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A Study of Agronomic, Genetic and Environmental Influences on Oat (Avena sativa L.) Grain Quality.

David Gavin Humphreys

A thesis presented to the Faculty of Graduate Studies and Research of McGill University in Partial Fulfilment of the degree of Doctor of Philosophy

Department of Plant Science

June, 1994

McGill University, Montréal, Canada

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Short Title:

Agronomic and Genetic Aspects of Oat Grain Quality.

D. Gavin Humphreys

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General Abstract

Agronomic, genetic and environmental influences on oat grain quality were investigated. Nitrogen application at the boot stage of crop development did not generally affect physical characteristics of oat grain. Groat protein content increased with the application of nitrogen at the boot stage of crop development but oil content tended to decrease while B-glucan content was generally not affected. Delayed seeding usually reduced oat grain quality and usually increased protein and ß-glucan contents. Heritability of B-glucan content, groat percentage and rust resistance were all low; hence, breeding to improve these traits may be difficult. Results of experiments on the inheritance of Bglucan were not conclusive but it appears that ß-glucan content is under the influence of the endosperm genome. Genotype by environment interactions were significant for grain yield and for protein, oil and B-glucan contents. The cultivars Ultima and Sylva were superior in mean grain yield across environments compared to other cultivars. The cultivars Laurent and Nova were superior for mean protein content across environments and the cultivar Marion QC was superior for mean oil and ß-glucan contents. AMMI (Additive main effects and multiplicative interaction) analysis would be favoured over the other genotype by environment analyses used in this study because AMMI afforded more information about the genotype by environment interactions.

Résumé

Les influences des pratiques agronomiques, de la génétique, et de l'environnement sur la qualité du grain d'avoine ont été étudiés. En générale, les caractéristiques physiques n'ont pas été affectés par l'application de l'azote au stage gonflement (Zadoks 40-43). Par contre, le semis tardif a eu pour l'effet de réduire la qualité du grain d'avoine. Cependant, cela ne fut pas le cas dans tous les environnements. Le contenu en protéines a augmenté avec l'application d'azote au stade gonflement alors que le contenu en huile et en ß-glucanes n'ont pas été généralement affectés. Le semis tardif a augmenté le contenu en protéines et B-glucanes mais encore, les effets de l'environnement ont joué un rôle important. L'héritabilité des contenu en B-glucanes, du pourcentage en amandes et de la résistance à la rouille ont été tous faibles ce qui suggère que l'amélioration génétique de ces caractères peut s'avérer très difficile. Des expériences traitant spécifiquement de l'hérédité des ß-glucanes n'ont pas été concluantes mais il semble que le contenu en ß-glucanes soit sous l'influence du génome de l'endosperme. Les interactions génotype par environnement ont été significatives pour le rendement en grains de même que pour le contenu en protéines, huile et ß-glucanes. Les cultivars Ultima et Sylva ont été supérieurs pour le rendement en grain moyen parmi tous les environnements comparé aux autres cultivars. Les cultivars Laurent et Nova ont été supérieurs aux autres cultivars dans cette étude en ce qui a trait au contenu moyen en protéines alors que Marion QC a été supérieur pour le contenu moyen en huile et ßglucanes. L'analyse AMMI serait préférable aux autres analyses statistiques des effets génotype par environnement utilisé dans cette étude parce que AMMI a fourni plus d'information sur les interactions génotype par environnement.

Acknowledgements

The acknowledgement section is often the last one to be written and this one is no exception. It is impossible to properly thank all the people who helped in their own way to make this research and thesis possible. Hence, I apologize in advance if I miss anyone.

First, I would like to thank Dr. Diane Mather for her support, guidance and erudite contributions to this thesis. This work would have never been accomplished without Diane's seemingly tireless ability to advance ideas on how to improve both the research and the manuscript.

I would like to thank Dr. Don Smith, Dr. Inteaz Alli and Dr. Bruce Coulman for serving on my supervisory committee. I must acknowledge the help of the crew at the Seed Farm in particular Thomas Mpoulimpassis who aside from being a good technical advisor became a good friend. I am indebted to several people in the "lab" who were always ready to help: my thanks to Stewart Liebovitch, Birgit Schultz and especially Nathalie Brière. Nathalie was present for most of the time the research was being conducted and her technical assistance, dedication to the task and her friendship are truly appreciated. I must also acknowledge the contribution of my friend, Dr. Nick Tinker, who tolerated me for over a year during the writing of this thesis and who was always ready when called upon with advice. I thank my parents for their boundless belief in me and my abilities and for making the road along the way that much smoother. Finally, I thank my family: Emma, William and most of all Sylvie. You made it tolerable and you were the reason I continued at those times when I thought that I would not. This accomplishment is in some ways your accomplishment too, for you have sacrificed alongside me during the completion of my doctorate. To you, I give my deepest thanks.

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Description of Thesis Format

The format of this thesis is consistent with conditions approved by the Faculty of Graduate Studies and Research of McGill University and as outlined in part B, section 2 "Manuscripts and Authorship", which are as follows:

2/ Manuscripts and Authorship: Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), providing that these copies are bound as an integral part of the thesis. If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory. The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published. The thesis must include, as separate chapters or sections: (1) a table of contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and/or summary. Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis. In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph.D. Oral Defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers. "

This thesis includes original manuscripts suitable for submission to refereed journals. Chapter 1 is a statement of objectives. Chapter 2 is a comprehensive literature review. Chapters 3 through 7 are manuscripts prepared for submission to refereed journals and these chapters are each preceded by a preface that is written to provide logical links between the different chapters and to outline the contributions of the authors to the work. Each manuscript contains a literature review pertinent to that particular study, while background literature can be found in the literature review section (chapter 2). The remaining sections, summarize research, outline contributions to knowledge and outline suggestions for future research. All literature cited in this thesis is listed in the a separate section at the end of the thesis.

Chapter 1: Objectives of Thesis Research

The objectives of the research were the following:

- (1) To determine the effects of a supplemental nitrogen fertilizer application and the effects of delayed seeding on various characteristics of oat grain quality.
- (2) To estimate the heritability of β-glucan, groat percentage and crown rust resistance in oat.
- (3) To investigate the inheritance of β -glucan in oat.
- (4) To determine the effects of genotype by environment interactions on grain yield, protein, oil and β -glucan contents in oat.

Chapter 2: Review of the Literature

2.1 Oat

Oat is an annual grass that belong to the genus Avena. Between 19 (Rajhathy and Thomas, 1974) and 27 species (Baum, 1977) have been identified within the genus Avena among three ploidy levels (2n=14, 28 and 42). Most cultivated oat belongs to the hexaploid group (2n=42).

Avena sativa L. (common white oat) is the best known and most important cultivated oat (Youngs et al., 1982). It is grown principally in temperate regions of the world and retains its hull after threshing. Naked oat is Avena sativa that lose their hull (lemma and palea) during threshing. However, the environment can significantly reduce the percentage of hull-less oat in the harvested crop (Lawes and Bowland, 1974). At present, naked oat is of limited commercial importance. Avena byzat. Ina C. Koch. (common red oat) is commonly grown as a winter oat but it is notably less cold tolerant than winter wheat or rye (Stoskopf, 1985). Significant acreage of this species in North America is found in the southern United States.

Oat is looked upon as a soil-conserving crop that can be useful to farmers as: (1) an alternative to row crops; (2) a crop that can help prevent the build up of soil-borne pathogens that attack other major crops such as wheat or potato; (3) a supplier of livestock bedding; and (4) a companion crop for the establishment of forage seedings (Forsberg and Shands, 1989).

Oat is grown on every continent in the world. Oat ranks sixth in world production among cereal grains (FAO, 1990). The major producing regions include the Europe,

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Usage	Tonnage (Mt)	Percentage of total
Seed	156	5%
Human Food	100	3%
Export	100	3%
Feed on Farm	2 584	78%
Domestic Feed	360	11%

Table 2.1: Usage of Canadian oat crop by category, tonnage and percent of total.

North America and the former Soviet Union (Youngs *et al.*, 1982). Canada ranks fifth in the world in total production (Schrickel, 1986). In the 1980's, Canadian oat production averaged 3.3×10^6 tonnes. Oat produced in Canada is used primarily for livestock feed (Table 2.1). Human consumption accounts for a small but growing proportion of total oat production.

Until approximately 1960, oat grain was in demand as feed for ruminants and workhorses. Oat straw was used for bedding animals. Oat could be cut for green feed or hay if especially pasture or hay crops failed. During the earlier portion of this century, millions of acres of oat were grown in Canada.

Farm mechanization has resulted in a decline in the demand for oat as a workhorse feed; and consequently, in oat acreage and importance. Moreover, corn (Zea *ssp.*) and barley (*Hordeum vulgare* L.) have assumed the role of feed grains for swine and poultry. Wheat (*Triticum aestivum* L.) is considered the primary human food grain

in Canada. All of these factors have contributed to reduce the importance of oat as a commodity in Canadian agriculture.

Today, most oat produced in Canada is consumed domestically, and a large portion is used for on farm feed (Table 2.1). Oat grain is useful in dairy cattle and horse rations because of its high fibre, 12-15% whole-grain protein, and superior oil content and composition. Moreover, the protein is of a superior quality, making oat grain a desirable fraction of feed rations for breeding animals and young stock. However, oat hulls add considerable amount of crude fibre to the diet which is deemed undesirable as it may reduce rate of gain (Hesselman and Åman, 1986).

The oat groat is the portion which remains after the hull (lemma and palea) is removed. Groats generally contain 16-21% protein whose nutritional quality is approximately 86% that of casein while wheat and corn proteins are only 60% and 40% that of casein, respectively. Oat grain lipid content is approximately 1.8 times that of maize or millet. Fractionation of oat groats could yield products of potential commercial value (i.e. high protein, lipid, starch or β -glucan fractions). Webster (1986) has suggested that the principal impediment to commercialization of oat fractionation is byproduct utilization. The cost of fractionation is too high to support individual fraction production; and the processor would require a market for the large by-product fraction.

The principal use of oat groats is as rolled oat in hot breakfast cereals (Webster, 1986). Oat flour is often mixed with flour of other cereals or soybean for use in cold breakfast cereal production. Oat flour and flakes are also major ingredients in many cookie mixes (McKechnie, 1983).

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Oat flour has been shown to have antioxidant properties (Peters and Musher, 1937). Oat antioxidants, such as Avenex and Aveeno (Youngs *et al.*, 1982), have not been particularly successful due to the availability and effectiveness of synthetic antioxidants like butyl hydroxytoluene (BHT). Oat flour could be revived as an antioxidant if the concern among health-conscious consumers and chemical regulatory bodies over specific chemicals in food continues to grow (Webster, 1986).

The manufacture of cosmetics products from different oat fractions is gaining in popularity. Commercial products from oatmeal and flour include soaps, shampoo and a bath preparation (Webster, 1986).

Presently, there is a great interest in oat due to the fact that oat bran has been linked to lower blood cholesterol (Hurt *et al.*, 1988), and bran may play a role in blood sugar regulation (Anderson, 1986). Furthermore, "oat gum", a portion of which is composed of β -glucan, has been found to have a remarkable thickening ability which suggests a potential value as a hydrocolloid by-product of any fractionation process (Wood *et al.*, 1989).

2.2 Oat Agronomy

In the northern hemisphere, *A. sativa* is usually planted in April or May and harvested in July or August (Schrickel, 1986). Well drained soils are preferred, and a seedbed that has been prepared through ploughing or disking is desirable. Oat is usually drill-planted at a maximum depth of 5 cm with 15 to 20 cm between rows depending on seeding equipment (Stoskopf, 1985). Seeding rate is normally 90 kg/ha. Generally, oat

yields well with only intermediate amounts of fertilizer. Excessive fertilization of oat can result in lodging which may reduce harvestable yields.

The choice of which cultivar to grow is very important since environmental conditions and prevalent diseases of a specific growing region will tend to favour some cultivars over others. Maximum oat yield is favoured through proper weed control. Broadleaf weeds can be readily controlled with application of recommended herbicides. Two harvest techniques for oat are used: direct combining or windrowing followed by combing of grain from the windrow after a drying period.

Production of milling oat for food purposes requires more careful production of the crop because milling oat must meet or exceed well-defined seed specifications. Most recommended cultivars can be used for milling purposes; however, care should be taken to reduce lodging and to control weeds and diseases. Harvesting should be done promptly to reduce weathering and grain discoloration.

Significant diseases of oat in Canada include: crown rust (*Puccinia coronata* Cda.); stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Erikss. and Henn.); Septoria avenae blotch and black stem (*Septoria avenae* f. sp. *avenae*); common root rot (*Fusarium* ssp.); bacterial blight (*Pseudomonas syringae* pv.) and yellow dwarf or red leaf (barley yellow dwarf virus).

In general, the rust diseases are the most widespread; hence, the most problematic for oat producers. In parts of Québec, crown rust can be particularly prevalent due in part to the presence of significant numbers of buckthorn (*Rhamnus* ssp.), its secondary host. Fortunately, numerous genes for resistance to crown rust are available which should aid to control this disease for the foreseeable future (Martens and Dyck, 1989). In Canada, red leaf is second only to the rusts in economic importance (Martens *et al.*, 1988). Schrickel (1986) reported that yellow dwarf was the most economically important disease of oat in the United States.

2.3 Oat Botany

The inflorescence of the oat plant is a panicle which may be compact with short branches and internodes; unilateral or "mane-shaped" as in the "side-oat"; or ramose as in the wild oat (*Avena fatua* L.). Cultivated oat typically have a central and open panicle with 5 to 7 nodes from which branches arise bearing spikelets. Each lateral branch ends in a single apical spikelet. From primary branches grow secondary branches which may give rise to tertiary branches. Second and third order branches also end in spikelets. A typical panicle has 20 to 50 spikelets.

An oat spikelet consists of two glumes on a rachilla which normally bears one to three fertile florets. Naked cat usually have between 4 and 8 fertile florets per spikelet. The oat floret is composed of a stiff lemma and papery palea which surround the reproductive parts of the flower. The pistil consists of an ovary and two styles which have feather-like stigmatic branches. Generally, three anthers and two lodiculae are present in a floret. The latter are located at the base of the floret and may swell during the time of pollination to open the glumes. This can cause the anthers to be extruded from the spikelets and may increase the possibility of cross-fertilization (Stoskopf, 1985). The oat grain is composed of a caryopsis (groat) to which the lemma and palea (hull) closely adhere. In naked oat, the groat threshes free of the lemma and palea. The oat caryopsis is generally spindle-shaped with a crease on one side. Variable amounts of fine hairs (trichomes) cover the groat. If the primary floret fails to develop properly, the lemma of the primary floret may envelop most of the secondary floret. The secondary floret is then referred to as a bosom kernel. The entire entity may be referred to as a double oat.

2.4 Oat Milling

Milling of grain may be civilization's oldest industry (Deane and Commers, 1986). Primitive inhabitants pounded grain upon a flat stone or boulder. Much of the history of milling relates to wheat but as early as 1840, a North American device for oat dehulling had been produced. Presently, oat milling is a highly automated process which includes cleaning, hulling, steaming, flaking and packaging.

Upon the receipt of an oat shipment at a mill, the cargo is weighed and evaluated for percent moisture, test weight, weed seed and insect contamination, groat coloration and theoretical milling yield. This information is used to establish the price of the shipment and allows grading of the oat for proper storage and usage later. Different grades of oat are often blended before milling in an effort to minimize variations in test weight and groat colour between lots of oat. The actual process of milling usually varies from one milling operation to another. A generalized description of oat milling is described here from a process presented by Deane and Commers (1986). Preliminary cleaning is often performed upon acceptance by the mill of an oat shipment. A receiving separator performs the task of removing dust, loose chaff, and coarse objects that could damage equipment. After the initial cleaning, the shipment is stored.

Milling of oat begins with mechanical cleaning of the grain which is initiated by the milling separator. Thus machine uses aspiration to remove multiple contaminants from the oat including: straw, dust, and loose hulls. Multiple levels of screens with different opening sizes separate large seeds (e.g. corn, soybeans) and stones from good oat. Small weed seeds fall through all screens and are discarded.

The next stage of cleaning uses a series of disk separators. These machines are designed to separate by length. The first disk separator remove sticks. These are followed by disk separators which divide the oat into large, medium and smah oat fractions. Smaller mills may only have two sizes. Grading by size is done so that each fraction, which contains its own particular types and sizes of impurities, may be given a cleaning treatment best tailored to its specific set of impurities. The result is a more thoroughly and efficiently cleaned product (Deane and Commers, 1986).

Following grading, oat is passed through width graders. Width graders separate seeds based on thickness (plumpness). Large grade oat is passed through 2 sets of width graders. The first set removes the larger double oat. The second set of width graders separates "pin oats". Pin oats, or thin oats, are very thin oat which often have no groat. Medium grade oat is also passed through a width grader to remove pin oats. Small oat

is passed through one width grader to remove larger wheat and short barley seeds, and another to remove pin oats.

Large oats are subsequently aspirated to remove light oats. A light oat is usually of normal size but possesses an undersized groat. Medium oats move from disk separators to gravity tables. Specific gravity separators, better known as gravity tables, clean good oat from impurities based on their specific gravity. Air is used to stratify the oat grains based on their specific gravity. Gravity tables are used to divide the heavier from the lighter fractions. Impurities removed from the medium grade oat include rodent droppings, stones and light oat.

At this stage in processing, oat is heat treated and dried to deactivate fat catabolizing enzymes (i.e. lipase). This is necessary because dehulled oat contains approximately 6.5% fat (Deane and Commers, 1986). Damage to oat groats during cleaning or hulling may activate lipase enzymes that can breakdown triglycerides into free fatty acids and glycerol. The free fatty acids can give rise to a product that is rancid and unpalatable.

Hulls are removed using impact dehullers. Oat is propelled against a carborundum or rubber ring inside the dehuller. The impact and abrasion cause the hulls to detach from the groat (caryopsis). A properly adjusted dehuller can function at 90-95% efficiency; in contrast, a poorly adjusted dehuller may only remove 55% of hulls in the first pass through the dehuller.

The dehuller outflow consists of hulled oat, groats, hulls, groat chips and fines (oat dust). This mixture must be separated. Aspiration is used to remove hulls, fines and

other light particles such as trichomes. Paddy separators which employ the difference in specific gravity, surface smoothness and grain shape to separate objects of similar size and shape, are used to separate hulled oat from groats. Hulled oat is returned to dehullers.

At this point in milling, the grain has been cleaned and the hull removed. The groats are now ready for processing into end products. Groats are cut into pieces so that flakes will be of a more uniform size which facilitates packaging. Following cutting, the oat pieces are moved to the rollers for flaking. To prevent disintegration of the groats into a fine flour under the heavy pressure of the rollers, the groats are steamed immediately prior to rolling. This also completes the enzyme deactivation process. The thinness of the flake depends on the desired end product. For example, instant or quick-cooking oatmeal consists of relatively thin flakes compared to regular whole-groat oatmeal.

Following rolling, flakes are placed in coolers where temperature and moisture is adjusted to ensure acceptable shelf life. Subsequently, flakes are packaged or passed to the grinders for whole oat flour production. To minimize handling of the finished product, packaging may be located just below the flaking equipment. Packaged flakes are ready for shipping and sale to the consumer.

Oat flour is produced by grinding flakes or enzyme-deactivated groats. Oat bran is a bran-rich fraction which is produced by sieving coarsely ground oat flour (Webster, 1986).

2.5 Oat Grain Milling Quality

Recently, the demand for oat of superior milling quality has increased. In Ontario, the cultivar 'Donald' has been recommended as a milling oat and the cultivar 'Newman' is now widely used for the same purpose. A superior, milling-quality oat cultivar adapted to Quebec could help Quebec farmers to compete for a greater share of the milling market.

Important milling seed characteristics include seed colour, groat colour, plumpness, percentage thin oat, test weight, kernel weight, percent hull, percent hull produced during threshing, theoretical milling yield, facility of dehulling and groat durability.

The 'Western Expert Committee on Oat Grain Quality' has compiled guidelines for milling characteristics. A white to yellow hull colour is preferable, and groats should be white to cream. Darker oat grains are undesirable since they resemble animal faecal matter. Plump uniform-sized oats are preferred for milling but thin oats are acceptable to a maximum of 2 percent through a 1.6 mm screen using a laboratory precision grader. Double oats are undesirable.

A minimum test weight of 47.4 kg/hl (or 38 lbs./bushel) has been suggested by processors. In Sweden, 55.1 kg/hl is the base test weight, and Swedish producers receive a 0.5 percent penalty per kg below the base test weight value on the price of their oat (Mattson, 1986). Test weight is the most commonly used quality trait because it is easy to measure and provides an indication of the plumpness of the groat.

The 'Western Expert Committee on Oat Grain Quality' suggested that a minimum 1,000 kernel weight of 28 g was acceptable. Percent hull of 22-26 is desirable, and over 30 percent is not recommended since high percent hull will likely result in lower milling groat yields for the food processor. Dehulling of oat grain during threshing can expose groats to pests and damage. A maximum of 5 percent dehulled oat grains from threshing is recommended.

Theoretical milling yield (TMY) is defined as the number of pounds of hulled oat required to produce 100 lbs. of groats. A maximum TMY of 160 lbs is suggested. Because theoretical milling yield is difficult to measure, other means of evaluating groat percentage are frequently used.

Finally, three other general grain characteristics may be important for milling. Removal of hulls should be moderately easy but very thin hulls will likely result in a higher percent of dehulled oats after threshing which would be undesirable. Groats that resist breakage during processing are desirable. Damage can result in lipase and lipoxygenase enzyme activity that may give rancid off flavours to the final product (Youngs, 1986).

Symons and Fulcher (1988) found that for a constant set of milling conditions, smaller kernels were damaged less in an impact dehuller than larger kernels. Therefore, for milling purposes oat grain can be overly plump because such oat grains may be subjected to more damage during milling than smaller grains.

It is evident from the above discussion that oat milling quality involves many characters. Superior values in one character (e.g. test weight) may result in reduced

values for another desirable trait (e.g. percent breakage). An acceptable compromise would likely be the best option in such cases.

High heritability for primary caryopsis percentage, test weight (kg/hl), primary kernel weight (mg), primary caryopsis weight (mg) and plump kernel percentage have been reported (Wesenberg and Shands, 1973). Hence, breeding for each of these characteristics is possible; however, due to the interrelationship of seed quality components simultaneous monitoring of several traits would be recommended to assure that an increase in one trait does not adversely affect others. Other characteristics (e.g. percent hull) are significantly influenced by the environment which could impede breeding progress.

2.6 Biochemical Characteristics of Cereal Grain:

2.6.1 Protein

Cereal grains supply a major portion of the world food protein requirements. Generally, cereal grains have low protein percentage compared to legumes and cereal proteins contain inadequate levels of some essential amino acids. Oat has a high protein percentage and good quality relative to other grains (Iwig and Ohm, 1978). Whole-oat protein content is similar to other cereals but groat protein levels are much higher and can range from 14 to 22 percent (Robbins *et al.*, 1971).

Enzymes, structural proteins and storage proteins are all present in seeds (Goodwin and Mercer, 1983). Seed proteins can be classified in a general fashion according to solubility (Table 2.2).

Table 2.2: General solubility classifications of proteins.

