The Genetics of Cadmium Accumulation

in

Durum Wheat (*Triticum turgidum* L. var *durum*) Grain

By

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THE GENETICS OF CADMIUM ACCUMULATION IN DURUM WHEAT (*Triticum turgidum* L. *var durum*) GRAIN

BY

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

This is a study of the genetics of grain cadmium (Cd) accumulation in durum wheat (*Triticum turgidum* L. var *durum*). Specific objectives were; inheritance of Cd accumulation ,allelism of different low Cd accumulating breeding lines, and the identification of a molecular marker linked to a gene(s) governing Cd accululation.

The inheritance of Cd was determined on the F_2-derived F_3 families from crosses derived between high and low grain Cd accumulating populations; Sceptre/Biodur (also F_2 derived F_4), DT618/DT627, and DT471/DT624. Selected Sceptre/Biodur F_2 derived F_4 were grown in the greenhouse to verify F_3 results, otherwise all lines were grown outdoors. F_2 populations of low by low Cd accumulating crosses DT471/DT637, Biodur/DT637, DT627/Biodur and Nile/DT471 were grown outdoors. F_3 families of a suspected group of high Cd accumulating F_2's were grown indoors. The DT471/DT624 population was also tested for linkage between Cd accumulation and tyrosinase activity, gliadins, and low molecular weight type-2 glutenins. Selected DT471/DT624 and Sceptre/Biodur families, Langdon(*T. dicoccoides*) substitution lines and Chinese Spring nullisomic-tetrasomics were searched for randomly amplified polymorphic DNA (RAPD) fragments linked to loci governing Cd accumulation.

The frequency distributions of Sceptre/Biodur and DT471/DT624 were skewed towards low Cd accumulation, indicating that low accumulation is partially dominant to

dominant. In the DT618/DT627 cross, the distribution was not normal. DT627 and DT471 are considered to have the same low Cd source. In the Sceptre/Biodur cross, the majority of selected F_4 families grown indoors were high Cd accumulating, supporting a single gene hypothesis. Heritability estimates of Cd accumulation were 68.6% (nearly narrow sense) in the Sceptre/Biodur and 88.94% (broad sense) in the DT618/DT627 populations. From the F_2 and F_3 family data of intercrosses, the two low sources, Biodur and DT637 (sib line DT627), are believed to be allelic. One RAPD fragment, previously found in Sceptre and linked to Cd accumulation, behaved accordingly with the Sceptre/Biodur progeny tested. Substitution line Langdon (*T. dicoccoides* 5B), a low Cd accumulator, had the same RAPD fragment as other substitutions, suggesting it may not be allelic to Biodur and DT637/DT627. The Chinese Spring nullisomic-tetrasomic lines gave no chromosome location clues. No linkage was detected between genes for Cd accumulation and molecular markers in this study.

1.0 INTRODUCTION

Triticum turgidum L. var *durum* is the cultivated allotetraploid wheat used primarily to make pasta. It is made up of two genomes, A and B, each containing seven pairs of chromosomes. Durum wheat ranks eighth in area devoted to cereals worldwide and is cultivated over more than 21.0 million hectares (Bozzini, 1988). Much of the durum wheat produced by Canada is exported. Of the approximately 5,344,000 tonnes of durum wheat Canada held in 1992-1993, 43% was exported and only 3.5% was used in Canada for human food. Canadian durum wheat is traded throughout the world. Major trade partners in 1992-1993 included the United States, India, Algeria, and Japan (Canada Grains Council, 1994).

As an exported food product durum wheat must meet heavy metal content limits set by the Codex Alimentarius Commission. Current heavy metal cadmium (Cd) intake from food and tobacco may put segments of certain human populations at a health risk. Now a limit of 0.1 mg/kg for the Cd allowed in grains has been proposed by the Codex Alimentarius Commission (Tkachuk, personal communication).

The first association of Cd and human health problems was the 1946 report of Itai-Itia disease among the population of the Jintzu River basin in Toyama Prefecture, Japan. Water and rice were contaminated by zinc mine effluents and people consumed more than ten times the daily average Cd uptake per day (Hallenbeck, 1984). Malnutrition and other environmental

factors were not considered at the time but may have also played a role (Goyer and Cherian, 1979).

People exposed to Cd through their occupations have suffered kidney damage, bone lesions, and emphysema among other ill effects. Cd has been suggested to cause cancer or hypertension but conclusive evidence is lacking (Bernard and Lauwerys, 1984: Hallenbeck, 1984). The validity of these early reports is questionable. Tobacco smoking greatly increases the amount of Cd stored in the body (Bernard and Lauwerys, 1984) but smoking habits were not always taken into account (Hallenbeck, 1984). Some studies involved very high Cd doses (Wagner, 1993). Certain segments of such groups as pregnant or postmenopausal women, smokers, and people with severe nutritional deficiencies may have health problems due to chronic Cd exposure (Wagner, 1993; Goyer and Cherian, 1979).

The now proposed Cd limits put Canadian durum exports at risk. There is a new Canadian plant breeding objective, the development of lower Cd accumulating durum wheat cultivars. Plant breeders, agronomists, soil scientists, cereal chemists and many other professionals are now tackling this problem. To date no reduced Cd accumulation agricultural crop varieties have been released from breeding programs (Wagner, 1993).

There are several difficulties hampering scientists. First, little historical information on levels of Cd accumulation and its genetic control in cereal food crops is available. Secondly, current methods to determine Cd levels in

plants are destructive, slow and very costly. Finally, Wagner (1993) has suggested that germplasm for low Cd accumulating cereals may be limited as there has been little selective pressure for mechanisms of Cd exclusion.

The purpose of this study was to gain insight into the genetics of Cd accumulation in durum wheat grains. Specific objectives were:

1) To study the inheritance of low levels of Cd in the grain through crosses between previously identified relatively low Cd grain accumulating (LCGA) and high Cd grain accumulating (HCGA) breeding lines of durum wheat.

2) To determine the allelic relationship of the LCGA trait in different lines.

3) To identify a distinct and economical genetic marker linked to the LCGA trait.

2.0 LITERATURE REVIEW

2.1 Cd in Our Diets

As a trace element, Cd was difficult to accurately measure by early analytical methods. The term 'trace elements' now refers to elements that occur at the milligram per kilogram level or below. Though occuring at low levels, trace elements can influence an animal's biochemistry or cell function (Crosby, 1977).

Cd accumulates in the body. For non-smokers and people who do not work with heavy metals the principal source of Cd is food. The types of foods consumed determines the amount of Cd stored in the body. Ordinary tap water contains less than 2 μg of Cd per litre. The total biological half-life of Cd in the liver and kidneys is estimated at 30 years (Hallenbeck, 1984).

Wheat products (particularly pasta), potato products and seafood are often identified as containing high Cd levels (Crosby, 1977; Van Dokkum et al, 1989, Dabeka and McKenzie, 1992). In a total diet study of average adult Canadians, the four highest sources of Cd in the bakery category were wheat and bran cereals, rice cereal, raisin pie, and plain pasta. Daily ingestion by Canadian adults was 14.5 μg/day (Dabeka and McKenzie,1992). The FAO/WHO has set a provisional tolerable weekly intake of 400-500 μg/person or 57-71 μg/person/day (FAO/WHO, 1989). In comparison, in Pakistan the estimated weekly intake of Cd from wheat flour alone is 374.0 μg/person.

This is 93% of the tolerance level set by WHO (Ahmad et al., 1994).

What an acceptable limit for Cd is in the human diet has been the source of current debate. The FAO Codex Alimentarius Commission (1991) has been attempting to draft new guidelines for the levels of Cd in certain food groups. Australia, Denmark, the Netherlands, and Hungary have set specific limits for Cd in foodstuffs. The Australian National Health and Medical Research Council has set the maximum permissible concentration of Cd in unspecified foodstuffs at 0.05 mg/kg fresh weight. Its limits for bran and wheat germ are 0.2 mg/kg fresh weight (Oliver et al., 1993).

2.2 Plant Influence on Uptake and Tolerance of Heavy Metals

2.2.1 Zinc, Cd & Copper: Important Kin?

Many of the studies on Cd uptake in plants include the heavy metals zinc (Zn) and copper (Cu). Zn and Cu are located near Cd on the periodic table. The three metals share many common properties, such as their binding characteristics. Cd has been assumed to behave like Zn in soil-water-plant systems because of these similarities (Mitchell et al., 1978). There is a positive correlation between Cd and Zn content in Swedish winter wheat grain (A. Andersson and O. Pettersson, 1981) and in lettuce (*Lactuca sativa* L. var. *crispa*) (Xue and Harrison, 1991). Furthermore, researchers have identified genes conferring resistance to Zn and Cd ions (the ZRC1 gene) as

well as Cu and Cd ions (the CUP1 gene) in the yeast *Saccharomyces cerevisiae* (Kamizono et al., 1989; Jeyaprakash et al., 1991).

Plants require Zn for many metabolic processes. Cd is not required and can disrupt plant enzymes (Hutchinson, 1979). The general order of metal accumulation in aerial plant tissue is Zn> Cd> nickel (Ni)> Cu (Mitchell et al., 1978). The rate of accumulation in the roots of sunflowers (*Helianthus annus* L.) grown in nutrient solutions was Cu> Cd> Zn> lead (Pb), but in the aerial tissue it changed to Zn> Cu> Pb >Cd (Kastori et al., 1992).

2.2.2 Different Genotypes = Different Cd Concentrations

Cd uptake varies among and within plant species. Tomato (*Solanum lycopersicum* L.) seedlings grown in nutrient solutions had over twice the Cd uptake of cucumber (*Cumumic sativus* L.) exposed to similar conditions (Pettersson, 1977). Over several Saskatchewan and Manitoba locations the bread wheat cultivar Katepwa had lower Cd concentrations than the durum wheat Kyle (Leisle, personal communication). The durum wheat cultivars grown in the United States surveyed by Meyer et al. (1982) had significantly higher Cd levels than other types of wheat grown in similar soil conditions.

Various corn and lettuce genotypes raised under similar conditions have been observed to take up different amounts of Cd (Hinesly et al., 1982; Thomas and Harrison, 1991). Genotypes were the most significant main effect accounting for

variation in Cd concentrations in a factorial analysis of variance of sludge rates, corn tissues, years and corn hybrids. Cd concentration in the tissues of a hybrid produced from two high Cd accumulators was much greater than a hybrid produced from two low Cd accumulators under all conditions tested (Hinesly et al., 1982). The cultivar Holme (*Triticum sativum* Lam) consistently had lower seed Cd concentrations than Starke in the six locations analyzed by Pettersson (1977). Yuran and Harrison (1986) had a significant genotype by year interaction term in their split-plot analysis of lettuce (*Lactuca sativa* L.) leaf Cd concentrations. While several of their 60 lines had consistent Cd concentration rankings over two years a few exhibited quite large changes. One cultivar rose from ranking 6th lowest in leaf Cd levels, to 54th. The grain Cd levels of the rice cultivars Hungarian and Taichung grown in pots of untreated soil were 0.01 ppm and 0.02 ppm respectively (Williams and David, 1972). After 200 ug of Cd was soil incorporated, grain Cd levels rose only slightly in Hungarian (0.05 ppm) compared to Taichung (0.14 ppm). High root Cd levels in both Hungarian (4.00 ppm) and Taichung (1.27 ppm) led to the suggestion that somehow the plants were retaining Cd in their root systems.

2.2.3 Genetics of Heavy Metal Tolerance

The term 'metal tolerant' has been used to identify an entire species, or individuals exhibiting the trait within a species (Antonovics et al., 1971). Many articles have been

written on the genetics of plants tolerant to heavy metal (Antonovics et al., 1971). However, the following question has not been addressed: "Do tolerant plants accumulate less metals in their edible portions than their non-tolerant brethren?"

Plants may tolerate metal rich environments by actively excreting metal ions out of their roots (Zhang and Taylor, 1989). Aluminum (Al) absorption by Al-tolerant *Triticum aestivum* and Cd absorption by *Lupinus albus* increased after treatment with 2,4-dinitrophenol (DNP), a metabolic inhibitor (Zhang and Taylor, 1989: Costa and Morel, 1993). Costa and Morel (1993) concluded that *Lupinus albus* has an active excretion mechanism, possibily inhibited by DNP, as well as an an active uptake mechanism. Sudhakar et al. (1992) observed the percent accumulation of lead in the roots and leaves of a tolerant legume, bengalgram (*Cicer arietinum* L. cv.'Annugiri'), was less than that of the other seven legumes grown in lead ore tailings.

Thomas and Harrison (1991) investigated whether four lettuce lines known to differ in leaf Cd concentrations also differed in Cd translocation or uptake efficiency. These lines had similar Cd concentration rankings during two years of field study. Their low-to-intermediate line ('Ruby') had the lowest overall Cd uptake rate in six of the seven Cd solutions they chose. At 60.0 nM and 100.0 nM, Cd uptake by a high Cd line (PI278080) was much greater than the two low Cd lines (PI 169693 and PI140398). However, there were significantly

greater translocation percentages in PI278080 and Ruby than in PI169493 or PI140398. In comparison, the cell walls of Zn tolerant *Agrostis tenuis* clones accumulated more Zn than the non-tolerant clones, but Turner and Marshall (1972) only studied root subcellular fractions. Also, some clones had a three-fold increase in Zn cell wall binding capacity, but little variation in their calculated tolerance values.

The parallel technique has been used to categorize plants as metal tolerant or non-tolerant (Walley et al., 1974). The parallel technique is the ratio of the length of the longest root grown in a metal containing solution to the longest root of a plant growing in control solution such as hydrated calcium nitrate. The tolerance index calculation is very similar but uses the mean rootlength instead (Turner and Marshall, 1972; Humphreys et al., 1984). The problem with these indexes is that the values are based on phenotypic observations. Root growth vigour may be under independent genetic control in culture solutions with and without metals (Humphreys and Nicholls, 1984).

Tolerance is dominant according to the many studies cited in a review by Antonovics et al. (1971). In 1974 Walley et al. screened for Zn and Cu tolerant *A. tenuis* on Cu waste and Zn waste soil mixtures. Upon observing the small number of survivors, they stated that tolerance is metal specific and tolerances to Cu and Zn are independent. Following analysis of a 10x10 diallel cross and an 8x8 diallel cross involving Zn

tolerant *Anthoxanthum odoratum*, researchers concluded that tolerance is partially dominant with a high additive component, polygenic, and under no maternal control (Gartside and McNeilly, 1974a). Finally, Gartside and McNeilly (1974b) stated tolerance was not a universal feature of species, but is restricted to certain genotypes within the species. They studied nine plant species, of which only one (*A. tenuis*) is commonly found on Cu contaminated British soils. Six generations of selection were required to attain fully Cu tolerant *A. tenuis* individuals, but 26 generations would be needed to select Cd tolerant *Arrhenatherum elatius*.

2.2.3 The Genetics of Cd Accumulation

There has been very little genetic research in heavy metal accumulation compared to the research on tolerance. In a paper, regarding Cd and Zn accumulation by corn hybrids, Hinesly et al. (1982) stated there was no maternal effect on metal uptake in reciprocal crosses between corn inbreds that accumulated high and low amounts of metals.

