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Crown Rust Resistance in Wild Oats: Presence, Inheritance, and Interspecific Gene Transfer to Common Oats

by

Stephen L. Fox

A thesis presented to the
University of Manitoba
in partial fulfillment of the requirements for the degree of
Master of Science
in the
Department of Plant Science

Winnipeg, Manitoba, Canada

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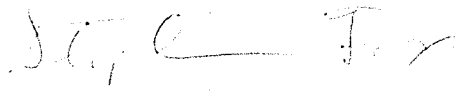

Stephen L. Fox

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In closing, the support of my family has been a mainstay. My only hope is that I can return the strength that they have given me.

Abstract

Fox, S.L. 1989. Crown rust resistance in wild oats: presence, inheritance, and interspecific gene transfer to common oats. University of Manitoba.

There were three objectives of this work:

(1) to find new sources of resistance to crown rust (*Puccinia coronata* Cda.)

(2) to characterize the inheritance of crown rust resistance in thirteen wild oat (*Avena* L.) accessions

(3) to transfer major genes for crown rust resistance from wild oats to common oats (*Avena sativa* L).

One hundred eleven accessions of the Iberian Wild Oat Collection were screened with six test races of crown rust: CR 25, CR 36, CR 50, CR 56, CR 77, and CR 107. Diploid, tetraploid, and hexaploid species were found that conferred resistance to some or all of the test races.

The inheritance of resistance was studied in eight hexaploid accessions using CR 13 and CR 50 as test races. Genetic relationships of resistance in the unknowns to each other and to certain Pc-genes were also investigated. Crown rust resistance genes, temporarily designated H, J and K, and L from IB 2433, IB 1454, and IB 3432 respectively, were identified as being new and independent of the known resistance genes tested with them.

Three tetraploid accessions were crossed and backcrossed twice with Rodney 0. SF 1402, a BC_2F_1 plant of IB 3220 parentage, was found to be highly fertile and resistant to CR 13 and CR 50. Two diploid accessions were crossed with Rodney 0, but no viable seed was obtained.

Autotetraploid plants were developed from the diploids using colchicine as a chromosome doubling agent. The partially sterile autotetraploids were crossed with Rodney 0 producing some normal appearing seeds.

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1.0. Introduction

Oats (*Avena sativa* L.) are an ancient cereal crop which has been cultivated at least since the time of Theophrastus (371-286 B.C.). One of the major diseases of this crop is crown rust (*Puccinia coronata* Cda.). Yield and quality characteristics are adversely affected by this disease. With narrow profit margins at the farm level and high quality demands of specialty markets, providing an effective disease protection strategy for oat production is desirable.

Genetically controlled disease resistance is the most effective, cost efficient, and environmentally friendly method. Crown rust resistance genes have been introduced into *A. sativa* from several related oat species. They have conferred specific resistance to naturally prevalent races of crown rust. The useful lifetime of a resistance gene can be relatively short due to the pathogen's ability to develop new virulence to an introduced resistance gene. Thus, the continued search and introduction of new resistance genes is necessary.

This research project had three main objectives:

(1) screening of part of a wild oat collection for new sources of crown rust resistance,

(2) studying the inheritance of resistance and the genetic relationships between unknown and known resistance genes in hexaploid *Avena*,

(3) attempting to transfer resistance from tetraploid and diploid accessions to *A. sativa*.

2.0. Review of literature

2.1. Oats today

Canada is the fourth largest producer of oats (2,993,000 tonnes in the 1988/89 crop year) after the U.S.S.R., the European Community, and the U.S.A. but is expected to be the world's largest exporter of oats (550,000 tonnes) in the 1988/89 crop year (National Grains Bureau, 1989a and 1989b).

Oats represent about six percent of Canadian domestic grains and oilseeds production (National Grains Bureau, 1989b). About 5-10 percent of the oat crop is used for human consumption (National Grains Bureau, 1989b; Dietz, 1988); the rest is used as seed and livestock feed.

In the last few years, a renewed interest in oats has occurred in the specialty markets of human food and race horse "pony" oats (Dietz, 1988). Both markets require a high quality crop. Oats appear to be a superior source of beta-glucan, a soluable fibre component of oat bran, which lowers serum cholesterol levels (Wilkins 1989). Oats are also the highest of the cereals in the essential amino acid lysine and in lipids (Lasztity *et al.* 1980).

2.2. The origin of oats

Coffman (1961) provides a brief history of the origin and early cultivation of oats. The difficulty in presenting this topic is due to the few writings that exist about oats prior to the seventeenth century. Until modern times, oats were grown mainly for local use and did not play any major

role in commerce. Thus, transactions of oats for other goods were rarely recorded.

The cultivation of oats as a crop is more recent than the cultivation of wheat or barley. The origin of oats is thought to be in Asia Minor (Coffman 1961). Vavilov concluded that the spread of common oats from this center was as a weed contaminant in Emmer wheat. Theophrastus, the ancient Greek philosopher and naturalist (371-286 B.C.), is usually considered the first to mention oats in writings. The beginning of pure oat culture was probably previous to this.

Oats were not indigenous to North America but were introduced when explorers arrived. An expedition in 1602 to New Hampshire and Massachusetts is credited for the introduction of oats into North America (Coffman 1961). Canada's first farmer Louis Herbert may have planted oats at Quebec City in 1607, and the Dutch were known to have cultivated oats in Newfoundland in 1622. Presumeably, the weed species of oats were also introduced at the same time.

The evolution of oats (*Avena sativa* L.) from progenitor species has been pondered by many workers. Coffman (1961) proposed that *A. sativa* arose from *A. byzantina* C. Koch which arose from *A. sterilis* L. *Avena fatua* L. was thought to have arisen from *A. sterilis* also.

2.3. The genus *Avena*

2.3.1. Species in the genus *Avena*

The genus *Avena* is made up of an allopolyploid series consisting of four genomes: A, B, C, and D. The basic chromosome number is seven. Therefore diploids are 2n=2X=14; tetraploids, 2n=4X=28; hexaploids, 2n=6X=42.

Baum (1977) presented twenty-eight taxomomic species (Table 2.3.1), and Ladizinsky (1988) presented fourteen biologic species (Table 2.3.2) to delineate different *Avena* types. Baum's (1977) taxonomic species key was based upon twenty-five morphologic and two cytogenetic features. The biologic species key by Ladizinsky (1988) was based upon fourteen morphologic features and chromosome number. How these species are related, regardless of their nomenclature, is still not clear.

Table 2.3.1. Taxonomic species of *Avena* according to Baum (1977)

species	genome	species	genome
A. *abyssinica*	AABB	A. *macrostachya*	?
A. *atherantha*	AACCDD	A. *maroccana*	AACC
A. *barbata*	AABB	A. *matritensis*	?
A. *brevis*	AA	A. *murphyi*	AACC
A. *canariensis*	AA	A. *nuda*	AA
A. *clauda*	CC	A. *occidentalis*	AACCDD
A. *damascena*	A_dA_d	A. *sativa*	AACCDD
A. *eriantha*	C_pC_p	A. *sativa fatuoid*	AACCDD
A. *fatua*	AACCDD	A. *sterilis*	AACCDD
A. *hirtula*	A_pA_p	A. *strigosa*	A_sA_s
A. *hispanica*	AA *	A. *trichophylla*	AACCDD
A. *hybrida*	AACCDD	A. *vaviloviana*	AABB
A. *longiglumis*	A_lA_l	A. *ventricosa*	C_vC_v
A. *lusitanica*	A_sA_s *	A. *wiestii*	A_sA_s/AABB

* presumably

The biologic species key groups some of the taxonomic species together under one name; however, variation in genomic structure between taxonomic species exists within

these groups. For example, the biologic species *A. clauda* Dur. contains two taxonomic species: *A. clauda* and *A. eriantha* Dur.

Table 2.3.2. Biologic species of *Avena* according to Ladizinsky (1988)

Biological species	2n	taxonomic species they include
A. atlantica	14	*A. atlantica*
A. canariensis	14	*A. canariensis*
A. clauda	14	*A. clauda, A. eriantha*
A. damascena	14	*A. damascena*
A. longiglumis	14	*A. longiglumes*
A. prostrata	14	*A. prostrata*
A. strigosa	14	*A. strigosa, A. brevis, A. hirtula, A. wiestii*
A. ventricosa	14	*A. ventricosa*
A. agadiriana	28	*A. agadiriana*
A. barbata	28	*A. barbata, A. abyssinica, A. vaviloviana*
A. macrostachya	28	*A. macrostachya*
A. magna	28	*A magna*
A. murphyi	28	*A. murphyi*
A. sativa	42	*A. sativa, A. sterilis, A. fatua*

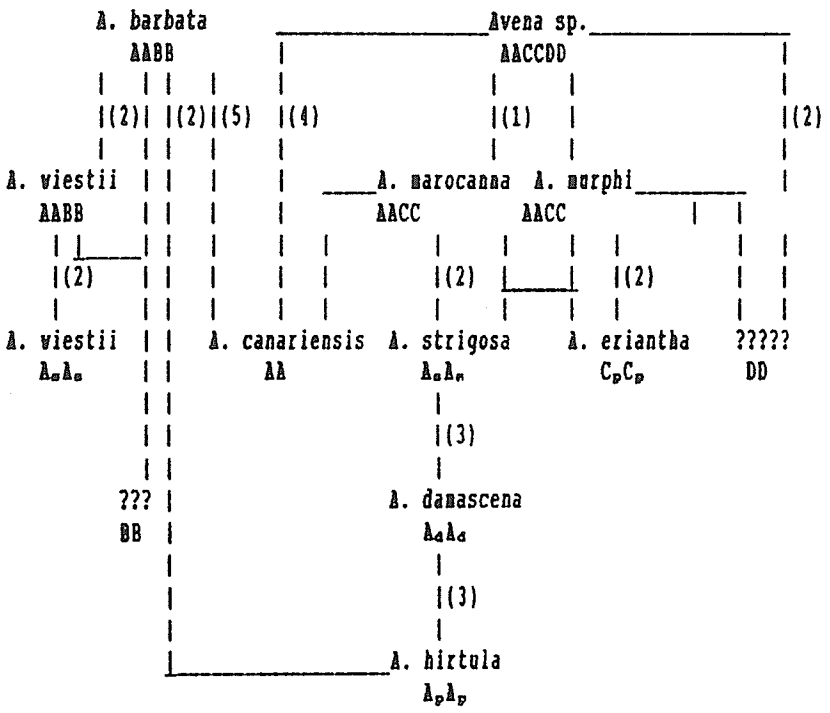
2.3.2. Possible genomic relationships

Thomas and Bhatti (1975) suggested that hexaploid oats probably originated from a natural hybrid between a tetraploid and a diploid followed by spontaneous doubling of chromosome number. So far , only tentative associations have been put forward (Figure 2.3.1).

Rajhathy and Thomas (1974) indicated that the identity of the diploid and tetraploid progenitors of hexaploid oats has not been established. The origin of the B and D genomes is still unknown. *A. barbata* Pott ex Link appears to be related to *A. hirtula* Lag. and *A. wiestii* Steud. morphologically, but the fertility of hybrids between them is low (Rajhathy and Thomas 1974). The low fertility is probably due to the triploid nature of hybrids. *A. barbata*

does not appear to be involved in the evolution of hexaploid oats (Leggett, 1980). A further discussion regarding the progenitor species of tetraploid oats is presented by in Fominaya *et al.* (1988).

Figure 2.3.1. Tentative genomic relationships in Avena



1. Thomas and Bhatti 1975
2. Rajhathy and Thomas 1974
3. Cahana and Ladizinsky 1978
4. Leggett 1980
5. Baum 1977

Another confusing problem is the placement of *A. canariensis* Baum in relation to other species. It is morphologically similar to *A. maroccana* Gdgr. and *A. sterilis* (Rajhathy and Thomas 1974). Cytologically, *A. canariensis* is not close to *A. maroccana* (Leggett 1980) as only partial homology between genomes has been shown. The question that arises is to what extent can the cytology of

the cell be used to categorize material in the face of obvious morphological similarities?

The relationships between *A. strigosa* Schreb., *A. damascena* Rajhathy et Baum, and *A. hirtula* has to do with the number of translocations each genome has accumulated (Cahana and Ladizinsky 1978). *A. damascena* has two translocations that differentiate it from *A. hirtula*, and *A. strigosa* has three more translocations that differentiate it from *A. damascena*.

The wild hexaploid species have the same basic chromosome structure as *A. sativa* with only minor structural differences (Thomas and Bhatti 1975). Hybrids between the different hexaploid species are usually fertile.

The relationships between many of the oat species has been hard to determine based on genome compatibility. It would appear that the oat genus has diversified to the extent that genetic differences between species may preclude determining what the relationships are.

2.4. *Puccinia coronata*

2.4.1. Taxonomic classification of *P. coronata*

The taxonomic classification of *Puccinia* spp. was presented by Littlefield (1981) as follows:

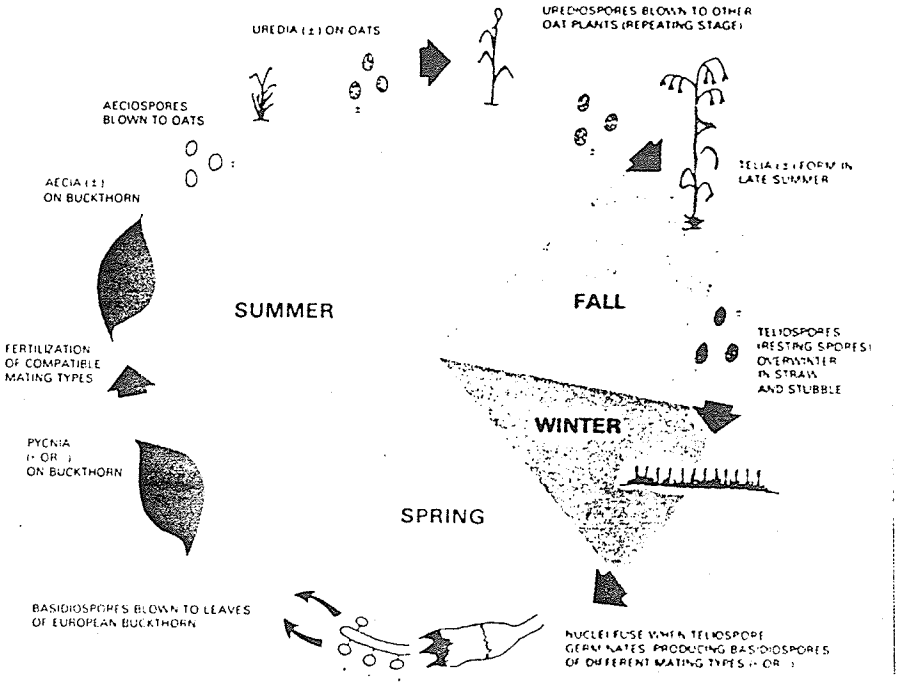
subdivision: Basidiomycotina
class: Basidiomycetes
subclass: Teliomycetidae
order: Uredinales
family: Pucciniaceae
genus: Puccinia

The species *coronata* has been divided into about sixteen *formae speciales* based on the host from which they were isolated (Eshed and Dinoor 1980). Work by Eshed and Dinoor (1980) showed that the host ranges of isolates collected from different host species overlapped extensively. They concluded that forms and races were essentially the same and should not be distinguished in the taxonomy of crown rust. As a result, it was felt that it was misleading to give *P. coronata* a formae specialis designation. Anikster (1984) noted, however, that the host range of the rusts appeared to be greater on artificially inoculated plants in the greenhouse or growth cabinet. Thus paralleling form and race for *P. coronata* may be a very conservative view for this pathogen.

2.4.2. Life cycle of *P. coronata*

Puccinia coronata is a heteroecious rust fungus whose main economic host is oats and alternate host is common buckthorn, *Rhamnus cathartica* L. It is an obligate parasite or biotroph: the fungus depends on living cells of a host plant for its nutrients. The chromosome number of this pathogen is unknown and there are few genetic markers available for genetic studies (Day 1974). A simplified diagram of the crown rust pathogen life cycle is presented by Martens *et al.* (1985) in Figure 2.4.1.

Figure 2.4.1. Life cycle of crown rust from Martens et al. (1985)



The thick-walled teliospore is the dormant overwintering structure of the pathogen. In the spring, a basidium forms when the two nuclei fuse forming a temporary diploid cell which then undergoes meiosis to form four basidiospores: two of the (+) mating type and two of the (-) mating type.

Basidiospores, which are attached to the basidium by sterigmata, are released and must land on the alternate host. With leaves wet with dew or light rain, a spore can germinate forming a germ tube which will terminate with the formation of an appressorium. The appressorium develops a penetration peg which can enter the host leaf at any point. Successful penetration results in the development of haploid mycelium in the area of the penetration peg. From this haploid thallus develops the pycnia.

Pycnia contain the male pycniospores and the female flexuous hyphae. The male and female components are generally not compatible within the same pycnium as they are of the same mating type. Pycniospores must be moved to a pycnia of opposite mating type by insects, rain splash, or leaf movement. Once a pycniospore successfully penetrates the flexuous haphae, the pycniospore nucleus migrates to the aecial primordia where the dikaryotic nature of the fungus is restored, and aecia are produced (Littlefield 1981).

Aeciospores are released from the buckthorn and will infect only the *Avena* host. Successful entry through a stomate is followed by the formation of a dikaryotic thallus

from which uredia form. Uredia give rise to urediospores which can reinfect the main host. When conditions become unfavourable for further development of new uredia, telia are formed to complete the life cycle.

In the prairie rust region, the greatest source of crown rust inoculum comes from urediospores blown in from the United States. Urediospores overwinter in the southern U.S. and migrate north on successively planted oat fields. The alternate host was introduced for use in hedges on the prairies and is found mainly in towns and around farm yards. As a result, the effects of the fungus' sexual cycle on the prairie crown rust population are minimal since the basidiospores from germinating telia usually have no alternate host to land on.

2.4.3. Sources of new pathogen virulence

P. coronata is heterokaryotic: each cell carries two genetically different nuclei in a common cytoplasm. This fungus has a great ability to develop new virulence combinations using several methods to recombine and create new genetic material.

Normal hybridization during the sexual cycle of *P. coronata* does not develop new virulence genes but recombines them such that new combinations of virulence genes can pressure host resistance sources.

Hyphal anastomosis is a process where dikaryotic haphae of different genotypes exchange nuclei (Day 1974). A

recombination of nuclei is possible without the fungus going through the sexual part of its life cycle.

The parasexual cycle involves the fusion of unlike nuclei to form a diploid. Crossing over in prophase I during meiosis results in new genetic recombinants (Agrios 1978).