Group Name	Solubility
Albumins	Soluble in H_2O and dilute salt
Globulins	Insoluble in H_2O ; soluble in dilute salt
Glutelins	Soluble in dilute acid and bases; insoluble in neutral solvents
Prolamins	Soluble in 70-80% ethanol _(aq) ; Insoluble in H_2O

(Goodwin and Mercer, 1983)

Most cereals have a high proportion of prolamins but oat prolamin content is low ranging between 4 and 14% of total protein (Peterson and Brinegar, 1986). However, researchers disagree as to the most prominent protein fraction. Previously, globulins were believed to make up the largest proportion of oat seed protein. Globulin content has been estimated to be as high as 80% (Brohult and Sandegren, 1954). Peterson (1976) reported a mean globulin level of 52% in oat samples from several cultivars and environments, and glutelins accounted for 21 to 27 percent of total protein. In contrast, German researchers have reported that glutelins are the major protein fraction, and that the globulin proportion is merely 12 to 19 percent (Micheal *et al.*, 1961; Wieser *et al.*, 1980). Differences in protein extraction protocol may explain this discrepancy in reported results (Peterson and Brinegar, 1986).

Protein level in cereals is highly susceptible to environmental influence. For example, Wiggans and Frey (1958) found a substantial increase in grain nitrogen percentage during a hot, dry season but little increase was reported for the same two varieties during a cool, humid year. Soil fertility and disease incidence have also been found to affect protein level in oat (Welch, 1986).

Application of nitrogen (N) fertilizer to soils has been shown to increase the protein levels of oat. Portch *et al.* (1968) reported that the application at seeding of 45 kg N/ha increased grain protein between 1.4 and 1.8 percent, depending on soil drainage. Welch and Yong (1980) compared the effects of application of 125 kg N/ha at the 2-3 leaf stage, and at heading. Early fertilization increased yield and slightly increased percent protein. Late application had little influence on yield but grain protein percentage increased more than the early treatment. Double application (both early and late) increased both yield and percent protein resulting in the highest protein yield. Nitrogen fertilizer has been shown to affect protein composition; globulin fractions appear to increase more than other fractions under fertilization (Peterson, 1976).

2.6.2 Oil

Oat groats have the highest lipid concentration among the cereal grains. A range of 3.6 to 11.6 percent oil has been reported in the world oat collection (Brown and Craddock, 1972) and recently oil contents of over 16 percent have been reported in specialized breeding lines (Schipper and Frey, 1991). In adapted oat cultivars, oil contents usually varies between 4 and 8 percent. Oat lipids are nutritionally significant since they are highly unsaturated and contain considerable amounts of linoleic acid (Youngs, 1986).

Classes of lipids in oat include: sterol esters and free sterols; triglycerides and partial glycerides; free and esterified fatty acids; as well as glycolipids and phospholipids. The proportion of total lipid that each class makes up will vary between cultivars (Youngs *et al.*, 1977) and due to effects of environment (Saastamoinen *et al.*, 1989). Moreover, the solvent employed for lipid extraction may affect the relative proportion of each class extracted (Sahasrabudhe, 1979). Nevertheless, triglycerides are accepted as the predominant fraction in oat lipids (Youngs, 1986).

Inheritance studies indicate that oil percentage is polygenically inherited, with a tendency for high oil percentage to be partially dominant (Frey and Hammond, 1975). Oil content is highly heritable, and is reportedly stable over a range of environmental conditions (Baker and MacKenzie, 1972). However, low temperatures during growth may increase oil content and can alter the fatty acid profile (Saastamoinen *et al.*, 1989). Fertilizer applications of 50 to 150 kg/ha slightly decreased oil levels (Lampinen, 1986).

Oat has been considered as a potential oilseed crop. In Iowa, 16 percent oil has been proposed as the required level for oat to be an economically feasible oilseed crop (Frey and Hammond, 1975). High-oil alleles from cultivated and wild oat have produced superior breeding material (Thro and Frey, 1985). Recurrent selection for oil content raised mean groat oil content from 84.96 to 113.30 g/kg (g oil per kg groats), and total oil yield from 0.18 to 0.21 Mg/ha. Recurrent selection for increased oil is believed to

have improved oil yields through increased grain and groat yield not necessarily higher oil content.

Oil content is often positively correlated with grain yield (Thro and Frey, 1984) and in some genotypes negatively correlated with protein percentage (Youngs and Forsberg, 1979). No consistently significant correlation has been reported between groat-oil level and kernel weight, kernel density, test weight, or groat percentage (Branson and Frey, 1989). However, a highly significant correlation between oil yield and grain yield, groat yield biomass as well as harvest index has been reported (Branson and Frey, 1989).

Lipid content can have a significant influence on oat quality. Lipid content changes little when whole oat, or undamaged groats are stored at normal temperatures, and a moisture content of less than 10 percent. However, at moisture levels of greater than 10 percent free fatty acids increase (Frey and Hammond, 1975). In oat flour, free fatty acids accumulate rapidly during storage; a condition that is exacerbated at ambient temperatures of $\geq 30^{\circ}$ C.

This rise in fatty acid during storage is due principally to the action of the enzyme: lipase. Lipase is a hydrolytic enzyme that produces free fatty acids from triglycerides and partial triglycerides. Frey and Hammond (1975) reported that a 20-fold variation in lipase activity has been found among 352 oat entries.

Generally, changes in oat lipids can be prevented by denaturing the lipolytic enzymes, especially lipase. While lipase activity is unaffected by freezing or freeze drying (Urquhart *et al.*, 1980), it is effectively negated through heat treatment; for this reason, groats are usually steamed prior to rolling (Youngs *et al.*, 1982).
2.6.3 Carbohydrates (excluding β -glucan)

Sugars most often reported in oat include: sucrose, raffinose, glucose, fructose, stachyose and fructosans (MacArthur-Grant, 1986). Compared to other cereal grains such as wheat or barley, the amounts of these sugars are low (MacLeod and Preese, 1954).

Whole oat contain approximately 14% by weight of pentosans which are made up principally of araban and xylan (Matz, 1969). The amount of pentosan present is higher in the hull (29%) (Whistler, 1950) than in the groat (4%) (Matz, 1969). In the past, oat hulls have been a source of pentosans for furfural production (Shulka, 1975); however, furfural is now principally produced from corn cobs. Furfural belongs to the furan (C_4H_40) family of chemicals (MacArthur-Grant, 1986). Furans are used extensively in both the petrochemical and chemical industries as well as tire production, brewing and cellulose pulp production.

The major carbohydrate in oat is starch. Oat starch content varies with cultivars and method of extraction (Youngs *et al.*, 1982). Paton (1977) reported that starch content ranged from 44 to 61 percent of the oat groat dry weight. Starch levels in oat groats are reportedly inversely proportional to protein content (Paton, 1977; MacArthur and D'Appolonia, 1979).

MacArthur and D'Appolonia (1979) have shown that most physicochemical characteristics of oat starch are similar to those of wheat. In contrast, Paton (1986) reported that variability of oat starch properties between oat cultivars existed, and that these properties may vary from those of wheat and corn.

2.6.4 ß-glucan

Cereal endosperm and aleurone cell walls frequently contain a high level of $(1\rightarrow3), (1\rightarrow4)-\beta$ -D-glucan (β -glucan) (Wood *et al.*, 1983). Oat and barley have relatively high portion of total β -glucan compared to other cereals. β -glucans are water soluble polysaccharides composed of β -D-glucopyranosy! units (Parrish *et al.*, 1960). The polysaccharide is unbranched, and contains about 70% 4-linked and 30% 3-linked β -D-glucopyranosy! units (Wood *et al.*, 1989). Woodward *et al.* (1983) found that barley β -glucan structure consisted of two or three 4-linked units separated by a single 3-linked unit but with approximately 5 areas of four or more consecutive (1-4) linkages. A similar structure has been proposed for oat β -glucan (Parrish *et al.*, 1960). Both soluble and insoluble β -glucan fractions exist in cereals. Solubility is affected by particle size and β -glucanase activity of flour; as well as temperature, pH and ionic strength of the extraction media (Wood *et al.*, 1978). A larger portion of the total β -glucan in oat is soluble than in barley (Åman and Graham, 1987).

In the brewing industry, a high β -glucan level in barley may result in a diminished rate of wort filtration, haze formation in the beer, and possibly reduced extraction efficiency (McCleary and Glennie-Holmes, 1985). β -glucans can have antinutritional properties, especially in chicken diets where their "gumminess" and indigestibility can affect food intake, cause sticky droppings and reduce feed conversion efficiency (Hesselman and Åman, 1986). Cave *et al.* (1990) reported that oat β -glucan could reduce the growth in young animals by impeding the absorption of fat soluble vitamins. Consumption of oat has been linked to reduced cholesterol levels in human beings. Degroot *et al.* (1963) were able to reduce serum cholesterol levels by 11% in hypercholesterolemic men fed 140 g of rolled as oat bread daily for 3 weeks. Subsequent studies over the past three decades have repeatedly demonstrated the cholesterol lowering effects of oat consumption in humans (Hurt *et al.*, 1988; Ripsin *et al.*, 1992). Mixedlinkage β -glucans of oat has been suggested as the agent that acts to reduce cholesterol levels.

The biochemical or physiological basis of oat dietary fibre induced changes in cholesterol metabolism is not fully understood. It is suggested that plant fibres bind bile acid and neutral sterol, and cause an increased excretion of these substances by preventing their reabsorption (Anderson and Chen, 1986). Neutral sterols include cholesterol and its bacterial metabolite. A decrease in bile acid reabsorption is thought to induce the liver to divert cholesterol from lipoprotein synthesis. Hence, less cholesterol-rich lipoproteins are available for secretion in the circulation, resulting in a lowered serum cholesterol level.

Dietary fibre has also been shown to reduce the glycemic index of food (Jenkins *et al.*, 1981). The "gummy" properties of oat β -glucans may create a viscous microclimate in the intestinal lumen that impedes the rate of glucose diffusion and adsorption (Hurt *et al.*, 1988).

2.6.5 β -glucan Quantification

Because of the negative effects of β -glucans on beer production, a number of methods have been developed for β -glucan quantification in barley. Previously, physical

methods such as precipitation techniques and viscosity measurements have been used; however, these techniques estimate β -glucan only for the particular conditions under which assays are carried out (Fleming *et al.*, 1974). Alternatively, β -glucan has been estimated following extraction and purification. Purified β -glucan was degraded to glucose, and estimated through quantification of the glucose (Wood *et al.*, 1978). β glucan has also been estimated indirectly using calcofluor dye. The carbohydrate is dyed and the amount of dye bound to the carbohydrate is quantified (Wood and Weisz, 1984).

A newer method of β -glucan quantification involves specific enzymatic degradation of the carbohydrate followed by quantification of the product. The carbohydrate is specifically degraded to β -gluco-oligosaccharides by lichenase. The β gluco-oligosaccharides are subsequently broken down into glucose molecules (McCleary and Glennie-Holmes, 1985). The glucose is colorimetrically quantified using a glucose oxidase/peroxidase procedure. The glucose measured is then converted to percent β glucan based on sample dry weight. This differs from previous techniques since enzymespecific degradation reduces the potential for contamination by other carbohydrates. Contamination by starch or other carbohydrates due to inadequate purification would bias β -glucan estimates when the sample was reduced to glucose. The enzymes for this technique are now commercially available (Biocon, 1987). This technique or its derivative has been successfully used in barley, rye, wheat and oat (McCleary and Glennie-Holmes, 1985; Henry, 1987). A streamlined version of the enzymatic technique has also been developed (McCleary and Codd, 1991). Henry (1985a) has attempted to quantify β -glucan in barley samples using nearinfrared analysis. Limited success has been reported to date as estimation of the carbohydrate can only reliably be done using starch-free samples of barley flour.

Oat cultivars with high fractions of soluble β -glucans may be favourable for human consumption. Therefore, it may be desirable to determine percent soluble and percent insoluble β -glucan in an oat sample. Åman and Graham (1987) used an enzymatic assay to determine total and percent insoluble μ glucan in Swedish oat. Soluble fractions were determined by subtraction.

Flow-injection analysis (FIA) has also been used to quantify β -glucan (Jørgensen, 1988). Calcofluor binds to β -glucan and the interaction is can be measured as an increase in the fluorescence intensity of the dye (Wood, 1993). The concentration of the β -glucan is proportional to the fluorescence intensity. The FIA technique must be calibrated with samples of known β -glucan content. Jørgensen (1988) found that β -glucan values obtained using FIA were highly correlated with those obtained using the enzymatic method of McCleary and Glennie-Holmes (1985).

2.6.6 Genetics of \beta-glucan

In oat, the genetic control of β -glucan has not been published. However, in barley a diallel cross analysis of gum content has been reported (Greenberg, 1977). Gum content was measured by extract viscosity which is closely correlated to β -glucan content. Diallel analysis showed that the trait was controlled by a simple additive-dominance genetic system with dominance for low gum. Two to three highly dominant genes were believed to be involved. Bogyo *et al.* (1988) found that both additive and additive x dominance effects were important in the genetic control of β -glucan in barley.

Barley β -glucan content is affected by both genotype and environment (Molina-Cano and Conde, 1982; Henry, 1986). There may be an inverse relationship between the level of moisture in the growth environment and extractable β -glucan content (Bendelow, 1975). Rain has been reported to affect β -glucan level in barley (Aastrup, 1979).

Significant genotype by environment effects have been found for β -glucan content in oat (Peterson, 1991; Lim *et al.*, 1992; Miller *et al.*, 1993); however, ranking of cultivars was generally consistent between locations and/or years. Brunner and Freed (1994) found that groat β -glucan content tended to increase with superior levels of N fertilizer and these authors concluded that environmental factors such as soil N and yearto-year climactic variability can have significant impact on oat β -glucan content. Miller *et al.* (1993) found that total precipitation was negatively correlated and temperature was slightly positively correlated with oat β -glucan content.

Hurt *et al.* (1988) reported a variation in β -glucan content among 35 commercial oat cultivars. β -glucan levels varied between 3.1 and 5.9 percent. Åman and Graham (1987) found that total β -glucan ranged from 3.0 to 6.9 percent in Swedish oat samples, and that there was variation for the proportion of soluble β -glucan among entries. The continuous range of variation of β -glucan content in oat may suggest that it is quantitatively inherited.

Selection for low β -glucan has been attempted in barley to improve brewing quality (Bendelow, 1975). Selection for high β -glucanase in barley has also been

proposed as a means of reducing β -glucan level (Bendelow, 1976). Selection for low β glucan and high β -glucanase could be done independently or concurrently. No selection protocol for β -glucan or β -glucanase has been reported for oat.

2.7 Interrelationships of Biochemical Components in Oat Grain

Generally, oil and protein contents of oat grain are not correlated (Gullord, 1980; Lampinen, 1986) but the relationship can vary between genotypes. Forsberg *et al.* (1974) reported that cultivar 'Dal' was high in both groat protein and lipid percentage. The relationship between protein and oil content may vary for the same genotype among locations and among years at the same location. This would not be unexpected since oil content and to a greater extent protein content are affected by the environment. Variation in the protein-oil relationship has been previously reported (Youngs and Forsberg, 1979).

Protein content is negatively correlated with yield (Forsberg *et al.*, 1974). Youngs (1972) showed an inverse relationship between percent bran and oat groat weight. As bran (including aleurone) was higher in protein than starchy endosperm, overall groat protein concentration increased as groat size decreased.

Saastamoinen *et al.* (1992) found that β -glucan content was positively correlated with grain yield, growing time, hectolitre weight and thousand-grain weight in many tests and significantly negatively correlated with protein content and hull percentage. Miller *et al.* (1993) reported a weak negative correlation between protein and β -glucan contents and no relationship was apparent between 1,000 kernel weight and β -glucan content. Brunner and Freed (1994) reported that protein content was positively correlated with β -

glucan content in two of three years but that test weight and hull percentage were not correlated with groat ß-glucan content.

2.8 Genetic Studies

Breeding schemes for improvement of quantitative traits often depend on the heritability of those traits. Heritability is the proportion of total phenotypic variation expressed among genotypes that can be attributed to genetic differences among them (Fehr, 1987). Narrow-sense heritability is defined as the proportion of phenotypic variance that is due to additive genetic variance among genotypes. Broad-sense heritability is equal to the genotypic variance divided by the phenotypic variance; this differs from narrow-sense heritability since dominance and epistatic effects are included in the numerator. Heritability estimates can be used to indicate the degree that the offspring will likely resemble their parents and these estimates can be used to predict gain from selection within the population for which the heritability value has been computed. Traits with high heritabilities can normally be improved through selection more easily than traits that have lower heritabilities.

Additive and phenotypic variances of a population can be obtained through such methods as parent-offspring regression (Fernandez and Miller, 1985), generation means analysis (Gamble 1962), and diallel analysis (Griffing, 1956). Genotypic variance can be calculated using the latter two designs. The parent-offspring technique has been employed to estimate the heritability of such seed characters as percent seed protein, and percent available methionine in *Phaseolus vulgaris* L. (Kelly and Bliss, 1975). In oat, parentoffspring regression has been used to determine the heritability of primary caryopsis percentage, test weight, primary kernel weight, primary caryopsis weight and percent plump kernels (Wesenberg and Shands, 1973).

Heritability estimates are the most useful when determined for the generations in which selection will be done since estimates are only applicable for the material from which they were derived. In most studies, F_2 and F_3 breeding lines are used. However, grain quality characteristics are rarely selected for in such early generations of a breeding program.

Estimation of genetic effects and variances associated with quantitative characters can be useful in providing information on the quantitative inheritance of the trait and in designing breeding strategies. Several genetic models have been proposed that can be used to estimate genetic components of both first and second order statistics (Mather, 1949; Mather and Jinks, 1982; Pooni *et al.*, 1992). The weighted least squares procedure has been widely used in the analysis of generation means as well as variances associated with different families. Normally, assumptions are made in order to employ genetic models which may include: (i) regular diploid and solely Mendelian inheritance; (ii) no environmental correlation among relatives; (iii) no linkage; (iv) the progenies or relatives are random members of their generation and family (Mather and Jinks, 1982). Additional assumptions may also be required with more complex models (Bogyo *et al.*, 1988). The appropriateness of the genetic model (e.g. a simple additive-dominance model) can be assessed by comparing the observed family means with those estimated using least squares analysis (Mather and Jinks, 1982). Mather (1949) employed least squares analysis to estimate the additive, dominance and error variances associated with oat grain length from parent, F_2 and F_3 data. Weighting of variance estimates is recommended when variances are not known with the equal precision.

Recently, genetic models have been proposed for the analysis of quantitative inherited characters which are expressed in the triploid endosperm of plants (Bogyo *et al.*, 1988; Pooni *et al.*, 1992). These models require data from Parental, F_1 , F_2 , F_3 and backcross populations. Bogyo *et al.* (1988) used their model to estimate additive, dominance and epistatic effects as well as the genetic variances for ß-glucan in barley. However, the existing models that estimate maternally controlled genetic variation may be biased because the models fail to fully consider maternal contributions or the models do not isolate the maternal effects arising from different phenomena (Foolad and Jones, 1992). For quantitative traits associated with plant seeds, genetic effects may be theoretically attributable to the triploid endosperm, diploid testa, embryo or cytoplasmic effects (Foolad and Jones, 1992). Moreover, epistatic effects may be present for these different sources of genetic contributions. Foolad and Jones (1992) have presented a comprehensive model to pinpoint the different sources of maternal contributions to quantitative traits.

When the embryo and/or endosperm genotype influences a seed characteristic, analysis of bulked seed of a genetically heterozygous plant will give biased information on inheritance. The bulk sample will not give a true characterization of the variation of the trait within the population because within-plant genetic variation will be lost.

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Positional effects of the seeds within a panicle or head may cause further within-plant variation of seed characteristics. Moreover, bulked seed-derived estimates are prone to sampling errors since the values are derived from a subsample of the bulked seed.

Kumar and Khush (1987) addressed these concerns in their study of the inheritance of amylose levels in rice. Amylose content of single seeds from Parents, F_1 , F_2 , and backcross plants was determined for high x low, high x intermediate and intermediate x intermediate crosses. Examination of the distributions of seed amylose content allowed genetic classification of generations. Bogyo *et al.* (1988) used single grain analysis to investigate the genetic effects that control of β -glucan content in barley.

Genotypes can be characterized as those that show little response to environmental changes and those that respond to changes in environmental conditions. Generally, stable genotypes are desirable but stability can be defined in different ways. Agronomic stability would refer genotypes that tend to give improved performance with ameliorated environmental conditions while biologically stable or homeostatic genotypes would tend to include those that give consistency of performance independent of changes to the growing environment. In an effort to evaluate the differing performances of cultivars across environments, a plethora of techniques have been published to measure stability (see Lin *et al.*, 1986; Peltonen-Sainio *et al.*, 1993). These techniques include stability statistics (Shukla, 1972; Lin and Binns, 1988), regression analysis (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), grouping and/or cluster analyses (Francis and Kannenberg, 1978; Lin and Butler, 1990), principal component analysis (Peltonen-Sainio *et al.*, 1993; Brown, 1991) and AMMI (additive main effects and multiplicative

interactions) analysis (Gauch, 1988; Zobel *et al.*, 1988). While many statistical techniques have been proposed, these stability measures are most useful when the nature of the stability being assessed and the environments included in the test are well understood (Lin *et al.*, 1986). The type of analysis performed and the nature of the environments included in the test can significantly influence the results of the analysis (Lin *et al.*, 1986).

Yield stability is a common breeding objective (Francis and Kannenberg, 1978; Bowley and McKersie, 1990; Peltonen-Saino *et al.*, 1993), but when grain quality is important, stability of grain quality (e.g. oil content; test weight) should be evaluated (Peltonen-Sainio and Peltonen, 1993) as a breeding objective.

2.9 Summary

Oat has been grown in Canada for centuries but in the past few decades the importance of the crop has diminished. The milling industry in eastern Canada is now growing due to increased human consumption, establishment of new milling facilities and expansion of existing mills in Ontario. Québec producers are shipping oat for milling to Ontario mills (Quaker Oats, pers. comm.). Further knowledge about the agronomics of, and the environmental effects on oat milling characteristics could aid producers to improve production practices and crop yields for this expanding market.

The recent popularity of oat food products is due at least in part to the reported cholesterol-lowering properties of β -glucan, which is found in high levels in oat grain. Oat food products with high β -glucan content are in demand from health-conscious

consumers. Webster (1986) has suggested that fractionation of oat may be commercially feasible if a market for the substantial by-product portion could be found. Oat breeding directed at increasing specific fractions, such as β -glucan or protein, may improve the economic viability of the fractionation process. Breeding for an oat cultivar with high β -glucan will require information on the inheritance, heritability and agronomics of this trait. Improvement of the quality of a crop can not be done piecemeal since quality traits tend to be interrelated. Breeding for improved β -glucan content in oat should be done such that other characters such as protein, oil and those affecting milling quality are also monitored.

Preface to Chapter 3

This chapter corresponds to a manuscript which will appear in the September-October volume of *Agronomy Journal*. The thesis version has been modified to conform to McGill University thesis guidelines. The purpose of this study was to evaluate the effect(s) of nitrogen fertilizer applied at the boot stage of crop development and delayed seeding on grain characteristics of oat quality. This experiment was designed, seeded and harvested by D. Gavin Humphreys. Technical assistance was obtained with machinery operations during field preparation, seeding and harvesting. Harvested materials were processed and grain characteristic data obtained by D. Gavin Humphreys with technical assistance from N. Brière. Data were analyzed and the manuscript was written by D. Gavin Humphreys. Dr. D.E. Mather and Dr. D.L. Smith provided supervisory guidance and contributed critical advice on the content of the manuscript. All literature cited in this manuscript can be found in a section at the end of this thesis. Tables are included at the end of the manuscript. Chapter 3: Nitrogen Fertilizer Application and Seeding Date Effects on Oat Grain Milling Quality.