Thomas and Harrison (1989) conducted an inheritance study of Cd concentrations in lettuce. Low and high Cd accumulating parental lines were crossed and F_1, F_2 and F_3 generations grown in pots. Mean F_1 and F_2 Cd concentrations were lower than the overall midparental mean (i.e. closer to the low Cd parental mean). They concluded that low Cd accumulation is partially dominant to high accumulation in lettuce. Their Cd accumulation division point was the midparental mean of the

parents grown with each population. Those lines above the mean were considered high Cd accumulators, and those below the mean as low Cd accumulators. Their F_2 generation fit a 3:1 low:high Cd accumulating ratio (using a midparental mean cutoff), but the F_3 plants did not fit a 5:3. Broad sense heritability, calculated using parental and F_2 variances, was 0.38 and 0.29 for their two crosses. Pearson correlation coefficients between the means of the F_3 on their F_2 parents were used to calculate narrow sense heritabilities. They were 0.05 and 0.30 respectively. Finally, there was a low correlation between leaf dry weight and Cd levels.

2.3 Environmental Effects

2.3.1 Amendments: Fertilizers, Manure, Sludge and Irrigation

Both increases and decreases in plant Cd levels have been observed by scientists using nitrogen (N), zinc (Zn) or phosphorous (P) fertilizers. Wheat Cd levels rose when fertilized with ammonium nitrate and rotated with legumes (Oliver et al., 1993). Wheat grown with nitrogenous fertilizers and in rotation with lupins had higher grain Cd than rotations of wheat-wheat, wheat-barley, wheat-peas, wheat-beans,wheat-pasture and wheat-fallow. An explanation for this may be that application of ammonium nitrate acidifies the soil (Foth, 1990) and legume roots excrete H^+. The form of nitrogen nutrition had a direct effect on Cd uptake and distribution in a lettuce study (Florijn et al., 1992). Four

lettuce varieties grown in NH_4 nutrient solutions had higher Cd and Zn concentrations than plants grown in NO_3 though the solution pH was constant.

In Australian field studies, Oliver et al. (1994) found that Zn fertilizer decreased wheat grain Cd at most sites tested. The relationship was affected by crop variety, soil characteristics and/or frequency of applications. Also, Zn fertilizer applications reduced durum wheat tissue Cd by 50% or more in a growth chamber experiment (Choudhary et al., 1993).

Manures, chemical fertilizers and the soil all interact and affect Cd availability. Wheat grain Cd levels were higher in NPK-fertilized plots (K=potassium) than farmyard manure plots in Rothamsted, England (Jones and Johnston, 1989). The manured soil held more Cd. Cd levels in hay fertilized with NPK and manure were similar though, when large amounts of organic matter were present. The experiments were confounded by changes in cultivars, Cd atmospheric deposition and herbage composition. Kim et al. (1988) tested 12 food plants including wheat and corn on two naturally Cd rich soils and four sludge treated soils. The plants sown in the naturally high Cd soils took up more Cd and Zn. No explanation was furnished. Sims and Kline (1991) though, saw no effect on wheat and soybean Cd, Cr and Pb tissue concentrations, using three soils, four rates of co-composted sewage sludge and four rates of lime.

Finally, a recent study found saline irrigation waters affect potato tuber Cd levels. Chlorine forms complexes with Cd, increasing its availability to plants; tuber Cd concentrations were positively correlated to chlorine concentrations in the soil (McLaughlin et al., 1993).

2.3.2 Soil Properties, Precipitation, and Heat

The cation exchange capacity (CEC), concentration and forms of other heavy metals present in the soil, pH, moisture levels, and temperature influence the amount of Cd available for uptake from the soil solution (Van Bruwaene et al., 1984). CEC of a soil refers to the total positive charges of adsorbed cations that its mineral and organic fractions will adsorb at a specific pH (Foth, 1990).

The different CEC properties of podzolic soil (low CEC) versus krasnozem (high CEC) resulted in a five-fold greater uptake of Cd by oats (Williams and David, 1972). Organic matter has a high CEC. It binds Cd allowing less to be bioavailable (Haighiri, 1974). As organic matter decomposes organic acids are formed increasing the CEC. The effects of organic matter are not permanent and are pH dependent (Foth, 1990).

The uptake of Cd was dependent on its own concentration and the availability of other elements in the case of a *Lactuca sativa* study (Thys et al., 1991). Lettuce Cd levels were significantly affected by Cd and Fe levels in the nutrient solution. The addition of 5 to 50 ppm Zn to soil

increased soybean Cd levels while 100 to 400 ppm Zn depressed the levels (Haghiri, 1974). In a solution culture study of durum wheat cultivars, as Cd levels reached 0.5 μM, root and shoot concentrations of Zn, Cu, K and manganese (Mn) in the cultivar Nile were more depressed than in Kyle (Jalil et al.,1993). Adding chromium acetate to sewage sludge/soil mixtures decreased tissue levels of Cu, Zn, Ni, Cd, and Mn in corn (*Zea mays* L.) and rye (*Seceale cereale* L.). Raising Zn concentrations of sludge/soil mixtures decreased the Cd/Zn plant tissue ratios but adding more Cu increased the ratio (Cunningham et al., 1975).

Brown et al. (1994) expressed the opinion that soil pH and natural soil Cd levels were important controllers of Cd bioavailability. Sludge amended soil pH did not significantly affect corn leaf Cd content, but grain Cd content declined above and below pH 6 (Jones et al., 1975). Cd levels in the seeds of sunflowers, wheat, peanuts, oats, and soybeans increased as field plots were treated with Cd rich sludge and soil pH was lowered (Chaney et al., 1993). Soil pH and wheat grain Cd were negatively related (r^2=0.47) in Australian field trials (Oliver et al., 1994). In a second study by Oliver (personal communication) wheat grain Cd levels decreased or did not change as soil pH increased. Dry matter production increased concomitantly with soil pH, possibly diluting wheat Cd levels. There was no consistent relationship between potato tuber Cd concentrations and soil pH (McLaughlin et al., 1993).

Also, cucumber leaf and fruit Cd and Zn levels were unchanged when the pH was lowered by adding granular sulfur into composts of peatmoss and sludge (Falahi-Ardakani et al., 1988).

Andersson and Pettersson (1981) observed that plant uptake of Cd is dependent on precipitation during the growing season. Low precipitation resulted in low Cd levels in the grain of winter wheat, the opposite occurred with high moisture levels.

Warm soils increased plant Cd levels on a few occasions. A significant increase in soybean dry matter production and Cd levels occurred as soil temperatures were raised from 15.5 to 26.6°C (Haghiri, 1974). The same relationship was observed between durum wheat tissue Cd concentration, and rising soil temperatures (Choudhary et al., 1993). Cd uptake by fresh roots and air dried roots of *Hordeum vulgare* L. Arivat increased linearly as the temperature of their nutrient solutions was raised from 3 to 50°C (Cutler and Rains, 1974). The authors suggested this implied that Cd uptake is mainly a physical process not dependent on plant metabolism.

2.4 Mechanisms of Control of Uptake/Tolerance

2.4.1 Cd Location Within the Plant

The location of Cd within the plant varies with plant species. Roots held most of the Cd measured in oat, wheat, and rice plants during a pot experiment using P fertilizers

(Williams and David, 1972). Jarvis et al. (1976) tested 23 species for Cd uptake in a solution culture, including winter wheat (*Triticum aestivum* L. cv. Capelle Desprez). Except for kale (*Brassica oleracea* ss. *acephala* L. cv. Maris Kestrl), lettuce (*Lactuca sariva* L. cv. Unrivalled), and watercress (*Rorippa nasturstium-aquaticum* L. Hayek) over 50% of the plants' total Cd was retained in the roots. Sela et al (1988) determined the aquatic fern *Azolla filiculoider* accumulates relatively equal amounts of Cd in the shoots and roots.

The second aspect of Jarvis's study was Cd localization and the types of roots. Fibrous roots of beets (*Beta vulgaris* L. cv. Pajbjerg Korsral), parsnips (*Pastinaca sativa* L. cv. Butter Cream), carrots (*Daucus carota* L. cv. Chantenay), and radishes (*Raphanus sativus* L. cv. French Breakfast) had higher Cd concentrations than the storage roots. Transport of Cd to the stem appeared to be restricted by some unknown mechanism.

There was no release of Cd from the margins of tomato leaves observed by Petit and Van de Geijn (1978). Older leaves had significantly higher Cd concentrations than younger leaves in six lettuce cultivars grown at two soil Zn and two soil pH levels (Xue and Harrison, 1991).

In oats 25% of total grain Cd is in the glumes and 60% of total straw Cd is in the leaves (Williams and David, 1972). Hexaploid wheat and durum wheat products were tested in Australian mills. The pollard and bran fractions had higher Cd concentrations than the flour or wheat grains. Bran is defined

as all structures of the kernel from the aleurone outward, and pollard is a mixture of endosperm, wheat germ and small bran particles. Two blends of durum wheat were tested. The blend containing the durum wheat with the highest protein content also had the higher Cd content (Oliver et al., 1993).

2.4.2 Cellular Control of Cd Accumulation

2.4.2.1 Metallothioneins

Cd accumulation was first studied in animals. The family of proteins referred to as metallothioneins (MT) which bind metal ions were discovered by Margoshes and Vallee (1957) in horse kidneys. Cd and Zn were the only heavy metals present in large amounts in the fractions of the kidney cortex separated by paper electrophoresis. Subsequently, the thiol-containing metal binding polypeptides were identified in other organisms and formally named. Metallothioneins have a low molecular mass, a high metal content (Cd is not specified), a high cysteine (Cys) content with the absence of aromatic amino acids and histidine, and many Cys-a second amino acid-Cys sequences (Rauser, 1990).

Plants, algae and certain fungi produce a specific class of metallothioneins - "CLASS III: atypical, nontranslationally synthesized metal thiolate polypeptides" (Rauser, 1990). There is no official name. Common names are phytochelatins or cadystin, but γ-glutamylcysteinyl isopeptides, yeast metallothionein, copper-metallothionein, copperthionein, and

copper-chelatin (among many others) have been used (Rauser, 1992).

As was previously mentioned, two genes conferring Cd resistance, CUP1 and ZRC1 have been found in *Saccharomyces cerevisiae*. The protein product of the CUP1 gene of a Cd and Cu resistant *S. cerevisiae* strain has two distinct metal-binding configurations. The first coordinates 8 binding sites/molecule for Cu or silver ions and the second 4 binding sites/molecule of Cd or Zn ions (Winge et al., 1985). Cells expressing the ZRC1 gene were resistant to Zn and Cd but not to Cu, Co, or Ni. THe ZRC1 product is not cysteine-rich and this gene offers a different resistance mechanism than CUP1 (Kamiziono et al., 1989).

Yeast plasmid constructs containing 2 copies of the CUP1 gene grew on both Cu and Cd-supplemented media while constructs with 1 copy only grew on Cu. Thus, resistance to Cd via CUP1 depends on the level of gene product (Jeyaprakash et al., 1991). Cd-resistant and Cu-resistant *S. cerevisiae* strains appear to produce the same metallothionein, CUP1. Only Cd induces CUP1 expression in the Cd-resistant strain. Based on their observations of the CUP1 gene, the researchers suggested that resistance (ie tolerance) is a partially dominant phenotype (Tohoyama et al., 1992).

The significance of metallothioneins in the tolerance of higher plants to heavy metals is uncertain. Transgenic tobacco (*Nicotiana tabacum*) containing a mouse metallothionein gene

accumulated less Cd in the leaves than untransformed plants. There was no evidence of exclusion or differential uptake of Cd by the transgenic, transformed or untransformed tobacco seedlings. A kanamycin resistant gene segregated 3:1 in the transformant progeny like a dominant Mendelian trait (Maiti et al., 1989).

Single-copy Zn metallothionein genes have been located on the long arm of chromosomes 1A, 1B, and 1D in hexaploid wheat. Using RNA blotting, Kawashima and coworkers (1992) determined that the accumulation of mRNA was greatest in immature embryos. Mature embryos were induced to accumulate mRNA by adding abscisic-acid to the culture. The early cysteine-labeled (E_c) protein might control Zn homeostasis during early embryogenesis. Only a small amount of the Zn in mature embryos is bound by the E_c protein.

2.4.2.2 Phytochelatins: Plant Cells

Current opinion is that plant cells synthesize metal-binding isopeptides by modulating glutathione and cysteine (Jackson et al., 1987; Steffens, et al., 1986; Rauser, 1993). Cd resistant tomato (*Lycopersicon esculentum* cv. VFNT Cherry) cell suspensions bound 90% of intracellular Cd by low molecular weight, heat-stable $(\gamma\text{-Glu-Cys})_3\text{-Gly}$ and $(\gamma\text{-Glue-Cys})_4\text{-Gly}$ peptides in the cytosol (Steffens, et al., 1986).

There was a direct correlation between the amounts of Cd and cysteine in peaks detected by chromatography of *Datura innoxia* cells. Considering current opinion this suggested a

correlation between metal-binding polypeptides and resistance levels (Jackson et al., 1987). As phytochelatins are not directly synthesized on ribosomes they can not simply be sequenced in order to make oligonucleotide probes to locate the genes for their synthesis (Tomsett et al., 1988).

There are problems when applying phytochelatin theory to whole plant systems. The majority of studies involve plant cells cultured under high metal conditions (Wagner, 1993). Secondly, though Cd is highly effective in many organisms in inducing poly-$(\gamma\text{-EC})_n$gly production (Rauser, 1992), a broad spectrum of metals will induce low levels of peptide production (Verkleij et al., 1990). These peptides, then, do not fit classical data on metal-specific heavy metal tolerance (Tomsett and Thurman, 1988). Finally, small amounts of peptides accumulated in resistant and sensitive lines when there were no excessive metals (Steffens et al., 1986). Outridge et al. (1991) suggested that tolerance differences observed in yeasts and plants may be the result of qualitative differences in phytochelatins.

2.4.2.3 Vacuoles, Cell Walls and Salts

Cellular Cd has been found in vacuoles, bound to the cell wall, and as salt deposits. Extracts of Cd-treated rice plants were separated into three fractions. Two fractions contained Cd nonspecificly bound to cell components, and a type of metallothionein. The third fraction held the majority of the Cd and it was further separated into an inorganic Cd

salt and an unknown material (Kaneta et al., 1986). In 1974 Cutler and Rains characterized the uptake of Cd by air-dried barley (*Hordeum vulgare* L. cv. Arivat) roots. The amount of Cd taken up and retained rapidly increased over time, then stabilized after 30 minutes. The majority of the Cd was freely diffusible but some was nonmetabolically bound to organic compounds of the cell walls.

Van De Geijn and Petit (1978) suggested Cd cations were bound to the polysaccharides of the collenchyma or retained as a salt deposit such as Cd phosphate in tomato plant stems. Dark grains high in Cd, P and calcium were found in bundle xylem cells in the upper stem of *Azolla filiculoides*. A two- to three-fold increase in the P content of roots grown in the presence of Cd was observed (Sela et al., 1988).

