POTENTIAL OF GRAIN AMARANTH PRODUCTION IN EASTERN CANADA

by

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This thesis has been written in the form of manuscripts to be submitted to scientific journals. The content of chapter 3, 4 and 5 correspond to three manuscripts that have been submitted for publication. The candidate was responsible for designing and conducting the field and laboratory research experiments, and for preparing the thesis dissertation and manuscripts. Dr. Philippe Seguin, Professor in the Department of Plant Science at McGill University, provided supervisory guidance, from the beginning of the project to the editing of the manuscripts before submission for publication.

ABSTRACT

The potential of grain amaranth production in southwestern Québec was studied. Twenty-nine cultivars were evaluated in single row plots, and seven in mechanically managed plots. Yields averaged 27 g $plant^{-1}$ in single row plots, and 649 kg ha^{-1} in mechanically managed plots. Another set of experiments evaluated different seeding dates, row spacings, seeding rates and nitrogen fertilization rates. Seeding date and nitrogen fertilization affected grain yield in one environment, and seeding rate and row spacing did not affect yield. Grains in all experiments required drying. These experiments suggest that grain amaranth could be cultivated in southwestern Québec. Oxalate concentration and form were determined in seeds from the cultivar, seeding date and nitrogen fertilization trials, and the effects of cooking on oxalate were evaluated. Fertilization increased oxalate concentration, seeding date had no effects, and cooking increased soluble oxalate. Grain amaranth is high in oxalate, but its absorbability is probably low.

RÉSUMÉ

Le potentiel de production d'amarante à graine dans le sud-ouest québécois fut étudié. Vingt-neuf cultivars furent évalués en parcelles d'un rang, et sept sous régie mécanisée. Les rendement moyens furent de 27 g $plant^{-1}$, dans les parcelles d'un rang et de 649 kg ha^{-1} sous régie mécanisée. Une deuxième série d'expériences évalua date de semis, espacement entre les rang, taux de semis et fertilisation azotée. La date de semis et la fertilisation azotée affectèrent le rendement dans un environnement, alors que le taux de semis et l'espacement entre les rang ne l'affectèrent pas. Le séchage des grains fut nécessaire pour toutes les expériences. Nos résultats suggèrent que la culture de l'amarante à graine est possible dans le sud-ouest québécois. La concentration et la forme d'oxalate furent déterminés dans les graines provenant des essais de cultivars, de date de semis et de fertilisation azotée. L'effet de la cuisson sur l'oxalate fut également évalué. La fertilisation augmenta la concentration en oxalate, la date de semis n'eu aucun effet, et la cuisson augmenta la proportion d'oxalate soluble. L'amarante à graine est élevée en oxalate, mais son absorbabilité est probablement faible.

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Chapter 1. GENERAL INTRODUCTION

1.1 Statement of the problem

The annual grain crops grown in eastern Canada consist largely of plants from 2 botanical families, poaceae and fabaceae. In Quebec, grain corn represents almost half the surface cultivated in annual grain (Fig. 1.1). Small species diversity in agro ecosystem is known to increase damage from insect pests, and requires greater use of pesticides than diversified systems (Matson et al., 1997). The incidence of plant diseases is also affected by species diversity, although the effect is not as consistent as with insect pests (Matson et al., 1997). For example, *Gibberalla zeae* can result in major yield losses by causing head blight in wheat, oats and barley and ear rot disease in maize and represent a threat to long-term yield stability (Goswami and Kistler, 2004). The incidence of *Fusarium* can be decreased by appropriate choice of preceding crop which has the effect of breaking the disease's cycle (Champeil et al., 2004). The investigation of the suitability of new crops to eastern Canada's conditions is a necessary step for the diversification of cropping systems.

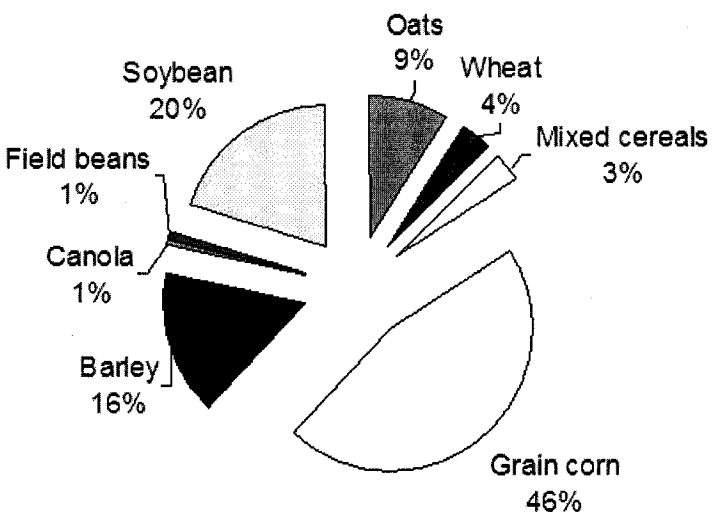


Figure 1.1. Proportions of the grain crops (surface cultivated) in Quebec for 2003.

1.2 Opportunities

Grain amaranth (*Amaranthus spp.*) is a good candidate for introduction in eastern Canada for several reasons. The crop belongs to the amaranthaceae, a botanical family to which none of Canada's field crop belong. Grain amaranth is known to be adaptable to a wide array of environments (National Academy of Science, 1984). Although markets are not fully developed for grain amaranth yet, the crop has the required properties to fill three distinct niche markets : i) people affected by celiac disease needing gluten-free food [grain amaranth is gluten free (Petr et al. 2003)], ii) people looking for highly nutritious food and, iii) people who desire more "exotic" food (Hackman and Myers 2003). Several commercial products can be prepared from grain amaranth, including snacks, bars, breakfast cereals, breads and pasta (Hackman and Myers 2003).

Grain amaranth comprises three species, *Amaranthus caudatus*, *A. cruentus* and *A. hypochondriacus*, and a great genetic diversity is present within each species (Sauer, 1967). As the feasibility of cultivating grain amaranth under eastern Canada's climatic and edaphic conditions is not known, conducting a cultivar screening is required in order to identify the most adapted genotypes.

The agronomic practices for the crop have been studied in several locations, with different conclusions regarding the optimal conditions under which the crop should be grown. Interactions between the factors studied and the environment were often observed, suggesting that grain amaranth reacted differently to the same agronomic practice in different environments. Optimal agronomic practices for eastern Canada remain to be defined.

Although grain amaranth was a traditional crop of pre-Hispanic Indian American cultures, its re-introduction raises concern about the safety of this "new" food, and an examination of its anti-nutritional factors content is appropriate and required. This was suggested by Stowe et al. (1977). Oxalate is an anti-nutritional compound that is linked to kidney-stone formation (Chai and Liebman, 2005b), and was reported in grain amaranth, but only in one cultivar. Substantial differences in leaf oxalate content have been observed amongst cultivars and amongst species (Prakash and Pal, 1991). It is not known whether a similar variability is present in the grain, or whether agronomic practices could affect grain amaranth oxalate concentration.

1.3 Thesis organization and objectives

A review of the literature on grain amaranth is presented in chapter 2, and the overall summary and conclusions to this research are presented in chapter 6. Chapter 7 contains recommendations for future research. Chapter 3, 4 and 5 present the research itself.

This research had two main objectives: an evaluation of the potential for grain amaranth production in eastern Canada and a study of the oxalate content of grain amaranth. The research was divided in three parts, each with its specific objectives.

1.3.1 Genotype screening

This part of the research is presented in chapter 3, under the title *Development and yield potential of grain amaranth genotypes grown in southwestern Québec.* It was submitted to the *Canadian Journal of Plant Science* for publication, and consists in the evaluation of 29 genotypes grown in replicated trials in Sainte-Anne-de-Bellevue over two growing seasons. Thirty genotypes were grown in the first growing season, but only 29 in the second, so only the results of those genotypes evaluated over two growing seasons are reported. The specific objectives were to determine i) the adaptation and yield potential of grain amaranth grown in southwestern Québec, and ii) the variation among selected genotypes for a range of agronomic traits.

1.3.2 Evaluation of optimal management practices

This part of the research is presented in chapter 4, under the title *Evaluation of optimal management practices for grain amaranth production in eastern Canada.* It was submitted to *Agronomy Journal* for publication and consists of three experiments, each replicated over three site-year in Sainte-Anne-de-Bellevue. The specific objectives were to determine appropriate i) seeding dates, ii) seeding rate and row spacing, and iii) nitrogen fertilization rate for grain amaranth grown in eastern Canada.

1.3.3 Oxalate

This part of the research is presented in chapter 5, under the title *Oxalate in grain amaranth.* It has been published in *Journal of Agricultural and Food Chemistry* (Gélinas and Seguin, 2007), and consists in several analyses of the oxalate content of seeds

collected from the first two parts of this research. The four specific objectives were to: i) determine the variability in oxalate concentration and form (soluble vs. insoluble) in seeds of 30 different grain amaranth genotypes representing three different species; and evaluate the effects of ii) calcium ammonium nitrate fertilization, iii) seeding date, and iv) cooking on oxalate concentration and form.

Chapter 2. REVIEW OF LITERATURE

2.1 General information

2.1.1 Taxonomy

The grain amaranths are dicotyledonous plants belonging to the amaranthaceae family. According to Sauer (1967), cultivated grain amaranth consists of three species, *Amaranthus caudatus* L., *A. cruentus* L. and *A. hypochondriacus* L. However, the taxonomy of the genus *Amaranthus* is not clarified to this day. For example, some authors (Drzewiecki, 2001; Greizerstein and Poggio, 1994; Zheleznov et al., 1997) consider *A. mantegazzianus* Pass., *A. edulis* L. and *A. paniculatus* L. as separate species. Sauer (1967) considers the first two as synonyms of *A. caudatus*, and the third as synonym of *A. cruentus*. For convenience, only the three species considered by Sauer (1967) will be used in this review. Certain *Amaranthus* species are also grown as a leafy vegetable (Singhal and Kulkarni, 1988). In Asia, *A. tricolor* L. is the species used for its leaves, whereas leaves of dark seeded *A. cruentus* L. are used in parts of Africa (Kauffman and Weber, 1990).

2.1.2 Morphology

Plant height ranges from 0.6 to over 2 m, and different genotypes present different degree of branching (Saunders and Becker, 1984). The leaves and stem can be green, red or purple. Grain amaranths have monoecious flowers (Brenner et al., 2000) arranged in large, often colourful (red, orange, pink or green) inflorescences whose shape have been compared to those of sorghum (Saunders and Becker, 1984). Each pistillate flower produces a single seed 1 to 1.5 mm in diameter. Thousand seeds weight ranges between 0.6 and 1 g (Saunders and Becker, 1984). Cultivars grown for seeds normally have beige seeds, commonly called "white". The embryo is described as campylotropous, i.e., it encircles the perisperm and the root tip nearly touches the tip of the cotyledons (Irving et

al., 1981). Starch is mostly contained in the perisperm, whereas lipids and proteins are concentrated in the embryo (Irving et al., 1981).

2.1.3 Physiology

Grain amaranth species are of tropical origin (Sauer, 1967) and have a C4 photosynthetic pathway (Johnson and Hatch, 1968). Like other plants possessing this pathway, grain amaranths have been observed to be drought tolerant (Kauffman and Haas, 1983). Studies were conducted to determine the water use pattern (Johnson and Henderson, 2002) and level of drought tolerance of amaranth (Myers, 1996). Johnson and Henderson (2002) found the effective rooting depth (maximum depth at which water is extracted) of grain amaranth to be similar to that of canola. The water use efficiency of amaranth in their study ranged from 2.8 to 11.8 kg ha^{-1} mm^{-1} with an average of 5.9 kg ha^{-1} mm^{-1}. This seems low, when compared to C3 crops, like wheat grown in Australia which can reach a grain water use efficiency of 20 kg ha^{-1} mm^{-1} (Passioura, 2004). However, as Passioura (2004) states, the photosynthetic efficiency is only one factor affecting grain water use efficiency of a crop and harvest index is just as important. The harvest index of grain amaranth is known to be low (see section 2.3). *A. caudatus* and *A. hypochondriacus* are photosensitive, which limits their adaptation to Northern latitudes. *A. cruentus*, however, is considered the most adaptable species and flowers under a wider range of daylengths (National Academy of Science, 1984).

2.1.4 History

It is usually accepted that *A. cruentus* and *A. hypochondriacus* were domesticated in Mexico and that *A. caudatus* was domesticated in Andean regions of South America (Sauer, 1967). *Amaranthus* seeds found together with grinding stones in New Mexico in 1953 were carbon dated and estimated to be 6 800 years old, making them the oldest known food grain of the US (Anonymous, 1957). At the time of the Conquest, the Aztec emperor was collecting about 200 000 bushels of amaranths (believed to be of the species *A. hypochondriacus*) from 17 provinces (Sauer, 1967). Because the grain was used in religious ceremonies, conquistador Cortez prohibited the cultivation of the crop in 1519

as a mean of repressing the native religion (Saunders and Becker, 1984). In the meantime, however, grain amaranth was introduced to Asia and was adopted by many peasant farmers of India, Nepal, Pakistan, Tibet and China (National Academy of Science, 1984). In the Americas, the crop declined in use to a point of near extinction (Sauer, 1967).

In the western world, research interest and the rediscovery of grain amaranth started with the publication of a report showing the high protein (14.5%) and high lysine (6.2 g 100 g^{-1} protein) content of *A. caudatus* (Downton, 1973). The Rodale Research Centre then started the assembly of a germplasm collection with landraces from several countries and initiated a breeding program to adapt the crop to modern agriculture (Lehmann, 1996). The cultivars released following the work of the Rodale Research Centre include 'Montana-3' (Schulzschaeffer et al., 1989a), 'Montana-5' (Schulzschaeffer et al., 1989b) and 'Plainsman' (Baltensperger et al., 1992). Grain amaranth is now grown on about 800 – 1200 ha in the USA (Sooby et al., 2005).

2.2 Grain composition and utilization

The proximate composition of *A. hypochondriacus* seeds is shown in table 2.1. There are differences in composition between cultivars of a same species and between species, however (Bressani et al., 1987).

2.2.1 Protein

Protein concentration of grain amaranth is between 12.4 and 16.8% (Bejosano and Corke, 1998). The protein composition of grain amaranth is one central argument in favor of its cultivation. Lysine level of grain amaranth's protein is twice that of wheat protein, three times that of corn and comparable to milk protein (National Academy of Science, 1984). When compared to the FAO standards, *A. cruentus* whole meal was found to be deficient only in leucine, which makes amaranth a good complement to cereal grains (Bejosano and Corke, 1998). The amino acid composition of soybean and *A. hypochondriacus* was found to be similar (Gorinstein et al., 2002).

Table 2.1. Proximate composition (%) of grain amaranth compared to common cereal grains.

Analysis	*A. hypochondriacus*	Maize	Rice	Wheat
Crude Protein	17.9	10.3	8.5	14
Fat	7.7	4.5	2.1	2.1
Fiber	2.2	2.3	0.9	2.6
Ash	4.1	1.4	1.4	1.9
Carbohydrate	57.0	67.7	75.4	66.9

Adapted from Segura-Nieto et al (1994).

2.2.2 Carbohydrates

Occurring at about 62% of the total weight, starch is the most abundant component of grain amaranth seeds (Becker et al., 1981). The starch is made of very small granules (0.75 – 3.50 μm), and has special properties such as high swelling power and high gelatinization temperature (Singhal and Kulkarni, 1988). There is also a wide diversity in starch properties among the different genotypes (Wu and Corke, 1999). Small diameter starches can be used in a wide array of food and non-food applications, such as fat replacement ingredient and the making of biodegradable films (Lindeboom et al., 2004).

2.2.3 Lipids

The oil content of *A. cruentus* was found to range between 1.9% and 5.0%, and that of *A. hypochondriacus* between 2.18% and 5.23% (He and Corke, 2003). The fatty acid profile of amaranth is very similar to that of wheat and barley (Leon-Camacho et al., 2001). Several studies have reported that hypercholesterolemic rodents fed grain amaranth had decreased cholesterol levels (Berger et al., 2003; Czerwinski et al., 2004; Plate and Areas, 2002). A similar effect was observed in chickens (Qureshi et al., 1996). It has been suggested that this cholesterol-lowering effect may be due to squalene, a lipophilic compound present at high concentration in amaranth oil (Shin et al., 2004). Squalene is present in the oil of other plant species at levels between 0.01 and 0.4%, whereas amaranth oil contains around 1.9 to 8.7%, depending on the genotype and species (He and Corke, 2003). Squalene is an important ingredient of skin cosmetics due to its photo protective properties and is used as a lubricant in high technology applications, such as computer disks, because of its thermostability. Squalene has traditionally been extracted from shark liver oil, but concerns about the sustainability of the practice has increased interest in finding renewable sources (He et al., 2002). The US demand for squalene is around 200-300 tons yr^{-1} and it could be extracted from amaranth grain as a co-product of starch, making the enterprise economically feasible (Leon-Camacho et al., 2001).

2.2.4 Anti-nutritional factors

Although grain amaranth was a traditional crop of pre-Hispanic Indian American cultures, its re-introduction raises concern about the safety of this "new" food, and an examination of its anti-nutritional factors content is appropriate and required. This was suggested by Stowe et al. (1977). Tannin and phytic acid levels in amaranth are similar to those in cereal grain (Singhal and Kulkarni, 1988). Saponin level in one cultivar on *A. caudatus* was undetectable (Cuadrado et al., 1995). However, Oleszek et al. (1999) found levels between 0.09 and 1% of dry matter in *A. cruentus*. The toxicity of the extracted saponin was tested in hamsters, and the authors concluded that given the low total concentration and the relatively high estimated lethal dose (1100 mg kg^{-1} of body weight), amaranth-derived products should pose no harm to its consumers. Bresler et al. (1995) isolated several mycotoxin-producing mould species from Argentinean grain amaranth sample. Although the mycoflora may differ amongst locations, this study stresses the importance of appropriate storage conditions. Recommended seed moisture for storage is 10-12% (Sooby et al., 2005). Amaranth grains were reported to be less susceptible to aflatoxin contamination than maize or rice (Fernando and Bean, 1985). Trypsin inhibitors were isolated from amaranth seeds and are considered highly heat stable by Koeppe et al. (1985). However, the seeds were only heated to 100°C, which is 35 – 71°C lower than temperatures used for extrusion cooking (Chavez-Jauregui et al., 2000) or popping, which can be done at temperatures up to 260°C (Konishi et al., 2004). Moreover, Bejosano and Corke (1998) point out that genetic variability exists for selecting lower trypsin inhibitor activity and that observed levels are relatively insignificant when compared to those in soybean. Oxalate concentration of *A. caudatus* was recently measured at about 228-236 mg 100 g^{-1} (Siener et al., 2005). This is higher than whole wheat flour or corn meal, which were reported to be 67 and 54 mg 100 g^{-1}, respectively (Chai and Liebman, 2005).

2.2.5 Uses of the grain

Traditionally, amaranth grains are used popped, cooked into a gruel, lightly roasted and milled to make a flour (Marx, 1977). Modern day uses of the grain include: crackers, sweet rolls, amaranth-containing spread, mixed grain pilaf, pancakes, hot cereals, bread,

tortillas, dumplings and muffins (Saunders and Becker, 1984). Bread including increasing amounts of amaranth flour gave satisfactory results at proportions up to about 15% of amaranth flour (Breene, 1991). At higher level, the paste structure collapses. Breads with amaranth were reported to have a pleasant, nutty taste and were preferred over a control white bread (Breene, 1991). Including amaranth flour in tortilla resulted in improved nutritional qualities and did not alter the sensory attributes (Sanchezmarroquin et al., 1987). Gluten-intolerant people could benefit from amaranth since it is a gluten-free grain (Petr et al., 2003). Cooked amaranth could be introduced in broiler feed at levels up to 40% of the total ration (Ravindran et al., 1996; Tillman and Waldroup, 1988). It was suggested that a lower cholesterol level in eggs could be obtained from amaranth-fed layers (Punita and Chaturvedi, 2000).

2.3 Genetic aspects

Kauffman and Weber (1990) identified several traits that are of crucial importance in the development of grain amaranth under modern commercial conditions. Of these, some have been isolated in breeding lines or cultivars: reduced plant stature, lack of branching, white or gold seeds, and early maturity (100-120 days). The remaining traits are: higher yield, increased seed size, tolerance to insects and diseases, seedling vigor, reduced shattering, resistance to lodging, synchronous drying of the plants and seed heads, ease of threshing, and high protein content. The USDA germplasm (North Central Regional Plant Introduction Station, Ames, IA) currently holds over 3300 different entries which are available free of charge to researchers around the world and can be used in breeding programs.

Amaranth is known to have a low harvest index (HI), which many researchers have pointed out to be a crucial component to improve in order to increase grain yields (Aufhammer et al., 1995; Elbehri et al., 1993; Erley et al., 2005). Table 2.2 shows a range of HI in some studies. In India, the cultivar 'Annapurna' (*A. hypochondriacus*) has been reported to have HI as high as 56% (Malligawad and Patil, 2001).

Grain amaranths are mostly self-pollinated crops, with various degrees of cross-pollination. Several studies on the rate of outcrossing of *A. cruentus* have been conducted, and estimates have ranged between 5-30% (Agong and Ayiecho, 1991; Drzewiecki, 2001;

Table 2.2. Summary of different studies evaluating grain amaranth management strategies.

Location	Grain yield range (kg ha^{-1})	Harvest index	Harvest method	Influencing variables	Reference
Minnesota	1063 – 1592	9.9 – 15.9	Plot combine	Cultivar, N fertilization	Elbehri et al. (1993)
North Dakota	254 – 1799	2.6 – 33.4	Hand	Cultivar × Year ; Density × Year	Henderson et al. (1998)
North Dakota	290 – 1720	6.8 – 26.4	Hand	Cultivar × Year; Sowing date × Year	Henderson et al. (2000)
North Dakota	831 – 2233	7.4 – 30.5	Hand	Cultivar × Year	Henderson et al. (2002)
South Germany	1900 – 2999	18 – 30	Hand	Sowing date; Cultivar	Aufhammer (1995)
South Germany	1986 – 2767	18 – 27	Hand	Cultivar, N fertilization	Erley et al. (2005)

Jain et al. 1982). The differences have been attributed to the ratio of staminate to pistillate flowers and to environmental variations such as the presence of pollinators (Brenner et al., 2000).