3.1 ABSTRACT

Management factors such as N fertilizer rate and seeding date can significantly influence oat (Avena sativa L.) grain milling quality. The effects of N fertilizer and seeding date on the grain yield and milling quality of four oat cultivars were evaluated using two N rates (40 kg ha⁻¹ applied at seeding or 40 kg ha⁻¹ applied at seeding plus 20 kg ha⁻¹ applied at the boot stage) and two seeding dates. Studies were conducted at Ste-Anne-de-Bellevue, Canada from 1990 to 1992 and at Ste-Rosalie, Canada in 1991. Nitrogen treatments had little effect on milling quality at Ste-Anne-de-Bellevue but hull percentage decreased and plump grain percentage increased with higher N in the Ste-Rosalie experiment. Delayed seeding reduced yields in all four experiments. In the Ste-Anne-de-Bellevue experiments, delayed seeding reduced test weight, 1000-grain weight, plump grain percentage and theoretical milling yield, and increased percentage of hull, thin grain and bosom grain. In the Ste-Rosalie experiment, opposite effects were observed; this may have resulted from reduced production of secondary and tertiary seeds in the late-seeded treatments. For most milling quality characteristics, significant differences between cultivars were observed and cultivars interacted with management treatments. In general, the higher application rate of N did not improve oat grain milling quality sufficiently to warrant its usage. Later seeding resulted in inferior yields at all locations but did not necessarily reduce milling quality. The correct choice of cultivar seems crucial for production of high quality milling oat grain.

3.2 INTRODUCTION

Crop management can play an important role in determining the quality of the harvested product. In small-grain cereals, management factors such as N fertilization and seeding date can have significant effects on milling quality attributes. These effects can be cultivar dependent (Ciha, 1983; Ohm, 1976); thus, the evaluation of the effects of management on modern cultivars could improve both oat production and milling quality. Superior cultivars with desirable milling quality may be sold to industrial processors since a portion of the oat crop is used for human consumption.

Delayed seeding of oat has been found to reduce grain yield and grain size (Gooding and Lafever, 1991; Anderson and Mclean, 1989; Ciha, 1983; Nass *et al.*, 1975; Frey, 1959a) and to reduce test weight (Nass *et al.*, 1975). Deschênes and St. Pierre (1980) found that earlier seedings resulted in superior yields on a clay soil relative to a loam soil. However, later seeding gave superior yields on the loam soil compared to the clay soil and resulted in higher weed biomass on both soil types (Deschênes and St. Pierre, 1980). Frey (1959a) and Ciha (1983) observed that cultivars differed in their sensitivity to seeding date. For example, the cultivar Andrew was able to yield more than other cultivars when planted late because it was able to develop more grains per panicle (Frey, 1959a). The effect of later seeding on the milling quality of modern cultivars grown in eastern Canada has not been reported.

Increased applications of N fertilizer to oat have been shown to increase grain yields, reduce test weight and slightly decrease grain weight (Brinkman and Rho, 1984; Ohm, 1976). Response to N fertilizer seems to be genotype specific for grain weight and/or yield (Brinkman and Rho, 1984; Welch and Yong, 1980; Ohm, 1976; Frey, 1959b). Schimdt (1960) reported that reduced yields resulting from delayed seeding could be only partially overcome by adequate fertilization.

The impact of delayed seeding on oat grain milling quality has received substantial attention (Gooding and Lafever, 1991; Anderson and Mclean, 1989; Ciha, 1983; Nass *et al.*, 1975; Frey, 1959a). However, there is little published regarding how the various oat grain milling characteristics can be affected by N fertility and its interaction with seeding date. The objective of this study was to evaluate the effects of two N fertilizer treatments and delayed seeding on grain yield and milling quality characteristics in four oat cultivars: Manic, Marion QC, Capital and Newman.

3.3 MATERIALS AND METHODS

The experiments were carried out at Ste-Anne-de-Bellevue, Québec from 1990 to 1992 and at Ste-Rosalie, Québec in 1991. In 1990, the soil type was Chateauguay clayloam (fine-silty, frigid, mesic, Typic Hapludalf). At Ste-Anne-de-Bellevue in 1991 and 1992, the soil type belonged to the Chicot series (fine-loamy, frigid, mesic, Typic Hapludalf) and at Ste-Rosalie the soil type was a Ste-Rosalie clay (fine, non-acid, frigid, Typic Humaquept). The experimental design used was a split-plot with four replicates. The main plot treatment was seeding date. Sub-plots consisted of a 2 (N levels) x 4 (cultivars) factorial arrangement of treatments. Plots were $0.9m \times 3.8m$ with 6 rows and 0.15 m between the rows. Plots were seeded at a rate of 350 seeds m⁻² in all experiments.

In 1990, the seeding dates were 24 April and 23 May. In 1991, the seeding dates were 1 May and 21 May at Ste-Anne-de-Bellevue, and 15 May and 3 June at Ste-Rosalie. In 1992, seeding dates were 3 May and 24 May. Two N levels were tested: the recommended level (40 kg ha⁻¹) applied at seeding and 40 kg ha⁻¹ applied at seeding plus 20 kg ha⁻¹ applied at the boot stage (Zadoks 40-43) as a side-dressing. In all cases, the N fertilizer used was granular NH_4NO_3 . The rationale for applying the supplemental N at a relatively late growth stage was to increase N supply during grain filling without greatly affecting the vegetative growth of the plant.

Four cultivars with contrasting characteristics were tested. Marion QC and Manic were included as high and low β -glucen cultivars, respectively. Oat grain β -glucan content can be a quality trait of interest in some markets. Capital was included because of its generally good agronomic performance and its low percent hull. Newman was included because it has very large groats and it has resistance to crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Eriks.).

Plots were harvested by hand in 1990 and 1992 as well as the 1991 experiment in Ste-Rosalie. Grain was threshed by manually passing the harvested material through a plot combine. At Ste-Anne-de-Bellevue in 1991 plots were harvested with a plot combine. Milling quality attributes were determined for two randomly-drawn samples of grain from each plot. For each sample, the mean was used as the datum value. Milling parameters were determined as follows:

a. Hectolitre weight = weight (g) of 100 ml of grain converted into kg hL⁻¹.

b. 1000-grain weight = number of grains in a 20 g sample, converted to its 1000grain weight equivalent. Bosom grains were removed from the sample prior to determining the 1000-grain weight.

c. Percent hull = the percentage by weight of hulls in a 5 g sample of grain.

d. Plumpness = the percentage by weight of 20 g of grain that does not pass through a 2.4 mm X 19 mm screen.

e. Thin grain = the percentage by weight of 20 g of grain that passes through a 0.8 mm X 19 mm screen.

f. Percent bosom = the percentage by weight of bosom grains in a 5 g sample of grain.

g. Theoretical milling yield = 50 g of grain of a known moisture was cleaned of foreign matter and twice passed through a laboratory-size impact dehuller (Quaker Oats Co., Barrington, Ill.). Groats were separated from the hulls using a South Dakota Seed Blower (Seedburo Equipment Co., Chicago, Ill.). Theoretical milling yield was calculated as the percent groat derived from 50 g of grain corrected for the foreign material (precleaning) and the moisture content before drying.

Homogeneity of variances across environments was tested using Bartlett's test, as described in Snedecor and Cochran (1980). Significant treatment effects were identified using analysis of variance procedures available in SAS (SAS Institute. Inc., 1990). When significant cultivar effects were found, cultivars were compared using an ANOVA-protected Duncan's multiple range test.

3.4 RESULTS

Data from the four environments could not be combined due to heterogeneity of error variances. Therefore, results of analyses of variance are presented separately for each environment (Table 3.1). Where interactions among treatment factors were significant (P<0.05), means are presented to illustrate simple effects (Tables 3.2, 3.3 and 3.6). Where interactions were not statistically significant, means are presented to illustrate significant main effects (Tables 3.4, 3.5 and 3.7)

3.4.1 Effects of N Treatments

Nitrogen treatments had no significant effects or interactions at Ste-Anne-de-Bellevue in 1990 or 1992 (Table 3.1). In 1991, the only significant (P < 0.05) N effect observed at Ste-Anne-de-Bellevue was a slight increase in theoretical milling yield, from 69.2 to 69.7%, when additional N was applied (Table 3.1). A significant seeding date x N x cultivar interaction was observed for theoretical milling yield and a significant cultivar by N interaction was observed for percentage of bosom grain at Ste-Anne-de-Bellevue in 1991 (Table 3.1). The cultivar Newman had a slightly higher theoretical milling yield at 40 + 20 kg ha⁻¹ N compared to 40 kg ha⁻¹ N when seeded early but not when seeded late (Table 3.2). The cultivar Marion QC had a marginally lower bosom grain percentage at the higher N rate (Table 3.3). For theoretical milling yield and percent bosom grain, the other cultivars did not show significant responses (Tables 3.2) and 3.3). At Ste-Rosalie in 1991, two significant N main effects and a significant seeding date by N interaction were observed (Table 3.1). At that site, the application of additional N resulted in lower percent hull and higher percent plump grain overall and a higher 1000-grain weight in late-seeded treatments (Table 3.4).

3.4.2 Effects of Seeding Date Treatments

Late seeding significantly reduced grain yields in all four environments (Table 3.5). At Ste-Anne-de-Bellevue, the average reduction was 48% in 1990 and 21.9% in 1991 (Table 3.5). At Ste-Rosalie in 1991 and Ste-Anne-de-Bellevue in 1992, there were significant seeding date by cultivar interactions for grain yield (Table 3.1). All cultivars yielded less when seeded late, but the extent of yield reduction varied among cultivars (Table 3.5). At Ste-Rosalie in 1991, the grain yield of Marion QC was reduced by only 5.7%; whereas, that of Manic was reduced by 33.6%. Manic was the highest yielding cultivar when seeded early, but the lowest yielding cultivar when seeded late (Table 3.5). At Ste-Anne-de-Bellevue in 1992, Manic was not significantly lower yielding than the best cultivar (Newman) when seeded early but was the lowest yielding cultivar when seeded late. Capital showed an even greater percentage yield reduction due to late seeding than did Manic in the 1992 experiment (Table 3.5).

Seeding date affected test weight in all four environments. At Ste-Anne-de-Bellevue, there were significant seeding date by cultivar interactions in all years (Table 3.1) and late seeding reduced test weight for all four cultivars (Table 3.6). The magnitude of this reduction varied among cultivars. Reductions in test weight were greatest for Manic and least for Newman. At Ste-Rosalie in 1991, late seeding increased average test weight (Table 3.7).

For grain size characteristics (1000-grain weight, percent plump grain, percent thin grain, percent bosom grain), seeding date by cultivar interactions were usually important (Table 3.1). The grain size characteristics of the cultivar Newman were not affected greatly by seeding date (Table 3.6). In the other cultivars, later seeding resulted in lower 1000-grain weights at Ste-Anne-de-Bellevue in all three years (Table 3.6), but average 1000-grain weight slightly increased with late seeding at Ste-Rosalie in 1991 (Table 3.7). Low 1000-grain weight was usually associated with high proportions of thin grain, but the relationship between 1000-grain weight and percent plump grain was less consistent. The cultivar Manic had high proportions of bosom grain, particularly when seeded late (Table 3.6). For percent hull, there were significant seeding date by cultivar interactions at Ste-Anne-de-Bellevue in 1990 and 1992 as well as at Ste-Rosalie in 1991 (Table 3.1). In the Ste-Anne-de-Bellevue environments, percent hull of all cultivars increased with delayed seeding, with greater increases in Manic, Marion QC, and Capital than in Newman (Table 3.6). At Ste-Rosalie, percent hull was reduced (Capital and Marion OC) or remained unchanged (Manic and Newman) with delayed seeding (Table 3.6). At Ste-Anne-de-Bellevue in 1991, later seeding resulted in an overall increase in percent hull (Table 3.7).

A significant seeding date by cultivar effect was observed for theoretical milling yield at Ste-Anne-de-Bellevue in 1990 and 1992 (Table 3.1). Delayed seeding reduced theoretical milling yield for all cultivars but the magnitude of the reduction varied among

cultivars (Table 3.6). At Ste-Rosalie in 1991, overall theoretical milling yield increased slightly with delayed seeding (Table 3.7) and a significant variation among cultivars for theoretical milling yield was detected (Table 3.7).

3.5 DISCUSSION

The various physical grain quality characteristics measured in these experiments are inter-related. Thousand-grain weight provides an assessment of grain size by weight, whereas the proportions of plump and thin grain provide size distribution of grains by thickness. Bosom (double) grains are thick and will tend to be included in the plump grain fraction. However, they are lighter than single grains of comparable thickness. In the absence of bosom grains, a close relationship would be expected between grain thickness and grain weight. In this study, the relationship between 1000-grain weight and percent plump grain was inconsistent possibly due to the variation among cultivars in the proportion of bosom grain (Table 3.6). The test weight (specific gravity) of a grain sample depends partly on the specific gravity of individual grains, and partly on how well grains. Percent hull is expected to be high for bosom grain and for thin grain but low for plump well-filled grain. Theoretical milling yield is largely a function of the percent hull of the sample but the sample is corrected for the percent moisture as well as the contaminants (i.e. chaff and weed seeds).

Theoretical milling yield is of primary importance to oat millers, because it is a direct expression of the amount of product that can be obtained from a given weight of

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grain. Test weight is of less direct consequence in the milling process, yet because it is easily assessed and is generally related to milling yield, it is used in determining the purchase price of oat grain.

In the experiments conducted at Ste-Anne-de-Bellevue, neither grain yield nor individual grain quality characteristics responded consistently to the application of additional N (Table 3.1). Apparently the basic amount of N fertilizer applied in the low-N treatment was sufficient for the crop, perhaps because of residual soil N from greenmanure alfalfa grown in previous years.

In 1991 at Ste-Anne-de-Bellevue, additional N significantly reduced the proportion of bosom grain in the cultivar Marion QC (Table 3.3). A drop in percent bosom grain is likely related to an avoidance of the stress(es) that causes bosom formation or improved grain-filling. Increases in N fertilizer have been found to delay maturity in some cultivars in some years (Marshall *et al.*, 1984), and a similar trend was observed in this study (data not shown). Delayed maturity could act to lengthen the grain-filling period. Marion QC would likely demonstrate the effects of extended grain-filling more readily because this cultivar generally matures earlier than the other cultivars in this study. Hence, the decrease in percent bosom with greater N fertilizer may be related to improved grain-filling in the cultivar Marion QC. Theoretical milling yield was affected by the application of additional N, possibly because groat percentage was generally greater and the correction for moisture content at harvest was generally lower at the higher N level (data not shown). The combined effect of larger groats and a reduced moisture correction in Newman resulted in a significantly superior theoretical milling yield for the higher N level in the early-seeded treatments (Table 3.2).

In the experiment conducted at Ste-Rosalie in 1991, application of additional N did not affect yield (Table 3.1), but did have minor effects on grain quality characteristics, altering the distribution of grain dry matter so that the 1000-grain weight increased (late seeding date only), the proportion of plump grain increased, and the percent hull decreased (Table 3.4). These effects, although small in magnitude, were all in the direction of superior milling quality. It is unlikely that the slight enhancement of physical grain quality observed at Ste-Rosalie would be sufficient to justify the added cost of N fertilizer. Thorough assessment of the effects of N fertilization on physical grain quality would require experimentation under N-limiting conditions, perhaps with additional N applied at seeding or early in plant development. By the time the additional N was applied in these experiments, sink size (grain number) was already established, and only grain filling could be affected.

As has been observed in other oat yield studies (Gooding and Lafever, 1991; Anderson and Mclean, 1989; Ciha, 1983; Nass *et al.*, 1975; Frey, 1959a), grain yields were lower in late-seeded treatments than in early-seeded treatments (Table 3.5). In the experiment conducted at Ste-Rosalie, the grain yield of the cultivar Marion QC was not affected by seeding date. Frey (1959a) and Ciha (1983) also reported differential sensitivity of cultivars to delayed seeding, but they observed substantial yield reductions in all cultivars tested.

Because of the yield losses that can be expected when seeding is delayed, early seeding is a recommended practice for oat (Anon, 1988). However, poor weather and other factors can cause delays in seeding; growers who wish to market their crop for milling purposes may want to know the effects of delayed seeding on physical grain quality. In the experiments conducted at Ste-Anne-de-Bellevue, later sowing had adverse effects on grain quality: reductions in test weight and 1000-grain weight (Table 3.6). These effects are similar to those reported by Frey (1959a). In contrast, later sowing resulted in increased test weight and 1000-grain weight in the Ste-Rosalie experiment (Table 3.7). The very late seeding date (June 3) used at the Ste-Rosalie site may have limited the numbers of secondary and tertiary seeds that were set. This is consistent with the observation that the proportion of thin grain was lower in the later seeded material compared to the early seeded material at Ste-Rosalie in 1991; the opposite trend was observed in the Ste-Anne-de-Bellevue environments. Gooding and Lafever (1991) speculated that a decrease in seeds per spikelet in later seeded oat resulted from plants flowering later in the season under conditions of higher temperatures resulting in increased sterility. Moreover, with very late seeding, the grain-filling season may be too short to permit proper filling of the secondary and tertiary seeds; hence, principally primary seeds are observed.

In recent years, part of the Québec oat crop has been purchased for milling, so information is needed on the milling characteristics of Québec oat cultivars. Newman has large grains, low hull percentage as well as superior test weight and theoretical milling yield. At Ste-Anne-de-Bellevue, it yielded well, but at Ste-Rosalie its yield was relatively

low (Table 3.5). Newman is a daylength-insensitive cultivar and is resistant to prevalent races of crown rust. These characteristics may partially explain its reduced sensitivity to the management treatments applied in these experiments. Among the Québec cultivars, Capital and Marion QC appeared to have greater potential for milling than Manic. When seeded early, Capital was superior with respect to yield, percent hull and percent bosom grain, and theoretical milling yield (Tables 3.5-3.7), but its test weight and 1000-grain weight were lower than those of Marion QC (Tables 3.6 and 3.7). Marion QC did not differ significantly from Newman for test weight and had only slightly lower 1000-grain weight (Table 3.6). With delayed seeding, Marion QC was able to sustain its yield, perhaps because it is earlier-maturing than the other cultivars (Table 3.5). The cultivar Manic exhibited a major disadvantage in these experiments: it had a high percentage of bosom grain, and this characteristic showed a marked sensitivity to differences among environments and to differences in management practices (Table 3.6).

3.6 CONCLUSIONS

Production practices can significantly influence the milling quality of oat grain produced. The effect of N varied among environments; in some environments split applications of N may improve milling quality. However, the improvement in grain quality is unlikely to justify the cost of the increased fertilizer. Later seeding will likely reduce the profitability of the crop through reduced yield. Milling quality may or may not suffer with delayed seeding depending on the growing environment and the cultivar, and an improvement in one milling quality characteristic may be offset by the deterioration of another. Choosing the correct cultivar appears to be crucial for the production of high quality oat grain.

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Table 3.1: Mean squares for oat grain quality characteristics tested at Ste-Anne-de-Bellevue (1990-1992) and Ste-Rosalie (1991).

		Yield	Test Weight	1000 Grain Wt.	Hull	Plump Grain	Thin Grain	Bosom Grain	TMY'
		Mg ha''	kg hL ^{.1}	g 			- x		
			Ste-A	nne-de-Bel 	levue 199	0 			
Planting Date	1	8.5	1622**	163	5.2	118	1582	48.8**	1343"
Cultivar	3	0.11	109	258	0.7	3384	1925**	9.4	193**
Nitrogen	1	0.02	10.9	4.6	0.0	0.0	1.3	0,0	2.1
DatexCultivar	3	0.04	47.7	39.4	0.3	1141	795	3.6	142
DatexN	1	0.00	0.0	1.1	0.0	0.8	6.3	0.2	1.3
CultivarxN	3	5.04	3.8	3.7	0.0	12.9	3,4	0.1	2.1
DatexCultivarxN	з.	0.0!	6.9	0.8	0.0	51.5	24.4	0.2	6.5
			Ste-A	nne-de-Bel	levue 199	91			
Planting Date	1	0.87**	424**	67.4	3.1"	— 0.9	40.3	47.8	0.2
Cultivar	3	0.09	323**	457**	107	61.9**	6171**	61.7	121
Nitrogen	1	0.00	2.0	0.3	2.4	0.3	49.4	1.6	5.3
DatexCultivar	3	0.01	16.5	5.9	0.5	3.6	7.1	8.2	0.2
DatexN	1	0.03	3.9	0.4	0.1	0,0	0.2	0.3	2.8
CultivarxN	3	0.01	3.0	0.6	1.2	0.1	1.9	4.4	0.5
DatexCultivarxN	3	0.02	0.9	1.0	1.2	0.2	16.6	3.1	4.1**
			:	Ste-Rosal1	e 1991				
Planting Date	1	1.7	- 76.9	71.0	16.1	960	401 ⁺⁺	5.0	7.0*
Cultivar	3	0.1	12.5	72.5	14.9	1425**	743	8.2	24.8
Nitrogen	1	0.06	1.8	1.0	5.5	110	30,5	0.0	2.1
DatexCultivar	3	0.18**	1.9	1.5	4.1**	394	51.6**	5.3**	1.4
DatexN	3	0.08	1.1	3.2	1.4	38.6	1.4	0.4	1.8
CultivarxN	3	0.04	4.1	1.0	0.9	13.7	13.8	0.5	1.3
DatexCultivarxN	3	0.01	0.7	1.1	0.6	23.1	8,9	0.9	0.6
			Ste-/	Anne-de-Be	llevue 19	92			
Planting Date	1	6.8	1098	683	922	 8802**	8274	93.1 ^{**}	1298
Cultivar	3	0.71	190**	357	152**	4881**	2606	109**	196**
Nitrogen	1	0.00	3.4	0.0	0.39	3.0	22.4	0.2	0.4
DatexCultivar	3	0.30"	48.7	26.1	57.2**	321	741**	101**	88.5
DatexN	1	0.00	0,1	4.0	0.07	67 R	51.5	0.6	0.7
CultivaryN	3	0 02	6.6	2.7	4.2	34.9	16.0	3.8	0.3
Dator () to and	2	0.02	9.5	0.2	9.6	τ 7 Ω	9 Q	3.0	0 4
DatexcultivarxN	3	0.01	9.5	0.2	9.5	7.8	9.9	3.0	0.4

". " Significant at the 0.05 and 0.01 probability levels, respectively.

' - Theoretical Milling Yield.

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Table 3.2: Theoretical milling yield of oat cultivars seeded early and late grown in Ste-Anne-de-Bellevue, Québec in 1991 with 40 kg ha⁻¹ N applied at seeding and 40 N kg ha⁻¹ applied at seeding plus 20 kg ha⁻¹ N applied at Zadoks growth stage(40-43).

	Early Seed	ing	Late Seedi	ng		
	N Treatmen	t (kg ha ^{·1})	N Treatment (kg haʻ')			
Cultivar	40	40 + 20	40	40 + 20		
	TMY'	(%)	TMY	(%)		
Capital	69.4	70.2	69.7	70.0		
Manic	67.0	67.3	66.6	67.2		
Marion QC	67.9	67.7	67.5	68.1		
Newman	71.9	74.8	73.4	72.6		

" = Means within a seeding date for the same cultivar are significantly (P<0.05) different according to a LSD.

' - Theoretical Milling Yield.

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	N Treatm	ent (kg ha ^{.1})
Cultivar	40	40 + 20
	Bosom	oat (%) ——
Capital	1.0c*	0.7Ь
Manic	5.3a	6.5a
Marion QC	2.3b	1.05*
Newman	2.2b	1.5b

Table 3.3: Percent bosom grain for four oat cultivars grown in Ste-Anne-de-Bellevue, Québec in 1991 with 40 kg ha⁻¹ N fertilizer applied at seeding and 40 kg ha⁻¹ N applied at seeding plus 20 kg ha⁻¹ N applied at Zadoks stage (40-43).

" = Means for the same cultivar are significantly (P<0.05) different according to a LSD.

[†] = Means within a column followed by the same letter are not significantly (P<0.05) different according to an ANOVA-protected Duncan's multiple range test. Table 3.4: Hull percentage, plump grain percentage and 1000-grain weight for oat grown at Ste-Rosalie. Québec in 1991 with 40 kg ha⁻¹ N fertilizer applied at seeding and 40 kg ha⁻¹ N applied at seeding plus 20 kg ha⁻¹ N applied at Zadoks growth stage (40-43).

