ALLELIC RELATIONSHIP OF *A. STRIGOSA* CROWN RUST RESISTANCE FACTORS AND THEIR RELATIONSHIP TO *PC38* SUPPRESSION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

An investigation of the suppression effect of *Pc38* on *Pc94* was conducted by developing F_2 segregating populations from genotypes possessing *Pc38* and *Pc94*, together with a population void of the *Pc38*. Segregating phenotypic ratios were used to determine the crown rust resistance suppression at the *Pc38* locus. A Leggett/Df-38 F_2 population, possessing no *Pc38* gene, produced resistant to susceptible ratios of 3:1 and 2:1. Leggett/Dumont and Leggett/Steele population possessing a copy of *Pc38* each, produced resistant to susceptible ratios of 1:3, 1:2 and 3:13. Leggett was the resistant parent possessing *Pc94* while Dumont and Steele were the susceptible parents possessing *Pc38*. Df-38, a susceptible parent, contained neither *Pc94* nor *Pc38*. CR91 was used to identify resistant and susceptible plants in the segregating populations. A factor closely linked to *Pc38* or the gene itself was confirmed to suppress the resistance effect of *Pc94* in this study. A second experiment was conducted to determine the allelic relationship of crown rust resistance genes in 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. The genotypes possessed resistance genes introgressed from *A. strigosa* similar to Leggett. Leggett is homozygous for *Pc94*. Four different F_2 segregating populations were developed from Leggett by 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. A crown rust race virulent to *Pc94* was used to determine resistant to susceptible ratios of the populations. Crown rust race 16MN (100-3) was used to discriminate between resistant and susceptible plants. The allelism test confirmed that the resistance gene present in 08BT26-2 and 08BT70-1 genotypes were the same as *Pc94* in Leggett while genotypes BT1020-1-1 and BT1021-1-1 were different.

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DEDICATION

This dissertation is dedicated to my family.

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GENERAL INTRODUCTION

Crown rust is a fungal disease incited by *Puccinia coronata* f. sp. *avenae*. Crown rust is considered to be the most extensive and destructive disease of oat with the capacity to reduce grain yield from 10 to 40%, depending on the disease severity and environmental factors. Total crop failure is possible in specific oat fields (Fetch *et al*., 2011; Simons, 1985). The extensive genetic diversity and complexity of the crown rust pathogen contributes to a grain yield loss of more than 50% in Brazil (Leonard and Martinelli, 2005; Martinelli *et al*., 1994).

Oat crown rust continually causes production losses, and breeding resistant cultivars conventionally is considered the most efficient method to control disease losses (Przystalski *et al*., 2013). Due to the changing races of oat crown rust (Leonard, 2003), pyramiding resistance genes has been used to provide protection against the spectra of races for a longer period of time in cultivars. However, interaction of resistance alleles and resistance inhibitors complicate pyramiding efforts. Wilson and McMullen (1997) reported a suppression effect of resistance gene *Pc38* or a closely linked factor on the resistance conferred by *Pc62*. Both *Pc38* and *Pc62* resistance genes were introgressed into *A. sativa* from *Avena sterilis*, a wild hexaploid species. A resistance gene *Pc94*, introgressed into lines/genotypes from a diploid species - *Avena strigosa* was also suppressed by *Pc38* (Chong and Aung, 1996).

Progeny of 'Steele' and 'Dumont' revealed *Pc38* resides at different loci in the genome in the F_2 segregating families. Susceptible individuals were observed in the segregating progeny even though both Steele and Dumont were homozygous for *Pc38* (Leach and McMullen, 1990). Progeny lines were identified with the *Pc38* locus duplicated, as well as lines deficient for the *Pc38* locus. We evaluated if the deficiency of the *Pc38* locus enhances the expression of *Pc94*. Understanding this phenomenon is important since many breeding lines in the NDSU oat

breeding project possess *Pc38*. *A. strigosa* is the source of three independently derived crown rust resistant lines produced in three different breeding programs and their identity needs to be verified. The three independently derived crown rust resistance factors from *A. strigosa* were introgressed at University of Minnesota St. Paul, Minnesota (Rines *et al*., 2007), University of Wisconsin, Madison (Duerst *et al*., 1999) and Winnipeg, Manitoba by Agriculture and Agri-food Canada Cereal Research Center (Mitchell Fetch *et al*., 2006).

The objective of this research was to determine the relationship between *Pc38* and *A. strigosa* derived crown rust resistance genes and to delineate the suppression of *A. strigosa* derived resistance effects when combined with factors at the *Pc38* locus. Specifically, we want to determine if *Pc38* suppresses *Pc94* and other *A. strigosa* derived genes as observed in crosses made between Leggett and *Pc38* genotypes. In addition, an allelism test was conducted to determine if *Pc94* in 'Leggett' was the same in 'Gem' and lines developed by Rines *et al*. (2007). Crown rust resistance in Leggett, Gem and lines developed by Rines *et al*. (2007) were independently introgressed from *A. strigosa*.

CHAPTER I: LITERATURE REVIEW

History and Domestication of Oat

Common oat *Avena sativa* L. (Coffman, 1977) is an important cereal crop that belongs to the genus *Avena L* in the *Gramineae* family. The origins of oat domestication are southwestern Asia and western Mediterranean for hexaploid oat; western Mediterranean for tetraploids; Middle East-Hindukush region, and the Iberian Peninsula-northwestern Africa region for some oat subsections (Murphy and Hoffman, 1992). Wild and weedy oat species have a wide diversity in their morphological characters (Travlos and Giannopolitis, 2010). They serve as a gene pool from which cultivated oats can be improved. Wild *Avena* species have different levels of ploidy, and hexaploid species exhibit easy crossability with cultivated species.

A. sterilis and other wild oat relatives have good sources of the genes for oat crown rust resistance. *A. sterilis* possesses a wide range of morphological and physiological variation. Wild oat, - *A. fatua*, also has weedy characteristics such as fruitfulness, seed shatter, and a large and persistent seed bank with variable degrees of primary seed dormancy. It can easily survive on its own and requires no special care as is the case of cultivated species (Beckie *et al*., 2012). All quantitative characters of *A. sterilis* showed variability in morphological characteristics such as plant height, number of leaves and tillers per plant, awn and floret length and lemma hairiness. *A. fatua* has less variability in the species as compared to *A. sterilis*. *A. sterilis* exhibits high variation in terms of open lemma color ranging from a yellowish shade to dark brown. *A. fatua* is prolific with great competitiveness (Travlos and Giannopolitis, 2010). *A. fatua* has been described as a highly polymorphic species with wide variability and low phenotypic variation among ecotypes (Allard, 1965; Jain and Marshall, 1967).

Beckie *et al*. (2012), cited sources that suggest distinguishing features between wild and cultivated oat. In the field, *A. fatua* (wild oat) compared to *A. sativa* (cultivated oat) is taller and more vigorous with whitish colored straw and chaff at maturity. Also, the wild species generally have more drooping panicles than the cultivated oat. *A. sativa* florets do not disarticulate at maturity as *A. fatua* does. *A. fatua* has long, twisted geniculate awns while *A. sativa* has near absence of awns. The varying seed colors of *A. fatua* contrast the yellowish-white seeds of *A. sativa*. *A. fatua* seed dormancy is characteristically intense and periodic with near absence of dormancy in the cultivated species. There are however less visible differences in the epiblasts and lodicules. The lodicule of *A. sativa* is differentiated from *A. fatua* by an attached side lobe. In *A. fatua* the lodicule is absent (Baum, 1968). The apex of the epiblast of *A. sativa* is almost whole, while the apex of the epiblast of *A. fatua* is irregularly crenate, eroded, or sinuate. Differences in habitat have led to a wide range of genotypic differences in populations of *A. fatua*, and those differences in agronomic practices could explain the variations in seed dormancy, germination, growth and herbicide responses (Beckie and Hall, 2012).

The domestication of oat follows a pattern similar to wheat and barley. Domestication of crops, notably grasses is one of the crucial events in the history of human evolution. The transition from wild plants to domesticated ones is more of an evolutionary adaptation resulting from human activities. Domestication of crop plants dates back to 5000-10,000 years ago. The most primitive trait of domestication is the loss of seed dispersing mechanisms in the grain crop plants (Ladizinsky, 1995). Rare mutations are the players for the traits marking domestication. However, hybridization is the method used much later in the modern era with the advancement of science and technology. An important trait present in domesticated varieties and absent in wild varieties is high grain yield, resulting from larger seed size with high harvest index

(Holland, 1997). Domestication of wild tetraploid oats *A. magna* and *A. murphyi* was not from selection of mutated genes but by transferring domesticated traits into them from *A. sativa* through interspecies hybridization (Ladizinsky, 1995). The genes for the domesticated traits transferred to these wild species include non-shedding spikelets, glabrous and yellow lemma, and reduced awn formation genes. These traits are controlled by a single gene each and hence easy to transfer to other species (Marshall and Shaner 1992). Useful traits also have been transferred from *A. sterilis* and *A. fatua* that resulted into newer and better evolved versions of *A. sativa*. Some of these genes are disease resistance, herbicide resistance genes, genes for high grain oil content, high grain protein content, and genes for high grain yield, large seed size and nematode resistance (Holland, 1997).

Dispersion of the Domesticated Oat Lines

There are diverse schools of thought concerning the origin of oat. Stanton (1953) indicated common oat was first discovered in different areas of Western Europe where it spread to other areas of the world. Stanton suggested that Mal'tzev, a different author, believes that *Avena fatua* is of Asiatic origin. It has likewise been suggested that *Avena sativa* was grown for grain and *Avena byzantina* (red oat) was grown as forage in Asia Minor. *Avena abyssinica* was found growing in barley fields as weeds in Ethiopia. Oat was brought along with barley, wheat and rye into America by Captain Bartholomew Gosnold. In the south coast of Massachusetts, specifically Elizabeth Island, he planted the grains in 1602. In 1611 the first production was carried out in Virginia with little success at first. In contrast, Murphy and Hoffman (1992), indicated that Coffman (1977) thought that oat was introduced into North America, through two different routes from different parts of Europe. The Spanish first brought *Avena byzantina* to the southern latitudes and the English and other Europeans transported *A. sativa* to Canada a newly

discovered land. These were also transported to Virginia, North Carolina and the northeastern USA in the 16th and beginning of the 17th centuries. *A. byzantina*, they believed was introduced to America from Spain by the Spaniards who came together with their Arabian-derived horses. Oat grain was therefore brought along to feed their horses (Youngs and Forsberg, 1987; Murphy and Hoffman, 1992).

As indicated by Coffman (1961); Vavilov (1926) and Malzew (1930) did not agree that Europe is the origin of *Avena sativa* L. Vavilov came across small areas of Emmer wheat (*Triticum dicoccum* Schlub.) during explorations in Central Persia in 1916. These seeds had been introduced from Turkish Armenia. Admixtures of *A. sativa* were present in the Emmer wheat. This he thought was interesting since in Persia, Afghanistan and Bokhara, oat cultivation was unknown. The presence of types of *A. sativa* as admixtures in Emmer led to the investigation of other grains among Emmer crops in other places. Samples of Emmer obtained from northern Caucasus, Transcaucasia, Armenia, Georgia, Azerbayijan, Asia Minor, Bulgaria, Crimea, the Basque region of the Pyrenees, and Abyssinia contained admixtures of oats. The samples contained grains that resembled common oats in different ways. Others had *A. byzantina* characteristics. Different ripening times, panicle forms, grain characteristics and spikelet separation were observed from plants grown from these various grains. These findings led Vavilov to conclude that the spread of *A. sativa*, from their original geographic center(s) of origin, was largely dependent on the spread of Emmer wheat. As a result, oat was carried northward as a weed admixture in Emmer wheat during its spread (Coffman, 1961).

Oats had been considered as a weed species in barley and wheat fields. They were later selected and domesticated as a crop. Archaeological deposits have shown evidence of non-domesticated *Avena* in Near East and eastern Mediterranean as far back as 7,000 to 12,000 years

ago. In addition, more archaeological evidence has shown domesticated oat in central Europe 3,000 to 4,000 years ago (Black *et al*., 2006). Most of the major crop plants were known to have been domesticated about 5,000 to 10,000 years ago as suggested through archaeological evidence. Characteristics like the loss of seed dispersion mechanism initiated the domestication and extension of grain crops. The raw materials for prehistoric and modern domestication include rare mutations. *Avena magna* and *A. murphyi*, wild tetraploid (2n = 28) oat species were domesticated through transferring non-shedding spikelets, glabrous, yellow lemma, and reduced awn formation characteristics from *A. sativa* (2n = 42) (Ladizinsky, 1995; Ladizinsky, 1971; Murphy *et al*., 1968; Piperno *et al*.**,** 2000; Smith, 2001; Thomas, 1992).

Common oat has been crossed with tetraploid species and the resulting pentaploid F_1 progeny backcrossed with pollen from the tetraploid wild parent, thereby achieving domestication (Ladizinsky, 1995). A and C genome diploid oat species originated in the western part of the Mediterranean region (Atlas Mountains/Pyrenees). Minor variants (Cp, Cv and Al, Ad, Ac, As) were obtained in transient from the A and C genome. Tetraploid oat species have the AC and AB genomes. The diploid species which has As genome is thought to be the origin of the species with AB genome. The origin of *A. magna* Murph. et Fed. is thought to result from overlapping areas of distribution of the diploid species *A. canariensis* Baum and *A. ventricosa* Baum. Tetraploids with the AC genome likely originated from an interspecific cross of diploid species with AC genome such as *A. canariensis* (Ac) and *A. ventricosa* (Cv). This was established from karyotype structure, cytogenetic features and interspecific hybridization data (Loskutov, 2008). The diploid cultivated species *A. strigosa* Schreb. occurs in Great Britain, Germany, Spain and Portugal, indicating the lines of dissemination of oats (Loskutov, 2008; Rajhathy and Thomas 1974).

Characteristics and Plant Requirements of Oat

Oat plants are a grass. The blade, sheath, collar, and ligule make up the leaf. The leaves are solitary, borne on the nodes with alternate and distichous leaf arrangements. Compared to other cereals the auricles are absent in oats which is an effective way of distinguishing oat from other small grains during the vegetative stage (Bonnett, 1961). Depending on the cultivar, day length and environmental conditions, more or less tillers can be produced with two to three tillers producing the most mature grains. More tillers produce a greater volume of biomass production that plays an important role in weed suppression. Axillary buds at the nodes of the crown below soil level generate the tillers. Each tiller develops into the inflorescence with a panicle arrangement that produce the oat grains. Two root systems occur in oats: the seminal root and the crown root system made up of adventitious roots. The smaller seminal root system is made up of the radicle (primary root) and a few adventitious roots that develop from the first nodal area during emergence and seedling growth. The larger crown root system is made up of adventitious roots that develop from the crown several nodes with very short internodes just below the soil level (Bonnett, 1961; McMullen, 2000).