Mutations are spontaneous heritable changes in the genetic material of an organism (Agrios 1978). Work by Bartos *et al.* (1969) suggested that mutation may be an important source of variation in crown rust. Mutations are the source of new virulence genes whereas the above mechanisms are ways in which virulence genes can be recombined.

2.4.4. Manipulation and storage of urediospores

Plant pathologists generally work with the urediospore stage of the fungus except when genetic studies of the pathogen are being done. Day (1974) describes urediospores as essentially binucleate conidia that provide a means of clonal propagation for the dikaryon.

Physiologic races of *P. coronata* are maintained in the urediospore stage for two reasons. A desired culture can be easily increased on susceptible oat lines, and the genetics of a particular culture are relatively stable since meiosis is avoided. A physiologic race may appear to become contaminated, however, if a mutation should occur. The clonal nature of the urediospore stage is very useful in genetic studies of the pathogen because heterozygous genotypes can be maintained.

There are several ways to store urediospores: vaccuum dried, ultralow temperature storage, and storage in liquid nitrogen (Rowell 1984). Sharp and Smith (1952) have shown that *P. coronata* urediospores store well and do not lose or change their pathogenicity with vacuum drying. Spores are dried for 2 to 3 hours under a vacuum of 10 to 250 micrometers of mercury. This process reduces spore moisture to about 2 percent. The spores are stored in sealed glass vials at 4 C to maintain viability. To use, spores must be rehydrated gradually so that the semipermeability of the spore plasmalemma is not disrupted. Rehydration is achieved by opening the tubes 16 to 24 hours ahead of use and placing the tubes in a sealed chamber with a relative humidity of 80 to 90 percent. Ultra low temperature freezing is perhaps the most convenient method of storage although the long term viability of spores is not known. Spores are frozen directly in sealed glass tubes at temperatures ranging from -55 to -85 C. To use these spores, they must be heat shocked by placing the sealed tubes in a water bath at 40-45 C for five minutes. Cryogenic storage of spores in liquid nitrogen at -196 C is very good for long term storage. Again, spores are prepared for use by heat shock, or by slow rehydration. This system is costly, somewhat hazardous to use, inconvenient for everyday use, and inconvenient for storing large quantities of spores that are required for initiating nursery epidemics.

2.5. A History of Pc-genes

The isolation and characterization of crown rust resistance genes in *Avena* has been carried out by scientists since the 1920s and will continue until a more economical method of crown rust control is found. Parker (1920) was one of the earliest workers to investigate crown rust resistance in oats. He recognized the importance of determining the inheritance of rust resistance to plant breeding and that the possible linkage of crown rust resistance with undesirable traits could be a hinderance to crop improvement. He was able to show that resistance was transferable from a resistant plant to a susceptible plant and that the seedling and adult plant rust reactions were similar. He also concluded that

> rust resistance and susceptibility hardly can be considered as simple characters or as being determined by a single factor difference (Parker 1920).

It was likely that the rust cultures Parker (1920) used to visualize the segregation of resistance and susceptibility were not of one physiologic race since physiologic race specialization had only been demonstated by Hoerner (1919) one year previous to Parker's work. It was noted by Parker (1920) that the resistant parent Burt in the Burt/60 Day cross was not homozygous for resistance; although, it appeared so for other agronomic traits. These two factors would have complicated the results making it difficult to show simple inheritance for crown rust resistance.

It was not until 1927 that the first example of a single gene for crown rust resistance was published (Simons *et al.* 1978).

Crown rust resistance genes have been found in four main sources: *Avena byzantina*, *A. sativa*, *A. sterilis*, and lower ploidy material - mainly *A. strigosa*. For convenience, the gene designations agreed upon by Simons *et al.* (1978) will be used throughout this discussion. Table 2.5.1 lists the resistance genes, their sources, and their relationships to each other.

Table 2.5.1. Pc-gene relationships

Gene	ref	source of gene	known relationships
Pc-1	1	Red Rustproof	
Pc-2	2,3	Victoria	linked to Hv-1
Pc-3	4	Bond	independent (IND) of Pc-2
Pc-4	4	Bond	IND of Pc-2,3
			complementary (COM) to Pc-3
Pc-5	5	Landhafer	IND of Pc-3,4
	6		IND of Pc-2
Pc-6	7	Sante Fe	IND of Pc-2,7,8
Pc-7	7	Sante Fe	IND of Pc-2,8
Pc-8	7,8	Sante Fe	IND of Pc-2, one of Sante Fe
			genes possibly allelic to Bond
Pc-3c	9	Ukraine	
Pc-4c	9	Ukraine	COM to Pc-3c, IND Pc-3c
Pc-6c	8	Ukraine	linked to Pc-9
Pc-6d	8	Trispernia	at least one allelic pair with
			Ukraine and Sante Fe
			IND of Pc-5
Pc-9	8	Ukraine	conflict with Weetman (1942)
			IND, Pc-2,5, at least one
			allelic pair with Sante Fe
Pc-10	8	Klein 69B	IND of Pc-2,5,6,6d,7,8
Pc-11	10	Victoria	IND of Pc-2
Pc-12	10	Victoria	IND of Pc-2,11
			Pc-11 epistatic to Pc-12
			Pc-12 epistatic to Pc-2
Pc-14	11	Ascencao	Ascencao also has Pc-2
			Pc-14 epistatic to Pc-2
			IND of Pc-2
Pc-15	12	*A. strigosa*	
Pc-16	12	*A. strigosa*	
Pc-17	12	*A. strigosa*	
Pc-18	13	*A. glabrota*	
Pc-19	13	*A. strigosa*	
Pc-20	13	*A. abysinica*	
Pc-22	14	Ceirch du Bach	hexaploid
Pc-23	15	*A. strigosa*	IND of Pc-15

[1]Dietz and Murphy 1930
[2]Murphy and Meehan 1946
[3]Litzenberger, 1949
[4]Cochran et al. 1945
[5]Kehr and Hayes 1950
[6]Rivers 1959
[7]Osler and Hayes 1953
[8]Finkner 1954
[9]Weetman 1942
[10]Welsh *et al.* 1954
[11]Simons 1956
[12]Murphy *et al.*1958
[13]Simons *et al.* 1959
[14]McKenzie 1961
[15]Dyck and Zillinsky 1963a

Table 2.5.1. Pc-gene relationships (continued)

Gene	ref	source of gene	known relationships
Pc-29	16	*A. glabrota*	
Pc-30	16	*A. strigosa*	
Pc-31	16	*A. strigosa* var *typica*	
Pc-32	16	Ceirch Llwyd	diploid
Pc-33	16	Ceirch Llwyd	diploid
Pc-34	17	*A. sterilis*	
Pc-35	17	*A. sterilis*	IND of Pc-34
Pc-37	18	*A. strigosa*	
Pc-38	19,22	*A. sterilis*	IND of Pc-35
Pc-39	19,22	*A. sterilis*	IND of Pc-35,38
Pc-40	19	*A. sterilis*	IND of Pc-38,39
Pc-41	19	*A. sterilis*	IND of Pc-40
Pc-42	19	*A. sterilis*	IND of Pc-40,41
Pc-43	19	*A. sterilis*	IND of Pc-40,41,42
Pc-44	20	Kyto	linked in repulsion to pg-9
Pc-45	21,22	*A. sterilis*	IND of Pc-35,38,39
Pc-46	21,22	*A. sterilis*	IND of Pc-35,38,39,45
Pc-47	22	*A. sterilis*	IND of Pc-35,38,39,45,46,
Pc-48	22	*A. sterilis*	IND of Pc-35,38,39,45,46,47
Pc-49	22	*A. sterilis*	IND of Pc-35,38,39,45,46,47,48
Pc-50	22	*A. sterilis*	IND of Pc-35,38,39,45,47,48,49
			linked or allelic to Pc-46
Pc-54	23	*A. sterilis*	linked or allelic to Pc-35
Pc-55	24	*A. sterilis*	linked or allelic to Pc-39
Pc-56	24	*A. sterilis*	IND of Pc-35,38,39,40,45,46,47
			and Pc-48,50,55
Pc-62	25	*A. sterilis*	linked or allelic to Pc-38
			IND of Pc-35,39,40,45,46,47,48
			and Pc-50,55,56
Pc-63	25	*A. sterilis*	linked or allelic to Pc-38,62
			IND of Pc-35,39,40,45,46,47,48
			and Pc-50,55,56
Pc-64	26	*A. sterilis*	IND of Pc-35,38,39,40,45,46,48
			and Pc-56
Pc-65	26	*A. sterilis*	IND of Pc-35,38,39,40,45,46,48
			and Pc-55,56,64
Pc-66	26	*A. sterilis*	IND of Pc-35,38,39,40,45,46,48
			and Pc-56,65,66
Pc-67	26	*A. sterilis*	IND of Pc-35,38,39,40,45,46,48
			and Pc-56
Pc-68	26	*A. sterilis*	linked or allelic to Pc-46,50
			IND of Pc-35,38,39,40,45,48,56

[16]Marshall and Myers 1961
[17]McKenzie and Fleischmann 1964
[18]Dyck, 1966
[19]Fleishmann and McKenzie 1968
[20]Martens *et al.* 1968
[21]Fleischmann *et al.* 1971a
[22]Fleischmann *et al.* 1971b
[23]Martens *et al.* 1980
[24]Kiehn *et al.* 1976
[25]Harder *et al* 1980
[26]Wong *et al.* 1983

2.5.1. Crown rust resistance genes from A. *byzantina*

The first early successes in finding crown rust resistance genes were from A. *byzantina* introductions. Pc-1, a dominant gene, was isolated from the cultivar Red Rustproof (Dietz and Murphy 1930; Welsh *et al.* 1953). This material may have originated in the Mediterranean, Mexico, or Georgia, U.S.A. (Coffman *et al.* 1961).

Victoria, an introduction from Uruguay, was found to contain three genes: Pc-2 (Litzenberger 1949), Pc-11 and Pc-12 (Welsh *et al.* 1954). Pc-2, and Pc-11 were dominant for resistance; Pc-12 was recessive for resistance. Welsh *et al.* (1954) showed that Pc-11 was epistatic to Pc-12, and Pc-12 was epistatic to Pc-2. The cultivars Rodney and Garry (reselected) carried Pc-11 and/or Pc-12. Simons (1956) identified two genes in the variety Ascencao: one was determined to be Pc-2 while the second, Pc-14, was epistatic to Pc-2. Whether Pc-14 was independent of Pc-11 and Pc-12 was not determined. One may speculate that Pc-14 is Pc-11 or Pc-12 because of its similar epistatic effects on Pc-2.

Bond, a Red Algerian/Gold Rain cross from Australia (Welsh *et al.* 1953), contained two dominant complementary genes for crown rust resistance: Pc-3 and Pc-4 (Cochran *et al.* 1945). Both genes had to be present in their dominant form for resistance to be expressed. Bond is the classical example of complementary gene action for disease resistance.

Pc-5 was a gene found in Landhafer. Landhafer was an introduction from Germany, but it probably originated in

South America (Welsh *et al.* 1953; Coffman *et al.* 1961). Pc-5 was a single dominant gene (Kehr and Hayes 1950; Finkner 1954; Rivers 1959).

Another South American introduction was Sante Fe which was the source of three crown rust resistance genes. Two of the genes were dominant and complementary to each other while the third was dominant and independent of the other two (Osler and Hayes 1953). The first two genes were designated Pc-7 and Pc-8; the latter gene, Pc-6 (Simons *et al.* 1978).

Klein was an *A. byzantina* introduction from Argentina which came to North America from Australia (Welsh *et al.* 1953). Klein 69b was found to contain Pc-10 (Finkner 1954).

2.5.2. Crown rust resistance genes from *A. sativa*

It would appear that *A. sativa* contains relatively few naturally occurring resistance genes useful against *P. coronata*.

Ukraine (Mutica), an introduction from Russia, contained two dominant complementary genes , Pc-3c and Pc-4c, (Weetman 1942). Finkner (1954) showed that two closely linked alleles, Pc-6c and Pc-9, conferred resistance in Ukraine. But later work by Sanderson (1960) showed that Ukraine resistance was due to a single dominant gene Pc-9. Discrepancies between workers often occurred with work involving introduced material because the material was not homogeneous for rust resistance. As a result, a number of resistance genes may have been present in the introduction.

Trispernia, an introduction from Rumania (Welsh *et al.* 1953), contained three genes for resistance (Finkner, 1954), one of which was Pc-6d (Simons *et al.* 1978).

Pc-22 was isolated from a Welsh variety Ceirch du Bach by McKenzie (1961). It would appear that that this material was a hexaploid; although, the species of oat involved was not clear.

Kyto oats was found to contain the dominant gene Pc-44 (Martens *et al.* 1968).

2.5.3. Crown rust resistance genes from *A. sterilis*

A. sterilis has been the richest source of crown rust resistance genes: Pc-34 , Pc-35 (McKenzie and Fleischmann 1964), Pc-38, Pc-39, Pc-40, Pc-41, Pc-42, Pc-43 (Fleischmann and McKenzie 1968), Pc45, Pc-46 (Fleischmann *et al.* 1971a), Pc-47, Pc-48, Pc-49, Pc-50 (Fleishchmann *et al.* 1971b), Pc-54 (Martens *et al.* 1980), Pc-55, Pc-56 (Kiehn *et al.* 1976), Pc-62, Pc-63 (Harder *et al.* 1980), Pc-64, Pc-65, Pc-66, Pc-67, Pc-68 (Wong *et al.* 1983). Unpublished crown rust resistance genes have been named up to Pc-74. Pc-38 and Pc-39 were used in combination for the current rust resistant oat cultivars Dumont, Riel, and Robert.

Most of the *A. sterilis* genes for crown rust resistance were dominant or incompletely dominant in their intra allelic interactions, but there were exceptions. Pc-54 was shown to be an incompletely recessive gene (Martens *et al.* 1980). Harder *et al.* (1980) noted from a personal communication from Dr. M.E. McDaniel that the dominance

behavior of Pc-45 varies with its host background. This could also be true for other Pc-genes.

2.5.4. Crown rust resistance genes from diploid and tetraploid oats

Diploid wild oats have been another source of crown rust resistance; although, transfer of this resistance has been only marginally successful. Pc-15, Pc-16, Pc17 were dominant genes recognized in *Avena strigosa* by Murphy *et al.* (1958). Pc-18 and Pc-19 (*A. strigosa*) were identified by Simons *et al.* (1959); Pc-23 (*A. strigosa*), by Dyck and Zillinsky (1963a); Pc-29, Pc-30 (*A. strigosa*), Pc-31 (*A. strigosa* var *typica*), Pc-32, Pc-33, by Marshall and Myers (1961); Pc-37 (*A. strigosa*), by Dyck (1966). Of these genes, only Pc-23 has been incorporated into a stable *A. sativa* background (Dyck and Zillinsky 1963a).

Pc-20 appears to be the only crown rust resistance gene that has been characterized and named from tetraploid oats (Marshall and Myers, 1961). No crown rust resistance genes appear to have been transferred from tetraploid sources into an *A. sativa* genetic background.

2.5.5. Pc-gene associations

There are five linkage groups that have been identified. Sante Fe, Ukraine, and Trispernia have exhibited allelism or close linkage for at least one gene (Finkner 1954). Pc-46, Pc-50, and Pc-68 are also associated (Wong *et al.* 1983). Pc-35 and Pc-54 are linked or allelic (Martens *et*

al. 1980). Similarly, Pc-39 and Pc-55 are associated (Kiehn et al. 1976) as is Pc-38, Pc-62 and Pc-63 (Harder et al. 1980).

Crown rust resistance genes have also been shown to be linked to genes which control other traits.

The Victoria type of resistance (Pc-2) and *Helminthosporium victoriae* susceptibility (Hv-1) have been shown to be closely associated. Cultivars containing the Victoria type of resistance were found to be susceptible to this blight. It was found that resistance conferred by Pc-2 and susceptibility to this blight was due either to two tightly linked genes in coupling (Murphy and Meehan 1946) or to a single gene exhibiting pleiotropy (Litzenberger, 1949). This was an important association since 75% of the 1945 U.S. oat acreage contained the Victoria type of resistance, and widespread attack by *H. victoriae* resulted in large yield losses (Litzenberger 1949).

Martens *et al.* (1968) suggested that there were three alleles for crown rust resistance and two alleles for stem rust resistance at or near the Pc-44 locus. Martens *et al.* showed that Pc-44 was linked in repulsion to pg-9. Pg-3 and pg-9 have been shown to be linked also (Simons *et al.* 1978). McKenzie *et al.* (1965) determined that pg-9 was associated with crown rust resistance in Ukraine oats. Ukraine was shown to have close linkage or allelism with crown rust resistance genes in Sante Fe and Trispernia (Finkner 1954).

Most studies that investigated possible linkages between crown rust resistance and other characters indicated independence. Torrie (1939) and Cochran *et al.* (1945) showed that loose smut, covered smut, stem rust, maturity, basal articulation, and basal hair length were independent of the factors conditioning Bond type resistance. Some association between crown rust reaction and heading date was detected (Torrie 1939). Landhafer type resistance was found to be inherited independently of genes for stem rust resistance, spikelet disarticulation, floret disjunction, and basal hair developement (Kehr and Hayes 1950). The genes conferring resistance in Sante Fe were shown to be independent of stem rust reaction, date of heading, number of basal hairs, length of basal hairs, percent florets awned, awn length, and kernel plumpness (Osler and Hayes 1953). The lack of association with heading date conflicted with Torrie's earlier findings. Later studies by Kiehn *et al.* (1976) showed that Pc-55 and Pc-56 were independent of genes governing seed color and awn type, and Wong *et al.* (1983) found no association between crown rust genes Pc-64, Pc-65, Pc-66, Pc-67, Pc-68 and genes for awns, lemma colour, and lemma pubescence.