Nitrogen		. Plump	1000-0	irain	
Treatment	Hull	Grain	Wei	ght	
			Early	Late	
	%	· · · · · · · · · · · · · · · · · · ·]	·
40	25.7a*	36.5b	33.1a	34.7Ь	
40 + 20	25.2b	39.2a	32.9a	35.4a	

' - Means within a column followed by the same letter are not significantly (P<0.05) different according to a LSD.

		Ste-Anne-de-B	ellevue		Ste-Ro	salie
	1990	1991	1992		199)1
Cultivar			Early	Late	Early	Late
			— Grain Yie	ld (Mg ha ^{.1}) ——		<u> </u>
Capital	4.72a ^t	3.66b	5.36a	2.98cb	4.59ab	3.45b
Manic	3.94b	3.46bc	4.82ab	2.71c	4.78a	3.18b
Marion QC	4.41ab	3. 33 c	4.41b	3.25b	4.40ab	4.15a
Newman	4.42ab	4.00a	5.40a	4.45a	4.05b	3.43b
Seeding Date						
<u> </u>						
Early	5.76a*	4.06a	-	-	-	-
Late	2.99b	3.17Ь	-		-	-

Table 3.5: Mean grain yield for four oat cultivars and for early and late seeding dates grown at Ste-Anne-de-Bellevue in 1990-92 and at Ste-Rosalie in 1991.

[†] = Cultivar means within a column with the same letter are not significantly (P>0.05) different according to an ANOVA-protected Duncan's multiple range test.

 * = Seeding date means within a column with the same letter are not significantly (P<0.05) different.

	Ste-Anne-de-Bellevue 1990													
	Te	st	1000-0	irain			Plur	πp	Thi	n	Bos	om.	Theoret	tical
<u>Cultivar</u>	<u>Wei</u>	<u>ght</u>	Wei	<u>ht</u>	Hu'	<u>11</u>	Gra	in	<u> </u>	in	Gra	<u>in</u>	Milling	<u>Yield</u>
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
	kg	'nL ^{∙1} ——			····				%					
Capital	51.8c [†]	41.8c*	30.2d	25.0d	22.4b	29.9b	48.0b	21.1d	16.5b	46.8a*	0.2c	2.2c*	73.4a	63.6b°
Manic	53.4b	38.5d*	32.6c	26.8c*	24.4a	33.7a*	37.7c	47.5b*	19.0b	27.0c*	1.4a	18.4a°	71.7b	54.0c*
Marion QC	53.5b	45.5b*	34.7b	32.0b	24.5a	28.2c*	23.6d	31.6c*	36.5a	35.3b	1.8ab	5.3b*	69.8c	65.5b [*]
Newman	55.3a	48.3a	36.1a	37.2a*	22.0b	25.1d*	62.2a	60.5a	9.8c	12.4d	0.8b	5.8b*	74.la	68.9a*

Table 3.6: Means of grain quality characteristics for early and late seeded oat cultivars grown at Ste-Anne-de-Bellevue, Québec (1990-1992) and Ste-Rosalie, Québec (1991).

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Table 3.6: (Continued).

					Ste-Anne-de-Be	llevue 1991			
	Tes	t	1000-0	Grain	bjawi	р	Bosc	ж	
<u>Cultivar</u>	Weig	<u>ht</u>	Weig	<u>ht</u>	Grain	<u>n</u>	Grat	in	
	Early	Late	Early	Late	Early	Late	Early	Late	
	kg	hL ^{.1}	·····	g	%		<u></u>	۲ <u>ــــــــــــــــــــــــــــــــــــ</u>	
Capita]	48.7c	42.8b*	27.5c	25.3c*	16.2b	8.5c*	0.4c	1.3c*	
Manic	47.8c	41.5c*	28.3c	25.7c*	18.4b	28.8b*	4.3a	7.5a*	
Marion QC	50.3b	44.4b	31.6b	28.4b [*]	10.8c	9.3c	0.4c	3.0b [*]	
Newman	55.6a	53.4a°	38.2a	37.9a	60.9a	50,9a [*]	1.8b	1.9bc	

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Table 3.6: (Continued).

			Ste-Rosa	lie 1991				
	Plump		Thin		Bos	om		
<u>Cultivar</u>	Hu	11	<u>Grain</u>		<u> </u>		Grain	
	Early	Late	Early	Late	Early	Late	Early	Late
				%				
Capital	25.4b	23.7c*	22.5b	44.la [*]	28.0b	18.8b*	1.0c	0.7b
Manic	26.7a	26.7a	44.5a	46.9a	20.6c	16.6bc	2.0b	2.4a
Marion QC	26.2ab	24.5b*	22.8b	30.7b*	33.2a	27.0a	1.9b	0.8b
Newman	25.5b	25.2b	46.la	45.2a	14.5d	13.9c	3.3a	1.4ab*

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Table 3.6: (Continued).

	Ste-Anne-de-Bellevue 1992													
	Tes	it	1000-0	Grain			Plu	mp	Thi	n	Bos	om	Theore	ical
<u>Cultivar</u>	<u>Weight</u>		Weight		<u>Hull</u>		Grain		<u> Grain </u>		<u>Grain</u>		Milling Yield	
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
	kg	hL ⁻¹ ——		g					%				·····	· <u> </u>
Capital	54.4ab	45.2c	32.5b	25.5c*	20.5d	29.6b	57.2b	25.2b [*]	15.9c	44.0b [•]	0.0b	0.1b	77.6a	66.4b
Manic	52.4b	40.1d	33.4b	24.8c	24.7a	35.7a°	47.2c	22.8b*	20.1b	43.5b*	1.1a	11.1a*	73.5d	60.1c*
Marion QC	56.4a	48.6b	39.2a	31.6b [*]	22.9b	30,95 [*]	36.3d	9.8c*	25.7a	61 4a	1.6a	1.7b	74.9c	66.0b
Newman	56.1a	52.2a	39.9a	37.1a	21.8c	24.0c	70.0a	59.1a	10.7d	14.5c*	1.7a	1.2b	76.5b	74.0a

* = Early and late treatment means within an attribute for the same cultivar and location are significantly (P<0.05) different according to a LSD.

¹ - Means within a column for the same seeding date and location that have the same letter(s) are not significantly (P<0.05) different according to an ANOVA-protected Duncan's multiple range test.

	Ste-	Anne-de-Bell 1991	Ste-Rosalie 1991			
Cultivar	Hull	Thin Grain	TMY ¹	Test Weight	1000- Grain Weight	тмү
·*		%		kg hL ^{.1}	g	~ %
Capital	27.7c1	48.6b	69.8b	50.0b	31.4d	73.2a
Manic	30.6a	38.2c	67.0d	50.2b	33.4c	70.7c
Marion QC	29.3b	57.9a	67.8c	51.0ab	34.8b	72.0b
Newman	24.6d	12.4d	73.2a	51.9 a	36.5a	73.4a
Seeding Date						
Early	27.8b ^{\$}	_	_	49.7b	33.0b	72.0b
Late	28.2a	-	-	51.8a	35.1a	72.7a

Table 3.7: Means of grain quality attributes for four oat cultivars grown in Ste-Anne-de-Bellevue.Québec (1991) and Ste-Rosalie, Québec (1991).

' = Theoretical Milling Yield.

' - Cultivar means within a column followed by the same letter are not significantly

(P>0.05) different according to an ANOVA-protected Duncan's multiple range test.

 5 = Seeding date means within a column followed by the same letter are not significantly (P<0.05) different.

Preface to Chapter 4

Chapter 4 corresponds to a manuscript that has been accepted for publication in the *Journal of Cereal Science*. The thesis version has been edited to conform to McGill University thesis guidelines. This chapter was designed to evaluate the effects of nitrogen fertilizer applied at the boot stage of crop development and delayed seeding on protein, oil and β-glucan contents of oat. The samples analyzed for these biochemical constituents came from the same field experiment for which grain characteristics were reported in chapter 3. This experiment was designed, seeded and harvested by D. Gavin Humphreys. Technical assistance was obtained with machinery operations during field preparation, seeding and harvesting. Harvested materials were processed and biochemical analyses were carried out by D. Gavin Humphreys with technical assistance from N. Brière. Data were analyzed and the manuscript was written by D. Gavin Humphreys. Dr. D.E. Mather contributed critical advice during data analysis and writing of the manuscript. Dr. D.L. Smith provided access to the Kjeldahl, Soxtec and NIR facilities and critically read the manuscript. All literature cited in this manuscript can be found in a section at the end of this thesis. Tables are included at the end of the manuscript. Chapter 4: Nitrogen Fertilizer and Seeding Date Induced Changes in Protein, Oil and ß-Glucan Contents of Four Oat Cultivars.

4.1 ABSTRACT

The application of nitrogen fertilizer at both seeding and the boot stage of plant development (Zadoks 40-43) and/or delayed seeding caused changes to the protein, oil and ß-glucan contents in four oat cultivars in field studies conducted in Québec, Canada between 1990 and 1992. Nitrogen fertilizer applied at the boot stage (Zadoks 40-43) tended to increase protein content and to decrease oil content; however, ß-glucan content did not respond significantly to additional N. Generally, delayed seeding increased protein and ß-glucan contents and decreased oil content. In 1992, however, protein and oil contents tended to increase with delayed seeding, while ß-glucan was not significantly affected, although not all cultivars responded in a similar fashion. Environmental conditions during the growing season seemed to influence the effects of delayed seeding on protein, oil and ß-glucan contents because results varied among years. While some cultivar x management treatment interactions were significant, the ranking of the four cultivars was generally consistent across experiments. Thus, if improvement in these characteristics is required, genotype x management interactions should not interfere unduly with the evaluation of late-generation breeding materials for protein, oil and ßglucan contents.

4.2 INTRODUCTION

Oat grain has long been recognized as a cereal of superior nutritive value. The protein content of oat groats (oat with the hull removed) is superior to that of other cereal grains (Peterson and Brinegar, 1986). Generally, protein content in cultivated oat varies between 13 and 22 percent (Youngs, 1972) and oat protein has a well-balanced amino acid content which varies little with changes in protein content (Robbins *et al.*, 1971). High-protein oat is valuable as a livestock feed and may be a useful raw material for the food industry.

The oil content of oat groats is the highest among the cereal grains (Youngs, 1986) and generally ranges from approximately 4 to 8 percent (Frey and Hammond, 1975) but oat genotypes with up to 16% oil have been reported (Schipper and Frey, 1991). Lipids can represent a significant energy source in oat grain used for livestock feed. Oat lipids, like those of other cereal grains, are nutritionally important because they are composed of a large proportion of unsaturated lipids including notable amounts of linoleic acid (Youngs, 1986). However, high oil content in oat may create rancidity problems for processors because enzymes (e.g. lipases) can be activated when oat is rolled or milled (Youngs, 1986).

The non-starch polysaccharide $(1\rightarrow 3), (1\rightarrow 4)$ - β -D-glucan (β -glucan) is usually present in cereal cell walls (Åman and Graham, 1987), and oat and barley have been found to contain particularly high levels of β -glucan compared to other cereal grains (Åman and Graham, 1987). A survey of oat lines gave β -glucan contents ranging from 3.0 to 6.4 percent (Lim *et al.*, 1992). In recent years, studies have demonstrated an association between the consumption of oat and lower serum cholesterol levels in humans (Ripsin *et al.*, 1992; Hurt *et al.*, 1988; Wood *et al.*, 1989) and it has been hypothesized that β -glucan is the chemical agent that is primarily responsible for the cholesterol lowering effect of oat (Hurt *et al.*, 1988; Wood *et al.*, 1989). Oat β -glucan may also have a therapeutic use in the management of diabetes (Hurt *et al.*, 1988) and "oat gum" of which β -glucan makes up a large component may have uses as a food hydrocolloid (Wood *et al.*, 1989). Beta-glucans also have anti-nutritional properties; for example, in chicken diets β -glucan "gumminess" and indigestibility can affect food intake, cause sticky droppings and reduce feed conversion efficiency (Hesselman and Åman, 1986). Cave *et al.* (1990) reported that increased β -glucan content in broiler chickens diets reduced rate of growth, and the availability of metabolizable energy and amino acids. In future, oat cultivars may be developed with either high or low β -glucan and/or oil contents to cater to the needs of different oat consumers.

The suitability of oat for feed or milling purposes may be influenced by production practices. Nitrogen (N) fertilizer and seeding date have been shown to influence the levels of biochemical constituents in oat grain (Youngs, 1986; Welch and Yong, 1980; Welch *et al.*, 1991; Nass *et al.*, 1975). Increased N fertilizer generally increases protein (Welch and Yong, 1980) and β -glucan (Welch *et al.*, 1991) contents but may reduce oil content (Youngs, 1986). Welch and Yong (1980) reported that the principal effect of early applications of N was to increase yield, whereas N application at heading increased grain protein content with little effect on yield. It has been observed that increased N at seeding can increase β -glucan content (Welch *et al.*, 1991) but there

have been no reports of the effect of late applications of additional N fertilizer on oat oil and β -glucan contents. Oat protein content has been found to increase with delayed seeding (Nass *et al.*, 1975), but the effect of delayed seeding on oil and β -glucan contents has not been reported. In barley, delayed seeding has been found to increase β -glucan content (Molina-Conde and Conde, 1982).

The objective of this study was to determine the effects of N fertilizer application at the boot stage (Zadoks 40-43) (Zadoks *et al.*, 1974) of plant growth and delayed seeding on the protein, oil and β -glucan contents of four oat varieties.

4.3 MATERIALS AND METHODS

4.3.1 Germplasm

Four commercial Canadian oat cultivars with contrasting milling quality characteristics were used in this study. Capital, Manic and Marion QC were recommended oat cultivars in Québec, Canada. Capital is a superior yielding, later maturing cultivar with low hull percentage and test weight (Humphreys *et al.*, 1994). Manic was chosen because it had been observed to be low in ß-glucan content (Hurt *et al.*, 1988). Manic is later maturing than the other cultivars in this study, and has been observed to yield poorly when seeded late (Humphreys *et al.*, 1994). Marion QC is earlier maturing than the other cultivars and has been found to be high in ß-glucan content (Hurt *et al.*, 1988). Newman is not a recommended cultivar in Québec but was included in this study because it has resistance to the prevalent races of crown rust (Puccinia coronata Cda. f. sp. avenae Eriks.) and has superior test weight and 1000grain weight compared to the other cultivars in this study (Humphreys et al., 1994).

4.3.2 Field experiments

Field experiments were carried out at Ste-Anne-de-Bellevue, Québec in 1990-92 and at Ste-Rosalie, Québec in 1991. In 1990, the soil type was Chateauguay clay-loam (fine-silty, frigid, mesic, Typic Hapludalf). At Ste-Anne-de-Bellevue in 1991 and 1992, the soil type belonged to the Chicot series (fine-loamy, frigid, mesic, Typic Hapludalf) and at Ste-Rosalie the soil type was a Ste-Rosalie clay (fine, non-acid, frigid, Typic Humaquept). The experimental design used was a split-plot with four replicates. The main-plot factor was seeding date. Sub-plot factors consisted of a 2 (N levels) x 4 (cultivars) factorial arrangement of treatments. Plots were 0.9m X 3.8m with 6 rows and 0.15 m between the rows.

In 1990, the seeding dates were 24 April and 23 May. In 1991, the seeding dates were 1 May and 21 May at Ste-Anne-de-Bellevue, and 15 May and 3 June at Ste-Rosalie. In 1992, seeding dates were 3 May and 24 May. Nitrogen applied was NH_4NO_3 33-0-0 (NPK) granular fertilizer. Two N levels were tested: 40 kg ha⁻¹ applied at seeding which is the recommended level of N fertilizer in Québec, Canada and 40 kg ha⁻¹ N applied at seeding plus 20 kg ha⁻¹ N applied at the boot stage (Zadoks 40-43) (Zadoks *et al.*, 1974). The rationale for applying the supplemental N at a relatively late growth stage was to increase N supply during grain filling without greatly affecting the vegetative growth of the plant.

Plots were harvested by hand in 1990 and 1992 in Ste-Anne-de-Bellevue and at Ste-Rosalie in 1991. Grain was threshed by manually passing the harvested material through a plot combine. At Ste-Anne-de-Bellevue in 1991, plots were harvested with a plot combine.

4.3.3 Grain preparation

The grain samples were dehulled using a laboratory-size dehuller and the groats were separated from hulls on a South Dakota Seed Blower. The oat groats were ground with a Wiley knife mill fitted with a 1 mm screen. Ground samples were stored at - 20°C. The day before analysis, groat samples were dried at 90°C overnight. In the case of oil content analysis, grain samples were dehulled, aspirated and ground the day before the analyses were done and the ground samples were dried overnight at 90°C.

4.3.4 Chemical Analyses

Protein content was estimated as Kjeldahl N x 6.25 determined on 0.5g of ground oat groats. The standard micro-Kjeldahl procedure was used (Anonymous, 1983). Flour samples were digested at 410°C in concentrated sulphuric acid plus 2 S-type catalyst Kjeltabs (Tecator Manual, Kjeltec System 1002 Distilling Unit) and the resulting ammonium was distilled into boric acid indicator and titrated with dilute HCl (Anonymous, 1983; Bradstreet, 1965).

Oil content was determined by near-infrared reflectance spectroscopy using a Percon Inframatic 8100 NIR. Calibration was performed using oil extraction data from 76 samples chosen using the 8100 NIR software. Oil was extracted from 3.0g of dried oat flour using a Soxtec System HT2. Extraction consisted of 30 minutes boiling and 30 minutes rinsing in 70 ml of extraction-grade diethyl ether. Oil content was determined gravimetrically.

Beta-glucan content was determined using the McCleary and Glennie-Holmes (1985) technique, except that 0.15g ground oat groats were used instead of the 0.5g sample originally published.

Beta-glucan determinations were done in duplicate and the mean taken as the datum. Protein and oil data were derived from single determinations. All protein, oil and ß-glucan contents are expressed as percentage of dry matter.

4.3.5 Statistical Analyses

Homogeneity of variances across environments was tested using Bartlett's test of homogeneity of variance as described in Snedecor and Cochran (1980). Significant treatment effects were identified using analysis of variance procedures available in SAS (1990). When significant cultivar effects were found, cultivars were compared using an ANOVA-protected Duncan's multiple range test (Steel and Torrie, 1980).

4.4 RESULTS

Results for protein from the four experiments are presented separately to show that treatment effects varied among locations. Oil and β -glucan data for the four experiments could not be combined due to heterogeneity of error variances. Results from the analyses of variance are presented separately for each experiment (Table 4.1). Where interactions were significant (P<0.05) means are presented to illustrate simple effects (Tables 4.3 and 4.4) and where interactions were not significant, means are presented to illustrate main effects (Table 4.2).

4.4.1 Effect of N treatments

In 1990, protein content did not increase with additional N (Table 4.1). At Ste-Rosalie in 1991 and at Ste-Anne-de-Bellevue in 1991 and 1992, protein content increased with additional N (Tables 4.1 and 4.2). In 1990, a significant seeding date x N x cultivar effect was observed for oil content (Table 4.1). Capital had a lower oil content at 40 kg ha⁻¹ N when planted late than when planted early but this was not observed at 40 + 20 kg ha⁻¹ N (Table 4.3). At Ste-Rosalie in 1991, oil content decreased with increased N (Table 4.2). Oil content was not significantly affected by N treatments at Ste-Anne-de-Bellevue in 1991 or 1992. Beta-glucan content was not significantly affected by N treatments in any experiment (Table 4.2).

4.4.2 Effect of seeding date

Protein content increased significantly with delayed seeding for experiments conducted in 1991 (Table 4.2). Seeding date x cultivar interactions were significant for protein content at Ste-Anne-de-Bellevue in 1990 and 1992. Response of groat protein content to delayed seeding varied among cultivars (Table 4.4). For example, groat protein content of the cultivar Manic tended to increase but Marion QC groat protein content tended not to increase with delayed seeding (Table 4.4).

Oil content tended to be lower for later planted material with the exception of 1992 when oil content was observed to increase with delayed seeding (Tables 4.2 and 4.4). In all Ste-Anne-de-Bellevue environments, seeding date x cultivar effects were significant for oil content (Table 4.1). At Ste-Anne-de-Bellevue in 1990 and 1991, oil content of the cultivars Capital and Marion QC tended to decrease more than Manic and Newman (Table 4.4) with delayed seeding. In 1992, oil content of Marion QC and Manic increased to a much greater extent than Capital or Newman (Table 4.4).

Seeding date x cultivar effects were significant for ß-glucan for all Ste-Annede-Bellevue experiments (Table 4.1). In general, delayed seeding increased ß-glucan contents but the increase in ß-glucan content with delayed seeding for a given cultivar varied among the Ste-Anne-de-Bellevue experiments (Table 4.4). For example, Marion QC increased from 5.0 to 6.9 percent ß-glucan at Ste-Anne-de-Bellevue in 1991 but did not significantly change in 1992 (Table 4.4). At Ste-Rosalie in 1991, no seeding date effect was observed but cultivars varied significantly for ß-glucan content (Table 4.2).

While cultivar performance varied between experiments, the oil and β -glucan contents of Marion QC were consistently among the highest of the four cultivars tested and Newman had the lowest oil and β -glucan contents in all experiments.

4.5 DISCUSSION

Considerations of oat grain quality have traditionally focused on physical properties of the grain such as test weight, grain weight and hull content. Biochemical components of the grain such as protein, oil and ß-glucan can also be important measures of oat grain quality in both the feed and milling industries.

4.5.1 Effects of N treatments

As in other studies (Welch and Yong, 1980), protein contents generally increased with late applications of N. At Ste-Anne-de-Bellevue in 1990, protein content did not respond to additional N fertilizer, possibly due to an abundance of residual soil N from green-manure alfalfa grown in the same field in 1989.

Oil content decreased with increased N at Ste-Rosalie in 1991 (Table 4.2). It has been observed that additional N can result in increased groat protein content and oil content is reportedly negatively correlated with protein content (Youngs and Forsberg, 1979; Brown *et al.*, 1966) although positive correlations have also been observed (Youngs and Forsberg, 1979; Peltonen-Sainio and Peltonen, 1993). At Ste-Rosalie in 1991, the increase in protein content with additional N fertilizer may have been related to the observed reduction in oil content. Cultivar and seeding date can also influence the response of oil content to N fertilizer. At Ste-Anne-de-Bellevue in 1990, late seeding reduced oil content of the cultivar Capital at 40 kg ha⁻¹ N but no response to late seeding was observed at 40+20 kg ha⁻¹ (Table 4.4).

Beta-glucan did not respond to additional N in any experiment (Table 4.1). Welch *et al.* (1991) reported that increased N resulted in increased β -glucan content; however, they applied additional N at seeding. In the study reported here, additional N was applied at the boot stage which may have been too late in plant development to significantly influence β -glucan synthesis. While Welch *et al.* (1991) noted an association between lower protein and lower β -glucan contents, other studies (Saastamoinen *et al.*, 1992) have reported negative relationships between β -glucan and protein contents. In this study, the high protein cultivar, Manic, had low ß-glucan content and the low protein cultivar, Marion QC, had high ß-glucan contents (Table 4.4).

4.5.2 Effects of seeding date

As has been observed previously (Nass *et al.*, 1975), late seeding resulted in increased protein content. The increase in protein content may be a reflection of reduced starch production resulting from a shortened grain-filling period and/or environmental conditions during grain filling. Peltonen-Sainio and Peltonen (1993) reported that a shortened grain-filling period resulted in lower grain protein content; however, an increase in hull content was also observed which would likely have contributed to the reduction in grain protein content. Response to late seeding varied among cultivars (Table 4.4). Manic and Capital consistently increased in protein content with late seeding; however, Marion QC did not increase in protein content at Ste-Anne-de-Bellevue in 1990 or 1992. Marion QC is an earlier maturing cultivar (Dubuc and Comeau, 1988) and may fiave been less affected by a shortened-growing season than later maturing cultivars such as Manic and Capital.