Oat is a cool season annual grass that grows best in areas of 35-50º latitudes north and 20-40º latitudes south (Schrickel, 1986). In the northern and southern hemisphere average temperatures between 16-23ºC and annual precipitation of 50-100 cm is required for optimal oat production (Hoffman, 1995). During heading and grain filling the plants become especially sensitive to dry and hot weather (Murphy and Hoffman, 1992). Apart from the requirement of cool conditions for growth, other environmental conditions include adequate moisture and well drained soils. The oat plant can reach heights of up to 150 cm depending on the cultivar, environmental conditions and time of seeding. Plant height has been classified into 60 to 90 cm -

short, 90 to 120 cm - medium and 120 to 150 cm - tall (Stanton, 1961). The crop can grow on different soil types but can develop well on acid, leached and podzolic soils when compared to other cereals. Depending on the cultivar maturity, the growing period of oat can be between 90-180 days (Murphy and Hoffman, 1992).

Importance of Oat

Oats are important to humans as food, and to livestock, poultry, horses and pigs as feed. The vegetative portions of the plant itself have been used as pasture, silage and hay. The straw also serves as bedding for livestock. Chemical components of oat grain include (1,3 and 1,4) β–glucan, twenty unique polyphenols and avenanthramides (Meydani, 2009). Vitamin E (tocols), phytic acid, flavonoids and sterols are also found in oats (Peterson, 2001). As reported by Litwinek *et al*. (2013), oat contains 2-3 times higher lipid concentration than found in other cereals, as well as high protein content, low carbohydrates, significant dietary fiber, vitamins, thiamine and pantothetic acid. Oat is an important source of high-quality plant protein that is comprised of a complete balance of the essential amino acids. Oat protein usually exceeds the protein nutritional requirements of monogastric animals. The only exceptions were the amino acids lysine and threonine (Peterson, 2011). The oat groat contains about 15-20% protein by weight as influenced by genotype and environment. Oat nutritional quality, functionality and health effects are associated with its storage protein so that protein quality remains constant when protein concentration is increased (Peterson, 1992). In the nutrition of humans and animals, globulin forms the abundant storage protein of oat and has a better amino acid composition than the storage protein prolamin contained in other cereals. In most instances, the quality and quantity of oat protein is considered sufficient for non-ruminant nutrition (Peterson, 2011). Human health benefits of oats include the reduction of serum blood cholesterol and regulation of

gastro-intestinal function from the consumption of the oat bran (Gibson and Benson, 2002). The water-soluble fiber, Beta glucan in oats reduces heart disease by improving HDL-cholesterol (good cholesterol) to LDL- cholesterol (bad cholesterol) ratio (Davy *et al*., 2002; Gibson and Benson, 2002). Beta glucan functions to moderate glycemic and insulin response, and helps boost the immune system against fungi, bacteria, viruses and parasites (Rondanelli *et al.,* 2009). In addition, oat food products increase appetite-control hormones (Beck *et al.,* 2009), as well as helping to reduce asthma risks in children (Virtanen, 2010).

Researchers have also found that oats may help reduce the risk of type 2 diabetes (Lammert *et al*., 2008) and improve insulin sensitivity (Maki *et al*., 2007). Another important oat health benefit is to help control blood pressure (Keenan *et al*., 2002). Research also revealed that intake of oat can help reduce the need for laxatives especially in the elderly (Sturtzel *et al*., 2009). Furthermore, oat has the potential to boost the nutritional profile of gluten-free diets (Kemppainen *et al*., 2010; Løvika *et al*., 2009). Oats are used as antioxidants, and in ice creams and other dairy products as stabilizers. Experiments revealed that consumption of oats reduced obesity and abdominal fat. In addition, the study showed that intake of oats improved lipid profiles and liver functions among the age groups of 18 to 65 with whom the experiment was conducted (Chang *et al*., 2013). In the chemical industry, the hull of the oat is used as a raw material for furfural, which is a refining material for making resin (Gibson and Benson, 2002).

In the cosmetic industry, oat has been used for centuries against various xerotic dermatoses as a soothing agent to relieve irritation and itch (Sur *et al*., 2008). Johnson & Johnson Consumer Companies, Inc., one of the leading cosmetic industries indicates that the Egyptians and Arabians used oats as a skin beauty product around 2000 B.C. Skin ailments were also healed using oat baths by the ancient Romans and Greeks. Colloidal oatmeal has been confirmed

to have moisturizing, barrier repair and anti-inflammatory properties. In an experiment conducted among the age groups of three months to sixty years, moisturizers and cleansers with colloidal oatmeal was shown to greatly improve atopic dermatitis when used daily (Fowler *et al*., 2012).

Crown Rust Disease of Oat

Crown rust incited by *Puccinia coronata* Corda var. *avenae* is the most damaging and widespread of oat diseases because severe infection especially of the flag leaves result in decreased photosynthesis and the transport of synthesized sugars from leaves to the maturing grain is restricted (Fetch *et al*., 2011). This results in shriveled grains which reduces the feed value. In instances of moderate to severe crown rust epidemics, yield loss can range between 10 to 40%. Total crop failure is possible for individual oat fields under heavy crown rust infestation. Certain environmental conditions that facilitate the spread of crown rust include the occurrence of frequent dews and mild temperatures of 15 to 25°C. This temperature range also is the most favorable for optimum oat growth. Therefore, greatest yield loss is experienced at these temperatures when oat yields are expected to be the highest (Simons, 1985).

The symptom of crown rust involves the formation of uredinia on the lower and upper leaf surfaces of infected plants. Heavy infestation of uredinia can occur on the leaf sheath under severe epidemic conditions. Uredinia consist of orange-yellow pustules which are round or oval and can be up to 5 mm in length. The leaf epidermis has to rupture to expose the orange-yellow spores. Black margins develop after 1 to 2 weeks around the uredinia and form teliospores (Simons, 1985).

Initial inoculum source and infestation occurs on oats grown in the fall and usually comes from uredinia infested oat plants that survive the heat of summer through the protection of moist

habitats around streams and/or irrigation channels. *Rhamnus* spp. serves as an alternate crown rust host and source of inoculum for oat in temperate regions of North America and Europe. In the spring, surviving teliospores on straw of infected plants from the previous summer begin to germinate. Basidiospores are formed which infect tender leaves of *Rhamnus*. Basidiospore infection of *Rhamnus* produces aecia, and oat plants are infected by the aeciospores released by the aecia. Free water on surfaces of leaves provide a favorable environment for urediniospores and aeciospores to germinate. At temperatures between 10 to 25°C, germination of urediniospores and aeciospores occur, and infection ensues on leaves through stomata. Temperatures above 30°C inhibit infection (Simons, 1985).

The uredinial stage of crown rust is unable to survive through the winter in the northern states of the United States but does survive in southern states. In the early summer in the south, while crown rust epidemics increase, urediniospores which are air-borne are capable of being blown to the north to infect oats planted in the spring. Nevertheless, a more important source of inoculum for the northern states are aeciospores from *Rhamnus cathartica* (buckthorn bushes). Teliospores, which usually remain on the straw, survive winter conditions in temperate areas. In regions with Mediterranean climates, teliospores can survive hot dry summers. Dormant teliospores are stimulated to germinate in mild wet weather. Tender leaves of *Rhamnus* are infected with basidiospores produced by the teliospores (Simons, 1985).

The crown rust infection of oat occurs across North America, but the incidence is more severe in the "Puccinia Pathway" (Frey *et al*., 1977). The "Puccinia Pathway" consists of a large epidemiological unit found in the central part of North America from the south into Canada. Urediniospores migrate along the "Puccinia pathway" from the southern United States into Canada but do not over-winter in Canada. In North America, the life cycle of crown rust is

mostly asexual, but buckthorn, which is an alternate host, functions as a sexual host for the development of new crown rust races (Fetch *et al*., 2011).

Plant Breeding Efforts on Crown Rust Resistance in Oat

Oat domestication likely resulted in weakened and breaking down of mechanisms in nature that kept a balance between the disease and host. The natural mechanisms include interspecific diversity, intraspecific diversity, host resistance and pathogen population stabilizing tendencies (Browning and Frey, 1969). Over the centuries, through selection, hosts resistant to disease were used in agriculture production and led to the development of uniform resistant cultivars. This uniform resistant cultivar development caused the interspecific and intraspecific heterogeneity of disease control to be lost in the early 1900s, especially in small grains (Frey *et al*., 1977).

Through the work of Stakman and Piemeisel (1917) and Stakman and Levine (1922), several races of stem rust were identified that could be distinguished by the host-pathogen interaction. This discovery led to the search for genes that conferred broad spectrum resistance against the races and developed resistance gene combinations to remain steps ahead of the developing new races (Frey *et al*., 1977). Around 1905 oat breeding in the Mid-west, was initiated along with breeding for crown rust resistance. This included assessing and selection of plants with good agronomic performance and resistance to crown rust from introduced land races from northern Europe, the selection of resistant pure-lines from land races and hybridization of cultivars to combine genes for resistance. Eventually oat cultivars failed to resist the evolving crown rust races (Frey *et al*., 1977).

Oat Resistance Genes

Among all the proposed ways of controlling oat crown rust, genetic resistance is considered the most economical and effective method (Harder and Haber, 1992). *P. coronata* utilizes both sexual and asexual means of reproduction with sexual reproduction responsible for the production of diverse virulence among races (Groth and Roelfs, 1982). Due to sexual reproduction, virulence to resistance often occurs, and new and more durable resistance requires introgression of effective resistance genes into existing cultivars. Pyramiding different and highly effective crown rust resistance genes is currently the technique used by breeders to prolong the effectiveness of the resistance genes in cultivars (McCartney *et al*., 2011; Penner *et al*., 1993). Over the years, many crown rust resistance genes and virulence pathotypes of *P. coronata* have been identified. *Pc2* and *Pc11* from 'Victoria', as well as *Pc3* and *Pc4* from 'Bond', are among the early resistance genes discovered in the 1940s that were important in oat breeding programs in North America (Simons, 1985; Simons, 1978; Chong *et al*., 2000). Over the years, more than 100 crown rust resistance genes, designated as *Pc*, have been identified (Simons *et al*., 1978; Marshall and Shaner 1992; Bush *et al*., 1994). Crown rust resistance can be a single dominant gene, partially dominant or recessive resistance genes (Nof and Dinoor, 1981; Simons *et al*., 1978). The need to search for resistance in wild species occurred when domestic hexaploid sources of resistance were no longer effective. Success has been achieved in the transfer of sources of resistance to cultivated species from wild relatives of lower ploidy levels. Special techniques have been helpful in transferring resistance genes across species of the same genus and even across genera (Dinoor, 1970).

Pc38 and *Pc94* are among the resistance genes present in cultivars of many breeding programs today. The most effective crown rust resistance gene in North America is considered to

be *Pc94* (Chong and Zegeye, 2004). *Pc94* was transferred from a diploid oat RL1697 (*Avena strigosa*) into SunII, a hexaploid oat. *Avena longiglumis* (CW57), a diploid oat, was used as a bridge species to allow pairing between the chromosomes of *A. strigosa* and *A. sativa* (Aung *et al*., 1996). The crown rust resistance genes *Pc38*, *Pc62* and *Pc63* are clustered in the oat genome (Harder *et al*., 1980). The accession CW 491-4, (*A. sterilis*) accession was the source of *Pc38* (McMullen and Patterson, 1992).

Mode of Crown Rust Resistance Genes Inheritance

The hexaploid oat genome of cultivated *Avena sativa* L. is known to be large and complex. It has a 14 Gb genome, the majority of which are repetitive sequences (Xiaomei *et al*., 2012). The chromosome number of *Avena sativa* L. is 2n = 6x = 42 with n = 7 as the basic chromosome number of cultivated, wild as well as weedy species (O'Mara, 1961; Leggett, 1992; Thomas, 1992; Leggett and Thomas, 1995). Three basic sub-genomes exist in the genome of hexaploid *Avena* species. These are genomes A, C, and D, and they contain seven pairs of chromosomes each (Rajhathy and Morrison, 1960; Thomas, 1992).

The inheritance of crown rust resistance genes takes into account the number of genes involved in the expression of the resistance. The inheritance of crown rust resistance is governed by nuclear resistance genes (Staletic *et al*., 2009). Oat resistance to *P. coronata avenae* inheritance can be recessive, intermediate or dominant. Dominant resistance is categorized into complete or incomplete. In complete dominance, resistance is fully expressed in the F_1s while resistance is partially expressed in the F_1s in the case of incomplete dominance. Genes controlling resistance can be monogenic, oligogenic or polygenic. A single gene governs monogenic resistance, a few genes govern oligogenic resistance while polygenic resistance is controlled by added effects of many genes from multiple loci. Resistance to *P. coronata avenae*

is mainly conferred by dominant genes. Different resistant to susceptible plant ratios have been observed and conclusions drawn on mode of resistance inheritance using conventional methods of breeding for hybrid generations F_1, F_2, BC_1 and BC_2, as referenced by Staletic *et al*., 2009.

Different segregation ratios of resistant to susceptible plants observed after crossing of genotypes serves as an indication of the number of genes involved in the resistance. In their experiment, Graichen *et al.*, 2010, tested for fit for 1:1, 3:1 and 1:3 resistant to susceptible ratios for oat crown rust resistance in $F_{5:6}$ and $F_{5:7}$ recombinant inbred lines. A 1AA:1aa ratio results with single gene resistance. In the second case, two effective genes acting independently are involved in the 3:1 ratio with (AABB,AAbb,aaBB):(aabb) genotypes. A 1:3 resistant to susceptible ratio is indicative of two resistance genes that interact with each other through epistasis. The genotypes involved are (AABB):AAbb,aaBB,aabb) or (aabb):(AABB,AAbb,aaBB). Also, a crown rust resistance ratio of resistant to susceptible plant of 9:7 and 7:9 was observed indicating two complementary resistant or susceptibility genes. The 9:7 ratio showed that the two major interacting genes were involved in the disease resistance. This is called duplicate negative recessive epistasis. A 7:9 F_2 segregation ratio on another cross was duplicate positive recessive epistasis, also showing two major interaction genes involved in the crown rust resistance (Staletic *et al.*, 2009).