Cd may be complexed with peptides/proteins or organic acids and then transported into vacuoles (Wagner, 1993). Using tobacco seedlings grown in nonphytotoxic Cd solution, Vogeli-Lange and Wagner (1990) showed most Cd is contained in vacuoles. The leaves were tested five to nine days after Cd exposure. Cd-binding peptides were produced and most were also in the vacuole. In a previous study using tobacco cells grown under non-growth-inhibiting Cd and Zn levels, Cd-binding peptides were not present until after three hours of cell exposure. Malic and citric acid already present in the vacuoles complexed with the Cd and Zn ions (Krotz et al., 1989). The mechanism by which Cd moves from the cytoplasm into

the vacuole is unknown. There is some evidence of a tonoplast Cd/H^+ antiporter activity (Salt and Wagner, 1993).

2.5 Molecular Markers

Morphological and molecular based markers are used to increase the efficiency of moving desirable traits through a breeding program. There are problems with morphological markers (Tanksley et al., 1989). They are often associated with deleterious effects or they mask the effects of linked minor genes. Seed proteins visualized by electrophoresis, monoclonal antibodies, and DNA polymorphisms are some types of the molecular markers that may be used in plant breeding without these problems.

2.5.1 Wheat Storage Proteins

Wheat storage proteins comprise a heterogeneous mix of protein types including, albumins, globulins, gliadins, and glutenins. Gliadins which are soluble in 70% ethanol, and glutenins which are soluble in acids, bases, hydrogen and hydrophobic bond-breaking solvents, are storage proteins and gluten forming (Feillet, 1988). Gliadins are small, single polypeptides mixtures (Pomeranz, 1988; Payne et al., 1984A) while glutenins are large molecules made of at least fifteen subunits. The glutenin subunits are further divided into those of low molecular weight (LMW) and those of high molecular weight (HMW) (Wall, 1979).

The gluten proteins are classified into the groups α, β, γ, and ϖ according to their mobility on starch gels (Jones et al., 1959; Woychik et al., 1961). Genes that code for the storage proteins have been located on chromosomes 1A, 1B, 1D, 6A, 6B, and 6D (Payne et al., 1984). Chromosome 6A mainly controls the α-gliadins and chromosome 6B the β-gliadins. Some components are controlled by both (Du Cros et al., 1983). The genes coding for the LMW-2 and LMW-1 glutenin subunits are located on the short arm of chromosome 1B (Payne et al., 1984B).

γ-Gliadin 45 and white glumes were early markers associated with gluten strength (Leisle et al., 1985). Payne et al. (1984) showed that durum wheat containing LMW-2 subunits and γ-gliadin 45 made strong pasta. There are at least two alleles of the LMW2 subunit, LMW-2 and LMW-2^- (Carrillo et al., 1990). Lower gluten strength in durum is associated with LMW-2^-.

The electrophoretic banding patterns directly reflect genotypes and are used as a method of varietal identification in wheat (Wrigley et al., 1982). Gliadin components can be separated by using polyacrylamide gel electrophoresis (Khan, 1982). The proteins separate according to size and charge while moving through the aqueous solution within the gel matrix (Conn et al., 1987; Khan, 1982). The genetic blueprint is stable under varying environmental conditions (Zillman and Bushuk, 1979). Gliadins are inherited as a group without

changes in composition. A block, or a group of bands detected by electrophoresis, is coded by a cluster of genes likely from a single ancestral gene. Inheritance is a codominant characteristic following simple Mendelian principles (Wrigley, 1982). A F_1 generation will contain the gliadins of both parents. Band intensity detected via electrophoresis is determined by gene dosage effect (Feillet, 1988).

2.5.2 Enzyme Linked Immunosorbent Assay

One method of detecting LMW subunits LMW-2 and LMW-2$^-$ is by using the monoclonal antibody (MAb) ***Gli*** 45-8. Durum wheat cultivars with the LMW-2 allele bind MAb ***Gli*** 45-8 (Howes et al, 1995). In an enzyme linked immunosorbent assay (ELISA) procedure a sandwich is made of antibody, antigen, enzyme-conjugated antibody against the antigen, and chromogenic substrate. Antigen titre is equivalent to the amount of coloured product released in a fixed time period (Nisonoff, 1984). Favourable aspects of ELISA are simultaneous testing of many seeds, computerized processed results and nondestructive testing. (Clarke et al., 1993).

Durum wheat lines have been screened for the presence of gliadin band 45, and strong gluten strength, using Mabs (Howes et al., 1989). In Australia, Howes et al (1991) screened 105 durum cultivars with a Gli-A2 specific Mab, a Glu-B1 specific Mab and two Gli-B1 specific Mabs and compared the results to mixograph tests. Two of the Mabs effectively identified half of the weakest 38 durum wheats. These Mabs could be used for

early generation durum line screening in the authors opinions.

2.5.3 Tyrosinase Test

According to Lamkin et al. (1981) tyrosinase activity is a genetic characteristic. Multiple forms of (poly)phenol oxidase, or tyrosinase have been identified in wheat. Tyrosinase production is dominant in tetraploid wheat, and controlled by one gene. The locus for production of tyrosinase is located on chromosome 2A and 2D of Chinese Spring substitution lines (Zeven, 1972). The addition of phenol, or a tyrosinate solution, to (poly)phenol oxidase cause melanin pigments to form (Zeven, 1972; Mahoney and Ramsay, 1992). The tyrosinase assay is one manner of distinguishing cereal germplasms which have high tyrosinase activity, an undesirable quality characteristic (Bernier and Howes, 1994).

2.5.4 Polymerase Chain Reaction and Random Amplified Polymorphic DNA

The polymerase chain reaction technique (PCR) was developed by Kary Mullin in 1987 (Barinaga, 1991). The PCR reaction exponentially amplifies a specific DNA sequence by using *Taq* DNA polymerase, an enzyme which was isolated from the thermophilic bacterium *Thermus aquaticus* (Erlich, 1989). Polymerases are enzymes that make the phosphodiester bonds of the sugar-phosphate backbone of DNA (Watson et al., 1992).

Random Amplified Polymorphic DNA markers (RAPD's) were suggested for use as genetic markers by Williams et al. (1990). RAPDS involve the amplification of random genomic DNA

segments (PCR products) using single, short oligonucleotide primers usually nine to ten nucleotides long (Waugh and Powell, 1992). Williams et al. (1990) stated that after analyzing RAPD samples taken from different individuals by electrophoresis not all segments (or bands) amplified were common. Polymorphisms result either from changes in the genomic DNA nucleotide sequence the primer binds to between individuals, or insertions, deletions or inversions of bases which may alter the size of the fragment or prevent amplication (Waugh and Powell, 1992)

Positive and negative points have been raised about the technical and theoretical aspects of RAPD analysis. RAPD analysis is being touted for identification of cereals. The PCR reaction is very sensitive and requires small sample sizes. Potentially, according to Ko et al. (1994), a standard, automated procedure can be set. RAPDs can be performed on crudely extracted DNA without the use of radioisotopes (Waugh and Powell, 1992). However, the concentration of the magnesium chloride, polymerase, and genomic DNA affect reproducible fragment amplification. Variations in the denaturing temperature (Devos and Gale, 1992) or slight changes in the annealing temperature alter RAPD banding patterns. Lastly, the type of PCR equipment used will affect the size range of fragments produced (Penner et al., 1993).

Michelmore and co-authors (1991) suggested that using RAPDs and bulk segregant analysis rather than near isogenic

lines or cytogenetic stocks to identify markers linked to particular genes. Bulk segregant analysis involves pooling the DNA from individuals that are identical for one particular trait, but otherwise potentially heterogeneous. The bulks are presumed genetically unalike only for the one locus, and are screened for polymorphisms. Ko et al. (1994) preferred RAPD analysis using 5S ribosomal gene primers, rather than primers of arbitrary sequences, for cereal species identification in pure samples. In seed mixtures not all fragments that had amplified in pure samples appeared. Generally individual plants of each cultivar had banding patterns consistent with bulk samples, but the scientists questioned the genetic consistency of individual plants within a cultivar grouping.

Simple and quick RAPD analysis was favoured by Fairbanks et al. (1993) to isozyme or Restriction Fragment Length Polymorphism analysis (RFLP) for screening a large number of samples to detect markers. Isozymes are plant enzymes that exist in multiple molecular forms and they have been shown to be different between and within species (McMillin, 1983). RFLP analysis involves probing Southern blots of restricted genomic DNA with a single copy DNA fragment (Gibson and Somerville, 1993). On one occasion both RAPDS and isozyme markers produced *Avena sterilis* L. dendographs of similar overall construction (Heun et al., 1994). However, RAPDs produced a more definitive separation of clusters than isozyme markers in the dendograph of genetic distances between accessions. In the researchers

opinion, DNA-based markers should be used to differentiate closely related accessions . Monte and co-researchers (1993) touted RFLP analysis above isozyme analysis during their studies of phylogenetic relationships in the tribe Triticeae because a larger number of loci were available. During RAPD analysis of wheat genomic DNA the frequency of polymorphisms was the same as RFLP analysis or lower (Penner, 1994; Devos and Gale, 1992). RAPDs were equivalent to RFLPs in determining genetic similarity among *Brassica oleracea* L. genotypes in one study. The differences in estimation of genetic relationships between RAPD or RFLP markers was mainly due to sampling errors (Dos Santos et al., 1994).

In polyploids recessive alleles may be masked by dominant homoeologous sequences making them difficult to identify. Segregation analysis may be used to confirm the identity of RAPD bands (Gale et al., 1989; Devos and Gale, 1992). The lack of polymorphism between closely related self-pollinating plant lines (Dweikat et al., 1993) and a high amount of repetitive sequences in the large wheat genome makes molecular mapping difficult (Gale et al., 1989, Penner, 1994). Penner (1994) stated the frequency of his ability to detect polymorphisms in Canadian durum wheat was less than 10%. The usefulness of RAPDs is also tempered by the inability to detect heterozygotes. RAPD markers show a dominant pattern (Devos and Gale, 1992) and may be scored incorrectly. The lack of a

fragment may be due to a mechanical failure in the PCR procedure, not an absent allele (Penner, 1994).

3.0 METHODS AND MATERIALS

3.1 Inheritance Study

Crosses were made between LCGA and HCGA durum wheat breeding lines and/or cultivars to study the genetics of low Cd. The crosses made were Sceptre/Biodur, DT471/DT624, and DT618/DT627. Pedigrees and grain Cd accumulation ratings are presented in Table 1. F_1 plants were grown in growth cabinets. Approximately 200 randomly selected F_2 seeds were planted in the greenhouse to produce F_3 families. All genetic crosses were planted in the field into non-replicated plots unless indicated otherwise.

The F_2 derived F_3 families of the crosses Sceptre/Biodur (148) and DT471/DT624 (126) were seeded as single rows in 1992 at Glenlea. Three rows of each parent were sown as LCGA/ HCGA pairs. Both crosses were planted with a Bolen seeder in 2.13 m (7') rows 0.30 m (1') apart, at about 40 seeds/row. Rows were individually bulk harvested.

In 1993 Sceptre/Biodur F_2 derived F_4 families (105) were seeded at Glenlea. The same seeding equipment, seeding rate, and row settings as in 1992 were used. Two rows of Sceptre/Biodur F_1, five rows of Sceptre, four rows of Biodur, two rows of Medora (a HCGA), and one row of DT471 were seeded as checks. Plants were hand weeded and sprayed once using a backpack sprayer with Tilt CIBA-Geigy (1.2 mL/L) to control Septoria and Tan Spot. Rows were individually bulk harvested in September.

One hundred and thirty-five DT618/DT627 F_2 derived F_3 families were planted at Morden in 1993. The Morden site was selected based on the high Cd levels observed in 1991 Durum Coop checks grown in that region, compared to their Cd levels at Glenlea, Stewart Valley or Elrose. The Morden material was arranged in the field as a 12X12 simple lattice with two replicates. Two lines each of DT618, DT627, Sceptre, and Medora and 1 line of DT627/DT618 F_1 were included per replicate. Lines were individually bulk harvested.

Twenty-eight selected F_4 families of Sceptre/Biodur (1992 F_3 Cd levels from 0.091 to 0.179 ppm) were planted into greenhouse beds February, 1994. Three rows per family, each with six randomly selected seeds, and two rows each of Sceptre and Biodur were planted. In addition, five randomly selected low Cd grain accumulating families from the same cross (0.028 to 0.070 ppm in 1992) were seeded. Rows were 71 cm (28") long and 8.9 cm (3.5") apart. The plants were sprayed once for control of aphids with Cygon-2E ICI Chipman (1.5mL/L). The greenhouse was set at 13°C (55°F) and 16 hours light per 24 hour cycle. Eight plants/family of eighteen of the F_4 families (originally 0.091 ppm to 0.114 ppm) and two of the randomly selected low Cd grain accumulating families were analyzed for grain Cd. Also, five plants of each parental check were analyzed.

3.2 Allelism Study

LCGA breeding lines and cultivars were grown in a 13°C growth cabinet and hand crossed (1992). Four seeds were planted per 5" pot and watered with 20-20-20 Plant Prod (3.3 mL/L or 1 tbs/gallon). Plants were treated with sulfur powder to control powdery mildew. The temperature was raised to 17°C after heading. Crosses made, and later harvested, were DT471/DT637, Biodur/DT637, DT627/Biodur, and Nile/DT471.

The F_1 seed was increased, two seeds/pot, in a growth cabinet (same settings as before). F_2 seeds were harvested, cleaned and placed into envelopes. To eliminate possible dormancy, seeds in envelopes were soaked in gibberellic acid in acetate ($6.5*10^{-4}$ M) for 15 - 20 min, then removed and left to dry overnight.

The F_2 of the intercrosses were planted at Glenlea June 1, 1993 (Figure 1). Blocks contained 192 seeds planted 0.30 m (1') apart into three paired rows with a 0.61 m (2') space between paired rows. Parents and F_1 were also planted.

In case of emergence problems extra F_2 seeds were planted into peat cups in the growth cabinet June 2. Where emergence was poor in the field the extra F_2 seedlings were transplanted (peat pots removed) to the field June 18. Plants were sprayed once with Tilt CIBA-Geigy (1.2 mL/L) for control of *Septoria* and tan spot. Mature plants were harvested individually. Grain from crosses which had over 72 mature plants were analyzed for Cd.

Seed from selected high Cd accumulating F_2 plants of the crosses DT627/Biodur and Biodur/DT637 were planted January 31, 1994 into 71 cm (28") rows 8.9 cm (3.5") apart in greenhouse beds. Rows, from a single F_2 plant, contained six seeds evenly spaced apart. Small or shrivelled seeds were started first in covered petri dishes in the fridge. One row each (six seeds) of Biodur, DT637, Sceptre and DT627 were planted as checks. The plants were sprayed once for aphid control with Cygon-2E ICI Chipman (1.5 mL/L). Mature single plants were harvested individually.