In 1990 and 1991, delayed seeding resulted in reduced groat oil content for all cultivars (Table 4.2 and 4.4). Oil concentration is reportedly positively correlated with days-to-heading and length of grain-filling period (Peltonen-Saino and Peltonen, 1993).

In 1992, oil content tended to increase with delayed seeding (Table 4.2) and several factors may have acted to give the observed results. The 1992 growing season was cool and wet compared to 1990 and 1991 and lower growing temperatures can increase oil content in oat (Saastmoinen *et al.*, 1989) particularly the free or unbound

lipids (Beringer, 1971). The solvent used in this experiment tends to extract free or unbound lipids more readily than bound lipids (Youngs, 1986). Further, later seeding increased thin oat content (Humphreys *et al.*, 1994) in 1992 more than in other years. Brown *et al.* (1966) observed that secondary kernels have higher oil content on a per weight basis than primary kernels. The increase in the proportion of thin grain may have also contributed to the higher oil contents observed in 1992.

Beta-glucan content tended to increase with delayed seeding. In barley, a similar trend has been observed (Molina-Cano and Conde, 1982). This may be partly due to a reduction in starch production caused by the shortening of the grain-filling period; however, a true increase in β -glucan production is also possible. In the Ste-Anne-de-Bellevue experiments, β -glucan response to delayed seeding seemed similar to that of protein (Table 4.2). In the Ste-Rosalie experiment, no significant seeding date effect was observed for β -glucan content; the reasons for this are unclear.

No cultivar was superior for all three biochemical characteristics but Marion QC was generally superior in oil and ß-glucan contents in all environments and was generally low in protein content. Days-to-heading and length of grain-filling have been positively correlated with oil concentration in oat and it has been suggested that early maturity may reduce lipid and protein syntheses more than carbohydrate synthesis (Peltonen-Sainio and Peltonen, 1993). Marion QC, a particularly early-maturing cultivar (Dubuc and Comeau, 1988), is low in protein compared to other cultivars but high in oil and ß-glucan contents. Newman tended to be low for all three characteristics; Newman has very large seeds which likely contain considerable starch which could reduce the

relative proportion of the other components. Although some cultivar x management treatments interactions were significant, the ranking of the four cultivars for protein, oil and β -glucan contents tended to be consistent. Hence, improvement of these biochemical characteristics might be possible with some confidence that genotype x management interactions would not unduly affect the superiority of a cultivar for protein, oil and β -glucan contents.

In conclusion, additional N applied at the boot stage of oat development increased protein content in three of four experiments and tended to decrease oil content although this was only statistically significant in one site-year. Beta-glucan content was not significantly affected by additional N. Additional N could be used to increase oat protein content but oat production costs would also increase. Delayed seeding increased protein and β-glucan contents and the later-maturing cultivars tended to give greater increases in protein content with delayed seeding than the earlier-maturing cultivars. Oil content decreased with later seeding in 1990 and 1991 but increased in 1992; hence, the response of groat oil content to delayed seeding seemed to depend on environmental conditions during the growing season. • •

Table 4.1: Hean squares for oat groat protein, oil and B-glucan contents (% of dry matter) from experiments conducted at Ste-Anne-de-Bellevue (1990-1992) and Ste-Rosalie (1991).

Effect	df	Protein	011	8-glucan
<u></u>		Ste-Anne-de-Belle	vue 1990	
Seeding Date	1	79.66	0.29	11.81
Error (Main Plot)	3	0.33	0.01	0.19
Cultivar	3	13.35	2.28	2,39
Nítrogen	1	0.20	0.11"	0.02
Date x Cultivar	3	2.80**	0.07*	0.28
Date x N	1	0.56	0.05	0.21
Cultivar x M	3	1,77	0.01	0.07
Date x Cultivar x N	3	0,59	0.14**	0.10
Error (Sub-plot)	42	0.44	0.04	0.23
		Ste-Anne-de-Belle	evue 1991	
Seeding Date	1	24.88	1.00"	11.28**
Error (Main Plot)	3	0.22	0.07	0.02
Cultivar	3	5.31**	7.85**	8.14
Nitrogen	1	2.29"	0.05	0.28
Date x Cultivar	з	1.31	0.12**	1.86"
Date x N	1	0.24	0.03	0.32
Cultivar x N	3	0.88	0.02	0.28
Date x Cultivar x N	3	0.15	0.03	0,18
Error (Sub-plot)	42	0.48	0.38	0.13
		Ste-Rosalle	1991	
Seeding Date	1	20,03	0.54**	0,31
Error (Main Plot)	3	0.01	0.01	0.09
Cultivar	3	11.55**	3.94**	7.82**
Nitrogen	1	6.13	0,22	0.01
Date x Cultivar	3	0.88	0.09	0.05
Date x N	3 .	0.68	0.12	0.03
Cultivar x N	3	0.41	0.01	0,08
Date x Cultivar x N	3	0.11	0.03	0.03
Error (Sub-plot)	42	0.85	0.04	0.05
		Ste-Anne-de-Bell	evue 1992	
Seeding Date	1	13.83	2.62**	0.29
Error (Main Plot)	3	1.73	0.06	0.08
Cultivar	3	16.15"	5.06	5.35**
Nitrogen	1	4.81**	0.06	0.01
Date x Cultivar	3	5.20	0.43**	0.17
Date x N	1	1.57	0.00	0.03
Cultivar x N	3	0.10	0,02	0.14
Date x Cultivar x N	3	0.07	0.04	0.09
From (Sub-plot)	42	0.06	0.02	0.05

*. T Significant at the 0.05 and 0.01 probability levels, respectively.

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Table 4.2: Mean groat protein, oil and B-glucan contents (% of dry matter) of four oat cultivars for two seeding dates and for two nitrogen levels (40 kg ha⁻¹ N fertilizer applied at seeding and 40 kg ha⁻¹ N applied at seeding plus 20 kg ha⁻¹ N applied at the boot stage (Zadoks 40-43)) from experiments conducted at Ste-Anne-de-Bellevue, Québec (1991 and 1992) and Ste-Rosalie, Québec (1991), (Cultivar, seeding date and N fertilizer values are means of 16, 32 and 32 observations, respectively).

	Ste-Anne-o	le-Bellevue	Ste-Rosalie						
	1991	<u> 1992 </u>		1991					
Cultivar	Protein	Protein	Protein	0i1	ß-glucan				
· <u>-</u>			%		·				
Capital	17.7bª	16.1b	17.4b	6.12b	5.28b				
Manic	18.5a	17,0a	18.5a	5.84c	4.69c				
Marion QC	17.2c	15.9b	17.2b	6.94a	5.99a				
Newman	18.2a	14,6c	16.4c	5.96c	4.42d				
Seeding Date	۵								
Early	17.3b	15.4a	16.8b	6.31a	5.02a				
Late	18.5a	16.4a	17.9a	6.12b	5.16a				
<u>N Fertilizer</u>)								
40	17.7b	15.6b	17.1b	6.27a	5.08a				
40 + 20	18.1a	16.2a	17.7a	6.16b	5.10a				

• = Cultivar means within a column for the same locations that have the same letter(s) are not significantly (P<0.05) different according to a ANOVA-protected Duncan's multiple range test.

^b = Seeding date and nitrogen treatment means within a column that have the same letter are not significantly different according to an analysis of variance.

Table 4.3: Mean groat oil content (% of dry matter) for four oat cultivars grown at Ste-Anne-de-Bellevue in 1990 at two seeding dates and with 40 kg ha⁻¹ N fertilizer applied at seeding and 40 kg ha⁻¹ N applied at seeding plus 20 kg ha⁻¹ N applied at the boot stage (Zadoks 40-43). (Oil content values are means of 4 observations).

		<u>Nitrogen Treatment (kg ha</u>				
Cultivars	Seeding Date	40	40+20			
<u></u>		Oil (%)	·····			
Capital	Early	6.43a*	6.39a			
	Late	5.93b	6.25a			
Manic	Early	5.89a	5.93a			
	Late	5.74a	5.83a			
Marion QC	Early	6.67a	7.00a			
	Late	6.34a	6.45a			
Newman	Early	5.74a	5.75a			
	Late	5.62a	5.67a			

* = Means within a cultivar for the same nitrogen treatment that have the same letter(s) are not significantly (P<0.05) different according to a ANOVA-protected t-test.

	Prot	<u>ein</u>	0	<u>i1</u>	<u> </u>		
Cultivar	Early	Late	Early	Late	Early	Late	
<u></u>			%				
			1990				
Capital	14.9b°	17.6b*	6.34b	6.16b	4.48ab	5.37a	
Manic	16.7a	19,4a*	5.83c	5.86c	4.11b	4.82ab*	
Marion QC	16.2b	17.1b	6.84a	6.40a	4.58a	5.20ab	
Newman	14.8b	17.2b*	5.75c	5.65d	3.51c	4.72b [*]	
			1991				
Capital	17.1b	18.3b	6.15b	5.71b [*]	5.12a	5.76b	
Manic	18.3a	18.8ab	5.85c	5.74b	4.90a	5.42b*	
Marion QC	16.5c	17.9b*	7.32a	6.97a"	5.04a	6.89a	
Newman	17.3b	19.2a"	5.64d	5.54b	4.08b	4.44c	
			1992				
Capital	15.4a	16.9b*	6.36c	6.68c	4.48b	4.57b	
Manic	15.9a	18.2a*	6.48b	6.96b [*]	4.02c	4.43b*	
Marion QC	16.0a	15.7c	7.15a	7.95a"	5.18a	5.10a	
Newman	14.5b	14.7d	6.23d	6.25d	3.73d	3.79c	

Table 4.4: Mean groat protein, oil, and B-glucan content (% of dry matter) of four oat cultivars for early and late seeding dates grown at Ste-Anne-de-Bellevue, Québec (1990-1992), (Protein, oil and B-glucan content values are means of 8 observations).

* = Means within a column for the same planting time and location that have the same letter(s) are not significantly (P<0.05) different according to a ANOVA-protected Duncan's multiple range test.

* - Means within an attribute for the same cultivar and location are significantly (P<0.05) different.

Preface to Chapter 5

Chapters 3 and 4 dealt with agronomic aspects of oat grain quality. Chapters 5 and 6 evaluate genetic aspects of β -glucan in oat. When breeding for improved grain quality knowledge of the genetics of β -glucan would be useful.

This chapter corresponds to a manuscript which has been submitted for publication in *Euphytica*. It has been modified to conform to McGill University thesis guidelines. In chapter 5, the heritability of β -glucan, groat percentage and crown rust resistance were determined. This experiment was designed, seeded and harvested by D. Gavin Humphreys. Technical assistance was obtained with field preparation, seeding and harvesting. Harvested materials were processed and grain characteristic data were obtained by D. Gavin Humphreys with technical assistance from N. Brière. Data were analyzed and the manuscript was written by D. Gavin Humphreys. Dr. D.E. Mather contributed critical advice during data analysis and writing of the manuscript. H. Cohen Rimmer aided with formatting of the figures for submission to *Euphytica*. All literature cited in this manuscript can be found in a section at the end of this thesis. Tables and figures are included at the end of the manuscript. Figure captions are printed on the page preceding the figure.

Chapter 5: Heritability of ß-glucan, Groat Percentage and Crown Rust Resistance in Two Oat Crosses.

5.1 ABSTRACT

Parent-offspring regression was used to estimate F_5 - F_6 , F_5 - F_7 , F_6 - F_7 heritabilities for β -glucan content and groat percentage and the F_6 - F_7 heritability for crown rust resistance in two oat populations that were composed of 99 random lines. Generally, heritabilities for all traits were low (0.07 - 0.35) although the F_6 - F_7 heritability estimate for β -glucan content was higher (0.45) in the Sylva/Marion QC population. The heritability of β -glucan content has not been previously reported. Lines with β -glucan contents superior to the parents were observed in both populations; hence, selection for improved β -glucan content should be possible although selection may be most effective in later generations due to low heritability. Heritable resistance to crown rust was observed in the Sylva/Marion QC population and several lines were identified which had relatively low mean crown rust rating scores in both the F_6 and the F_7 generations.

5.2 INTRODUCTION

Heritability is a measure of the resemblance between parents and offspring for a given trait. Narrow-sense heritability (h^2) is equal to the ratio of additive genetic variance to phenotypic genetic variance of individuals in a population (Nyquist, 1991). Heritability estimates can be used to indicate the degree that the offspring will likely resemble their parents and these estimates can be used to predict gain from selection within the population for which the h^2 value has been computed. Traits with high heritability can

normally be improved through selection more easily than traits that have lower heritability.

Methods for calculating heritability estimates have been thoroughly reviewed (Nyquist, 1991). Parent-offspring regression is often used to compute h^2 . In oat, heritability estimates have been calculated for many traits including kernel weight, caryopsis percentage, test weight (Wesenberg and Shands, 1973), protein percentage (Takeda and Frey, 1979; Frey, 1975), oil percentage (Baker and McKenzie, 1972; Brown et al., 1974) and crown rust resistance (Simons, 1969; Simons, 1975; Simons, 1985). However, heritability estimates have not been published for the non-starch polysaccharide $(1\rightarrow 3), (1\rightarrow 4)$ -B-D-glucan (B-glucan).

Oat and barley have been found to contain particularly high levels of β -glucan compared to other cereal grains (Åman and Graham, 1987). In recent years, studies have demonstrated an association between the consumption of oat and lower serum cholesterol levels in humans (Ripsin *et al.*, 1992; Hurt *et al.*, 1988; Wood *et al.*, 1989), and it has been hypothesized that β -glucan is the chemical agent that is primarily responsible for the cholesterol lowering effect of oat (Hurt *et al.*, 1988; Wood *et al.*, 1989). "Oat gum" of which β -glucan makes up a large component may have uses as a food hydrocolloid (Wood *et al.*, 1989). Beta-glucans also have anti-nutritional properties; for example, in chicken diets β -glucan "gumminess" and indigestibility can affect food intake, cause sticky droppings and reduce feed conversion efficiency (Hesselman and Åman, 1986). Cave *et al.* (1990) reported that increased β -glucan content in broiler chickens diets reduced rate of growth, and the availability of metabolizable energy and amino acids. In future, oat cultivars may be developed with either high or low β -glucan contents to cater to the needs of different oat consumers.

Groat percentage is an important oat grain quality characteristic (Wesenberg and Shands 1973). The groat-to-hull ratio and protein content essentially control the nutritive value of oat grain because oat hulls contain very little protein (Youngs, 1972). Thus, increasing oat groat percentage can improve the value of oat as animal and human food. Breeding for lower hull and/or increased groat percentages is a common goal of oat breeding programs (Burrows, 1986). Realized heritability estimates of groat percentage range from 34 to 72% (Stuthman and Granger, 1977).

Oat crown rust may be the single most damaging disease in oat (Harder and Haber, 1992). This disease is caused by the fungus *Puccinia coronata* Cda. *f. sp. avenae* Eriks. In southwestern Québec, important epiphytotics of crown rust can be experienced due at least in part to the prevalence of the alternate host, buckthorn (*Rhamnus spp.* L.). Severe rust infection during the grain filling period may result in decreased dry matter accumulation and reduced groat accumulation. Simons *et al.* (1979) concluded that groat percentage was reduced in direct proportion to crown rust susceptibility. Singleton *et al.* (1982) reported a similar result for 250-grain-weight ratios (rusted/unrusted 250-grain-weights) although the 250-grain-weight ratio of some moderately resistant entries were equal to or exceeded highly resistant entries under similar crown rust severity.

Numerous simply inherited but broadly effective resistance genes to crown rust have been identified (Harder and Haber, 1992). This resistance is also referred to as specific 'seedling' resistance (Simons, 1985). However, such resistance tends not to be durable (Frey *et al.*, 1977). Fortunately, multiple sources of quantitative resistance (general resistance) to crown rust are available (Simons, 1985). General resistance may be less susceptible to breakdown resulting from the appearance and spread of new forms of the fungus than is specific 'seedling' resistance. The use of general resistance in conjunction with specific resistance may increase the agronomic lifetime of resistant cultivars (Simons, 1985). The cultivar 'Sylva', which is well adapted to Québec, has been identified as a potential source of general resistance to crown rust (Brière *et al.*, 1993).

Simons (1975) estimated narrow-sense heritability of general (field) resistance to *P. coronata* in oat between 0.18 and 0.74 using standard unit heritability. Resistance was evaluated as yield reduction under disease conditions. Slightly higher standard unit heritabilities were observed when disease stress was evaluated as reduction in oat grain weight. Simons (1985) found mean standard unit heritabilities that were evaluated by reductions in grain yield and in seed weight were 0.41 and 0.58 respectively. In wheat, narrow sense heritability of leaf rust resistance estimated using parent-offspring regression ranged between 0.21 and 0.77 among 6 crosses (Bjarko and Line, 1988).

The objectives of this study were to estimate the narrow-sense heritabilities using parent-offspring regression of percent ß-glucan, groat percentage, and crown rust resistance for late generation populations from two oat crosses; and to evaluate two oat populations for the presence of general resistance to crown rust.

5.3 MATERIALS AND METHODS

5.3.1 Germplasm

Two populations were used in this study. The oat cultivar 'Marion QC' was crossed to oat cultivars 'Nova' and 'Sylva'. The two crosses were advanced as bulks without selection to the F_4 generation.

5.3.2 Field experiments

Two populations of ninety-nine randomly chosen lines were established in autumn 1989 from the unselected bulks of two oat crosses: (1) Nova/Marion QC; and (2) Sylva/Marion QC. F_5 seeds were sown in 20 cm pots and plants were grown in a greenhouse with a 16 hour photoperiod. Plants were harvested by hand and heads threshed. F_6 seeds were sown at Ste-Anne-de-Bellevue, Québec on May 9 in 1990. Experimental design used was a split-plot with four replicates. Main plots consisted of populations and sub-plots were genotypes within populations. Plots were composed of 10 plants seeded 5 cm apart. Plots were separated by a row of winter wheat (cv. Frankenmuth) and the alternating rows of oat and winter wheat were grown 20 cm apart. All seeding and harvesting was done by hand. Harvested plants from each plot were bound together and hung to dry for 4-6 weeks. Dried plants were threshed in a head thresher and the grain kept for analysis. In 1991, the experiment was repeated with F_7 seed using the same experimental and planting designs. The lines were sown by hand on May 6.

5.3.3 Field data

At Zadoks growth stage 75 (Zadoks *et al.*, 1974) the oat crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Eriks.) symptoms on the flag leaf were scored visually using a scale of 0 to 3:

0 - No visible rust infection or <1% of flag leaf covered with pustules.

1 - 1% to 20% of flag leaf covered with pustules and/or lesions.

2 - 21% to 50% of flag leaf covered with pustules and/or lesions.

3 - >50% of flag leaf covered with pustules and/or lesions.

These four categories correspond approximately to categories 5 through 8 on the scale of Couture (1980).

5.3.4 Grain analysis

Grain material used for determination of groat and ß-glucan percentages was derived by bulking grain from across the four replicates for each line in both crosses. Bulking across replicates reduced the number of ß-glucan assays required and was acceptable for parentoffspring regression analysis which does not require replicated data. From each bulked sample, a subsample of 20 grains was weighed and dehulled by hand. Mass of the groat fraction was taken and these data were used to compute the groat percentage.

The groat samples were ground for four minutes in a Retsch Vibratory Mill, Type MM2 using 5 ml cylinders containing 2 steel ball bearings (2g size). Flour was stored in a paper envelope at -20°C. Oat flour ß-glucan content was determined using the McCleary and Glennie-Holmes technique (McCleary and Glennie-Holmes, 1985) except that only 0.1g oat flour was used.

5.3.5 Statistical calculations

Heritability estimates of β -glucan, groat percentage and crown rust rating score were calculated using parent-offspring regression. Residuals of F₇ crown rust rating scores were not normally distributed. A Box-Cox transformation of F₇ residuals was performed using The R Package (Legendre and Vaudor, 1991) and the F₇ rating score values were adjusted using transformed residuals. Analysis of variance was used to determine whether significant inter- and intra-population variation existed for crown rust infection. Calculations were performed using the regression and ANOVA procedures available in SAS (SAS Institute Inc., 1990).

5.4 RESULTS

 F_5 - F_6 , F_5 - F_7 and F_6 - F_7 parent-offspring regression heritabilities for β -glucan content in oat two crosses are given in Figures 5.1a-f. Heritabilities for β -glucan were low, especially in the earlier generations. In the Nova/Marion QC population, all heritability estimates were quite low but in the Sylva/Marion QC population, the F_6 - F_7 heritability estimate was intermediate (Figure 5.1f). In both populations, mean β -glucan contents were higher in later generations and the ranges of β -glucan contents were narrower (Figures 5.1a-f). Generally, lines with the lowest β -glucan content were observed in the Sylva/Marion QC populations. Lines with greater β -glucan contents than the parents were found in both populations and all generations (Figure 5.1a-f).

 F_5 - F_7 , F_5 - F_6 and F_6 - F_7 parent-offspring regression heritabilities for groat percentage are given in Table 5.1. Heritability estimates were low in both populations. Mean groat percentages were lowest and variances were highest in the F_6 generation for both populations. Groat percentage variance was lowest in the F_7 generation for both populations.

 F_6 - F_7 parent-offspring regression heritability for crown rust resistance were 0.07 (P>0.05) for the Nova/Marion QC population and 0.31 (P<0.01) for the Sylva/Marion QC population. Crown rust rating scores varied significantly between populations and within both populations there was significant variation among genotypes in the F_6 and F_7 generations (Table 5.2). Rust rating scores in the F_6 generation were generally lower than in the F_7 generation in both populations (Figures 5.2a-b). More resistance was observed in the Sylva/Marion QC population than the Nova/Marion QC population (Figures 5.2a-b). Disease severity varied between generations. In the Nova/Marion QC population, no

lines averaged 3.0 in the F_6 generation but 14 lines had mean disease scores of 3.0 in the F_7 generation (Figure 5.2a). The mean disease scores in the F_7 generation often differed from those for the same line in the F_6 generation. For example, the lines in the Nova/Marion QC population that averaged 2.25 in the F_6 generation had mean rating scores between 1.75 and 2.75 in the F_7 generation (Figure 5.2a).

In the Sylva/Marion QC population, crown rust rating scores tended to be higher in the F_7 generation than the F_6 generation; for example, 37 percent of the lines in the F_6 generation scored on average 1.0; however, these same lines had average rating scores of between 0.75 and 3.0 in the F_7 generation (Figure 5.2b). F_6 rating scores were generally lower than F_7 rating scores and 75 percent of lines had mean disease scores between 1.0 and 1.5 (Figures 5.2b); whereas, 87 percent of lines in the F_7 generation had mean disease scores of 2.0 or greater. More resistance appears to be present in the Sylva/Marion QC population; 13 lines were identified that had mean rating scores of less than 2.0 in both generations (Figure 5.2b) compared to only 3 such lines in the Nova/Marion QC population (Figure 5.2a).

5.5 DISCUSSION

Narrow-sense heritabilities estimated for β -glucan, groat percentage and crown rust infection were low to intermediate. The heritability of β -glucan in oat has not previously been reported. In barley, β -glucan content has been hypothesized to be controlled by two to three major genes with dominance for low β -glucan and some modifier genes may also be present (Greenberg, 1977). Because domestic oat is hexaploid (AACCDD) (Forsberg and Shands, 1989), there may be multiple copies of the genes governing β -glucan content in oat which could reduce heritability of the trait. Generally,

 β -glucan heritability was higher in the Sylva/Marion QC population than the Nova/Marion QC population. Based on cultivar means (data not shown) Sylva/Marion QC could be considered a low x high cross and Nova/Marion QC an intermediate x high cross. These results would suggest that cross selection is an important consideration when breeding for β -glucan content. Selection for improved β -glucan content should be possible because lines with higher β -glucan content than the parents were found in both generations. Selection may be most effective if carried out in late generations since the heritabilities for the trait were low to intermediate (Figures 5.1a-f).