Inhibitor Genes

Inhibition of the effect of resistance genes has been reported not only in oat but in other crops as well (McIntosh *et al*., 2011). Kerber and Green (1980) described a suppressor of stem rust resistance in the hexaploid wheat (*Triticum aestivum* L.) which was believed to have been derived from *Aegilops squarrosa* L., a diploid ancestor progenitor of common wheat. Suppression of *Pm8* gene for the resistance of powdery mildew (*Blumeria graminis* f. sp. *tritici*

(*Bgt*)) in wheat introgressed from cereal rye (*Secale cereale*) has also been reported (Hanusova, 1996 and McIntosh *et al*., 2011). The suppression of other resistance genes reported in wheat include leaf rust, stripe rust, mildew blotch, glume blotch and tan spot (Trottet *et al*., 1982; Gill *et al*., 1986 and Siedler *et al*., 1994). Also, in an experiment with flax, *L2* and *L10* which confer resistance independently to flax rust produced no resistance when the two alleles were combined into an allele called *suL10* (Shepherd and Mayo, 1972 and Luck *et al*., 2000). In oat, suppression of resistance genes has been identified since 1930 for *Pc1* by Dietz and Murphy (1930). Other inhibition of crown rust resistance genes has been reported for *Pc3* and *Pc4*, *Pc10*, *Pc13* and *Pc14*, *Pc26* by Cochran *et al*. (1945), Finkner (1954), Chang (1959), and Upadhyaya and Baker (I960) respectively. In addition, *Pc38* is among the resistance genes known to by itself or a closely linked factor suppress *Pc62* (Wilson and McMullen, 1997). Chong and Aung (1996) also reported the suppression of *Pc94* by *Pc38*.

A. strigosa Derived Resistance Genes

A. strigosa is a source of resistance genes for crown rust. *A. strigosa* is a diploid (2n = 2x = 14) oat that requires special techniques to transfer resistance into hexaploid (2n = 6x = 42) cultivars (Rines *et al*., 2007 and McMullen, 2000). In the transfer of a resistance gene from *A. strigosa* CI6954SP, Rines *et al*. (2007) used two different methods where in one the *A. strigosa* was directly crossed to *A. sativa* cv. Black Mesdag (hexaploid) and embryo rescue was carried out, after which colchicine was used to double the chromosome number and subsequent crosses were made to the hexaploid oat - *A. sativa* cv. Ogle. In the second method, *A. murphyi* P12, a tetraploid was used as a bridge species between the *A. strigosa* CI6954SP diploid and hexaploid *A. sativa* cv. Ogle (Rines *et al*., 2007). Rines *et al*. (2007), identified the source of resistance genes to be from *A. strigosa*.

Leggett possesses an *A. strigosa* (diploid oat RL1697) derived crown rust resistance gene designated *Pc94*. Leggett was developed by Agriculture and Agri-food Canada Cereal Research Center, Winnipeg, Manitoba. It is resistant to crown rust with the combination of *Pc94*, *Pc68* and possibly *Pc39* resistance genes (Mitchell Fetch *et al*., 2006). Leggett was produced from the cross involving OT294 and a combination of crosses from which *Pc94* was derived. The cross, AC Medallion/OT268 produced OT294 while *Pc94* was derived from Dumont*3/4/SunII*5/3/SunII*2/RL1697//SunII/CW57. An *Avena longiglumis* CW5 line was used as a bridge species to enable easy pairing between *A. strigosa* chromosomes and *A. sativa* chromosomes (Mitchell Fetch *et al*., 2006 and Aung *et al*., 1996).

Gem is a spring oat produced from the pedigree MO0768/6/'Holden'/Irr.4/'Garland'-/2/6x amphiploid/2* CIov6936/3/Garland/5/'Froker'/7/'Ogle' parentage. The final cross, X6051/Ogle was made in 1984. Gem was developed at University of Wisconsin, Madison. At its registration, Gem was resistant to crown rust races CR36, CR152, and CR169. The progenitors that produced crown rust resistance to Gem was a 6x amphiploid from the cross of a tetraploid and diploid (Duerst *et al*., 1999). *Avena abyssinica* (C.I. 2108 and C.I. 2109) was the tetraploid and either *Avena strigosa* (C.I. 3436) or *Avena strigosa* var. Saia (C.I. 4639) were the diploids used (Brown and Shands, 1954; Forsberg and Shands, 1969).

References

Allard, R. 1965. Genetic systems associated with colonizing ability in predominantly self-pollinated species. Genet colonizing spec: Proceedings of 1st biology science symposium genetic biology, pp. 49–76.

Aung, T., J. Chong, and M. Leggett. 1996. The transfer of crown rust resistance gene Pc94 from a wild diploid to cultivated hexaploid oat. p. 167–171. In Proc. European and Mediterranean Cereal Rusts and Powdery Mildews Conf. 9th, Lutheran, Netherlands. 2–6 Sept. 1996. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands.

Baum, B.R. 1968. On some relationships between *Avena sativa* and *A. fatua* (Gramineae) as studied from Canadian material. Can. J. Bot. 46:1013-1024.

Beck, E.J., L.C. Tapsell, M.J. Batterham, S.M. Tosh, and X.F. Huang. 2009. Increases in peptide Y-Y levels following oat beta-glucan ingestion are dose-dependent in overweight adults. Nutr. Res. 29(10):705-709.

Beckie, H.J., A. Francis, and L.M. Hall. 2012. The biology of Canadian weeds. 27. *Avena fatua* L. (updated). Can. J. Plant Sci. 92:1329-1357.

Black, M., J.D Bewley, and P. Halmer. 2006. The encyclopedia of seeds science technology and uses. p. 447-450. In, M. Black, J.D Bewley and P. Halmer (ed.) Oat. CAB International, Cambridge, MA.

Bonnett, O.T. 1961. Morphology and development. p. 41-74. *In* F.A. Coffman (ed.) Oats and Oat Improvement. American Society of Agronomy, Madison, WI.

Brown, C.M., and H.L. Shands. 1954. Behavior of the interspecific hybrid and amphiploid of *Avena abyssinica* x *A. strigosa*. Agron. J. 46:557-559.

Browning, J.A., and K.J. Frey. 1969. Multiline cultivars as a means of disease control. Ann. Rev. Phytopathol. 7:355-382.

Bush, A.L., R.P. Wise, P.J. Rayapati, and M. Lee, 1994. Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome. 37:823-831.

Chang, H.C., C.N. Huang, D.M. Yeh, S.J. Wang, and C.H. Peng, C.J. Wang. 2013. Oat prevents obesity and abdominal fat distribution, and improves liver function in humans. Plant Foods Human Nutri. 68(1):18-23.

Chang, T.T. 1959. Analysis of genes conditioning resistance of oat varieties to races of *Puccinia coronata* Cda. var. *avenae* F. and L. Dissertation Abstracts 20:1133. Ph.D. thesis. University of Minnesota, St. Paul.

Chong, J., K.J. Leonard, and J.J. Salmeron. 2000. A North American System of Nomenclature for *Puccinia coronata f. sp. avenae*. Plant Dis. 84:580-585.

Chong, J., and T. Aung. 1996. Interaction of the crown rust resistance gene Pc94 with several Pc genes. p. 172-175. In G.H.J. Kema et al (ed.) Proc. European and Mediterranean Cereal Rusts and Powdery Mildews Conf. 9th, Lunteren, Netherlands. 2-6 Sept. 1996. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands.

Chong, J., and T. Zegeye. 2004. Physiologic specialization of *Puccinia coronata* f. sp. *avenae* in Canada from 1999 to 2001. Can. J. Plant Pathol. 26:97–108.

Cochran, G.W., C.O. Johnston, E.G. Heyne, and E.D. Hansing. 1945. Inheritance of reaction to smut, stem rust, and crown rust in four oat crosses. J. of Agric. Res. 70:43-61.

Coffman, F.A. 1977. Oat history, identification and classification. USDA-ARS Tech. Bull. 1516. U.S. Gov. Print. Office, Washington, DC.

Coffman, F.A. 1961. Origin and history. p. 15-40. *In* F.A. Coffman (ed.) Oats and oat improvement. Agron. Monogr. 8. ASA, Madison, WI.

Davy, B.M., K.P. Davy, R.C. Ho, S.D. Beske, L.R. Davrath, and C.L Melby. 2002. High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men. Am. J. Clin. Nutr. 76(2):351-358.

Dietz, S.M., and H.C. Murphy. 1930. Inheritance of resistance to *Puccinia coronata avenae*, p. f. III. (Abstract) Phytopathology. 20:120.

Dinoor, A. 1970. Sources of oat crown rust resistance in hexaploid and tetraploid wild oats in Israel. Can. J. Bot. 48:153-161.

Duerst, R.D., H.F. Kaeppler, and R.A. Forsberg. 1999. Crop Sci. 39:879-880.

Fetch, T., B. McCallum, J. Menzies, K. Rashid, and A. Tenuta. 2011. Rust diseases in Canada. Prairie Soils & Crops J. 4:86-96.

Finkner, V.C. 1954. Genetic factors governing resistance and susceptibility of oats to *Puccinia coronota* Corda var. *avenae* F. and L. Race 57. Iowa Agricultural Exp. Stat., Res. Bull. 411:1039-1063.

Forsberg, R.A., and H.L. Shands. 1969. Breeding behavior of two *Avena abyssinica* x *A. strigosa* Amphiploid. Crop Sci. 9:64-67.

Fowler, J.F., J. Nebus, W. Wallo, and L.F. Eichenfield. 2012. Colloidal oatmeal formulations as adjunct treatments in atopic dermatitis. J. Drugs Dermatol. 11(7):804-807.

Frey, P.H., J.A. Browning, and M.D. Simon. 1977. Management systems for host genes to control disease loss. Annals of the New York Academy of Sci. 287:255–274.

Gibson L., and G. Benson. 2002. Origin, History, and Uses of Oat (*Avena sativa*) and Wheat (*Triticum aestivum*). Site sponsor. http://agron-www.agron.iastate.edu/Courses/agron212/Readings/Oat_wheat_history.htm. Accessed October 28, 2013. Department of Agronomy, Iowa State University.

Gill, B.S., H.C. Sharma, W.J. Raupp, L.E. Brouder, J.H. Hatchett, T.L. Harvey, J.G. Moseman, and J.G. Waines. 1986. Resistance in *Aegilops squarrosa* to wheat leaf rust, wheat powdery mildew, green bug and Hessian fly. Plant Dis. 70:553-556.

Graichen, F.A.S., J.A. Martinelli, L.C. Federizzi, M.T. Pacheco, M.S. Chaves, and C. de L. Wesp. 2010. Inheritance of resistance to oat crown rust in recombinant inbred lines. Sci. Agric (Piracicaba, Braz.). 67(4):435-440.

Groth, I.V., and A.P. Roelfs. 1982. Effect of sexual and asexual reproduction on race abundance in cereal rust fungus populations. Phytopathology. 72:1503-1507.

Hanusova, R., L.K. Hsam, P. Bartos, and F.J.Zeller. 1996. Suppression of powdery mildew resistance gene *Pm8* in *Tritium aestivum* L. (common wheat) cultivars carrying wheat-rye translocation T 1BL1RS. Heredity. 77:383-387.

Harder, D.E., R.I.H. McKenzie, and J.W. Martens. 1980. Inheritance of crown rust resistance in three accessions of *Avena sterilis*. Can. J. Genet. Cytol. 22:27–33.

Harder, D.E., and S. Haber. 1992. Oat diseases and pathological techniques. p. 307–425. *In*: H.G. Marshall and M.E. Sorrells (ed.) Oat Science and Technology. Agronomy Monograph No. 33. American Society of Agronomy, Inc., Madison, WI.

Hoffman, L.A. 1995. World production and use of oats. p. 34-61. *In* Welch RW (ed.) The oat crop production and utilization. Chapman and Hall, London.

Holland, J.B. 1997. Oat Improvement. Crop Improvement for the 21st century. Iowa Agriculture and Home Economics Experiment Station. Ames, IA. J-16942.

Jain, S.K., and D.R. Marshall. 1967. Population studies in predominantly self-pollinating species. X. Variation in natural populations of *Avena fatua* and *A. barbata*. Am. Nat. 101:19-34.

Keenan, J.M., J.J. Pins, C. Frazel, A. Moran, and L. Turnquist. 2002. Oat ingestion reduces systolic and diastolic blood pressure in patients with mild or borderline hypertension: a pilot trial. J. Fam. Pract. 51(4):369.

Kemppainen, T.A, M.T. Heikkinen, M.K. Ristikankare, V.M. Kosma, and R.J. Julkunen. 2010. Nutrient intakes during diets including unkilned and large amounts of oats in celiac disease. Eur. J. Clinical Nutr. 64:62-67.

Kerber, E.R., and G.J. Green. 1980. Suppression of stem rust resistance in the hexaploid wheat cv. Canthatch by chromosome 7DL. Can. J. Bot. 58:1347-1350.

Ladizinsky, G. 1971. *Avena murphyi*: a new tetraploid species of oat from southern Spain. Isr. J. Bot. 20:24–27.

Ladizinsky, G. 1995. Domestication via hybridization of the wild tetraploid oats *Avena Magna* and *A. Murphyi*. Theor. Appl. Genet. 91:639-646.

Lammert, A., J. Kratzsch, J. Selhorst, P.M. Humpert, A. Bierhaus, R. Birck, K. Kusterer, and H.P. Hammes. 2008. Clinical benefit of a short term dietary oatmeal intervention in

patients with type 2 diabetes and severe insulin resistance: a pilot study. Exp. Clin. Endocrinol Diabetes. 116(2):132-134.

Leach, G.D., and M.S. McMullen. 1990. Genetics of a Reciprocal Translocation Involving the Crown Rust Resistance Gene Pc-38. P. 29-30. *In* Oat Newsletter Vol.41 abstracts. 14-17 Aug. 1990. American Oat Workers' Conference, Jackson Hole, WY.

Leggett, J.M. 1992. Classification and speciation in *Avena*. p. 32-52. *In* H.G Marshall and M.E. Sorrels (eds.) Oat Science and Technology. American Society of Agronomy, Madison, WI.

Leggett, J.M., and H. Thomas. 1995. Oat evolution and cytogenetics. p. 120-149. *In* R.W. Welch (ed.) The Oat Crop: Production and Utilization. Chapman and Hall, London.

Leonard, K.J. 2003. Regional frequencies of virulence in oat crown rust in the United States from 1990 through 2000. Plant Dis. 87(11):1301-1310.

Leonard, K.J., and J.A. Martinelli. 2005. Virulence of oat crown rust in Brazil and Uruguay. Plant Dis. 89:802-808.

Litwinek, D., H. Gambuś, G. Zięć, R. Sabat, A. Wywrocka-Gurgul, and W. Bersk. 2013. The comparison of quality and chemical composition of breads baked with residual and commercial oat flours and wheat flour. J. Microbiol, Biotechnol. Food Sci. 2(1):1734-1743.

Loskutov, I.G. 2008. On evolutionary pathways of *Avena* species. Genet. Resour. Crop Evol. 55:211–220.

Løvika, A., A.U. Gjøenb, L. Mørkridc, V. Guttormsend, T. Uelande, and K.E.A. Lundina. 2009. Oats in a strictly gluten-free diet is associated with decreased gluten intake and increased serum bilirubin. Eur. Society Clin. Nutr. Metabol. 4(6):315–320.