3.3 Cd Analysis

Five gram samples of bulk lines and two gram samples of single plants were analyzed for Cd levels. Whole, clean seed was weighed out. Number of seeds in all the two gram samples and the Sceptre/Biodur F_4 were determined using an electronic counter. Samples were ground in a Moulinex Coffee and Spice Mill with stainless steel blades. The samples were ground for 20 sec. The mill was physically shaken, one shake per second, to provide a more uniform grind for the small samples. Cd analysis was done using a graphite furnace atomic absorption spectrophotometer with microwave digestion at the Grain Research Laboratory, Agriculture and AgriFood Canada, Winnipeg (Gowalka and Tkachuk, 1994).

TABLE 1. Pedigrees and LCGA Ratings of Durum Wheat

Durum Line/ Cultivar	LCGA	Pedigree
DT471	yes[a]	Wascana/Hercules[b]//Cando/Edmore
Sceptre	no[cd]	6811/Ward/3/DT188[e]/DT224//DT182[e]
DT624	no[f]	S7838/S7986
DT618	no[a]	D66761/Quilafen//Macoun/SC7061-121-2
Biodur	yes[c]	French Cultivar
DT627 or DT637	yes[d]	Hercules/DT310//Mexican 2/3/Ward/Macoun/4/Edmore/Ward//Wakooma
Nile	yes[g]	ICARDA Line

[a] Based on Cd ppm ratings 1991 Durum Coop Test

[b] Low Cd ppm levels recorded in 1991 Glenlea Durum Increases, 1991 and 1992 Durum Coop Tests

[c] Based on Cd ppm ratings Fresh Water Institute, Glenlea 1989

[d] Based on Cd ppm ratings 1992 Durum Coop Test

[e] Sister selections of c.v. Hercules

[f] Based on Cd ppm ratings 1992 Durum Preliminary Test

[g] Based on Cd ppm ratings of diverse germplasm, Swift Current 1992

Figure 1. Field Plan of F_2 LCGA Intercrosses, Parents and F_1 Hills (1993, Glenlea)

Biodur/DT637[a] Bulk F_2	SC84/Biodur[a] Bulk F_2	Two rows of checks (Parents and F_1)	DT618/DT627[b]
DT637/DT473[a] Bulk F_2	DT637/DT471[a] Bulk F_2		Nile/DT637[a] Bulk F_2
DT471/DT637[a] Bulk F_2	DT637/Biodur[a] Bulk F_2		DT473/DT471[a] Bulk F_2
Biodur/DT471[a] Bulk F_2	Nile/DT471[a] Bulk F_2		DT627/Biodur[a] Bulk F_2

[a] Each F_2 bulk consists of three paired rows with a two foot space between pairs. Thirty two seeds/row for a total of 192 seeds.

[b] Planted for D. Leisle, not part of the study

3.3 Molecular Markers

3.3.1 Acid Polyacrylamide Gel Electrophoresis (APAGE)

The gliadins of all the DT471/DT624 F_3 families were separated by APAGE (Bushuk and Zillman, 1978). DT624 and DT471 have a β1 and β2 gliadin, respectively. A single seed of each family was tested.

Proteins were extracted from whole seeds set overnight at 40°C in 150 μl 50% (v/v) aqueous propan-2-ol in rigid, flat-bottomed 96-well ELISA plates (Dynatech), then squashed with a glass rod. A 6% acrylamide gel with 1.25% bis-acrylamide was prepared using aluminum lactate buffer (pH 3.1). One seed of each parent was included per gel. Gels ran 105 min at 520 V 7°C with a 8.5 mM aluminum lactate top buffer (pH 3.1) and a bottom buffer composed of 0.113% (v/v) of a 60% (w/w) sodium lactate solution (pH 3.1). The gels were stained overnight in 12% trichloroacetic acid to which a few drops of ethanol dissolved Brilliant Blue R (Sigma) had been added. It was destained in water until the gliadin bands could be scored visually and photographed on a UV transilluminator with a Polaroid type 55 positive/negative film in a Polaroid MP4 Copy Camera.

3.3.2 Tyrosinase

Five seeds from all 126 DT471/DT624 F_3 families were assayed for tyrosinase activity (Bernier and Howes, 1994). DT624 is high in tyrosinase and DT471 is low. Single kernels were placed into flat-bottomed 96-well microtitre plates, 22

μL of substrate solution was added (0.01 M disodium L-tyrosine, 0.1 M Tris-HCL buffer, 0.2% (w/v) Tween 80 pH 9.0). Checks, two seeds each of DT471 and DT624, were included per plate. The plates were incubated three hours (37°C). The first visual scoring was made after the 37°C incubation. The plates were left, covered, 24 hrs at 20°C and scored again.

3.3.3 Enzyme Linked Immunosorbent Assay (ELISA)

Ninety-four DT471/DT624 F_3 families were tested with monoclonal antibody (MAb) ***Gli*** 45-8 to distinguish LMW-2 and LMW-2$^-$ glutenin alleles (Howes et al., 1989). DT624 has the LMW-2 allele and DT471 has LMW-2$^-$. ELISA was performed on five seeds per family. The protein extracts were the same ones used in APAGE. Four seeds of each parent were included in each plate. The propan-2-ol extracts were diluted 1:300 in 70% (v/v) ethanol and bound for 20 min at 20°C or 12 hrs at 4°C. The plates were rinsed three times in TBS-Tween (0.01 M Tris-HCL pH 7.5, 0.9% NaCl, 0.05% Tween 80), blocked 20 min at 40°C with TBS-Tween containing 1.0% milk (Carnation brand), and rinsed again. (All rinses use TBS-Tween). Mab ***Gli*** 45-8 was diluted 1:200 in Blotto (0.05% milk), 50 μl/well added, bound for 60 min at 40°C, and rinsed 4 times (5 min between washes). A 1:2000 dilution of affinity purified goat anti-mouse IgG phosphatase (BioRad Laboratories, Richmond, CA) was added (50 μl/well) and incubated for 90 min at 40°C. After 5 rinses, the final substrate solution (0.5 mg/mL p-nitrophenyl phosphate, 1 M diethanolamine pH 9.6, 0.5 mM $MgCl_2$) was added and the

plates were left, covered, at 4°C overnight. The plates were scanned using a Titre Tek plate reader with a 405 nm filter. Each plate, which held a total of 80 seeds, was scored independently.

3.3.4 Polymerase Chain Reaction (PCR)

DT471, DT624, 30 individuals from the 15 highest and 15 lowest Cd accumulating DT471/DT624 F_3 families, Sceptre, Biodur, individuals from the 5 highest and 5 lowest Sceptre/Biodur F_4 families, Langdon-16, 13 Langdon(*T. dicoccoides*) disomic substitutions and 11 Chinese Spring nullisomic-tetrasomic substitutions were examined for randomly amplified polymorphic DNA (RAPD).

Three procedures were used to extract genomic DNA (gDNA). Rapid leaf disc gDNA extraction was performed using 1.5 mL microfuge tubes to punch the tissue (Edwards et al, 1991). Extraction buffer consisted of 200 mM Tris pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS. In certain cases proteins were removed by adding 0.05 mg/mL Proteinase K (Boehringer Mannheim). The mixture was incubated at 55°C for 30 min. gDNA was resuspended in 100 μl of 0.1 X TE (1 mM Tris pH 7.5, 0.1 mM EDTA) and stored at 4°C. Secondly, larger scale leaf gDNA extraction was performed using the method of McCouch et al., (1988) with the following modifications. DNA was precipitated overnight at -20°C with two volumes of cold 95% ethanol. DNA was rinsed three to four times with 70% ethanol. The DNA was precipitated using two volumes of cold 95% ethanol and 0.1

volumes 3 M sodium acetate overnight at -20°C. Samples were resuspended in 1 mL TE. The third method was seed gDNA extraction (Penner et al., 1994). Embryos were cut off 5 seeds/family. The endosperms, in a small amount of liquid nitrogen, were ground in a cold mortar with a pre-chilled pestle. The ground seed was scraped into a sterile 1.5 ml tube containing 500 µl 0.1 X TE. An equal volume of 1:1 phenol:chloroform was immediately added. The mixture was shaken, then centrifuged (6 min), resulting in two layers. In a new tube the top aqueous layer and an equal volume phenol:chloroform was re-centrifuged. The DNA was precipitated with 0.1 volumes 3M sodium acetate and 2.5 volumes of cold 95% ethanol at -20°C for minimum of one hour. Pelleted DNA was washed with 70% ethanol, dried, and resuspended in 200 µL 0.1*TE.

All parental and substitution line gDNA was extracted from the leaves of plants grown in either growth cabinets or greenhouses. Leaf discs from DT471/DT624 families were collected prior to heading from the field into ice filled coolers, then kept at -20°C until DNA extraction. The gDNA of the F_4 Sceptre/Biodur was seed extracted.

Concentrations of gDNA were determined by optical densities (ODs) with spectrophotometers, visual comparisons to standard ladders, and with a TKO 100 Mini-Fluorometer. (The fluorometer was only used once.) ODs were measured with a DMS 100 UV Visible Spectrophotometer or a Spectronic Genesys 5.

The concentration of gDNA = (OD 260 nm)(50 μg DNA/ml per OD unit)(total volume DNA sample and 0.1*TE loaded/volume DNA sample loaded). A λ EcoRI Hind III ladder and samples were run on a 1% agrose 0.5 μg/ml ethidium bromide mini-gel. The gDNA band was visually matched to a ladder band of comparable brightness. Concentration of gDNA = (Number of basepairs(bp) per band/total bp of ladder)(ladder concentration)(volume of ladder loaded into gel).

The PCR with arbitrary (RAPD) primers was carried out in a 25 μl reaction mixture containing one unit of Taq DNA Polymerase (Promega or Gibco BRL), 2mM $MgCl_2$ (Promega or Gibco BRL), 1 X PCR buffer (Promega or Gibco BRL), 0.8 mM dNTP (200 μM each of dATP, dCTP, dGTP and dTTP from Pharmacia), 30-40 ng gDNA and 20 pM primer (Penner et al., 1994). This procedure was later modified to use 1.5 mM $MgCl_2$ and 0.75 units of Taq. The reaction mixtures were overlaid with 15μL mineral oil. PCR was performed in a Thermolyne Temptronic thermocycler and was initiated by one cycle at 94°C denaturing (2 min), 36°C annealing (30 s), 72°C extension (1 min), followed by 35 cycles of 94°C (5 sec), 36°C (30 s), and 72°C (1 min). Reactions were held at 5°C until removed.

The RAPD fragments were first separated by agrose-gel electrophoresis (1.8% or 1.6% agrose and 0.5 μg/ml ethidium bromide). The gels ran for approximately 3.5 hours at 70 V in 2 L of 1 X TAE (0.04 M Tris-acetate, 0.001 M EDTA). Later, fragment separation was done by modified temperature sweep gel

electrophoresis (TSGE) which yielded more polymorphisms (Penner and Bezte, 1994). Gels were made of 8% acrylamide (37.5:1 acrylamide:bis-acrylamide), 3.5 M Urea, 20 % (v/v) deionized formamide, 1 X TAE, 0.05% (v/v) ammonium persulfate, and 0.01 % (v/v) TEMED. Four litres of 1 X TAE running buffer were brought to 25°C in a buffer tank with a circulating water bath. PCR amplified gDNA samples and 300 ng λ EcoRI Hind III ladder were loaded into the gel. The gel was placed into the buffer tank. The upper tank was filled with 350 mL of 1 X TAE. A peristaltic pump (Buchler) recirculated the upper and lower buffers at a rate of 80 mL/hour. A 300 V current was passed through the gel while the temperature was increased in 10°C increments/hour to 55°C. The gel ran for one hour at 55°C, then removed. A thermometer in the buffer tank generally recorded an increase from approximately 25°C to 43°C during the run. The gels were cooled under cold, running tap water, stained in a dilute ethidium bromide solution (0.5 μg/ml) for 10 min and rinsed for three minutes in water before photographing on a UV transilluminator using Polaroid type 57 4X5 Instant Sheet Film in a Polaroid MP4 Copy Camera. Agarose gels were exposed for 1.5 sec. Acrylamide gels were exposed for four seconds.

3.4 Statistical Analysis

Data was analyzed using the Statistical Analysis System, Version 6.09 (SAS, 1991). Frequency bar chart distributions based on Cd ppm were made for all populations. Divisions based

on the Cd concentrations of the HCGA parent, the midparental mean and arbitrary divisions of frequency distributions were made of LCGA, segregating, and HCGA families. Chi-square tests were used to test goodness of fit to hypothetical genetic ratios. Midparental Cd means were compared to the F_3 Cd means. Correlations of seed number to Cd levels were performed on the Sceptre/Biodur F_4 and the allelism crosses.

Results of the A-PAGE on the DT471/DT624 F_3 families were run through an exact two-sample t-test after determining that the populations the samples were drawn from were normally distributed. Data collected from the DT471/DT624 tyrosinase test were analyzed using chi-square. Two groups, low tyrosinase and high tyrosinase, were tested with an exact two-sample t-test. DT471/DT624 families which were segregating, or only expressing the LMW-2 or the LMW-2^- allele were analyed using chi-square. The LMW-2 types were tested with the Wilcoxon Rank Sum test.

Two heritability estimates were calculated. Narrow sense heritability and standard error were determined for the Sceptre/Biodur generations (Falconer, 1989). The DT618/DT627 F_3 was analyzed as a simple partially balanced lattice. After all checks were removed the data set became a randomly complete block design which was also analysed. Blocks and genotypes were considered random. Broad sense heritability was estimated using expected means squares (Dudley and Moll, 1969) and confidence intervals (Knapp et al., 1985) were calculated.

4.0 RESULTS AND DISCUSSION

4.1 Inheritance Study

TABLE 2. Means, Standard Errors, Sample Size and Range of the Sceptre/Biodur F_3 Families and Checks (Glenlea, 1992)

	Mean (ppm)	Standard Error (ppm)	Sample #	Range (ppm)
Biodur[a]			2	0.055-0.048
Sceptre[b]	0.113	0.002	3	0.109-0.116
Midparent	0.088	0.015	5	
F_3 Families	0.071	0.003	105	0.028-0.179

[a] Biodur sample was 0.039 ppm, Fresh Water Institute, Glenlea 1989
[b] Sceptre sample was 0.119 ppm, Fresh Water Institute, Glenlea 1989

4.1.1 Sceptre/Biodur Population

The 1992 field parental Cd concentrations for Biodur (0.055 to 0.048 ppm) and Sceptre (0.113 +/- 0.002 ppm) (Table 2) were similar to those previously observed for the Glenlea location. Cd values for the 105 F_3 Sceptre/Biodur families ranged from 0.028 to 0.179 ppm. The frequency distribution (Figure 2) is skewed towards the lower Cd values (skewness 0.882044). The F_3 mean (0.071 +/- 0.003 ppm) is less than the

midparental mean (0.088 +/-0.015) indicating that low Cd accumulation tends to be partially dominant to dominant.