2.6. Specific disease resistance in cereals

2.6.1. Terminology of specific disease resistance in cereals

aegricorpus:
the living manifestation of the genetic interaction in and between pathogen and host (Loegering 1984)

biotype:

a population of individuals of the same genotype such as the progeny of a single aeciospore or urediospore (Roelfs 1984)

Note that a race is not a biotype.

disease:

a biological process that damages plants (Browder 1985) It is a departure from normal metabolism, reducing the normal potentiality for growth and reproduction of the host, caused by the presence of the pathogen (Day 1974).

parasite:

an organism that lives in or on another organism from which it obtains nutrient (Day 1974)

pathogen:

a parasite which produces disease in its host (Day 1974)

pathogenicity:

describes the pathogen that can successfully invade a host (Day 1974)

the capacity of one organism to parasitize a species of another organism and cause disease (Dinoor *et al.* 1988) Dinoor *et al.* (1988) distinguishes three levels of genetic control for pathogenicity: host species, host cultivar, and efficiency of infection. The various species that a particular pathogen can attack defines the pathogenicity of the pathogen. In the case of *Puccinia graminis*, there are three physiologic forms: each attacks one species of cereal. Although, these are morphologically similar, they are distinguished into formae specialis *tritici*, f. sp. *secalis*, and f. sp. *avenae*. When determining the physiologic form of a pathogen, it is important to inoculate it to host genotypes that lack any known resistance genes. This helps avoid resistant or immune reaction phenotypes that would lead to misclassification of the pathogen's form. Within a host species where the pathogen exhibits differential reactions to different resistance genes, the pathogenicity of a pathogen is narrowed further into physiologic races. At this level, the terms avirulence and virulence are used to describe the activity of the pathogen on resistant and susceptible plants respectively. The term aggressiveness is used where the efficiency of infection between races to attack a particular host genotype varies.

physiologic race:

represents a collection of fungal biotypes with the same avirulence/virulence formula which is based ideally on a set of resistant lines containing different resistance genes

Race should be distinguished from the terms isolate or culture, however. The isolate or culture represents a specific genotype of the fungus while the race

represents any group of isolates or cultures that may have in common only their avirulence/virulence formula.

resistance:
any genetically determined characteristic of a host plant that in any way limits the damage produced by disease (Browder 1985)

susceptible:
describes a host which can be successfully invaded by the pathogen (Day 1974)

2.6.2. Defenses against disease

Wahl *et al.* (1984) lists four types of defense against crown rust infection: conventional resistance, slow rusting, tolerance, and escape. Dyck and Kerber (1984) break down conventional resistance into four types: hypersensivity, immunity, moderate, and adult. Hypersensitivity is observed as the collapse and death of host cells at the infection site. This will appear as chlorotic or necrotic lesions. Immunity is one form of hypersensitive reaction where a lesion fails to form. This is a symptomless phenotype (Day 1974). Moderate hypersensitivity does not prevent colonizations by the pathogen, but it does restrict its spread. Adult plant resistance is manifested at the adult plant stage. Adult plant resistance genes are poorly or not expressed in the seedling growth stage. Slow rusting (Hooker 1967) describes a plant which exhibits slow development of rust although the final infection would appear equal to that of a fully susceptible plant. Tolerance is defined by Simons (1969) as the ability of a variety to show signs and symptoms similar to those on a susceptible variety, but the tolerant variety is affected less by the infection than is the susceptible variety. Slow rusting and tolerance are

distinguished from each other since the former type of resistance slows down the development of an epidemic while the latter does not. Escape describes susceptible plants that fail to become infected with rust either by being planted in areas not subjected to sources of inoculum or due to very dry conditions which do not promote spore germination. Early maturity is a method of escape also (Hooker 1967).

2.6.3. The gene-for-gene concept

Flor (1971) presented a definition from Person *et al.* (1962) which he felt best described the gene-for-gene concept:

> a gene-for-gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized.

Flor (1956) was the first to propose the gene-for-gene concept as a result of his work on the *Linum usitatissimum* L.:*Melampsora lini* host:parasite system. This concept has been shown to hold in a number of host:pathogen systems. Only recently has the gene-for-gene relationship been shown in the oat:crown rust system (Dinoor *et al.* 1988).

Two oat lines and two cultures of crown rust which reacted differentially on the hosts (Table 2.6.1) were crossed to generate a F_2 population for plant and pathogen.

Table 2.6.1. Host:pathogen differential reactions

	P_1	P_2
H_1	+	-
H_2	-	+

The host F_2 population segregated in a 3 resistant:1 susceptible ratio when P_1 or P_2 races were applied on first and second leaves of the same plants. When the reactions of both races were considered, a good fit to a 9 R/R : 3 R/S : 3 S/R : 1 S/S was obtained. The pathogen F_2 segregated 3 avirulent:1 virulent when inoculated on H_1 and segregated 13 avirulent: 3 virulent when inoculated on H_2. Looking at the combined set of inoculations, a 39 A/A:9 A/V: 13 V/A: 3 V/V ratio was obtained. Dinoor *et al.* (1988) put representative cultures from the F_2 of the pathogen cross onto representative genotypes from the F_2 of the host cross. Table 2.6.2 displays their results which supports the gene-for-gene hypothesis. In all cases, a resistance reaction was obtained only when resistance genes and avirulence genes were matched.

Table 2.6.2. F_2 host:F_2 pathogen genotype interactions

F_2 host / F_2 pathogen	9 R/R	3 R/S	3 S/R	1 S/S
39 A/A	R	R	R	S
9 A/V	R	R	S	S
13 V/A	R	S	R	S
3 V/V	S	S	S	S

summary of data by Dinoor *et al.* (1988)

The work by Dinoor *et al.* (1988) supports the view by Browder (1985) that resistance is the active phenomenon where specific genes for resistance in the host interact with matching genes for avirulence in the pathogen leading to a low infection type. This implies that if a resistance

gene produces a gene product, then the matching avirulence gene in the pathogen must do the same.

Resistance genes are defined by their phenotypic effects and genotypic properties (Ellis *et al.* 1988). They have not been identified specifically since gene products associated with host plant resistance have not been identified. Ellis *et al.* (1988) indicated that it is not known whether resistance in a plant is constitutive or whether it is induced by pathogen attack.

3.0. Materials

3.1. Source and selection of materials

All of the wild oat accessions involved in this project were part of the Iberian Wild Oat Collection made by Dr. J.W. Martens and others during the late 1970s. Accessions were collected from four countries: Spain, Portugal, Morocco, and the Canary Islands. A preliminary screening of many of the accessions (Chong, unpublished) using crown rust races CR 25, CR 36, CR 50, CR 56, CR 77, and CR 107 revealed some accessions that have good crown rust resistance. One hundred and eleven accessions that lacked rust reaction data for some or all of the six test races were screened using the required test races. Thirteen accessions were selected for use in genetic studies based on diverse geographic source (Table 3.1.1, Figure 3.1.1), chromosome number (Table 3.1.1),and good resistance to crown rust (Table 3.1.2).

Table 3.1.1. Geographic source and chromosome number of resistant accessions

Accession number	Geographic source	Chromosome number
IB 1454	Marrakech, Morocco	42
IB 1487	Kasba Tadla, Morocco	42
IB 2402	Aljezur, Portugal	42
IB 2428	Figueira, Portugal	42
IB 2433	Almansil, Portugal	42
IB 2434	Figueira, Portugal	42
IB 2465	Tunes, Portugal	42
IB 3432	Vejer de la Frontera, Spain	42
IB 691	Santa Cruz de la Palma, La Palma	28
IB 845	Valle de Guerra, Teneriffe	28
IB 3220	Santa Olalla, Spain	28
IB 2353	Rio Maior, Portugal	14
IB 2404	Odeceixe, Portugal	14

Figure 3.1.1. Map of Iberian Wild Oat Collection area (after Grosvenor 1975)

Table 3.1.2. Infection types[a] produced on seedlings or adults of the selected accessions by 15 races of *Puccinia coronata*

Collection number	Race of rust applied														
	CR11	CR12	CR13	CR25	CR36	CR50	CR56	CR77	CR103	CR107	CR120	CR123	CR146	CR160	CR169
IB 1454	2,3	;,1	0,;	0	;	;	;	0	0?	;	;,2	2	2	1	4
IB 1487	1	1,2	1	0	;	;	;	0	1	;	2,3	1	2,3	1	1
IB 2402	0	0	0,;-	0	0	0	0	0	0	0	0	0	0	0	0
IB 2428	1	1-	1-	;	0	0	0	;	0?	0	;,1+	;,1	1-	;,1	;
IB 2433	;	;1-	1	;-	0	0	;	;	0?	;	;,1	;-	1-	;	;-
IB 2434	1	1	1-	;	;	;	;	;	0?	;	1	;	;	;	;
IB 2465	0	0	0	;-	0	0	0	0	0?	0	0,;-	0	0	0	0
IB 3432			0,;	;	;-	;	;,1	;	0,;						
IB 691				;	;	0	;	3	0						
IB 845			4	4	0	0,;	0	;	;-						
IB 3220			;	;	0	;	;	1-	;						
IB 2353				0	0	;-	;	0	;-						
IB 2404				0	0	;	;	4	;-						

Rusts applied on first leaf: CR 11, CR 12, CR 13, CR 56, CR 107 (Chong, unpublished)
Rusts applied on second leaf: CR 36, CR 50, CR 123, CR 146, CR 169
Rusts applied on flag leaf: CR 25, CR 77, CR 103, CR 120, CR 160
[a]See Table 4.4.2 for the explanation of infection types.

To conduct inheritance studies on the resistant accessions, additional lines were selected that were susceptible to CR 13 and CR 50 (Table 3.1.3). In a cross between resistant and susceptible parents, the resistance exhibited in a segregating F_2 population was attributed to the resistant parent.

The pedigree of Rodney 0 is Rodney*5/Exeter, and it is an *Avena sativa* L. species.

IB 1000-128 was originally selected based on previous work which showed that it had a chromosome number of 28. A

check of this revealed that it had 42 chromosomes. Hybrids with the tetraploid accessions yielded only sterile plants.

Table 3.1.3. Geographic source and chromosome number of susceptible accessions and cultivars used in genetic studies

Accession number	Geographic source	Chromosome number
Rodney 0	Agriculture Canada	42
IB 1000-128	San Bartolome, Gran Canaria	42
IB 1212	Tiznit, Morocco	28
IB 390	Tinajo, Lanzarote I.	14
IB 1229	Tafraoute, Morocco	14

3.2. Identification of *Avena* species

The *Avena* species used in the following studies (Table 3.2.1) were identified using a taxonomic species key (Baum 1977) and a biologic species key (Ladizinsky 1988). The biologic species designations were more useful as they helped to indicate genetic compatibility between species and thus the ability to make a successful hybridization.

Table 3.2.1. Identity of *Avena* species used in studies

Accession Number	Designated Species Name taxonomic[1]	biologic[2]
IB 1454	*A. trichophyla*	*A. sativa* ssp. *sterilis*
IB 1487	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 2402	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 2428	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 2433	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 2434	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 2465	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 3432	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 691	*A. barbata*	*A. barbata*
IB 845	*A. barbata*	*A. barbata*
IB 3220	*A. barbata*	*A. barbata*
IB 2353	*A. hispanica*	*A. strigosa* ssp. *strigosa*
IB 2404	*A. hispanica*	*A. strigosa* ssp. *strigosa*
Rodney 0	*A. sativa*	*A. sativa* ssp. *sativa*
IB 1000-128	*A. sterilis*	*A. sativa* ssp. *sterilis*
IB 1212	*A. barbata*	*A. barbata*
IB 1229	*A. matritensis*	*A. damascena*
IB 390	*A. canariensis*	*A. canariensis*

[1] from Baum (1977)
[2] from Ladizinsky (1988)

3.3. Pc-gene lines

Nine single gene crown rust resistant lines (Table 3.3.1) were used to identify resistance genes in the unknown hexaploids. These Pc-gene lines were selected based on their current use in the Agriculture Canada Winnipeg Research Station oat breeding program. They were also selected because they reacted like the unknown resistance sources to the 15 test races which suggested the possibility that the unknowns and the Pc-gene lines contained similar genes for crown rust resistance. These Pc-gene lines also make up part of the current crown rust differential set which is used to determine the avirulence/virulence combinations of crown rust isolates and to evaluate the viability and purity of particular rust cultures used. Appendix 1 lists the sources and pedigrees of the Pc-gene lines.

Table 3.3.1. Infection types produced on selected Pc-gene lines

	crown rust races															
line	CR11	CR12	CR13	CR25	CR36	CR50	CR56	CR77	CR103	CR107	CR120	CR123	CR146	CR160	CR169
Pc-38	;	;	;	;	4	0	;	0	-	0	;	0	;	4	0
Pc-39	;	;	4	4	;	;-	;	0	-	0	;	4	;	;	4
Pc-55	1	;	4	4	;	;	;-	0	-	0	;	2	;	;	4
Pc-56	;	;	;	;	;	4	4	0	-	0	4	0	4	;	;
Pc-58	0	;	1	;	;-	;	0	1	-	0	0	;	;-	0	;
Pc-61	;	;	0	;	;	0	0	0	-	0	;	0	0	;	;
Pc-63	;	1	0	0	4	0	0	0	-	0	0	0	0	4	0
Pc-64	;	;	;	;	;	3	0	0	-	4	;	;	4	;	;
Pc-68	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0

(Chong, unpublished)

3.4. Crown rust races

Fifteen crown rust races were used in the various studies (Table 3.4.1). CR 25, CR 36, CR 50, CR 56, CR 77, and CR 107 were used in the initial screening of the Iberian collection. The other races were used on material that showed some resistance. CR 13 and CR 50 were used throughout the genetic studies. CR 13 was a highly virulent race being able to attack eight resistance genes. CR 50 was relatively less virulent being able to attack four resistance genes. These two races attack different Pc-genes except for Pc-40 and Pc-46 which they both attack.

Table 3.4.1. Virulence of crown rust test races.

crown rust race	ineffective Pc-genes
CR 11	40, 66
CR 12	35, 40, 65, 66
CR 13	39, 40, 45, 46, 48, 54, 55, 60
CR 25	39, 40, 45, 46, 48, 54, 55, 60
CR 36	38, 40, 63, 65, 67
CR 50	35, 46, 50, 56
CR 56	56
CR 77	35, 40, 46, 59
CR 103	35, 40, 45, 46, 48, 50, 62, 63
CR 107	40, 46, 62
CR 120	46, 50, 65, 66
CR 123	39, 40, 45, 48, 54, 56, 57
CR 146	35, 56, 62, 64
CR 160	38, 40, 63, 67
CR 169	35, 39, 40, 46, 55, 59, Fidler

(Chong, unpublished)

4.0. Methods

4.1. Introduction

The improvement of crown rust resistance in the agronomic species *A. sativa* has required the transfer of resistance genes from related wild oat species. This objective has been approached by making collections of wild material from the centers of origin of the crop and screening these collections for crown rust resistance as demonstrated in chapter five. Once a resistance source has been identified, studies of inheritance of resistance and genetic relationships to known sources of resistance are conducted as demonstrated in chapter six.

Transferring resistance from tetraploid or diploid lines (chapter seven) to hexaploid lines require different techniques than transferring resistance between hexaploid species.

The following is a discussion of the methods common to all of the work in this project. Methods specific to a particular situation are discussed in their appropriate chapter.

4.2. Growing oats in the growth cabinet and the greenhouse

Growing healthy plants was important to the success of this project for two reasons: production of sufficient high quality seed and good crown rust infections.

Plants to be grown to the adult stage were grown in 15 cm diameter fibre pots filled with one of several proven soil mixes (3 soil : 1 turface : 1 sand, 3 soil : 1 peat :

1 turface : 1 sand, 3 soil : 1 peat: 1 sand). The number of plants per pot varied depending on the intended use of the plants: one or two plants/pot when maximum seed production was desired, two plants/pot when plants were used as females in a crossing program, three plants/pot when plants were used as males in a crossing program, and a maximum of five plants/pot when only 20-50 seeds per plant were required.

Plants were fertilized every two weeks with a 20-20-20 fertilizer (Plant Products Co. Ltd., Bramalea, Ontario) at a rate of 3 g/L and 225 mL of solution applied per pot. Fertilizing usually began one week after planting. To avoid or remedy micronutrient deficiency symptoms, most often observed as purpling of leaves, a 12% manganese chelate and a 9% iron chelate were applied with the first fertilization and again when plants entered the boot stage (Zadoks' growth stage 41). Both minerals were applied at 0.07 g/L (1/4 teaspoon/7L) applied at 225 mL solution per pot.

Aphid control, important in maintaining plant health, was achieved using various methods. Aldicarb (Temik (R) Brand 15G, Union Carbide Agricultural Products Co. Inc.), applied as a granular one week after planting, controlled aphids for 30-50 days. Malathion 50% EC (Later Chemicals, Ltd., Richmond, B.C.) and pirimicarb (Pirimor 50 WP, Chipman Inc., Stoney Creek, Ontario) were sprayed as required on adult plants.

The growth cabinet day-night cycle was set at 18 hours light - 6 hours dark and 20-12 C (approximate). The banks of

fluorescent and incandescent lights were kept at 15 to 50 cm above the plants. In the greenhouse, lighting periods were similar to those in the growth cabinets but at a reduced intensity. Greenhouse temperatures were more variable and were seasonal.

4.3. Cross-pollinating oats

Oats are a self-pollinating crop with natural outcrossing occurring at about 0.5 to 1 % (Poehlman 1987). To cross-pollinate oats, pollen must be transferred from a desired male parent to an emasculated female parent. The techniques used by breeders to pollinate oats vary with available facilities and with the manipulation of the oat spikelet. The following is a description of the method used to cross-pollinate oats in this study.

All crossing was done in growth cabinets. From six to eight hours after the light cycle started, mature anthers would dehisce readily upon removal from the oat floret. For convenience, male and female parents were placed in adjacent growth cabinets with their cycles staggered by six hours so the male parents received six hours of light before the beginning of the light cycle in the female's cabinet. Two hours of pollinating per day can be done with this arrangement.

To make a pollination, mature anthers from a male parent were exposed by pulling back the inner glume, the secondary floret and the palea of the main floret. Bright, creamy yellow anthers were extracted with tweezers and placed on a blue paper pad so dehiscence could be easily viewed. If care in handling the spikelet was taken, the secondary floret anthers could be used the next day. On the female plant, the secondary floret and the primary floret anthers were removed and discarded. While holding the palea of the primary floret back with one finger, pollen from the male parent was applied to the stigmatic branches of the female by shaking an anther above them. The palea and inner glume were then pushed back into place. Floret pollination was marked by removing the tip of one glume. After two or three days, the floret was checked to see if pollination had succeeded. If the stigmatic branches still appeared feathery, the floret was pollinated again.

After pollination, the panicle was enclosed by a wet, glasine bag to improve seed set (Marshall 1962) and prevent contamination from stray pollen. Panicles were labelled using a crossing tag.

Many factors affected the success of hand pollination. Growing healthy plants and gentle handling of the oat spikelet were considered key technical aspects. Daily, light watering of plants involved in crossing was considered helpful in avoiding any minor water stress that could lead to poor seed set.

Accidental pollination from other pollen sources was a

problem that was addressed. When emasculating the female parent, care was taken not to rupture anthers. Florets were discarded if there was any indication of this. Licking the ends of the tweezers before approaching a new floret was a convenient way to avoid outside pollen contamination.