Luck, J.E., G.J. Lawrence, P.N. Dodds, K.W. Shepherd, and J.G. Ellis. 2000. Regions outside of the leucine-rich repeats of flax rust resistance proteins play a role in specificity determination. The Plant Cell. 12:1367–1377.

Maki, K.C., R. Galant, P. Samuel, J. Tesser, M.S. Witchger, J.D. Ribaya-Mercado, J.B. Blumberg, and J. Geohas. 2007. Effects of consuming foods containing oat beta-glucan on blood pressure, carbohydrate metabolism and biomarkers of oxidative stress in men and women with elevated blood pressure. Eur. J. Clin. Nutr. 61(6):786-95.

Malzew, A.I. 1930. Wild and Cultivated Oats (sectio Euavena Griseb). Bul. Appl. Bot., Gen., and Plant Breeding, Suppl. 38. Leningrad. (English translation: pages 473-506).

Marshall, H.G., and G.E. Shanar. 1992. Genetics and inheritance in oat. p. 510–571. *In* H.G. Marshal and M.E. Sorrells (ed.) Oat science and technology. ASA, and CSSA, Madison WI.

Martinelli, J.A., L.C. Federizzi, and A.C. Bennedetti. 1994. Yield reductions of oat grains due leaf rust severity. Summa Phytopathologica. 20:110-113. (in Portuguese, with abstract in English).

McCartney, C.A., R.G. Stonehouse, B.G. Rossnagel, P.E. Eckstein, G. J. Scoles, T. Zatorski, A.D. Beattie, and J. Chong. 2011. Mapping of the oat crown rust resistance gene Pc91. Theor. Appl. Genet. 122:317–325.

McIntosh, R.A., P. Zhang, C. Cowger, R. Parks, E.S. Lagudah, and S. Hoxha. 2011. Rye-derived powdery mildew resistance gene Pm8 in wheat is suppressed by the Pm3 locus. Theor Appl Genet. 123:359-367.

McMullen, M.S. 2000. Oats. p. 127-148. *In* K. Kulp and J.G.Jr. Ponte (ed). Handbook of cereal science and technology. 2nd edition revised and expanded. Marcel Dekker, Inc., Madison Avenue, NY.

McMullen, M.S., and F.L. Patterson. 1992. Oat cultivar development in the USA and Canada. p. 573–612. *In* H.G. Marshall and M.E. Sorrells (ed.) Oat Science and Technology. Agron Monogr 33. ASA and CSSA, Madison, WI.

Meydani, M. 2009. Potential health benefits of avenanthramides of oats. Nutr Rev. 67(12):731-735.

Mitchell Fetch, J.W., S.D. Duguid, P.D. Brown, J. Chong, Jr.T.G. Fetch, S.M. Haber, J.G. Menzies, N. Ames, J. Noll, T. Aung, and K.D. Stadnyk. 2006. Leggett oat. Can. J. Plant Sci. 87:509–512.

Murphy, H.C., K. Sadanaga, F.J. Zillinsky, E.E. Terrell, and R.T. Smith. 1968. *Avena magna*: An important new tetraploid species of oats. Sci. 159:103–104.

Murphy, J.P., and Hoffman, L.A. 1992. The origin, history, and production of oat. p. 1–28. *In* H.G. Marshall, and M.E. Sorrells (ed.) Oat science and technology. ASA, and CSSA, Madison WI.

Nof, E., and A. Dinoor, 1981. The manifestation of gene-for-gene relationships in oats and crown rust. Phytoparasitica. 9:240.

O'Mara, J.G. 1961. Cytogenetics. p. 112-124. *In* F.A. Coffman (ed.). Oat and oat improvement. American Society of Agronomy, Madison, WI.

Penner, G.A., J. Chong, C.P. Wight, S.J. Molnar, and G. Fedak. 1993. Identification of an RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome. 36:818-820.

Peterson, D.M. 1992. Composition and nutritional characteristics of oat grain and products. p. 265-292. *In* H.G. Marshall and M.E. Sorrells (ed.) Oat Science and Technology. American Society of Agronomy and Crop Science Society of American, Madison, WI.

Peterson, D.M. 2001. Oat Antioxidants. J. Cereal Sci. 33(2):115–129.

Peterson, D.M. 2011. Storage Proteins. p. 123-142. *In* F.H. Webster and P.J. Wood (ed.) Oat chemistry and technology. AACC International Inc. St. Paul, MN.

Piperno, D.R., A.J. Ranere, I. Holst, and P. Hansell. 2000. Starch grains reveal early root crop horticulture in the Panamanian tropical forest. Nature. 407:894–897.

Przystalski, M., P. Tokarski, and W. Pilarczyk. 2013. A method for identifying oat varieties with improved resistance to oat crown rust from a series of field trials. Field Crops Research 149:49-55.

Rajhathy, T., and H. Thomas. 1974. Cytogenetics of oats (Avena L.). Miscellaneous Publications of Genetics Society of Canada. 2. Ottawa Ontario.

Rajhathy, T., and J.W. Morrison. 1960. Genome homology in *Avena*. Can. J. Genet. Cytol. 2:278-285.

Rines, H.W., H.L. Porter, M.L. Carson, and G.E. Ochocki. 2007. Introgression of crown rust resistance from diploid oat *Avena strigosa* into hexaploid cultivated oat *A. sativa* by two methods: direct crosses and through an initial 2x.4x synthetic hexaploid. Euphytica. 158:67-79.

Rondanelli, M., A. Opizzi, and F. Monteferrario. 2009. The biological activity of beta-glucans. Minerva Med. 100(3):237-45.

Schrickel, D.J. 1986. Oat production, value, and uses. p. 1-11. *In* F.H. Webster (ed.) Oats: Chemistry and Technology. American Association of Cereal Chemists, Inc., St. Paul, MN.

Shepherd, K.W., and G.M.E. Mayo. 1972. Genes conferring specific plant disease resistance. Sci. 175:375–380.

Siedler, H., A. Obst, S.L.K. Hsam, and R.J. Zeller. 1994. Evaluation for resistance to Pyrenophora tritici-repentis in *Aegilops tauschii* Coss. and synthetic hexaploid wheat amphiploids. Genet. Res. Crop Evol. 41:27-34.

Simons, M.D. 1985. Crown rust. p. 131-172. *In* A.P. Roelfs and W.R. Bushnell (ed.) The Cereal Rusts. Vol. II, Diseases, Distribution, Epidemiology and Control. Academic Press, Orlando, FL.

Simons, M.D., J.W. Martens, R.I.H. McKenzie, I. Nishiyama, K. Sadanaga, J. Sebesta, and H. Thomas. 1978. Oats: A standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters. U.S. Dep. Agric. Agric. Handb. 509.

Smith, B.D. 2001. Documenting plant domestication: The consilience of biological and archaeological approaches. Proc Natl Acad Sci USA. 13 Feb 2001. 98(4):1324–1326. Washinton DC.

Stakman, E.C., and N.N. Levine. 1922. The determination of biologic forms of Puccinia graminis on Triticum spp. Minn. Agr. Ext. Sta. Tech. Bill. 8.

Stakman, E.C., and R.J. Piemeisel. 1917. A new strain of Puccinia graminis. Phytopathology. 7:73.

Staletic, M.D., M.S. Milovanović, A.I. Marković, and G.T. Đelić. 2009. Inheritance of spring oat resistance to *Puccinia coronata avenae*. Kragujevac J. Sci. 31:75-83.

Stanton, T.R. 1953. Production, Harvesting, Processing, Utilization and Economic Importance of Oats. Economic Botany. 7(1):43-64.

Stanton, T.R. 1961. Classification of Avena. p. 75-95. *In* F.A. Coffman (ed.) Oat and oat improvement. ASA, Madison, WI.

Sturtzel, B., C. Mikulits, C. Gisinger, and I. Elmadfa. 2009. Use of fiber instead of laxative treatment in a geriatric hospital to improve the wellbeing of seniors. J. Nutr. Health Aging. 13(2):136-139.

Sur, R., A. Nigam, D. Grote, F. Liebel, and M.D. Southall. 2008. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. Dermatological Res. 300(10):569-574.

Thomas, H. 1992. Cytogenetics of *Avena*. p. 473-507. *In* H.G Marshall and M.E. Sorrells (ed.) Oat Science and Technology. American society of Agronomy, Madison, WI.

Travlos, I.S., and C.N. Giannopolitis. 2010. Assessment of distribution and diversity of *Avena sterilis* L. and *Avena fatua* L. in cereal crops of Greece based on a 3-year survey and selected morphological traits. Genetic Resources Crop Evol. 57:337–341.

Trottet, J., J. Jahier, and A.M. Tanguy. 1982. A study of an amphiploid between *Aegilops squarrosa* Tausch. And *Triticum dicoccum* Schubl. Cereal Res. Comm. 10:55-59.

United States Department of Agriculture National Agricultural Statistics. Crop Production 2012 Summary. Site sponsor. http://usda01.library.cornell.edu/usda/current/CropProdSu/CropProdSu-01-11-2013.pdf. Accessed October 30, 2013.

Upadhyaya, Y.M., and E.P. Baker. 1960. Studies on the mode of inheritance of Hajira type stem rust resistance and Victoria type crown rust resistance as exhibited in crosses involving the oat variety Garry. Linnean Soc. of New South Wales Proc. 85:157-179.

Vavilov, N.I. 1926. Studies on the origin of cultivated plants. Bul. Appl. Bot. and Plant Breeding. 17(2): 139-245.

Virtanen, S.M., M.Kaila, J. Pekkanen, M.G. Kenward, U. Uusitalo, P. Pietinen, C. Kronberg-Kippilä, T. Hakulinen, O. Simell, J. Ilonen, R. Veijola, and M. Knip. 2010. Early introduction of oats associated with decreased risk of persistent asthma and early introduction of fish with decreased risk of allergic rhinitis. Br. J. Nutr. 103(2):266-273.

Wilson, W.A, and M.S. McMullen. 1997. Dosage dependent genetic suppression of oat crown rust resistance gene *Pc-62*. Crop Sci. 37:1699-1705.

Youngs, V.L., and R.A. Forsberg. 1987. Oat. p. 457-499. *In* R.A. Olson and K.J. Frey (ed.) Nutritional quality of cereal grains: Genetic and agronomic improvement. Agron. Monogr. 28. ASA, Madison, WI.

Xiaomei L., C.P. Wight, Y. Zhou, and N.A. Tinker. 2012. Characterization of chromosome-specific genomic DNA from hexaploid oat. Genome 55:265–26.

CHAPTER II: *A. STRIGOSA* DERIVED CROWN RUST RESISTANCE SUPPRESSION AT THE *PC38* LOCUS

Abstract

F_2 segregating populations were developed from genotypes possessing *Pc38* and *Pc94*, as well as a population void of the *Pc38* to investigate the suppression effect of *Pc38* on *Pc94*. Leggett was the resistant parent containing *Pc94* and Dumont and Steele were the susceptible parents possessing *Pc38*. Dumont has *Pc38* in an interchanged position on the chromosome relative to Steele. Df-38 was a susceptible parent that contained neither *Pc94* nor *Pc38*. CR91, a crown rust race virulent on *Pc38* and avirulent on *Pc94* was used to inoculate segregating populations to identify plants as resistant or susceptible. A Leggett/Df-38 F_2 population, which did not possess the *Pc38*, produced resistant to susceptible phenotypic ratios of 3:1 and 2:1. Two other populations (Leggett/Dumont and Leggett/Steele), which possess a copy each of *Pc38*, produced resistant to susceptible phenotypic ratios of 1:3, 1:2 and 3:13. The suppression effect of *Pc38* on *Pc94* was therefore confirmed.

Introduction

The effect of gene interaction has its positive and negative sides. Pyramiding of resistance genes is used by plant breeders to strengthen the resistance against evolving races of crown rust (Wilkins, 1975a; Leonard, 2003). On the negative side, *Pc38*, or a factor close to it, has been identified to suppress the resistance effect of *Pc62* (Wilson and McMullen, 1997).

Most breeding lines in the NDSU oat breeding program possess *Pc38*. Leggett is a Canadian cultivar that possesses *Pc94*, a potent source of crown rust resistance derived from *A. stigosa* (Mitchell Fetch *et al*., 2006). When *Pc94* was introgressed into genotypes having *Pc38* in the background, crown rust resistance in the breeding line was inhibited. The interaction of these

2 resistance genes, instead of enhancing the resistance to crown rust, confers susceptibility due to suppression of *Pc94*. The objective of this research was to determine the suppression of *Pc94* by *Pc38* as observed in crosses made between Leggett and genotypes possessing *Pc38* and to explain the suppression of *A. strigosa* derived resistance in the combination of factors at the *Pc38* locus.

Materials and Methods

Parental Genotypes

The experimental genotypes used in the evaluation of the presumed suppression effect of *Pc38* were Leggett, Steele, Dumont, Duplication 38 (Dp-38) and Deficiency 38 (Df-38). Leggett was developed by Agriculture and Agri-food Canada Cereal Research Center Winnipeg, MB. It possesses crown rust resistance conferred by *Pc94*. *Pc94* was transferred from *Avena strigosa* (a diploid oat RL1697) into a hexaploid oat, SunII (Mitchell Fetch *et al*., 2006). Leggett is homozygous for *Pc94* and it is resistant to crown rust CR91 pathotype. Steele was developed at NDSU using the RL 3038 germplasm line. RL 3038 was the source of crown rust resistance genes *Pc38*, *Pc39* and stem rust resistance gene *Pg-13*. The resistance genes in RL 3038 were derived from *A. sterilis* accessions CW 491-4 for *Pc38*, accession F 366 for *Pc39* and accession CW 490-2 for *Pg-13*. The pedigree of Steele was RL 3038/Dal//Noble (McMullen and Patterson, 1992). Steele is susceptible to CR91. Dumont is a Winnipeg line developed at Agriculture Canada Research Station, Winnipeg, Manitoba. The cross that produced Dumont is 'Harmon HAM' /Double Cross 7 and contains the *Pc38* derived from *A. sterilis* (McKenzie *et al*., 1984; Wilson and McMullen, 1997). Dumont is susceptible to CR91. Dumont and Steele differ by a chromosome interchange involving the *Pc38* locus. Dp-38 and Df-38 are NDSU lines with Dp-38 having four copies of *Pc38* and Df-38 deficient for the *Pc38* locus (Figure 1. by Wilson and

McMullen, 1997). Df-38 is a susceptible line in the crosses. Dp-38 and Df-38 are both susceptible to CR91.