Recent research works have set the basis for the development of high yielding grain amaranth cultivars. Male-sterile amaranths were found to occur in *A. hypochondriacus* (Gudu and Gupta, 1988; Peters and Jain, 1987), which makes possible the large-scale production of hybrid amaranth seeds. Interspecific hybrids between *A. cruentus* and *A. hypochondriacus* were created, and exhibited high-parent biomass heterosis of up to 40%. Biomass production in the same range as hybrid sorghum were obtained (Lehmann et al., 1991). *In vitro* culture techniques including plant regeneration from callus cells were developed for *A. caudatus* and *A. hypochondriacus* (Bennici et al., 1992), which opens possibilities for biotechnological applications of the plant such as genetic engineering. Non-shattering populations have been isolated in *A. hypochondriacus* and *A. cruentus* and the breeding lines are now available from the USDA germplasm (Brenner, 2002). Shattering represents a major problem in grain amaranth. In Northern latitudes, the plants are often harvested several weeks after the seeds have matured (see section 2.4.3), which increases shattering losses (Fitterer et al., 1996).

2.4 Agronomic and management aspects

Over the past 25 years, grain amaranth has attracted the interest of many researchers around the world. The crop is now cultivated on small scale commercial basis in many parts of the United States (Sooby et al., 2005). Appreciable yields are obtained as far north as North Dakota and Minnesota (Henderson et al. 2000). Table 2.2 gives a summary of studies in different temperate environments.

Grain amaranth has been successfully grown in many different countries with contrasting environmental conditions. These include Australia (Angus et al., 1982), Argentina (Peiretti and Gesumaria, 1998), South Germany and Poland (Kaul et al., 1996), China (Wu et al., 2000), Puerto Rico and Sweden (Saunders and Becker, 1984), Hungary (Mihalik and Szocs, 1993), Chile (Berti et al., 1996), Guatemala (Calderon et al., 1991), Mexico (Espitia, 1992), Bolivia (Apaza-Gutierrez et al., 2002), Thailand (Senthong et al., 1992), India (numerous studies, but interestingly a cultivar, 'Annapurna' was released in

1985 (Joshi, 1985) and yielded an average of 2230 kg ha^{-1}) and Kenya (Gupta and Thimba, 1992).

2.4.1 Stand establishment

2.4.1.1 Seeding method

The seeding equipment used must be able to handle small seeds. Weber (1987) stated three general requirements that the equipment must meet: ability to i) seed at shallow depth, ii) deliver small amount of seeds per hectare [2.2 kg is usually the recommended rates (Myers, 1996)] and iii) provide adequate contact between seed and soil. Grain drills, the insecticide box of corn planter, and vegetable planters have been used successfully (Weber, 1987). Grain amaranth seems to be well suited to a broad range of soil types, but good drainage is an important factor (Williams and Brenner, 1995).

Amaranth seeds are very small (0.6 – 0.8 g 1000 $seeds^{-1}$) and therefore have very limited energy reserve to support emergence (Aufhammer et al., 1998). Bavec and Mlakar (2002) studied the effect of seeding depth on sand, sandy loam and loam, and recommended a seeding depth of 15 mm for all 3 soil types. They stated the importance of well-structured soils; non-structured soils (such as soil low in organic matter) are prone to crusting, which can deter emergence. Satisfactory field emergence is attained with temperatures ranging between 18.5 and 24°C (Webb *et al.* 1987). Depending on the species, emergence of 50% can still be obtained at 11°C, when using a sandy loam at a seeding depth of 5 mm. The different response to temperature of the different species suggests the possibility for selecting cultivar with higher emergence under lower soil temperature.

2.4.1.2 Seeding date in temperate zones

In temperate zones, there must be a balance between late and early seeding. Early seeding lengthens the growing period and guarantees moisture availability for germination, but low temperatures may inhibit germination and favor seed rotting. Later seeding ensures adequate soil temperature and prevents seed decay, but shortens the

growing season (Bavec & Mlakar 2002). Seeding date is determined by soil temperature, soil moisture, cultivar and average date of first fall frost and last spring frost (Henderson et al., 1998). Due to the requirements for both sufficient moisture and high soil temperature, the time where optimal emergence conditions are met is more limited for amaranth that for larger seeded crops (Weber, 1987). In Missouri, seeding from mid-May to mid-June had no effect on yield, whereas seeding in early July decreased yield up to 60%. In Northern latitudes, growers have to plant early enough so that the plant can mature before frost. In South Germany, Aufhammer et al. (1995) evaluated three seeding dates (6 May, 20 May and 15 June) and obtained the highest yields under the mid-May date. In North Dakota, seeding in mid-June has been recommended (Henderson et al., 1998).

2.4.1.3 Plant population

Plant population density studies conducted with amaranth often show contradictive results. The diverging environments probably account for a great part of the differences observed. It has been suggested that water availability should determine plant population, as higher plant population will require greater amount of water (Weber, 1987).

In North Dakota, Henderson et al. (2000) found a significant environment × density effect on grain yield, which suggests that different population densities should be adopted in different environments. They nevertheless made a recommendation of 173 000 plants ha^{-1} (the range studied was 74 000 to 272 000 plants ha^{-1}), since it allowed for a uniform stand, and resulted in smaller stem diameter which eases mechanical harvesting. In the more humid environment, they obtained a slightly higher yield with the highest density. Interestingly, they observed a decrease in height at increasing plant density, which is desirable for mechanical harvesting. Malligawad and Patil (2001) observed increasing yield (from 1858 to 3242 kg ha^{-1}) when increasing plant population from 55 000 to 222 222 plant ha^{-1}. They also observed decreasing stem diameter with increasing plant population. In Bolivia, yield was positively correlated with increasing plant density at levels up to 220 000 plants ha^{-1} (Apaza-Gutierrez et al., 2002). The authors hypothesized that higher populations could still increase yields. In Missouri, seeding rates

ranging from 0.28 to 4.4 kg, were evaluated and no differences in grain yield were observed (Myers, 1996). Self-thinning is often observed at higher population rates, i.e. the established population differs from the population at the end of the season (Myers, 1996; Henderson et al., 2000). This could account for the lack of response to seeding rate.

2.4.1.4 Row spacing

As no herbicide is so far available for weed control in grain amaranth, mechanical weed control must be practiced, which limits the options for row spacing (Sooby et al., 2005). The effects of row spacing have nevertheless been studied. Interaction effects between row spacing and environment were observed, stressing the importance of environment (Henderson et al., 2000). In India, Bansal et al. (1995) obtained higher grain yield at decreasing row width. In an Argentinean study, there was no difference in yield between four different row spacings ranging from 30 to 70 cm (Peiretti and Gesumaria, 1998). The authors nevertheless suggested a narrower row spacing of 30 or 45 cm, which they claim to help control weeds by increasing the rate of canopy closure. This recommendation is in contradiction to Myers' (1996) observation that reduced self-thinning in narrower rows resulted in increased plant competition and reduced plant height and maturity. Wider row spacing have been reported to increase lodging (Henderson et al., 2000).

2.4.2 Fertilization

Fertilization requirements, particularly nitrogen, were studied in diverse environments. Different conclusions were drawn, according to the different environments where the studies were conducted. It was suggested that more nitrogen could be applied in areas of higher rainfall (Stallknecht and Schulz-Schaffer, 1993).

In Minnesota, Elbehri et al. (1993) obtained yield response to nitrogen, but no responses were observed to phosphorus or potassium. Yield increases were obtained with applications of 45 – 180 kg N ha^{-1}. Yields went from 1094 kg ha^{-1} without nitrogen application to 1428 kg ha^{-1} at 180 kg N ha^{-1}. In some sites and year, no responses to

nitrogen were observed, however. Different cultivars gave maximum grain yield at different N levels. Seed nitrogen content was significantly higher when more nitrogen was applied. Nitrogen use efficiencies of 3.5 – 7.9 kg grain per kg soil N were obtained; in corn, nitrogen use efficiency is around 20 – 32 kg grain per kg soil N (Elbehri *et al.* 1993). The authors attribute this low nitrogen use efficiency to a low nitrogen harvest index and to low harvest index. They obtained no yield response to phosphorus, except on one site where the available phosphorus was very low. No interaction effects between the nitrogen, phosphorus and potassium were observed.

In Missouri, Myers (1998) evaluated nitrogen rates between 0 and 180 kg ha^{-1}. Yields increased with increasing N rate up to 90 kg ha^{-1}. Nitrogen fertilization also increased seed moisture at harvest and the number of days to anthesis. In South Germany, grain yields increased from 1986 kg ha^{-1} without nitrogen to 2767 kg ha^{-1} with 120 kg N ha^{-1} (Erley et al., 2005). The authors also observed increases in grain nitrogen concentration in one cultivar. In India, significant yield increases were observed with increasing nitrogen rates (Bhaskar et al., 1996; Saini and Shekhar, 1998). Bhaskar et al. (1996) observed the highest yield at 25 kg N ha^{-1} during the rainy season, whereas Saini and Shekhar (1998) observed maximum yield at 90 kg ha^{-1} under dry conditions.

Lodging is a problem with grain amaranth. Elbehri et al. (1993) found an increase in lodging with increasing nitrogen rate; observed lodging increased from 1.7 to 7.8 out of 10. Myers (1998) also observed increasing lodging (mostly root lodging) with increasing nitrogen rate.

2.4.3 Harvesting

The crop was initially thought to be limited to hand harvesting (Marx, 1977), but mechanical harvesting proved to be possible with properly adjusted equipment (Krishnan et al., 1994; Majewski et al., 1994). Due to the external position of the embryo in *Amaranthus* seeds, damage and reduced germination rate may occur upon combine harvesting (Krishnan et al., 1994). The usual harvesting method for amaranth under northern latitudes in the USA consists in harvesting approximately 10 days after the first killing frost, which allows for a good dry down of the plant (Sooby et al. 2005). Fitterer et al. (1996) evaluated the harvest loss due to mechanical harvesting before a killing frost in

northern latitudes to be around 25-30%. In southern latitudes, plants usually dry down before fall frosts (Sooby et al. 2005).

2.4.4 Pathogens

So far, no major diseases have been reported to affect grain amaranth, but increasing cultivation areas will most likely increase selection pressure on pathogenic agents. Also, diseases may potentially arise from the wild relatives. *A. hybridus* is thought to be a progenitor of all three grain amaranth (Chan and Sun, 1997). Knowing what the potential threats are is a useful mean to insure quicker reaction, should problems arise.

Grain amaranth is susceptible to damping-off caused by *Pythium* and *Rhizoctonia*, and to stem canker caused by *Phoma* and *Rhizoctonia* (Williams and Brenner, 1995). Damping-off can be controlled by proper soil drainage, nitrogen fertilization and temperature at sowing (Williams and Brenner, 1995). The following genera and species have been reported to cause leaf spot in the USA: *Colletotrichum, Alternaria alternantherae* and *Cercospora* (Williams and Brenner, 1995). A stem canker caused by *Pythium aphanidermatum* has been isolated (Block et al., 2002; Mihail and Champaco, 1993). The disease causes dry, dark coloured cankers to develop, that can spread 15-45 cm on the bottom of the stem (Block et al., 2002). The infected plants eventually lodge and die. *Fusarium oxysporum* have been observed in South Africa on *A. hybridus* (Chen and Swart, 2002). The symptoms include root rot, crown necrosis, discoloration of root vessels, stem canker, and eventually wilt and death of the plant. Chen and Stewart (2002) performed screening for resistance on 5 genotypes of *A. hybridus*, and found varying levels of resistance between the genotypes. Tobacco ring spot virus was observed in *A. hybridus*, and a virus similar to cucumber mosaic virus have been reported to affect and *A. caudatus* and *A. hypochondriacus* (Williams and Brenner, 1995). Nematodes of the genera *Meloidogyne* and *Nacobbus* have been reported, although not causing serious damages (Williams and Brenner, 1995).

2.4.5 Insect pests

The main pest of grain amaranth is the tarnished plant bug, *Lygus lineolaris.* The insect feeds on immature seeds and flowers and can cause major yield losses (Olson and Wilson, 1990). The amaranth weevil, *Conotrachelus seniculus*, bores into roots and can cause lodging. Another weevil, *Cylindrocopturus adspersus*, was observed in North Dakota (Williams and Brenner, 1995). An extensive study of potential problematic insects on grain amaranth was published (Wilson, 1989).

2.4.6 Weed control

Although amaranth has a fast growth rate, its seedlings grow slowly and can suffer from weed competition (Tucker, 1986). Few studies have been published on weed control in amaranth, since it is mostly done mechanically. *Amaranthus* weeds tend to be a major problem in grain amaranth fields, due to their similar ecological requirement. It is recommended to avoid fields already contaminated with them (Weber, 1987). Endres and Longer (1987) conducted an herbicide selectivity study to identify compounds that selectively control the weedy amaranths *A. hybridus* and *A. palmeri*, without affecting the grain amaranths *A. cruentus* and *A. hypochondriacus.* Metolachlor, bentazon, and naptalam combined with dinoseb exhibited some level of selectivity. This indicates potential for development of chemical weed control in grain amaranth.

Chapter 3. DEVELOPMENT AND YIELD POTENTIAL OF GRAIN AMARANTH GENOTYPES GROWN IN SOUTHWESTERN QUÉBEC

3.1 Abstract

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous pseudocereal, characterized by high protein and lysine content, and a lack of gluten. This crop is adaptable to a wide range of environments and has been successfully introduced in a range of regions worldwide, it has however not been evaluated in southwestern Québec. To evaluate the feasibility of grain amaranth cultivation locally and determine variation for a range of agronomic traits, twenty-nine grain amaranth genotypes were evaluated in Sainte-Anne-de-Bellevue, QC, in single row plots (hand harvested) over two growing season (i.e., 2005 and 2006), and out of these, seven were selected for further evaluation in mechanically managed plots at two sites over one growing season (i.e., 2006). Considerable variation between cultivars and years were observed for all variables studied (i.e., grain DM yield, total DM biomass, harvest index, branch number, plant height, number of days to anthesis, lodging, and grain crude protein content). In single row plots, grain yield per plant ranged between 13 and 73 g DM plant^{-1}, while in mechanically managed plots grain yield ranged between 432 and 979 kg DM ha^{-1}. In both years and for both experiments, plants from all genotypes under evaluation matured before the first killing frost, however grain moisture at harvest was high (avg. of 25.8%) and drying was necessary. Although the present study demonstrated that grain amaranth could be produced in southwestern Québec, it will remain a very marginal crop in the foreseeable future. For its production to increase, there is a need for major food processors to integrate grain amaranth in widely distributed products. Also a dedicated breeding program would likely be necessary to improve grain yields.

3.2 Introduction

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous (Johnson and Hatch, 1968) pseudo-cereal crop which was widely cultivated in pre-Columbian America (Sauer,

1950). Due to pressure from the conquistadors, who disliked its use in native ceremonies, grain amaranth's cultivation in the Americas decreased to a point of near extinction at the beginning of the 20th century (Sauer, 1950). Since the discovery of its high lysine content (Downton, 1973), food scientists and agronomists around the world have shown sustained interest in developing grain amaranth. Nutritionally, grain amaranth is interesting due to its high protein (i.e., 15 to 18%), lysine, and calcium concentrations and its lack of gluten (Petr et al., 2003). It is estimated that over 2.1 million people in the USA alone are affected by the celiac disease, which renders affected people intolerant to gluten (Celiac Spruce Association, 2004). This makes grain amaranth a crop with a great market potential. Grain amaranth is currently a niche market crop, with most sales coming from health food stores. Several commercial products can be prepared with grain amaranth, including snacks, bars, breakfast cereals, breads and pasta. Hackman and Myers (2003) identified three key reasons motivating consumers to purchase grain amaranth: i) people affected by celiac disease needing gluten-free food, ii) the favourable nutritional profile of the grain and iii) the desire for more "exotic" food.

Grain amaranth is adaptable to a wide range of environments and thus has been successfully introduced in several countries of Europe, Asia and Africa (National Academy of Science, 1984). The crop had been introduced in Asia and Africa prior to its cultivation decrease in the Americas (Sauer, 1967), which allowed for the preservation of a considerable diversity. Two major *ex situ* collections have been built over the years (i.e., in the USA and India), each containing over 3000 accessions (Brenner et al., 2000). In the USA, possible range of cultivation of the crop extends as far north as North Dakota (Henderson et al., 1998), with most production being concentrated in Nebraska.

The introduction of grain amaranth in Canada has been described as "risky", mainly due to the fact that grain amaranth is a warm climate crop that may not mature in most regions (Small, 1995). The main obstacle for the growth of amaranth in Canada is the season length and the resulting high seed moisture content at harvest. The usual harvesting method for amaranth under northern latitudes in the USA consists in harvesting approximately 10 days after the first killing frost, which allows for a good dry down of the plant (Sooby et al., 2005). Several regions of Canada successfully support the growth of warm climate crops such as corn and we believe that the introduction of grain

amaranth in southwestern Québec and southern Ontario could be successful and would represent an opportunity for crop diversification. The growing season should be sufficient in these regions to support grain amaranth production. To our knowledge grain amaranth has not been evaluated in southwestern Québec. The objectives of the present study were thus to: i) determine the adaptation and yield potential of grain amaranth grown in southwestern Québec, and ii) determine the variation among selected genotypes for a range of agronomic traits.

3.3 Materials and methods

3.3.1 Plant material and field management

Field experiments were conducted in 2005 and 2006 at the Emile A. Lods Agronomy Research Centre of McGill University in Sainte-Anne-de-Bellevue, QC, Canada (45°25′ 45″N lat., 73°56′00″ W long.). Temperature and precipitation for the two growing seasons and 30-yr averages are presented in Table 3.1.

A first experiment consisting in the evaluation of 29 genotypes from three grain amaranth species (*A. cruentus, A. hypochondriacus* and *A. hybrid*) was conducted over two consecutive years. Genotypes were obtained from the US National Genetic Resources Program (North Central Regional Plant Introduction Station, Ames, IA) (Table 3.2). Field A and B were used in 2005 and 2006, respectively (Table 3.3). Nitrogen, phosphorus and potassium fertilization was done based on soil tests according to local recommendations for sorghum (CRAAQ, 2003), as there are currently no recommendations available locally for grain amaranth. Plants were grown in a randomized complete block design with three blocks. Each plot consisted in one 2 m row. Rows were 80 cm apart. Seeds were placed manually 4 cm apart and plants were subsequently thinned to one plant every 20 cm in the row. Weed control was done manually throughout the season. Due to time constraints, seeding and harvesting were sometimes carried out over more than one day. In these cases, whole blocks were completed in the same day. In 2005, seeding was done on June 9 and June 10 and harvesting was done on October 30. In 2006, seeding was done on June 6, 13, and 14 and harvesting was done on November 1 and 3. In 2005, all plants were hand harvested, and in 2006 5 plants per plot were harvested, due to greater biomass

production. Harvesting was done in both years approximately 2 weeks after the first killing frost, as recommended for production in northern USA (Sooby et al., 2005). All harvested seeds were dried at 50 °C until moisture content stabilized to express yields on a DM basis.

Using data from 2005, 7 genotypes were chosen for evaluation in larger plots. This second experiment was set up as a randomized complete block design with four blocks per site and was replicated at two sites in 2006; all field operations were mechanized. Field C and D were used (Table 3.2). Plots were 5 m long with four rows spaced 76 cm apart. Seeding was done on 2006 May 31 at a rate of 2 kg ha^{-1} using a disk drill (Fabro, Swift Current, SK, Canada). Weed control was done three times during the season using a row crop cultivator (Kongskilde, Denmark). Harvesting was done mechanically using a self-propelled harvester (Wintersteiger, Saskatoon, SK, Canada) on 2006 October 30, approximately 10 days after the first killing frost. Only the middle two rows of each plot were harvested. All harvested seeds were dried at 50 °C until moisture content stabilized to express yields on a DM basis and determine grain moisture at harvest.

3.3.2 Data collection

Total plant biomass in the first experiment (i.e., single row plots evaluation of 29 genotypes) was determined by harvesting all above ground material and drying it at 50 °C until moisture content stabilized. The harvest index was calculated as the contribution of seeds to total aboveground biomass. Plant height and branch number were measured shortly prior harvesting, on 5 plants per plot in the first experiment and on 10 plants per plot in the second experiment (i.e., four row plots evaluation of 7 genotypes). Plant height was measured as the distance between soil level and the top of the main inflorescence and branch number was the number of side shoots having at least one visible node. Lodging was recorded on the day of harvest and was the fraction of the total number of plants in the row bending at an angle greater than 30°. In the first experiment, lodged plants in the entire plot were counted and expressed out of 10, whereas in the second experiment, a visual evaluation was done using a 0 to 5 scale, 0 referring to absence of lodging and 5 to all plants in the plot being lodged. The number of days to anthesis was the number of

days between seeding and the day when 50% of the plants were showing first signs of anthesis. Crude protein concentration of seeds of the 29 genotypes evaluated in the first experiment was determined upon harvest. Seeds were ground through a 1-mm screen using a Tecator Cyclotec sample mill (Tecator Corporation, Sweden). Nitrogen content was then determined using a Leco Nitrogen Analyzer (FP-428, Nitrogen Determinator System, Leco Corporation, Saint Joseph, MI, USA). Crude protein was calculated as %N × 5.85 (Becker et al., 1981).

3.3.3 Statistical analyses

Statistical analyses were performed using PROC GLM of SAS 9.1 (SAS Institute Inc., 2003). Statistical models for both experiments were elaborated after McIntosh (McIntosh, 1983), using combined analyses over years (i.e., experiment 1) or sites (i.e., experiment 2). When there was a significant year × genotypes effect, genotypes were re-analyzed by year. LSD values were calculated when there were significant model and treatment effects. In the second experiment, Dunnett's multiple comparison test was performed using 'Plainsman' (PI 558499) as a control, this cultivar being the most commonly grown commercially in North America (Thomas Jefferson Agricultural Institute, 2006). Significance level was set at 0.05 for all analyses.