The distribution is not clearly bimodal, but attempts were made to fit the distribution to various two gene pair and one gene hypotheses with two phenotypic groups. First, assuming that the families with Cd concentrations equal to, or above the range of the HCGA parent (0.109-0.116 ppm) are homozygous HCGA, the observed ratio is 93 LCGA/segregating:12 HCGA families. This fits the two gene pair 13:3 hypothetical ratio (P=0.10-0.05). One gene when dominant, is epistatic to the second, and the second gene, when homozygous recessive, is epistatic to the first. The data did not fit the hypothetical ratio for two gene pairs 15:1, 11:5 or 9:7, nor a one gene 3:1 ratio ($p<0.05$). The two gene epistatic duplicate dominance model (15:1 ratio) would still be rejected if one includes as HCGA only those families equal to and above the Sceptre mean (0.113 ppm). Secondly, for comparison's sake, the method used by Thomas and Harrison (1989) was applied. They chose to split their lettuce populations into two groups, high Cd accumulating and low Cd accumulating, using the midparental mean and then fit a 3:1 single, completely dominant gene model to the data. By their method, there are 77 LCGA/segregating:28 HCGA families which does fit a 3:1 ratio (P=0.90-0.70). Finally arbitrary divisions were created in the frequency distribution bar chart based on the frequency distribution between 0.095 and 0.100 ppm. This division of the F_3 families

into 82 LCGA/segregating:23 HCGA families fit the 3:1 hypothetical ratio of a single dominant gene (p=0.70-0.50). While each of these methods of dividing up the families has flaws, they helped define upper and lower limits of Cd concentrations, and thus a specific group of families, to investigate further.

Figure 2. Sceptre/Biodur F_3 Frequency Histogram (Glenlea,1992)

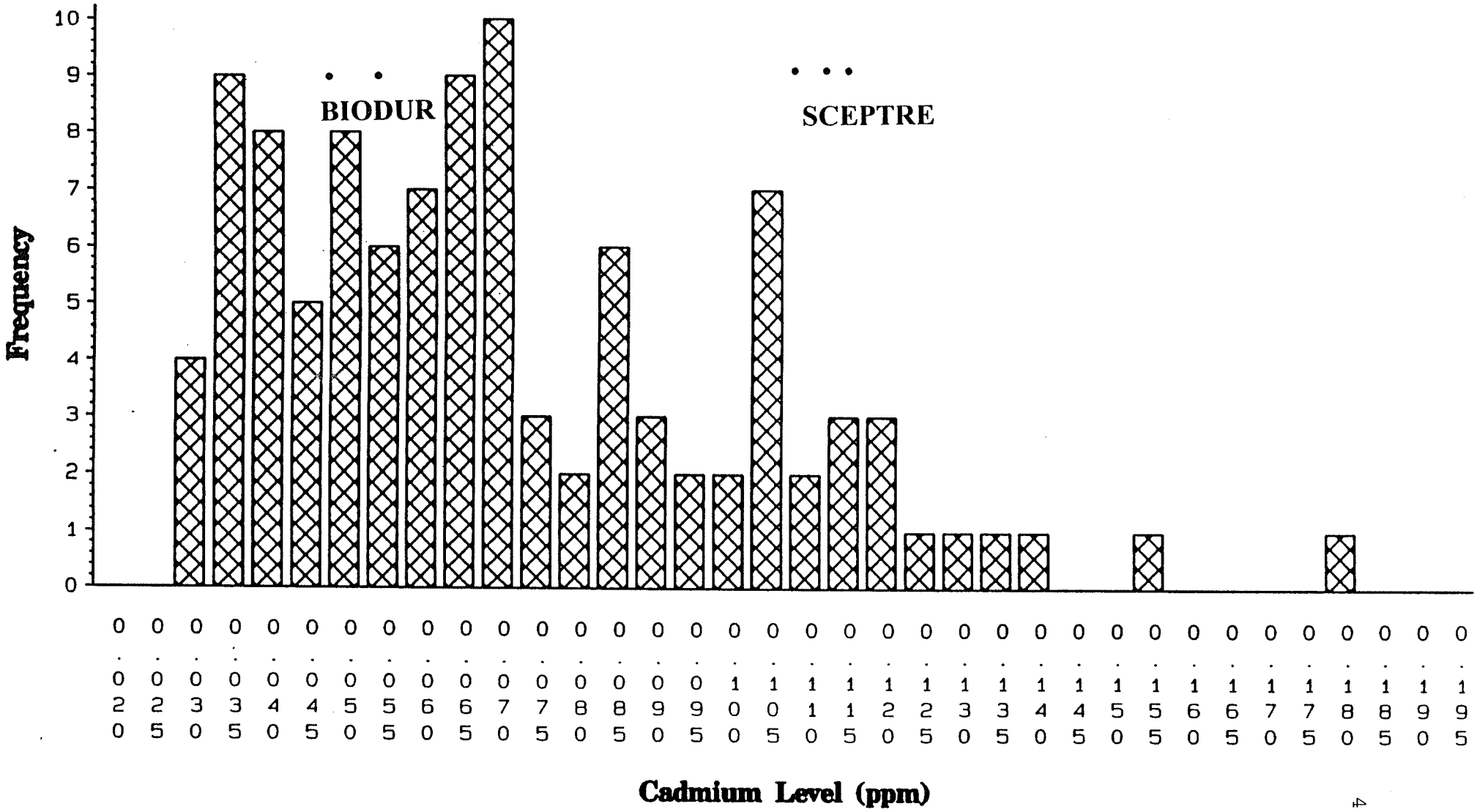

BIODUR
SCEPTRE
Frequency
0
1
2
3
4
5
6
7
8
9
10
0.020 0.025 0.030 0.035 0.040 0.045 0.050 0.055 0.060 0.065 0.070 0.075 0.080 0.085 0.090 0.095 0.100 0.105 0.110 0.115 0.120 0.125 0.130 0.135 0.140 0.145 0.150 0.155 0.160 0.165 0.170 0.175 0.180 0.185 0.190 0.195
Cadmium Level (ppm)

TABLE 3. Mean, Standard Error, Sample Size and Range of the Sceptre/Biodur F_4 Families and Checks (Greenhouse)

Family	Mean (ppm)	Standard Error (ppm)	Sample #	Range (ppm)	F_3 Cd Level (Glenlea)
Biodur	0.094	0.008	5	0.072-0.113	
Sceptre	0.188	0.015	5	0.148-0.241	
Midparent	0.141	0.018	10		
GK1.1	0.086	0.008	7	0.056-0.119	0.028
GK1.39	0.072	0.006	7	0.052-0.094	0.056
GK1.78	0.232	0.011	7	0.200-0.270	0.091
GK1.79	0.234	0.015	7	0.174-0.277	0.092
GK1.80	0.228	0.014	7	0.179-0.284	0.092
GK1.81	0.227	0.012	7	0.176-0.269	0.095
GK1.82	0.241	0.026	7	0.177-0.388	0.097
GK1.83	0.160	0.011	7	0.121-0.207	0.101
GK1.84	0.242	0.021	7	0.172-0.302	0.102
GK1.85	0.214	0.018	7	0.156-0.279	0.103
GK1.86	0.303	0.035	7	0.205-0.454	0.104
GK1.87	0.241	0.032	7	0.097-0.360	0.104
GK1.88	0.213	0.012	7	0.185-0.265	0.105
GK1.89	0.173	0.014	7	0.126-0.235	0.106
GK1.90	0.191	0.015	7	0.126-0.263	0.106
GK1.91	0.207	0.010	7	0.183-0.249	0.107
GK1.92	0.250	0.017	7	0.200-0.301	0.108
GK1.93	0.199	0.031	7	0.056-0.341	0.108
GK1.94	0.164	0.006	7	0.143-0.189	0.113
GK1.95	0.191	0.022	7	0.074-0.256	0.114

To further test whether a single gene or a gene pair controls Cd accumulation, single seeds from F_3 families with Cd concentrations within the range of 0.091-0.114 ppm were planted in the greenhouse with parental checks. In addition, single seeds of two randomly selected F_3 families from the lower end of the Cd grain concentration range were planted. Seven plants of each family were analyzed.

Grain Cd concentrations were relatively higher in the greenhouse than the 1992 field grown material (Table 3) . Parental means and standard errors were 0.094 +/- 0.008 ppm and 0.188 +/- 0.015 ppm for Biodur and Sceptre respectively. The F_4 single plant grain Cd levels of the two "lower" families had mean values of 0.072 +/- 0.006 ppm and 0.086 +/- 0.008 ppm. All individual plants, except one in the latter family, were within one standard error of a mean Biodur concentration. The one plant had a grain Cd level of 0.119 ppm, greater than two standard errors from Biodur. All members of these two F_4 families were below the midparental mean (0.141 +/- 0.018 ppm). These two families are considered to be homozygous LCGA.

Of the 18 families with higher F_3 Cd concentrations, 15 had mean concentrations above the Sceptre mean. One family had a mean concentration equivalent to one standard error below the Sceptre mean. The other two F_4 families had mean values within two standard errors of Sceptre. Based on mean

concentrations it is likely that at least 15 of the 18 F_4 families tested were homozygous recessive HCGA.

A single F_4 plant of five families, and two plants from one family, were below the midparental mean. In three of these families all the individual plant values were above two standard errors from the mean Biodur concentration. Of the three remaining families (GK1.87, GK1.93 and GK1.95) only a single plant within two of the three families had Cd concentrations below the Biodur mean. GK1.87 had a single plant within one standard error of the Biodur mean. Therefore of 126 F_4 individuals tested 3 may be low Cd accumulators.

If grain Cd accumulation is controlled by a single gene with LCGA partially dominant, then most of the 18 families which were selected would be expected to be homozygous recessive HCGA. However, if two genes control grain Cd levels, most of the families in the region of the frequency distribution which were selected would be expected to be either heterozygous or homozygous LCGA. For example if the 13:3 ratio was correct and the high Cd accumulating F_3 families had been accurately identified and planted, one sixth of F_4 individuals (21 plants in this case) should be low Cd accumulators. Since most of the families were HCGA, the F_4 data tends to support the single gene, not the two gene pair, hypothesis.

TABLE 4. Means, Standard Errors, Sample Size and Range of the Sceptre/Biodur F_4 (1993), Checks and F_3 Families (1992, Glenlea)

Genotypes	Mean (ppm)	Standard Error (ppm)	Sample #	Range (ppm)
Biodur	0.042	0.001	4	0.039-0.044
Sceptre	0.084	0.005	4	0.074-0.093
Midparent	0.063	0.009	8	
F_1	0.047	0.002	2	0.042-0.052
F_4 Families	0.057	0.003	87	0.020-0.108
F_3 Families[a]	0.070	0.003	87	0.028-0.138

[a] Results of the F_3 families which were parents to F_4 families harvested in 1994

In the 1993 field grown material, Cd concentrations of Biodur and Sceptre were 0.042 +/- 0.001 ppm and 0.084 +/- 0.005 ppm respectively (Table 4). Though all F_2 derived F_3 lines were replanted at Glenlea in 1993, several died before maturing, likely stressed by the excessive rainfall and high levels of disease (Table 5). As some of the seed was shrivelled, seed number per five gram sample was obtained to determine if Cd concentration was affected. There was no relationship evident through calculation of the correlation coefficient (-0.142) between Cd levels and seed number (P=0.19).

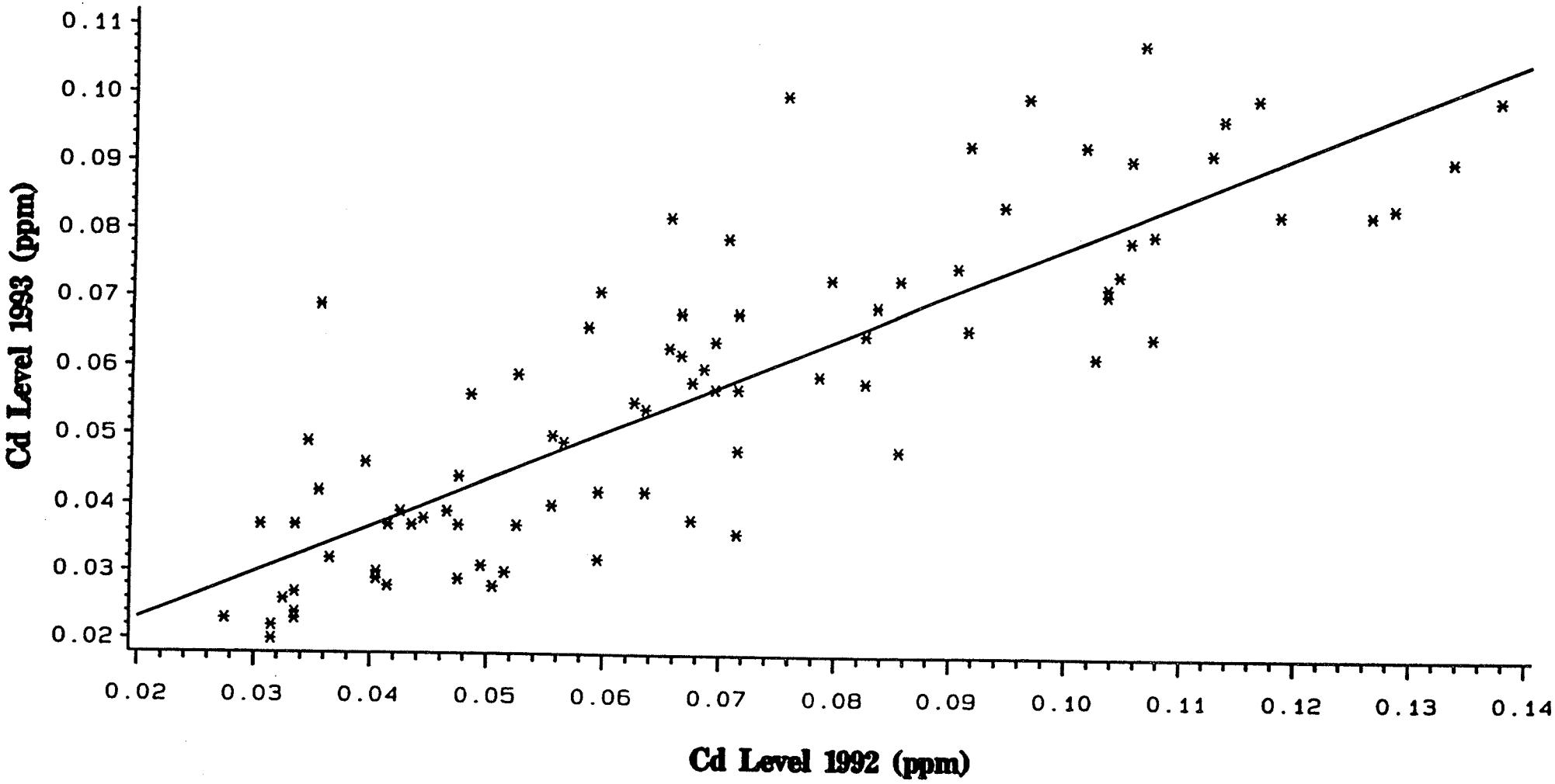
TABLE 5. Precipitation Throughout the Two Growing Seasons at Glenlea[a]

	Monthly Precipitation Totals (mm)					
	May	June	July	August	September	October
1992	28	106.6	87.8	69.2	70.0	3.8
1993	41.0	72.8	246.0	160.0	31.8	-

[a] Information received from the University of Manitoba, Department of Plant Science.