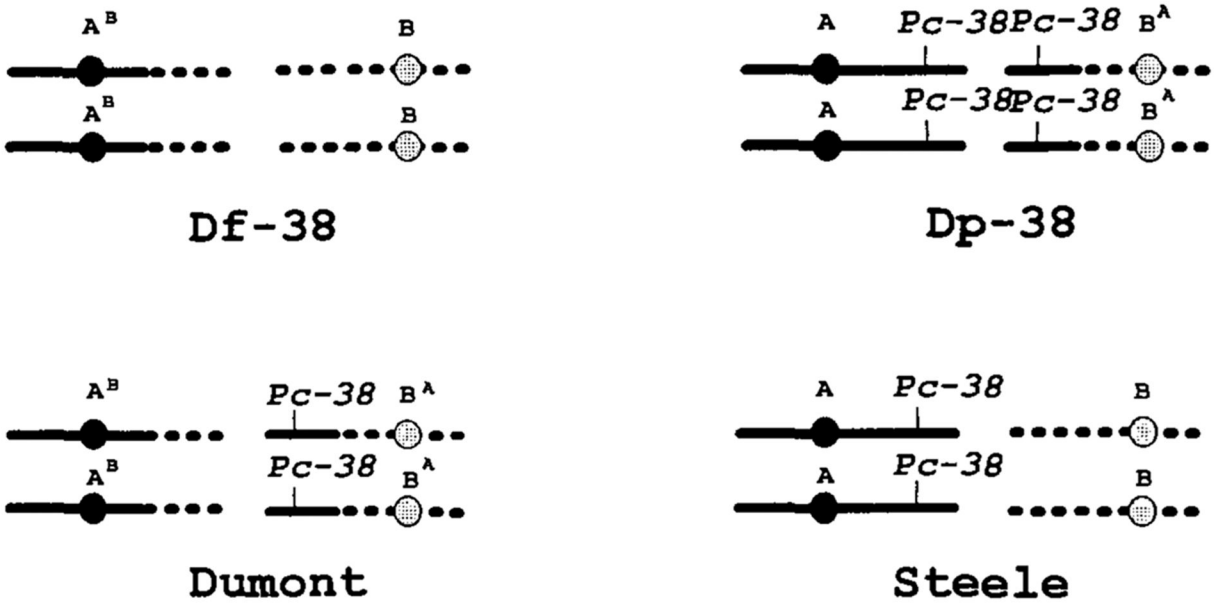

Figure 1. Crown rust resistance locus of *Pc38* for parental genotypes (Wilson and McMullen, 1997)

Emasculation and Pollination

Df-38, Dp-38, Dumont, Steele and Leggett were planted in 18 by 13 cm black round plastic pots. The pots were filled with ¾^{ths} of Sunshine LCI mix. Five seeds of each genotype were planted in the pots and thinned to 3 plants per pot after 3 weeks, depending on the germination percentage. More vigorous and healthy plants were left to grow. Three sets of 3 pots per genotype were planted at 1 week intervals to ensure adequate production of mature inflorescence at the time of emasculation and pollination. N.P.K (20-20-20) liquid fertilizer was applied at about 2 weeks after planting and ¾ spoon full of multicote granules applied after 5 weeks of planting. Multicote is a controlled release fertilizer consisting of 15-7-15 (N.P.K percentages), 1.2 % Mg, 7 % S and some minor nutrients (B, Cu, Fe, Mn, Mo and Zn). When all the plants had adequate inflorescence, emasculation and pollination were done. The temperature

in the greenhouse was set between 15-18ºC at planting and was increased to 22-26ºC after 7 weeks.

Reciprocal crosses were made. Pollination was done after 2 days of emasculation. Plump, yellow and ready to dehisce anthers were independently collected from the pollen parents (Df-38, Dp-38, Dumont and Steele) to pollinate the emasculated florets. Two to three anthers were used to pollinate each floret. The genotype number of the pollen parent was then indicated on the tag and a large 'X' put at the back of the tag indicating completion of the cross. Many crosses were done to ensure adequate seed formation, since the rate of successful fertilization can vary depending on time of day, temperature, pollen maturity and condition of stigma at pollination (Frey and Caldwell, 1961). Emasculation and pollination were done in the greenhouse.

Leggett/Dp-38 F_1 and F_2 progenies were inoculated with CR91 to determine if the expression of *Pc94* was suppressed. CR91 is virulent on *Pc38, Pc39* and *Pc91* but avirulent on *Pc94*. Leggett/Df-38 progenies inoculated with CR91 were evaluated to determine if the expression of *Pc94* was enhanced. Resistant to susceptible phenotypic ratios were used to evaluate the F_1 and F_2. The evaluation of the effect of *Pc38* in two different genomic positions on *Pc94* was carried out; Steele containing *Pc38* was crossed with Leggett containing *Pc94*, and the F_1 and F_2 progenies were evaluated by inoculating with CR91. In addition, Dumont containing *Pc38* was crossed with Leggett containing *Pc94* and the F_1 and F_2 progenies evaluated by inoculating with CR91. Leggett was the pollen parent for the reciprocal F_1 crosses.

F_2 Population Development

The F_2 populations were developed by crossing a resistant parent with a susceptible parent to produce the F_1 seeds. Leggett was the resistant parent while Df-38, Dp-38, Dumont and Steele were the susceptible parents. F_1 seeds were obtained from successful crosses of each cross

and advanced to the next generation through self-pollination. Five seeds were planted per pot and at 2 weeks, 2 of the 5 F_1 plants were transplanted into another 18 by 13 cm plastic pots for each genotype. By the 4th week, ¾ spoon full of multicote slow release granular fertilizer was applied to each pot. Watering was done when necessary, as determined by moisture content of the Sunshine LCI mix. In the greenhouse, temperatures were set between 15-18ºC at planting and increased to 22-26ºC after 7 weeks. Plants were allowed to go through all the developmental stages until maturity and F_2 seeds were harvested from each genotype. The F_2 seeds were obtained from 1 to 7 tillers depending on the genotype. Once panicles were dried on the plants, they were cut off with a scissors and seeds were hand threshed, put in paper envelops and labelled. Seeds were kept at room temperature for the next phase of the experiment.

Increase of Crown Rust Urediniospore in the Greenhouse

Four seeds of the oat cultivar Jury (a cultivar susceptible to CR91) were planted into 9 by 9 by 8 cm green containers filled up with ¾ sunshine LCI mix. Thirty containers were used for the urediniospore increase. 'Jury' possesses *Pc91* and is used to increase CR91 spores for inoculum. Week old plants with fully expanded first leaves were inoculated. Initial inoculum consists of urediniospore derived from a single pustule isolate from 'HiFi'. HiFi possess crown rust resistance gene *Pc91*. Ashcroft spray equipment was used to apply the inoculum on the plant. The CR91 spores were mixed with soltrol in the spray container. Once the mixture was thoroughly shaken, the nozzle with the pressure tube was connected to the spray container. The pressure was set at 6 psi and the content of the spray bottle released through the front part of the nozzle onto the plant by stopping the top of the nozzle with a finger. The nozzle was held about 20 cm away from the plants to apply the inoculation mixture. Once the application was done, the plants were allowed 5-10 mins for the soltrol to dry.

The inoculated plants were placed in the mist chamber. To produce near 100% humidity in the chambers for fast spore germination, the mist was allowed to continuously run for 30-45 mins. The regulator was then changed to a mist time of 20 seconds of on cycle and 4 mins between cycles. The plants were left overnight in the dark for 16-20 hours. The mist chambers were shut off and the doors opened the next day for the plants to dry. The plants were then transferred to the greenhouse with the temperature set at 22ºC. The plants were left in the greenhouse for 2 weeks to allow the development of the crown rust pustules. Liquid fertilizer was applied to the plants to keep them green and healthy, and watering of the plants was done as and when needed.

When sufficient urediniospores developed on the leaves, they were collected by tilting the containers then tapping to release the spores onto an aluminum foil. The spores on the aluminum foil were poured into a glass petri dish and placed in a desiccator jar for 3 days to dry. The desiccator jar contained 80% Ammonium Sulfate. After drying, the spores were packaged by scooping the spores with a small metallic spatula into gelcap capsules. The spores covered ¼ of the capsule. The capsules were covered and put into a 0.5 ml microcentrifuge tubes and labelled with the date and crown rust race. The 0.5 ml microcentrifuge tubes containing the spores were then stored in a -80ºC REVCO freezer until needed. A second collection of inoculum from the same plants occurred after 2 weeks following the same procedure as the first collection.

Planting of F_2 Seeds

Six and eight-inch containers were filled with ¾ potting mix and a set of F_2 seeds were planted per pot. The first set of F_2 populations were planted in 6" pots with up to 10 seeds per pot depending on the quantity of seeds in each F_2 population. A second set of F_2 populations were

planted in 8" pots with up to 50 seeds per pot. The sets of F_2 populations consisted of Leggett/Df-38, Leggett/Dp-38, Leggett/Dumont and Leggett/Steele. A total of 182, 216, 159 and 218 seeds were planted for Leggett/Df-38, Leggett/Dp-38, Leggett/Dumont, and Leggett/Steele F_2 segregating populations respectively. Five to seven 6" pots were used depending on the number of F_2 seeds in each. The second set of F_2 segregating populations planted consisted of 248 seeds for Leggett/Df-38, 268 seeds for Leggett/Dp-38, 443 seeds for Leggett/Dumont and 228 seeds for Leggett/Steele. Five or ten 8" pots per F_2 population were used. All the seeds produced from each F_1 plant were used in the experiment for each specific cross. Plants were grown in the greenhouse with temperature between 21-25ºC and a photo period of 16 hours of light and 8 hours of darkness.

Inoculation with Crown Rust and Infection Type Classification

Germination of the frozen urediniospores was promoted through heat shock treatment by placing the capped frozen crown rust spores in 42ºC water for 5-6 minutes. The heat shocked crown rust spores were then ready for the inoculation process and can be used for up to 2 weeks when kept in the refrigerator. When the F_2 seedlings were 9 days old, the primary leaves were inoculated with urediniospores suspended in a light mineral oil (soltrol). Plants were then placed in mist chambers with near 100% relative humidity at 22-25ºC for 16-20 hours in the dark. Inoculated seedlings were transferred to the greenhouse at 20-22ºC for the development of the infection type (IT). The IT, developed by Murphy (1935), of 0 (immune) to 4 (susceptible) were assigned to the inoculated seedlings. The symbol and infection type of host reaction were as follows: Host reaction 0 – nearly immune; showed no formation of uredia but the presence of necrotic areas or chlorotic flecks. Host reaction 1 – Highly resistant; showed either small and few uredia in necrotic areas constantly or the production of less necrotic areas without uredia

development. Host reaction 2 – Moderately resistant; showed the presence of fairly abundant small to medium size uredia always in necrotic or very chlorotic areas; necrotic areas are rarely without uredia. Host reaction 3 – showed abundant medium size uredia enclosed by chlorotic areas; necrotic areas are entirely absent. Host reaction 4 – showed large abundant uredia without chlorotic or necrotic areas immediately enclosing uredia. The scores of 0, 1 and 2, designated as resistant were assigned to seedlings showing chlorotic flecking and small uredia spots. Scores of 3 and 4 were assigned to disease reaction on leaves showing broken epidermis with orange uredia of varying sizes. These two scores were grouped as one and seedlings designated as susceptible.

CR91 was used to inoculate the seedlings. The source of CR91 was from a single pustule isolated on HiFi at the North Dakota State University Oat program. This race was collected from oat fields when virulence for crown rust resistance gene *Pc91* was no longer effective. Leggett was a resistant check because it contains *Pc94* which is resistant to CR91 and Df-38 was a susceptible check because it contains neither *Pc94* nor *Pc38*.

Phenotypic Data

Uredia developed 2-3 weeks after being removed from the mist chamber. Data were collected by individually checking each fully expanded coleoptile of each plant. Leaves were observed and depending on the absence, presence and quantity of uredia with necrosis at the infection site, plants were assigned an infection type. Plants rated as a 3 or 4 were considered susceptible. The remaining plants which were 0, 1 and 2 were considered resistant.

Statistical Analysis

Chi-square goodness-of-fit test was used to estimate segregating populations for resistant to susceptible ratios of 1:15, 3:1 and 1:3. The ratios were determined by checking if the observed

number of resistant and susceptible category of inoculated plants fit the expected number of resistant and susceptible category calculated to determine significant values (Mather, 1951). A resistant to susceptible phenotypic ratio of 1:15 denotes the presence of 2 dominant suppressor genes. In the 1:15 scenario, all homozygous dominant and heterozygous allele combinations will produce susceptible plants with the suppressor gene except for the homozygous recessive, which will produce resistant plants. A 1:3 or 3:1 resistant to susceptible ratio indicates a single dominant gene, that is one homozygous dominant and two heterozygous alleles will produce one phenotypic characteristic and the homozygous recessive allele will produce another phenotypic characteristic. Two families were developed for each F_2 population, values pooled, and heterogeneity chi square tests determined for families that fit the same ratios.

Results and Discussions

Reaction of Parental lines to CR91

In the evaluation of the crown rust resistance suppression at the *Pc38* locus, the parental genotypes were tested for their responses to the CR91. The parental genotypes were inoculated and visually scored for their responses to the crown rust race. The 5 parental lines Df-38, Dp-38, Dumont, Leggett and Steele contain specific or none of the pc-gene used to determine the suppression effect of *Pc38*. As expected Df-38 which is deficient in the *Pc38* gene was susceptible to CR91 (Table 1). Dumont, Dp-38 and Steele, possessing 2 copies of *Pc38*, 4 copies of *Pc38* and 2 copies of *Pc38* respectively, were all susceptible to CR91. Leggett, which is the resistant parent was resistant to CR91 since it contains 2 copies of *Pc94* genes (Aung, 1996). These are the resistance gene backgrounds from which the F_2 segregating populations were developed to estimate the suppression effect of *Pc38*. Crown rust 91 is virulent to *Pc38*, *Pc39*, *Pc91* but it is avirulent to *Pc94* (Figure 2).