3.4 Results and discussion

3.4.1 Climate data

The two years of experimentation differed significantly in terms of climatic conditions (Table 3.1). Compared with 2006, the 2005 season was somewhat warmer, and precipitations were about the same, except for May, which was dryer, and September, which was wetter. Both years received more precipitation than the 30-yr average in October, which is the month where harvest took place. The last spring frosts were May 13 and April 28, for 2005 and 2006, respectively and the date of first fall frosts were October 21 and October 22 for 2005 and 2006, respectively. Despite the fact that both years of experimentation received more precipitation than average, in both years and for both experiments, plants from all genotypes under evaluation matured before the first

killing frost, which indicates that grain amaranth production in southwestern Quebec is possible.

3.4.2 Experiment 1. Single row plots

Analysis of data combined over years indicated significant genotype effects ($P < 0.05$) for all variables studied, with considerable variation being observed between genotypes (Table 3.4). Year main effects were significant ($P < 0.05$) for grain yield per plant, harvest index, branch number, number of days to anthesis, lodging score, and crude protein content. There were also significant year by genotype interactions ($P < 0.05$) for grain yield per plant, harvest index, and plant height.

Grain yield per plant ranged between 13 and 73 g plant^{-1}, with averages of 20 and 39 g plant^{-1} in 2005 and 2006, respectively (Table 3.4). The year by genotype interaction ($P < 0.01$) revealed that differences between cultivars were only significant in 2006, during which yield per plant ranged between 18 and 73 g plant^{-1} (Table 3.5). Grain yield per plant was positively correlated ($P < 0.001$) with plant height, harvest index, total dry matter biomass per plant, as well as with lodging (Table 3.6). The positive correlation between lodging and grain yield may be explained by the fact that all plants, lodged or not, were hand harvested; mechanical harvesting does not always allow harvesting of lodged plants. Seed quality of lodged plants may also be reduced due to contact with soil. It is therefore important to develop lines that are resistant to lodging. Shattering was not quantified, but we observed the phenomenon starting in early September. Non-shattering trait has been found in grain amaranth (Brenner, 2002), but it remains to be included in commercial varieties. Yields per plant we observed are in the range of those previously reported for several genotypes grown in China (Wu et al., 2000) and Mexico (Espitia, 1992). For example, the mean yield per plant reported by Wu et al. (2000) for the same amaranth species as those included in our study was 28 g per plant.

Harvest indices were relatively low in both years, ranging between 8 and 28%, with means of 17 and 21% in 2005 and 2006, respectively (Table 3.4). Data was analyzed for each year, due to the presence of a significant ($P < 0.001$) year × genotype interaction, and in both years genotype had a significant effect ($P < 0.001$) on harvest index (Table

3.5). Total dry matter per plant averaged 149 g plant^{-1}, and ranged between 105 and 188 g plant^{-1} (Table 3.4). As expected there was a strong correlation ($P < 0.001$) between harvest index and yield (Table 3.6). The low harvest index of grain amaranth has been identified by many as a limiting aspect that could be improved by breeding and selection (Elbehri et al., 1993; Aufhammer et al., 1995; Erley et al., 2005). In India, the cultivar 'Annapurna' (*A. hypochondriacus*) has been reported to have harvest indices as high as 57%, with yields ranging between 1900 and 3500 kg ha^{-1} (Malligawad and Patil, 2001).

Anthesis occurred somewhat late in the season in both years, spanning from late July to mid-August. The number of days to anthesis ranged between 56 and 82 with an average of 67 (Table 3.4) and was negatively correlated ($P < 0.001$) to grain yield (Table 3.6). Negative correlations between number of days to anthesis and yield have been reported by Kulakow and Jain (1987), who suggested that selection for shorter number of days to anthesis should result in rapid yield improvement. The heritability of this trait was evaluated to be between 0.35 and 0.66. It has been proposed that early flowering is governed by only one gene (Brenner et al., 2000). In the present study, three genotypes reached the anthesis stage in less than 60 days after seeding. Out of these, PI 538323 (K432) and PI 538324 (K433) seem particularly valuable, as they are the only genotypes that started to dry down before the fall frost. They are short (avg. of 109 cm) and had a low lodging score (avg. of 3 out of 10). They also had among the highest harvest indices (avg. of 22%) and protein levels (avg. of 16.4%). The use of these genotypes in breeding programs interested in extending the range of cultivation of grain amaranth into short season environments such as southwestern Québec should be considered. One genotype, PI 568179 an ornamental that was grown only in 2005, reached anthesis stage in about 40 days and also dried down before the first fall frost (data not shown). It has white seeds (a desired characteristic in grain amaranth) and could also be potentially used in a breeding programs aiming at developing grain amaranth cultivars for short season environments.

Plant height averaged 158 cm in 2005 and 199 cm in 2006, with values ranging between 99 cm and 254 (Table 3.4 and 3.5). As noted by Myers (1998), the location of the seed head on top of the plant makes tall genotypes more prone to lodging and harder to harvest mechanically. Genotypes PI 538323 and PI 538324 are two noticeable exceptions in terms of height, with an average height of 109 cm over the two years (Table

3.5). Combined with their resistance to lodging and early dry down characteristic, this makes these two genotypes well suited for mechanical harvesting. Plant height was greater on average than what was observed in Mexico for *A. cruentus* (Espitia, 1992) and in North Dakota for *A. cruentus* and *A. hypochondriacus* × *A. hybridus* hybrids (Henderson et al., 2000). This is most likely due to the greater available moisture in southwestern Québec, as soil moisture is known to be a factor limiting plant height in grain amaranth (Williams and Brenner 1995). This suggests that under southwestern Québec conditions, genotypes will tend to express their full height potential.

Lodging scores ranged between 2 and 10 and averaged 5 in 2005 and 8 in 2006, respectively (Table 3.4). Difference between years ($P < 0.05$) is probably due to the greater height and grain yield (and thus head weight) in 2006 (Table 3.4), which probably both contributed to lodging. Lodging was positively correlated ($P < 0.01$) with grain yield, total biomass, harvest index, height, and branch number (Table 3.6). Lodging is one of the most unfavorable aspects of the crop and reducing it should be a prime objective of any breeding program. Plants with more developed branches, like PI 576447, PI 538323 and PI538324 seem to be less prone to lodging. This is reflected in the significant correlation ($P < 0.01$) between these two traits (Table 3.6). In both years, lodging happened later in the season, after the filling of the seed heads, which occurs in early to late September, depending on the genotype. Root lodging was the most common type of lodging and in both seasons occurred after the heavy rains of October. The month of October in both years was substantially rainier than the 30-yr average with 120 and 101 mm more rain in 2005 and 2006, respectively (Table 3.1). We suppose that lodging scores would be lower during a more typical drier fall.

Crude protein concentrations ranged between 12.5 and 16.9%, with an average of 14.3% (Table 3.4). These values are similar to those reported by Bressani et al. (1987), but slightly inferior to Becker et al. (1981). Along with the absence of molded and germinated seeds, this confirms the potential to produce in southwestern Québec grain amaranth of quality comparable to that obtained in other regions. Mycotoxins should however be quantified. Toxinogenic species of *Fusarium* have been isolated from grain amaranth (Bresler et al., 1995) and *Fusarium* is known to be have a greater incidence in more humid environments (Soriano and Dragacci, 2004).

Some of the genotypes evaluated did not have white seeds (Table 3.2), which are preferred by the food industry. Although dark seeds are usually not used for human consumption, these genotypes could be used in breeding programs, as a source of specific traits. PI 451711, a black seed genotype, had the lowest number of days to anthesis of all genotypes evaluated. PI 576447, a brown seed genotype, had the lowest lodging incidence. It is a highly branched genotype with sturdy stems, which also had a high protein content (i.e., 16.1%). PI 619250 has unusual and attractive golden colored seeds; its acceptance for human consumption should be investigated.

No precise data were recorded as to the diversity and incidence of insects among the different genotypes. However, aphids were present in some genotypes and tarnished plant bugs were observed in all genotypes, with some appearing to be more tolerant than others. No fungal or bacterial diseases were observed.

3.4.3 Experiment 2. Mechanically managed four row plots

Significant differences ($P < 0.05$) were observed between the most commonly grown cultivar in the USA, 'Plainsman', and at least one other genotype for grain yield, grain moisture at harvest, days to anthesis, branch number, plant height, and lodging (Table 3.7). Grain yield ranged between 432 and 979 kg ha^{-1} with an average of 649 kg ha^{-1}. 'Plainsman' yielded 801 kg ha^{-1}, and only one genotype, D70-1, yielded more, with 979 kg ha^{-1}. The overall low yields we observed are probably due to high weed pressure, particularly in field C. Weeding being done mechanically, complete control was difficult to achieve, weeds being problematic particularly within the rows.

Grain moisture at harvest ranged between 23.8 and 28.1%, with an average of 25.8%. The grain moisture at harvest of 'Plainsman' (28.1%) was higher than that of all other genotypes, except for RRC 1386 which also was 28.1%. After the first killing frost in the fall, a dry down period of at least 10 days is needed in order for plants to become harvestable mechanically (Sooby et al., 2005). The harvesting conditions in both years were suboptimal, due to very wet conditions in October (Table 3.1). There were only 15 days without rain in October 2005 and 13 in October 2006 which restricted time where harvest was possible. We noticed that once plants have been killed by a frost and dried to

a certain point, they re-dry quickly even after a heavy rain (i.e., in about 1 day). This is certainly an advantage when only few consecutive days of dry weather are available. It was the case in this experiment: there were 43 mm of rain on October 28, 2 mm on October 29, with rain stopping in the middle of the night, and harvesting was possible in the afternoon of October 30, after only 8 hours of clear weather. Under dryer, more typical fall conditions (Table 3.1), seed moisture could be expected to be lower. However, drying will always be necessary to bring moisture content down to approximately 10-12% (Sooby et al. 2005).

The number of days to anthesis ranged between 63 and 69 days, with an average of 66 days (Table 3.7). This is a short range and probably does not affect the yield potential to any great extent. 'Plainsman' did not differ significantly from the genotypes with the shortest number of days to anthesis. Plant height ranged from 143 cm ('Plainsman') to 168 cm (RRC 1027 and RRC 1386) (Table 3.7). The three genotypes (i.e., R-158, RRC 1027, and RRC 1386) that yielded less than 'Plainsman' were also the tallest and the ones that lodged the most; their use as cultivar under southwestern Québec conditions should probably be discouraged. On the other hand, the height and lodging score of 'Plainsman' were amongst the lowest. All genotypes evaluated had either no branch or few undeveloped branches.

This experiment demonstrates the feasibility of entirely mechanized production of grain amaranth under southwestern Québec climatic conditions. Yields we observed are similar to those achieved in grain amaranth trials conducted in Delhi, Ontario from 1988 to 1990 (Peter White, personal communication, 2007). In these trials, grain yields of 'Plainsman' ranged between 249 kg ha^{-1} in 1988 and 988 kg ha^{-1} in 1990. This increase in yields was attributed to the development of a better expertise in weed control and overall management of the crop over the years. The highest grain yield observed was 1272 kg ha^{-1} with K432 in 1990.

It has been suggested that "dramatic improvement in yield [...] should be possible with a dedicated breeding program, [since] currently available cultivars are the result of just first or second generation breeding cycles from ecotype selections" (Brenner et al. 2000). Three elements have set the basis for the development of high-performance grain amaranth cultivars: 1) the discovery of male-sterile *A. hypochondriacus* (Peters and Jain,

1987; Gudu and Gupta, 1988); 2) the possibility of interspecific hybrids exhibiting high-parent biomass heterosis of up to 40% (Lehmann et al., 1991); and 3) the discovery of non-shattering lines (Brenner, 2002). Given the adaptability and yields that we observed at this point for grain amaranth in southwestern Québec, we think that it should be possible to make grain amaranth competitive with at least small cereals in terms of yields. The genotypes evaluated in this study were all obtained through the USDA, but as noted by Brenner et al. (2000), several other lines have recently been developed around the world (e.g., in Europe). The performance of these lines under southwestern Québec conditions should now be evaluated and compared to the ones developed in the USA.

3.5 Conclusion

Although all the genotypes studied over two years of experimentation matured, some demonstrated more potential for southwestern Québec. PI 538323 (K432), PI 538324 (K433), PI 538326 (D70-1) and PI 558499 ('Plainsman') are genotypes that seem particularly adapted to local conditions. Although 'Plainsman' was only compared in small plot trials to six other genotypes, it performed relatively well under local climatic conditions, combining several desirable characteristics including high yield potential, low incidence of lodging, early anthesis, and a relatively high harvest index. It is also the most widely available grain amaranth cultivar. Thus at this point it should be considered as a potential cultivar by anyone considering commercial production of grain amaranth in southwestern Québec. Mechanical combining of the crop was possible, even under wet fall conditions we experienced, which suggest that the crop should be harvestable without major problems in most years. Drying of the seeds is however necessary. As no herbicide is currently available for grain amaranth, good mechanical weed control is essential. A study of the mycotoxin production potential should be conducted to determine the safety of grain amaranth grown under more humid conditions. Although the present study demonstrated that grain amaranth could be produced in southwestern Québec, it will remain a very marginal crop in the foreseeable future. For its production to increase there is a need for major food processors to integrate grain amaranth in widely distributed products. Also a dedicated breeding program would likely be necessary to improve yields to levels similar to at least small grain cereals.

3.6 Tables

Table 3.1. Average temperature and precipitation in Montreal[z] in 2005 and 2006 and 30-year averages.

Month	30 year average		2005		2006	
	Temperature (°C)	Total precipitation (mm)	Temperature (°C)	Total precipitation (mm)	Temperature (°C)	Total precipitation (mm)
May	13.4	76	11.9 (-1.5)[y]	43 (-33)	14.5 (1.1)	173 (97)
June	18.2	83	21.5 (3.3)	129 (46)	19.2 (1.0)	104 (21)
July	20.9	91	22.2 (1.3)	126 (34)	22.6 (1.7)	135 (44)
August	19.6	93	21.7 (2.1)	134 (41)	19.3 (-0.3)	154 (61)
September	14.6	93	17.4 (2.8)	113 (20)	15.0 (0.4)	65 (-28)
October	8.1	78	10.1 (2.0)	198 (120)	7.9 (-0.2)	179 (101)

[z] Weather station located about 12 km east of the Sainte-Anne-de-Bellevue study site.
[y] Deviation from 30-year average

Table 3.2. Description of grain amaranth genotypes grown in Sainte-Anne-de-Bellevue, QC, Canada.

Genotype[z]	Common descriptive	Species	Origin[y]	Seed color	Flower color
PI 451711		Amaranthus cruentus	Mexico	Black	red
PI 477912	RRC 416	*A. cruentus*	Mexico	White	green
PI 477913	RRC 1011	*A. cruentus*	Mexico	White	green
PI 515959	Montana-3	*A. cruentus*	Montana	White	green
PI 525498	MT-5	*A. cruentus*	Montana	White	green
PI 538255	Amont	*A. cruentus*	Montana	White	green
PI 538319	K266	*A. cruentus*	Pennsylvania	White	green
PI 538320	K283	*A. cruentus*	Pennsylvania	White	pink
PI 538321	K436	*A. cruentus*	Pennsylvania	White	red
PI 538323	K432	*A. hybrid*	Pennsylvania	White	green
PI 538324	K433	*A. hybrid*	Pennsylvania	White	green
PI 538325	K593	*A. hybrid*	Pennsylvania	White	red
PI 538326	D70-1	*A. hybrid*	Pennsylvania	White	red
PI 538327	D136-1	*A. hybrid*	Pennsylvania	White	green
PI 558499	Plainsman	*A. hypochondriacus*	Pennsylvania	White	red
PI 568179	Ames 12991	*A. hybrid*	Iowa	White	red
PI 576447	Ames 13446	*A. cruentus*	Nigeria	Brown	green
PI 604666	RRC 1027	*A. cruentus*	Pennsylvania	White	orange
PI 605354	R-158	*A. cruentus*	Pennsylvania	White, translucent	red
PI 606767	Ames 8272	*A. cruentus*	Pennsylvania	White	orange
PI 606797	A200D	*A. cruentus*	Illinois	White	green
PI 606799	RRC 1017	*A. cruentus*	Pennsylvania	White	red & green
PI 618962	Ames 2015	*A. cruentus*	Benin	Dark brown	green
PI 619250	Ames 2265	*A. hypochondriacus*	Pennsylvania	Golden	red, green & pink
PI 628780	RRC 423	*A. cruentus*	Mexico	White	purple-red
PI 628781	RRC 444	*A. cruentus*	Mexico	White	green
PI 628782	RRC 446	*A. cruentus*	Mexico	White	green
PI 628783	RRC 776	*A. cruentus*	Mexico	White	red & green

Table 3.2. continued

PI 633584	RRC 27	*A. cruentus*	China	Dark brown	red
PI 636182	RRC 1386	*A. cruentus*	Argentina	White	dark pink

[z] PI number refers to plant introduction number from the US National Genetic Resources Program.
[y] Origin is that of the donor to the US National Genetic Resources Program.

Table 3.3. Description of fields used in 2005 and 2006 in Sainte-Anne-de-Bellevue, QC, Canada.

Field	P (kg ha^{-1})	K (kg ha^{-1})	pH	Texture
A	335	408	6.70	Loamy sand
B	195	193	5.85	Sandy clay loam
C	193	226	6.58	Sandy loam
D	155	270	5.50	Clay loam

Table 3.4. Agronomic traits of 29 genotypes grown in single row plots in 2005 and 2006 in Sainte-Anne-de-Bellevue, QC, Canada. Results illustrate year and genotypes main effects.

Main factor	Grain yield (g DM plant^{-1})	Total biomass (g DM plant^{-1})	Harvest index	Days to anthesis	Branch number	Height (cm)	Lodging (0-10)[z]	Crude protein (%N × 5.85)
Year								
2005	22	129	17	71	9	158	5	15
2006	39	188	21	64	7	199	8	14
$LSD_{0.05}$	3.2	59.0	1.0	1.3	ns	41.0	0.7	0.3
Genotype[y]								
PI 451711	30	164	19.4	57	6	169	8	14.4
PI 477912	28	160	18.5	62	1	196	9	14.7
PI 477913	30	174	18.4	68	4	196	6	14.1
PI 515959	31	175	17.9	69	3	194	5	12.7
PI 525498	24	143	17.6	64	4	186	10	13.7
PI 538255	27	140	21.1	68	3	190	8	13.5
PI 538319	33	155	21.9	69	6	184	8	13.8
PI 538320	26	137	19.7	62	4	168	3	13.8
PI 538321	26	153	17.5	69	6	177	4	14.6
PI 538323	23	110	22.3	56	12	110	3	16.9
PI 538324	22	105	21.7	56	12	108	3	15.9
PI 538325	38	172	22.2	68	17	158	8	14.7
PI 538326	22	106	21.5	71	13	127	5	14.8
PI 538327	16	135	12.3	79	16	138	3	14.5
PI 558499	28	131	22.1	63	13	143	7	14.0
PI 576447	24	134	17.8	64	21	167	2	16.1
PI 604666	23	151	16.0	75	4	202	8	15.0
PI 605354	29	150	19.9	69	17	179	9	12.5
PI 606767	21	127	16.9	73	1	198	6	14.9
PI 606797	31	163	19.6	65	2	200	7	12.8
PI 606799	28	180	16.0	71	1	217	8	13.7
PI 618962	22	130	18.1	72	18	180	4	14.9

Table 3.4. continued.

PI 619250	23	188	13.2	68	15	160	7	14.4
PI 628780	24	141	17.8	71	2	206	8	14.6
PI 628781	33	174	20.5	64	2	203	8	13.3
PI 628782	26	130	21.7	64	2	181	8	13.6
PI 628783	32	167	20.1	69	4	201	7	13.3
PI 633584	15	148	9.3	82	15	220	7	14.4
PI 636182	34	182	19.3	70	3	217	8	14.3
Average	27	149	18.6	67	8	179	6	14.3
Range	15 - 38	105 -188	9.3 - 22.3	56 - 82	1 - 21	108 - 220	2 - 10	12.5 - 16.9
$LSD_{0.05}$	12.2	56.4	3.93	4.8	3.8	17.7	2.7	1.32
ANOVA[x]	---------- P-value ----------							
Year	*	*	*	*	ns	*	*	*
Genotype	*	*	***	***	***	***	***	***
G × Y	**	ns	***	ns	ns	*	ns	ns

[z] Proportion of plants lodged

[y] PI numbers refer to plant introduction numbers from the US National Genetic Resources Program.

[x] ns, not significant ($P > 0.05$), and statistically significant at the * 0.05, ** 0.01, and ***0.001 levels.

Table 3.5. Agronomic traits of 29 genotypes grown in single row plots in 2005 and 2006 in Sainte-Anne-de-Bellevue, QC, Canada. Results illustrate significant year × genotype interactions.

Genotype	Year					
	2005			2006		
	Grain yield (g DM plant^{-1})	Harvest Index	Height (cm)	Grain yield (g DM plant^{-1})	Harvest Index	Height (cm)
PI 451711	18	13.2	162	49	25.6	177
PI 477912	19	13.2	189	43	23.9	202
PI 477913	18	12.3	176	50	24.7	216
PI 515959	24	15.4	183	45	20.5	205
PI 525498	16	13.8	165	39	21.5	208
PI 538255	18	17.2	165	45	24.9	215
PI 538319	33	24.0	156	38	20.5	213
PI 538320	16	15.1	151	42	24.4	184
PI 538321	17	13.2	150	42	21.9	204
PI 538323	15	17.9	99	36	26.6	120
PI 538324	16	16.7	102	30	26.6	114
PI 538325	39	23.3	146	37	21.4	171
PI 538326	22	22.1	112	24	21.6	142
PI 538327	14	14.5	105	18	10.2	170
PI 558499	23	19.6	123	36	24.7	163
PI 576447	21	19.5	136	33	16.0	198
PI 604666	20	16.3	177	30	16.3	227
PI 605354	34	22.8	160	26	17.3	199
PI 606767	18	16.8	182	25	18.0	214
PI 606797	20	14.0	189	47	25.3	212
PI 606799	19	13.7	200	48	18.4	234
PI 618962	27	23.6	150	24	12.7	210
PI 619250	20	12.4	139	32	14.0	181
PI 628780	19	17.1	179	32	19.0	234
PI 628781	15	14.3	181	73	26.7	224

Table 3.5. continued.