4.4. Testing oats for crown rust resistance

4.4.1. Growth and inoculation of oats

Wild oats sometimes exhibit dormancy; they also often have large awns and hairy lemmas. These factors can lead to uneven germination which can be a problem when trying to synchronize first and second leaf rust inoculations of large numbers of plants. Every effort was made to ensure that an even growth of test material was achieved.

To screen wild oat seedlings, seeds were dehulled, treated with Agrox N-M 50% maneb and placed on wetted filter paper in a petri dish. The dish was kept in a refrigerator at approximately 4 C for seven days in an attempt to break any seed dormancy. On day five, each seed was treated with a drop of 0.01 % GA_3 solution. It was found that seeds with *A. sativa* parentage did not require treatment with the growth regulator. The seeds were left for two more days in the refrigerator to imbibe the growth regulator. The seeds were then placed in a dark cabinet at room temperature for 24 hours to germinate. Those seeds that did not germinate were treated with GA_3 again and cycled through the refrigerator and cabinet again. Germinated seeds were planted in fibre pots or flats.

Rust testing of plants was done by finger inoculating

first, second, or flag leaves (Table 4.4.1) with the appropriate crown rust culture. Inoculation was also done by applying a 1:50-60 spore-talc mixture using a compressed air duster. A differential set containing 20 different single gene lines, one 2 gene line, and four oat varieties was also inoculated to verify the identity, viability, and purity of the rust culture used (Appendix 1).

Table 4.4.1 Crown rust cultures used in screening and the growth stage applied (Zadok et al. 1974)

crown rust race	growth stage Zadok-Chang-Konzak scale
CR 13	11
CR 56	11
CR 107	11
CR 36	12
CR 50	12
CR 25	39
CR 77	39

After inoculation, the plants were placed in a closed box (incubator) for 20 hours at greenhouse temperature (20 +/- 5 C) to allow the urediospores to germinate. The interior of the box, the plant pots, and the plants were sprayed with a 0.1% solution of Tween 20, polyoxyethylene (20) sorbitan monolaurate. High humidity or free water on the leaf surface enhances urediospore germination and host infection.

After 20 hours, the plants were removed from the box so plant growth could resume. Twelve to fourteen days after inoculation, rust reactions were rated using the rust reaction scale found in Murphy (1935) (Table 4.4.2).

4.4.2. Rust evaluation

Crown rust reactions were evaluated using the 0 to 4 scale. This scale has been in use with slight variations since the inception of cereal rust pathology. Below is the scale as was applied in this project. Variations in the symbolism used include using 0 for I, and the use of "+" or "-" within each class for noting smaller differences.

Table 4.4.2. Crown rust infection types (after Murphy 1935).

Host reaction	symbol	description of infection type
immune	0	no macroscopic evidence of infection
nearly immune	;	no uredia formed; necrotic areas or chlorotic areas produced without development of uredia
highly resistant	1	uredia few, small, always in distinct necrotic areas; also more or less necrotic areas produced without the development of uredia
moderately resistant	2	uredia fairly abundant, small midsized, always in necrotic or very chlorotic areas; necrotic areas seldom without uredia
mesothetic	M	apparently a combination of two or more extreme types in varying proportions
moderately susceptible	3	uredia abundant, midsized, and surrounded by chlorotic areas; necrotic areas entirely absent.
susceptible	4	uredia abundant, large; no necrosis or chlorosis immediately surrounding the uredia .

4.5. Cytogenetic work with oats

4.5.1. Counting chromosomes from root tips

Some cytogenetic work was done with the oat material to determine chromosome number and meiotic stability.

Chromosome counts were obtained using root tip squashes. Root tips were collected from seeds that were germinated on filter paper in a petri dish or from plants

that were rapidly growing and thus undergoing cell division. One centimeter long tips were given a twenty-four hour cold pretreatment in ice water before being fixed in Farmer's solution (3 95% ethanol:1 glacial acetic acid). The cold treatment was used to arrest cell division at late prophase. This is achieved since spindle formation is stopped at this temperature (Darlington and La Cour, 1950c).

Root tips were left in the Farmer's fix for at least two days to soften cell walls and matrices before squashing (Darlington and La Cour, 1950a). Farmer's fix was used to arrest growth; the acetic acid maintained chromosome structure but caused excessive swelling which was countered by using ethanol which had a shrinking and hardening effect (Sharma and Sharma, 1972a).

In preparation for squashing, root tips were hydrolyzed for eleven minutes in one normal HCl at 60 C. This treatment time differed slightly from Sun and Sadanaga (1968) who recommended a hydrolysis time of 10 minutes. Tips were then put in Feulgen's stain and left for at least 45 minutes before being squashed. Tips could be stored in the Feulgen's stain in the fridge for a week although the tips tended to become rubbery with time.

To squash a root tip, a drop of acetic carmine stain was put on a dust free slide. A root tip was selected; its muscilagenous cap sliced off with a razor blade; and a thin slice cut and placed in the drop of stain. A cover slip was then placed over the drop with one corner of the cover slip

resting on the razor blade. While holding the cover slip firmly on one side with a piece of blotting paper, the root tip was gently squashed using repeated tapping with a pencil tip eraser. Tapping was continued until the material had spread. The slide was then gently heated using a hot plate. The purpose of heating was to flatten the cells, stick them to the slide and cover slip, and to spread the chomosomes appart (Darlington and La Cour, 1950b). The slide was then placed between blotting paper and pressed very firmly using a thumb.

Chromosomes were found using 160 X magnification, and counted under 400 X. The chromosome number for the plant was decided upon after four identical counts were obtained.

4.5.2. Analysis of pollen mother cells (PMCs)

Pollen mother cells were used to determine the meiotic stability of the material. Metaphase I was the preferred meiotic stage to observe chromosome pairing as chromosomes are quite compact and form bivalents of homologous chromomsomes. Anaphase I and anaphase II were used to observe chromosomes that were lagging or were not associated with the spindle apparatus. Presence of micronuclei at the tetrad stage was also indicative of univalents. Late anaphase I could also be used to determine the chromosome number of the parental material by counting the gametic chromosome number at one end of the dividing cell.

Oat PMCs were collected when the plant was exposing three to five centimeters of the boot. The primary panicle

was collected where possible since it usually contained more florets and thus more meiotic stages. As a result, the possibility of finding the desired stage in meiosis was improved. Panicles were fixed in Farmer's solution or in Carnoy's solution (6 ethyl alcohol : 3 chloroform : 1 glacial acetic acid). PMCs were observed using acetic carmine stain under the same magnifications as above.

In making slides for PMC analysis, anthers were dissected from their spikelets. Using tweezers, scalpel, and a dissecting microscope proved to be the easiest method to remove immature anthers. Once extracted, an anther was put on a slide in a drop of acetic carmine where it was cut in half. This allowed the microspores to escape from the anther when being squashed as described above.

The oat panicle matures from top to bottom and outside to inside of the panicle. Thus earlier stages of meiosis will be found near the bottom of the panicle; later stages, near the top.

5.0. Screening of the Iberian Wild Oat Collection

5.1. Introduction

Crown rust (*Puccinia coronata*) resistance is important for reliable oat production in areas where this disease is prevalent. Since the field virulence of crown rust changes with time, it is useful to find new sources of resistance that can be used in future oat cultivars.

The Iberian Wild Oat Collection was made in the late 1970s by Dr. J.W. Martens and others. Accessions were collected from Spain, Portugal, Morocco, and the Canary Islands. Although unpublished, much work has been done with this collection in screening for crown and stem rust resistance.

The purpose of screening this collection was to find accessions of wild oats which may contain new resistance genes not already identified in previous work.

5.2. Materials and methods

One hundred eleven wild oat accessions (Table 5.3.1) were screened with six races of crown rust: CR 25, CR 36, CR 50, CR 56, CR 77, and CR 107 (Table 3.4.1). These accessions were selected for screening because they lacked crown rust reaction data from previous work. The races that were used covered a broad spectrum of virulence: fifteen resistance genes could be attacked. These genes including Pc-38 and Pc-39 which were used in the current oat cultivars Dumont, Riel, and Robert.

Seedling and adult plants were grown, inoculated, and evaluated as discussed in the general methods. Seeds were also collected for future use. Panicles were bagged with perforated, plastic bakery bags so shattering seed could be collected. These bags provided only incomplete protection against outcrossing.

5.3. Results and discussion

Table 5.3.1. Country of origin, chromosome number, and rust reactions to selected accessions from the Iberian Wild Oat Collection

Accession	Country	Town	Chro #	CR 25	CR 36	CR 50	CR 56	CR 77	CR 107
IB 10	Puerteventura I.	Tetir	14	4	4	0,;	0,4	4	4
IB 19	"	Del R to Tetir	14	4	4	4	4	4	4
IB 21	"	"	14	4	4	4	4	4	4
IB 22	"	"	14	4	;,1	4	;,1	2,4	4
IB 23	"	"	14	4	4	4	4	4	4
IB 26	"	Tetir	14	4	4	4	4	4	4
IB 30	"	"		4	4	4	0,4	4	;
IB 30S	"	"		4	4	4	4	3,4	3,4
IB 31	"	"	14	4	4	4	4	4	3,4
IB 34	"	La Matilla	28	4	3	2	3	4	4
IB 37	"	Tetir	14	0,;	2	4	;	4	4
IB 38	"	"	42	4	2	4	;	4	4
IB 44	"	Tindaya	14	0,;	2	4	2	;	4
IB 79	"	Corralejo	14	0,;	0,1	4	;,1	0,4	4
IB 86	"	"	42	4	4	2	4	4	4
IB 92	"	"	14	4	4	4	4	4	4
IB 95	"	"	14	4	2	4	1	4	0,2
IB 96	"	Majanicho	14	4	4	4	4	4	4
IB 101	"	Corralejo	14	4	4	4	4	4	4
IB 109	"	"	42	4	4	4	4	0	4
IB 124	"	"	42	4	4	2	4	4	4
IB 134	"	Puerto del Rosario	14	4	4	4	4	4	3
IB 135	"	"	14	4	4	4	4	4	0
IB 137	"	"	14	4	4	4	4	4	4
IB 147	"	"	42	4	4	4	2,3	0,4	4
IB 149	"	"	14	4	4	4	4	4	4
IB 150	"	La Ampuyenta	14	4	4	4	0,4	4	4
IB 151	"	"	42	1,2	4	4	4	4	4
IB 152	"	"	14	4	4	4	0,4	4	4
IB 162	"	Puerto del Rosario	14	4	4	4	4	0,4	4
IB 163	"	"	42	4	4	4	0,4	4	4
IB 166	"	Casillas del Angel		4	4	4	0,4	4	4
IB 166S	"	"		3,4	4	4	4	0,4	4
IB 169	"	La Ampuyenta	14	4	4	4	4	0,4	4
IB 175	"	Tiscamanita	28	4	0,1	2	;,1	4	1,2
IB 217	"	?? unknown		2,3	;	4	;	4	4
IB 222	"	Gran Tarajal	14	0,4	4	4	4	0,4	1,4

Table 5.3.1. Country of origin, chromosome number, and rust reactions to selected accessions from the Iberian Wild Oat Collection (continued)

Accession	Country	Town	Chro	CR 25	CR 36	CR 50	CR 56	CR 77	CR 107
IB 228	"	Toto	14	0,4	4	4	4	0,4	4
IB 229	"	"	14	4	4	4	3,4	4	4
IB 230	"	"	14	4	4	4	4	2,3	;
IB 234	"	Vega de Rio Palma	14	4	1,3	0	2,3	2	1
IB 238	"	"				0,4		1,3	0,1
IB 238S	"	"				4		4	0,1
IB 244	"	Toto	14					4	
IB 245	"	"	14	4	0,4	4	3,4	4	3
IB 246	"	Vega de Rio Palma	14			4		0,4	0
IB 259	"	"	42	4	4	4	4	4	4
IB 264	"	Llanos de la Concepcion	42	0,4	4	4	;		0,4
IB 266	"	Vega de Rio Palma	42	4	4		1		
IB 270	"	Betancuria	14	0,4	4	4	4	0,;	
IB 274	"	Llanos de la Concepcion	14	4	4	4	;	4	;,4
IB 279	Fuerteventura I.	Tefia	42	4	4	4	4		0,4
IB 282	"	"	42	0,4	4	4	3	3	4
IB 283	"	"	14	4	4	4	3,4		4
IB 299	"	Betancuria	28	4	3,4		2,3		
IB 305	"	Majada Blanca	14	3	4	4	3,4	4	4
IB 309	"	"	14	4	4	4	4	3	3
IB 317	"	"	14	4	4	4	3,4	4	
IB 322	"	"	14	4	4	4	4	2	4
IB 335	"	Pocetas	42	4	0,4	2,4	3,4		4
IB 337	"	Triquivijate	14	0,4	3,4		3,4	3	
IB 347	"	Rosa del Toro		4	4	4	3	4	4
IB 348	"	Triquivijata	14	4	4	4	4	4	4
IB 349	"	Pocetas	14	0,4	4	4	3,4	4	;,3
IB 350	"	Rosa del Toro	14	0,4	4	3,4	4	4	4
IB 352	"	"	14	4	4	4	4	4	1,4
IB 359	"	"	14	0,4	4	4	3,4		
IB 378	Lanzarote I.	Tinajo	42	4	4	4	4	2	4
IB 379	"	Tinquaton	28	4	;	0	;	;	0
IB 394	"	Parque Nat. de Timanfaya	14	4	4	0,4	4	4	0,4
IB 395	"	"	14	4	4		4		0,4
IB 396	"	"	28	0,4	0,2	4	1	4	4
IB 397	"	"	28	4	0,1	4	0,1	4	4
IB 431	"	Carmen	14	4	4		3,4		
IB 438	"	Yaiza	28	2	2,4		;,1		
IB 442	"	Uga	28	1	3		1		
IB 480	"	Cirecife	28	1,4	0	;	0	0	0
IB 610	La Palma I.	Puntallanna	28	;	;	1,4	;	0,;	0,1
IB 942	Gran Canaria I.	Santa Brigida	28	;,3	;	4	;	1,4	0,;
IB 959	"	Valleseco	28	4	;	4	;	1	1,3
IB 988	"	Firgas	28	4	;	0	;	0,;	0
IB 1005	Morocco	Sidi Bettache		;	4	;	4	0,4	3
IB 1020	"	Ben Slimane	14	;	2,3	1	3	0	4
IB 1022	"	"	42	4	0,4	4	4	0	4
IB 1024	"	Sidi Bettache	14	0	0	;	0,;	0	0
IB 1057	"	El Jadida	28	4	0,4	;,1	1	0,2	;,1
IB 1058	"	"	14	4	3	2,4	4	0,4	0,4
IB 1072	"	Sidi si Bennour	42	4	0,3	4	2	0,4	4 .

Table 5.3.1. Country of origin, chromosome number, and rust reactions to selected accessions from the Iberian Wild Oat Collection (continued)

Accession	Country	Town	Chro	CR 25	CR 36	CR 50	CR 56	CR 77	CR 107
IB 1075	"	"	14	3	0,;	;,3	;,1	1	0,4
IB 1091	"	Tleta Sidi Bouguedra	42	0,4	0,4	4	3	0,4	4
IB 1539	"	Boulemane	14	4	2,3	4	;		4
IB 2000	Portugal	Lisboa	28	3	4	4	3,4	;,4	0,4
IB 2012	"	Elvas	28	;	;	0	4	0	0,;
IB 2014	"	"	42	4	4	4	4	4	4
IB 2016	"	"		0	0	0	;	0	0
IB 2031	"	"	28	0	0,;	0	0	0	0
IB 2100-110	"	Reguengos		;	0,;	;	0	0,;	0
IB 2100-123	"	Evora	14	0	0,;	0	0	0	0,;
IB 2100-135	"	Elvas	28	2,3	;	1	;	0,1	;
IB 2246	"	Alter do Chao	14	0	;	0	0	0	1
IB 2249	"	Monforte	14	;		0	;	0	0
IB 2390	"	Senera	14	0	0,1	3	0	0	4
IB 2406	"	Odeceixe	14	0	;	;	;	;	;
IB 2435	"	Figueira	42	0	0	;	0	0	0,;
IB 2536	"	Sao Marcos da Serra	42	3	4	4	0,2	0,4	0,4
IB 3071	Spain	Jerez de la Frontera	42	;	;	0	;	0,4	1
IB 3076	"	"	42	0	0	0	0	0	0
IB 3081	"	"	28	0	0,1	;	0	0	0,;
IB 3251	"	Ecija	28	0,2	;	;	0	0	;
IB 3254	"	La Carlata	28	0,;	;	;	;	;	;
IB 3788	"	Elda	28		;	1	;	2	;

Rusts applied on first leaf: CR 56, CR 107
Rusts applied on second leaf: CR 36, CR 50
Rusts applied on flag leaf: CR 25, CR 77
(some rust data from Chong, unpublished)

Accessions from all collection areas (Figure 3.1.1) and ploidy levels could be found that exhibited good resistance to the six test races. Since the material analyzed represents about 3% of the total collection, remarks regarding location and frequency of resistant sources could not be made.

Accessions that were susceptible to all test races lacked any effective crown rust resistance. However, accessions IB 1024, IB 2016, IB 2031, IB 2100-110, IB 2100-123, IB 2246, IB 2249, IB 2406, IB 2435, IB 3076, IB 3081, IB 3251, IB 3254, and IB 3788 exhibited resistance to the six races. IB 2249 and IB 3788 exhibited resistance to five

of the test races, but data was not available for reaction the CR 36 and CR 25 respectively. Inheritance studies would be required to determine the number resistance genes in each accession. Only IB 2435 and IB 3076 had 42 chromosomes. The other accessions were of lower ploidy level which makes the resistance more difficult to incorporate into the common oat.

Certain differential reactions between the test races and the accessions suggested particular gene combinations that may be present. As an example, IB 2012, a tetraploid oat, was found to be susceptible to CR 56 but resistant to the other test races. CR 56 was known to be virulent only on Pc-56, but CR 50 was also virulent on Pc-56. Therefore, IB 2012 likely contained a gene(s) other than Pc-56. Also, virulence of CR 56 on IB 2012 suggests that CR 56 has additional virulence not detected with the present Pc-gene lines.

Susceptibility to only CR 25 was found in six accessions: IB 379, IB 480, IB 988,IB 1057, IB 1075, IB 2100-135. This differential reaction suggested the presence of Pc-39, Pc-45, Pc-48, Pc-54, Pc-55, Pc-60, or possibly a new gene. Although CR 25 is virulent on Pc-40 and Pc-46, these genes were not likely to be present in these accessions because CR 36, CR 50, and CR 77 were not virulent on them.

Susceptibility to CR 25, CR 36, CR 77 and CR 107 and resistance to CR 50 and CR 56 suggests the presence of Pc-40. IB 10, a diploid, matched this differential pattern.

