UMBL-2.2F28	0	0	1	1	0	0	0	0	0	0	1	0	0	0	2	3	2	1	0	0	0	1	0	0	0	0.46	
849	98		1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	3	0	1	1	0	0	0	1	0	0	0.36	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating	
1065	99		0	0	0	1	0	0	0	2	0	0	0	0	0	0	1	1	0	2	0	0	0	1				0.36		
1205	99		1	1	1	1	1	1	1	1	0	0	1	3	3	2	4	4	4	5	2	2	1	1	0	0	1	1.64	S	
826	100	UMBL-2 2F29	0	0	0	0	0	0	1	1	0	0	0	1	1													0.31		
967	100		0	0	1	0	0	0	0	0	0	0	1	0	1	2	1	0	1	0	0	0	1	0	0	0	0	0.32		
1111	100		0	0	1	0	0	0	1	0	0	0	1	4	4	0	1											0.80		
1119	100		0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0.28	S	
724	74	UMBL-2 2F3	2	0	1	0	0	1	0	2	2	0	1	0	2	0	2	4	1	2	1	0						1.05		
951	74		0	0	1	0	0	1	0	0	1	0	0	4	0	0	3	0	1	1	1	0	0	0	1	1	1	0.64		
1063	74		0	3	0	0	0	0	0	1	0	0	1	0	2	0	1	0	1	2	0	2	1	0	0	0	0	0.59		
1211	74		0	0	1	1	0	0	1	3	0	0	0	0	0	0	0	1	0	2	1	0	0	3	0	0	1	0.59	S	
701	101	UMBL-2 2F30	0	0	2	0	0	2	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.39	
942	101		1	1	0	1	1	1	0	1	0	1	1	0	0	1	1	1	1	0	1	1	0	0	1	1	0	0.64		
1002	101		0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	1	0	2	3	1	0	0	1	1	0	0.52		
1232	101		0	0	1	0	0	0	1	2	1	1	0	1	1	1	0	1	1	0	1	0	0	1	1	0	0	0.56	R	
727	102	UMBL-2 2F31	2	0	0	1	0	0	2	0	2	1	0	0	3	0	2	1	2	2	2	0	3	2	3	0	0	1.12		
967	102		0	0	1	1	1	0	0	1	2	0	0	5	0	0	4	3	1	0	0	0	0	0	0	3	0	0.89		
1092	102		1	0	1	0	0	1	0	0	2	0	3	0	4	1	0	2										0.94		
1115	102		0	0	0	1	0	1	0	2	2	1	0	0	1	0	0	0	1	1	1	1	1	0	0	1	0	0.59	S	
836	75	UMBL-2 2F4	0	0	0	0	0	0	1	0	0	0	1															0.18		
904	75		0	0	2	2	0	0	1	0	0	2	0	1	0	0	0	0	1	0	0	0	1	1	0	1	1	0.52		
1082	75		1	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	2	0	0	0	2				0.36		
1196	75		1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.28	R	
778	76	UMBL-2 2F5																												
989	76		0	0	1	1	1	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	1	0	0	0	0.39		
1014	76		0	1	0	1	2	2	0	0	0	0	0	1	2	0	0	2	0	2	1	0	0	0	2	0	0	0.64		
1181	76		0	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.24	R	
716	77	UMBL-2 2F6	0	5	5	1	5	4	2	0	1	2	0	1	0	0	0	1	5									1.66		
968	77		0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0.24		
976	77		0	0	2	4	0	0	2	0	2	1	0	0	1	1	0	0	2	0	2	0	0	1	0	0	0	0.72		
1140	77		0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0.32	S	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating	
788	78	UMBL-2 2F7																												
885	78		1	2	0	0	0	0	2	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	1	0.44		
1025	78		0	0	2	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	2	0	1	1	3	3	1	0.64		
1118	78		1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0	0					0.29	R	
810	79	UMBL-2 2F8	0	0	1	0	2	0	2	1	0	1	0	2	0	2	0	0	0	1	0	0	0	3	1	1	1	0.72		
952	79		0	0	2	1	1	0	0	0	0	1	0	0	0	0	0	2	1	0	0	1	2	0	0	1	0	0.48		
1020	79		0	1	2	0	3	3	1	0	1	1	1	0	0	0	0	0	0	0	0	2	1	0	0	1	2	0.76		
1201	79		0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0.20	R	
702	80	UMBL-2 2F9	1	1	1	0	0	0	0																			0.43		
928	80		0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1								0.22		
1012	80		0	0	1	1	0	0	1	0	0	0	0	2	0	0	0	2	1	0	0	0	0	0	0	0	0	0.32		
1171	80		0	1	0	0	0	1	0	0	0	0	3	0	0	0	1	0	0	0	1	0	0	0	3	2	1	0.52	R	
821	103	UMBL-2 4H1	0	1	0	0	0	1	0	0	0	1	1	0	1	1	0	1	1	0	0	1	0	0	1	1	0	0.44		
921	103		0	0	0	1	0	0	1	2	0	0	0	2	0	0	0	0	0	0	1	1	0	1	1	2	2	0.60		
1024	103		0	1	0	1	0	0	0	1	0	2	0	0	1	0	1	0	2	1	0	1	0	1	0	0	0	0.48		
1154	103		1	0	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	0	0	1	0	0.32	R	
753	104	UMBL-2 8B1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0.28		
957	104		1	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0	1	0	0	0	0.38		
1085	104		1	0	2	0	0	0	0	0	0	0	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0.38		
1127	104		1	2	1	0	4	4	3	2	4	4	2	1	0	2	1	2	0	0	0	0	1	0	0	1	0	1.40	S	
707	105	UMBL-2 8B2	0	0	0	0	1	1	2	0	2	0	0	0	0	0	2	0	0	2	0	1	0	0	0	2	0	1	0.58	
888	105		0	2	0	2	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0.38		
1076	105		0	1	2	0	0	1	0	1	2	0	2	2														0.92		
1224	105		0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	2	0	1	0	0	0	1	2	1	0.44	R	
728	108	UMBL-2 8B3	4	2	0	0	1	0	0	0	2	4	0	0	2	1	0	4	0	2	0	4	0	0	2	0	4	1.28		
928	108		0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0.28		
1086	108		0	0	0	0	1	0	0	3	2	1	0	0	2	2	1	0										0.75		
1143	108		1	0	0	2	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.24	S	
763	107	UMBL-2 8B4	0	0	0	0	0	4	0	0	3	0	4	2	4	1	0	2	2	1	1	1	0	0	0	0	0	1.00		
880	107		0	0	0	0	0	4	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0.36		