PI 628782	13	15.2	161	53	28.3	201
PI 628783	18	15.7	178	57	24.5	225
PI 633584	15	12.4	186	20	7.8	254
PI 636182	21	15.9	190	56	22.9	244
Average	20	17	158	39	21	199
Range	13 – 39	12 – 24	99 - 200	18- 73	8 – 28	114- 254
$LSD_{0.05}$	ns[y]	6.06	28.6	17.9	5.15	21.4
P-value	ns	***	***	***	***	***

[z]PI numbers refer to plant introduction numbers from the US National Genetic Resources Program.
[y] ns, not significant ($P > 0.05$), and statistically significant at the * 0.05, ** 0.01, and ***0.001 levels.

Table 3.6. Correlations among agronomic traits of 29 genotypes of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada.

	Grain yield	Total biomass	Harvest index	Days to anthesis	Branch number	Plant height	Lodging score
Grain yield	1.00	0.83***[z]	0.71***	-0.34***	-0.14[ns]	0.40***	0.34***
Total biomass		1.00	0.24**	-0.14 [ns]	-0.07[ns]	0.56***	0.28**
Harvest index			1.00	-0.44***	-0.10[ns]	-0.02[ns]	0.22**
Days to anthesis				1.00	0.20**	-0.08[ns]	-0.60 [ns]
Branch number					1.00	-0.46***	-0.21**
Plant height						1.00	0.38***
Lodging score							1.00

[z]ns, not significant ($P > 0.05$), and statistically significant at the * 0.05, ** 0.01, and ***0.001 levels.

Table 3.7. Agronomic performance of 7 grain amaranth genotypes grown in four rows plots in 2006 at two sites in Sainte-Anne-de-Bellevue, QC, Canada.

Genotype	Common descriptive	Yield (kg DM ha^{-1})	Grain moisture at harvest (%)	Days to anthesis	Branch number	Plant height (cm)	Lodging (0-5)	Population (1000 plants ha^{-1})
PI 558499	Plainsman[z]	801	28.1	63.0	0	143	0.8	553
PI 515959	Montana-3	720	**25.7**	**65.8**	0	153	0.9	704
PI 538325	K593	641	**23.8**	**69.3**	**0.6**	146	0.9	449
PI 538326	D70-1	**979**	**25.3**	**68.5**	0.5	143	0.8	577
PI 604666	RRC 1027	**432**	**25.4**	**68.4**	0	**168**	**2.0**	458
PI 605354	R-158	**466**	**25.2**	63.3	0	156	**2.1**	465
PI 636182	RRC 1386	**503**	28.1	64.2	0	**168**	1.6	612
	Average	649.0	25.8	66.1	0.2	154.5	1.3	545.5
	Range	432 — 979	23.8 — 28.1	63.0 — 69.3	0 — 0.6	143 — 168	0.8 — 2.1	458 — 704
	$LSD_{0.05}$	132.9	1.28	1.43	0.44	13.44	0.83	182.1

[z] 'Plainsman' being the most commonly grown cultivar in the USA, it was considered the control in Dunnett multiple comparison tests; means in bold are significantly different from 'Plainsman' ($P < 0.05$).

PREFACE TO CHAPTER 4

In chapter 3, the adaptation and yield potential of grain amaranth grown in southwestern Québec were evaluated. It was concluded that grain amaranth cultivation is possible in southwestern Québec. Yields were low, mainly because of the low harvest index of grain amaranth. Among the 29 genotypes evaluated, variation was found for all of the agronomic traits studied. It was suggested that a dedicated breeding program would likely be necessary to improve yields to levels similar to at least small grain cereals. Chapter 3 was submitted to the *Canadian Journal of Plant Science* for publication under the title *Development and yield potential of grain amaranth genotypes grown in southwestern Québec.*

Chapter 4 presents the results of three experiments conducted in Sainte-Anne-de-Bellevue, each replicated over three site-years. Given that cultivation of grain amaranth is considered possible, this part represents the next logical step in the introduction of the crop, i.e., the evaluation of different management practices. Chapter 4 was submitted to *Agronomy Journal* for publication under the title *Evaluation of optimal management practices for grain amaranth production in eastern Canada.*

Chapter 4. EVALUATION OF OPTIMAL MANAGEMENT PRACTICES FOR GRAIN AMARANTH PRODUCTION IN EASTERN CANADA

4.1 Abstract

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous pseudo-cereal crop which was widely cultivated in pre-Columbian America. It was successfully introduced in many regions with contrasting environmental conditions. The introduction of grain amaranth in eastern Canada would represent an opportunity for diversification. This paper presents an evaluation of management practices for grain amaranth grown in this region. Three field experiments replicated in three environments were conducted to evaluate the following factors: i) seeding date (mid-May, early-June and mid-June) and cultivar ('K432', 'K593' and 'Plainsman'); ii) row spacing (38, 58 and 76 cm) and seeding rate (1, 2 and 4 kg ha^{-1}) and iii) nitrogen fertilization rate (0, 50, 100, 150 and 200 kg N ha^{-1}) and cultivar ('D136' and Plainsman). Seeding date affected grain yield in only one out of three environments, with the earlier date resulting in the highest yields. Cultivars differed in yield in only one of three environments, with Plainsman resulting in highest yields. Later seeding dates resulted in higher seed moisture at harvest in all environments. Seeding rate and row spacing did not affect grain yield, but row spacing affected grain moisture at harvest, with narrower rows resulting in grains with lower moisture content. Nitrogen fertilization increased yield and lodging in only one environment. Seed moisture and plant height were positively related to nitrogen fertilization in all environments. Cultivar D136 yielded more than Plainsman in 2005, and less in 2006. Grain amaranth production in eastern Canada thus seems possible, management practices having limited impact on grain yield, which averaged 923 kg ha^{-1} across all experiments and environments.

4.2 Introduction

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous pseudo-cereal crop which was widely cultivated in pre-Columbian America (Sauer, 1950). Its cultivation however decreased to a point of near extinction in the early 20th century due to pressure from the conquistadors, who disliked its use in native ceremonies (Sauer, 1950). Interest for grain amaranth was revived in the 1970's, after reports of its high protein and lysine content (Downton, 1973). This plant is adaptable to a wide range of environments and has been successfully introduced in several regions with contrasting environmental conditions (National Academy of Science, 1984). In the USA, range of cultivation of the crop extends as far north as North Dakota (Henderson et al., 1998), with most production being concentrated in Nebraska.

Seeding of grain amaranth in northern USA usually takes place from mid-May to mid-June (Henderson et al., 1998). Due to the requirements for sufficient moisture, high soil temperature, and shallow seeding depth, the time where optimal emergence conditions are met is more limited for grain amaranth than for larger seeded crops (Weber et al., 1987). It has been suggested that seeding should be done approximately two weeks after the last spring frost, seeding in early-June being recommended for the Northern Great Plains (Henderson et al., 1998). Under northern latitudes in the USA, the usual harvesting method for grain amaranth consists in harvesting approximately 10 days after the first killing frost, which allows for a good dry down of the plant (Sooby et al., 2005). Freezing therefore acts as a desiccant.

Plant population density studies conducted with grain amaranth often reports conflicting results; environmental factors most likely accounting for a great part of the differences observed. It has been suggested that water availability should determine optimal plant population, higher populations requiring greater amounts of water (Weber et al., 1987). In North Dakota, Henderson et al. (2000) found a significant environment × plant density effect on grain yield suggesting that different population densities should be adopted in different environments. The plasticity of the plant's morphology may limit its response to seeding rate and row spacing (Henderson et al., 2000). As no herbicide is currently available for weed control in grain amaranth, mechanical weed control must be practiced, which limits the options for row width (Sooby et al., 2005). Narrower row

spacing has been suggested as a means of reducing weed pressure, due to the resulting earlier canopy closure (Peiretti and Gesumaria, 1998), this however makes mechanical weed control difficult. Others have however argued that wider rows result in increased competition in the row, reduced plant height, later maturity (Myers, 1996) and increased lodging (Henderson et al., 2000).

Soil fertility requirements of grain amaranth must be defined for the different environments where it is cultivated. It was suggested that more nitrogen may be required in higher rainfall areas (Stallknecht and Schulz-Schaffer, 1993). In Missouri, Myers (1998) evaluated nitrogen fertilization rates between 0 and 180 kg ha^{-1}; yields increased with N fertilization up to 90 kg ha^{-1}. Nitrogen fertilization however increased seed moisture at harvest and number of days to anthesis. In Minnesota, Elbehri et al. (1993) obtained yield response with nitrogen fertilization up to 180 kg ha^{-1}, no responses being observed for phosphorus and potassium. Yields increased from 1094 kg ha^{-1} without nitrogen application, to 1428 kg ha^{-1} with N applied at 180 kg N ha^{-1}.

Grain amaranth belongs to a different family than all of the main crops grown in eastern Canada and could thus be a good addition to existing crop rotations. Grain amaranth seems adapted to eastern Canada, as demonstrated by preliminary trials in southwestern Québec and southern Ontario, where yields comparable to those reported from northern USA were obtained (Gélinas and Seguin, 2007, submitted). However, management strategies for the region have not been researched. The objectives of this research were therefore to determine appropriate i) seeding dates, ii) seeding rate and row spacing and iii) nitrogen fertilization rate for grain amaranth when grown in eastern Canada.

4.3 Materials and methods

4.3.1 Seeding date and cultivar experiment

This experiment was conducted at two sites in 2005 and one in 2006 (Table 4.1). A randomized complete block design with split-plot restriction and four blocks was used. Main plot consisted of three seeding dates (mid-May, early-June and mid-June) and sub-plot, three cultivars ['K432' (*Amaranthus hybrid*), 'K593' (*A. hybrid*) and 'Plainsman'

(*A. hypochondriacus*)]. Seeding dates were targets and actual dates varied (Table 4.1). Seeding was done at a depth of approximately 2.5 cm and a rate of 2 kg ha^{-1} using a disk drill (Fabro, Swift Current, SK, Canada). Plots were 5 m long, with four rows spaced at 76 cm. The middle two rows of each plot were harvested mechanically using a self-propelled harvester (Wintersteiger, Saskatoon, SK, Canada). Weed control was done manually. Nitrogen, phosphorus and potassium fertilization was done based on soil tests according to local recommendations for sorghum (CRAAQ, 2003), no recommendations being available locally for grain amaranth. Plots were harvested on average 10 days after the first killing frost (Table 4.1). All harvested seeds were dried at 50 °C until moisture content stabilized, to express yields on a DM basis and determine grain moisture at harvest. Weather data was retrieved from a nearby station (Table 4.2).

4.3.2 Effects of row spacing and seeding rate

This experiment was conducted at one site in 2005 and two in 2006 (Table 4.1). A randomized complete block design with split-plot restriction and four blocks was used, main plot consisting in three row spacings (38, 58 and 76 cm) and sub-plot, three seeding rates (1, 2 and 4 kg ha^{-1}). Plots were 5 m long, number of rows differing in different row spacing treatments. Plots at 38, 58 and 76 cm row spacing respectively had four, six and four rows. Harvested rows were, the middle two for the 76 cm plots, three in the 58 cm plots, and all four in the 38 cm plots. Seeding, harvesting, seed drying methods and fertilization were as previously described for the seeding date and cultivar experiment. The cultivar Plainsman was used.

4.3.3 Nitrogen fertilization rate and cultivar experiment

This experiment was conducted at two sites in 2005 and one in 2006 (Table 4.1). The experiment was laid out as a randomized complete block design with split-plot restriction and four blocks. Main plot consisted in five nitrogen fertilization rates (0, 50, 100, 150 and 200 kg ha^{-1}) and sub-plot, two cultivars ('D136' (*A. hybrid*) and Plainsman). Nitrogen fertilizer was applied at seeding in the form of calcium ammonium nitrate (27.7 %N, 4.6% Ca, and 2.4% Mg). Seeding, harvesting, seed drying methods, and phosphorus

and potassium fertilization were as previously described for the seeding date and cultivar experiment.

4.3.4 Data collection

The number of days to anthesis was defined as the number of days between seeding and the day when 50% of the plants were showing first signs of anthesis. The remaining data was collected shortly prior to harvesting. Plant height was measured as the distance between soil level and the top of the main inflorescence. Number of branches per plant was the number of side shoots having at least one visible node. Measurements were made on 10 plants per plot. Plant population was determined by doing three random counts in the middle two rows of each plot on a 36 cm length. Lodging was recorded on the day of harvest and was determined as the fraction of the total number of plants in the plot bending at an angle greater than 30°. A visual evaluation was done using a 0 to 5 scale, 0 referring to absence of lodging and 5 to all plants in the plot being lodged.

4.3.5 Statistical analyses

All statistical analyses were done using SAS 9.1 (SAS Institute Inc., 2003). Statistical models and appropriate F-tests were elaborated after McIntosh (1983). Every site-year was considered an environment, each experiment being replicated in three environments (Table 4.1). When the model and main effects or interactions were significant, multiple comparisons were carried out with Scheffe's test. Regression analyses were carried out for the nitrogen rate experiment; when a nitrogen rate × environment interaction was significant, analysis was done separately for each environment. Linear and quadratic regression coefficients were tested. When the quadratic coefficient was significant, both linear and quadratic were kept in the equation. Statistical significance level was set at 0.05 for all tests.

4.4 Results and discussion

4.4.1 Climate data and general observations

The two years of experimentation differed in terms of climatic conditions (Table 4.2). Compared with 2006, 2005 was warmer; precipitations were generally comparable, except for May which was dryer, and September which was wetter in 2005. Precipitations in both years were greater than the 30-yr average in October, the month during which harvest took place. The last spring frosts were on May 13 and April 28, in 2005 and 2006, respectively, and first fall frosts were on October 21 and October 22 in 2005 and 2006, respectively. In both years and for both experiments, plants from all cultivars under evaluation matured before the first killing frost, indicating that grain amaranth production in eastern Canada is possible.

No precise data was recorded on the diversity and incidence of insects among the different cultivars. However, aphids were present in some cultivars and tarnished plant bugs were observed in all cultivars, some appearing to be more tolerant than others. Tarnished plant bugs were more prevalent in 2005 than 2006. In the more humid fields, cultivar K593 was sometimes affected by a fungal disease hypothesized to be *Pythium aphanidermatum* stem canker (C. Block, personnal communication). The disease caused dry, dark colored cankers to develop, which spread 15-45 cm on the bottom of the stem. The infected plants eventually lodged and died. These symptoms are characteristic of *Pythium aphanidermatum* stem canker (Block et al., 2002)

4.4.2 Seeding date and cultivar experiment

4.4.2.1 *Environment*

Environment main effects were observed for grain yield, grain moisture, branch number and days to anthesis, however it affected variables mainly through interactions with seeding date and genotype (Table 4.3). Grain yield was highest in 2006 at site A (i.e. 1113 kg ha^{-1}), intermediate at site B in 2005 (i.e. 876 kg ha^{-1}) and lowest at site A in 2005 (i.e. 647 kg ha^{-1}). Grain moisture at harvest also differed significantly across environments. The difference between site A and site B in 2005 is due to the later harvest

date of site B, which allowed for four extra days of drying (Table 4.1). Fitterer et al. (1996) reported decreasing seed moisture with later harvesting dates, the difference being the greatest after a killing frost. They also reported yield losses with later seeding dates, which they attributed to seed shattering. Yield losses with later harvesting however have to be weighed against reduced drying costs.

4.4.2.2 Seeding date

The effect of seeding date varied depending on the environment for all variables except plant height, which was not affected by seeding date (Table 4.3). Grain yield was only affected by seeding date at site A in 2006, where the highest yield occurred with the earliest seeding date (i.e., mid-May, 1398 kg ha^{-1}). Henderson et al. (1998), in North Dakota and Aufhammer et al. (1995), in southern Germany, observed an effect of seeding date on grain yield in all of the five environments they evaluated, the seeding date resulting in greatest yield not always being the same in different environments.

In all environments, later seeding dates resulted in higher grain moisture at harvest (Table 4.4). More humid grain at harvest can decrease grain quality, by favoring microbial growth. Gimplinger et al. (2007) reported a positive correlation between grain amaranth moisture at harvest and number of colony forming units of aerobic mesophilic bacteria. They also observed differences between cultivars, which they attributed to differing environmental conditions during maturation and differences in inflorescence architecture. As climate in eastern Canada is particularly humid during the fall, it would be important to conduct studies on the microbial status of the grain produced. Mycotoxin contamination should also be examined, as toxinogenic species of *Fusarium* have been isolated from grain amaranth (Bresler et al., 1995). *Fusarium* is known to have a greater incidence in more humid environments (Soriano and Dragacci, 2004).

In all three environments, we noted that unlike plants seeded in mid-May, plants seeded in mid-June had not started to dry down by the date of first frost. The first and second seeding dates at site B in 2005 are the only ones that resulted in grain moisture lower than 20%. Interestingly, a significant negative correlation was observed between

grain yield and grain moisture at harvest ($r = -0.39$, $p < 0.0001$), indicating that cultivars and seeding date favoring high yields might also result in lower drying costs.

Plant height was not affected by seeding date in any environment. This suggests that plants seeded in mid-June grew at a much faster rate than plants sown in mid-May. It appeared that this faster growth rate of later seeded plants resulted in weaker stems, as later seeding dates resulted in increased lodging in all environments (Table 4.4). Another indicator of this was the significant negative correlation found between days to anthesis and lodging ($r = -0.50$, $P < 0.0001$). Aufhammer et al. (1995) reported a higher nitrogen concentration in vegetative parts with later seeding. Grain nitrogen concentration was also lower with later seeding dates, thus reducing seed quality. They attributed this effect to a lack of nitrogen translocation with later seeding dates. The increased lodging with later seeding dates we observed was probably due to the fact that the stems were less mature and lignified, and thus were weaker than with earlier seeding dates. Henderson et al. (1998) also reported increased lodging with later seeding date in one out of two environments in North Dakota. Later seeding dates also markedly reduced number of days to anthesis in all environments (Table 4.4). Similar results were observed in North Dakota by Henderson et al. (1998).

There was a strong seeding date × environment crossover interaction for plant population at harvest (Table 4.3). At both sites in 2005, the early-June seeding date resulted in much better stand establishment and hence higher plant population. However, in 2006, the reverse was observed, late-June and mid-May seedings resulting in the best stand establishment (Table 4.4). Branch number was also affected by a seeding date × environment crossover interaction. This may indirectly reflect a strong negative correlation between branch number and plant population ($r = -0.59$, $p < 0.0001$), which suggests that the greater interplant space at lower populations promoted branching. Henderson et al. (1998) also observed a seeding date × environment interaction for plant population, with later dates however consistently resulting in higher plant population at harvest.

Soil temperature for optimal grain amaranth germination has been estimated to range between 18.5 and 24°C, while seeding depth should in general not exceed 2.5 cm (Webb et al., 1987). Ideal germinating conditions are therefore a warm and moist soil at

the surface. In spring in eastern Canada, such conditions typically happen on a few occasions, lasting only few days each time. This suggests that it is preferable to wait for the proper field conditions, rather than trying to seed on a particular, recommended date. Nevertheless, when possible earlier seeding is preferable in that it allows for better seed drying, minimizes lodging, and may result in higher grain yields.

4.4.2.3 *Cultivar*

There was a significant difference in yield among cultivars in only one environment, with Plainsman yielding more than either K432 or K593 at site A in 2005 (Table 4.5). Absence of difference in grain yield between cultivars has been reported in other regions (Pospisil et al., 2006; Gimplinger et al., 2007), however several studies reported yield differences among cultivars, or environment × cultivar interactions (Elbehri et al., 1993; Henderson et al. 1998; Henderson et al., 2000). Cultivars however differed in grain moisture at harvest, Plainsman producing the most humid seeds, followed by K432, and K593. Interestingly, K593 had the lowest grain moisture in all three environments, and also had the highest number of days to anthesis (Table 4.5). Cultivar K432 was shorter and easier to harvest mechanically than Plainsman and K593 at both sites in 2005, differences in height in 2006 were not significant. However, there were no differences in lodging between the cultivars. Cultivars K432 and K593 produced significantly more branches than Plainsman and this difference was more pronounced at site A in 2006.

4.4.3 Row spacing and seeding rate experiment

4.4.3.1 *Environment*

Environment was the factor affecting the most variables in this experiment (Table 4.6). Lower grain yields at site A in 2005 and at site C in 2006 were probably due to compaction and poor drainage in these two environments. Poor drainage at site C in 2006, combined with heavy rainfall in October (Table 4.2) also delayed harvesting. This resulted in significantly lower grain moisture at site C in 2006 compared to the other two environments (Table 4.6). Lower yielding environments also produced the shortest plants.

The higher yielding environment, site A in 2006, also had the highest incidence of lodging, but lodging was overall low. Branch number was affected by a significant environment × row spacing interaction. This interaction reflected that branches were observed in only one environment, site A in 2006, where row spacing was positively related to branch number (Table 4.6). The number of days to anthesis was also considerably affected by environment (Table 4.6). Experiments seeded later had fewer days to anthesis (Table 4.2 and 4.6). This is in agreement with results from our seeding date experiment. Finally, site A in 2006 had a plant population approximately half of that observed in the other two environments.

4.4.3.2 Row spacing and seeding rate

Row spacing and seeding rate did not affect grain yield (Table 4.6). Such results are in accordance with Aufhammer et al. (1995), Myers (1996), Henderson et al. (2000), and Gimplinger et al. (2007), who also all failed to observe yield response to row spacing or seeding rate. Myers (1996) evaluated seeding rates ranging from 0.28 to 4.4 kg ha^{-1} at row spacing of 76 cm and attributed the lack of grain yield response to the fact that plants responded to high densities by self-thinning and lowering seed production per plant. On the other hand, Bhaskar et al. (1996) and Malligawad and Patil (2001) in India reported grain yield increases with increasing established plant population. Malligawad and Patil (2001) reported yield increases from 1858 to 3242 kg ha^{-1} with increase in plant population from 55 000 to 222 222 plant ha^{-1}. Such densities are extremely low and would be difficult to obtain using mechanical seeding under our conditions. For example, the lowest plant population observed in our experiments was 778 000 plants ha^{-1}, obtained with a seeding rate of 1 kg ha^{-1}.

Doubling seeding rate also nearly doubled plant population (Table 4.6). At a seeding rate of 4 kg ha^{-1}, plant population was slightly above 2 million plants ha^{-1}, which probably promoted interplant competition and thus might explain the resulting reduced plant height (Table 4.6). Henderson et al. (2000) observed a similar height reduction when manipulating plant population up to 272 000 plants ha^{-1}. Seeding rate also affected

the number of days to anthesis (Table 4.6), although differences were biologically insignificant.