Table 1. Reaction of parental lines with specified Pc-gene to CR91

Parental genotype	Pc-gene	Infection type	Response to CR91
Df-38	deficient	4	Susceptible
Dp-38	4 *Pc38*	4	Susceptible
Leggett	2 *Pc94*	0	Resistant
Dumont	2 *Pc38*	4	Susceptible
Steele	2 *Pc38*	4	Susceptible

IT Classifications: (0, 1, 2) – Resistant; (3, 4) – Suscep tible

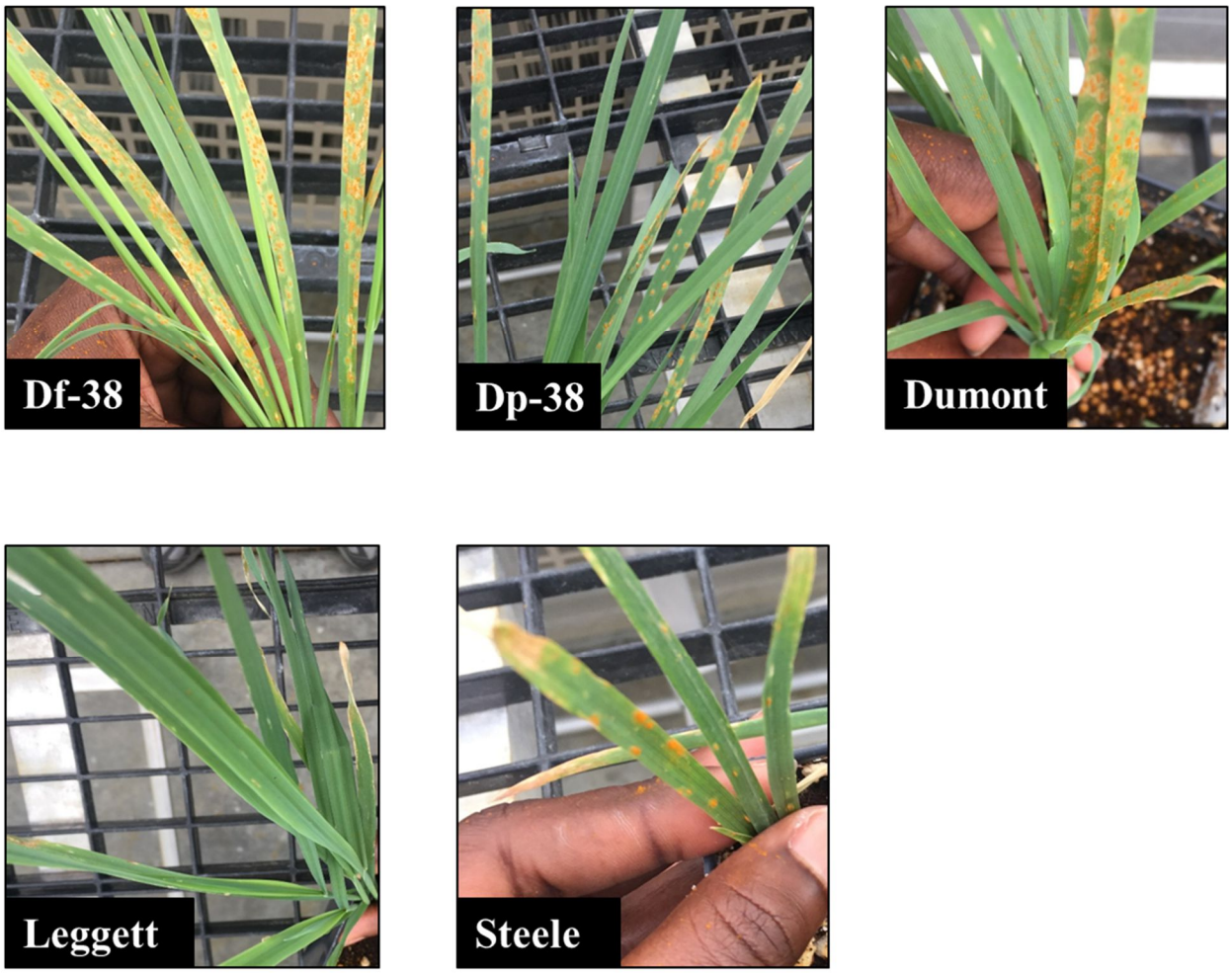

Figure 2. Visual parental genotype responses to CR91

Suppression Effect of Pc38 on F_1 Plants

Crosses with parental lines producing F_1 plants reacted as expected (Table 2). Leggett was the common parent between the crosses. The presence of *Pc38* within the F_1 was expected to be one copy in Dumont and Steele, whereas two copies of the *Pc38* were in the Leggett/Dp-38

crosses. Leggett contributed a copy of *Pc94* to each F_1. With the crossing that occurred between the parents, the F_1 will contain a copy of *Pc94* from Leggett and 2 copies of *Pc38* from Dp-38. The suppression effect of *Pc38* was demonstrated in the F_1 generations of Leggett/DP-38, Leggett/Dumont and Leggett/Steele because the plants were susceptible to CR91 irrespective of the fact that the F_1s possessed *Pc94*. In their experiments, Wilson and McMullen (1997) and Dietz and Murphy (1930) reported that susceptibility to F_1 seedlings were conferred by a suppressor gene, which was also observed in this experiment.

Table 2. Seedling reaction of F_1 plants and their reciprocal crosses to CR91

F_1s with reciprocal lines	**Infection type**	**Disease response to CR91**
Leggett/Df-38	0	3 Resistant
Df-38/Leggett	0	3 Resistant
Leggett/DP-38	4	3 Susceptible
DP-38/Leggett	4	3 Susceptible
Leggett/Dumont	4	3 Susceptible
Dumont/Leggett	4	3 Susceptible
Leggett/Steele	4	3 Susceptible
Steele/Leggett	4	3 Susceptible

IT Classifications: (0, 1, 2) – Resistant; (3, 4) – Susceptible

Since maternal effect can significantly contribute to offspring phenotype, reciprocal crosses were made to verify if maternal effect was a contributing factor in the resistance gene inheritance or suppression effect. Maternal effect is the phenotypic contribution of the female parent to the offspring, irrespective of the equal contribution of chromosomes from the male and female parents (Roach and Wulff, 1987). However, no maternal effects were detected since all the crosses and reciprocal crosses reacted the same way to CR91. A similar result was obtained in crown rust resistance inheritance in perennial ryegrass (Kimbeng, 1999).

Scoring of F_2 Segregating Populations

The F_2 segregating populations were scored based on the IT developed by Murphy (1935). The segregating F_2 population developed to estimate the suppression effect of *Pc38* produced 247

seedlings for the Leggett/Df-38 population with 169 resistant plants and 78 susceptible plants when inoculated with CR91 (Table 3). *Pc94* produced a single dominant gene effect, since *Pc38* was not segregating in the F_2 population. This result confirmed our expectation with excess resistant plants in both instances. On the other hand, the Leggett/Dp-38 population (1) did not confirm our expected results. The population had 186 resistant plants and 76 susceptible plants. A phenotypic ratio of 3:1 instead of 1:15 resistant to susceptible ratio was obtained, because the F_1 was expected to contain two copies of the *Pc38* genes and suppress the effect of the *Pc94* gene.

Table 3. F_2 segregating populations seedling scoring to CR91

F_2 repeated exp.	**Infection type**					**Total**
	Resistant			**Susceptible**		
F_2 populations (1)	*0*	*1*	*2*	*3*	*4*	
	94	56	19	27	51	
Leggett/Df-38		**169**			**78**	**247**
	101	75	10	11	65	
Leggett/Dp-38		**186**			**76**	**262**
	90	27	7		315	
Leggett/Dumont		**124**			**315**	**439**
	5	17	10		195	
Leggett/Steele		**32**			**195**	**227**
F_2 populations (2)						
	99	18	13	21	31	
Leggett/Df-38		**130**			**52**	**182**
	106	1			108	
Leggett/Dp-38		**107**			**108**	**215**
	49	2			106	
Leggett/Dumont		**51**			**106**	**157**
	31	5	3		171	
Leggett/Steele		**39**			**171**	**210**

The Leggett/Dumont population (1) had 124 resistant plants and 315 susceptible plants that fit a ratio of 1:3 resistant to susceptible ratio. There were 32 resistant plants and 195

susceptible plants in the Leggett/Steele population (1) when the F_2 plants were inoculated with the CR91 which fit a 3:13 resistant to susceptible ratio.

The Leggett/Df-38 F_2 population (2) produced 130 resistant plants and 52 susceptible plants when seedlings were inoculated with CR91 and fit a ratio of 3:1 resistant to susceptible plants. When plants were inoculated with CR91, 107 resistant plants and 108 susceptible plants were observed for Leggett/Dp-38. Similar results of excess susceptible plants were observed in Leggett/Dumont (1 and 2) and Leggett/Steele (1 and 2) F_2 populations except for Leggett/Dp-38 (1 and 2) F_2 population. Even though the Leggett/Dp-38 population (1) fit a 3:1 ratio, the Leggett/Dp-38 population (2) fit a 1:1 ratio, making the results inconsistent. The two families did not fit the expected ratios. The expected result was a 1:15 resistant to susceptible ratio, because 2 copies of *Pc38* was expected to segregate in the developed populations. However, excess resistant plants were observed in the first F_2 population and not enough susceptible plants in the second F_2 population. Since Leggett was used as the female parent in both cases, *Pc38* from Dp-38 may have been only partially or may have not been transmitted to the progeny through the pollen.

The Leggett/Dumont F_2 population (2) produced 51 resistant plants and 106 susceptible plants when inoculated with CR91 and fit a 1:2 resistant to susceptible ratio. The Leggett/Steele F_2 segregating population fit a 3:13 resistant to susceptible ratio confirming dominant suppression epistasis. Thirty-nine plants were resistant while 171 plants were susceptible.

X^2 of F_2 Segregating Populations

Chi-square goodness-of-fit tests (X^2) were calculated for the individual experiments to evaluate if the number of observed individuals in either the resistant or susceptible category fit the number of expected individuals calculated. Heterogeneity chi-square tests were done to pool

the individual experiments within each F_2 population together that were less than the critical value. Populations that did not fit the projected ratios were tested for other ratios. (Table 4). An α of 0.05 and a critical value of 3.84 for 1 degree of freedom (df) was used to determine the significant values for each X^2. The presence of a single dominant gene controlling the crown rust resistance was confirmed by obtaining a 3:1 resistant to susceptible ratio in the Leggett/Df-38 F_2 population (2), Leggett/Df-38 F_2 population (1) fit a 2:1 ratio. X^2 values of 0.34 and 1.24 were obtained for the Leggett/Df-38 F_2 segregating populations (1) and (2) respectively. *Pc94* is segregating in the Leggett/Df-38 F_2 segregating population. Rines *et al.* (2007) also observed inconsistent crown rust resistance to susceptible 2:1 and 3:1 ratios from F_2 progenies of BC_3 lines. The pedigree for the cross was (*A. strigosa* CI6954SP/Black Mesdag) C_1//Ogle*3, and further testing of their BC_2F_1 plants revealed between 33-57% micronuclei among the sampled plants. They explained that the presence of micronuclei indicates lagging chromosomes from either lack of homologous meiotic pairing or incomplete homologous meiotic pairing (McMullen *et al*., 1982). Even though different ratios were obtained between the first and second populations, excess resistant plants were demonstrated in both cases. The X^2 were 2.24 and 0.0047 for Leggett/Dp-38 F_2 population (1 and 2) and fit ratios of 3R:1S and 1R:1S respectively.

Table 4. Chi-square tests across F_2 population families

F_2 populations (1)	**X^2**	**Ratios**
Leggett/Df-38	0.34	2(R):1(S)
Leggett/Dp-38	2.24	3(R):1(S)
Leggett/Dumont	2.47	1(R):3(S)
Leggett/Steele	2.68	3(R):13(S)
F_2 Populations (2)		
Leggett/Df-38	1.24	3(R):1(S)
Leggett/Dp-38	0.0047	1(R):1(S)
Leggett/Dumont	0.05	1(R):2(S)
Leggett/Steele	0.0044	3(R):13(S)

In the absence of *Pc38*, the single dominant gene effect of *Pc94* was expressed in a resistant to susceptible phenotypic ratio of 3:1 in the Leggett/Df-38 population (1). The single dominant gene effect of *Pc94* however, was suppressed in both Leggett/Dumont and Leggett/Steele F_2 populations in the presence of the *Pc38* gene. The X^2 for Leggett/Dumont populations (1 and 2) and Leggett/Steele populations (1 and 2) were (2.47 and 0.05) and (2.68 and 0.0044) respectively. These results confirm that the *Pc94* single dominant gene effect was suppressed and a resistant to susceptible phenotypic ratio of 1:3 instead of 3:1 was observed in Leggett/Dumont population (1) which possesses a copy of *Pc38* each. Both Leggett/Steele populations demonstrated a dominant suppression and fit a 3(R):13(S) ratio with a homogeneity X^2 value of 0.89. In a dominant suppression, a single *Pc38* allele produces a dominant phenotype and suppresses the expression of *Pc94*. The suppression effect of *Pc38* on a different resistance gene *Pc62* has also been confirmed by Wilson and McMullen (1997).

Crown rust resistance gene inheritance has been demonstrated to be complex since different models of most of the developed populations varied. Other factors could be involved in crown rust genes inheritance that have not yet been discovered. Nof and Dinor (1981) and Simons *et al*. (1978) reported that crown rust resistance genes can be inherited as a single dominant, partially dominant or recessive gene. Rines *et al*. (2017), identified a crown rust resistance gene that expressed IT 1 and 2 at the seedling stage and produced adult resistant plants without the presence of any uredia.

References

Aung, T., J. Chong, and M. Leggett. 1996. The transfer of crown rust resistance gene *Pc94* from a wild diploid to cultivated hexaploid oat. p. 167–171. In Proc. European and Mediterranean Cereal Rusts and Powdery Mildews Conf. 9th, Lutheran, Netherlands. 2–6 Sept. 1996. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands.

Dietz, S.M., and H.C. Murphy. 1930. Inheritance of resistance to *Puccinia coronate avenae*, p.f III. Phytopathology 20:120.

Frey, K.J., and R.M. Caldwell. 1961. Oat breeding and pathologic techniques. p. 227-262. *In* F.A. Coffman (ed.) Oats and Oat Improvement. American society of Agronomy, Madison, WI.

Kimbeng, C.A. 1999. Genetic basis of crown rust resistance in perennial ryegrass, breeding strategies, and genetic variation among pathogen population: a review. Australian J. Exp. Agric. 39:361-378.

Leonard, K.J. 2003. Regional frequencies of virulence in oat crown rust in the United States from 1990 through 2000. Plant Dis. 87(11):1301-1310.

Mather, K. 1951. The measurement of linkage in heredity. John Wiley & Sons, NY.

McKenzie, R.I.H., P.D. Brown, J.W. Martens, D.E. Harder, J. Nielsen, C.C. Gill, and G.R. Boughton. 1984. Registration of Dumont oats. Crop Sci. 24:207.

McMullen, M.S., and F.L. Patterson. 1992. Oat cultivar development in the USA and Canada. p. 573–612. *In* H.G. Marshall and M.E. Sorrells (ed.) Oat Science and Technology. Agron Monogr 33. ASA and CSSA, Madison, WI.

Mitchell Fetch, J.W., S.D. Duguid, P.D. Brown, J. Chong, T.G. Fetch, S.M. Haber, J.G. Menzies, N. Ames, J. Noll, T. Aung, and K.D. Stadnyk. 2006. Leggett oat. Can. J. Plant Sci. 87:509–512.

McMullen, M.S., R.L. Phillips, and D.D. Stuthman. 1982. Meiotic irregularities in *Avena sativa* L./*A. sterilis* L. hybrids and breeding implications. Crop Sci. 22:890–897.

Murphy, H.C. 1935. Physiology specialization in *Puccinia coronata avenae*. USDA Tech. Bull. 433. U.S. Gov. Print. Office, Washington, DC.