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Rating
989	107		0	0	0	0	3	0	0	2	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	2	1	0.46	
1124	107		0	0	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0.32	8
824	108	UMBL-2 8B5	0	0	0	1	4	1	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0.44	
918	108		0	0	0	0	1	0	0	0	1	0	0	1	1	0	1	1	0	2	2	0	0	0	1	0	0	0.44	
1053	108		1	2	0	1	0	1	0	2	1	0	0	0	0	0	2	1	1	0	0	3	1	0	0	0	0	0.64	
1128	108		2	3	2	1	1	0	1	4	2	0	0	0	0	1	0	0	0	0	1	0	3	2	1	0	0	0.66	8
715		Westar	2	1	1	1	2	0	0	1	1	4	3	1	0	2	1	2	4	0	1	1	0	0	1	0	1	1.20	
730			0	0	2	1	3	1	1	0	4	0	0	3	2	2	3	3	1	2	2	2	1	2	3	2	1	1.64	
745			1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.26	
760			0	1	0	0	2	0	0	0	0	0	0	0	1	0	1	0	0	2	0	1	1	1	1	1	0	0.46	
775			2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0.24	
790			0	0	0	2	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1	2	2	2	0.52	
805			1	0	1	0	0	1	0	2	0	0	0	0	0	0	0	1	1	0	1	1	2	0	0	0	0	0.44	
820			0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0.16	
835			2	1	2	2	1	1	1	1	1	1	0		0	1	2	4	3	3	2	0	0	5	4	2	4	1.63	
850			3	2	1	2	1	1	2	0	2	0	2	0	1	2	1	0	1	2	1	0	2	2	2	4	0	1.36	
865			1	3	1	0	0	0	1	3	2	1	0	0	0	2	0	1	0	1	1	2	0	3	1	3	4	1.20	
880			0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0.24	
895			1	0	1	2	2	1	0	1	0	0	2	2	2	2	0	5	3	1	2	0	1	0	0	2	2	1.26	
910			2	1	1	1	1	2	2	1	1	1	0	0	0	2	0	2	2	4	0	0	0	4	0	2	2	1.24	
925			1	1	4	0	0	1	0	0	0	0	4	0	0	0	1	0	0	0	1	0	0	2	1	0	0	0.64	
940			2	2	0	0	1	2	2	2	4	4	2	0	1	0	4	3	3	2	1	0	1	1	0	2	1	1.60	
955			0	1	0	4	2	2	0	0	0	2	2	1	2	4	4	4	4	4	4	2	4	2	2	4	2	2.32	
970			2	2	1	1	0	2	1	0	0	1	0	1	1	1	2	2	1	0	0	0	0	1	1	0	1	0.64	
985			0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	1	1	1	0.36	
1000			1	0	0	0	2	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0.24	
1015			0	0	0	0	0	0	0	0	0	0	2	0	2	0	3	0	0	1	0	2	1	0	0	2	1	0.56	
1030			3	4	4	3	1	3	3	4	0	2	3	0	2	0	0	0	0	2	0	2	0	1	2	0	0	1.56	
1045			0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0.24	
1060			2	1	0	0	2	0	0	0	0	1	0	2	0	2	0	2	2	5	2	0	1	0	2	0	1	1.00	