Row spacing affected grain moisture at harvest, with narrower rows resulting in dryer grains (Table 4.6). This might be due to a more even plant distribution at narrower row spacing, allowing for a better airflow. The row spacing × seeding rate interaction observed for plant population reflects that with 38 cm row spacing and a seeding rate of 4 kg ha^{-1}, plant population was higher than at the same seeding rate for the other two row spacings. Interestingly, plant population was significantly higher with narrower rows, suggesting a weaker self-thinning effect at a row spacing of 38 cm than with spacings of 58 or 76 cm (Table 4.6). Self-thinning at narrow row spacing has been reported by Myers (1996).

4.4.4 Nitrogen rate and cultivar experiment

4.4.4.1 *Environment*

As in the seeding rate and row spacing experiments, environment affected all variables studied, through environment main effect and nitrogen rate × environment interactions, but mostly through cultivar × environment interactions (Table 4.7). Site A yielded more than site B, which was had a more fertile but lighter soil (Table 4.1). The difference in grain moisture between the two sites in 2005 was most likely due to the fact that the first fall frost was more severe at site A, resulting in a better dry down (Table 4.7). Plant height differed among environments, with plants at site A in 2006 being significantly taller. Lodging was overall low and was completely absent at site B in 2005. There was a slight, biologically insignificant difference in number of days to anthesis between environments (Table 4.7).

4.4.4.2 Nitrogen rate

Regression analyses were carried out with all environments pooled only for those variables not affected by a significant interaction involving environment (i.e., grain moisture at harvest, plant height and number of days to anthesis). For grain yield, lodging

and branch number, which were affected by a nitrogen rate × environment interaction, regression analysis was run for each environment separately. The regression coefficient for all environments pooled was only significant for plant height (Fig. 4.1), with increasing nitrogen fertilization rate resulting in increased plant height in a linear fashion. Such relation between nitrogen fertilization and plant height was also reported by Myers (1998) and Elbehri et al. (1993).

For the variables affected by a nitrogen rate × environment interaction, significant regression coefficients were found for grain yield (linear) and lodging (quadratic), at site A in 2006 only (Fig. 4.1). Elbehri et al. (1993) also reported a linear effect of nitrogen fertilization on grain yield and a quadratic effect on lodging, while Myers (1998) reported a linear effect of nitrogen fertilization on grain yield and lodging. The increased lodging caused by nitrogen fertilization makes mechanical harvesting less efficient and could decrease seed quality when inflorescences are in direct contact with the soil. Our results are in contradiction with Erley et al. (2005) who observed no effects of nitrogen fertilization on lodging; they however only evaluated nitrogen fertilization rate up to 120 kg N ha^{-1}. The nitrogen rate × environment interaction for branch number illustrates that branch number was not affected by N fertilization at either sites in 2005, but was positively affected by N at site A in 2006 (data not shown). Finally, increasing nitrogen fertilization resulted in slightly greater grain moisture (Table 4.7). This has economic importance, as the extra costs of drying and fertilizer have to be weighed against yield gain.

4.4.4.3 *Cultivar*

Cultivar main effects and/or cultivar × environment interactions affected several variables (Table 4.7). Cultivar D136 had lower yields than Plainsman at site A in 2006, the reverse was observed in 2005 (Table 4.8). Cultivar D136 is later maturing than Plainsman, it has superior grain moisture at harvest, greater number of days to anthesis and exhibited less dry down after frost. Its lower yield at site A in 2006 might therefore be explained by the cooler temperature prevailing in most months of that year (Table 4.2). D136 also has more branches and leaves, which when plants are not properly dried down,

can interfere with mechanical harvesting. This cultivar may therefore not be recommendable for use in eastern Canada due the short growing season and prevailing humid conditions.

The cultivar × nitrogen rate × environment interaction for grain yield illustrates the fact that at site A in 2005 and B in 2005 there were no effects of nitrogen fertilization on yield and that site A in 2006, there was a positive effect of N on yield (Fig. 4.1). Also at site A in 2006, cultivar D136 responded to N only up to 150 kg N ha^{-1}, whereas Plainsman kept responding up to 200 kg N ha^{-1}.

4.5 Summary and conclusions

According to our results, earlier seeding dates seem preferable, as they resulted in higher grain yield in one environment, and lowered seed moisture at harvest and minimized lodging in all three environments. Seeding rate and row spacing did not affect grain yield. However, row spacing affected grain moisture at harvest, with narrower rows resulting in dryer grains. Seeding in narrow rows might thus be preferable when better weed management strategies become available, however, as no herbicide is currently available for grain amaranth, the use of wider rows remains the only practical choice. Nitrogen fertilization increased grain yield in only one environment, however it also increased lodging. Seed moisture and plant height were also increased by nitrogen fertilization in all environments.

Optimal management practices for grain amaranth production in eastern Canada are mainly dictated by the relatively short growing season and prevailing humid conditions. Grain yield averaged 923 kg ha^{-1} across experiments and environments, which is comparable to yields obtained in North Dakota (Henderson et al., 1998). Eastern Canada could therefore be considered a potential area for grain amaranth production. However, unless markets can be developed, with major food processors integrating grain amaranth in widely distributed products, its production will remain marginal.

4.6 Tables and figure

Table 4.1. Description of field conditions and seeding and harvest dates of three grain amaranth field experiments conducted in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 and 2006.

Study	Year	Location	Soil characteristics					Seeding date	Harvest date
			Type	P ($kg\ ha^{-1}$)	K ($kg\ ha^{-1}$)	pH	Organic matter (%)		
1†	2005	Site A	Sandy clay loam	224	378	6.0	3.1	20, 31 May & 30 June	27 Oct.
1	2005	Site B	Sandy	524	408	6.7	4.0	20, 31 May & 30 June	31 Oct.
1	2006	Site A	Sandy clay loam	196	193	5.8	2.9	25 May, 7 & 22 June	27 Oct.
2‡	2005	Site A	Sandy clay loam	210	466	5.9	3.1	1 June	29 Oct.
2	2006	Site A	Sandy clay loam	274	354	6.3	3.0	13 June	27 Oct.
2	2006	Site C	Sandy clay loam	96	205	6.0	3.0	16 June	7 Nov.
3§	2005	Site A	Sandy loam	238	290	6.0	3.0	31 May	28 Oct.
3	2005	Site B	Sandy	524	408	6.7	4.0	31 May	29 Oct.
3	2006	Site A	Sandy clay loam	196	193	5.8	2.9	30 May	1 Nov.

† Seeding date and cultivar
‡ Seeding rate and row spacing
§ Nitrogen rate and cultivar

Table 4.2. Average temperature and precipitation in Montreal†, QC, Canada, in 2005 and 2006 and 30-year averages.

Month	30 year average		2005		2006	
	Temperature (°C)	Total precipitation (mm)	Temperature (°C)	Total precipitation (mm)	Temperature (°C)	Total precipitation (mm)
May	13.4	76	11.9 (-1.5)‡	43 (-33)‡	14.5 (1.1)	173 (97)
June	18.2	83	21.5 (3.3)	129 (46)	19.2 (1.0)	104 (21)
July	20.9	91	22.2 (1.3)	126 (34)	22.6 (1.7)	135 (44)
August	19.6	93	21.7 (2.1)	134 (41)	19.3 (-0.3)	154 (61)
September	14.6	93	17.4 (2.8)	113 (20)	15.0 (0.4)	65 (-28)
October	8.1	78	10.1 (2.0)	198 (120)	7.9 (-0.2)	179 (101)
May-Oct.		514		743		810

† Weather station located about 12 km east of the Sainte-Anne-de-Bellevue study site.
‡ Deviation from 30-year average

Table 4.3. Effects of seeding date and cultivar on grain yield and several agronomic traits of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 (2 sites) and 2006 (1 site). Results illustrate main effects, with means averaged over other factors.

Source of variation	Grain yield	Grain moisture	Plant height	Lodging	Branch number	Days to anthesis	Plant population
	kg ha^{-1}	%	cm	0 to 5‡	plant^{-1}	days	1000 plants ha^{-1}
Environment							
Site A 2005	647 b	31.9 a	123	1.3	5.5 b	62.1 a	595
Site B 2005	876 ab	20.3 c	131	1.9	3.4 b	59.4 b	608
Site A 2006	1113 a	26.3 b	135	1.3	9.9 a	62.0 a	490
Seeding date							
Mid-May	960	24.5	131	0.3	5.7	68.8 a	484
Early June	871	25.6	127	1.2	6.0	61.3 b	653
Mid-June	805	28.4	131	2.9	7.1	53.4 c	556
Cultivar							
K432	847	25.5 b	115	1.4	7.6	57.2 b	479
K593	805	24.1 b	136	1.5	7.8	66.0 a	505
Plainsman	984	29.0 a	137	1.5	3.4	60.3 ab	709
ANOVA	P value						
Environment (E)	**	***	NS	NS	***	***	NS
Seeding date (D)	NS	NS	NS	NS	NS	**	NS
D × E	***	***	NS	***	***	***	***
Cultivar (C)	NS	**	NS	NS	*	*	NS
C × E	NS	*	***	NS	**	***	NS
D × C	NS	NS	NS	NS	NS	*	NS
D × C × E	NS	*	NS	*	NS	*	NS

† Means within each column for a given main effect followed by different letters are significantly different from each other ($P \leq 0.05$).
‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table 4.4. Effects of seeding date on grain yield and agronomic traits of grain amaranth grown in three different environments in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 and 2006.

Environment	Seeding date	Grain yield	Grain moisture	Plant height	Lodging	Branch number	Days to anthesis	Plant population
		kg ha^{-1}	%	cm	0 to 5‡	plant^{-1}	days	1000 plants ha^{-1}
Site A 2005	mid-May	661	31.0 b	124	0.2 c	5.0 b	71.1 a	465 b
	early-June	643	30.7 b	117	0.9 b	2.8 b	61.9 b	1022 a
	mid-June	638	34.1 a	129	4.5 a	8.6 a	53.2 c	297 b
Site B 2005	mid-May	820	19.8 b	135	0.4 b	4.3	67.9 a	437 b
	early-June	964	18.8 b	126	0.8 b	2.2	60.1 b	857 a
	mid-June	844	22.4 a	131	2.8 a	3.8	50.2 c	531 b
Site A 2006	mid-May	1398 a	22.8 c	134	0.4 b	7.6	67.5 a	549 ab
	early-June	1007 ab	27.4 b	138	2.0 a	13.1	61.8 b	80 b
	mid-June	933 b	28.8 a	132	1.5 a	8.9	56.8 c	842 a

† Means within each environment for a given variable followed by different letters are significantly different from each other ($P \leq 0.05$).

‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.

Table 4.5. Effects of cultivar on grain yield and on agronomic traits of grain amaranth grown in three different environments in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 and 2006.

Environment	Cultivar	Grain yield	Grain moisture	Plant height	Lodging	Branch number	Days to anthesis	Plant population at harvest
		kg ha^{-1}	%	cm	0 to 5‡	$plant^{-1}$	days	1000 plants ha^{-1}
Site A 2005	K432	540 b	31.9 b	102 b	1.9	5.4 b	56.9 c	585
	K593	650 a	29.8 c	132 a	1.8	7.2 a	67.4 a	528
	Plainsman	751 a	34.1 a	136 a	1.8	3.8 b	61.8 b	671
Site B 2005	K432	848	18.7 b	110 b	1.3	5.2 a	53.7 c	533
	K593	780	18.2 b	138 a	1.3	4.0 a	64.3 a	587
	Plainsman	1000	24.0 a	143 a	1.3	1.2 b	60.1 b	705
Site A 2006	K432	1153	26.0 b	134	1.1	12.0 a	60.9 b	320 b
	K593	986	24.2 b	137	1.4	12.2 a	66.2 a	399 b
	Plainsman	1200	28.9 a	134	1.3	5.4 b	59.0 b	752 a

† Means within each environment for a given variable followed by different letter are significantly different from each other ($P \leq 0.05$).

‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.

Table 4.6. Effects of row spacing and seeding rate on grain yield and several agronomic traits of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 (1 site) and 2006 (2 sites). Results illustrate main effects, with means averaged over other factors.

Source of variation	Grain yield	Moisture	Height	Lodging	Branch number	Days to anthesis	Plant population
	kg ha^{-1}	%	cm	0 to 5‡	plant^{-1}	days	1000 plants ha^{-1}
Environment							
Site A 2005	754 b†	27.6 a	115 b	0.8 a	0.1 b	60.3 a	1688 a
Site A 2006	1189 a	28.1 a	135 a	1.0 a	1.9 a	52.0 b	874 b
Site C 2006	780 b	21.3 b	104 b	0.1 b	0.0 b	49.1 c	1604 a
Row spacing							
38 cm	919	24.8 b	111	0.4	0.3	53.3	1767 a
58 cm	897	26.0 ab	122	0.7	0.8	53.8	1228 b
76 cm	907	26.3 a	122	0.8	1.0	54.4	1171 b
Seeding rate							
1 kg ha^{-1}	875	25.9	119 ab	0.5	0.8	53.8 b	778 b
2 kg ha^{-1}	932	25.9	122 a	0.7	0.7	53.7 b	1302 ab
4 kg ha^{-1}	916	25.3	114 b	0.8	0.5	54.0 a	2086 a
ANOVA	P value						
Environment (E)	***	***	**	***	***	***	***
Row spacing (R)	NS	*	NS	NS	NS	NS	*
R × E	NS	NS	NS	NS	***	NS	NS
Seeding rate (S)	NS	NS	*	NS	NS	*	*
S × E	NS	NS	NS	NS	NS	NS	***
R × S	NS	NS	NS	NS	NS	NS	**
R × S × E	NS	NS	NS	NS	NS	NS	NS

† Means within each column for a given main effect followed by different letters are significantly different from each other ($P \leq 0.05$).
‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table 4.7. Effects of nitrogen fertilizer rate and cultivar on grain yield and agronomic traits of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 (2 sites) and 2006 (1 site). Results illustrate main effects, with means averaged over other factors.

Source of variation	Grain yield	Grain moisture	Plant height	Lodging	Branch number	Days to anthesis	Plant population
	kg ha^{-1}	%	cm	0 to 5‡	plant^{-1}	days	1000 plants ha^{-1}
Environment							
Site A 2005	1111 a	31.1 b	132 b	0.4	3.8 b	69.8 ab	721 a
Site B 2005	796 b	35.3 a	138 ab	0.0	3.0 b	69.3 b	623 a
Site A 2006	1039 a	28.4 c	156 a	0.4	7.0 a	70.9 a	368 b
Nitrogen rate							
0	957	31.0 b	130 b	0.1	3.8	71.0	636
50	976	31.2 ab	138 ab	0.1	3.8	69.8	566
100	946	31.5 ab	145 a	0.2	4.3	69.8	576
150	1027	31.8 ab	149 a	0.2	5.1	69.4	566
200	1004	32.3 a	148 a	0.6	5.8	69.9	509
Cultivar							
D136	984	32.6	144	0.1	6.7	76.7 a	419 b
Plainsman	980	30.6	140	0.4	2.4	63.3 b	722 a
ANOVA	P value						
Environment (E)	***	***	*	*	**	**	***
Nitrogen rate (N)	NS	*	**	NS	NS	NS	NS
N × E	*	NS	NS	*	*	NS	NS
Cultivar (C)	NS	NS	NS	NS	NS	**	**
C × E	***	**	NS	**	***	**	NS
C × N	NS	NS	NS	NS	NS	NS	NS
C × N × E	*	NS	NS	NS	NS	NS	NS

† Means within each column for a given main effect followed by different letters are significantly different from each other ($P \leq 0.05$).
‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

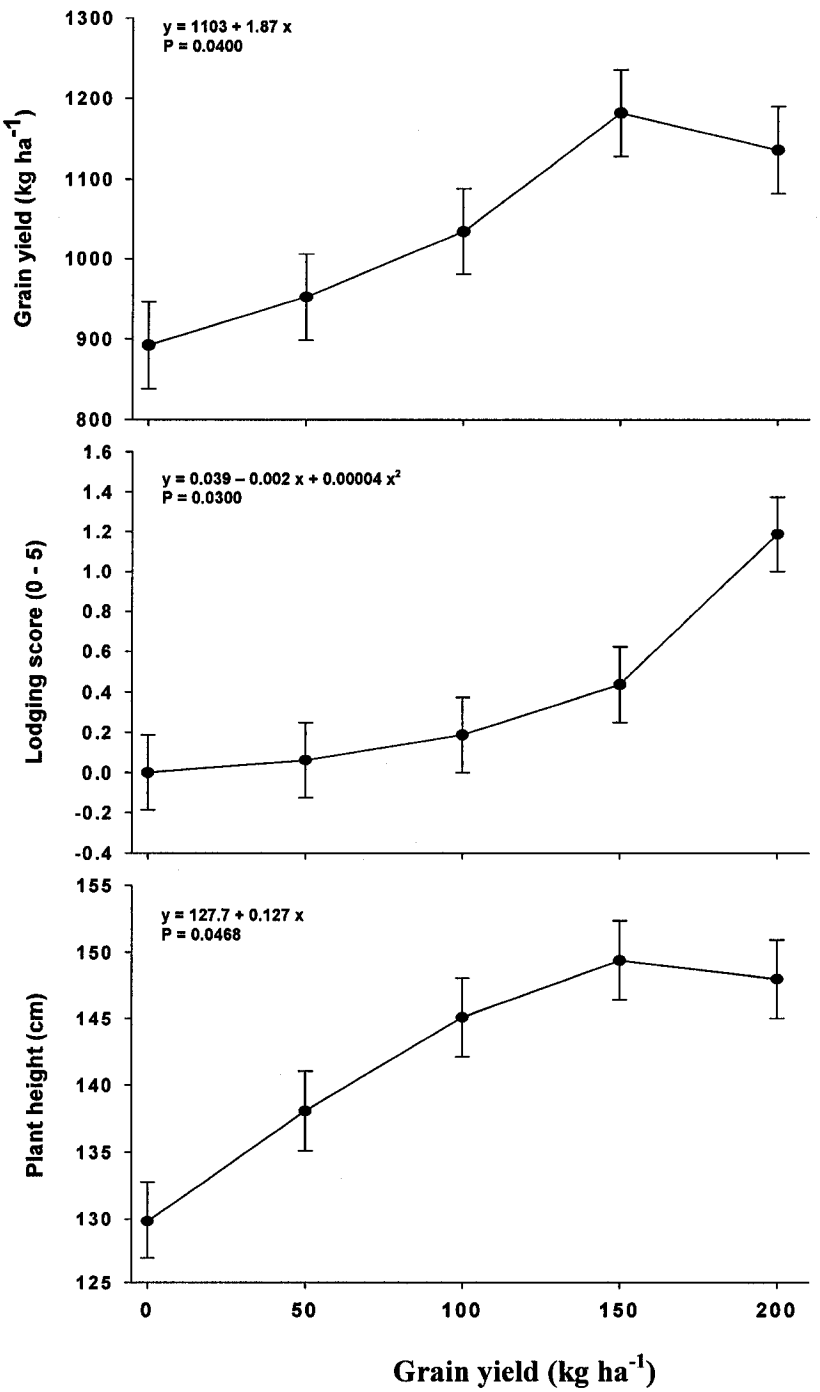
Table 4.8. Effects of cultivar on grain yield and agronomic traits of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada, in 2005 and 2006.

Environment	Cultivar	Grain yield	Grain moisture	Plant height	Lodging	Branch number	Days to anthesis	Plant population
		kg ha^{-1}	%	cm	0 to 5‡	plant^{-1}	days	1000 plants ha^{-1}
Site A 2005	D136	1339 a	31.6 a	131	0.1 b	5.5 a	76.0 a	552 b
	Plainsman	883 b	30.6 b	133	0.7 a	2.0 b	63.6 b	889 a
Site B 2005	D136	823	36.3 a	142 a	0.0	4.3 a	76.1 a	473 b
	Plainsman	768	34.3 b	133 b	0.0	1.8 b	62.6 b	772 a
Site A 2006	D136	789 b	29.8 a	158	0.2 b	10.4 a	78.1 a	231 b
	Plainsman	1290 a	26.9 b	155	0.5 a	3.5 b	63.6 b	504 a

† Means within each environment for a given variable followed by different letter are significantly different from each other ($P \leq 0.05$).

‡ 0 refers to absence of lodging and 5 to all plants in the plot being lodged.

Figure 4.1. Effects of nitrogen fertilization on grain yield, lodging score and plant height of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada. Grain yield and lodging are for one environment in 2006, whereas plant height represents plant response averaged across three environments in 2005 and 2006. Lodging, 0 refers to absence of lodging and 5 to all plants in the plot being lodged. Vertical bar represent one standard error.


y = 1103 + 1.67 x
P = 0.0400
Grain yield (kg ha-1)
1300
1200
1100
1000
900
800
y = 0.039 – 0.002 x + 0.00004 x2
P = 0.0300
Lodging score (0 - 5)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
y = 127.7 + 0.127 x
P = 0.0468
Plant height (cm)
155
150
145
140
135
130
125
0
50
100
150
200
Grain yield (kg ha-1)

PREFACE TO CHAPTER 5

Chapters 3 and 4 focused on agronomic aspects of grain amaranth. Chapter 3 evaluated the adaptability of 29 genotypes to southwestern Québec, while chapter 4 evaluated different management strategies. Based on this research, the culture grain amaranth in southwestern Québec should be possible, and management strategies are better defined.

Although grain amaranth was a traditional crop of pre-Hispanic Indian American cultures, its re-introduction raises concern about the safety of this "new" food, and an examination of its anti-nutritional factors content is appropriate and required. The research presented in chapter 5 was initiated to study the safety aspect of grain amaranth. One study had been published on the oxalate concentration of *A. caudatus,* which was about 228-236 mg $100g^{-1}$ (Siener et al., 2005). This is higher than whole wheat flour or corn meal, which were reported to be 67 and 54 mg $100g^{-1}$, respectively (Chai and Liebman, 2005). Oxalate is an anti-nutritional compound that is linked to kidney-stone formation and can lead to calcium and magnesium deficiencies when consumed in large amounts.