Stuthman and Granger (1977) found that realized heritability estimates for caryopsis percentage ranged between 0.34 and 0.72. Takeda and Frey (1979) estimated mean broad-sense heritability of groat weight at 38 percent. In barley, parent-offspring regression estimated heritability for kernel weight ranged from 25.3 to 25.9 percent (Borthakur and Poehlman, 1970). Heritability estimates for groat percentage in this study were slightly lower than those published previously but heritability estimates are specifically applicable to the germplasm sampled and the environments used to grow the germplasm (Nyquist, 1991). Hence, heritability estimates determined for different populations are not necessarily comparable.

Simons (1969) estimated crown rust tolerance heritability in oat using F_3 - F_4 parent-offspring regression. Disease incidence was scored by evaluating yield reduction under disease conditions. Heritability estimates were 0.19 for Markton (susceptible)/Andrew (tolerant) and 0.26 for Richland (susceptible)/Cherokee (tolerant) and standard unit heritabilities were 0.24 and 0.38 for the two crosses. Simons (1985) found mean standard unit heritabilities for crown rust resistance were 0.41 and 0.58 for backcross progeny of a resistant x susceptible cross. Heritability estimates of crown rust

resistance found in this study for the Sylva/Marion QC population were within the range of estimates published previously. Hence, heritable crown rust resistance is likely present in the Sylva/Marion QC population but no heritable resistance was detected in the Nova/Marion QC population.

Disease observed in this experiment was caused by natural infection of the plants. Disease severity was higher in 1991 (F_7 generation) than 1990 (F_6 generation). Possibly, the level of inoculum and/or environmental conditions present during the 1991 growing season were more conducive to rust infection than in 1990. Mean crown rust rating scores in the Sylva/Marion QC population were generally superior to the Nova/Marion QC population. The Québec oat cultivar 'Sylva' has been previously observed to have general resistance to crown rust (Brière *et al.*, 1994). In this study, we found that F_6 and F_7 offspring of this cultivar possessed general resistance to crown rust. While many lines scored were highly suscentible to crown rust (Figures 5.2a-b), resistant lines were identified in both populations although more resistant lines were identified in the Sylva/Marion QC population than the Nova/Marion QC population.

Crown rust rating scores did not seem to be related to ß-glucan or groat percentage. Singleton *et al.*(1982) found that yield and 250-grain-weight ratios (rusted/non-rusted plots) were negatively correlated with crown rust intensity but some moderately resistant entries were identified that gave 250-grain-weight ratios equal or superior to highly resistant entries. Similarly, some lines in our study may have been capable of supporting significant amounts of crown rust without undue loss in groat percentage.

In conclusion, heritabilities determined in this study were generally low which would suggest that improvement of these traits will be slow. However, heritability of ß-

glucan for these random lines was similar to heritability estimates for protein and improvement in protein content in oat has been reported (Takeda and Frey, 1979). Further, improvement may be enhanced with careful choice of breeding population because β -glucan heritability estimates varied between crosses. Lines with β -glucan contents superior and inferior to the parents were observed in both generations. Resistance to crown rust has been observed. While the heritability was low, lines that showed resistance in both the F₆ and the F₇ generations were identified.

Table	e 5.1:	F_{5} - F_{7} , F_{5}	$_{5}$ -F ₆ and	1 F ₆ -F7 h	eritabil	ities.	populat	tior	n mean,	range a	nd varia	nce for	groat pei	rcentage
from	three	generat	ions o	if two o	at popu	lations	grown	at	Ste-Anr	ne-de-B	ellevue,	Québec .	Canada	between
1989	and 19	991.												

	·	Nova/	<u>Marion QC</u>		Sylva/Marion QC				
Generation	Heritability'	Mean	Range	Variance	Heritability'	Mean	Range	Variance	
				· <u> </u>					
F ₅	0.23	78.8	66.4-82.9	7.0	0.26	78.4	69.3-82.7	6.4	
F₀	0.26*	67.4	58.3-72.8	8.8	0.32	66.4	55.3-74,8	13.6	
F,	0.32*	71.1	62.5-75.6	5.2	0.26*	71.5	66.5-79.3	4.9	

' = F_5 , F_6 and F_7 heritabilities refer to F_5 - F_7 , F_5 - F_6 and F_6 - F_7 parent-offspring regression heritabilities, respectively.

• = significantly (P<0.05) different from zero.

Effect	df	Crown Rust Rating
	Ste-Anne-de-Bellevue 1	1990 (F ₆)
Population	1	123.7**
Genotypes within Population	196	0.7**
Genotypes(Nova/Marion QC)	98	0.5
Genotypes(Sylva/Marion QC)	98	0.9"
	Ste-Anne-de-Bellevue :	1991 (F,)
Population	1	10,5
Genotypes within Population	196	3.4**
Genotypes(Nova/Marion QC)	98	2.6**
Genotypes(Sylva/Marion QC)	98	4.2**

Table 5.2: Mean squares for crown rust ratings for two populations of oat grown at Ste-Anne-de-Bellevue. Québec, Canada in 1990 and 1991.

"." = Significant at 0.05 and 0.01 levels, respectively.

Figure 5.1: Scatter plots with regression lines and heritability estimates (b) for F_5 - F_6 , F_5 - F_7 and F_6 - F_7 parent-offspring regressions for two oat populations (Figures 5.1a-5.1c: Nova/Marion QC; Figures 5.1d-5.1f: Sylva/Marion QC) grown at Ste-Anne-de-Bellevue, Québec, Canada between 1989 and 1991. Population means are denoted by vertical and horizontal lines on graphs (* = Heritability estimate significant at P<0.05 level.).



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Figure 5.2: Frequency distributions of mean crown rust rating scores for F_6 and F_7 generations in two oat populations ((a) Nova/Marion QC; (b) Sylva/Marion QC) grown at Ste-Anne-de-Bellevue, Québec, Canada between 1989 and 1991.



Preface to Chapter 6

Devising breeding strategies for selection for ß-glucan content would be facilitated by knowledge of the inheritance of this trait. In chapter 6, the inheritance of ß-glucan content in oat was evaluated.

This chapter corresponds to a manuscript which will be submitted for publication in *Crop Science*. The manuscript has been written to conform to McGill University thesis guidelines. This experiment was designed and carried out by D. Gavin Humphreys. Technical assistance was obtained from N. Brière during crossing and β -glucan analysis. Data were analyzed and the manuscript was written by D. Gavin Humphreys. Dr. D.E. Mather contributed critical advice during data analysis and writing of the manuscript. All literature cited in this manuscript can be found in a section at the end of this thesis. Tables and figures are included at the end of the manuscript. Figure captions are printed on the page preceding the figure. Chapter 6: The inheritance of mixed-linkage ß-glucan content in oat (Avena sativa L.)

6.1 ABSTRACT

Breeding for β -glucan content in oat could be facilitated through an improved understanding of the genetic control of β -glucan in oat. Reciprocal crosses were made with two sets of low β -glucan and high β -glucan parents (Don and IL83-80922; Manic and Larry). Groat β -glucan content was determined for individual F₂ grains from F₁ plants and for samples of 20 F₃ grains from F₂ plants. Oat β -glucan content appeared to be controlled by the endosperm genome because significant reciprocal differences were observed for the F₂ grains from F₁ plants but reciprocal differences were less pronounced for the F₂ grain from F₃ plants. Further, variation among F₂ plants was not significantly greater than variation among parental plants. In general, grain position within the panicle did not affect β -glucan content. Nevertheless, significant intra-panicle variation for β glucan content was observed; hence, single-grain evaluation is not likely the appropriate manner to differentiate high and low β -glucan genotypes.

6.2 INTRODUCTION

An oat cultivar that is high in β -glucan for human food and/or industrial usage may be an objective of oat breeders in the future (Webster, 1986). Achieving this goal would likely be facilitated by an understanding of the inheritance of this carbohydrate because the inheritance cf a character can have a significant effect on the choice of parents for crossing and the breeding techniques used.

Beta-glucan is a complex polysaccharide found in high levels in the oat endosperm and particularly in the aleurone and subaleurone layers. At least two enzymes are reportedly involved in mixed-linkage β -glucan synthesis in oat (Tsai and Hassid, 1971 and 1973) but details on the inheritance of this carbohydrate have not been reported in the literature. Expression of seed chemical components that are found principally in the endosperm tissue may be influenced by the genotype of the endosperm cells, by the genotype of the embryo, by the genotype of the maternal plant and/or a combination of these. Thus, the expression of this carbohydrate may be controlled by the 3*n* endosperm genome which is derived from the triple fusion of two maternal polar nuclei and one paternal sperm nucleus. Alternatively, it may be influenced by the 2*n* embryo genome or the 2*n* genome of the maternal plant on which the seed develops. Furthermore, cytoplasmic effects could be important.

In barley, β -glucan content is reportedly controlled by a simple additive (Powell *et al.*, 1985) or additive-dominance genetic system with dominance for low β -glucan content (Greenberg, 1977). Bogyo *et al.* (1988) reported that additive x dominance interactions were also important. Bogyo *et al.* (1988) estimated genetic variances for the genetic effects. While none were significant the largest ones were the additive and environmental variances. Powell *et al.* (1985) found that linkage disequilibrium was not important in the genetic control of β -glucan in barley.

Single-grain analysis within individual plants should allow one to determine the distribution of phenotypes in a population independent of any positional effects within a plant inflorescence. Kumar and Khush (1987) used single-grain analysis in their study of the inheritance of amylose in rice and Bogyo *et al.* (1988) used single-grain analysis in their investigations into the genetic effects controlling ß-glucan content in barley.

The objectives of this study were: to evaluate the effect of position within the panicle on the β -glucan content of individual grains of oat and to evaluate the inheritance of β -glucan content in oat.

6.3 MATERIALS AND METHODS

6.3.1 Genetic Material

Reciprocal crosses were made between the oat cultivars Manic and Larry and between the cultivar Don and the breeding line IL83-80922 (IL83). Larry had been previously characterized as a high β -glucan cultivar and Manic and Don as low β -glucan cultivars (Hurt et al., 1988). IL83 had been found to be high in B-glucan content (F. Kolb, pers. comm.). Parental and F_1 seeds were sown in a randomized complete block design with 3 replicates. Each replicate consisted of four plants: one plant from each of the two parents, and one F_1 plant of each of the high x low and low x high crosses. Seeds were germinated on moistened Whatman #1 filter paper in petri dishes on growth benches at 20° C with a 16 hour photoperiod. After 5 days, germinated seedlings were transferred to 15-cm pots containing Metro-mixTm. Plants were grown on growth benches at 20°C with a 16 hour photoperiod. F_2 seed was harvested by hand from the F_1 plants. Each panicle was divided into 3 approximately equal sections: bottom, middle and top. The bottom was defined as the bottom node of the panicle, the middle section was defined as 1 to 2 nodes above the bottom section and the remainder was defined as the top section of the panicle. Seeds for analysis were harvested from the primary panicle. Secondary panicles were harvested in a similar fashion to primary panicles and the seeds from secondary panicles were kept for sowing the F₃ generation. Within each floret, primary seeds were separated from secondary and tertiary seeds. Principally primary seeds were used for the β -glucan analysis of the F₂ generation; however, in some instances, large secondary seeds were used for β -glucan analysis because insufficient primary seeds were available.

 F_2 seeds were sown in Metro-mixTm in 15-cm pots in a randomized complete block design with 3 replicates. Each block contained 80 plants. Ten plants each of Manic, Larry, Don and IL83 and 10 plants each of F_2 Manic/Larry, F_2 Larry/Manic, F_2 Don/IL83 and F_2 IL83/Don. Each block contained 40 pots with 2 plants per pot. Plants were grown on a growth bench at 20°C with a 16 hour photoperiod. Parental, F_2 and F_3 plants were each harvested by hand. Primary F_3 seeds from the main panicle of each F_2 plant were bulked and a subsample was used for β-glucan determination of F_3 seeds from F_2 plants.

6.3.2 Seed Preparation

Grains were dehulled by hand and groats were ground in a Retsch Vibratory Mill, Type MM2 using 5 ml cylinders containing 2 steel ball bearings (2g size). Flour was carefully collected in a clean microfuge tube and stored at -20°C. F_2 groats were each ground individually for two minutes and samples of 20 F_3 groats were ground for four minutes.

6.3.3 B-glucan Determination

The β -glucan contents of 30 individual F_2 groats per F_1 plant (10 groats each from the top, middle and bottom sections) were determined using a modification of the McCleary and Glennie-Holmes (1985) technique. Each ground groat (approximately 25 mg) was added to a pre-weighed glass culture tube and was dried overnight at 90°C. The tubes were removed from the oven, and allowed to cool and weighed. To each tube, a small stir bar was added, along with 0.05 ml aqueous ethanol (50% v/v) and 2.0 ml NaH₂PO₄ buffer (20 mM, pH 6.5). The slurry was gently mixed on a stir plate at low speed for approximately 30 seconds. Tubes were transferred to a boiling water bath for 2 minutes. The water bath was mounted on stir plates so that stirring of the solution occurred during heating to prevent the flour from forming into lumps. Following heating, tubes were cooled to 40°C and 0.1 ml lichenase [10U] in a 20 mM NaH₂PO₄ buffer (pH 6.5) was added to each tube. Tubes were capped and the mixture was stirred on a stir plate then placed in a 40°C shaking water bath for 30 minutes. After 30 minutes the tubes were removed and stirred gently on a stir plate, then returned to the 40°C water bath for an additional 30 minutes.

Following lichenase digestion, the volume in each tube was increased to 12.0 ml using double distilled H₂O. The contents were thoroughly mixed and the solution was filtered through Whatman #41 filter paper. A 0.2 ml aliquot of filtrate was carefully transferred to 3 small test tubes. To one of the tubes, 0.1 ml NaC₂H₂OOH buffer (50mM, pH 4.0) was added while to each of the other two tubes 0.1 ml β-glucosidase ([0.2 U] in 50 mM NaC₂H₂OOH buffer, pH 4.0) and reagent blanks with 0.2 ml dd H₂O, 0.1 ml β-glucosidase and NaC₂H₂OOH buffer were included with the sample. Following incubation, 3.0 ml glucose oxidase/peroxidase reagent (Megazyme (Aust) Pty. Ltd., North Rocks, Australia) were added to each tube and the tubes were incubated at 40°C for 20 minutes. Absorbance of a 1.8 ml aliquot of each tube was recorded at 510 nm using a LKB Ultraspec II fitted with a LKB Autofill autosampler.

Percent ß-glucan was calculated using the following equation:

% ß-glucan =
$$\triangle_{ABS} \div W \times F$$
;

where \triangle_{ABS} = Sample Absorbance - Blank Absorbance,

W = Dry weight of flour sample,

 $\mathbf{F} = (50 \ \mu g \ \text{glucose} \div \text{Absorbance of } 50 \ \mu g \ \text{glucose}) \ x \ 27$

6.3.4 Statistical Analysis

Analysis of variance was used to determine whether significant differences existed for β -glucan contents of seeds between the top, middle and bottom sections of the panicle. Variances and means were calculated using SAS (SAS Institute Inc.).

6.4 RESULTS AND DISCUSSION

For both sets of parents, significant differences (P < 0.05) were observed between the parents and between the reciprocal crosses for the F₂ grains harvested from F₁ plants. Mean groat β-glucan content was higher for Larry than for Manic (Figure 6.1) and higher for IL83-80922 than for Don (Figure 6.2). These results were not unexpected because Larry and IL83-80922 were chosen for their high β-glucan contents and Manic and Don were chosen for their low β-glucan contents. In both crosses, the average groat β-glucan of F₂ grains harvested from F₁ plants more closely resembled the maternal parent than the paternal parent except for Larry/Manic which was closer to the paternal parent (Figures 6.1 and 6.2). Averaged across the reciprocal crosses, the F₂ groat βglucan content was significantly (P < 0.001) below the midparent value for the Manic/Larry cross and significantly (P < 0.05) above the midparent value for the Don/II83 crosses. These results suggest that there is partial dominance for low β-glucan content in the Manic/Larry population; whereas, partial dominance for high β-glucan content is present in the Don/IL83 crosses.

There was considerable variation for β -glucan content among oat groats within panicles, even for the parents (Figures 6.1 and 6.2). Thus, a sample of oat grain is required to differentiate between high and low β -glucan genotypes; and single-groat

assessment would not be useful in a selection program. In both the Larry/Manic and the IL83-80922/Don crosses, the variation in β -glucan content among groats within panicles tended to be higher for the high β -glucan parent than for the low β -glucan parent, and the variation among F₂ groats was similar to the among-groat variation of the more variable parent (Figures 6.1 and 6.2). For most genotypes (Larry, Manic, Larry/Manic, Manic/Larry, IL83-80922 and IL83-922/Don), there were no significant differences in groat β -glucan content for F₂ grains harvested from the upper, middle and lower portions of the panicle (Table 6.1). For Dcn and Don/Il83-80922, β -glucan contents were slightly higher for groats from the lower portion of the panicle compared to from the upper portion of the panicle but these within-panicle differences were small relative to differences observed among genotypes (Table 6.1). Possibly, Don was more sensitive to small variations in the environment than the other genotypes.

In the experiment involving F_3 grains harvested from F_2 plants, significant (P<0.05) differences in groat β-glucan content between parental and cross populations were only observed for the Manic/Larry cross (Figures 6.3 and 6.4). As observed in the previous experiment, Larry had higher groat β-glucan content than Manic (Figure 6.3), and the groat β-glucan contents of the reciprocal cross combinations Larry/Manic and Manic/Larry were more similar to those of their maternal parents than to those of their paternal parents (Figure 6.3). Variation in β-glucan content among F_2 plants was similar to that among parental plants (Figures 6.3 and 6.4).

Possible explanations for similarity of crosses to the maternal parents include: cytoplasmic inheritance, maternal inheritance and influence of the endosperm genome. In this study, influence of the endosperm genome seems the most plausible for the following reasons: (1) the trait of interest is an endospermic characteristic; (2) the

reciprocal differences were stronger for F_2 grain than for F_3 grain; (3) there was relatively little variation among F_2 plants for the progeny. With cytoplasmic inheritance, reciprocal differences should be consistent over generations. For a maternally influenced trait, one would expect high variation among F_2 plants.

6.5 CONCLUSIONS

Generally, positional effects in oat panicles did not affect β -glucan contents of the grain. While some small differences between top and bottom positions for Don and Don/II83-80922 were found, these differences were small compared to differences in β -glucan contents among genotypes. Substantial intra-panicle variation for β -glucan content was found even for the parents. Thus, a sample of grain from a panicle should be used to determine whether a plant is high or low β -glucan. It would appear that oat β -glucan is under the control of the endosperm genome because β -glucan is an endospermic trait; stronger reciprocal differences were observed for the F₂ grains from F₁ plants than the F₂ grain from F₃ plants; and little variation was found among F₂ plants.

6.6 ACKNOWLEDGEMENTS

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Effect	df	IL83	IL83/Don	Don	Don/1L83	Larry	Larry/Hanic	Nanic	Manic/Larry
					———— Mean Squares ———				
Position	2	0.12	0.04	0.03	0.11*	0.29	0.16	0.01	0.03
Error	4	0.05	0.03	0.003	0.02	0.12	0,08	0.004	0.03
Posit'en					— B-glucan (%) ————				
Bottom		5.64a'	5.50a	4.63a	4.95a	5.74a	4.67a	3,66a	3,64a
Middle		5.36a	5.26a	4.51ab	5.04a	6.00a	4.26a	3.76a	3.50a
Тор		5.16a	5.38a	4.43b	4.66b	5.38a	4.67a	3.64a	3.69a

Table 6.1: Hean squares and mean β -glucan contents for β -glucan content of grains from top, middle and bottom positions of the oat panicle for parental and F_2 genotypes derived from two reciprocal oat crosses.

* - Significant at the P<0.05 level.

⁴ - Due to missing values error mean square of 1L83 has 3 df.

 $^{\circ}$ - Means with the same letters within the same column are not significantly (P > 0.05) different according to a ANOVA-protected LSD.

Figure 6.1: Frequency plots for β -glucan contents of Don and IL83-80922 and of F₂ seeds from F₁ plants for Don/IL83-80922 and IL83-80922/Don crosses.

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Figure 6.2: Frequency plots for β -glucan contents of Manic and Larry and of F_2 seeds from F_1 plants for Manic/Larry and Larry/Manic crosses.



Figure 6.3: Frequency plots for β -glucan contents of Don and IL83-80922 and of F_2 grain samples (20 grains/sample) from F_3 plants for Don/IL83-80922 and IL83-80922/Don crosses.



Figure 6.4: Frequency plots for β -glucan contents of Manic and Larry and of F_2 grain samples (20 grains/sample) from F_3 plants for Manic/Larry and Larry/Manic crosses.

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Preface to Chapter 7

In chapter 3 and 4, the effects of crop production practices on oat grain quality were evaluated and in chapters 5 and 6, genetic aspects of oat quality were considered. In chapter 7, genotype by environment interactions were evaluated for oat grain yield as well as protein, oil and ß-glucan contents. Because some oats destined for milling are grown in Québec, it would be useful to know whether genotype by environment interactions could adversely affect oat grain quality.

This chapter corresponds to a manuscript which will be submitted for publication in *Crop Science*. The manuscript has been written to conform to McGill University thesis guidelines. This experiment was designed by D. Gavin Humphreys. Grain samples for biochemical analysis were obtained from the CPVQ oat trials. Grain yield data were obtained from the Québec oat trial records. Technical assistance was obtained from N. Brière during biochemical analysis. Data were analyzed and the manuscript was written by D. Gavin Humphreys. Dr. D.E. Mather contributed critical advice during data analysis and writing of the manuscript. All literature cited in this manuscript can be found in a section at the end of this thesis. Tables and figures are included at the end of the manuscript. Figure captions are printed on the page preceding the figure. Chapter 7: Analysis of genotype by environment interactions for grain yield and protein, oil and ß-glucan contents in oat.

7.1 ABSTRACT

Interest in oat food products has increased the demand for high quality milling oat. The objective of this study was to evaluate genotype by environment interactions for grain yield and protein, oil and B-glucan contents of seven oat cultivars. For each cultivar, samples of grain were gathered in six environments in 1990 and four environments in 1991 of the Québec regional oat cultivar trial. Yield data were obtained from the regional trials. Protein, oil and B-glucan contents were determined for samples of ground oat groats of each cultivar in each replicate of each environment. Significant environment, genotype and genotype by environment effects were observed for all traits. AMMI (Additive Main effects and Multiplicative Interactions) analysis found that the grain yield and oil data fit an AMMI1 model while the protein and B-glucan data fit an AMMI2 model. Genotype by environment interaction for grain yield seemed to be related to cultivar performance in the Ste-Rosalie versus the St-Hyacinthe environments. The cultivars Laurent and Nova had higher protein contents other cultivars across environments these cultivars interacted positively with different environments. The cultivar Marion QC had higher oil and B-glucan contents across environments and had particularly high oil contents in cooler environments. For B-glucan, no trend was evident for the genotype by environment interaction. AMMI analysis was more informative than other statistical techniques used in this study for genotype by environment analysis.