Appendix C: Summary of Blackleg Adult Doubled Haploid Line Scores

PLOT	ENTRY	NAME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean	Ranking
1075			0	0	2	1	0	0	1	2	1	2	1	2	2	2	2	2	0	0	0	1	0	1	1	1	0	0.06	
1080			1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.08	
1105		Westar	2	1	0	0	1	2	1	1	0	1	2	2	2	2	1	0	0	1	2	2	1	2	1	0	1	1.12	
1120			3	2	2	3	3	1	1	0	0	0	1	0	0	1	4	2	0	3	2	0	1	3	2	0	1	1.40	
1135			0	0	1	2	0	0	2	2	1	0	2	1	0	0	0	2	1	2	3	3	2	2	3	2	2	1.32	
1150			1	0	3	1	0	0	1	0	0	0	0	0	0	0	3	2	1	0	1	0	0	0	0	0	0	0.82	
1165			1	1	1	1	0	1	1	1	0	0	1	0	0	1	3	2	4	3	2	1	1	3	3	3	3	1.48	
1180			0	0	2	1	0	0	2	1	2	2	0	1	1	0	1	2	2	1	0	2	2	2	3	3	1	1.24	
1185			2	2	1	3	0	1	2	2	2	1	1	1	1	0	2	2	0	0	2	3	2	2	1	2	2	1.48	
1210			0	0	0	0	0	0	0	1	2	0	0	1	1	1	0	2	2	2	1	1	1	0	1	1	1	0.72	
1225			0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.20	
1240			0	0	0	2	3	3	1	0	1	1	0	0	0	1	0	2	2	2	1	0	0	0	0	0	0	0.76	
1248			2	2	2	1	0	0	1	2	2	2	0	2	1	2	2	2	3	1	2	2	1	2	3	2	2	1.04	
1250			0	0	2	3	2	2	2	2	2	2	2	1	1	2	2	2	1	3	2	3	2	1	1	4	0	1.76	8

R - RESISTANT
MR - MODERATELY RESISTANT
MS - MODERATELY SUSCEPTIBLE
S - SUSCEPTIBLE

Appendix D

Appendix D: Summary of White Rust Doubled Haploid Line Cotyledon Inoculation Scores

[illegible]