I therefore decided to investigate whether there was variability in oxalate concentration among the genotypes harvested, and whether seeding date, nitrogen fertilization rate and cooking had an influence on oxalate concentration. This investigation is presented in chapter 5. This chapter was published in *Journal of Agricultural and Food Chemistry*, under the title *Oxalate in grain amaranth* (Gélinas and Seguin, 2007).

Chapter 5. OXALATE IN GRAIN AMARANTH

5.1 Abstract

Grain amaranth (*Amaranthus* spp.) is a widely adaptable C4 pseudo-cereal crop which has interesting nutritional characteristics including high protein and calcium concentrations, and a lack of gluten. To date no anti-nutrient has been found at problematic levels in grain amaranth; however, oxalate has not been thoroughly studied. Dietary oxalate is a potential risk factor of kidney stone development and its presence in food lowers calcium and magnesium availability. Oxalate concentration and forms, and calcium and magnesium concentrations were determined in 30 field-grown grain amaranth genotypes from the species *A. cruentus*, *A. hybrid*, and *A. hypochondriacus*. The effects of seeding date and fertilization with calcium ammonium nitrate were evaluated in field experiments conducted in multiple environments; the effects of cooking were also evaluated. Mean total oxalate concentration in the 30 genotypes analyzed was 229 mg 100 g^{-1}, with values ranging between 178 and 278 mg 100 g^{-1}; the greatest proportion being insoluble (avg. of 80%). Calcium concentration averaged 186 mg 100 g^{-1} and ranged between 134 and 370 mg 100 g^{-1}, whereas magnesium averaged 280 mg 100 g^{-1} and ranged between 230 and 387 mg 100 g^{-1}. Fertilization only marginally increased total oxalate concentration and had no effects on other variables. Seeding date had no effects on any of the variables studied. Boiling increased the proportion of soluble oxalate but did not affect total oxalate concentration. Grain amaranth can be considered a high oxalate source, however as most is in insoluble form, and due to its high calcium and magnesium concentrations, oxalate absorbability could be low. This should be confirmed by bioavailability studies.

5.2 Introduction

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous (Johnson and Hatch, 1968) pseudo-cereal crop which was widely cultivated in pre-Columbian America (Sauer, 1950). Its cultivation however decreased to a point of near extinction in the early 20th century due to pressure from the conquistadors, who disliked its use in native ceremonies (Sauer, 1950). Interest for grain amaranth was revived in the 1970's and emphasis was

put in adapting the crop to mechanized agriculture (National Academy of Science, 1984). This plant is adaptable to a wide range of environments and has been successfully introduced in several countries of Europe, Asia and Africa (National Academy of Science, 1984). Grain amaranth has interesting nutritional properties such as high content of lysine rich protein (Downton, 1973) and an absence of gluten (Petr et al., 2003). It is estimated that over 2.1 million people in the USA alone are affected by celiac disease, which renders affected people intolerant to gluten (Celiac Spruce Association, 2004). This represents a great potential market for grain amaranth. Calcium in amaranth was reported to be as high as 308 mg 100 g^{-1} (Bressani et al, 1987), and its grain has thus been proposed as a weaning food ingredient (Rathod and Udipi, 1991). Most anti-nutrients that have been studied to date in grain amaranth appear to be non-problematic. Levels of tannin and phytic acid are comparable to those observed in grain cereals (Lorenz and Wright, 1984), while trypsin and chymotrypsin activity were reported to be lower (Bressani, 1994). Potential for aflatoxin and zearalenone production was also reported to be comparable or lower than other grains (Bresler et al., 1998). Saponin content is low and those produced are of low toxicity (Oleszek et al., 1999). However, there are concerns regarding grain amaranth oxalate concentration, given that amaranth leaves have are known to contain high oxalate concentrations (Vityakon and Standal, 1989).

Oxalate is a naturally occurring anti-nutrient present in several cultivated plants such as spinach (*Spinacia oleracea* L.) and rhubarb (*Rheum rhabarbarum* L.). High oxalate intake can lead to mineral deficiency, most notably calcium and magnesium, as oxalic acid binds these two elements to form insoluble calcium or magnesium oxalate (Libert and Franceschi, 1987; Bohn et al., 2004). Sodium and potassium oxalate are more soluble and are therefore considered more absorbable (Grentz and Massey, 2002). Urinary oxalate excretion, which necessarily follows oxalate absorption, is known to be an important risk factor in the formation of kidney stones (Jaegger and Robertson, 2004). Calcium oxalate stone formers usually have higher urinary oxalate excretion (Holmes and Assimos, 2004) and are recommended to avoid oxalate-rich foods (Zimmermann and Hesse, 2005). The amount of urinary oxalate reported to be derived from the diet varies, ranging from 5 to 50 percent, the remaining being endogenously synthesized (Jaegger and Robertson, 2004; Holmes and Assimos, 2004). Strawberries, chocolate and soynuts are

examples of common foods that have been shown to increase urinary oxalate levels (Grentz and Massey, 2002). Calcium or magnesium ingestion along with oxalate is known to decrease oxalate absorption, as they form insoluble complexes (Liebman and Costa, 2000). Therefore, the oxalate to calcium molar ratio of foodstuff is believed to influence the amount of oxalate absorbed (Jaegger and Robertson, 2004). The proportion of soluble vs. insoluble oxalate present in foodstuff is often regarded as an indicator of the bioavailability of oxalate (Grentz and Massey, 2002; Jaegger and Robertson, 2004), although this theory has been criticized (Holmes and Assimos, 2004).

Oxalate levels in a given crop can be controlled by cultivar selection and breeding, field management, and processing (Libert and Franceschi, 1987). Variation in oxalate concentrations and forms have been reported among soybean (*Glycine max* (L.) Merr.) cultivars (Horner et al., 2005). Oxalate concentration in spinach was manipulated by varying nitrogen levels and forms (Zhang et al., 2005) and in bean leaves (*Phaseolus vulgaris* L.) by varying calcium supply (Zindler-Frank et al., 2001). Calcium fertilization however did not affect oxalate concentration in soybean seeds (Streeter, 2005). Boiling or steaming was found to decrease oxalate concentration in several vegetables (Chai and Liebman, 2005a).

To our knowledge, there has been only one report of grain amaranth oxalate concentration, with concentrations in one cultivar of *A. caudatus* ranging between 228 and 236 mg total oxalate 100 g^{-1}, with 35% in the soluble form (Siener et al., 2006). Such levels are similar to those reported for soybean and soy products and could be problematic for calcium oxalate stone-formers (Massey et al., 2001). It is already known that oxalate is present in high concentrations in grain amaranth leaves, variation being observed between cultivars (Prakash and Pal, 1991). It is not known whether a similar variability is present in the grain, or whether agronomic practices could affect grain amaranth oxalate concentration.

If grain amaranth is to be consumed in appreciable amounts by people affected by celiac disease, vegetarians looking for alternative protein sources, or due to its use as an ingredient in weaning food, it is important to minimize oxalate concentration in seeds. Therefore, our main objectives were to: i) determine variability in oxalate concentration and forms in seeds of 30 different grain amaranth genotypes representing 3 different

species; and evaluate the effects of ii) calcium ammonium nitrate fertilization, iii) seeding date, and iv) cooking on oxalate concentration and forms.

5.3 Materials and methods

5.3.1 General description and field conditions

Field experiments were conducted in 2005 and 2006, at the Emile A. Lods Agronomy Research Center in Sainte-Anne-de-Bellevue, QC, Canada (45°25′ 45″N lat., 73°56′00″ W long.). Soils used are described in Table 5.1. Except for the fertilization experiment, nitrogen, phosphorus and potassium fertilization was done based on soil tests according to local recommendations for sorghum (CRAAQ, 2003), no recommendations existing for grain amaranth. All harvested seeds were dried at 50 °C until moisture content reached about 9% and were stored at room temperature until analysis. Seeds were then ground through a 1-mm screen using a Tecator Cyclotec sample mill (Tecator Corporation, Sweden).

5.3.2 Genotype evaluation

Thirty grain amaranth genotypes (Table 5.2) were obtained from the US National Genetic Resources Program (North Central Regional Plant Introduction Station, Ames, IA). Plants were grown in 2005 in field A in a randomized complete block design with three blocks. Each plot consisted in one 2 m row. Plants were spaced 20 cm in the row and 80 cm between rows. The field was hand-seeded on June 9th and hand-harvested on October 30th (i.e, one week after the first killing frost). All plants were harvested.

5.3.3 Fertilization experiment

Cultivar 'Plainsman' (Albert Lea Seedhouse, Albert Lea, MN) was grown in a randomized complete block design with three blocks, replicated over two growing seasons with two sites each season. Fields A and B were used in 2005 and fields C and D in 2006 (Table 5.1). Plots were 5 m long with four rows spaced 76 cm apart. Nitrogen was broadcast applied on the day of seeding in the form of calcium ammonium nitrate (27.5% N; 4.6% Ca ; 2.4% Mg) at rates of 0, 50, 100, 150 and 200 kg N ha^{-1}. Depending

on the year and site, seeding was done between May 30th and June 1st at a rate of 1.5 kg ha^{-1} using a disk drill (Fabro, Swift Current , SK, Canada). Only the middle two rows of each plot were harvested. Harvesting was done mechanically using a self-propelled harvester (Wintersteiger, Saskatoon, SK, Canada) between October 28th and November 1st.

5.3.4 Seeding date experiment

Field layout, plot size, seeding method, cultivar and harvesting were as in the nitrogen fertilization experiment. The experiment was conducted in 2005 at two sites (i.e., fields A and B; Table 5.1). Seeding dates were May 20, May 31 and June 30. Harvesting was done on October 31st in field A and October 27th in field B.

5.3.5 Cooking experiment

Seeds of the cultivar 'Plainsman' were used for this experiment, because it is currently the most widely grown grain amaranth cultivar in North America (Thomas Jefferson Agricultural Institute, 2006) and therefore the most consumed. Cooking was done by boiling at atmospheric pressure (uncovered) 50 ml of non-ground seeds in 100 ml of distilled water until complete water absorption. The resulting porridge was spread as a thin layer on aluminium foil, dried for 3 days at 60 °C, ground through a 1-mm screen using a Tecator Cyclotec sample mill (Tecator Corporation, Sweden) and stored at room temperature until analysis. The cooking experiment was repeated on two different days, with 3 replicates on the first day and 4 on the second.

5.3.6 Oxalate quantification

Oxalate was quantified with an enzymatic kit (procedure no. 591, from Trinity Biotech, Newark, NJ) according to the method of Horner et al. (Horner et al., 2005). Ground seeds were dried for three days at 60 °C and 100 mg was placed in a 15 ml centrifuge tube. For each sample, 4 tubes were prepared: 2 tubes A (total oxalate, duplicates) and 2 tubes B (insoluble oxalate, duplicates). Tubes were then autoclaved for 20 minutes. Soluble oxalates were then removed from tubes B in the following way. First,

2 ml sterile deionized (DI) water were added, content was mixed by gentle vortexing and tubes were sonicated for 6 minutes in a sonicator bath. Another 2 ml sterile DI water was added and ground seeds were resuspended. Tubes were then centrifuged at 2000 g for 5 min and the supernatant was discarded. Three subsequent washings were completed after this initial one, by adding 4 ml DI water. All tubes were then dried at 60 °C for 3 days. Then 2 ml EDTA solution (included in procedure no. 591) were added to tubes A and B and the ground seeds were resuspended. Tubes were sonicated for 6 min, after which an additional 2 ml EDTA solution was added. Tubes were then incubated at 55 °C for 24 h and centrifuged at 3000 g for 5 min. Fifty µl of supernatant was used for enzymatic determination of oxalate. In each batch, a blank consisting of DI water and a standard were analyzed, as well as tubes A and B. First, 1 ml oxalate reagent A (included in procedure no. 591) was transferred in 2 ml polypropylene centrifuge tubes. Then, 50 µl of supernatant (or DI water for blank, and 0.5 mmol oxalate L^{-1} for standard) were added to each tube. Finally, 100 µl of oxalate reagent B were added, and tubes were closed and mixed by inversion. After 5 min incubation, content was transferred to cuvettes and absorbance read at 590 nm using an Ultrospec II spectrophotometer (Biochrom LTD, Cambridge, UK). A blank was used to set the spectrophotometer to zero. Oxalate concentrations in mg 100 g-1 were determined as follows: (absorbance of A or B/absorbance of standard) × (1/sample weight in mg) × 18×10^6 = mg oxalate/100 g dried seeds. Soluble oxalate was determined by subtraction.

5.3.7 Calcium and magnesium determination

Ground dry seeds were digested using the method of Parkinson and Allen (1975). Calcium and magnesium concentrations were determined using a Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer, Waltham, MA). Only seeds from the genotype experiment were analyzed for Ca and Mg concentrations.

5.3.8 Statistical analyses

All statistical analyses were performed with SAS 9.1 (SAS Institute, 2003). Residuals were tested for normality, using the Shapiro-Wilk test in PROC

UNIVARIATE. Logarithmic transformation was applied to non-normal data. Heterogeneity of variance was evaluated using the BIC criteria. Statistical analyses for the genotype and the cooking experiments were performed using PROC GLM. Multiple comparison tests were performed with Scheffé's procedure. In the genotype experiment, given the objective of finding whether variation exists among genotypes, and due to the large number of entries, multiple comparison tests were not performed. The data from the seeding date and the nitrogen experiments were analyzed using procedure MIXED in SAS (SAS Institute Inc., 2003), because block and environment were considered random effects. Statistical models were elaborated according to McIntosh (1983). Regression analysis was performed with the data from the fertilization experiment. Using PROC CORR, Pearson product–moment correlation coefficients were calculated among variables from the genotype experiment. For all analyses, level of statistical significance was set at 0.05.

5.4 Results and discussion

5.4.1 Genotypes

Among the 30 genotypes, there were significant differences ($p < 0.05$) in total and insoluble oxalate, as well as in calcium and magnesium concentrations, and the oxalate: calcium ratio (Table 5.3). Mean total oxalate concentration of the 30 genotypes analyzed was 229 mg 100 g^{-1}, with values ranging between 178 and 278 mg 100 g^{-1} (Table 5.2), representing a 56% variation. Mean total oxalate concentration observed is comparable to the value of 232 mg 100 g^{-1} reported previously for one genotype of A. caudatus (Siener et al 2006). Insoluble oxalate averaged 182 mg 100 g^{-1} (80% of total oxalate) and ranged between 151 and 224 mg 100 g^{-1}, representing a 48% variation. Soluble oxalate averaged 47 mg 100 g^{-1} and ranged between 26 and 82 mg 100 g^{-1}, but differences between genotypes were not significant ($p > 0.05$). These values are comparable to values reported for buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) (Siener et al., 2006; Chai and Liebman, 2005b), two pseudo-cereals also recommended as gluten-free alternatives to cereals (Petr et al., 2003). However, buckwheat and quinoa have a higher proportion of soluble oxalate than grain amaranth,

thus representing potentially a greater risk of kidney stone formation. 'Plainsman', the most widely grown grain amaranth cultivar in the United States (Myers, 1998), had 203 mg of total oxalate 100 g^{-1} with 156 of mg insoluble oxalate 100 g^{-1}. If amaranth is to be consumed on a regular basis by people affected by celiac disease, or those seeking an alternative protein source, such oxalate concentrations might be problematic, as foods with more than 10 mg oxalate per 125 ml serving are considered high oxalate foods (Chicago Dietetic Association, 2000); 125 ml of dry grain amaranth weighs approximately 100 g and would contain 203 mg oxalate per serving. However, grain amaranth has much lower oxalate concentrations than vegetable amaranth, which have been reported to contain between 8.57 and 9.57 g of total oxalate 100 g^{-1} on a dry matter basis (Vityakon and Standal, 1989), and most is found as insoluble oxalate (i.e., avg. of 80% of the total oxalate).

Calcium concentrations averaged 186 mg 100 g^{-1} and ranged between 134 and 370 mg 100 g^{-1}, whereas magnesium concentrations averaged 280 mg 100 g^{-1} and ranged between 230 and 387 mg 100 g^{-1}. The oxalate:calcium molar ratio averaged 0.59 and ranged between 0.30 and 0.77. Finally, the oxalate:magnesium molar ratio averaged 0.23 and ranged between 0.19 and 0.27. Mean calcium and magnesium concentrations were almost identical to those previously reported in grain amaranth by Becker et al. (1981), but slightly inferior to those by Bressani et al. (1987). When comparing the results from the present study to those reported elsewhere for grains and legumes (Chai and Liebman, 2005), grain amaranth contains roughly 4 to 5 times more total oxalate than conventional cereals and legumes, the grains for which amaranth can be used as an alternative. However, due to the high calcium and magnesium concentration of grain amaranth (Table 5.3), oxalate absorbability might be relatively low. Ingestion of calcium or magnesium along with oxalate rich food is known to reduce oxalate absorption, as these minerals bind to free oxalic acid and produce insoluble oxalate complexes (Liebman and Costa, 2000). The average oxalate:calcium ratio reported here is much lower than that of spinach, which was reported to be around 1.08 and to have only 5% of the total Ca available (Heany and Weaver, 1988). Grain amaranth can probably be viewed as a good source of calcium, since on a molar basis there is nearly twice as much calcium than oxalate. We are not aware of any study evaluating the bioavailability of calcium in grain amaranth.

Several significant correlations were observed between variables measured (Table 5.4). Total oxalate was positively correlated with both calcium ($r = 0.44$, $p < 0.0001$) and magnesium ($r = 0.51$, $p < 0.0001$) concentrations. As shown in figure 5-1, although total oxalate is positively correlated to calcium, the oxalate: calcium ratio decreases with increasing calcium. This suggests that breeding for higher calcium could be beneficial, because although oxalate increases with increasing calcium, proportionally more calcium is present to bind oxalate in genotypes with higher calcium concentration.

Variability observed in oxalate, calcium and magnesium concentrations as well as for the oxalate:calcium ratio, suggests that breeding for these traits might be possible. Mode of inheritance of oxalate in grain amaranth is however currently unknown. In soybean, it was suggested that breeding for low oxalate would be possible (Horner et al., 2005). It must be noted that only 1% of the nearly 3000 grain amaranth genotypes available from theUS National Genetic Resources Program (North Central Regional Plant Introduction Station, Ames, IA) were evaluated in the present study, and that more extreme values would most likely be found in a more thorough screening. The recent isolation of *Medicago truncatula* mutants showing altered oxalate crystal patterns and oxalate concentrations has shown that oxalate concentration in plants is at least partially genetically controlled (Nakata and McConn, 2000). Some important genetic tools, like male-sterility (Gudu and Gupta, 1988) and non-shattering traits (Brenner, 2002) are likely to contribute to the development of the next, high yielding generation of grain amaranth cultivars. Oxalate bioavailability studies are required before the initiation of new grain amaranth breeding programs to determine whether oxalate concentration should be a consideration.

5.4.2 Fertilization and seeding date

Averaged over four environments (i.e., two years and two sites), there was a significant ($p < 0.05$) effect of fertilization with calcium ammonium nitrate on total oxalate concentration ($y = 304 + 0.07x$). However, with a regression coefficient of only 0.07, this means that only a slight decrease in oxalate concentration could be achieved by considerably reducing calcium ammonium nitrate fertilization, which however in turn

would reduce grain yield (Myers, 1998). Adapting calcium ammonium nitrate fertilization levels is thus not a viable means of reducing oxalate concentration in grain amaranth. Other components measured (i.e., insoluble oxalate, soluble oxalate, percents of insoluble and soluble oxalate, calcium, magnesium, oxalate:calcium ratio) all remained unaffected by fertilization ($p > 0.05$). Finally, seeding date had no effect on all variables measured ($p > 0.05$, data not presented).

Increased oxalate concentration was reported in spinach with increases in both calcium and nitrogen fertilization (Libert and Franceschi, 1987). Also in spinach nitrate was reported to increase oxalate more than ammonium (Zhang et al., 2005). In the present experiment, we used calcium ammonium nitrate, which supplied half the nitrogen as nitrate and half as ammonium, and also provided some calcium and magnesium. We therefore simultaneously varied levels of nitrogen (0 to 200 kg N ha^{-1}), calcium (0 to 33 kg Ca ha^{-1}) and magnesium (0 to 17 kg Mg ha^{-1}). Since we applied all the fertilizer once at seeding, it is reasonable to assume that most of the applied ammonium had been oxidized to nitrate before uptake. However, we only observed a weak increase in total oxalate concentration with increasing fertilization, which could be attributed to: i) the high calcium levels prior experimentation of soils used (Table 5.1), with further calcium fertilization not affecting plants; ii) nitrogen leaching (although this is unlikely because a grain yield response was observed, data not shown) or iii) the fact that grain amaranth may not be as responsive as spinach to fertilization. It is also possible that the oxalate concentration of seeds is less affected by environmental factors than that of leaves or vegetative material. As pointed out by Libert and Franceschi (1987), oxalate formation is not simply a crystallization process, but involves the development of specialized membranes or cells. Plants therefore exert a certain degree of control over the amount of oxalate and calcium accumulated. Another explanation for the weak response to fertilization we observed in grain amaranth is a possible interaction between nutrients. Libert and Franceschi (1987) indeed reported that nitrogen increased oxalate in spinach under low phosphorus status, but had no effect under higher phosphorus status.

5.4.3 Cooking

Boiling amaranth seeds at atmospheric pressure in a 2:1 water to seeds ratio until complete absorption changed the form but not the total concentration of oxalate (Table 5.5); soluble oxalate proportion being greater after cooking ($p < 0.05$). The absence of a decrease in total oxalate concentration following boiling is in contradiction with results observed in a range of vegetables (Chai and Liebman, 2005), legumes (Quinteros et al., 2003) and cereals (Judprasong et al., 2006). Differences might be attributed to the fact that in our study, cooking water was completely absorbed by the grains; therefore any solubilized material was kept and detected in analyses. Beetroot (*Beta vulgaris* L.) was reported to have a greater proportion of soluble oxalate after pressure cooking for 45 min at 15 psi (Savage et al., 2000). Cooking methods for grain amaranth found in the popular literature often recommend (with water to grain ratio varying slightly) boiling until complete absorption of water. If soluble oxalate is, as previously reported, more absorbable than insoluble oxalate (Jaegger and Robertson, 2004), this method of cooking could increase the total amount of oxalate absorbed.