Nof, E., and A. Dinoor. 1981. The manifestation of gene-for-gene relationships in oats and crown rust. Phytoparasitica. 9:240.

Rines, H.W., M.E. Miller, M. Carson, S. Chao, T. Tiede, J. Wiersma, and S.F. Kianian. 2017. Identification, introgression, and molecular marker genetic analysis and selection of a highly effective novel oat crown rust resistance from diploid oat, *Avena strigosa*. Theor Appl Genet. 131(3):721-733.

Rines, H.W., H.L. Porter, M.L. Carson, and G.E. Ochocki. 2007. Introgression of crown rust resistance from diploid oat *Avena strigosa* into hexaploid cultivated oat *A. sativa* by two methods: direct crosses and through an initial 2x.4x synthetic hexaploid. Euphytica. 158:67-79.

Roach, D.A., and R.D. Wulff. 1987. Maternal effects in plants. Ann. Rev. Ecol. Syst. 18:209-235.

Simons, M.D., J.W. Martens, R.I.H. McKenzie, I. Nishiyama, K. Sadanaga, J. Sebesta, and H. Thomas. 1978. Oats: A standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters. U.S. Dep. Agric. Agric. Handb. 509.

Wilkins, P.W. 1975a. Implications of host-pathogen variation for resistance breeding in the grass crop. Ann. Appl. Biol. 81:257-261.

Wilson, W.A, and M.S. McMullen. 1997. Dosage dependent genetic suppression of oat crown rust resistance gene *Pc-62*. Crop Sci. 37:1699-1705.

CHAPTER III: ALTERNATE CROWN RUST RESISTANCE GENE TO *PC94* INTROGRESSED INTO HEXAPLOID OAT FROM *A. STRIGOSA*

Abstract

An allelism test was done to determine the allelic relationship of crown rust resistance genes in 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. These genotypes possess resistance genes that were introgressed from *A. strigosa* sources, the resistance source of *Pc94* in Leggett. Four different F_2 segregating populations were developed from Leggett by 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. Crown rust race 16MN (100-3) which is virulent to *Pc94* was used to determine resistant to susceptible phenotypic ratios of the developed populations. Two of the genotypes, 08BT26-2 and 08BT70-1, had the same allele as Leggett since all the F_2 plants were susceptible to 16MN (100-3). On the other hand, resistant to susceptible ratios of 3:1 and 13:3 were obtained from populations developed using BT1020-1-1 and BT1021-1-1. Therefore, the resistance genes in BT1020-1-1 and BT1021-1-1 were confirmed to be different from that present in Leggett.

Introduction

The effect of crown rust on cultivated oat (*Avena sativa*) ranges from reduced grain yield and grain quality such as groat percentage and weight of kernel (Doehlert *et al*., 2001). Endo and Boewe (1958) reported that lodging is more prevalent in oat plants affected by crown rust, since the production of straw is reduced thereby weakening the straw strength as a result. The introgression of crown rust resistance genes into cultivated oat to curtail the adverse effects of crown rust cannot be overemphasized. This has been the objective of many oat breeders where disease resistance is concerned, as crown rust races evolve and overcome resistant cultivars (Carson, 2011). *A. strigosa* is one of the recent sources from where resistance genes have been

introgressed into cultivated oat (Nazareno *et al*., 2018). *Pc94* which was introgressed into cultivated oat from *A. strigosa* provides crown rust resistance in Leggett (Mitchell Fetch *et al*., 2006). The resistance genes in Gem, 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1 were also introgressed from *A. strigosa* (Brown and Shands, 1954; Rines *et al.*, 2007 and Rines *et al*., 2017).

The objective of this research was to determine the allelic relationship of independently introgressed crown rust resistance factors from *A. strigosa* into 4 genotypes and Leggett. The study is to verify if the *A. strigosa* derived crown rust resistance factor *Pc94* is the same or different in Leggett, Gem and lines developed by Rines *et al*. (08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1).

Materials and Methods

Parental Lines

The parental genotypes used for the allelism tests were Leggett, Gem, 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. All the crown rust resistance genes present in these lines were introgressed into the genotypes from an *A. strigosa* source. Leggett was developed by Agriculture and Agri-food Canada Cereal Research Center in Winnipeg, Manitoba. The crown rust resistance gene *Pc94* was introgressed into hexaploid oat from *Avena strigosa* (a diploid oat RL1697) into SunII - a hexaploid oat, and Leggett was developed (Mitchell Fetch *et al*., 2006). Leggett is resistant to crown rust race CR91 and it is homozygous for *Pc94*. Gem was developed at the University of Wisconsin, Madison (Duerst *et al*., 1999). The crown rust resistance factors in Gem and lines from Rines *et al*. (2007) were also transferred from *A. strigosa* C.I. 3436 / C.I. 4639, CI6954SP and PI 258731, all of which are diploid sources.

To determine the relationship of the resistance factor in 08BT26-2, 08BT70-1, BT1020-1-1, BT1021-1-1, Gem, and Leggett, genotypes were inoculated initially with CR91 which is virulent on *Pc38, Pc39* and *Pc91*, and seedling reactions were evaluated (Table 5). CR91 is avirulent on *Pc94*. Secondly, the parental genotypes were inoculated with a crown rust race 16MN (100-3) which was obtained from the USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN. *Pc94* in Leggett is susceptible to crown rust 16MN 100-3. Inoculation was carried out and the seedlings scored for resistance or susceptibility based on the IT developed by Murphy (1935).

Table 5. Reaction of Pc-genes to crown rust races

Crown rust race	Virulence	Avirulence
CR91	*Pc38, Pc39, Pc91*	*Pc94*
16MN (100-3)	*Pc94*	

Crossing of Parental Lines and F_2 Seedling Growth

Leggett was the female parent and pollen parents were 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1 to produce the F_1 seeds. Reciprocal crosses were also done with pollen from Leggett and crossed with 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1. Three plants for each genotype were grown in 6" pots. Three sets of each genotype were planted a week apart. The potting mix was Sunshine LCI mix and pots were filled with ¾ of the potting mix before seeds were planted. A ¾ spoon full of multicote slow release fertilizer was applied at 2 weeks of planting. Pots were watered as needed, usually every other day. The emasculation and pollination procedure were as described in chapter II. To verify if the resistance gene *Pc94* present in Leggett was the same or different from the crown rust resistance factors in 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1, F_2 segregating populations were developed from the selfed F_1 plants. The F_2 segregating populations were Leggett/08BT26-2, Leggett/08BT70-1, Leggett/BT1020-1-1 and Leggett/BT1021-1-1. The F_1s were selfed and the resulting segregation

ratios of the F_2 plants with CR16MN (100-3) were evaluated. The crossing was done in the NDSU greenhouse and temperatures were set between 15-18ºC at planting and between 22-26ºC after 7 weeks.

All seeds obtained from the F_1 plants (F_2 seeds) were planted and used for the study. Two families of each F_2 segregating population were planted. A total of 237, 286, 256 and 351 seeds were planted for the Leggett/08BT26-2, Leggett/08BT70-1, Leggett/BT1020-1-1 and Leggett/BT1021-1-1 F_2 populations (1) respectively. An average of 50 seeds were planted in 8" pots for each F_2 segregating population. Seeds from each segregating population were either planted using 5, 6 or 7 of the 8" pots. The second round of planting was done in 6" pots. Leggett/08BT26-2 F_2 segregating population (2) had 105 seeds which were planted in 2 pots. Two 6" pots of 68 seeds of the Leggett/08BT70-1 F_2 populations (2). Three pots each contain 157 and 166 F_2 seeds were planted from the Leggett/BT1020-1-1 and Leggett/BT1021-1-1 F_2 segregating population (2) respectively.

Inoculation of F_2 seedling

Once the F_2 populations were 9 days old, the frozen 16MN (100-3) spores were heat shocked and inoculation was performed as described in Chapter II. Following inoculation, plants were left to dry for 10 mins before they were placed in the mist chamber. The mist chamber was set for 20 second mist time separated by 4 mins between misting. The plants remained in the mist chamber for 16-20 hours in the dark. The inoculated plants were placed back in the greenhouse at a temperature of 22ºC for the development of the infection types. After 2 weeks, when the crown rust spores had developed, the plants were scored using the IT developed by Murphy (1935) as described in Chapter II.

Crown rust 16MN (100-3) is a race that is virulent on *Pc94*. Inoculum was obtained from the USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN. The inoculum was increased by planting and using Leggett as the susceptible host. The procedure outlined in Chapter II was followed to increase the crown rust inoculum. Once the spores were collected, they were dried, packaged and stored in a -80ºC REVCO freezer.

Results and Discussions

Parental Lines Inoculation with Crown Rust Races CR91 and 16MN (100-3)

The crown rust resistance genes present in Gem, Leggett, 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1 were all introgressed from the diploid oat *A. strigosa*. Even though the source of the resistance genes for these cultivars and lines were *A. strigosa*, the individuals who introgressed the genes and the location where the introgression of the genes were done varied. An allelism test was conducted to verify the resistance genes present in the various lines. The first crown rust race inoculation on the parents was CR91. This race is virulent on *Pc38*, *Pc39* and *Pc91* but avirulent of *Pc94*. Leggett possesses *Pc94*. Upon inoculation, Gem was found to be susceptible to CR91, but Leggett, 08BT26-2, 08BT70-1, BT1020-1-1 and BT1021-1-1 were resistant to the CR91 race (Figure 3). Due to the susceptibility of Gem to CR91, it was discontinued from the experiment.

Figure 3. Visual responses of parental genotypes to CR91

The next inoculation done on the parental lines was with crown rust 16MN (100-3), which is virulent on *Pc94*. Leggett is homozygous for *Pc94* and it appears that 08BT26-2 and 08BT70-1 were also homozygous for *Pc94* due to response to the pathotypes (Table 6). However, BT1020-1-1 and BT1021-1-1 were resistant to the 16MN (100-3) (Figure 4). The IT classification of BT1020-1-1and BT1021-1-1 was scored as 1 because of the presence of tiny and few uredia in necrotic areas found on the leaves indicating the 2 genotypes are highly resistant but not immune at the seedling stage. Rines *et al*. (2017) also reported that these 2 genotypes were moderately susceptible to composite urediniospores from Matt More buckthorn nursery as seedlings, but resistant at adult stage.

Table 6. Reaction of parental lines to crown rust race 16MN 100-3

Parental genotype	Pc-gene	Infection type	Response to 16MN 100-3
Leggett	2 *Pc94*	4	Susceptible
08BT26-2	-	4	Susceptible
08BT70-1	-	4	Susceptible
BT1020-1-1	-	1	Resistant
BT1021-1-1	-	1	Resistant

IT Classifications: (0, 1, 2) – Resistant; (3, 4) – Susceptible

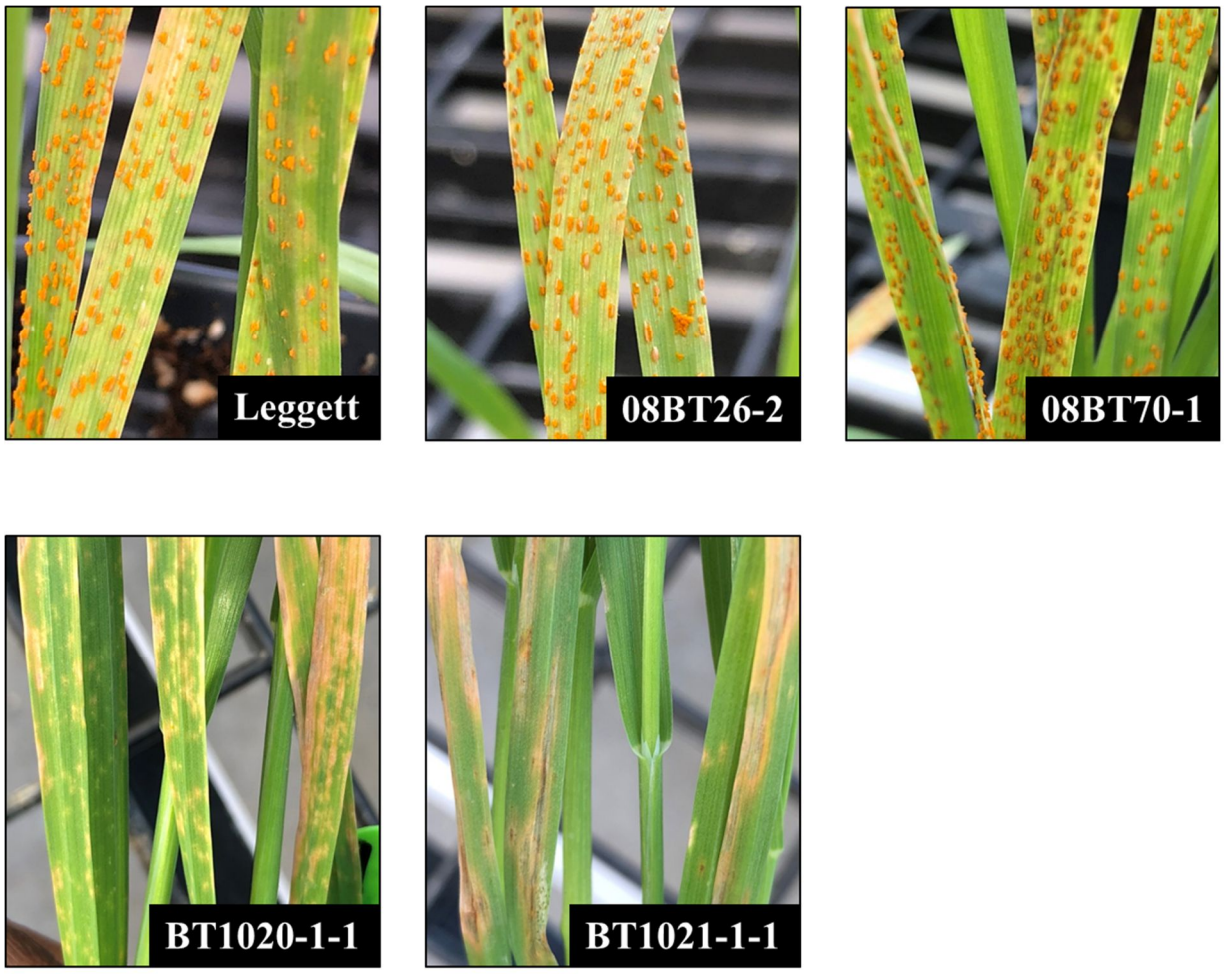

Figure 4.Visual parental genotype responses to 16MN 100-3

F_1 Plant Reaction to Crown Rust 16MN (100-3)

F_1 plants and corresponding reciprocal crosses of Leggett and the lines from Rines *et al*. (2007) reacted in the same way to 16MN (100-3) (Table 7). This indicates the absence of maternal effect in the responses of the genotypes to 16MN (100-3). Leggett/08BT26-2 and Leggett/08BT70-1 were susceptible, while Leggett/BT1020-1-1 and Leggett/BT1021-1-1 were

resistant. Leaves of BT1020-1-1 and BT1021-1-1 inoculated with the crown rust inoculum became necrotic.