The presence of Pc-46 was suggested in the accessions IB 22, a diploid, and IB 217 (chromosome number unknown) by the differential reaction of susceptibility to CR 25, CR 50, CR 77, and CR 107 and resistance to CR 36 and CR 56.

IB 610, a tetraploid, may be another source of Pc-50 as some of the test plants were susceptible only to CR 50.

IB 3071, a hexaploid, may be another source of Pc-59 since it was susceptible only to CR 77.

No accessions were found to be susceptible only to CR 36. This differential reaction would have suggested the presence of Pc-38, Pc-63, Pc-65, or Pc-67.

No accessions had differential reactions that suggested the presence of Pc-35 or Pc-62.

Screening a collection of material is very laborious and time consuming, but the results may be very rewarding if a good strategy is used. The selection of test races is very important. A range of virulence from low to high is desirable. Races with a narrow specificity such as CR 56 will allow detection of more resistance genes. CR 25 has a wide range of virulence in that it can attack eight known crown rust genes. Expression of resistance towards a race such as CR 25 might be of some interest since it would indicate that the source of resistance is not one of the eight known genes.

The virulence range of the races used to screen an unknown collection should be as broad as possible. For six test races which range in their ability to attack one to six genes, a maximum of twenty-one resistance genes could be tested for. The test races used in this study had a virulence range of fifteen crown rust resistance genes. This can be attibuted to virulence overlaps for Pc-35, Pc-40, Pc-46, and Pc-56.

There were a few difficulties with this work associated with wide differences in plant types. Narrow leaved plants or plants with miniscule flag leaves made rust inoculations more difficult. Some plant types faired poorly in the greenhouse. Purpling of leaves, excessive height and weak stems were common problems. Most accessions had spikelets which disarticulated from the panicle at maturity. This required that plants be bagged so seed could be collected.

6.0. Inheritance of crown rust resistance in eight accessions of *Avena sterilis* L.

6.1. Introduction

In the Canadian prairie rust region of Manitoba and eastern Saskatchewan, oats, *Avena sativa* L., are subject to attack by crown rust, *Puccinia coronata* Corda. Griffiths (1953) wrote that "there is no effective practical means of controlling crown rust other than by the cultivation of resistant varieties". This statement is still valid. The relatively low cost and neutral environmental impact of incorporating disease resistance into cultivars makes this type of control measure still the most desirable. The crown rust pathogen has many physiologic forms and has the ability to develop virulence on the many crown rust resistance genes that have been introduced into oat cultivars over the years. Finkner (1954) expressed the need for the continued investigation of new sources of crown rust resistance that could be incorporated into commercial cultivars.

Avena sterilis L. has been a source of over thirty new crown rust resistance genes (Fleischmann and McKenzie 1968; Kiehn *et al.* 1976; Harder *et al.* 1980; Wong *et al.* 1983). Of these new genes, Pc-38 and Pc-39 have been combined into the current oat cultivars Dumont (McKenzie, *et al.* 1982), Riel (McKenzie *et al.* 1986), and Robert (unpublished). Although these cultivars are highly resistant in the prairie rust area, it is necessary to continue searching for new sources of resistance that can be incorporated into future

cultivars. Isolates of crown rust have been found in Manitoba and eastern Saskatchewan that are virulent on Pc-39 alone and on the combination of Pc-38 and Pc-39 (Chong 1988).

The aims of this study were to determine the inheritance of crown rust resistance in eight *A. sterilis* accessions and to determine their genetic relationships to each other and to nine known crown rust resistance genes.

6.2. Materials and methods

The resistant material used in this study was selected based on diverse geographic distribution (Table 6.2.1) and low infection types to fifteen crown rust races (Table 6.2.2 and Table 6.2.3).

Table 6.2.1. Geographic source of the selected resistant accessions.

Accession number	Geographic Location
IB 1454	Marrakech, Morocco
IB 1487	Kasba Tadla, Morocco
IB 2402	Aljezur, Portugal
IB 2428	Figueira, Portugal
IB 2433	Almansil, Portugal
IB 2434	Figueira, Portugal
IB 2465	Tunes, Portugal
IB 3432	Vejer de la Frontera, Spain

Three types of crosses were made to analyze the crown rust resistance found in the above hexaploid accessions: resistant/universal suscept, resistant/resistant, and resistant/Pc-gene line. Crosses between each wild oat line and the universal suscept, Rodney 0, were made so that the number and mode of inheritance of resistance genes could be determined. Each cross was tested with two races of rust on

110 to 150 F_2 progeny seedlings. CR 13 was applied on the first leaf, and CR 50 was applied on the second leaf. To determine allelism and the total number of different resistance genes, crosses between selected pairs of wild oat lines were screened in the F_2 generation on populations of usually 350 seedlings or more. CR 13 and CR 50 were similarly used to screen this material. F_2 progeny of crosses between the selected accessions and lines containing known single genes for crown rust resistance (Pc-lines) were similarly tested with CR 13 and CR 50 to determine the relationships between the unknown genes and known Pc-genes.

Table 6.2.2. Infection types produced on seedlings or adults of the selected accessions by 15 races of *Puccinia coronata*

Collection	Race of rust applied															
number	CR11	CR12	CR13	CR25	CR36	CR50	CR56	CR77	CR103	CR107	CR120	CR123	CR146	CR160	CR169
IB 1454	2,3	;,1	0,;	0	;	;	;	0	0?	;	;,2	2	2	1	4
IB 1487	1	1,2	1	0	;	;	;	0	1	;	2,3	1	2,3	1	1
IB 2402	0	0	0,;-	0	0	0	0	0	0	0	0	0	0	0	0
IB 2428	1	1-	1-	;	0	0	0	;	0?	0	;,1+	;,1	1-	;,1	;
IB 2433	;	;1-	1	;-	0	0	;	;	0?	;	;,1	;-	1-	;	;-
IB 2434	1	1	1-	;	;	;	;	;	0?	;	1	;	;	;	;
IB 2465	0	0	0	;-	0	0	0	0	0?	0	0,;-	0	0	0	0
IB 3432			0,;	;	;-	;	;,1	;	0,;						

Rusts applied on first leaf: CR 11, CR 12, CR 13, CR 56, CR 107 (Chong, unpublished)
Rusts applied on second leaf: CR 36, CR 50, CR 123, CR 146, CR 169
Rusts applied on flag leaf: CR 25, CR 77, CR 103, CR 120, CR 160

Table 6.2.3. Virulence combinations of 15 crown rust test races.

crown rust race	ineffective Pc-genes
CR 11	40, 66
CR 12	35, 40, 65, 66
CR 13	39. 40, 45, 46, 48, 54, 55, 60
CR 25	39, 40, 45, 46, 48, 54, 55, 60
CR 36	38, 40, 63, 65, 67
CR 50	35, 46, 50, 56
CR 56	56
CR 77	35, 40, 46, 59
CR 103	35, 40, 45, 46, 48, 50, 62, 63
CR 107	40, 46, 62
CR 120	46, 50, 65, 66
CR 123	39, 40, 45, 48, 54, 56, 57
CR 146	35, 56, 62, 64
CR 160	38, 40, 63, 67
CR 169	35, 39, 40, 46, 55, 59, Fidler

(Chong, unpublished)

The choice of whether to use the resistance sources as male or female parents depended on the type of cross being made. Rodney 0 was used as the male parent because of its excellent pollen production. In the crosses between resistant accessions, decisions as to which accessions were used as males or females were arbitrary. In general, if a healthy first culm were available, it had priority for use as a female. In crosses with the single gene Pc-lines, the Pc-lines were used as females to avoid the problems of seed dehiscence that were associated with the wild oats.

To screen wild oat seedlings, seeds were dehulled, treated with Agrox N-M 50% maneb and placed on wetted filter paper in a petri dish. The seeds were kept in a refrigerator at approximately 4 C for seven days in an attempt to break any dormancy. On day five, each seed was treated with a drop of 0.01 % GA_3 solution. It was found that seeds with *A. sativa* parentage did not require treatment with the growth regulator. The seeds were left for

two more days in the refrigerator to imbibe the growth regulator. The seeds were then placed in a dark cabinet at room temperature for 24 hours to germinate. Those seeds that did not germinate were treated with GA_3 again and were cycled through the refrigerator and cabinet. Germinated seed was planted in fibre pots or flats with a 3 soil : 1 sand : 1 peat moss potting mixture. This method of seed handling ensured that hybrid seed was not lost and seedlings were synchronized for ideal rust inoculation.

Rust screening of the plants was done by finger inoculating the first and second leaves with a particular race of rust or by applying a 1 spore : 50-60 talc mixture using a compressed air duster. A differential set containing 20 different single gene lines (Pc-35, Pc-38, Pc-39, Pc-40, Pc-45, Pc-46, Pc-48, Pc-50, Pc-54, Pc-55, Pc-56, Pc-58, Pc-59, Pc-60, Pc-62, Pc-61, Pc-63, Pc-64, Pc-67, Pc-68), one line containing the gene combination Pc-55/56, and four oat varieties (Ascencau, Hudson, Fidler, Dumont) was also inoculated to verify the identity, viability, and purity of the rust culture used.

After inoculation, the plants were placed in a moist chamber for 20 hours at greenhouse temperature (20 $\pm$ 5 C) to allow the urediospores to germinate. The chamber was kept moist by periodic spraying with a 0.1% solution of Tween 20 [polyoxyethylene (20) sorbitan monolaurate (MCB Manufacturing Chemists Inc. 2909 Highland Ave., Cincinnati, Ohio, 45212)]. A thin film of free water was maintained on the leaf surfaces to attain good urediospore germination.

After 20 hours, the plants were removed from the chamber so plant growth could resume. Twelve to fourteen days after inoculation, rust reactions were read using the rust reaction scale described by Murphy (1935).

The chi square statistic was calculated to determine goodness of fit to various proposed genetic models as well as to test for homogeneity of pooled data.

6.3. Results and discussion

6.3.1. Inheritance of resistance in IB 1487

Table 6.3.1. Segregation of F_2 seedling populations from crosses of IB 1487 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 1487/Rodney 0	CR 13	84	34	3:1	0.250 - 0.500
	CR 50	39	79	1:3	0.025 - 0.050
IB 1454/IB 1487	CR 13	207	10	61:3	0.950 - 0.975
	CR 50	196	13	61:3	0.250 - 0.500
IB 2402/IB 1487	CR 13	359	27	15:1	0.100 - 0.250
	CR 50	371	15	61:3	0.250 - 0.500
IB 1487/IB 2428	CR 13	374	0		
	CR 50	363	8	63:1	0.250 - 0.500
IB 2433/IB 1487	CR 13	357	0		
	CR 50	341	10	63:1	0.050 - 0.100
IB 2434/IB 1487	CR 13	200	0		
	CR 50	190	9	15:1	0.250 - 0.500
IB 2465/IB 1487	CR 13	338	25	15:1	0.500 - 0.750
	CR 50	360	2	63:1	0.100 - 0.250
IB 3432/IB 1487	CR 13	340	22	15:1	0.750 - 0.900
	CR 50	362	0		
Pc-38/IB 1487	CR 13	353	14	15:1	0.050 - 0.100
	CR 50	346	20	15:1	0.500 - 0.750
Pc-39/IB 1487	CR 13	303	93	3:1	0.250 - 0.500
	CR 50	376	19	15:1	0.100 - 0.250
Pc-56/IB 1487	CR 13	375	0		
	CR 50	354	21	15:1	0.500 - 0.750
Pc-63/IB 1487	CR 13	260	25	15:1	0.050 - 0.100
	CR 50	279	5		
Pc-64/IB 1487	CR 13	377	13	15:1	0.010 - 0.050@
	CR 50	323	63	13:3	0.100 - 0.250
Pc-68/IB 1487	CR 13	410	8	15:1	<0.001@
	CR 50	396	23	15:1	0.500 - 0.750.

@ Inoculation was very light.

When the F_2 population of the cross IB 1487/Rodney 0 was tested with CR 13, 84 resistant plants and 34 susceptible plants were observed which fitted a single, dominant gene ratio (Table 6.3.1). Testing the same plants with CR 50 revealed 39 that were resistant and 79 that were susceptible. The results gave a poor fit to a single, recessive gene model. When the F_2 seedlings were categorized based on their reaction to both test races, it was found that two independent genes were present (Table 6.3.2). One gene, designated "A" (for the purpose of this study), was dominant and resistant to CR 13 but susceptible to CR 50; the second gene, designated "B", was recessive and resistant to CR 50 but susceptible to CR 13. It was noted that gene B acted as a dominant gene in all other crosses (Table 6.3.1).

Table 6.3.2. Tests of gene independence

Cross	CR 13/CR 50 expected ratio				X^2	df	P of X^2
	R/R	R/S	S/R	S/S			
IB 1487/Rodney 0	3	9	1	3	3.5744	3	0.250 - 0.500
IB 2402/Rodney 0	12	-	1	3	1.1445	2	0.250 - 0.500
IB 2465/Rodney 0	12	-	3	1	4.1365	2	0.100 - 0.500
IB 3432/Rodney 0	12	-	3	1	0.2723	2	0.750 - 0.900
Pc-55/IB 1454	52	-	9	3	1.6338	2	0.250 - 0.500
IB 1454/IB 1487	235	9	9	3	1.9569	3	0.500 - 0.750
IB 2402/IB 1487	235	9	9	3	7.2132	3	0.050 - 0.100
IB 2465/IB 1487	237	3	15	1	2.9682	3	0.250 - 0.500
Pc-38/IB 1487	57	3	3	1	3.7986	3	0.250 - 0.500
Pc-39/IB 1487	45	3	15	1	1.9294	3	0.750 - 0.900
Pc-38/IB 2433	249	3	3	1	1.7755	3	0.500 - 0.750
Pc-39/IB 2433	237	3	15	1	6.0566	3	0.100 - 0.250
Pc-64/IB 2433	57	3	3	1	4.4033	3	0.100 - 0.250
Pc-68/IB 2433	249	3	3	1	2.4199	3	0.250 - 0.500
Pc-63/IB 2465	60	-	3	1	2.4586	2	0.250 - 0.500
Pc-38/IB 3432	60		3	1	0.4434	2	0.500 - 0.750
Pc-68/IB 3432	60	-	3	1	1.7042	2	0.250 - 0.500.

When tested with CR 13, the F_2 populations derived from the crosses of IB 1487 with IB 2428, IB 2433, and IB 2434

did not segregate for resistance (Table 6.3.1). This indicated that gene A of IB 1487 was closely linked or allelic to a gene in each of the other three accessions. Testing with CR 50 resulted in a three gene segregation ratio of 63 resistant : 1 susceptible for the crosses involving IB 2428 and IB 2433. However, with IB 2434, a two gene segregation ratio of 15 resistant : 1 susceptible was found. This indicated that gene B was independent of the genes conferring resistance in IB 2428, IB 2433, and IB 2434. This also indicated that two genes conferred resistance to CR 50 in IB 2428 and IB 2433 and one gene in IB 2434.

Crossing IB 1487 to IB 1454, IB 2402, IB 2465, and IB 3432 resulted in the segregation of the F_2 progeny to race CR 13 (Table 6.3.1). This indicated that gene A in IB 1487 was independent of the resistance factors in these other four wild oat lines. When tested with CR 50, segregation occurred with populations involving IB 1454, IB 2402, and IB 2465 thus indicating the independence of gene B from factors in these lines. The cross with IB 3432 did not segregate when tested with CR 50. This suggested close linkage or allelism of gene B in IB 1487 with a gene in IB 3432.

F_2 progenies from crosses involving IB 1487 and six Pc-lines: Pc-38, Pc-39, Pc-56, Pc-63, Pc-64, and Pc-68 (Table 6.3.1) were tested with CR 13. Those derived from crosses involving Pc-38, Pc-63, Pc-64, and Pc-68 segregated in a 15 resistant : 1 susceptible ratio. This indicated that gene A

was independent of these Pc-genes. The progeny from crosses involving Pc-64 and Pc-68 fit poorly to this two gene model due to a very light inoculation which made detection of susceptible plants difficult. Testing with CR 50 yielded similar results for gene B regarding Pc-38, Pc-39, Pc-56, and Pc-68. With Pc-64, a 13 resistant : 3 susceptible ratio was obtained with CR 50. This indicated that gene B and Pc-64 were independent of each other but interacted in a dominant recessive epistatic manner. With Pc-63, only 5 susceptible plants were obtained out of 284 plants in the F_2 progeny. Gene B was likely linked to Pc-63. Harder *et al.* (1980) found that Pc-38 and Pc-63 were closely linked or allelic to each other. The fact that gene B was shown to be independent of Pc-38 and linked to Pc-63 supported the hypothesis that Pc-38 and Pc-63 were linked - rather than allelic - to each other. Similar results with crosses involving IB 3432 with Pc-38 and Pc-63 supported this hypothesis also (Table 6.3.7).

The cross of Pc-39/IB 1487 (Table 6.3.1), when tested in the F_2 with CR 13, gave an expected single gene ratio. This was because CR 13 was virulent on the gene Pc-39, but not on gene A (Table 6.2.3); thus, only the expression of gene A was expected. Screening with CR 50 resulted in a two gene ratio (15 resistant : 1 susceptible). This indicated that gene B and Pc-39 were independent of each other. The independence of gene A from Pc-39 could not be shown using

CR 13 or CR 50 since the expression of resistance by both genes at the same time using either race could not occur.

The F_2 progeny from the cross Pc-56/IB 1487 were all resistant when tested with CR 13. This indicated that gene A in IB 1487 and gene Pc-56 were either closely linked or allelic. The close genetic association and similar differential reactions to CR 13 and CR 50 suggested that gene A was Pc-56. When tested with CR 50, a single gene ratio was expected since Pc-56 was susceptible to this test race (Table 6.2.3), and only gene B would be expressed. Instead, a 15:1 ratio was obtained. This finding was explained by testing nineteen progeny of the Pc-56 parent. This parent was expected to be susceptible to CR 50, but it segregated eighteen resistant plants to one susceptible plant. It was thought that a second gene was present in some plants of this parent. The second gene was probably Pc-55 and occurred as a seed source contaminant from the line containing both gene Pc-55 and Pc-56.

6.3.2. Inheritance of resistance in IB 2402

Table 6.3.3. Segregation of F_2 seedling populations from crosses of IB 2402 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 2402/Rodney 0	CR 13	108	35	3:1	0.750 - 0.900
	CR 50	114	29	13:3	0.500 - 0.750.
IB 2402/IB 1487	CR 13	359	27	15:1	0.100 - 0.250
	CR 50	371	15	61:3	0.250 - 0.500
IB 2402/IB 2465	CR 13	439	0		
	CR 50	439	0		.
Pc-39/IB 2402	CR 13	355	64	13:3	0.025 - 0.050
	CR 50	403	13	61:3	0.100 - 0.250.