5.5 Summary and conclusions

Results presented herein underline the need for conducting grain amaranth oxalate absorbability studies. Grain amaranth has high total oxalate concentrations, however most is found as insoluble oxalate. If oxalate absorbability is low, then substituting amaranth for regular cereals could be done with fewer concerns for those people with predisposition to kidney stone development. If absorbability is high, selection for low-oxalate genotypes will be necessary. Heritability studies for oxalate concentration and forms will also be required. Based on our results, management appears not to represent an effective way of manipulating oxalate concentration and forms in grain amaranth. Because of the low oxalate:calcium ratio and the high calcium concentration of grain amaranth, we suggest that it might represent a good source of dietary calcium that could benefit vegetarians, although it should be confirmed by bioavailability studies.

5.6 Tables and figure

Table 5.1. Description of soils used for field experiments conducted in Sainte-Anne-de-Bellevue, QC, Canada.

Field	Soil type	pH	P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	Organic matter %
A	Loamy sand	6.70	335	408	3700	292	4.00
B	Sandy clay loam	5.93	210	466	2331	235	3.10
C	Sandy clay loam	5.85	195	193	2362	318	2.87
D	Sandy clay loam	6.00	96	205	4927	641	4.25

Table 5.2. Description of grain amaranth genotypes grown in Sainte-Anne-de-Bellevue, QC, Canada.

Genotype[a]	Common descriptive	Species	Origin[b]	Seed color	Flower color
PI 451711		*Amaranthus cruentus*	Mexico	Black	red
PI 477912	RRC 416	*A. cruentus*	Mexico	White	green
PI 477913	RRC 1011	*A. cruentus*	Mexico	White	green
PI 515959	Montana-3	*A. cruentus*	Montana	White	green
PI 525498	MT-5	*A. cruentus*	Montana	White	green
PI 538255	Amont	*A. cruentus*	Montana	White	green
PI 538319	K266	*A. cruentus*	Pennsylvania	White	green
PI 538320	K283	*A. cruentus*	Pennsylvania	White	pink
PI 538321	K436	*A. cruentus*	Pennsylvania	White	red
PI 538323	K432	*A. hybrid*	Pennsylvania	White	green
PI 538324	K433	*A. hybrid*	Pennsylvania	White	green
PI 538325	K593	*A. hybrid*	Pennsylvania	White	red
PI 538326	D70-1	*A. hybrid*	Pennsylvania	White	red
PI 538327	D136-1	*A. hybrid*	Pennsylvania	White	green
PI 558499	Plainsman	*A. hypochondriacus*	Pennsylvania	White	red
PI 568179	Ames 12991	*A. hybrid*	Iowa	White	red
PI 576447	Ames 13446	*A. cruentus*	Nigeria	Brown	green
PI 604666	RRC 1027	*A. cruentus*	Pennsylvania	White	orange
PI 605354	R-158	*A. cruentus*	Pennsylvania	White, translucent	red
PI 606767	Ames 8272	*A. cruentus*	Pennsylvania	White	orange
PI 606797	A200D	*A. cruentus*	Illinois	White	green
PI 606799	RRC 1017	*A. cruentus*	Pennsylvania	White	red & green
PI 618962	Ames 2015	*A. cruentus*	Benin	Dark brown	green
PI 619250	Ames 2265	*A. hypochondriacus*	Pennsylvania	Golden	red, green & pink
PI 628780	RRC 423	*A. cruentus*	Mexico	White	purple-red
PI 628781	RRC 444	*A. cruentus*	Mexico	White	green
PI 628782	RRC 446	*A. cruentus*	Mexico	White	green
PI 628783	RRC 776	*A. cruentus*	Mexico	White	red & green
PI 633584	RRC 27	*A. cruentus*	China	Dark brown	red
PI 636182	RRC 1386	*A. cruentus*	Argentina	White	dark pink

[a] PI numbers refer to plant introduction numbers from the US National Genetic Resources Program.
[b] Origin is that of the donor to the US National Genetic Resources Program.

Table 5.3. Oxalate, calcium and magnesium concentration of 30 grain amaranth genotypes grown in Sainte-Anne-de-Bellevue, QC, Canada (n=3).

Genotype[a]	Total Oxalate (mg $100g^{-1}$)	Insoluble Oxalate (mg $100g^{-1}$)	Soluble Oxalate (mg $100g^{-1}$)	% Insoluble Oxalate	% Soluble Oxalate	Calcium mg $100g^{-1}$)	Magnesium (mg $100g^{-1}$)	Oxalate: Calcium (molar ratio)	Oxalate: Magnesium (molar ratio)
PI 451711	247	191	56	78	22	370	301	0.30	0.23
PI 477912	197	162	35	82	18	157	249	0.58	0.22
PI 477913	234	179	56	76	24	167	277	0.64	0.23
PI 515959	202	164	39	81	19	134	247	0.70	0.23
PI 525498	227	191	37	84	16	188	288	0.55	0.22
PI 538255	214	171	43	80	20	144	264	0.70	0.23
PI 538319	218	153	65	71	29	165	254	0.62	0.24
PI 538320	178	152	26	86	14	140	264	0.58	0.19
PI 538321	234	190	45	82	18	159	280	0.68	0.23
PI 538323	216	186	30	86	14	168	300	0.59	0.20
PI 538324	251	206	46	82	18	191	289	0.64	0.26
PI 538325	262	206	57	78	22	156	289	0.77	0.26
PI 538326	266	224	43	84	16	158	271	0.77	0.27
PI 538327	247	201	47	81	19	185	276	0.61	0.25
PI 558499	203	156	47	77	23	165	242	0.56	0.23
PI 568179	241	195	46	81	19	208	317	0.53	0.21
PI 576447	278	213	65	78	22	349	387	0.36	0.20
PI 604666	247	208	40	84	16	193	281	0.59	0.24
PI 605354	216	177	39	82	18	154	230	0.64	0.26
PI 606767	236	189	48	80	20	199	301	0.54	0.22
PI 606797	199	165	34	83	17	149	244	0.61	0.23
PI 606799	206	161	45	79	21	156	244	0.61	0.23
PI 618962	248	194	54	78	22	252	340	0.45	0.20
PI 619250	258	206	52	81	19	160	272	0.73	0.26
PI 628780	207	168	39	81	19	184	291	0.52	0.20
PI 628781	215	177	39	82	18	168	246	0.58	0.24

Table 5.3 Continued

PI 628782	209	166	43	80	20	165	267	0.58	0.22
PI 628783	229	151	79	66	34	170	255	0.62	0.25
PI 633584	256	175	82	69	31	244	344	0.48	0.21
PI 636182	211	173	38	83	17	172	275	0.56	0.21
SEM	19.8	14.3	12.9	4.5	4.5	15.5	18.5	0.052	0.019
p-value	0.0457	0.0090	0.4880	0.4482	0.4482	<0.0001	<0.0001	<0.0001	0.1605
Mean	229	182	47	80	20	186	280	0.59	0.23
Range	178–278	151–224	26–82	66–86	14–34	134–370	230–387	0.30–0.77	0.19 – 0.27

[a] PI numbers refer to plant introduction numbers from the US National Genetic Resources Program.

Table 5.4. Correlations coefficients of oxalate, calcium and magnesium concentrations, and their molar ratios in 30 grain amaranth genotypes grown in Sainte-Anne-de-Bellevue, QC, Canada (n=90).

	Total oxalate	Insoluble oxalate	Soluble oxalate	Calcium	Magnesium	Oxalate: calcium	Oxalate: magnesium
Total oxalate	1.00	0.80***	0.63***	0.44***	0.51***	0.20^{ns}	0.55***
Insoluble oxalate		1.00	0.05^{ns}	0.33**	0.41***	0.20^{ns}	0.44***
Soluble oxalate			1.00	0.31**	0.32**	0.09^{ns}	0.35***
Calcium				1.00	0.70***	-0.74***	-0.20^{ns}
Magnesium					1.00	-0.46***	-0.43***
Oxalate:calcium						1.00	0.66***
Oxalate:magnesium							1.00

ns, not significant ($p > 0.05$).
Correlation significant at the *, 0.05; **, 0.01; and ***, 0.001 levels.

Table 5.5. Effects on oxalate concentration and form of boiling grain amaranth at atmospheric pressure in a 2:1 water to grain ratio (n=7).

Treatment	Total oxalate (mg 100 g^{-1})	Insoluble oxalate (mg 100 g^{-1})	Soluble oxalate (mg 100 g^{-1})	% Insoluble oxalate	% Soluble oxalate
Raw	268	235	33	87.8	12.2
Cooked	271	215	56	79.4	20.6
SEM	4.1	3.1	3.3	1.0	1.0
p-value	0.6731	0.0007	0.005	0.0001	0.0001

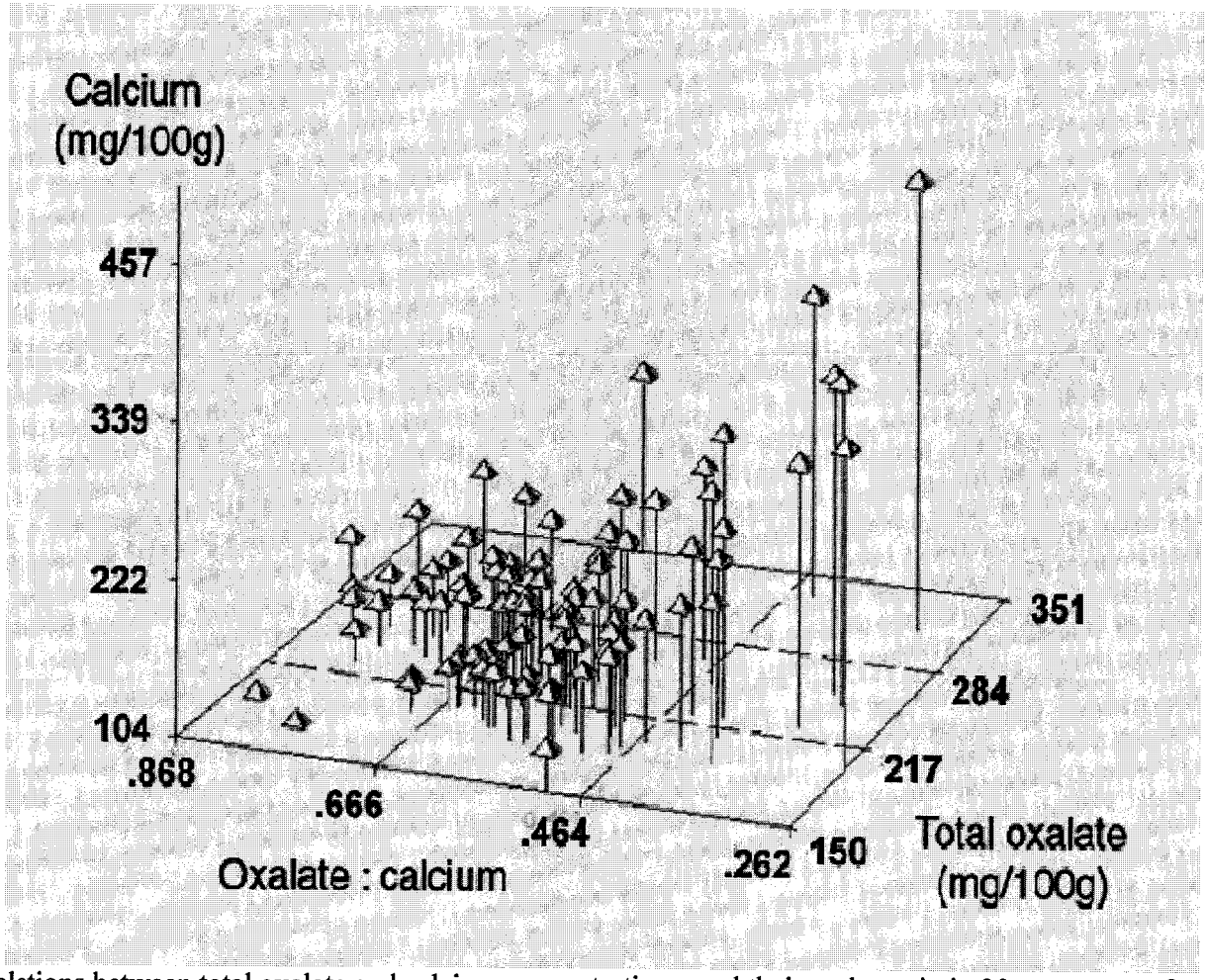


Figure 5-1. Correlations between total oxalate and calcium concentrations, and their molar ratio in 30 genotypes of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada (n=90). Correlation coefficients were: calcium and total oxalate ($r = 0.44$, $p < 0.001$); calcium and oxalate:calcium ($r = -0.74$, $p < 0.001$); oxalate:calcium and oxalate ($r = 0.2$, $p = 0.05$).

Chapter 6. SUMMARY AND OVERALL CONCLUSIONS

No study has been published on the feasibility of grain amaranth cultivation in eastern Canada. The research presented in this thesis strongly suggests that grain amaranth could be produced in southwestern Québec. Although all of the genotypes studied over the two years of experimentation matured, some seemed more promising. PI 538323 (K432), PI 538324 (K433), PI 538326 (D70-1) and PI 558499 (Plainsman) seemed particularly adapted to local conditions. Plainsman was probably the best cultivar evaluated, combining several desirable characteristics including high yield potential, low incidence of lodging, early anthesis, and a relatively high harvest index. It is also the most widely available grain amaranth cultivar. Thus at this point it should be considered as a potential cultivar by anyone considering commercial production of grain amaranth in southwestern Québec. Mechanical combining of the crop was possible, even under the wet fall conditions experienced during the field experiments, which suggest that the crop should be harvestable without major problems in most years. Drying of the seeds is however necessary. As no herbicide is currently available for grain amaranth, proper mechanical weed control is essential.

Optimal management practices for grain amaranth production in eastern Canada are mainly dictated by the relatively short growing season and prevailing humid conditions. According to our results, earlier seeding dates seem preferable, as they resulted in higher grain yield in one environment, lowered seed moisture at harvest and minimized lodging in all environments. Seeding rate and row spacing did not affect grain yield. However, row spacing affected grain moisture at harvest, with narrower rows resulting in dryer grains. Seeding in narrow rows might thus be preferable when better weed management strategies become available; however, as no herbicides are currently available for use in grain amaranth, wider rows remain the only practical choice. Nitrogen fertilization increased grain yield in only one environment, however it also increased lodging. Seed moisture and plant height were also increased by nitrogen fertilization in all environments. Grain yield averaged 923 kg ha^{-1} across experiments and environments, which is comparable to yields obtained in North Dakota (Henderson et al., 1998). Eastern Canada could therefore be considered a potential area for grain amaranth production.

Grain amaranth has a high total oxalate concentration; however it is mostly present in the insoluble form. The absorbability of oxalate from grain amaranth is not known. If oxalate absorbability is low, then substituting amaranth for regular cereals could be done with fewer concerns for those people with predisposition to kidney stone development. However, if absorbability is high, selection for low-oxalate genotypes will be necessary. Based on our results, management appears not to represent an effective way of manipulating oxalate concentration and forms in grain amaranth. Because of the low oxalate:calcium ratio and the high calcium concentration of grain amaranth, we suggest that it might represent a good source of dietary calcium which could benefit vegetarians.

Although no economic analysis was conducted during this research, the yields obtained during this experiment seem not to be sufficient to create an incentive for producers to consider grain amaranth production. Therefore, unless significant yield increase can be achieved, grain amaranth will remain a marginal crop in the foreseeable future.

Chapter 7. RECOMMENDATIONS FOR FUTURE RESEARCH

There is a need for a dedicated breeding program to improve yields to levels similar to at least small grain cereals. Based on the results presented here, traits to improve are harvest index, resistance to lodging, early dry down and larger seeds. *Amaranthus pumilus* has seeds twice the size of current grain amaranth cultivars and its use in breeding program has been suggested (Marcone, 2000).

The development of grain amaranth-based food products would increase the potential for development of the crop. Research trips in countries where grain amaranth is consumed traditionally, like Mexico and India, could provide basis for the development of industrial products.

Since the climate of southwestern Québec is more humid than that of most regions where grain amaranth is cultivated, fungal diseases should be closely monitored. Some of them can produce mycotoxins, which can considerably reduce the quality of the crop. A study of the mycotoxin production potential should be conducted to determine the safety of grain amaranth grown under the more humid conditions of eastern Canada. A cultivar screening to identify resistance should be considered. The impact of management on mycotoxin production should also be studied.

Although grain amaranth has a high oxalate content, its absorbability should be low due to the high calcium and magnesium content of the seed. This should be investigated by an absorbability study, in order to determine whether oxalate content should be a concern in breeding programs. Because genetic variability is present for oxalate concentration and form, heritability studies are required.

REFERENCES

Anonymous. 1957. Pigweed seeds dated oldest U.S. food grain. Sci. News Lett. 72:345.

Agong, S.G., and P.O. Ayiecho. 1991. The rate of outcrossing in grain amaranths. Pl. Breeding 107:156-160.

Angus, J.F., D.H. Mackenzie, R.J.K. Myers, and M.A. Foale. 1982. Phasic development in field crops 3. The pseudocereals nuckwheat (*Fagopyrum esculentum*) and grain amaranth (*Amaranthus mantegazzianus*). Field Crop. Res. 5:305-318.

Apaza-Gutierrez, V., A. Romero-Saravia, F.R. Guillen-Portal, and D.D. Baltensperger. 2002. Response of grain amaranth production to density and fertilization in Tarija, Bolivia, p. 107-109, *In* J. Janick and A. Whipkey, eds. Trends in new crops and new uses. ASHS Press, Alexandria.

Aufhammer, W., D. Czuczorova, H.P. Kaul, and M. Krusse. 1998. Germination of grain amaranth (*Amaranthus hypochondriacus* x *A. hybridus*): effects of seed quality, temperature, light, and pesticides. Eur. J. Agron. 8:127-135.

Aufhammer, W., H.P. Kaul, P. Herz, E. Nalborczyk, A. Dalbiak, and M. Gontarczyk. 1995. Grain yield formation and nitrogen uptake of amaranth. Eur. J. Agron. 4:379-386.

Baltensperger, D.D., L.E. Weber, and L.A. Nelson. 1992. Registration of 'Plainsman' grain amaranth. Crop Sci. 32:1510-1511.

Bansal, G.L., Rana, M.C. and R.G. Upadhyay. 1995. Response of grain amaranth (*Amaranthus hypochondriacus*) to plant density. Indian J. Agric. Sci. 65:818-820.

Bavec, F. and Mlakar, S.G. 2002. Effects of soil and climatic conditions on emergence of grain amaranths. Eur. J. Agron. 17:93-103.

Becker, R., E.L. Wheeler, K. Lorenz, A.E. Stafford, O.K. Grosjean, A.A. Betschart, and R.M. Saunders. 1981. A compositional study of amaranth grain. J. Food Sci. 46:1175-1180.

Bejosano, F.P., and H. Corke. 1998. Protein quality evaluation of *Amaranthus* wholemeal flours and protein concentrates. J. Sci. Food Agric. 76:100-106.

Bennici, A., S. Schiff, and R. Bovelli. 1992. In vitro culture of species and varieties of four *Amaranthus* L. species. Euphytica 62:181-186.

Berger, A., I. Monnard, F. Dionisi, D. Gumy, K.C. Hayes, and P. Lambelet. 2003. Cholesterol-lowering properties of amaranth flakes, crude and refined oils in hamsters. Food Chem. 81:119-124.

Berti, M., H. Serri, R. Wilckens, and I. Figueroa. 1996. Field evaluation of grain amaranth in Chile, p. 223-226, *In* J. Janick, ed. Progress in new crops: Proceedings of the Third National Symposium Indianapolis, Indiana, 22-25 October, 1996. American Society for Horticultural Science, Alexandria.

Bhaskar, J., G.M. Bharad, and S.N. Patil. 1996. Effect of plant population, nitrogen and phosphorus on grain amaranth (*Amaranthus* species). Indian J. Agron. 41:181-182.

Block, C.C., J.W. Van Roekel, and L. Robertson. 2002. Pythium stem canker on grain amaranth. Phytopathology 92:S8.

Bohn, T.; Davidsson, L.; Walczyk, T.; Hurrell, R. F. 2004. Fractional magnesium absorption is significantly lower in human subjects from a meal served with an oxalate-rich vegetable, spinach, as compared with a meal served with kale, a vegetable with a low oxalate content. Br. J. Nutr.91:601-606.

Breene, W.M. 1991. Food uses of grain amaranth. Cereal Foods World 36:426-430.

Brenner, D.M., D.D. Baltensperger, P.A. Kulakow, J.W. Lehman, R.L. Myers, M.M. Slabbert, and B.B. Sleugh. 2000. Genetic Resources and Breeding of Amaranthus. Pl. Breeding Rev. 19:227-285.

Brenner, D.M. 2002. Non-shattering grain amaranth populations, p. 104-106, *In* J. Janick and A. Whipkey, eds. Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Bresler, G., S.B. Brizzio, and G. Vaamonde. 1995. Mycotoxin-producing potential of fungi isolated from amaranth seeds in Argentina. Int. J. Food Microbiol. 25:101-108.

Bresler, G.; Vaamonde, G.; Degrossi, C.; Pinto, V. F. 1998. Amaranth grain as substrate for aflatoxin and zearalenone production at different water activity levels. Int. J. Food Microbiol. 42:57-61.

Bressani, R., J.M. Gonzales, J. Zuniga, M. Breuner, and L.G. Elias. 1987. Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. J. Sci. Food Agric. 38:347-356.

Bressani, R. 1994. Composition and nutritional properties of amaranth, p 185-205. *In* Amaranth: biology, chemistry and technology; Paredes-Lopez, ed.; CRC Press: Irapuato, Mexico.

Calderon, E., J.M. Gonzalez, and R. Bressani. 1991. Agronomic, physical, chemical and nutritional characteristics of fifteen amaranth varieties. Turrialba 41:458-464.

Celiac Spruce Association. 2004. Celiac disease defined [Online] Available: http://www.csaceliacs.org/celiac_defined.php [26 September 2006].

Chai, W. W.; Liebman, M. 2005a. Effect of different cooking methods on vegetable oxalate content. J. Sci. Food Agric.53:3027-3030.

Chai, W.W., and M. Liebman. 2005b. Oxalate content of legumes, nuts, and grain-based flours. J. Food Comp. Anal. 18:723-729.