7.2 INTRODUCTION

Grain protein, oil and β -glucan contents are biochemical characteristics which can affect the quality of oat grain for food and feed. Protein can be an important constituent of oat grain; high-protein oat is valuable as a livestock feed and may be useful as a raw material for the food industry (Welch and Yong, 1980). Lipids can represent a significant energy source in oat grain used for livestock feed. However, overly high oil content in oat may create rancidity problems for processors (Youngs, 1986). In recent years, studies have demonstrated an association between oat consumption and lower serum cholesterol levels in humans (Hurt *et al.*, 1988; Wood *et al.*, 1989; Ripsin *et al.*, 1992) and it has been hypothesized that β -glucan is the chemical agent that is primarily responsible for the cholesterol lowering effect of oat (Hurt *et al.*, 1988;Wood *et al.*, 1989). However, β -glucans also have anti-nutritional properties; for example, in chicken diets β -glucan "gumminess" and indigestibility can affect food intake, cause survacy droppings and reduce feed conversion efficiency (Hesselman and Åman, 1986).

Genotypes can give variable phenotypic responses in different environments. Genotype by environment interactions can have significant effects on oat grain quality (Peltonen-Sainio and Peltonen, 1993; Peterson, 1991; Saastamoinen *et al.*, 1989; Saastamoinen *et al.*, 1990; Saastamoinen *et al.*, 1992) and grain yield (Peltonen-Sainio *et al.*, 1993). Peltonen-Sainio and Peltonen (1993) found that genotype by environment interactions were significant for oat grain protein content and that cultivars with higher protein content tended to have greater variability for protein content across environments. Oat oil content has been found to vary among cultivars (Saastamoinen *et al.*, 1989) and among environments (Saastamoinen *et al.*, 1990). Oil content was higher in environments with lower temperatures during the growing season (Saastamoinen *et al.*, 1990). Peterson

(1991) found significant genotype by environment interactions for oat β -glucan contents but the variance ratio for the interaction effect was much smaller than those of the main effects and the rank order of the cultivars studied was generally similar across locations. Similarly, Lim *et al.*(1992) reported that the rankings of oat lines on the basis of β glucan content were similar between years. Again, a significant genotype by year interaction was detected but the interaction was deemed of little importance for variety development.

In efforts to evaluate the differing performances of cultivars across environments, a plethora of techniques have been published to measure stability (see Lin *et al.*, 1986; Peltonen-Sainio *et al.*, 1993). In this study, three statistical methods will be used to evaluate performance across environments: a superiority measure (Lin and Binns, 1988; a genotype grouping technique (Francis and Kannenberg, 1978); and the Additive main effects and Multiplicative interaction (AMMI) analysis (Gauch, 1988; Zobel *et al.*, 1988).

Lin and Binns (1988) devised a superiority measure to assess general adaptability across environments. The general adaptability of a genotype is measured as the distance mean square between the genotype's response and the maximum response. Lin and Binns (1988) also proposed the calculation of a pairwise genotype by environment mean square between the maximum and each test cultivar in order to detect narrowly adapted cultivars. If the pairwise mean square is not significantly larger than the error mean square, parallelism between the maximum and the test cultivar's responses across environments is implied (i.e. the differences from the maximum responses are about the same for all locations) (Lin and Binns, 1985).

The superiority measure is useful for ranking cultivars based on their performance across environments but does not give information about a cultivar's variability across environments. Francis and Kannenberg (1978) developed an method for grouping genotypes which is based on mean yield across environments and variability as measured by coefficient of variation. Genotypes are classified into one of four groups:

Group I : high yield, low variation

Group II: high yield, high variation

Group III: low yield, low variation

Group IV: low yield, high variation

Francis and Kannenberg (1978) suggested that Group I genotypes would be the most desirable because such genotypes would tend to give high and consistent performance relative to the other genotypes in the experiment.

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Multivariate statistical techniques such as principal component analysis (PCA) can also be used to study genotype by environment interaction (Brown, 1991). A disadvantage of this approach is that the components are mathematical artifacts which may not have obvious direct relationships to environmental conditions (Lin and Thompson, 1975). Peltonen-Sainio *et al.* (1993) found that the PCA and Finlay-Wilkinson analyses ranked cultivars in a similar fashion; when the data fit a linear model, multivariate analyses may be unnecessary. However, if the data did not adequately fit a linear model, multivariate analysis can be an effective means to analyze genotype by environment data. Bradu and Gabriel (1978) suggested that initial analysis using the AMMI analysis may permit identification of the appropriate model.

Zobel *et al.* (1988) proposed the use of the AMMI (Additive Main effects and Multiplicative Interaction) model to analyze data from many genotypes grown in a

number of environments. Genotype by environment data can be partitioned into genotype and environment main effects and genotype by environment interaction. By definition, main effect data are additive and the interaction data (the residual from the additive model) are nonadditive (Snedecor and Cochrane, 1980). The AMMI model analyses the genotype and environment main effects using the ordinary ANOVA procedure and the nonadditive residual (i.e. the genotype by environment interaction) using principal component analysis (PCA). Often much of the interaction effect can be summarized in just a few interaction principal component axes resulting in a reduced AMMI model with a residual term (Gauch, 1990). The reduced AMMI model can account for most of the sums of squares in the model with fewer degrees of freedom (Gauch, 1990). AMMI analysis has also been referred to as "biplot" analysis (Kempton, 1984) because AMMI results can be used to construct a "biplot" with main effects on the abscissa and interaction PCA scores on the ordinate. This provides a graphical display of the genotype by environment interaction for the genotypes and environments analyzed (Zobel *et al.*, 1988).

The objectives of this study were: (1) to rank seven oat cultivars by their protein, oil and β -glucan contents and grain yield across environments; (2) to evaluate the genotype by environment interaction for protein, oil and β -glucan contents and grain yield of seven oat cultivars grown in Québec; and (3) to compare the results of the analysis of genotype by environment data using the superiority measure (Lin and Binns, 1988), the genotype mean-CV grouping (Francis and Kannenberg, 1978) and the AMMI analysis (Zobel *et al.*, 1988).

7.3 MATERIALS AND METHODS

7.3.1 Experimental Material

Samples of 150g of grain from each replicate for seven cultivars were gathered from six environments in 1990 and four environments in 1991 of Québec regional oat trials. Oat trials were conducted using a randomized complete block design with three replicates. In 1990, grain samples of the oat cultivars Appalaches, Capital, Laurent, Marion QC, Nova, Sylva and Ultima were obtained from the following environments: Normandin (NR90), St-Eugène (SE90), Pintendre (PI90), Deschambault (DE90), St-Hyacinthe (Early seeding) (SH90e) and St-Hyacinthe (Late seeding) (SH90l). In 1991, grain samples of the same cultivars were obtained from the following environments: Normandin (NR91), Deschambault (DE91), Ste-Rosalie (SR91) and Ste-Anne-de-Bellevue (SA91).

7.3.2 Grain preparation

For protein and ß-glucan analyses, subsamples of 50g of oat grain were dehulled using a laboratory-size impact dehuller (Quaker Oats Co., Barrington, Ill.). Groats were separated from the hulls using a South Dakota Seed Blower (Seedburo Equipment Co., Chicago, Ill.). The oat groats were ground with a Wiley (Thompson Scientific, U.S.A.) knife mill fitted with a 1 mm screen. Ground samples were stored at -20°C. The day before analysis, groat samples were dried at 90°C overnight. For oil content analysis, separate subsamples of 15g of oat grain were dehulled, aspirated and ground the day before the analyses were done and the ground samples were dried overnight at 90°C.

7.3.3 Biochemical analyses

Protein content was estimated using Kjeldahl-calibrated near-infrared reflectance values (PerCon Inframatic 8100; PerCon Prfgerte GmbH, Hamburg, Germany) on 15g of ground oat groats. The standard micro-Kjeldahl procedure was used (Anonymous,

1983). Samples of 0.5g dried oat groats were digested at 410°C in concentrated sulphuric acid plus 2 S-type catalyst Kjeltabs (Tecator Manual, Kjeltec System 1002 Distilling Unit) and the resulting ammonium was distilled into boric acid indicator and titrated with dilute HCl (Anonymous, 1983; Bradstreet, 1965).

Oil content was determined by near-infrared reflectance. Calibration was performed using oil extraction data from 76 samples chosen using the 8100 NIR software. Oil was extracted from 3.0g of dried oat flour using a Soxtec System HT2 (Tecator AB, Höganäs, Sweden) 30 minutes boiling and 30 minutes rinsing in 70 ml of extraction-grade diethyl ether. Oil content was determined gravimetrically.

Beta-glucan content was determined using the McCleary and Glennie-Holmes (1985) technique, except that 0.15g ground groats were used instead of the 0.5g sample originally published. Beta-glucan determinations were done in duplicate and the mean taken as the data value.

All protein, oil and ß-glucan contents are expressed as percentage of dry matter.

7.3.4 Statistical Analysis

Site-years were considered environments in the analysis. Analysis of variance was used to evaluate main effects and interactions. Cultivar superiority (Lin and Binns, 1988) for protein, oil and β -glucan content was measured as the square distance between the genotype's response and the maximum response at each environment average over all environments. To identify specifically adapted cultivars, a pairwise genotype by environment interaction mean square between the maximum in each environment and each genotype was calculated (Lin and Binns, 1988).

Cultivars were classified into one of four groups based on performance and variability (Francis and Kannenberg, 1978). Performance was measured by mean protein,

oil and ß-glucan contents and mean grain yield across environments and variability was measured by the mean coefficient of variation.

The AMMI analysis was performed using Matmodel (Version 2.0) (Gauch, 1990). Additive effects for genotype and environment were analyzed by analysis of variance, then multiplicative effects for the genotype by environment interactions were analyzed using principal component analysis (Gauch, 1988; Zobel *et al.*, 1988). Genotype and environment PCA scores were scaled as a unit vector times the square root of the eigenvector (Zobel *et al.*, 1988). AMMI models were chosen based on the F-tests of the PCA axes, the proportion of pattern estimated to be present in the model and assessments of predictive accuracy using Matmodel's validating mode. The degrees of freedom for F-tests used to determine the significance of the IPCA mean squares were calculated as: df = G + E - 1 - 2n; where n = the number of PCA axes (Gollob, 1968 as cited in Zobel *et al.*, 1988).

7.4 RESULTS

Analysis of variance of grain yield and protein, oil, and β -glucan contents gave highly significant environment and genotype main effects as well as genotype by environment interaction effects (Table 7.1). The environment sums of squares (SS) were greatest for grain yield and protein content. Cultivar SS were greatest for oil and β glucan contents.

7.4.1 Superiority of Grain Yield and Composition

Superiority measures (P_i) and pairwise genotype by environment interaction mean squares (MS_{ij}) for each cultivar are given in Table 7.2. Ultima, Sylva and Capital were better than other cultivars for grain yield across environments. Laurent and Nova had

higher protein content across environments compared to other cultivars. Marion QC had higher oil and β -glucan contents compared to other cultivars across environments. None of the pairwise genotype by environment interaction mean squares were significant (Table 7.2). Superiority measures ranked cultivars in almost the same order as their mean grain yields and mean protein, oil and β -glucan contents across environments (Table 7.3).

Superiority of β -glucan was negatively correlated with superiority of yield (r= -0.79; P<0.05). Here β -glucan cultivars such as Marion QC were lower yielding across environments and cultivars with better yield across environments (i.e. Ultima) had lower β -glucan contents. A similar trend was observed for protein and yield (r= -0.69; P<0.10). Oil content and yield superiorities were not correlated (r= 0.14 NS) but Ultima and Sylva had both high mean oil content and high grain yield (Table 7.2).

7.4.2 Yield Stability

Genotype mean-CV (GMCV) grouping for yield (Francis and Kannenberg, 1978) is shown in Figure 7.1. Ultima and Sylva had above average grain yield and low variability while Capital had higher than average grain yield but was more variable than average. Marion QC and Laurent yielded below average and were less variable than average (Figure 7.1).

Results of the AMMI analysis for grain yield are given in Table 7.3 and illustrated in Figure 7.2. It was estimated that the treatment variance components contained 98.6% pattern and the AMMI1 model accounted for 98.0% of the treatment SS. Laurent and Sylva had large, positive IPCA1 (Interaction Principal Component Analysis) scores and Capital, Appalaches and Nova had large, negative IPCA1 scores.

The Ste-Rosalie and St-Eugène environments had large, positive IPCA1 scores and the St-Hyacinthe environments had large negative IPCA1 scores.

In the biplot of mean grain yield against IPCA1 scores, the range of mean grain yields for cultivars is relatively low compared to the range of mean grain yields for environments (Figure 7.2). The contribution to genotype by environment interaction as measured by the IPCA1 scores seemed to separate the cultivars into three groups. Laurent and Sylva seemed to perform well in the Ste-Rosalie and St-Eugène environments and Capital, Appalaches and Nova performed relatively better in the St-Hyacinthe environments. Marion QC and Ultima contributed less than the other cultivars to the genotype by environment interaction as measured by IPCA1 scores.

7.4.3 Stability of Biochemical Composition

Genotype mean-CV (GMCV) groupings (Francis and Kannenberg, 1978) for protein, oil and ß-glucan contents are shown in Figure 7.3a-c. Both protein content and variability across environments were higher than average for the cultivars Laurent and Nova while Marion QC had lower than average protein content and variability (Figure 7.3a). Higher than average oil contents and lower than average variability were found for Sylva, Ultima and Appalaches. Marion QC had the highest average oil content and showed higher than average variability (Figure 7.3b). Marion QC and Nova had higher than average ß-glucan content and low variability (Figure 7.3c).

Results of the AMMI analysis for protein, oil and β -glucan contents are given in Table 7.3 and illustrated in Figures 7.4a-c. For protein, the first and second IPCA axes were retained (P<0.001) and the remainder was assigned to the residual. It was estimated that the treatment variance components contained 93.5% pattern and the AMMI2 model accounted for 94.5% of the treatment SS. IPCA1 scores were large and

positive for Nova and Appalaches while Laurent had large negative scores (Table 7.3). IPCA1 scores for environments suggest that St-Eugène, Deschambault in 1991 contributed strongly to the genotype by environment interaction for the IPCA1 axis (Table 7.3). IPCA2 scores were large and positive for Marion QC and large and negative for Laurent (Table 7.3). IPCA2 scores were large and positive for Deschambault 1990 and large and negative for Normandin 1990 (Table 7.3). Deschambault 1990 and Deschambault 1991 had opposite IPCA1 scores.

In the biplot of protein content means against IPCA1 scores, Laurent has the highest protein content across environments among cultivars and Normandin (1991) was the environment with the highest protein content (Figure 7.4a). In 1990, protein contents were generally lower at Deschambault than in 1991 and the contribution to the genotype by environment interaction was similar level in magnitude but opposite in direction (Figure 7.4a).

For oat oil content, the first IPCA axis was retained (P < 0.001) and the remainder allocated to the residual (Table 7.1). It was estimated that the treatment variance components contained 95.8% pattern and the AMMI1 model accounted for 94.3% of the treatment SS. Marion QC had a large, positive IPCA score that was much greater than the other cultivars and Ultima had an intermediate and negative IPCA1 score (Table 7.3). The St-Eugène environment had the largest, positive IPCA1 scores and the St-Hyacinthe environments had important, negative IPCA1 scores (Table 7.3).

In the biplot of oil content means against IPCA1 scores, Marion QC contributed quite strongly to the genotype by en_v contents interaction (Figure 7.4b). The Deschambault location gave higher oil contents in 1991 than in 1990 and in both years

these environments contributed at an intermediate level to the genotype by environment interaction but in opposite directions (Figure 7.4b).

For β -glucan content, the first two IPCA axes were retained (P<0.001) and the remaining SS were assigned to the residual (Table 7.1). It was estimated that the treatment variance components contained 92.5% pattern and the AMMI2 model accounted for 92.3% of the treatment SS. IPCA1 scores were positive and large for Appalaches and Laurent but negative and large for Sylva (Table 7.3). IPCA2 scores were large and opposite for Sylva and Ultima while most other cultivars had rather low IPCA2 scores (Table 7.3). St-Eugène had the largest IPCA1 score and Normandin 1990 had a large, negative IPCA2 score compared to other environments. IPCA1 and IPCA2 scores for Deschambault 1990 and 1991 were all negative.

In the biplot for β-glucan content, cultivar performance in the St-Eugène environment appeared to be an important factor in the genotype by environment interaction since this environment had a high IPCA1 score (Figure 7.4c). Appalaches, Laurent and Capital interacted positively with St-Eugène; whereas, Marion QC, Nova Sylva and Ultima interacted negatively with this environment (Figure 7.4c).

7.5 DISCUSSION

Significant genotype by environment interactions were found for all traits. Oat grain yield and composition varied significantly among cultivars within and across environments in Québec.

Yield is often the most important agronomic characteristic for a cultivar and genotype by environment interactions for yield are usually important. Ultima was the best yielding cultivar overall and had a small genotype by environment interaction; hence,

Ultima could be characterized as high yielding and stable which would be desirable for producers. Laurent could be characterized as a less stable cultivar because it was lower yielding relative to other cultivars except in Ste-Rosalie and St-Eugène where it yielded well. The genotype by environment effect seemed to be dominated by the performance of cultivars in the Ste-Rosalie environment versus the St-Hyacinthe environments (Figure 7.2). These results may be related to year-to-year environmental variability; these locations are geographically close and would be expected to be environmentally similar but the data are from two different years.

Yield superiority was inversely related to protein and β -glucan superiority. Cultivars that had better grain yield generally had lower protein and β -glucan contents. While an inverse relationship between protein content and grain yield has been previously reported (Brown *et al.*, 1966; Forsberg *et al.*, 1974; Peltonen-Sainio and Peltonen, 1993), an inverse relationship between β -glucan content and grain yield in oat has not. Saastamoinen *et al.* (1992) reported both positive and negative correlation between β glucan content and grain yield although only the positive correlation coefficients were significant. Superiority measures for oil content was not necessarily correlated to yield superiority measures but cultivars that combined higher than average oil contents and high grain yield were identified (Table 7.2).

Protein can be an important constituent of oat grain and factors that can influence groat protein percentage can have an notable effect on oat grain quality. Environmental effects appear to be more important than cultivar effects for protein content because environment SS were considerably larger than either cultivar or genotype by environment SS. The cultivars, Laurent and Nova, had higher protein contents than the other cultivars and higher protein contents seemed to be associated with higher than average variability

for protein content (Figure 7.3a). A similar result has been previously reported (Peltonen-Sainio and Peltonen, 1993). IPCA1 scores suggest that in environments where Nova had relatively high protein (i.e. St-Eugène), Laurent had relatively low protein content. Environmental factors that acted to reduce protein content for the cultivar Laurent did not have the same effect on the cultivar Nova. IPCA2 scores seemed dominated by cultivar performance, particularly Marion QC and Nova, in the Deschambault 1990 compared to the Normandin 1990 environments.

IPCA1 scores for the two Deschambault environments (1990 and 1991) had opposite signs. Apparently, environmental conditions at Deschambault varied such that the contribution to the genotype by environment interaction was different between 1990 and 1991 (Figure 7.4a). Cultivars with high protein content at Deschambault in 1990 had relatively low protein contents in 1991 (Table 7.3). The St-Hyacinthe and Ste-Rosalie environments plotted to similar locations in the biplot which might be expected since these environments are geographically close. Shorter season environments (i.e. Normandin and St-Eugène) tended to give higher protein contents. A shorter growing season could reduce starch production which could raise the relative proportion of groat protein.

Generally, Marion QC had high oil content compared to the other cultivars while Capital had the lowest oil content across locations (Table 7.3). Marion QC may be a better feed oat based solely on its oil content; whereas, the low oil content of Capital could favour its use by millers. Marion QC had its highest oil contents in the northerly environments with which it interacted positively (Figure 7.4b), whereas Ultima had higher oil contents in the longer season environments (i.e. St-Hyacinthe). Marion QC was more variable across environments than other cultivars which seemed to be related

to a greater increase in oil content for Marion QC in cooler growing conditions compared to other cultivars. Cooler environments have been previously shown to favour high oil contents in oat (Saastamoinen *et al.*, 1990). The Deschambault environments gave opposite IPCA1 scores for 1990 and 1991 but the Normandin environments gave positive IPCA1 scores in both years. It could be more difficult to predict oil contents in cultivars grown at Deschambault than at Normandin because ranking of cultivar for oil content tended to change more between years at the Deschambault site than at the Normandin site.

High ß-glucan contents may be desirable in oat destined for human consumption; however, low B-glucan may be preferred in oat destined for monogastric livestock. Therefore, genotype by environment effects that influence ß-glucan contents could influence the suitability of grain for specific end uses of the grain. Generally, Marion OC had high ß-glucan contents compared to all other cultivars and had lower than average variability for B-glucan content across environments; hence, Marion QC could be considered a stable, high B-glucan cultivar. Ultima had low B-glucan content and lower than average variability across environments suggesting this cultivar could be considered a stable, low B-glucan cultivar. Sylva contributed more than the other cultivars to the genotype by environment interaction when both IPCA axes were considered (Table 7.3) which suggests that Sylva is less stable for β -glucan content relative to other cultivars. The Deschambault environments contributed in a similar manner to the genotype by environment interaction for ß-glucan content (Figure 7.4c) but the opposite was observed for protein and oil contents (Figures 7.4a-b). Generally, 8-glucan contents did not change between years at the Deschambault site; whereas, increases in protein and oil contents were observed in 1991 compared to 1990.
The genotype by environment interaction for β -glucan content seemed to be related most strongly to the performance of a particular cultivar in a specific environment rather than to an obvious environmental factor. IPCA1 scores seemed to be based most strongly on the β -glucan contents in the St-Eugène environment while β -glucan contents in the Normandin 1990 environment was important in the IPCA2 scores but it is unclear what factor(s) in these environments influenced β -glucan contents. As in previous studies (Peterson, 1991; Lim *et al.*, 1990), genotype by environment interactions for β -glucan contents did not result in important rank changes across environments.

All three genotype by environment analyses permitted one to distinguish more desirable from less desirable cultivar(s). The superiority measure pinpointed the best cultivars for grain yield and protein content but only one cultivar was distinguished from the others for oil and ß-glucan contents (Table 7.2). It appears that the dominant cultivar, Marion QC, limited the ability of the superiority measure to distinguish between other cultivars. The superiority measure supplies little information about the nature or structure of the genotype by environment interaction. The GMCV technique was simple to use and separated cultivars based on yield and coefficient of variation. This technique identified the cultivars that had low average variability which was not necessarily possible with the other methods. Cultivars in Group I are usually considered the most desirable because these cultivars combine high mean performance and low variability (Francis and Kannenberg, 1978). However, in the case of oil and protein contents, the cultivar with the highest mean was a Group II cultivar. The GMCV grouping may place too much emphasis on variability.

High variability genotypes that have high mean performance (e.g. high mean yield) may be preferred to genotypes with higher than average performance and low

variability because the more variable genotypes may still outperform the less variable genotypes. Moreover, high variability may indicate that a cultivar responds to the environment which can be agronomically desirable because increased inputs (e.g. nitrogen fertilizer) are applied to improve plant performance through amelioration of the growing environment. However, cultivars that are overly sensitive to environmental changes are unlikely to be desirable.

The AMMI analysis determined the cultivars with favourable performance across environments and quantifies the contribution of the different genotypes and environments to the genotype by environment interaction using principal component analysis scores. Hence, AMMI analysis permitted one to characterize genotype and environments based on their performance and their contribution to the genotype by environment interaction. AMMI models are designed to capture the pattern associated with the treatment effects and place noise in a residual (Gauch, 1988; Gauch and Zobel, 1988). The biplot of means versus IPCA1 scores gives a graphical representation of the genotype by environment interaction which was not possible with the other analyses. The biplot graphically displays the positive and negative interaction between cultivars and environments as measured by the IPCA scores. However, the analysis is more difficult to comprehend when multiple IPCA axes are involved in the AMMI model and the IPCA scores do not always have clear agronomic interpretability.