Appendix D: Summary of White Rust Doubled Haploid Line Cotyledon Inoculation Scores

UMBL-1 6F																													Mean	Rating												
1	0	0	0	3	3	0	0	0	0	0	0	0	0	1	1	3	1	3	1	1	0.85	R																				
2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
3	7	7	7	NG	NG	9	9	9	7	7	9	9	7	9	9	9	9	9	9	9	8.33	S																				
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
5	9	9	9	NG	NG	9	9	9	9	9	9	9	9	9	9	9	9	9	NG	NG	9.00	S																				
6	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
7	0	0	3	3	NG	0	0	1	1	0	0	0	0	3	3	1	1	1	0	3	1.05	R																				
8	9	9	9	9	NG	9	9	9	9	9	9	9	9	NG	NG	9	9	9	9	9	9.00	S																				
9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
10	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
11	7	7	9	9	9	9	9	7	9	9	9	9	9	9	9	9	9	7	9	9	8.60	S																				
12	1	1	1	0	3	3	3	0	1	1	1	1	1	3	1	1	1	3	3	3	1.60	R																				
13	9	9	9	7	9	9	9	7	7	9	9	9	9	9	7	7	7	7	9	9	8.30	S																				
14	9	9	9	7	9	9	9	9	7	9	9	9	9	9	9	9	9	9	7	7	8.60	S																				
15	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
16	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
17	NG	9	9	9	9	9	9	9	NG	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
18	NG	NG	9	9	9	9	9	9	NG	NG	9	9	9	9	9	9	9	9	9	9	9.00	S																				
19	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
20	NG	NG	7	7	7	7	7	NG	7	7	7	7	7	NG	7	NG	7	7	7	7	7.00	MS																				
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
22	NG	0	0	0	0	0	0	0	0	0	0	0	0	NG	0	0	0	0	0	NG	0.00	R																				
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
27	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S																				
28	7	7	7	9	9	7	9	9	9	9	9	9	9	9	7	7	7	9	7	9	8.20	S																				
29	5	5	9	9	5	9	9	9	7	7	9	9	9	9	9	5	7	9	9	9	7.90	MS																				
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
UMBL-1 7C																																										
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
UMBL-2 2F																																										
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R																				

Appendix D: Summary of White Rust Doubled Haploid Line Cotyledon Inoculation Scores

UMBL-2 2F					
6	0	0	0	NG	NG
7	9	9	9	9	9
8	9	9	9	9	9
9	5	5	5	NG	NG
10	5	5	5	5	5
11	5	5	5	5	5
12	NG	NG	7	9	9
13	9	9	9	9	9
14	0	1	0	5	3
15	1	1	1	NG	NG
16	3	0	0	3	3
17	7	7	7	NG	9
18	5	5	5	5	5
19	NG	5	5	7	5
20	0	0	0	0	0
21	NG	NG	NG	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	NG	NG	NG	NG
25	9	9	9	NG	NG
26	9	9	NG	NG	9
27	0	0	0	0	0
28	9	9	9	9	9
29	9	9	9	9	9
30	0	0	0	0	0
31	0	0	0	0	0
UMBL-2 4H					
1	0	0	0	0	NG
UMBL-2 8B					
1	9	9	9	9	9
2	9	9	9	9	9
3	NG	9	9	9	9
4	9	9	9	NG	9
5	9	9	9	9	9
SYL ACB 2H					
1	0	0	0	0	0
2	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG

Appendix D: Summary of White Rust Doubled Haploid Line Cotyledon Inoculation Scores

SYL ACB 2H																					Mean	Rating
4	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S
5	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R
9	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
10	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
11	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
12	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
13	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
14	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
15	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	R
17	9	9	9	NG	9	9	9	NG	NG	9	9	9	9	NG	NG	9	9	9	9	NG	9.00	S
18	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9.00	S
SYL UM 2A																						
1	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

NG - NO GERMINATION
 R - RESISTANT
 MR - MODERATELY RESISTANT
 MS - MODERATELY SUSCEPTIBLE
 S - SUSCEPTIBLE