The 87 surviving F_4 families (Appendix 1) ranged from 0.020 to 0.108 ppm with a mean grain Cd concentration (0.057 +/- 0.002 ppm) which was lower than the midparental mean (0.063 +/- 0.009 ppm) (Table 4). This supports the F_3 data that for this cross LCGA is partially dominant to dominant. Heritability calculated by regression of offspring on mid-parent (Figure 3) was 68.6% +/- 4.75 (Falconer, 1989). The covariance among offspring and parents in this self-pollinated species (assuming one locus) defined relative to the F_2 population contains additive genetic variance and 1/8 dominance genetic variance. Therefore the regression is a very close estimate to narrow sense heritability.

Figure 3. Regression of F_4 Offspring on F_3 Parental Mean for Sceptre/Biodur



Regression Equation:
CAD = 0.009326 + 0.686278*CPM

R-square value = 0.71

TABLE 6. Means, Standard Errors, Sample Size and Range of the DT471/DT624 F_3 Families and Checks (Glenlea, 1992)

	Mean (ppm)	Standard Error (ppm)	Sample #	Range (ppm)
DT471	0.041	0.001	3	0.039-0.042
DT624	0.126	0.013	3	0.099-0.142
Midparent	0.083	0.020	6	
F_3 Families	0.094	0.003	126	0.039-0.194

4.1.2 DT471/DT624 Population

The DT471 and DT624 had mean Cd concentrations of 0.041 +/- 0.001 ppm and 0.126 +/- 0.013 ppm respectively, with a midparental mean of 0.083+/- 0.020 ppm (Table 6). One of the DT624 check lines had a much lower Cd concentration than the other lines, thus lowering the midparental mean. The F_3 population has a non-normal distribution (Prob<W = 0.0001) and is skewed towards lower Cd ppm values (Figure 4). F_3 Cd values ranged from 0.039 ppm to 0.194 ppm with a mean of 0.094 +/- 0.003 ppm. While the midparental mean was lower than the F_3 mean the skewness of the frequency distribution toward lower Cd concentrations would again suggest partial dominance of LCGA over HCGA.

If there is no dominance in the grain Cd accumulation trait and if it is monogenically inherited, then theoretically the F_2 distribution should be normally shaped. The ease with

which the homozygotes could be distinguished from the heterozygotes would depend on the degree of environmental effect on phenotype. Secondly, if there is no dominance and grain Cd accumulation was controlled by several genes, each with an equal effect, again the F_2 distribution should resemble a normal distribution (Allard, 1960).

The continuous nature of the frequency distribution, further complicated by the wide range in Cd values for the DT624 parent, does not lend itself readily to estimating the number of genes. If one assumes those families within and about the range of the DT624 parent to be homozygous HCGA, the observed ratio is 76 LCGA or segregating:50 HCGA. This does not fit a 3:1 ratio for a single gene ($P<0.05$) but it does fit a 9:7 ratio for two complementary genes ($P=0.50-0.30$). By comparison, as the lowest DT624 appears an outlier, division of the population at the second lowest DT624 (0.136 ppm) gives 109 LCGA or segregating to 17 HCGA which fits a 13:3 two gene pair ratio ($P=0.20-0.10$). It does not fit a 3:1 one gene pair ratio. If a division is made at the midparental mean there are more HCGA (68) than LCGA families (57), the opposite of the frequency distribution. As it was difficult where to decide to make any arbitrary breaks, none were made.

Figure 4. DT471/DT624 F_3 Frequency Histogram (Glenlea, 1992)

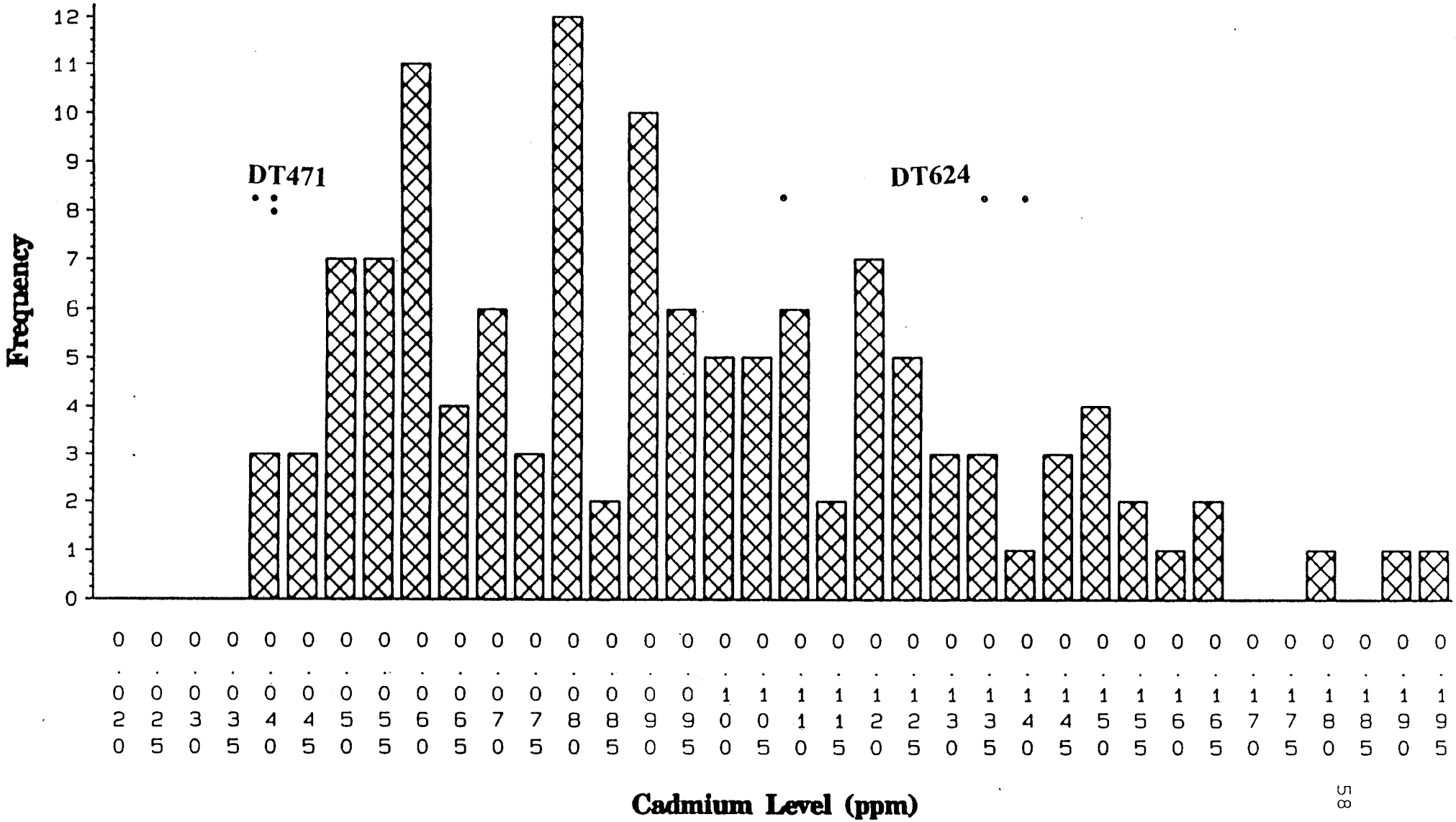

DT471
DT624
Frequency
0
1
2
3
4
5
6
7
8
9
10
11
12
0.020
0.025
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
0.105
0.110
0.115
0.120
0.125
0.130
0.135
0.140
0.145
0.150
0.155
0.160
0.165
0.170
0.175
0.180
0.185
0.190
0.195
Cadmium Level (ppm)

TABLE 7. Analysis of Variance, Lattice, DT618/DT627 F_3 Families and Checks (Morden, 1993)

Sources of Variation	D.F.	Mean Square
Replications	1	103209.4
Entries (unadj.)	143	27055.6*
Blocks within Replication (adj.)	22	10906.0
Intra-Block Error	121	1621.9
Coefficient of Variation		14.49
Relative Efficiency		166.29

* Significant at the 0.01 probability level

4.1.3 DT618/DT627 Population

The analysis of variance (Table 7) indicates highly significant differences in grain Cd concentrations were found among the DT618/DT627 F_3 families and the parental checks. The lattice design was more efficient than a randomized complete block design (RCBD). As previously observed (Leisle, personal communication) grain Cd concentrations were much higher at the Morden location than at Glenlea. A frequency distribution of adjusted means is bi-modal (Figure 5). Unlike the previous crosses' frequency distributions, the DT618/DT627 distribution has two almost equal groups. Between the two replicates there were some very noticeable differences in the progeny and

checks. Re-analysis of the data as an RCBD (table 9) shows significant differences between blocks and entries.

Figure 5. DT618/DT627 F_3 Adjusted Means Frequency Histogram (Morden, 1993)

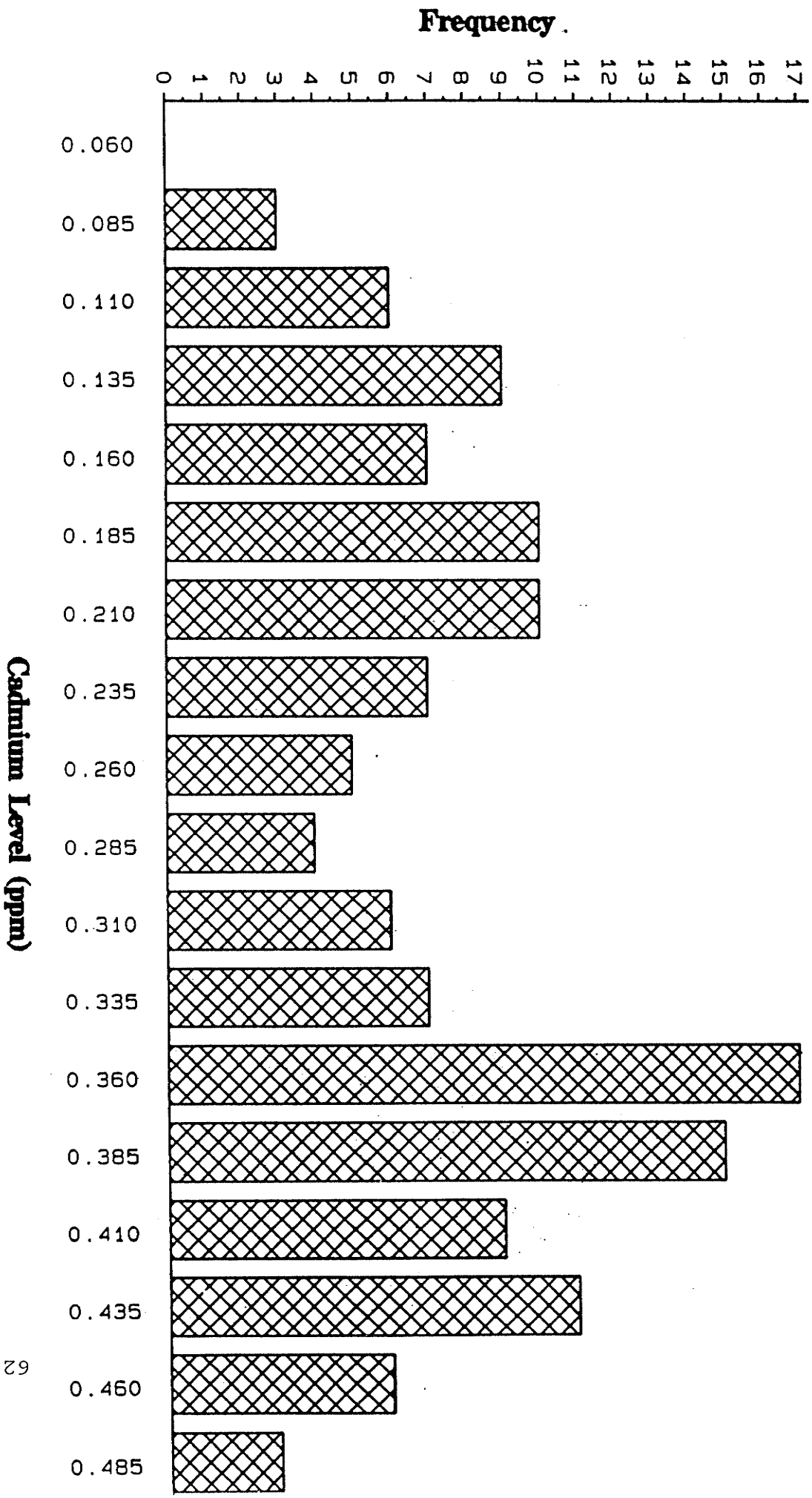

Frequency
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Cadmium Level (ppm)
0.060
0.085
0.110
0.135
0.160
0.185
0.210
0.235
0.260
0.285
0.310
0.335
0.360
0.385
0.410
0.435
0.460
0.485

TABLE 8. Grain Cd Concentrations of Checks Planted with DT618/DT627 F_3 Families, Midparental Means and Standard Errors

	Replicate 1	Replicate 2
All Cd concentrations in ppm		
DT618	0.288 (0.304, 0.285)[a]	0.419
DT627[b]	0.095	0.127 (0.125)[c]
Sceptre	0.225	0.329
Medora	0.379	0.429
F_1 DT627/DT618	0.160	0.190
DT618	0.469	0.517 (0.508)[c]
DT627	0.124	0.117
Sceptre	0.196	0.349
Medora	0.242	0.500
Midparental Mean	0.244 +/- 0.086	0.295 +/- 0.102

[a] Cd levels of a second 5 g sample taken from the same family
[b] LCGA line identified in 1991,1992 Durum Coop
[c] Sample was analyzed twice

The grain Cd means of the F_3 families in replicate one (0.279 +/- 0.011 ppm) and two (0.313 +/- 0.011 ppm) were greater than the individual replicate midparental means, 0.244 ppm and 0.295 ppm respectively (Table 8). These F_3 averages were also greater than the Cd concentration of the single rows

of the F_1. This indicates that in this cross there is no dominance of LCGA as was observed in the Sceptre/Biodur or DT471/DT624 crosses.

Cd values in the first replicate range from 0.081 to 0.564 ppm, and in the second from 0.083 to 0.566 ppm. Between the two replicates parental DT627 values ranged from 0.095 to 0.127 ppm, a difference of 0.032 ppm. However, values of the HCGA parent, DT618 ranged from 0.288 ppm to 0.517 ppm, a difference of 0.229 ppm. Upon sorting the grain Cd concentrations of both replicates it becomes quite noticeable that grain Cd levels increased in many F_3 families (Figures 6 and 7) and the checks between replicates one and two. However the opposite occurred with F_3 families which had relatively high Cd levels in replicate one (Figure 8).

Cd concentrations in 18 of the 20 lowest F_3 families, ranked by Cd levels of replicate one, rose between the first and second replicate. Cd levels decreased though, in 17 of the 20 highest families (also ranked by replicate one). In replicate one, 15 of the highest 20 grain Cd accumulating families occurred within four adjacent blocks. Also, 11 of the highest 20 families in replicate two occurred within four adjacent blocks. Many of the highest Cd accumulating families in both replicates were located quite close together in the field. The low families were much more scattered throughout the field. This indicates the presence of variability in the

soil affecting Cd accumulation between and within replicates as was discussed in section 2.3.2.