When the F_2 population of IB 2402/Rodney 0 was tested with CR 13, 108 resistant plants and 35 susceptible plants were observed (Table 6.3.3). This segregation for resistance fitted a single gene ratio indicating the presence of a single, dominant gene, designated "C". When the same plants were tested with CR 50 on the second leaf, the segregation for resistance occurred in a 13 resistant : 3 susceptible ratio which indicated the presence of two independent genes interacting in a dominant recessive epistatic manner. Categorizing the seedlings based on their reaction to both test races indicated the presence of only two genes: gene C and a second gene designated "D" (Table 6.3.2).

The F_2 progeny from the cross IB 2402/IB 2465 did not segregate when tested with either CR 13 or CR 50 (Table 6.3.3). This indicated that these two accessions had genes that were allelic or closely linked to each other.

The F_2 progeny of IB 2402/IB 1487 segregated in a 15 resistant : 1 susceptable ratio when tested with CR 13 (Table 6.3.3). This indicated the presence of two independent genes: gene A of IB 1487 and gene C of IB 2402. Testing with CR 50 resulted in a three gene segregation ratio of 61 resistant to 3 susceptible plants. Gene B of IB 1487 and genes C and D of IB 2402 were independent and effective against CR 50 in this segregating population with gene C and gene D interacting in a dominant recessive epistatic manner.

A 13 resistant : 3 susceptible ratio (Table 6.3.3) was obtained from the F_2 progeny of Pc-39/IB 2402 when tested with CR 13. This indicated the presence of two independent genes. This was not expected since CR 13 was virulent on Pc-39 (Table 6.2.3). It appears that gene Pc-39, although ineffective against CR 13, may interact with gene C in a dominant recessive epistatic manner. When tested with CR 50, a 61 resistant : 3 susceptible ratio was observed. This indicated that Pc-39 was independent of both genes C and D, but dominant recessive epistasis was detected between genes C and D.

6.3.3. Inheritance of resistance in IB 2465

Table 6.3.4. Segregation of F_2 seedling populations from crosses of IB 2465 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 2465/Rodney 0	CR 13	103	47	3:1	0.050 - 0.100
	CR 50	137	12	15:1	0.250 - 0.500@
IB 2465/IB 1454	CR 13	381	18	61:3	0.750 - 0.900
	CR 50	394	2	253:3	0.100 - 0.250
IB 2465/IB 1487	CR 13	338	25	15:1	0.500 - 0.750
	CR 50	360	2	63:1	0.100 - 0.250.
Pc-39/IB 2465	CR 13	342	76	13:3	0.750 - 0.900
	CR 50	409	8	63:1	0.500 - 0.750
Pc-63/IB 2465	CR 13	409	26	15:1	0.750 - 0.900
	CR 50	430	3	63:1	0.100 - 0.250
Pc-64/IB 2465	CR 13	282	67	13:3	0.750 - 0.900
	CR 50	345	6	63:1	0.750 - 0.900
Pc-68/IB 2465	CR 13	448	0		
	CR 50	448	0		.

@light infection

The F_2 progeny of the cross IB 2465/Rodney 0 segregated 103 resistant : 47 susceptible plants when tested with CR 13 (Table 6.3.4). This segregation ratio fitted a single gene ratio which indicated the presence of a dominant gene,

designated "E". Testing with CR 50 resulted in a 15 resistant : 1 susceptible two gene segregation ratio indicating the presence of gene E and a second resistance gene, designated "F". Categorizing the seedlings based their reactions to both test races indicated that two dominant genes were present: gene E conferred resistance to both races of rust while gene F conferred resistance only to CR 50 (Table 6.3.2).

IB 2465 was crossed with IB 1454 and IB 1487 (Table 6.3.4). In both cases, segregation for resistance to both test races occurred which indicated the independence of genes E and F from resistance factors in IB 1454 and from genes A and B of IB 1487.

IB 2465 was also crossed with Pc-39, Pc-63, and Pc-64 (Table 6.3.4). The F_2 populations of these crosses gave two gene segregation ratios of 15 R : 1 S and 13 R : 3 S when tested with CR 13. The dominant recessive epistatic interaction found with the cross involving Pc-39 was not expected because of the known susceptibility of Pc-39 to CR 13 (Table 6.2.3). This type of epistasis was also noted with the cross involving Pc-64. Pc-64 usually acted as an effective dominant gene when tested with CR 13. F_2 progeny of Pc-63/IB 2465 segregated in a 15 resistant : 1 susceptible ratio since gene E and Pc-63 were both effective against CR 13. When tested with CR 50, F_2 progenies from the crosses of IB 2465 with Pc-39, Pc-63, and Pc-64 segregated in the ratio 63 resistant : 1 susceptible. This indicated

the presence of three dominant genes in each case. Thus, genes E and F of IB 2465 were independent of Pc-39, Pc-63, and Pc-64. The independence of genes E and F and Pc-63 was further substantiated by categorizing the F_2 progeny for reaction to both test races. A three dominant gene model fitted well to the observed values (Table 6.3.2).

The F_2 population of Pc-68/IB 2465 did not segregate for resistance when tested with CR 13 or CR 50 (Table 6.3.4). This indicated that gene E of IB 2465 was closely linked or allelic to Pc-68. Since IB 2402/IB 2465 did not segregate when tested with CR 13 or CR 50 (Table 6.3.3), it appeared that gene C and gene E were also closely linked or allelic to each other and to Pc-68. Although Pc-46 and Pc-50 were known to be closely associated to Pc-68 (Fleischmann *et al.* 1971b; Wong *et al.* 1983), gene C and gene E cannot be either of these alleles since Pc-46 and Pc-50 were ineffective against CR 50 whereas genes C and E are effective (Table 6.2.3).

It was important to note that Pc-64 segregated with IB 2465 while Pc-68 did not. This is evidence that Pc-64 was independent of Pc-68.

6.3.4. Inheritance of resistance in IB 2433

When tested with CR 50, the F_2 population of IB 2433/Rodney 0 segregated 115 resistant : 28 susceptible plants. This was indicative of two independent genes interacting in a dominant recessive epistatic manner (13

resistant : 3 susceptible) (Table 6.3.5). Unfortunately, testing of this cross with CR 13 was not done.

The F_2 population of IB 2433/IB 1454 segregated for resistance when tested with CR 13 and CR 50 (Table 6.3.5). This indicated that these two wild oat lines did not share any resistance genes in common .

Table 6.3.5. Segregation of F_2 seedling populations from crosses of IB 2433 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 2433/Rodney 0	CR 13	-	-		
	CR 50	115	28	13:3	0.750 - 0.900.
IB 2433/IB 1454	CR 13	425	3	253:3	0.250 - 0.500
	CR 50	429	2	253:3	0.100 - 0.250
IB 2433/IB 1487	CR 13	357	0		
	CR 50	341	10	63:1	0.050 - 0.100
IB 2433/IB 2434	CR 13	396	0		
	CR 50	396	0		.
Pc-38/IB 2433	CR 13	393	7	63:1	0.750 - 0.900!
	CR 50	390	8	63:1	0.250 - 0.500#
Pc-39/IB 2433	CR 13	376	30	15:1	0.250 - 0.500
	CR 50	384	10	63:1	0.100 - 0.250
Pc-56/IB 2433	CR 13	404	0		
	CR 50	376	30	15:1	0.250 - 0.500
Pc-58/IB 2433	CR 13	378	1	63:1	0.025 - 0.050
	CR 50	374	2	63:1	0.100 - 0.250
Pc-61/IB 2433	CR 13	384	9	63:1	0.100 - 0.250
	CR 50	365	0		
Pc-64/IB 2433	CR 13	289	20	15:1	0.500 - 0.750
	CR 50	273	11	15:1	0.050 - 0.100
Pc-68/IB 2433	CR 13	399	10	63:1	0.100 - 0.250
	CR 50	397	7	63:1	0.750 - 0.900@

! $X^2_{homogeneity}$ = 11.1409, 0.025<P<0.050
$X^2_{homogeneity}$ = 13.4446, 0.010<P<0.025
@ $X^2_{homogeneity}$ = 11.1688, 0.010<P<0.025

IB 2433 was crossed to Pc-38, Pc-39, Pc-58, and Pc-68 to determine the relationship of the resistance in IB 2433 to these known genes. In crosses involving Pc-38, Pc-58, and Pc-68, segregation ratios indicating the presence of three genes were obtained when the F_2 progenies were tested with CR 13 and CR 50. In each case, the activity of one gene

could be attributed to the known Pc-gene; the other two genes could then be attributed to IB 2433. When the progenies were categorized using both test races (Table 6.3.2), it was found that four genes were present in each segregating population of which one was the known Pc-gene. Based on this evidence, there were three genes in IB 2433 with the following characteristics. One gene, designated "G", was resistant to CR 13 and susceptible to CR 50. A second gene, designated "H", conferred resistance to both test races. A third gene, designated "I", was susceptible to CR 13 and resistant to CR 50. The F_2 data from Pc-38/IB 2433 was pooled from six F_1 plants. When screened with either test race, the F_2 progeny from each F_1 plant gave results which were not homogeneous. This was also true of the F_2 data from Pc-68/IB 2433 when tested with CR 50. Although these data were supportive of the presence of genes G, H, and I, it cannot stand alone.

The cross Pc-39/IB 2433 also supported the presence of three genes in IB 2433. When tested with CR 13, the F_2 population segregated into 376 resistant plants and 30 susceptible plants which fitted a two dominant gene model (Table 6.3.5). This would be expected since Pc-39 was ineffective against CR 13 (Table 6.2.3). When tested with CR 50, a three dominant gene ratio was obtained. The reactions to both CR 13 and CR 50 were used to categorize the seedlings, and they supported the presence of four genes in

this F_2 population: one gene was Pc-39 while genes G, H, and I were active in IB 2433 (Table 6.3.2).

The F_2 population of the cross Pc-56/IB 2433 did not segregate for resistance and susceptibility when tested with CR 13. This indicated that the gene Pc-56 was closely linked or allelic with a resistance factor in IB 2433. When tested with CR 50, a 15 resistant : 1 susceptible ratio was observed. Thus the presence of two genes was indicated which was expected since Pc-56 was ineffective against CR 50 (Table 6.2.3). It was important to note that the progeny from the Pc-56 parent were tested with CR 13 and CR 50 and reacted as expected: the line was homozygous for Pc-56. The cross IB 2433/IB 1487 did not segregate in the F_2 generation when tested with CR 13 which indicated that these two wild oat lines contained genes that were closely linked or allelic to each other (Table 6.3.5). Testing with CR 50 revealed a three gene segregation ratio (Table 6.3.5). One effective gene was attributed to IB 1487 (gene B) and two effective genes were attributed to IB 2433 (genes H and I). Thus, gene B was independent of genes H and I. Recalling that Pc-56/IB 1487 did not segregate for CR 13 either, it appeared that gene A of IB 1487, gene G of IB 2433, and Pc-56 were closely linked or allelic to each other. Also, these genes all had the same differential reactions to CR 13 and CR 50 which suggested that they may be the same gene.

The F_2 population of Pc-61/IB 2433 segregated into 384 resistant plants and 9 susceptible plants (Table 6.3.5). The

data best fitted a three gene segregation ratio (63 resistant : 1 susceptible). This indicated that genes G and H of IB 2433 were independent of Pc-61. When tested with CR 50, no segregation occurred. Thus, Pc-61 is closely linked or allelic with gene I of IB 2433. Pc-61 could not be associated with gene H since segregation occurred with the CR 13 screening.

Since it appeared that Pc-56 was associated with gene G and Pc-61 was associated with gene I and genes G and I were independent of each other, it could be concluded that Pc-56 and Pc-61 were independent of each other. Independence of these genes means that they can be combined in the same genotype. This is important when trying to pyramid resistance genes.

The cross of Pc-64/IB 2433 did not perform as expected in the F_2 generation. Testing with CR 13 and CR 50 both resulted in a two dominant gene segregation ratio (Table 6.3.5). This was difficult to explain since the expression of one resistance gene appeared to be absent in each case.

6.3.5. Inheritance of resistance in IB 1454

The mode of inheritance of the resistance in IB 1454 was difficult to determine based on the information available. The majority of the data was best explained if it was assumed that IB 1454 contained two genes for resistance, designated gene "J" and gene "K", which acted in a dominant recessive epistatic manner in segregating F_2 populations.

The F_2 population of IB 1454/Rodney 0 was tested only with CR 50. The population segregated into 86 resistant plants and 27 susceptible plants (Table 6.3.6). Although the data fitted a single gene ratio best, it also fitted a 13 resistant : 3 susceptible model. Because of the small population size, it was difficult to distinguish between the two models. At this size, there was about a 1/3 chance of making the wrong decision (Steel and Torrie 1980).

Table 6.3.6. Segregation of F_2 seedling populations from crosses of IB 1454 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 1454/Rodney 0	CR 13	-	-		
	CR 50	86	27	13:3	0.100 - 0.250.
IB 1454/IB 1487	CR 13	207	10	61:3	0.950 - 0.975
	CR 50	196	13	61:3	0.250 - 0.500
IB 1454/IB 2428	CR 13	426	3	253:3	0.250 - 0.500
	CR 50	428	2	253:3	0.100 - 0.250
IB 2433/IB 1454	CR 13	425	3	253:3	0.250 - 0.500
	CR 50	429	2	253:3	0.100 - 0.250
IB 2434/IB 1454	CR 13	395	5	253:3	0.750 - 0.900
	CR 50	397	1	253:3	0.050 - 0.100
IB 2465/IB 1454	CR 13	381	18	61:3	0.750 - 0.900
	CR 50	394	2	253:3	0.100 - 0.250
IB 3432/IB 1454	CR 13	330	48	61:3	
	CR 50	377	5	253:3	0.750 - 0.900.
Pc-38/IB 1454	CR 13	358	37	61:3	< 0.001
	CR 50	358	41	61:3	< 0.001
Pc-55/IB 1454	CR 13	345	71	13:3	0.250 - 0.500
	CR 50	387	14	61:3	0.250 - 0.500
Pc-64/IB 1454	CR 13	328	27	15:1	0.250 - 0.500
	CR 50	261	96	3:1	0.250 - 0.500.

IB 1454/IB 1487 was tested in the F_2 generation with CR 13 and CR 50. In both cases, the segregation data could be fitted to either a 15 resistant : 1 susceptible ratio or to a three gene segregation ratio where two loci are interacting in a dominant recessive epistatic manner (61 resistant : 3 susceptible). Again, it was not possible to

distinguish which was the most appropriate model; although, it was apparent that genes A and B were independent from those in IB 1454 since susceptible plants were obtained in the F_2 progenies.

F_2 populations from crosses of IB 1454 with IB 2428, IB 2433, and IB 2434 resulted in similar segregations for resistance and susceptibility when tested with CR 13 and CR 50 (Table 6.3.6). In all cases, only a small number of susceptible plants were obtained. The data could be explained using a three gene model (63 resistant : 1 susceptible), but it was better explained with a four gene model with genes J and K in IB 1454 behaving in a dominant recessive epistatic manner (253 resistant : 3 susceptible).

When tested with CR 13, the F_2 population of IB 2465/IB 1454 segregated into 381 resistant plants and 18 susceptible. The data could be fitted to a two gene segregation model where one of the genes was gene E of IB 2465. The data was better explained if a 61 resistant : 3 susceptible model was used where the three genes involved were E, J, and K. Testing with CR 50 resulted in 394 resistant plants and only 2 susceptible plants. Again, these results were best explained using a 253 resistant : 3 susceptible model with genes J and K acting in a dominant recessive epistatic manner. Therefore, genes E and F of IB 2465 and gene J and K of IB 1454 were independent of each other.

The ratio of 330 resistant : 48 susceptible plants was obtained in the F_2 progeny from the cross involving IB 3432 and IB 1454, when tested with CR 13 (Table 6.3.6). The data did not fit any of one, two, or three gene models. Because there was segregation for susceptibility to CR 13, it was likely that the genes in these two accessions were independent of each other. When tested with CR 50, this population gave a very close fit to a 253 resistant : 3 susceptible segregation ratio. Therefore, genes J and K were independent of resistance factors in IB 3432.

The segregation ratios obtained when testing the F_2 generation of Pc-38/IB 1454 with CR 13 and CR 50 were difficult to explain (Table 6.3.6). They gave a very poor fit to the expected 61 resistant : 3 susceptible model because of the large number of susceptible plants that were obtained. Genes J and K were probably independent of Pc-38.

The F_2 progeny from the cross Pc-55/IB 1454 segregated 13 resistant : 3 susceptible (Table 6.3.6). This segregation ratio was the strongest evidence that genes J and K were present in IB 1454 and that they interacted in a dominant recessive epistatic manner. If only one gene were present in IB 1454, then a single gene segregation ratio would have been expected since Pc-55 was ineffective against CR 13 (Table 6.2.3). When tested with CR 50, 345 resistant plants and 14 susceptible plants were observed (Table 6.3.4). This segregation for resistance could only be explained using the 61 resistant : 3 susceptible model.

The cross Pc-64/IB 1454, when tested with CR 13 and CR 50, gave results that could not be reconciled with the rest of the data (Table 6.3.6). With CR 13, the F_2 population segregated into 328 resistant plants and 27 susceptible plants. This segregation only fit a 15 resistant : 1 susceptible model thus not supporting the hypothesis that two genes for crown rust resistance existed in IB 1454. With CR 50, a single gene segregation ratio was obtained. This could also not be explained since both IB 1454 and Pc-64 were known to be effective against CR 50.

Since IB 1454/IB 1487 segregated for resistance (Table 6.3.6) but Pc-56/IB 1487 did not (Table 6.3.1), it was concluded that Pc-56 and gene A were independent of genes J and K of IB 1454. Similarly, IB 1454 genes were independent of Pc-68 and gene E since IB 2465/IB 1454 segregated for resistance in the F_2 (Table 6.3.6) while Pc-68/IB 2465 did not (Table 6.3.4). Also, since Pc-68 was previously shown to be closely linked or allelic to Pc-46 and Pc-50 (Wong *et al.* 1983), it is probable that genes J and K are independent of Pc-46 and Pc-50.

6.3.6. Inheritance of resistance in IB 3432

When tested with CR 13, the F_2 population of IB 3432/Rodney 0 segregated into 105 resistant plants and 42 susceptible plants (Table 6.3.7). A single dominant gene model best explained these results. When tested with CR 50, a 15 resistant : 1 susceptible ratio was obtained. When the seedlings were categorized based on their reaction to both

test races, it was found that only two genes were present in this F_2 population (Table 6.3.2). One gene governed resistance to both races of crown rust and was designated "L". The second gene conferred resistance only to CR 50 and was designated "M".