Champeil, A., Doré, T. and J.F. Fourbet. 2004. *Fusarium* head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Pl. Sci. 166:1389-1415.

Chan, K.F., and M. Sun. 1997. Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. Theoret. App. Genet. 95:865-873.

Chavez-Jauregui, R.N., M. Silva, and J.A.G. Areas. 2000. Extrusion cooking process for amaranth (*Amaranthus caudatus* L.). J. Food Sci. 65:1009-1015.

Chen, W.Q., and W.J. Swart. 2002. The *in vitro* phytoxicity of culture filtrates of *Fusarium oxysporum* to five genotypes of *Amaranthus hybridus*. Euphytica 127:61-67.

Chicago Dietetic Association. 2000. Manual of clinical dietetics; American Dietetic Association: Chicago, IL.

CRAAQ. 2003. Millet japonais, millet perlé et sorgho-soudan. p 237. *In* Centre de Référence en Agriculture et Agroalimentaire du Québec, ed. Guide de Référence en Fertilisation, 1st ed., Sainte-Foy, QC, Canada.

CRIACC. 2005. Centre de ressources en impacts et adaptation au climat et à ses changements [Online] http://www.criacc.qc.ca/ (verified November 30).

Cuadrado, C., G. Ayet, C. Burbano, M. Muzquiz, L. Camacho, E. Cavieres, M. Lovon, A. Osagie, and K.R. Price. 1995. Occurrence of saponins and sapogenols in Andean crops. J. Sci. Food Agric. 67:169-172.

Czerwinski, J., E. Bartnikowska, H. Leontowicz, E. Lange, M. Leontowicz, E. Katrich, S. Trakhtenberg, and S. Gorinstein. 2004. Oat (Avena sativa L.) and amaranth (*Amaranthus hypochondriacus*) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets. J. Nut. Biochem. 15:622-629.

Downton, W.J.S. 1973. *Amaranthus edulis*: a high lysine grain amaranth. World Crop. 25:20.

Drzewiecki, J. 2001. Similarities and differences between *Amaranthus* species and cultivars and estimation of outcrossing rate on the basis of electrophoretic separations of urea-soluble seed proteins. Euphytica 119:279-287.

Elbehri, A., D.H. Putnam, and M. Schmitt. 1993. Nitrogen-fertilizer and cultivar effects on yield and nitrogen-use efficiency of grain amaranth. Agron. J. 85:120-128.

Endres, C.S. and D.E. Longer. 1987. Herbicide selectivity among grain and weedy *Amaranthus* species. Agron. J. 79:824-826.

Erley, G.S., H.-P. Kaul, M. Kruse, and W. Aufhammer. 2005. Yield and nitrogen utilization efficiency of the pseudocereals amaranth, quinoa, and buckwheat under differing nitrogen fertilization. . Eur. J. Agron 22:95-100.

Espitia, E. 1992. Amaranth germplasm development and agronomic studies in Mexico. Food Rev. Int. 8:71-86.

Fernando, T., and G. Bean. 1985. Production of aflatoxins on amaranth seeds by *Aspergillus* spp. Food Chem. 17:33-39.

Fitterer, S.A., B.L. Johnson, and A.A. Schneiter. 1996. Grain amaranth harvest timeliness in eastern North Dakota, p. 220-223, *In* J. Janick, ed. Progress in new crops: Proceedings of the third national symposium, Indianapolis, Indiana, 22-25 October, 1996. American Society for Horticultural Science, Alexandria, VA.

Gélinas, B. and Seguin, P. 2007. Oxalate in grain amaranth. J. Agric. Food Chem. 55:4789-4794.

Gimplinger, D.M., G. Dobos, Shönlechner, and H.P. Kaul. 2007. Yield and quality of grain amaranth (*Amaranthus* sp.) in eastern Austria. Plant Soil Environ. 53:105-112.

Gorinstein, S., E. Pawelzik, E. Delgado-Licon, R. Haruenkit, M. Weisz, and S. Trakhtenberg. 2002. Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. J. Sci. Food Agric. 82:886-891.

Goswami, R.S. and C. Kistler. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Pl. Path. 5:515-525.

Greizerstein, E.J., and L. Poggio. 1994. Karyological studies in grain amaranths. Cytologia 59:25-30.

Grentz, L.; Massey, L. 2002. Contribution of dietary oxalate to urinary oxalate in health and disease. Top. Clin. Nutr. 17:60-70.

Gudu, S., and V.K. Gupta. 1988. Male-sterility in the grain amaranth (*Amaranthus hypochondriacus* Ex-Nepal) variety Jumla. Euphytica 37:23-26.

Gupta, V.K., and D. Thimba. 1992. Grain amaranth - A promising crop for marginal areas of Kenya. Food Rev. Int. 8:51-69.

Hackman, D., and R. Myers. 2003. Market Opportunities for Grain Amaranth and Buckwheat Growers in Missouri, Report to the Federal-State Marketing Improvement Program, Washington, D.C.

Heaney, R. P.; Weaver, C. M.; Recker, R. R. 1988. Calcium absorbability from spinach. Am. J. Clin. Nutr. 47:707-709.

Hackman, D., and R. Myers. 2003. Market opportunities for grain amaranth and buckwheat growers in Missouri, Report to the federal-state marketing improvement program, Washington, D.C.

He, H.P., and H. Corke. 2003. Oil and squalene in *Amaranthus* grain and leaf. J. Agric. Food Chem. 51:7913-7920.

He, H.P., Y.Z. Cai, M. Sun, and H. Corke. 2002. Extraction and purification of squalene from *Amaranthus* grain. J. Agric. Food Chem. 50:368-372.

Henderson, T.L., B.L. Johnson, and A.A. Schneiter. 1998. Grain amaranth seeding dates in the Northern Great Plains. Agron. J. 90:339-344.

Henderson, T.L., B.L. Johnson, and A.A. Schneiter. 2000. Row spacing, plant population, and cultivar effects on grain amaranth in the Northern Great Plains. Agron. J. 92:329-336.

Holmes, R. P.; Assimos, D. G. 2004 The impact of dietary oxalate on kidney stone formation. Urol. Res. 32:311-316.

Honow, R., D. Bongartz, and A. Hesse. 1997. An improved HPLC-enzyme-reactor method for the determination of oxalic acid in complex matrices. Clin. Chim. Acta 261:131-139.

Horner, H. T.; Cervantes-Martinez, T.; Healy, R.; Reddy, M. B.; Deardorff, B. L.; Bailey, T. B.; Al-Wahsh, I.; Massey, L. K.; Palmer, R. G. 2005. Oxalate and phytate concentrations in seeds of soybean cultivars [*Glycine max* (L.) Merr.]. J. Agric. Food Chem. 53:7870-7877.

Irving, D.W., A.A. Betschart, and R.M. Saunders. 1981. Morphological studies on *Amaranthus cruentus*. J. Food Sci. 46:1170-1174.

Jaegger, P.; Robertson, W. G. 2004. Role of dietary intake and intestinal absorption of oxalate in calcium stone formation. Nephron. Physiol.98:64-71.

Jain, S.K., H. Hauptli, and K.R. Vaidya. 1982. Outcrossing rate in grain amaranths. J. Hered. 73:71-72.

Johnson, B.L., and T.L. Henderson. 2002. Water use patterns of grain amaranth in the Northern Great Plains. Agron. J. 94:1437-1443.

Johnson, H.S., and M.D. Hatch. 1968. Distribution of the C4-dicarboxylic acid pathway of photosynthesis and its occurence in dicotyledonous plants. Phytochemistry 7:375-380.

Joshi, B.D. 1985. Annapurna - a new variety of grain amaranth. Indian Farming 35:29-31.

Judprasong, K.; Charoenkiatkul, S.; Sungpuag, P.; Vasanachitt, K.; Nakjamanong, Y. 2006. Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. J. Food Compos. Anal. 19:340-347.

Kauffman, C.S., and P.W. Haas. 1983. Grain amaranth: a crop with low water requirements and high nutritional value, p. 299-314, *In* W. Lockeretz, ed. Environmentally sound agriculture: selected papers, 4th conference, International

Federation of Organic Agriculture Movements, Cambridge, Mass., August 18-20, 1982. Praeger, New York.

Kauffman, C.S., and L.E. Weber. 1990. Grain amaranth. Janick, J. And J. E. Simon (Ed.). Advances in new crops; First National Symposium, New crops: Research, development, economics, Indianapolis, Indiana, Usa, October 23-26, 1988. Xxii+560p. Timber Press, Inc.: Portland, Oregon, Usa. Illus. Maps. 1990. 127-139.

Kaul, H.P., W. Aufhammer, B. Laible, E. Nalborczyk, S. Pirog, and K. Wasiak. 1996. The suitability of amaranth genotypes for grain and fodder use in central Europe. Bodenkultur 47:173-181.

Koeppe, S.J., J.H. Rupnow, C.E. Walker, and A. Davis. 1985. Isolation and heat-stability of trypsin-inhibitors in amaranth (*Amaranthus hypochondriacus*). J. Food Sci. 50:1519-1521.

Konishi, Y., H. Iyota, K. Yoshida, J. Moritani, T. Inoue, N. Nishimura, and T. Nomura. 2004. Effect of moisture content on the expansion volume of popped amaranth seeds by hot air and superheated steam using a fluidized bed system. Biosci. Biotech. Bioch. 68:2186-2189.

Krishnan, P., T.A. Evans, and W.G. Pill. 1994. Threshing cylinder speed affects germination of *Amaranthus cruentus* L. seeds. Hortscience 29:652-654.

Kulakow, P.A. and Jain, S. 1987. Variation and early generation response to selection in *Amaranthus cruentus* L. Theoret. Applied Genet. 74: 113-120.

Lehmann, J.W. 1996. Case history of grain amaranth as an alternative crop. Cereal Food. World 41:399.411.

Lehmann, J.W., R.L. Clark, and K.J. Frey. 1991. Biomass heterosis and combining ability in interspecific and intraspecific matings of grain amaranths. Crop Sci. 31:1111-1116.

Leon-Camacho, M., D.L. Garcia-Gonzalez, and R. Aparicio. 2001. A detailed and comprehensive study of amaranth (*Amaranthas cruentus* L.) oil fatty profile. Eur. Food Res. Tech. 213:349-355.

Libert, B.; Franceschi, V. R. 1987. Oxalate in crop plants. J. Agric. Food Chem. 35:926-938.

Liebman, M.; Costa, G. 2000. Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. J. Urol. 163:1565-1569.

Lindeboom, N., C. P.R., and R.T. Tyler. 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch-Starke 56:89-99.

Lorenz, K.; Wright, B. 1984. Phytate and tannin content of amaranth. Food Chem. 14:27-34.

Majewski, Z., J. Chlebowski, M. Sypula, and D. Wierzbicki. 1994. Application of standard agricultural machinery to harvesting and post-harvest handling of amaranth. Acta Hort. 355:95-98.

Malligawad, L.H., and V.C. Patil. 2001. Effect of plant density and planting geometry on growth and yield of grain amaranths. Karnataka J. Agric. Sci. 14:11-17.

Marcone, F. 2000. First report of the characterization of the threatened plant species *Amaranthus pumilus* (Seabeach Amaranth). J. Agric. Food Chem. 48:378-382.

Marx, J.L. 1977. Amaranth: A comeback of the food of the Aztecs? Science 198:40.

Massey, L. K.; Palmer, R. G.; Horner, H. T. 2001. Oxalate content of soybean seeds (*Glycine max*: leguminosae), soyfoods, and other edible legumes. J. Agric. Food Chem. 49:4262.

Matson, P.A., Parton, W.J., Power, A.G. and M.J. Swift. 1997. Agricultural intensification and ecosystem properties. Science 277:504-509.

McIntosh, M.S. 1983. Analysis of combined experiments. Agron. J. 75:153-155.

Mihail, J.D., and E.R. Champaco. 1993. Diseases of *Amaranthus* spp. caused by *Pythium aphanidermatum* and *Macrophomina phaseolina*. Can. J. Bot. 71:1219-1223.

Mihalik, E., and Z. Szocs. 1993. Cultivated *Amaranthus* species as food and medicinal plants: results and problems in introduction in Hungary. Acta Hort. 344:215-218.

Myers, R.L. 1996. Amaranth: new crop opportunity, p. 207-220, *In* J. Janick, ed. Progress in new crops: Proceedings of the third national symposium. Indianapolis, Indiana, 22-25 October, 1996. American Society for Horticultural Science, Alexandria, VA.

Myers, R.L. 1998. Nitrogen fertilizer effect on grain amaranth. Agron. J. 90:597-602.

Nakata, P. A.; McConn, M. M. 2000. Isolation of *Medicago truncatula* mutants defective in calcium oxalate crystal formation. Plant Physiol. 124:1097-1104.

National Academy of Science. 1984. Amaranth : modern prospects for an ancient crop. Washington, DC.

Oleszek, W.; Junkuszew, M.; Stochmal, A. 1999. Determination and toxicity of saponins from *Amaranthus cruentus* seeds. J. Agric. Food Chem. 47:3685-3687.

Olson, D.L., and R.L. Wilson. 1990. Tarnished plant bug (Hemiptera: Miridae): effect on seed weight of grain amaranth. J. Econ. Entomol. 83:2443-2447.

Parkinson, J. A.; Allen, S. E. 1975. Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological material. Comm. Soil Sci. Plant Anal. 5:1-11.

Passioura, J. 2004. Water use efficiency in the farmers' fields, p. 302-321, *In* M. A. Bacon, ed. Water use efficiency in plant biology. Blackwell Publishing Ltd, Boca Raton, Fl.

Peiretti, E.G., and J.J. Gesumaria. 1998. Effect of interrow spacing on growth and yield of grain amaranth (*Amaranthus* spp). Invest. Ag. Prod. Prot. Veg. 13:139-151.

Petr, J., I. Michalik, H. Tlaskalova, I. Capouchova, O. Famera, D. Urminska, L. Tukova, and H. Knoblochova. 2003. Extension of the spectra of plant products for the diet in celiac disease. Czech J. Food Sci. 21: 59-70.

Peters, I., and S. Jain. 1987. Genetics of grain amaranths. III. Gene-cytoplasmic male sterility. J. Hered. 78:251-256.

Plate, A.Y.A., and J.A.G. Areas. 2002. Cholesterol-lowering effect of extruded amaranth (*Amaranthus caudatus* L.) in hypercholesterolemic rabbits. Food Chem. 76:1-6.

Pospisil, A., Pospisil, M., Varga, B. and Z. Svecnjak. 2006. Grain yield and protein concentration of two amaranth species (*Amaranthus* spp.) as influenced by the nitrogen fertilization. Eur. J. Agron. 25:250-253.

Prakash, D.; Pal, M. 1991. Nutritional and antinutritional composition of vegetable and grain amaranth leaves. J. Sci. Food Agric. 57:573-583.

Punita, A., and A. Chaturvedi. 2000. Effect of feeding crude red palm oil (*Elaeis guineensis*) and grain amaranth (*Amaranthus paniculatus*) to hens on total lipids,

cholesterol, PUFA levels and acceptability of eggs. Plant Food. Hum. Nut. 55:147-157.

Quinteros, A.; Farre, R.; Lagarda, M. J. 2003. Effect of cooking on oxalate content of pulses using an enzymatic procedure. Int. J. Food Sci. Nutr. 54:373-377.

Qureshi, A.A., J.W. Lehmann, and D.M. Peterson. 1996. Amaranth and its oil inhibit cholesterol biosynthesis in 6-week-old female chickens. J. Nut. 126:1972-1978.

Rathod, P.; Udipi, S. A. 1991. The nutritional quality and acceptability of weaning foods incorporating amaranth. Food Nutr. Bull. 13:98-102.

Ravindran, V., R.L. Hood, R.J. Gill, C.R. Kneale, and W.L. Bryden. 1996. Nutritional evaluation of grain amaranth (*Amaranthus hypochondriacus*) in broiler diets. Anim. Feed Sci. Tech. 63:323-331.

Saini, J.P., and J. Shekhar. 1998. Effect of nitrogen fertilizer on growth and yield of grain amaranth (*Amaranthus hypochondriacus*) cultivars under dry-temperate condition. Indian J. Agron. 43:743-746.

Sanchezmarroquin, A., A. Feriamorales, S. Maya, and V. Ramosmoreno. 1987. Processing, nutritional quality and sensory evaluation of amaranth enriched corn tortilla. J. Food Sci. 52:1611-1614.

SAS Institute Inc. 2003. SAS software. Version 9.1. SAS Institute, Inc., Cary, NC.

Sauer, J.D. 1950. The grain amaranths: a survey of their history and classification. Ann. Mo. Bot. Gard. 37: 561-632.

Sauer, J.D. 1967. The grain amaranths and their relatives: a revised taxonomic and geographic survey. Ann. Mo. Bot. Gard 54:103-137.

Saunders, R.M., and R. Becker. 1984. Amaranthus - A potential food and feed resource. p 357-396 *In* Pomeranz, Y., Ed. Advances in cereal science and technology, Vol. 6. American Association Of Cereal Chemists Inc., St. Paul, MN.

Savage, G. P.; Vanhanen, L.; Mason, S. M.; Ross, A. B. 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. J. Food Compos. Anal. 13:201-206.

Schulzschaeffer, J., G.F. Stallknecht, D.E. Baldridge, and R.A. Larson. 1989a. Registration of Montana-3 grain amaranth germplasm. Crop Sci. 29:244-245.

Schulzschaeffer, J., D.M. Webb, D.E. Baldridge, G.F. Stallknecht, and R.A. Larson. 1989b. Registration of Montana-5 grain amaranth germplasm. Crop Sci. 29:1580-1581.

Senthong, C., S. Julsrigival, D. Tiyawalee, and P. Wivutvongvana. 1992. Germplasm screening of grain amaranth in Chiang-Mai, Thailand. Food Rev. Int. 8:143-157.

Segura-Nieto, M., Barba de la Rosa, A. P. and O. Paredes-López. 1994. Biochemistry of amaranth proteins. p. 76-106. In: Paredes-López, O., Ed. Amaranth: biology, chemistry and technology. CRC Press, Boca Raton, FL.

Shin, D.H., H.J. Heo, Y.J. Lee, and H.K. Kim. 2004. Amaranth squalene reduces serum and liver lipid levels in rats fed a cholesterol diet. Brit. J. Biomed. Sci. 61:11-14.

Siener, R., R. Honow, A. Seidler, S. Voss, and A. Hesse. 2006. Oxalate contents of species of the polyganaceae, amaranthaceae and chenopodiaceae families. Food Chem. 98:220-224.

Singhal, R.S., and P.R. Kulkarni. 1988. Amaranths - an underutilized resource. Int. J. Food Sci. Tech. 23:125-140.

Small, E. 1995. Crop diversification in Canada with particular reference to genetic-resources. Can. J. Plant Sci. **75**: 33-43.

Sooby, J., R. Myers, D. Baltensperger, D. Brenner, R. Wilson, and C. Block. 2005. Amaranth, production manual for the central United States. University of Nebraska Cooperative Extension. Lincoln, NE.

Soriano, J.M., and S. Dragacci. 2004. Occurrence of fumonisins in foods. Food Res. Int. 37:985-1000.

Stallknecht, G.F., and J.R. Schulz-Schaffer. 1993. Amaranth rediscovered, p. 211-218, *In* J. Janick and J. E. Simon, Eds. New Crops. Wiley, New York, NY.

Stowe, C.M., T.P.O. Leary, and T. Kommedahl. 1977. Amaranth: use as food. Science 198:668.

Streeter, J. G. 2005. Effects of nitrogen and calcium supply on the accumulation of oxalate in soybean seeds. Crop Sci. 45:1464-1468.

Thomas Jefferson Agricultural Institute. 2006. Grain amaranth, a lost crop of the Americas [Online] Available: http://www.jeffersoninstitute.org/pubs/amaranth.shtml [20 September 2006].

Tillman, P.B., and P.W. Waldroup. 1988. Assessment of extruded grain amaranth as a feed ingredient for broilers 1. Apparent metabolizable energy values. Poultry Sci. 67:641-646.

Tucker, J.B. 1986. Amaranth: the once and future crop. Bioscience 36:9-13.

Vityakon, P., B.R., Standal. 1989. Oxalate in vegetable amaranth (*Amaranthus gangeticus*) - forms, contents, and their possible implications for human health. J. Sci. Food Agric. 48:469-474.

Webb, D.M., C.W. Smith, and J. Schulz-Schaeffer. 1987. Amaranth seedling emergence as affected by seeding depth and temperature on a thermogradient plate. Agron. J. 79:23-26.

Weber, E.L. 1987. Amaranth grain production guide. Rodale Research Center. Rodale Press, Inc. Emmaus, PA.

Williams, J.T., and D. Brenner. 1995. Grain amaranth (*Amaranthus* species). p. 129-186, *In* J. T. William, ed. Cereals and Pseudocereals. Chapman & Hall, London.

Wilson, R.L. 1989. Studies of insects feeding on grain amaranth in the midwest. J. Kansas Entomol. Soc. 62:440-448.

Wu, H.X., and H. Corke. 1999. Genetic diversity in physical properties of starch from a world collection of Amaranthus. Cereal Chem. 76:877-883.

Wu, H.X., M. Sun, S.X. Yue, H.L. Sun, Y. Cai, R.H. Huang, D. Brenner, and H. Corke. 2000. Field evaluation of an *Amaranthus* genetic resource collection in China. Genet. Resour. Crop Evol.47:43-53.

Zhang, Y. P.; Lin, X. Y.; Zhang, Y. S.; Zheng, S. J.; Du, S. T. 2005. Effects of nitrogen levels and nitrate/ammonium ratios on oxalate concentrations of different forms in edible parts of spinach. J. Plant Nutr. 28:2011-2025.

Zheleznov, A.V., L.P. Solonenko, and N.B. Zheleznova. 1997. Seed proteins of the wild and the cultivated *Amaranthus* species. Euphytica 97:177-182.

Zimmermann, D. J.; Hesse, A.; von Unruh, G. E. 2005. Influence of a high-oxalate diet on intestinal oxalate absorption. World J. Urol. 23:324-329.

Zindler-Frank, E.; Honow, R.; Hesse, A. 2001 Calcium and oxalate content of the leaves of *Phaseolus vulgaris* at different calcium supply in relation to calcium oxalate crystal formation. J. Plant Physiol. 158:139-144.