Figure 6. The First 48 DT618/DT627 F_3 Families of Replicate One Sorted by Cd Concentrations, and Their Cd Concentrations in Replicate Two

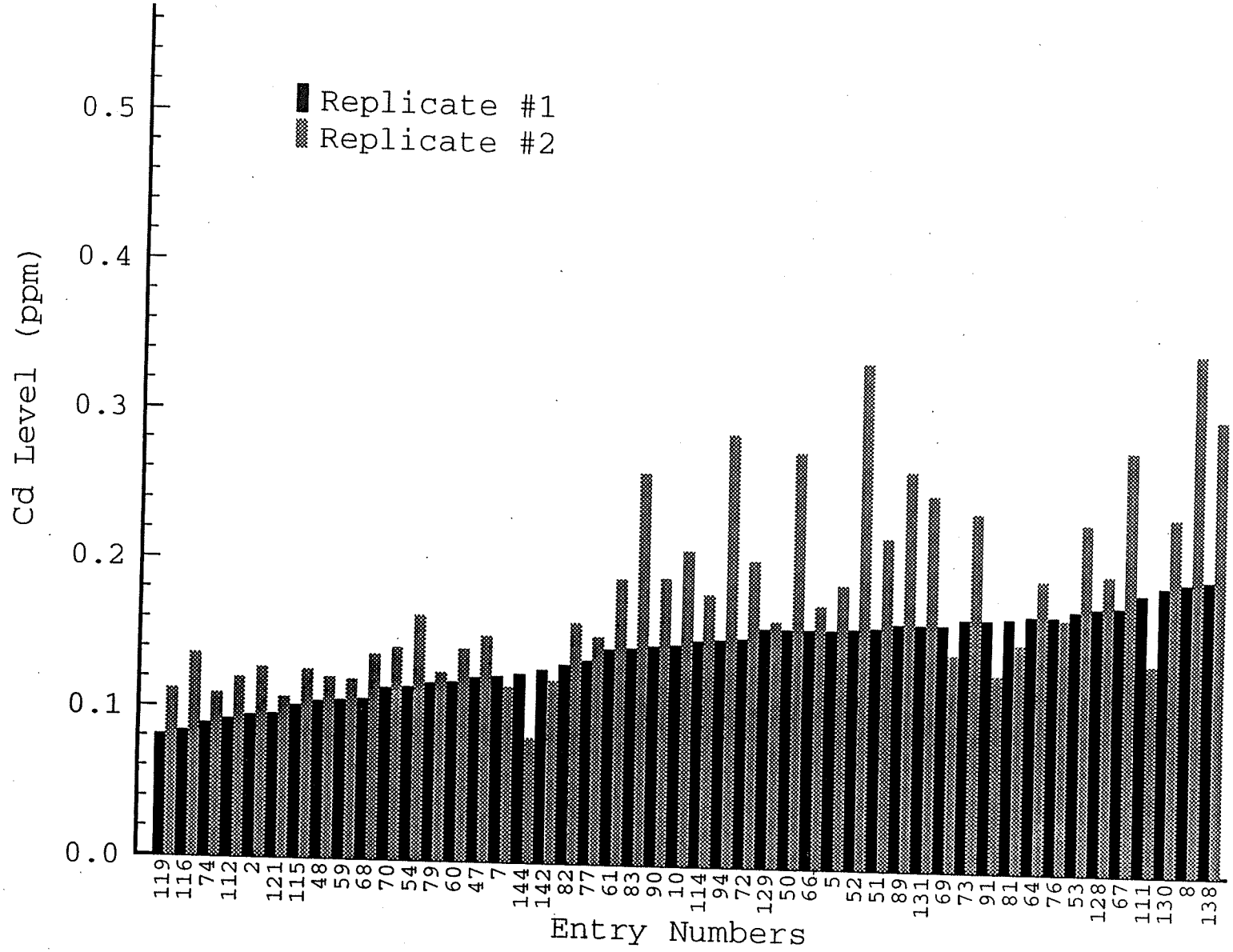

Replicate #1
Replicate #2
Cd Level (ppm)
0.0
0.1
0.2
0.3
0.4
0.5
119 116 74 112 2 121 115 48 59 68 70 54 79 60 47 7 144 142 82 77 61 83 90 10 114 94 72 129 50 66 5 52 51 89 131 69 73 91 81 64 76 53 128 67 111 130 8 138
Entry Numbers

Figure 7. The Second 48 DT618/DT627 F_3 Families of Replicate One Sorted by Cd Concentrations, and Their Cd Concentrations in Replicate Two

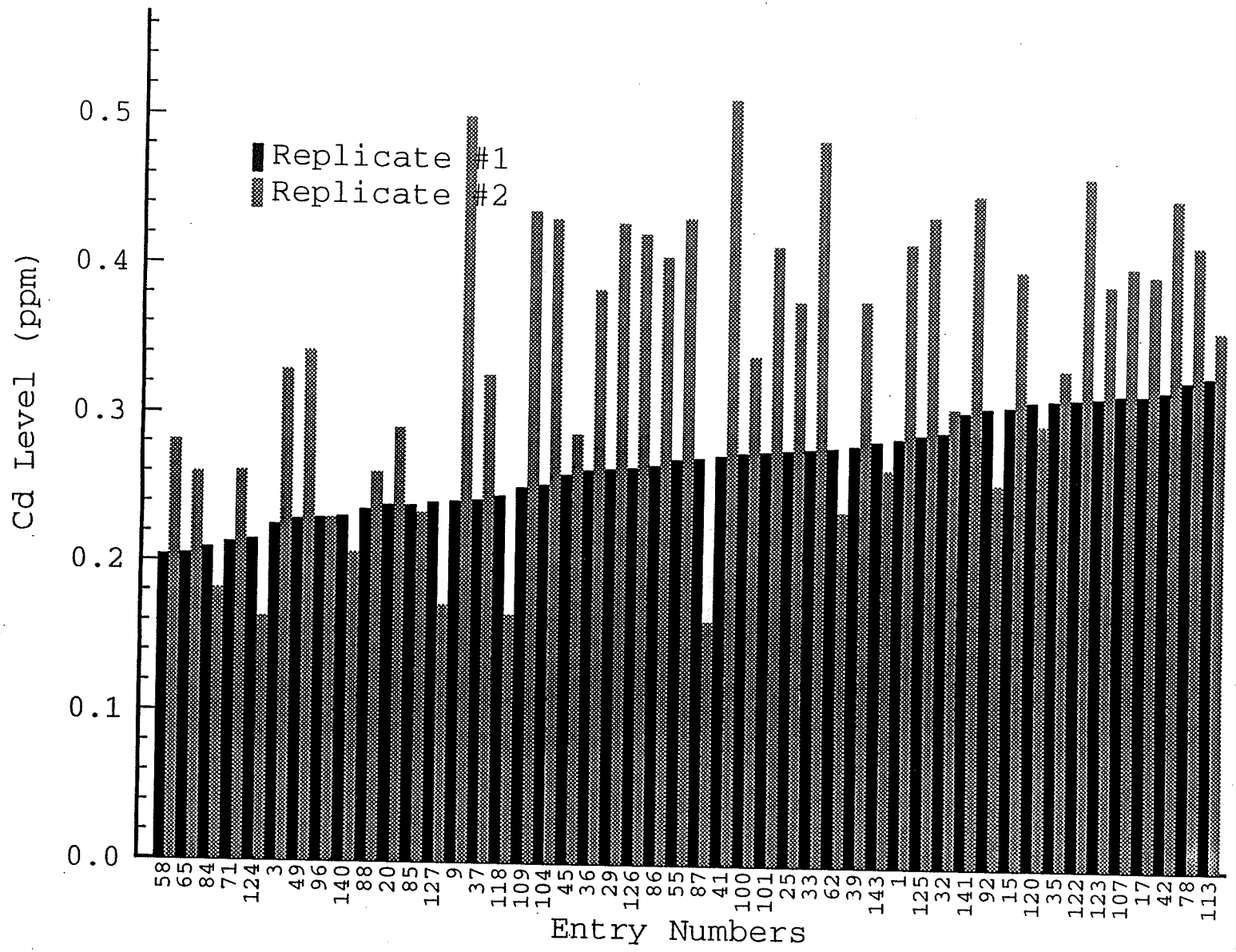

Replicate #1
Replicate #2
Cd Level (ppm)
0.0
0.1
0.2
0.3
0.4
0.5
58 65 84 71 124 3 49 96 140 88 20 85 127 9 37 118 109 104 45 36 29 126 86 55 87 41 100 101 25 33 62 39 143 1 125 32 141 92 15 120 35 122 123 107 17 42 78 113
Entry Numbers

Figure 8. The Last 48 DT618/DT627 F_3 Families of Replicate One Sorted by Cd Concentrations, and Their Cd Concentrations in Replicate Two

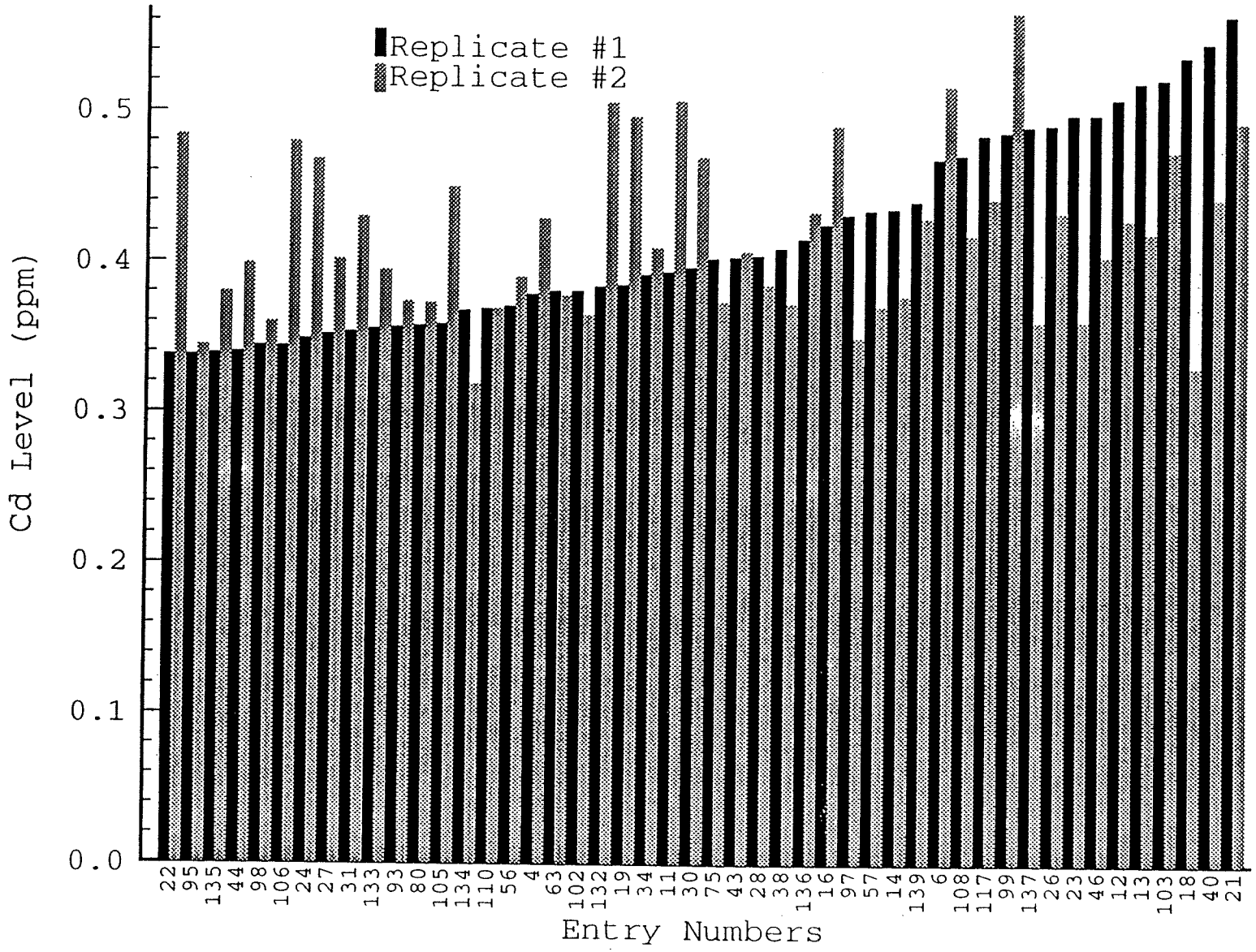

Replicate #1
Replicate #2
Cd Level (ppm)
0.0
0.1
0.2
0.3
0.4
0.5
22 95 135 44 48 106 24 27 31 133 93 80 105 134 110 56 4 63 102 132 19 34 11 30 75 43 28 38 136 16 97 57 14 139 6 108 117 99 137 26 23 46 12 13 103 18 40 21
Entry Numbers

TABLE 9. Analysis of Variance, RCBD, Grain Cd Concentrations of DT618/DT627 F_3 Families, No Checks

Source	df	Mean Square
Blocks	1	80186.7000**
Entries	134	26783.7918**
Error	134	396865.8000
R^2		0.90
C.V.		18.37

** Significant at the 0.01 level of probability

The analysis of variance as a RCBD, excluding the checks (Table 9) indicates the variances represented by this random sample of the DT618/DT627 F_3 is not equal to zero. There is a significant variation in the grain Cd concentrations of DT618/DT627 progeny. Broad sense heritability and upper and lower 90% confidence intervals calculated from the RCBD was 88.94% within 91.69% to 85.29%. A problem with this estimate is that an individual's phenotypic expression is the sum of a general population mean, a genotypic effect, a composite error, replicate, location and year effects, as well as interactions between or among the main factors. The genetic variance component of the heritability estimate is biased by interaction variances as data was collected for only one year in one location (Allard, 1960; Dudley and Moll, 1969).

4.2 Allelism Study

TABLE 10. Correlations of Seed Number and Cd Concentration of F_2 Single Plants

Cross	Correlation Coefficient	P-value
DT471/DT637	0.115	0.338
Nile/DT471	-0.289	0.014
DT627/Biodur	-0.147	0.219
Biodur/DT637	0.097	0.418

Seventy-two plants from each population of DT471/DT637, Nile/DT471, Biodur/DT637 and DT627/Biodur were analyzed for grain Cd concentration. There was an approximate 99% probability of observing a homozygous HCGA plant if two genes, either gene being epistatic to the other, were segregating. Due to excess moisture and disease in 1993, seed was shrivelled and of variable size. To determine if this had any effect on Cd concentration, correlations between number of seeds per sample and Cd concentration were calculated (Table 10). There were no significant correlations for any of the crosses for these traits .

For comparison purposes Table 11 lists Cd levels of all harvested parents and F_1's, as well as the Cd levels of check cultivars planted adjacent with the F_4 Sceptre/Biodur

population. Individual plant Cd values are available in Appendix 2. No DT637 or Nile parents were harvested due to growing conditions. Data for the HCGA cultivars Sceptre and Medora are shown as comparisons to the Cd concentration of the LCGA parents. Values for Sceptre and Medora ranged lower than generally observed, while the LCGA parents had similar values to those generally observed. DT471 had some plants which had values overlapping with the HCGA checks. This overlap of values for LCGA and HCGA cultivars does not generally occur, and is probably the result of adverse growing conditions.