Table 6.3.7. Segregation of F_2 seedling populations from crosses of IB 3432 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 3432/Rodney 0	CR 13	105	42	3:1	0.250 - 0.500
	CR 50	132	10	15:1	0.500 - 0.750!
IB 3432/IB 1454	CR 13	330	48	61:3	< 0.001
	CR 50	377	5	253:3	0.750 - 0.900
IB 3432/IB 1487	CR 13	340	22	15:1	0.750 - 0.900
	CR 50	362	0		
IB 2402/IB 3432	CR 13	396	33	15:1	0.100 - 0.250
	CR 50	415	12	253:3	< 0.001
IB 3432/IB 2465	CR 13	419	29	15:1	0.750 - 0.900
	CR 50	437	6	63:1	0.500 - 0.750.
Pc-38/IB 3432	CR 13	374	21	15:1	0.250 - 0.500
	CR 50	350	4	63:1	0.500 - 0.750
Pc-39/IB 3432	CR 13	340	66	13:3	0.100 - 0.250#
	CR 50	398	7	63:1	0.250 - 0.500
Pc-58/IB 3432	CR 13	367	23	15:1	0.250 - 0.500
	CR 50	365	0		
Pc-61/IB 3432	CR 13	350	42		@
	CR 50	360	3	63:1	0.250 - 0.500
Pc-63/IB 3432	CR 13	365	22	15:1	0.500 - 0.750
	CR 50	385	0		
Pc-64/IB 3432	CR 13	329	99	3:1	0.250 - 0.500
	CR 50	421	8	63:1	0.500 - 0.750
Pc-68/IB 3432	CR 13	433	29	15:1	0.975 - 0.990
	CR 50	441	10	63:1	0.250 - 0.500.

! light infection
$X^2_{homogeneity}$ = <0.001
@ data highly variable

F_2 segregation ratios of crosses of IB 3432 with Pc-38 and Pc-68 indicated the same genetic model when tested with CR 13 and similarly with CR 50 (Table 6.3.7). With CR 13 a 15 resistant : 1 susceptible segregation ratio was obtained which indicated that gene L of IB 3432 was independent of Pc-38 and Pc-68. With CR 50, a three dominant gene model

fitted the data which indicated that gene M of IB 3432 also was independent of Pc-38 and Pc-68. Categorizing the seedlings based on their reaction to both test races confirmed the existance of three effective genes operating in each of the two segregating populations (Table 6.3.2).

When the F_2 generation of Pc-39/IB 3432 (Table 6.3.7) was tested with CR 13, a 13 resistant : 3 susceptible ratio was observed which was not expected since Pc-39 was known to be ineffective against CR 13 (Table 6.2.3). The pooled data were also not homogeneous. In any case, independence of gene L and Pc-39 was indicated when the cross was screened with CR 50. A segregation ratio of 398 resistant : 7 susceptible fit a three dominant gene model very well. One gene could be attributed to Pc-39; the other two genes could be attributed to genes L and M from IB 3432.

The F_2 generation of Pc-58/IB 3432 segregated as two independent genes when tested with CR 13 (Table 6.3.7). This showed that gene L and Pc-58 were independent of each other. However, when tested with CR 50, no segregation occurred. This suggested that Pc-58 and gene M of IB 3432 were closely linked or allelic to each other.

When tested with CR 13, the F_2 generation of Pc-61/IB 3432 segregated such that no common Mendelian ratio could be applied to the data (Table 6.3.7). Independence between gene L and Pc-61 was suggested by the data. When tested with CR 50, 360 plants were resistant and 3 were susceptible. This result fitted a three dominant gene model and indicated that

genes L and M were independent of Pc-61. Recalling that Pc-58 appeared to be closely associated with gene M, it can be concluded that Pc-58 and Pc-61 occupy different loci.

The F_2 generation of Pc-63/IB 3432 segregated in a 15 resistant : 1 susceptible ratio when screened with CR 13, indicating that gene L of IB 3432 and Pc-63 were independent of each other. When tested with CR 50, no segregation occurred. It was concluded that Pc-63 and gene M were closely linked or allelic to each other. Since Pc-58 appeared to be associated with gene M it was concluded that Pc-63 and Pc-58 were also closely associated.

When tested with CR 13, the F_2 population of Pc-64/IB 3432 segregated for a single gene (Table 6.3.7). This was not expected because CR 13 was avirulent on Pc-64 and IB 3432. With CR 50, a three gene segregation ratio was obtained as was expected. This showed that genes L and M of IB 3432 and Pc-64 were independent of each other.

The results of crosses between IB 1487, IB 3432, Pc-38, and Pc-63 suggested that these genes may make up a complex locus in the order Pc-38, Pc-63, gene M, and gene B. Pc-38 and Pc-63 were found to be closely associated by Harder *et al* (1980). Gene B and gene M appeared to be closely linked or allelic since no susceptible plants were found in the F_2 generation when it was tested with CR 50 (Table 6.3.7). It was found that gene B and gene M were independent of Pc-38 (Tables 6.3.1 and 6.3.7), but they were closely associated with Pc-63. Gene M was considered located closer to Pc-63 as

no susceptible plants were found in the segregating F_2 generation of Pc-63/IB 3432, but there were some susceptible plants found in the Pc-63/IB 1487 F_2 population. Since Pc-58 did not segregate with gene M, it appeared that Pc-58 was somehow associated with this complex locus also.

6.3.7. Tests of gene independence

The categorization of F_2 progeny based on their reaction to both test races was very useful in determining the number of effective genes for resistance and whether they were independent of each other. Applying CR 13 to the first leaf and CR 50 to the second leaf of the same plants allowed for these categorizations to be made. One disadvantage of this method was that second leaf rust reactions can be different from those of the first leaf (personal communication, Dr. A.P. Roelfs) thus leading to different interpretations. In order to avoid this situation, genes found in each line must be separated into single gene lines. These lines can then be crossed and examined in the F_2 generation for segregation. This latter method is rather more labour intensive and thus fewer genes can be studied over the same period.

6.3.8. Problems with Pc-64

Crosses of Pc-64 with IB 1454, IB 1487, IB 2465, and IB 3432 did not segregate in the F_2 generation as expected. Although this also occurred with Pc-38/IB 1454 and IB 2402/IB 3432, the fact that these abnormal segregations were

mainly associated with Pc-64 suggested that Pc-64 was sensitive to certain gene combinations. Some sort of gene interaction was occurring that effected the expression of Pc-64 or the other resistance genes that were involved in these crosses. Progeny of parental lines of Pc-64 used for crosses with IB 1454, IB 2465, and IB 3432 were uniformly resistant to both CR 13 and CR 50 which confirmed the homozygosity of the parents.

6.3.9. Summary of results

Table 6.3.8 summarizes what genes were found in the *A. sterilis* lines that were studied and what relationships thoses genes had to each other and to certain known Pc-genes. Genes A, C, E, F, and L are dominant genes. Genes B, G, H, I, and M may be either dominant or incompletely dominant. Genes D and J or K may be recessive.

Table 6.3.8. Summary of genetic results involving eight *A. sterilis* lines

line	genes effective for resistance to races: CR 13	CR 50	Relationships
IB 1487	A	B	A and B are independent A is allelic with Pc-56 B is linked with Pc-63, but not Pc-38
IB 2402	C	C,D	C and D independent C and D interact dom - rec epistasis C allelic with Pc-68
IB 2465	E	E,F	E and F independent E allelic with Pc-68
IB 2433	G,H	H,I	G allelic to Pc-56 and gene A I allelic to Pc-61
IB 1454	J,K	J,K	two genes acting dom-rec epistasis independent of all other genes tested
IB 3432	L	L,M	M allelic to gene B, Pc-58 and Pc-63 L independent of all genes tested

Genes C, E, H, J, K, and L may be of most use in oat breeding programs because they could be used to replace or augment crown rust resistance genes that are already in use. However, it is necessary that these genes be isolated such that their effectiveness on a wide range of rust races can be evaluated. Genes C and E may only be new sources of Pc-68. The isolation of the genes in IB 1454 should clarify whether two genes are conferring resistance or whether it is only one gene that is being modified by other gene(s). Gene H will need to be isolated also so that its rust spectrum can be determined without the complicating effects of the presence of other resistance genes.

6.3.10. Uses of resistance genes

There are three main ways that these new genes can be used in a crop improvement program. They can be incorporated singly into new crop cultivars. This is a fairly straight forward process, but the single gene is likely to be defeated in a short period of time. Past history of the crown rust pathogen would indicate that it has a large genetic capacity to develop virulence to new resistance genes.

The second way that these genes could be used is to incorporate the new resistance genes into the existing genetic background of current resistant cultivars. The gene combination would provide a broader spectrum of resistance to the pathogen and be more difficult for the pathogen to develop new virulence to. This idea is based on the gene-

for-gene concept of disease resistance where resistance in the host must be matched by an avirulence gene in the pathogen (Flor 1971). In order for the pathogen to become virulent on a host, it must develop virulence alleles that overcome all the various resistance alleles in the host. The larger the number of resistance genes in the host, the more difficult it will be for the pathogen to develop a new virulence combination that can overcome the resistance gene combination. The drawbacks to gene pyramiding include time to build the pyramid and finding physiologic races that can screen for the presence of the various resistance genes when they are combined with other resistance genes. There is also a concern that a super race of the pathogen could develop that is virulent on all members of the pyramid.

The third use of these genes could be in a multiline where the genes are incorporated singly or in pairs in isogenic lines. Seed of these lines can then be mechanically mixed such that the field population of resistance genes that is experienced by the pathogen is heterogenous. The advantages of this method are that the resistance genes used in the mixture can be changed each season should a major change in the virulence of the pathogen population occur. Multilines put less selection pressure on the pathogen population so the development of new virulence combinations is not encouraged as much. The disadvantages of using multilines includes the large breeding effort in generating

these lines and the lack of uniformity in the crop and related quality problems.

7.0. Transfer of crown rust resistance in three tetraploid and two diploid wild oat accessions to common oats

7.1. Introduction

There is a great number of sources of crown rust resistance from wild oat species with chromosome numbers of 2n=4X=28 and 2n=2X=14. In wheat, there has been good success in transferring resistance from lower ploidy material to the agronomic hexaploid species (Knott, 1987). Similar success with crown rust resistance has not been realized within *Avena*. Beside the differences in chromosome number, there are seven different genomes that have been identified in the diploids and at least three in the tetraploids (Rajhathy and Thomas 1974). These genomes have differing affinities for the *A. sativa* genome.

Pc-20 appears to be the only crown rust resistance gene that has been characterized and named from a tetraploid oat species (Marshall and Myers, 1961). Developing meiotically stable materials from interploidy crosses has been difficult. No crown rust genes from tetraploid material have been transferred to common oats. This is only partially indicative of the problem. Breeders and pathologists have had great success in finding and transferring resistance from wild hexaploid material, thus avoiding the difficulties in making interploidy gene transfers.

Marshall and Myers (1961) crossed *A. strigosa* (a diploid) with *A. sativa* and had no difficulty in obtaining seeds. However, these seeds were only water filled and were

extremely shrivelled when dry. Zillinsky and Derrick (1960) created an autotetraploid oat by doubling the chromosome number of diploid material using colchicine. These autotetraploids were then crossed to *A. sativa*. Zillinsky and Derrick (1960) were the first to show that transfer of genes from a wild diploid to common oats was possible; however, these transfers were genetically unstable. Also, it was found that the autetraploids were partially sterile (Sadanaga and Simons 1960; Dyck and Zillinsky 1962).

Much work was done with CD 3820, an *A. strigosa* line, where several genes for crown rust resistance were found. Pc-15, Pc-16, and Pc-17 were identified by Murphy *et al.* (1958). Dyck and Zillinsky (1963a) showed the presence of two independent genes: Pc-15 and Pc-23. Pc-15 proved to be located on a chromosome that failed to pair with any *A. sativa* chromosomes (Dyck and Zillinsky 1963b). In lines homozygous for Pc-15, the chromosome number was 44 where Pc-15 was found on the extra chromosome pair. Pc-23 appeared to be completely incorporated into normal *A. sativa* (Dyck and Zillinsky 1963a). This is the only example of a successful transfer of genetic resistance from diploid to hexaploid oats.

The objective of this work was to transfer crown rust resistance from three tetraploid accessions and two diploid accessions to *A. sativa*.

7.2. Materials and methods

Three tetraploid lines were used in this study: IB 691, IB 845, and IB 3220. Two diploid lines were also used: IB 2353, and IB 2404. The source, rust reactions, and species of these lines are given in tables 3.1.1, 3.1.2, and 3.2.1.

7.2.1. *A. barbata/A. sativa* crosses

Previous workers have had success in making initial crosses between *A. sativa* and *A. barbata* so this method was used in an attempt to transfer disease resistance from these tetraploid sources to the cultivated species.

The wild oat accessions were used as females in crosses with Rodney 0. Rodney 0 was used as the pollen donor because of its superior pollen production. Because male sterility in the F_1 was complete, it was necessary to backcross the F_1 plants to Rodney 0. This was also desirable since the *A. barbata* genetic background had many poor agronomic traits. The resulting BC_1F_1 plants were screened with CR 13 on the first leaf and CR 50 on the second leaf, but conditions in the greenhouse were too hot for reliable readings. As a result, all of these plants (21) were grown out and backcrossed again since fertility was still a problem. Resulting BC_2F_1 and any BC_1F_2 seedlings were tested with CR 13 and CR 50 as before. Plants that exhibited good resistance to these two races of rust were again backcrossed. Chromosome counts were done at each backcross on the plants that were retained for further backcrossing. Panicles were collected from tillers with three to five

centimeters of the boot extended. These were used to observe pollen mother cells (PMCs) for meiotic stability.

7.2.2. Embryo culture of BC1F1 seeds

Of the many pollinations made between the two species, only five F_1 sterile plants resulted. These plants were backcrossed to Rodney 0. To improve the chances of obtaining viable plants from this backcross, embryos from immature seeds were excised and cultured (using a method developed by Dr. C.C. Chu, Plant Science Dept., University of Manitoba (pers. com.)).

A modified N6 culture medium was used (Chu and Hill 1988) and was further altered with the addition of agar, 2,4-D, and kinetin, and a reduction in sucrose from 90000 mg/L to 60000 mg/L.

Embryos were cultured 10 to 14 days after pollination under a sterilized laminar air flow hood. Embryos were excised from sterilized caryopses and placed scutellum down on the culture medium. Embryos were kept under a day night light regime and transplanted when a small shoot and root had developed.

7.2.3. *A. barbata/A. barbata* crosses

The *A. barbata* accessions were crossed with the susceptible *A. barbata* IB 1212 so that an inheritance study could be carried out at the tetraploid level. The tetraploid lines were also crossed with IB 1000-128 (selected as a

tetraploid) before it was discovered that it was a hexaploid.

7.2.4. A. *hispanica*/Rodney 0 crosses

IB 2353 and IB 2404 were used as females in direct crosses with Rodney 0, but no viable seed was recovered. Because of the failure in making a direct 2X/6X cross, another approach was tried.

Based on the success in crossing tetraploids to a hexaploid, autotetraploids were developed from these two diploid lines. A method for chromosome doubling using colchicine ($C_{22}H_{25}NO_6$) was adapted from Dorsey (1939). Germinated seeds with roots 0.5 to 1.0 cm long were treated for 1, 2, 4, and 8 hour periods in a 0.1% colchicine solution. After the treatment, the seeds were washed in cold tap water for ten minutes to rid the seeds of any residual colchicine. These seeds were planted. Since the resulting seedlings were expected to be weakened by the treatment, they were covered with clear plastic covers to ensure a moist environment until the seedlings were established. Seeds from these colchicine treated plants were collected and chromosome counts were made using root tips of seeds germinated in a petri dish. Those seeds that exhibited a doubled chromosome number of 28 were then grown for seed and crosses attempted with Rodney 0.

Zillinsky and Derick (1960) were able to obtain hybrid seed only when the autotetraploid was used as the female. As

a result, the autotetraploid was used as the female in crosses since the viability of pollen was probably low.

To determine the fertility of the autotetraploid plants, selfing was enforced by covering the panicles with glasine bags.

7.2.5. *A. hispanica/A. canariensis* and *A. matritensis* crosses

The two diploid lines were also crossed with two other diploids so that an inheritance study could be undertaken. The crosses were not successfully made.

7.3. Results and discussion

7.3.1. *A. barbata/A. sativa* crosses

F_1 plants were obtained from the crosses with all three tetraploids. The five F_1 plants were sterile. BC_1F_1 and BC_2F_1 plants tended to be fully or partially sterile although they displayed some of the phenotypic traits of Rodney 0.

Twenty-one BC_1F_1 plants were grown: three from cultured embryos and the rest from naturally maturing seed. This indicated that embryo culturing of this material was unnecessary.

Of 152 BC_2F_1 and 104 BC_1F_2 plants that were screened with CR 13 and CR 50, eight plants were selected based on resistant reactions to seedling inoculations of CR 13 and CR 50 (Table 7.3.1). Seven plants (SF 1401 - SF 1407) had IB 3220 parentage and were resistant to both test races; one

plant (SF 1408) had IB 845 parentage and gave resistance to both races, but it was expected to confer resistance only to CR 50. One BC_2F_1 plant (SF 1402), although not vigorous, was highly fertile.

Although a chromosome count from root tips was not available for SF 1402, a chromosome count of 42 was expected in the progeny of this plant. Instead, a chromosome count of 2n=44 was obtained from one progeny of SF 1402. This indicates that SF 1402 was aneuploid - probably 2n=43 or 2n=44. The next steps will be to test all the progeny of SF 1402 with CR 13 and CR 50, to determine the inheritance of crown rust resistance, to backcross a few of the resistant progeny to Rodney 0, and to monitor the meiotic stability of the material. It would be expected that the inheritance of crown rust resistance would involve only one or two dominant genes since the chances of transferring multigenic resistance is highly unlikely given the few plants that were involved in this work.

Table 7.3.1 Rust reaction, phenotype, fertility, and chromosome number of selected BC_2F_2 plants.

key #	pedigree	rust reaction		height	tiller	presence	fertility	chromosome
		CR 13	CR 50	(cm)	number	of awns	% (# florets)	number
SF 1401	IB 3220/3*Rodney 0	;	;	83	6	no	0 (40)	41
SF 1402	IB 3220/3*Rodney 0	0	;,1	95	8	no	90 (70)	no count
SF 1403	IB 3220/3*Rodney 0	0	0	115	12	no	0 (50)	40
SF 1404	IB 3220/3*Rodney 0	;	;-	80	12	single	0 (50)	no count
SF 1405	IB 3220/3*Rodney 0	;	;-		>15	single	0 (100)	40
SF 1406	IB 3220/3*Rodney 0	;	1	115	>15		2 (50)	43
SF 1407	IB 3220/3*Rodney 0	;	1	85	>15	single	0 (100)	41
SF 1408	IB 845/3*Rodney 0	1 sp	1-		1			no count

sp - single pustule

The other 248 plants that were not carried on were generally susceptible to one or both of the test races. It

was felt that carrying on material that had low or no resistance was of little value.