Table7. Reaction of F_1 lines and their reciprocal crosses to crown rust race 16MN (100-3)

F_1s with reciprocal lines	**Infection type**	**Disease response to 16MN (100-3)**
Leggett/08BT26-2	4	Susceptible
08BT26-2/ Leggett	4	Susceptible
Leggett/08BT70-1	4	Susceptible
08BT70-1/Leggett	4	Susceptible
Leggett/BT1020-1-1	1	Resistant
BT1020-1-1/Leggett	1	Resistant
Leggett/BT1021-1-1	1	Resistant
BT1021-1-1/Leggett	1	Resistant

IT Classifications: (0, 1, 2) – Resistant; (3, 4) – Susceptible

Segregation Ratios of Resistant to Susceptible F_2 Plants

The F_2 populations (1) Leggett/08BT26-2 had 84 seeds planted (Table 8). All the 84 plants were susceptible when inoculated with crown rust 16MN (100-3). All 63 plants of Leggett/08BT70-1 population (1) were susceptible also. Leggett/BT1020-1-1 and Leggett/BT1021-1-1 F_2 populations (1) both fit a 3:1 phenotypic ratio of resistant to susceptible plants. A total of 155 seeds were planted for Leggett/BT1020-1-1 population (1). Resistant plants in this population were 118 while 37 plants were susceptible. One hundred and sixty-one seeds were planted for Leggett/BT1021-1-1 F_2 population (1), with 118 resistant and 43 susceptible.

F_2 populations (2) of Leggett/08BT26-2 and Leggett/08BT70-1 consisted of 236 and 284 plants respectively. All plants in these two populations were susceptible to crown rust 16MN (100-3). On the other hand, Leggett/BT1020-1-1 and Leggett/BT1021-1-1 F_2 populations (2) produced 183 and 279 resistant plants, and 73 and 69 susceptible plants, respectively. The resistant to susceptible ratio obtained for the Leggett/BT1020-1-1 population fit 3:1, while Leggett/BT1021-1-1 fit a 13:3 ratio.

Table 8. F_2 segregating populations to crown rust 16MN (100-3)

F_2 repeated exp.	**Infection type**					**Total**
	Resistant			**Susceptible**		
F_2 populations (1)	*0*	*1*	*2*	*3*	*4*	
Leggett/08BT26-2				2	82	**84**
Leggett/08BT70-1				2	61	**63**
Leggett/BT1020-1-1	101	6	11	7	30	
		118			**37**	**155**
Leggett/BT1021-1-1	106	8	4	13	30	
		118			**43**	**161**
F_2 populations (2)						
Leggett/08BT26-2					236	**236**
Leggett/08BT70-1					284	**284**
Leggett/BT1020-1-1	75	30	78	12	61	
		183			**73**	**256**
Leggett/BT1021-1-1	205	29	45	18	51	
		279			**69**	**348**

All the plants in both Leggett/08BT26-2 and Leggett/08BT70-1 F_2 populations were susceptible to 16MN (100-3), therefore, it did not fit the expected ratio of 3:1 resistant to susceptible plants. The heterogeneity X^2 was 0 for Leggett/08BT26-2 and 0 for Leggett/08BT70-1 populations. The resistance gene present in Leggett is the same as the resistance genes present in 08BT26-2 and 08BT70-1 because the allele for resistance did not segregate in the F_2 populations. The parental lines Leggett, 08BT26-2 and 08BT70-1 reacted the same to 16MN (100-3).

On the other hand, Leggett/BT1020-1-1 F_2 populations (1 and 2) segregated 3:1 for the resistant to susceptible ratio. Leggett/BT1021-1-1 segregated 3:1 for population (1) and 13:3 for population (2). The allelism test showed that the resistance gene present in Leggett is different from the resistance genes present in BT1020-1-1 and BT1021-1-1. This is because the allele for disease resistance was different from that of Leggett causing segregation in the F_2 populations. Rines *et al*. (2017) noted in their experiment that BT1020-1-1 and BT1021-1-1 were F_2 family

sisters and the resistance genes they possess were different genetically from *Pc94* which Leggett possesses. Their result confirms our allelism test showing that the resistance genes present in BT1020-1-1 and BT1021-1-1 are different from the resistance gene present in Leggett. A 3:1 and 13:3 resistant to susceptible phenotypic ratios were obtained in the F_2 segregating populations.

References

Brown, C.M., and H.L. Shands. 1954. Behavior of the interspecific hybrid and amphiploid of *Avena abyssinica* x *A. strigosa*. Agron. J. 46:557-559.

Carson, M.L. 2011. Virulence in Oat Crown Rust (*Puccinia coronata* f. sp. *avenae*) in the United States from 2006 through 2009. Plant Dis. 95(12):1528-1534.

Doehlert, D.C., M.S. McMullen, and J.J. Hammond. 2001. Genotypic and environmental effects on grain yield and quality of oat grown in North Dakota. Crop Sci. 41:1066–1072.

Endo, R.M., and G. Boewe. 1958. Losses caused by crown rust of oats in 1956 and 1957. Plant Dis. Rep. 42:1126–1128.

Mitchell Fetch, J.W., S.D. Duguid, P.D. Brown, J. Chong, Jr.T.G. Fetch, S.M. Haber, J.G. Menzies, N. Ames, J. Noll, T. Aung, and K.D. Stadnyk. 2006. Leggett oat. Can. J. Plant Sci. 87:509–512.

Murphy, H.C. 1935. Physiology specialization in *Puccinia coronata avenae*. USDA Tech. Bull. 433. U.S. Gov. Print. Office, Washington, DC.

Nazareno, E.S., F. Li, M. Smith, R.F. Park, S.F. Kianian, and M. Figueroa. 2018. *Puccinia coronata* f. sp. *avenae*: a threat to global oat production. Mol. Plant Pathol. 19(5):1047–1060.

Rines, H.W., M.E. Miller, M. Carson, S. Chao, T. Tiede, J. Wiersma, and S.F. Kianian. 2017. Identification, introgression, and molecular marker genetic analysis and selection of a highly effective novel oat crown rust resistance from diploid oat, *Avena strigosa*. Theor Appl Genet. 131(3):721-733.

Rines, H.W., H.L. Porter, M.L. Carson, and G.E. Ochocki. 2007. Introgression of crown rust resistance from diploid oat *Avena strigosa* into hexaploid cultivated oat *A. sativa* by two methods: direct crosses and through an initial 2x.4x synthetic hexaploid. Euphytica. 158:67-79.

CHAPTER IV: GENERAL CONCLUSIONS AND SUGGESTIONS

The series of experiments conducted were designed to evaluate the suppression effect of *A. strigosa* derived crown rust resistance at the *Pc38* locus, and confirm the type of resistance gene present in Leggett and other *A. strigosa* derived crown rust resistance genes lines. The objective of the first experiment was to confirm the suppression effect of *Pc38* on *Pc94*. *Pc38* had been identified earlier to suppress *Pc62*. The experiment confirmed the suppression of *Pc94* by *Pc38* as well. Dumont and Steele possess two copies each of *Pc38*. Dumont has an interchanged *Pc38* locus on the chromosome relative to Steele, and both populations suppressed the effect of the resistance gene present in Leggett. Resistant to susceptible ratios of 1:2, 1:3 and 3:13 were produced from the F_2 populations. Resistant and susceptible plants were distinguished by inoculation with CR91. The single dominant gene effect of *Pc94*, which had been identified in other crosses, was suppressed in the presence of *Pc38*. Excess production of susceptible plants upon inoculation with CR91 affirmed that. Genotypes identified in the NDSU breeding program having *Pc38* in their genetic make-up should not be crossed with other genotypes with *Pc94*, since the purpose of strengthening and prolonging the effectiveness of the resistance gene to evolving crown rust races will be defeated.

Secondly, the objective to identify the crown rust resistance genes present in Leggett and 4 other genotypes developed by Rines *et al*. (2007; 2017) was confirmed. Two of the 4 genotypes were confirmed to possess different crown rust resistance gene from Leggett through the allelism test. The F_2 populations from the cross between Leggett/BT1020-1-1 and Leggett/BT1021-1-1 fit 3:1 and 13:3 resistant to susceptible ratios when plants were inoculated with the crown rust race 16MN (100-3).

APPENDIX

Table A.1 Chi square analysis of Leggett/Df-38 individual F_2 population (1)

Phenotypic class of Leggett/Df-38 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	169	164.67	+ 4.33	0.11
Susceptible	78	82.33	- 4.33	0.23
Total	247	247	0	0.34

Table A.2. Chi square analysis of Leggett/Df-38 individual F_2 population (2)

Phenotypic class of Leggett/Df-38 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	130	136.5	- 6.5	0.31
Susceptible	52	45.5	+ 6.5	0.93
Total	182	182	0	1.24

Table A.3. Chi square analysis of Leggett/Dp-38 individual F_2 population (1)

Phenotypic class of Leggett/Dp-38 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	186	196.5	- 10.5	0.56
Susceptible	76	65.5	+ 10.5	1.68
Total	262	262	0	2.24

Table A.4. Chi square analysis of Leggett/Dp-38 individual F_2 population (2)

Phenotypic class of Leggett/Dp-38 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	107	107.5	- 0.5	0.00233
Susceptible	108	107.5	+ 0.5	0.00233
Total	215	215	0	0.0047

Table A.5. Chi square analysis of Leggett/Dumont individual F_2 population (1)

Phenotypic class of Leggett/Dumont F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	124	109.75	+ 14.25	1.85
Susceptible	315	329.25	- 14.25	0.62
Total	439	439	0	2.47

Table A.6. Chi square analysis of Leggett/Dumont individual F_2 population (2)

Phenotypic class of Leggett/Dumont F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	51	52.33	- 1.33	0.03
Susceptible	106	104.67	+ 1.33	0.02
Total	157	157	0	0.05

Table A.7. Chi square analysis of Leggett/Steele individual F_2 population (1)

Phenotypic class of Leggett/Steele F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	32	42.5625	- 10.5625	0.61
Susceptible	195	184.4375	+ 10.5625	2.62
Total	227	227	0	3.23

Table A.8. Chi square analysis of Leggett/Steele individual F_2 population (2)

Phenotypic class of Leggett/Steele F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	39	39.375	- 0.375	0.000824
Susceptible	171	170.625	+ 0.375	0.00357
Total	210	210	0	0.00439

Table A.9. Chi square analysis of Leggett/Steele pooled F_2 population

Phenotypic class of Leggett/Steele pooled F_2 Populations	Observed number	Expected number	Deviation	X^2
Resistant	71	81.9375	- 10.9375	1.46
Susceptible	366	355.0625	+ 10.9375	0.34
Total	437	437	0	1.80

Table A.10. Heterogeneity chi square analysis of Leggett/Steele

Source	Chi square	df
Summed Experiments	2.69	2
Pooled	1.80	1
Heterogeneity	0.89	1

Table A.11. Chi square analysis of Leggett/08BT26-2 individual F_2 population (1)

Phenotypic class of Leggett/08BT26-2 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	0	63	- 63	63
Susceptible	84	21	+ 63	189
Total	84	84	0	252

Table A.12. Chi square analysis of Leggett/08BT26-2 individual F_2 populations (2)

Phenotypic class of Leggett/08BT26-2 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	0	177	- 177	177
Susceptible	236	59	+ 177	531
Total	236	236	0	708

Table A.13. Chi square analysis of Leggett/08BT26-2 pooled F_2 populations

Phenotypic class of Leggett/08BT26-2 pooled F_2 Populations	Observed number	Expected number	Deviation	X^2
Resistant	0	240	- 240	240
Susceptible	320	80	+ 240	720
Total	320	320	0	960

Table A.14. Heterogeneity chi square analysis of Leggett/08BT26-2

Source	Chi square	df
Summed Experiments	960	2
Pooled	960	1
Heterogeneity	0	1

Table A.15. Chi square analysis of Leggett/08BT70-1 F_2 individual population (1)

Phenotypic class of Leggett/08BT70-1 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	0	47.25	- 47.25	47.25
Susceptible	63	15.75	+ 47.25	142.02
Total	63	63	0	189

Table A.16. Chi square analysis of Leggett/08BT70-1 individual F_2 populations (2)

Phenotypic class of Leggett/08BT70-1 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	0	213	- 213	213
Susceptible	284	71	+ 213	639
Total	284	284	0	852

Table A.17. Chi square analysis of Leggett/08BT70-1 pooled F_2 populations

Phenotypic class of Leggett/08BT70-1 pooled F_2 Populations	Observed number	Expected number	Deviation	X^2
Resistant	0	260.25	- 260.25	260.25
Susceptible	347	86.75	+ 260.25	780.75
Total	347	347	0	1041

Table A.18. Heterogeneity chi square analysis of Leggett/08BT70-1

Source	Chi square	df
Summed Experiments	1041	2
Pooled	1041	1
Heterogeneity	0	1

Table A.19. Chi square analysis of Leggett/BT1020-1-1 individual F_2 population (1)

Phenotypic class of Leggett/BT1020-1-1 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	118	116.25	+1.75	0.03
Susceptible	37	38.75	-1.75	0.08
Total	155	155	0	0.11

Table A.20. Chi square analysis of Leggett/BT1020-1-1 individual F_2 populations (2)

Phenotypic class of Leggett/BT1020-1-1 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	183	192	- 9	0.42
Susceptible	73	64	+ 9	1.27
Total	256	256	0	1.69

Table A.21. Chi square analysis of Leggett/BT1020-1-1 pooled F_2 populations

Phenotypic class of Leggett/BT1020-1-1 F_2 pooled Populations	Observed number	Expected number	Deviation	X^2
Resistant	301	308.25	-7.25	0.17
Susceptible	110	102.75	+7.25	0.51
Total	411	411	0	0.68

Table A.22. Heterogeneity chi square analysis of Leggett/BT1020-1-1

Source	Chi square	df
Summed Experiments	1.78	2
Pooled	0.68	1
Heterogeneity	1.10	1

Table A.23. Chi square analysis of Leggett/BT1021-1-1 individual F_2 population (1)

Phenotypic class of Leggett/BT1021-1-1 F_2 Populations (1)	Observed number	Expected number	Deviation	X^2
Resistant	118	120.75	-2.75	0.06
Susceptible	43	40.25	+2.75	0.19
Total	161	161	0	0.25

Table A.24. Chi square analysis of Leggett/BT1021-1-1 individual F_2 populations (2)

Phenotypic class of Leggett/BT1021-1-1 F_2 Populations (2)	Observed number	Expected number	Deviation	X^2
Resistant	279	282.75	- 3.75	0.05
Susceptible	69	65.25	+ 3.75	0.22
Total	348	348	0	0.27