7.6 CONCLUSIONS

Significant environment and genotype main effects as well as genotype by environment interactions were recorded for grain yield and for protein, oil and ß-glucan contents. Ultima, Sylva and Capital were superior to the remaining cultivars for grain

yield across environments. Ultima could be a high-yielding, stable cultivar because it had a small genotype by environment contribution as measured by the IPCA1 score. Laurent and Nova were found to be superior to other cultivars for protein contents across environments and Nova gave high protein contents in environments where Laurent tended to be lower in protein content. Marion QC was superior to all other cultivars for β-glucan and oil contents across environments. Marion QC had above average variability for oil content and tended to have higher oil contents than the high oil low variability cultivars. Generally, protein and β-glucan contents were higher in lower yielding cultivars but this was not the case for oil content. The AMMI analysis provided much more information than the other two techniques and would be favoured for the analysis of genotype by environment interaction data. However, the superiority measure was useful to separate cultivars with high mean performance across environments from the other cultivars and could be useful in a breeding program. The GMCV technique seemed to place too much emphasis on variability and it should be used with care. Table 7.1: ANOVA and AMMI principal component analysis mean squares for grain yield (kg ha') and groat protein. oil and B-glucan contents (% of dry matter) for seven oat cultivars grown at six environments in 1990 and at four environments in 1991 in Québec. Canada.

Effect	df	Yield	Protein	0i1	8-glucan	
Environment	9	65180630***	46.1***	2.4***	1.0**	
Genotype	6	1239642***	17.3	5.9***	4.2***	
Genotype*Environment	54	395040***	2.0***	0.1***	0.2***	
AMMI1						
IPCA1	14	650551***		0.3***		
Residual	40	305611***		0.1***		
AMM12						
IPCA1	14		2.9		0.5***	
IPCA2	12		2.7		0.2	
Residual	28		1.2**		0.1	

". " Significant at the P<0.01 and P<0.001 probability level.

Table 7.2: Superiority measures (P_1) and pairwise genotype by environment mean squares (MS_{13}) indices (Lin and Binns. 1988) for grain yield and groat protein, oil and B-glucan contents for seven oat cultivars grown at six locations in 1990 and at four locations in 1991 in Québec. Canada.

	Grain Yield		<u>Protein</u>			<u> 0i1 </u>			<u>ß-glucan</u>		
Cultivar	P,' Ra	nk ^t MS _{1j} s	Ρ, f	tank	MS ₁	Ρ, Β	lank	MSij	Pi	Rank	MSij
Appalaches	397642	5 119034	2.00*	4	0.53	0.23	4	0.04	0.14	2	0.05
Capital	210294	3 75577	1.68"	3	0.30	0.96	7	0.05	0.21*	3	0.04
Laurent	360621*	5 93258	0.31	1	0.10	0.48*	5	0.08	0.33*	5	0.07
Marion QC	339421* 4	4 5957	2.69	6	0.66	0.01	1	0.01	0.03	1	0.03
Nova	398886* 7	7 68656	1.04	2	0.37	0.71	6	0.04	0.27*	4	0.05
Sylva	143830 2	2 70194	2.28	5	0.38	0.13	2	0.05	0.52*	6	0.10
Ultima	81447	1 37030	4.42*	7	0.19	0,20*	3	0.07	0.88*	7	0.06

* = Superiority measure significantly different from zero at P<0.05 level.

 t = Superiority measure of cultivar where P, is equal to the distance mean square between the cultivar and the maximum at a location averaged over all locations.

* = Rank of cultivar according to its superiority measure.

⁶ = Pairwise genotype by environment interaction mean square.

				<u>Yield Mg ha</u>	a ^{•1}	<u>_</u>				
							En	vironment		
Environment ¹	Appalaches	Capital	Laurent	MarionQC	Nova	Sylva	Ultima	Mean	IPCA1	
DE90	3.94	4.07	3.89	4.22	3.50	4,45	3.95	4.00	4.4	
DE91	5.32	5.52	5.37	5.63	5.76	5.57	5.89	5.58	-2.9	
SA91	3.86	3.65	3.28	3.21	3.46	4.10	3.83	3.63	-3.7	
NR90	5,04	4.36	4.24	4.43	3.72	4.87	4.47	4.45	0.8	
NR91	3.82	3.97	4.36	4.27	4.21	4.47	4.85	4.28	9.7	
SE90	4.05	4.36	5.06	4.29	3.90	4.62	4.93	4.46	16.5	
SH90e	8.92	9.44	8.45	8.43	9.04	8.58	9.21	8.87	-20.2	
SH901	7.77	8.70	6.99	7.73	7.91	8.07	8.11	7.90	-21.9	
SR91	5.66	6.12	6.52	6.04	6.18	7,45	6.70	6.38	20.9	
P190	4.30	4.79	4.69	4.48	4.37	4.14	5.25	4.57	-3.6	
MEAN	5.27	5.50	5,29	5.27	5.21	5.63	5.72	5.41 [*]		
IPCA1	-13.9	-22.1	24.3	3.6	-11.7	17.9	1.9			
-				Protein	(¥)				-	
			Cultiv	ar			E	nvironment	t	
Environment	Appalaches	Capital	Laurent	MarionQC	Nova	Sylva	Ultima	Mean	IPCA1	IPCA2
DE90	13.6	13.7	14.8	15.3	12.9	12.7	12.4	13.6	-0.6	1.0
DE91	16.1	15.8	16. 1	15.3	17.6	15.9	13.9	15.8	0.8	0.0
SA90	16.9	18.4	17.2	16.6	18.2	17.0	15.5	17.1	0.7	0.2
NR90	16.3	17.0	20.3	16.2	19.1	16.6	17.7	17.6	-0.5	-1.5
NR91	18.8	19.0	21.0	18.5	18,8	18.4	17.9	18.9	-0.4	-0.1
SE90	19.6	17.8	18.0	16.4	19.3	16.5	16.3	17.7	1.2	-0,2
SH90e	16.4	15.2	17.1	16.7	16.5	16.2	15.7	16.2	-0.2	0.2
SH901	17.1	16.8	18.5	16.2	16.3	16.7	15.9	16.8	-0.4	0.1
SR91	15.8	16.8	18.2	16.4	17.0	16.7	15.1	16.6	-0.3	0.0
PI90	18.0	18.6	19.9	18.0	18.4	18.8	16.4	18.3	-0.2	0.3
MEAN	16.8	16.9	18.1	16.6	17.4	16.6	15.7	16.9 ¹		
IPCA1	0.9	0.4	-1.1	-0.6	1.0	-0.1	-0.5			
IPCA2	0.4	0.3	-0.7	1.1	-0.9	0.3	-0.6			

Table 7.3: Mean grain yield and groat protein, oil and B-glucan contents and AMMI analysis principal component scores (IPCA) for seven oat cultivars grown at 6 locations in 1990 and 4 locations in 1991 in Québec, Canada.

Table 7.3: (Continue).

_	<u></u>	<u></u>		<u>B-gluca</u>	n (¥)					
								Environment		
Environment'	Appalaches	Capital	Laurent	MarionQC	Nova	Sylva	Ultima	Mean	IPCA1	IPCA2
DE90	4.53	4.27	4.38	5.34	4.41	3.96	3.67	4.37	-0.10	-0.24
DE91	4.53	4.62	4.10	5.17	4.72	4.57	3.76	4.50	-0.38	-0.26
SA91	4.45	4.40	4,06	4.36	4.35	3.92	3.55	4.16	0.10	-0.13
NR90	4.64	4.33	4.59	4.92	4,50	3.58	4.10	4.38	0.23	-0.66
NR91	4.16	4.01	3.40	4.77	4.22	3.89	3.80	4.04	-0.43	-0.25
SE90	5,72	5.26	4.89	4.91	4.43	3.86	3.81	4.70	0.96	0.14
SH90e	4.38	4.18	4.31	5.14	4.48	4.23	3.77	4.36	-0.28	-0.12
SH901	4.65	4.24	4,63	4.71	3,95	4.14	3.57	4.27	0.24	0.20
SR91	4.36	4.58	4.32	5.05	4.47	4.44	3.51	4.39	-0.23	0.37
P190	4,96	4.82	4,64	5.37	4.50	4.77	4.25	4.76	-0.12	0.17
MEAN	4.64	4.47	4.33	4.97	4.40	4.14	3.78	4.39'		
IPCA1	0.69	0.34	0.51	-0.43	-0.31	-0.61	-0.18			
IPCA2	0,05	0.34	-0.05	-0.23	-0.20	0.62	-0.53			
-	<u></u>			011 (*)						
								Environment		
Environment	Appalaches	Capital	Laurent	MarionQ	C Nova	Sylva	Ultima	Mean	IPCAL	
DE90	6.8	6.3	6.7	7.0	6.4	7.2	7.0	6.8	-0.4	
DE91	7.5	6.3	7.0	8.2	6.9	7.3	7.5	7.2	0.3	
SA91	6.5	5.9	6.7	7.2	6.2	7.0	6.8	6.6	0.0	
NR90	6.7	6.1	6.3	7.5	6.3	7.0	6.8	6.7	0.2	
NR91	6.7	6.4	6.7	7.6	6.4	7.2	6.7	6.8	0.2	
SE90	7.2	6.3	6.6	8.3	6.5	7.3	7.0	7.0	0.6	
SH91e	6.5	5.5	6.0	6.7	6.0	6.5	7.0	6.3	-0.3	
SH901	6.5	5.4	5.6	6.2	5.5	6.6	6.4	6.0	-0.4	
SR91	6.7	6.2	6.9	7.2	6.4	7.0	7.1	6.8	-0.3	
P190	6.9	6.4	6.7	7.6	6.2	7.1	6.9	6.8	-0.1	
MEAN	6.8	6.1	6.5	7.3	6.3	7.0	6.9	6.7'		
IPCA1	0.0	0.0	-0.2	0.9	-0.1	-0.2	-0.4			

t = Envi: onment refers to the location-year:

* = Grand mean.

-

Figure 7.1: Mean grain yield versus coefficient of variation for seven cultivars grown in 6 environments in 1990 and 4 environments in 1991 in Québec, Canada. The vertical line is the mean coefficient of variation and the horizontal line is the grand mean.



Figure 7.2: Biplot of the AMMI1 model of unadjusted means for grain yield for seven cultivars grown in 6 environments in 1990 and 4 environments in 1991 in Québec, Canada. The vertical line is the grand mean and the horizontal line is IPCA=0.



Figure 7.3: Mean (a) protein, (b) oil and (c) ß-glucan contents versus coefficient of variation for seven cultivars grown in 6 environments in 1990 and 4 environments in 1991 in Québec, Canada. The vertical line is the mean coefficient of variation and the horizontal line is the grand mean.



Figure 7.4: Biplot of the AMMI1 model of unadjusted means for (a) protein, (b) oil and (c) β -glucan contents for seven cultivars grown in 6 environments in 1990 and 4 environments in 1991 in Québec, Canada. The vertical line is the grand mean and the horizontal line is IPCA=0.





Summary

The research reported in this thesis is included in chapters 3 to 7. Investigations were made into agronomic, genetic and environmental aspects of oat grain quality. In chapters 3 and 4, the effects of nitrogen fertilizer applied at the boot stage of plant growth and the effects of delayed seeding were evaluated on physical and biochemical characteristics of oat grain quality. Nitrogen treatments had little effect on milling quality at Ste-Anne-de-Bellevue but hull percentage decreased and plump grain percentage increased with higher N in the Ste-Rosalie experiment. Delayed seeding reduced yields in all four experiments. In the Ste-Anne-de-Bellevue experiments, delayed seeding reduced test weight, 1000-grain weight, plump grain percentage and theoretical milling yield, and increased percentage of hull, thin grain and bosom grain. In the Ste-Rosalie experiment, opposite effects were observed; this may have resulted from reduced production of secondary and tertiary seeds in the late-seeded treatments. In general, the higher application rate of N did not improve oat grain milling quality sufficiently to warrant its usage. The correct choice of cultivar seems crucial for production of high quality milling oat grain.

Nitrogen fertilizer applied at the boot stage (Zadoks 40-43) tended to increase protein content and to decrease oil content; however, ß-glucan content did not respond significantly to additional N. Generally, delayed seeding increased protein and ß-glucan contents and decreased oil content. Results of the 1992 experiment differed slightly from the 1990-91 results, both protein and oil contents tended to increase with delayed seeding and ß-glucan was not significantly affected. Environmental conditions during the growing

season seemed to influence the effects of delayed seeding on protein, oil and β -glucan contents because results varied among years.

In chapters 5 and 6 genetic aspects of oat grain quality were considered. Parentoffspring regression was used to estimate F_5 - F_6 , F_5 - F_7 , F_6 - F_7 heritabilities for β -glucan content and groat percentage and the F_6 - F_7 heritability for crown rust resistance in two oat populations that were composed of 99 random lines. Generally, heritabilities for all traits were low (0.07 - 0.35) although the F_6 - F_7 heritability estimate for β -glucan content was higher (0.45) in the Sylva/Marion QC population.

In the experiments investigating the inheritance of β -glucan, results were not conclusive. It appears that oat β -glucan content is controlled by the endosperm genome because significant reciprocal differences were observed for the F₂ grains from F₁ plants while reciprocal differences were less pronounced for the F₂ grain from F₃ plants and variation among F₂ plants were similar to among parental plants variation. These results seem reasonable because the polysaccharide is principally found in the endosperm. In general, grain position within the panicle did not affect β -glucan content. Significant within-panicle variation for β -glucan content was observed; hence, single-grain evaluation is not likely the appropriate manner to differentiate high and low β -glucan genotypes.

In chapter 7, genotype by environment interactions for grain yield as well as protein, oil and ß-glucan contents were investigated. Significant environment, genotype and genotype by environment effects were observed for all traits. AMMI (Additive Main effects and Multiplicative Interactions) analysis found that the grain yield and oil data fit an AMMI1 model while the protein and ß-glucan data fit an AMMI2 model. Genotype by environment effects for grain yield seemed to be related to cultivar performance in the Ste-Rosalie versus the St-Hyacinthe environments. Laurent and Nova were superior

to other cultivars for protein content but these cultivars interacted positively with different environments. Marion QC was superior to all other cultivars for oil and β -glucan contents across environments and Marion QC had particularly high oil contents in cooler environments. For β -glucan, no trend was evident for the genotype by environment interaction. AMMI analysis seemed better than other statistical techniques used in this study for genotype by environment analysis.

General Discussion

This thesis includes reports on a diverse set of experiments, all centred around a common theme: oat grain quality. Quality can be defined in many ways. Simmonds (1979) considered quality as a measure of "fitness for purpose" or the suitability of the crop for its intended use. Both fitness and purpose are subjective terms. The guidelines that define quality will be designated in a general sense by the end use but the end user will apply the quality guidelines and the application of the quality guidelines is likely to vary between end users. Hence, quality can be defined in terms of both the end use and the end user.

Milling oat standards are usually rather strict and the standards (i.e. minimum test weight and theoretical milling yield) tend to evolve with the improvement of oat cultivars. In chapters 3, 4 and 7, no cultivar combined optimal levels of all quality characteristics. The "perfect" milling oat cultivar probably does not exist, so milling companies must choose from what is available. Plant breeders can help both producers and consumers of milling oats by improving milling quality of future oat cultivars, but

care must be taken to ensure that the improvement of one aspect of quality is not done at the expense of another because many quality traits are interrelated.

Oat grain quality can be influenced by more than just the cultivar specifications. The measurement of oat quality can be a function of not only the criteria used but also the rigour with which the quality guidelines are imposed. For example, when oat grain suitable for milling is plentiful, oat millers may demand grain with higher test weight or theoretical milling yield than when the quality of the oat crop is generally lower.

From a plant breeding perspective, quality can be considered a combination of two factors: *inherent quality* and *condition* (Simmonds, 1979). *Inherent quality* refers to the influence of a cultivar's genotype on its quality. In chapters 3, 4 and 7, significant variation between cultivars was observed for many quality traits (e.g. test weight, hull percentage, protein and β-glucan contents). A cultivar that lacks inherent quality will be unlikely to produce milling quality oats even in a favourable environment. Cultivars must also be well adapted to the particular growing region. For example, the cultivar Newman has desirable milling quality but is poorly adapted to Québec conditions and as a result it is not recommended for growing in Québec. Poor adaptation can result in large genotype by environment interactions and high variability in quality for producers and industry. Genotype by environment interactions can have an impact on the end use of the grain. While traits such as yield were highly affected by genotype by environment interactions, other traits (i.e. ß-glucan) were less affected.

Condition refers to the state of the harvested product. In order to obtain oat grain in the best possible "condition", appropriate production practices, superior cultivars and favourable environmental conditions (i.e. soil, rainfall, growing temperature etc.) are required. The quality of the harvested grain will likely suffer if any these three criteria

are not met. Agronomic factors such as seeding date can have a important negative impact on oat grain quality. In chapter 3, delayed seeding reduced the cultivar Manic from a high yielding cultivar of acceptable milling quality to a low yielding cultivar unacceptable for milling purposes. Cultivars that are overly variable would be unsuitable for production of quality oats. Stability for grain quality could be a breeding objective for future cultivars because grain quality is easily influenced by the environment. However, breeding for stable oat grain quality would a formidable task because so many characteristics are involved.

Improvement of quality traits can be simplified if evaluation of quality traits can be done in a quick, economical and efficient manner. The McCleary and Glennie-Holmes technique (McCleary and Glennie-Holmes, 1985) facilitated the estimation of ß-glucan contents in oat lines but grain processing and grinding were relatively slow and laborious. Beta-glucan evaluation would be more efficiently accomplished if ß-glucan analysis were included in the evaluation of oat flour for several traits (e.g. protein, oil). Thus, more information could be garnered from the resources spent on grain processing and grinding.

When breeding for quality traits, the plant breeder must ensure that efforts are not wasted. Traits that are trivial or no longer important should likely be avoided. In the late 1980's, the positive role of soluble dietary fibre that could play in modulating human blood cholesterol levels encouraged health-conscious consumers to include products high in dietary fibre in their diets. The documented hypocholesterolemic effects of oat consumption (Hurt *et al.*, 1988; Ripsin *et al.*, 1992) increased the demand for this crop. While the initial euphoria associated with the cholesterol-lowering effect of oat has waned, the component that is hypothesized to be primarily responsible for the cholesterol lowering effect of oat: β -glucan (Hurt *et al.*, 1988; Wood *et al.*, 1989) remains a useful

breeding objective. Aside from the cholesterol lowering properties associated with β glucan, this polysaccharide may also have a therapeutic use in the management of diabetes (Hurt *et al.*, 1988) and "oat gum" of which β -glucan makes up a large component may have uses as a food hydrocolloid (Wood *et al.*, 1989). Beta-glucans also have anti-nutritional properties; for example, in chicken diets β -glucan "gumminess" and indigestibility can affect food intake, cause sticky droppings and reduce feed conversion efficiency (Hesselman and Åman, 1986). Cave *et al.* (1990) reported that increased β glucan content in broiler chickens diets reduced rate of growth, and the availability of metabolizable energy and amino acids. Future oat cultivars will likely be developed with either high or low β -glucan contents to cater to the needs of different oat consumers.

Mixed-linkage β -glucan is a new entry to the already lengthy list of oat grain quality characteristics. Future breeding efforts in oat may endeavour to produce oat cultivars that are high in β -glucan content for human consumption and low β -glucan oat cultivars that could be used as feed, particularly for monogastric livestock. Oat β -glucan content can be influenced by agronomics, genetics and the environment. While β -glucan content tended to increase with delayed seeding it was unaffected by nitrogen application at the boot stage. Genotype and environmental effects seem to be the most important factors influencing β -glucan content in oat. The genotype by environment interaction for β -glucan content seemed to be related most strongly to the performance of a particular cultivar in a specific environment rather than to an obvious environmental factor. It was found that the cultivar Marion QC is high β -glucan content and Marion QC was stable for high β -glucan content across environments. Ultima was found to be stable across environments for low β -glucan content. Breeding for β -glucan contents may be facilitated by using such stable genotypes and testing could be done at a minimum of sites. Heritability of the trait is low; hence, selections should likely be carried out in later generations. Lines with β -glucan contents above and below the parents were observed in both the Sylva/Marion QC and the Nova/Marion QC populations; hence, selection for improved β -glucan content should be possible. A high x high β -glucan cross would likely be best for production of progeny with high β -glucan contents because maternal effects seemed to be important and a small F_2 variation were observed in the wider high x low β -glucan crosses used in chapter 6. Although a high x high cross could have even less variation than the high x low crosses reported in chapter 7, such a cross would likely have a high mean β -glucan value. Within-cross variation might be increased by using unrelated parents. If parents of different origins are crossed they might complement one another for genes for high β -glucan. Dominance for low β -glucan may exist in some genotypes; this could make selection more difficult in early generations. Variability for β -glucan content within the panicle was significant so that a sample of grain should be used to characterize the genotype of a plant or line.

Oat grain quality is a composite of many characteristics which can be influenced by many different factors. The studies reported in this thesis have evaluated the nature of some of the factors that can affect oat grain quality have been evaluated. Further, the genetic aspects of oat ß-glucan content. These findings should aid future breeding efforts to improve oat grain quality. It is difficult to accurately predict what oat grain quality characteristics will be important in ten years time because quality is defined by the market and the market is always changing. Nevertheless, plant breeders will continue to produce new oat cultivars and it seems assured that these cultivars will possess improved grain quality.

Contributions to Knowledge

Oat grain quality has been previously researched but various components of this research are contributions to original knowledge:

- 1. This study evaluated the effects of nitrogen fertilizer applied at the boot stage of crop development on several characteristics of oat grain quality for which the effect of N had not previously been evaluated, including percentage of plump, thin and bosom grains, theoretical milling yield, and oil content.
- 2. This is the first report of the effect of delayed seeding on oil and ß-glucan contents in oat. Moreover, this study afforded the evaluation of the interaction between nitrogen fertilizer and delayed seeding on various physical and biochemical characteristics oat grain quality which has not been reported previously.
- 3. This is the first report of the heritability of B-glucan in oat.
- 4. This is the first report of a protocol for single-grain evaluation of oat B-glucan and assessment of the intra-panicle variation of B-glucan in oat.
- 5. This is the first report on the genetics of B-glucan in oat.
- 6. This is the first report that combined evaluation of genotype by environment interactions of protein, oil and B-glucan contents and grain yield and it is the first report to employ AMMI in the analysis of protein and B-glucan genotype by environment data.

Suggestions for Future Research

Various research initiatives could be pursued to continue the research reported in this thesis. Evaluation of oat grain quality characteristics under Nlimiting conditions could help to improve the understanding of nitrogen fertilizer use for the production of superior oat grain quality.

Plant breeding efforts could focus on the production of high and low β -glucan germplasm that can be used to produce high and low β -glucan cultivars with desirable physical grain quality characteristics (i.e. superior test weight and 1,000 kernel weight, low hull percentage) and grain yield. Hulless oats are presently being developed as a feed for monogastrics such as chickens. It would appear the hulless cultivars should be low in β -glucan because increased β -glucan content in broiler chickens diets has been found to reduce rate of growth, and the availability of metabolizable energy and amino acids (Cave *et al.*, 1990).

Greater detail on inheritance of oat β -glucan could be generated. Synthesis of necessary generations to employ the genetic model of Bogyo *et al.*(1988) should allow one to estimate genetic effects controlling β -glucan in oat and the corresponding genetic variances.

Finally, an evaluation of the importance of genotype by environment interactions for physical characteristics of oat grain quality (i.e. test weight, 1,000 grain weight, hull percentage) would be useful. Data from the Québec oat trials could be used and results from such a study should improve knowledge of the stability of grain quality in modern oat cultivars. Stable genotypes could serve as germplasm for future breeding efforts.

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