One of the difficulties in this work lay in the differing genomic structures of both parents. *A. barbata* is made up of genomes A and B; *A. sativa* is made up of genomes A, C, and D. Transfer of resistance was most likely to occur only if the resistance gene were located in the A genome. Transfer of resistance could occur when the chromosomes of the A genomes of each parent paired homologously and thus could exchange genetic material during crossover events. Rajhathy and Thomas (1974) showed that the A genomes of both species were not completely homologous; they found loose chromosome pairing in pentaploids from crosses of the two species. If crown rust resistance genes were located on the B genome of *A. barbata*, the chance of a successful crossover event was very low since only homeologous pairing could be expected.

PMCs were observed for some of the BC_2F_1 plants. Later tillers were used so only cursory observations were made. There were two main indications of meiotic instablility. Univalents were observed at metaphase I for SF 1403, SF 1404 and SF 1406. The univalents in SF 1406 were observed to be separating into their component chromatids during anaphase I. Micronuclei were present at the tetrad stage in SF 1401 and SF 1407. All of these factors are indicators of chromosome losses.

7.3.2. *A. barbata/A. barbata* crosses

The attempts at crossing the resistant wild oat accessions with a susceptible accession of the same ploidy level were unsuccessful. This was due to two reasons. The *A. barbata* florets were smaller and more delicate such that lethal damage of the floret was more likely during manual emasculation. A second reason was that the ideal time of anther dehiscence for *A. barbata* was different from *A. sativa* (Nishiyama 1970). The proper timing for pollination using these small *barbata* anthers was not realized. As a result, delivering viable pollen to the female was much less likely. Since no F1 seeds were generated, an F2 population for studying the inheritance of crown rust resistance in these tetraploid accessions was not available.

7.3.3. *A. hispanica*/Rodney 0 crosses

The initial crosses between the diploid accessions and Rodney 0 (2n=6X=42) resulted in the same type of shrunken, inviable seeds as described by Marshall and Myers (1961). Dissection of the developing seeds at 12 to 14 days after pollination revealed that the seeds contained only a cloudy liquid rather than being filled with thick milky endosperm as is normal. No embryo could be found as would be expected with a normally developing caryopsis.

Table 7.3.2 displays the results of developing autotetraploid lines from diploid material. It appeared that as treatment time increased, the rate of formation of autotetraploids increased but plant survival decreased. Two

to four hours treatment appeared appropriate for chromosome doubling of this oat material.

Table 7.3.2. Results of colchicine treatment on IB 2353 and IB 2404.

Accession	colchicine treatment time (hours)	plants producing seed	seeds/ plant	4X plants/ seeds tested
IB 2353	1	10/10	> 100	0/7
IB 2404	2	10/10	30	4/10
IB 2353	4	6/10	22	3/6
IB 2404	8	2/10	4	0/1

Seven autotetraploid plants were identified. Chromosome counts were done twice to confirm the chromosome number of the selected plants: once on germinated seeds and later on late juvenile plants. Table 7.3.3 lists these plants, their chromosome number, and phenotype. It was noted that the crowns of SF 1342 and SF 1343 were above ground which made these plants very prone to lodging.

Table 7.3.3. Chromosome number and phenotype of seven doubled diploid plants.

key number	source	chromosome number	height (cm)	tiller number	crown location	fertility %
SF 1340	IB 2353	28	130	> 15	below ground	44
SF 1342	IB 2353	28	90-120	> 15	above ground	10-20
SF 1343	IB 2353	28	140	> 15	above ground	10-20
SF 1358	IB 2404	28	100	> 15	below ground	0-5
SF 1360	IB 2404	28	105	> 15	below ground	0-5
SF 1361	IB 2404	28		> 15	below ground	0
SF 1369	IB 2404	27		> 15	below ground	0-5

Meiotic instability of the autotetraploids was a problem. This was expressed as low fertility in these plants and abnormal chromosome configurations in the pollen mother cells. In SF 1340, micronuclei were seen in many cells at the tetrad stage. Univalents, trivalents, and quadrivalents were observed in SF 1343 and SF 1361. Univalents appeared to separate into chromatids at anaphase I. This separation lagged behind the separation of bivalents. Cells at anaphase

I also displayed uneven chromosome reduction. Some reductions were equal with 14 chromosomes being found at either cell pole, but others were unequal with 13 chromosomes gravitating to one pole and 15 to the other. SF 1358 displayed lagging chromosomes at anaphase II and micronuclei at the tetrad stage. Observations for SF 1342, SF 1360, and SF 1369 were not made.

Six of the seven plants were successfully hybridized with Rodney 0, SF 1360 being the exception (Table 7.3.4). The rate of successful pollinations was higher than the natural self pollination rate of the autotetraploid parent (Table 7.3.3 and 7.3.4).

Table 7.3.4. Autotetraploid/Rodney 0 crosses and pollination success rate.

Cross	# seeds/# pollinations attempted	% seed set
SF 1340/Rodney 0	30/55	55
SF 1342/Rodney 0	2/	
SF 1343/Rodney 0	20/48	42
SF 1358/Rodney 0	29/96	30
SF 1360/Rodney 0		
SF 1361/Rodney 0	20/64	31
SF 1369/Rodney 0	8/43	19

At this stage of the work, it is hoped that some of the hybrid seeds are viable and resulting F_1 plants still exhibit crown rust resistance. It is hoped that further backcrossing will allow crossover events to occur between the resistance donor and the Rodney 0 genomes such that the gene(s) for disease resistance can be tranferred to the *A. sativa* background.

7.3.4. *A. hispanica/A. canariensis* and *A. matritensis* crosses

A. hispanica was crossed with two other susceptible diploids in order to generate segregating F_2 populations such that the inheritance of resistance could be determined. These crosses were not obtained for two main reasons. Differing genomes were probably a major reason why crosses could not be made. Using Baum's (1977) taxomomic key, *A. hispanica* may have an A genome; *A. canariensis*, an A genome; the genome of *A. matritensis* was not classified. As a result, chromosome pairing in hybrids may have been poor. It was not realized that differences in flowering times existed with these lines. Nishiyama (1970) found that the genome A diploid species were evening blooming types. Crossing was done using the afternoon blooming period which was suitable for the hexaploid material.

8.0. Miscellaneous topics

8.1. A haploid oat

There are a few reports of allopolyhaploids in *Avena sativa* L.: the first being by Nishiyama and Tabata (1964) and the latest by Leggett (1977).

Of approximately 12,000 F_2 seedlings from crosses involving *A. sterilis*/Pc-lines that were observed in this study, six sets of twin plants from single seeds were isolated. Of these six, one set of twins consisted of a normal plant with 42 chromosomes and a second plant with 21 chromosomes - a haploid oat.

These twins came from the cross Pc-64/IB 1487 and were evaluated for several traits (Table 8.1.1). As similarly observed by Nishiyama and Tabata (1964), the haploid plant was less vigorous and had smaller plant parts than its normal twin. The haploid maintained the expression of simply inherited traits such as rust reaction. It was interesting to note that spikelet number on the primary culms was the same. This may indicate the simple inheritance of this character.

Table 8.1.1. Morphology, fertility, rust reaction, and chromosome number of SF 10679 and SF 10679H.

Trait	SF 10679	SF 10679H
height (spikelet extended) (cm)	85	71
stem diameter (base) (mm)	2.5	2.5
length of spikelet (cm)	3.7	2.6
length of spikelet + awn (cm)	6.6	3.6
flag leaf width (mm)	6.0	4.5
flag leaf length (cm)	13.3	7.5
spikelets/primary panicle	10	10
fertility	100	0
CR 13 rust reaction (first leaf)	0	0
CR 50 rust reaction (second leaf)	1	4
chromosome number	42	21

Figure 8.1.1. Panicles of SF 10679 and SF 10679H

SF 10679H is an example of a spontaneously occurring haploid oat (Figure 8.1.1). An abberation during the early development of the zygote was probably the cause of its formation. Since both plants came from the same seed, it would appear that fertilization was normal. During early embryo development, a cell may have undergone two cell divisions in a row without going through a complete cell cycle. This would have led to reductional division in the second mitotic event and thus the haploid chromosome number.

8.2. Sterility of two hexaploid interspecific crosses.

The crosses Pc-56/IB 1454 and Pc-55/IB 1487 were both highly sterile and produced only a few seeds. These two crosses were of interest because they were the only two of 64 similar crosses made that were sterile. The sterility of these two crosses may have been due to translocation heterozygosity of the F1 hybrid seed. McKenzie *et al.* (1970) indicated that segmental differences between species of oats is common. Structural differences between parents could cause difficult pairing arrangements which may result in the formation of duplication and deficiency gametes. Sufficient structural differences due to translocations may create a reproductive barrier. If structural differences exist, their effect will be maximal in the F_1 and may be expressed as sterility (McKenzie *et al.* 1970).

8.3. A morphologic abnormality observed in an A. *barbata*/ A. *sativa* cross

The plant, SF 55, derived from the IB 3220/2*Rodney 0 cross, exhibited ovaries with three stigmatic branches attached to it instead of the usual two branches.

9.0. Recommendations for future research

1. It has proven to be useful to use two races of rust on the same plants in these genetic studies. The rusts used had differing ranges of virulence: CR 13 being virulent on many genes and CR 50 being virulent on fewer and different genes. Differences in range of virulence allowed for the possibility of differential reactions to occur.

2. Work in the area of genomic structure and evolution of oats should be further encouraged. Oat species appear to be more distinct from each other than wheat. Making interspecific crosses is more difficult. If genes for resistance are to be transferred from lower ploidy material to common oats, it is important to know which species can be used for gene transfer and how they might be used.

3. Crown rust resistance and floret characters have been shown to be independent in a number of studies over the years. Further work in this area seems unnecessary.

4. Looking for enhancement of disease reaction through interaction of resistance gene pairs might be of interest. Genes in combination with genes already incorporated into registered cultivars would be the most useful gene combinations to investigate.

5. The Iberian collection has only been evaluated for crown rust and stem rust resistance. Other characters such as protein percent, amino acid variation, bran percent, drought and salt tolerance could be evaluated. Work is required to maintain this collection. The seed sources are

aging and require rejuvenation. Ideally, certain characters would be evaluated while being grown for new seed.

6. Currently, there are two main methods for inoculating flats of seedlings: using a spore-talc mix applied with a duster or using an oil-spore mix applied with a mister. Although both methods provide adequate inoculation of the plants, they could be improved. Talc is not phytotoxic to young oat seedlings, but reading highly resistant plant reactions can be hampered by the presence of talc residue. The oil that is used as a carrier has some phytotoxic effects on oat seedlings causing some leaf edge burning or whole leaf necrosis depending on the genetic background of the material. A nonphytoxic carrier should be found. With either method, a suitable inoculation area is required which can scrub stray rust spores and carrier out of the air so that contamination of other research materials is minimized. An enclosed work area set up with overhead fine spray water nozzles would be suitable for this.

7. The oat gene nomenclature book (Simons *et al.* 1978) needs to be updated. This is a very useful reference to anyone working in oat genetics.

8. F_3 lines now exist for all of the crosses (*A. sterilis*/Rodney 0) made in the inheritance study. Those lines could be used as a source of material to isolate the newly identified crown rust resistance genes. IB 2433 was identified in screening work as containing some stem rust resistance. F_3 lines of the cross IB 2433/Rodney 0 could be

used to determine the inheritance of this stem rust resistance.

9. Progeny from SF 1402 should be tested for rust resistance and resistant plants backcrossed again with Rodney 0. Similarly, the progeny from the autotetraploid/Rodney 0 crosses should be evaluated and backcrossed to Rodney 0. There is potential in this material for the incorportation of disease resistance from this lower ploidy material into an *A. sativa* background.

10.0. References

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Appendix 1. The Differential Sets

A. The Standard Differential Set

cultivar	source and pedigree	Pc-genes
Anthony Victoria	White Russian/Victory (1) Introduction of *A. byzantina* from Uruguay (2)	2,11,12 (3)
Appler	Red Rustproof (1)	1 (3)
Bond	Red Algerian/Gold rain (4)	3,4 (3)
Landhafer	Introduction of *A. byzantina* from Germany (4)	5 (3)
Sante Fe	A pureline selection from an unnamed commercial oat in South America (4)	6,7,8,9c,21 (3)
Ukraine	Introduction of *A. sativa* from Russia (4)	3c,4c,6c,9 (3)
Trispernia	Introduction of *A. sativa* from Rumania (4)	6d (3)
Bondvic Saia	Introduction of *A. strigosa* from Brazil	15,16,17 (3)

1 - Coffman *et al.* 1961
2 - Torrie 1939
3 - Simons *et al.* 1978
4 - Welsh *et al.* 1953

B. The Current Differential Set

cultivar or single gene line	source (1)	pedigree
Pc-35	*A. sterilis* D-137	Pendek*4/D137 (2)
Pc-38	*A. sterilis* CW 491-4	CAV 2648-4/4*Pendek (2)
Pc-39	*A. sterilis* F-366	CAV 5165/4*Pendek (2)
Pc-40	*A. sterilis* F-83	Pendek*2/CAV 4997 (2)
Pc-45	*A. sterilis* F-169	Pendek*4/CAV 5050 (2)
Pc-46	*A. sterilis* F-290	Pendek*4/CAV 5115 (2)
Pc-48	*A. sterilis* F-158	Pendek*2/CAV 5041 (2)
Pc-50	*A. sterilis* CW-486	CAV 2643/4*Pendek (2)
Pc-54	*A. sterilis* CAV 1830 and CAV 1832	Pendek*2/CAV 1832 (2)
Pc-55	*A. sterilis* CAV 4963	CAV 4963/2*Pendek (2)
Pc-56	*A. sterilis* CAV 1964	CAV 1964/4*Pendek (2)
Pc-58	*A. sterilis* TAM-O-301	(1)
Pc-59	*A. sterilis* TAM-O-312	(1)
Pc-60	*A. sterilis* Coker 227	(1)
Pc-61	*A. sterilis* Coker 234	(1)
Pc-62	*A. sterilis* CAV 4274	Fraser*4/CAV 4274 (6)
Pc-63	*A. sterilis* CAV 4540	Fraser*4/CAV 4540 (7)
Pc-64	*A. sterilis* CAV 4248	Makuru *2//CAV 4248/ 2* Sun II (8)
Pc-67	*A. sterilis* CAV 4656	Makuru *2//CAV 4656/ 2* Sun II (9)
Pc-68	*A. sterilis* CAV 4904	Makuru *2//CAV 4904/ 2* Sun II (10)
Pc-55/56	CAV 4963/2*Pendek//4*Harmon Ham /3/ CAV 1964/2*Pendek//4*Harmon Ham (11)	
Ascencau Hudson	contains Pc-2,14	CI 6792/Rodney/2/OT 174 /3/RL 2877/4/Pendek/ Lodi (3)
Fidler	contains Pc-39	Random/RL 3013 (4)
Dumont	contains Pc-38,Pc-39	Harmon Ham/Double cross 7 (5)

1. Simons *et al.* 1978
2. Harder *et al.* 1980
3. McKenzie *et al.* 1976
4. McKenzie *et al.* 1981
5. McKenzie *et al.* 1982
6. Brown 1977-78 N.Z. hybrid nursery 3048-3114
7. Brown 1977-78 N.Z. hybrid nursery 3115-3187
8. Brown 1984 Glenlea hybrid nursery 6253-
9. Brown 1984 Glenlea hybrid nursery 6337,6338,6342
10. Brown 1984 Glenlea hybrid nursery 6344,6351,6355
11. Brown 1978 Glenlea prelim key 553

Appendix 2. Inheritance of crown rust resistance in IB 2428 and IB 2434

Table 1. Segregation of F_2 seedling populations from crosses of IB 2428 and IB 2434 with Rodney 0, selected wild oat accessions, and lines possessing known (Pc) resistance genes

Cross	Test race	Observed R	Observed S	Model R:S	P of X^2
IB 2428/Rodney 0	CR 13	100	44	3:1	0.100 - 0.250
	CR 50	42	102	1:3	0.100 - 0.250
IB 1454/IB 2428	CR 13	426	3	253:3	0.250 - 0.500
	CR 50	428	2	253:3	0.100 - 0.250
IB 1487/IB 2428	CR 13	374	0		
	CR 50	363	8	63:1	0.250 - 0.500
Pc-56/IB 2428	CR 13	396	0		
	CR 50	306	86		&
IB 2434/Rodney 0	CR 13	92	57	9:7	0.100 - 0.250
	CR 50	83	66	9:7	0.750 - 0.900
IB 2434/IB 1454	CR 13	395	5	253:3	0.750 - 0.900
	CR 50	397	1	253:3	0.050 - 0.100
IB 2434/IB 1487	CR 13	200	0		
	CR 50	190	9	15:1	0.250 - 0.500
Pc-56/IB 2434	CR 13	417	0		
	CR 50	374	39	15:1	0.005 - 0.025.

& number of susceptible plants was extremely variable between progeny of individual F_1 plants

Crosses with IB 2428 resulted in conflicting results (Table 1). The F_2 progeny from the cross IB 2428/Rodney 0 segregated for resistance and susceptiblity for a single dominant gene when screened with CR 13. When tested with CR 50, a single recessive gene appeared to control resistance to crown rust. The F_2 progeny of the cross Pc-56/IB 2428 did not segregate when tested with CR 13 which indicated that a gene in IB 2428 was allelic or closely linked to Pc-56. Screening the same cross with CR 50, however, gave variable results. F_2 progeny from individual F_1 plants indicated the presence of one or two genes. It was possible that the seed source for IB 2428 was not pure or that contamination during pollination occurred.

The F_2 progeny form the cross IB 2434/Rodney 0 segregated in the F_2 generation in a 9 resistant : 7 susceptible ratio when screened with CR 13 and CR 50 (Table 1). When the reaction to both test races was considered the progeny fell into four phenotypic resistance classes in the ratio 27 R/R:12 R/S:12 S/R:13 S/S (X^2=5.0828, 0.100<P<0.250). The F_2 progeny of the cross Pc-56/IB 2434 did not segregate when tested with CR 13 indicating that IB 2434 contained one gene either allelic or closely linked to Pc-56.

There is not enough information available to make conclusions as to the actual number of resistance genes in IB 2428 or IB 2434.