TABLE 11. Means, Standard Errors, Sample Size, and Range of F_2 Populations of Various LCGAs Intercrosses and Checks

	Mean (ppm)	Standard Error (ppm)	Plant #	Range (ppm)
DT 627			2	0.046-0.047
Biodur			2	0.041-0.043
DT471	0.065	0.007	6	0.048-0.097
F_1 DT471/Nile			1	0.070
F_1 DT627/Biodur			2	0.062-0.068
Sceptre[a]	0.084	0.005	4	0.074-0.093
Medora[a]			2	0.087-0.112
Biodur[a]	0.042	0.001	3	0.039-0.044
DT471[a]			1	0.043
F_1 Sceptre/Biodur[a]			2	0.042-0.052
F_2 DT471/DT637	0.058	0.002	72	0.028-0.090
F_2 Nile/DT471	0.063	0.002	72	0.032-0.101
F_2 DT627/Biodur	0.056	0.001	72	0.035-0.088
F_2 Biodur/DT637	0.069	0.002	72	0.035-0.119

[a] Cd values are for bulked rows, not single plants

Both DT471 and DT637 have Hercules in their ancestry and are presumed to carry the same LCGA gene(s). The range and distribution in Cd concentration of the DT471/DT637 F_2 population (Figure 9) may be considered to be due to environmental effects on the LCGA source. The population had a normal distribution (Pr<W 0.1000). The mean of the F_2 population (0.058 +/- 0.002 ppm) was within one standard error of the LCGA DT471 mean (Table 11). The F_2 plants ranged from 0.028 to 0.090 ppm; a few plants were lower, but none were higher than the range of the LCGA parent.

DT627/Biodur and Biodur/DT637 are expected to behave as reciprocal crosses since these two DT lines are sibs. Both populations have a normal distribution (pr<W=0.0359, 0.4601 respectively). For the DT627/Biodur cross the F_2 mean Cd concentration was 0.056 +/- 0.001 ppm (Table 11). For further reference, a frequency distribution of DT627/Biodur is shown in Appendix 3. The range of DT627 parent plants, and F_1 DT627/Biodur were within two standard errors of the F_2 mean; Biodur values were slightly lower. Individuals for this cross ranged from 0.035 to 0.088 ppm. While this is beyond the range of the parents, it is similar to the distribution of the DT471/DT637 F_2 distribution; the latter is considered the effect of environment.

The F_2 mean Cd concentration for the Biodur/DT637 cross (0.069 +/- 0.002 ppm) was slightly higher than the F_2 DT627/Biodur cross (Table 11). Again the Biodur plants' Cd

levels were lower than two standard errors from the F_2 mean. The range was shifted towards higher grain Cd concentrations as well, with a maximum of 0.119 ppm and a minimum of 0.035 ppm. The frequency distribution (Figure 10) showed a small group of plants with high Cd concentrations beyond the range of the DT471/DT637 cross.

Figure 9. DT471/DT637 F_2 Frequency Distribution (Glenlea, 1993)

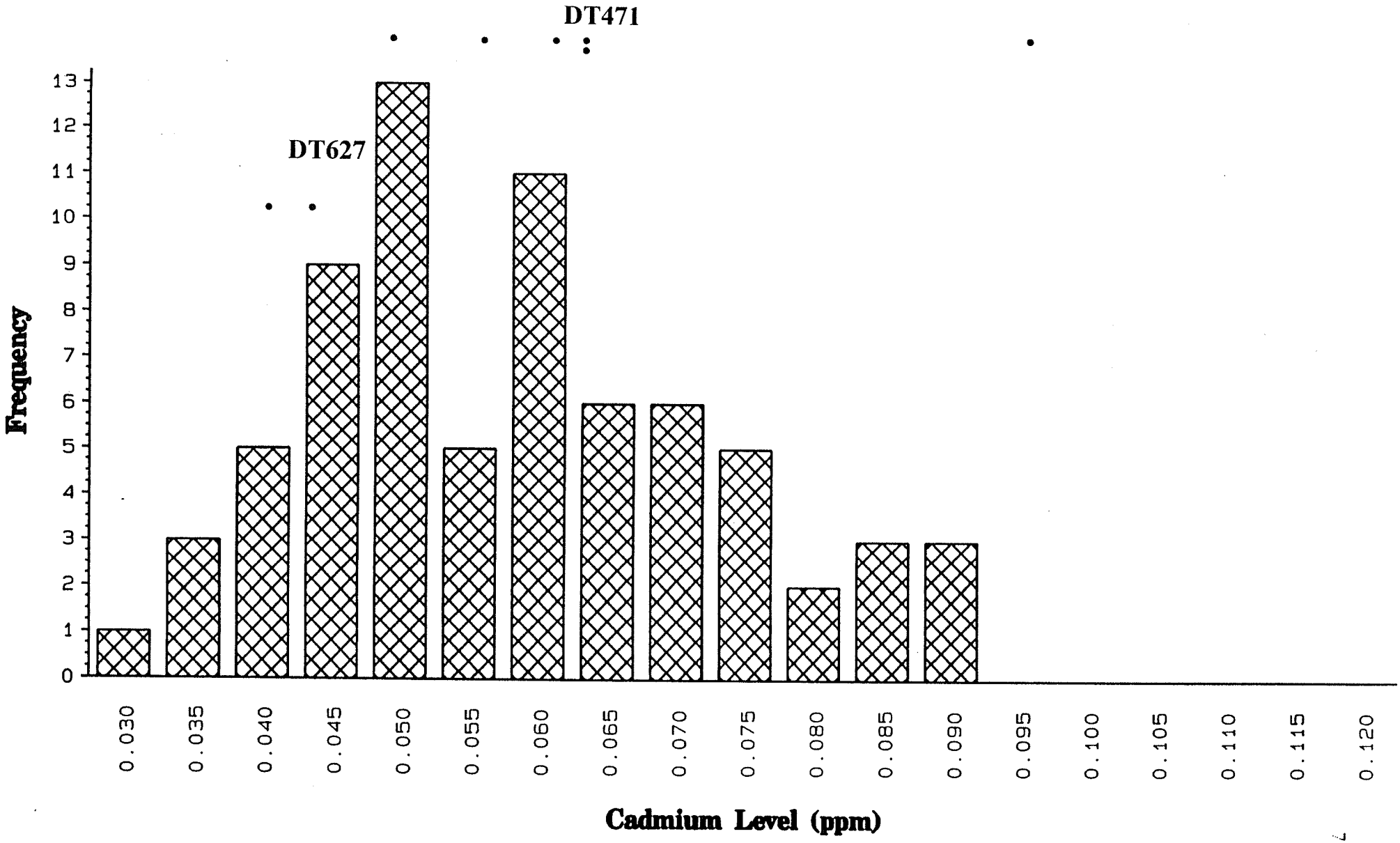

DT471
DT627
Frequency
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
0.105
0.110
0.115
0.120
Cadmium Level (ppm)

Figure 10. Biodur/DT637 F_2 Frequency Distribution (Glenlea, 1993)

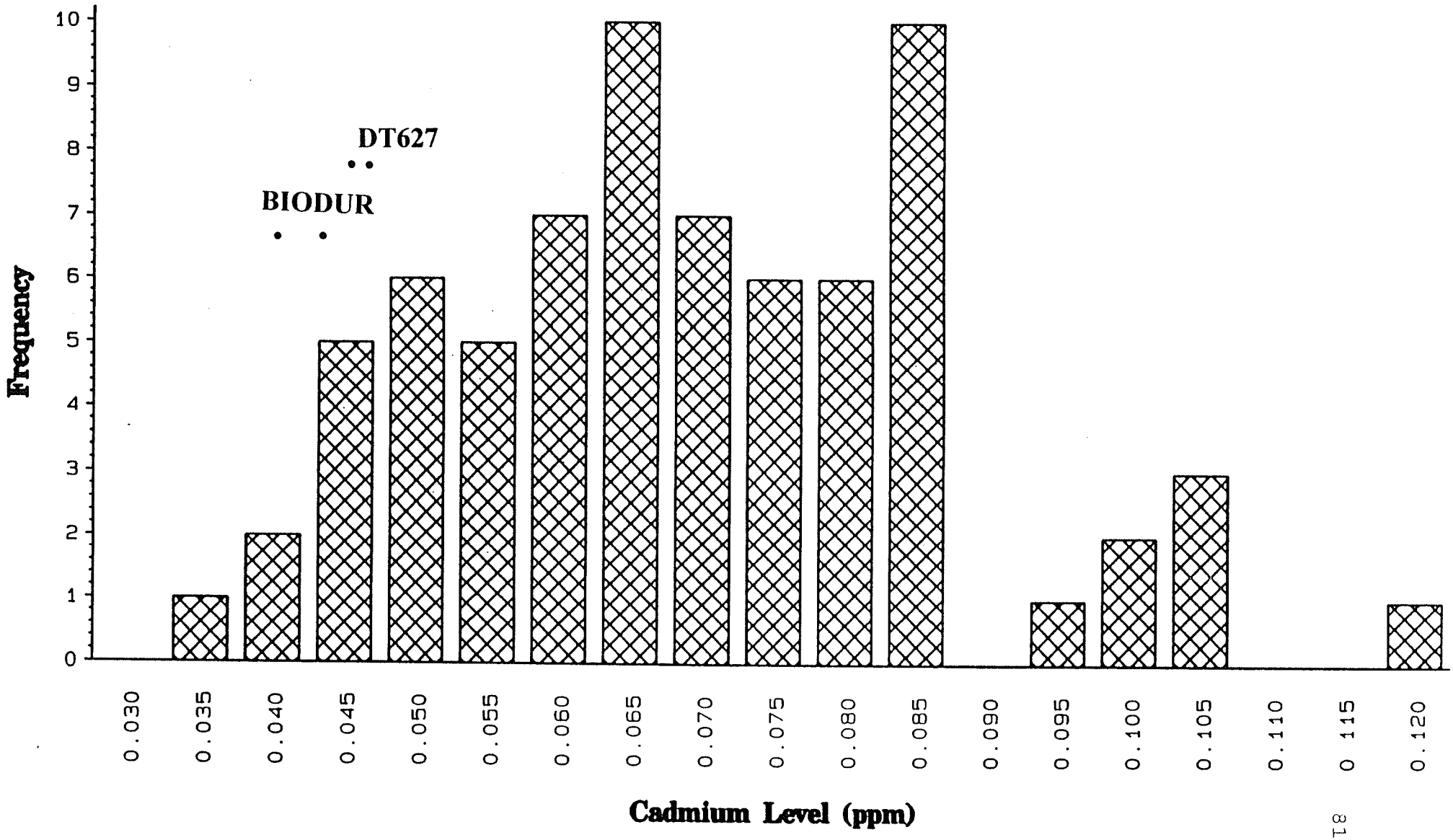

DT627
BIODUR
Frequency
0
1
2
3
4
5
6
7
8
9
10
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
0.105
0.110
0.115
0.120
Cadmium Level (ppm)

TABLE 12. Means, Standard Errors, Sample Size and Range of Select DT627/Biodur F_3 and Biodur/DT637 F_3 (Greenhouse)

Genotype	Mean (ppm)	Standard Error (ppm)	Plant #	Range (ppm)	Parent F_2 Cd ppm
Sceptre	0.144	0.021	4	0.096-0.194	
Biodur	0.076	0.012	5	0.039-0.106	
DT627	0.076	0.007	5	0.061-0.096	
DT637	0.064	0.011	5	0.042-0.099	
5.692[a]	0.087	0.005	5	0.070-0.099	0.075
5.693[a]	0.091	0.011	5	0.062-0.126	0.078
5.126[a]	0.114	0.008	5	0.098-0.141	0.080
5.131[a]	0.080	0.002	5	0.074-0.086	0.082
5.143[a]	0.075	0.003	5	0.066-0.082	0.087
5.337[a]	0.061	0.009	5	0.040-0.085	0.088
12.366[b]	0.068	0.006	5	0.055-0.090	0.094
12.343[b]	0.067	0.004	5	0.061-0.08	0.098
12.597[b]	0.063	0.001	2	0.062-0.064	0.098
12.353[b]	0.078	0.004	5	0.065-0.088	0.104
12.617[b]	0.104	0.011	2	0.092-0.115	0.107
12.371[b]	0.046	0.004	5	0.038-0.059	0.119

[a] 5.___ identifies a DT627/Biodur F_2 individual

[b] 12.___ identifies a Biodur/DT637 F_2 individual

The seven highest Cd accumulating F_2 families of both crosses were selected to be advanced in the greenhouse. Unfortunately there was no seed left of some of these families and the seed that was available did not germinate well. Many durum wheat plants harvested in 1993 were infected with disease. In the greenhouse *Fusarium avenaceum* resulted in no germination or the wilt of seedlings from 'apparently' normal seeds (Gilbert, personal communication). Thus, F_3 plants from only six F_2 plants from each of the DT627/Biodur and Biodur/DT637 high Cd accumulating groups, and checks, grew to maturity. Five plants from each F_3 family, and the checks, were analyzed for Cd concentrations when available (Table 12). Individual plant Cd levels are given in Appendix 4. The average Cd levels of the LCGA parents Biodur (0.076 +/- 0.012 ppm), DT627 (0.076 +/- 0.007 pm) and DT637 (0.064 +/- 0.011 ppm) were all lower than the HCGA check Sceptre (0.144 +/- 0.021 ppm). Of the six DT627/Biodur families, four are within one standard error of their LCGA parents and one is within two standard errors. Five families are more than two standard errors, that is significantly different, from Sceptre .

In the Biodur/DT637 cross, five of the six F_3 families had mean Cd concentrations lower, or within one standard error of the LCGA parents. For the remaining family only two plants were available. The range of Cd levels (0.092-0.115 ppm) is within two standard errors of the Biodur mean and within two standard errors of Sceptre. These F_3 plants came from seed of

very poor quality that was infected and shrivelled. The quality of the seed used could result in weak plants which may have negatively affected their Cd accumulation mechanisms, although the mechanisms have not been identified in wheat.

Since the two crosses may be expected to behave as reciprocals and do not differ in their F_2 means, they can be considered together. Thus, based on the F_2 and F_3 family data, 140 of 143 F_2 plants (one high Cd F_2 plant not verified in F_3) may be considered homozygous LCGA. Two F_2 plants might be homozygous LCGA. The assumption from the F_2 data was that the high Cd concentrating plants, in particular from the Biodur/DT637 cross, were possibly homozygous HCGA. This would indicate that Biodur and DT627/DT637 have different genes for low Cd accumulation. However, based on the F_3 data, at least 11 of these high Cd F_2 plants might be homozygous LCGA. The high F_2 values may have been the result of other environmental effects on Cd accumulation such as perhaps root length. The data suggests that the two LCGA sources may be allelic, though further verification is required.

The source of LCGA in Nile is unknown. The Nile/DT471 F_2 population was normally distributed (PR<W=0.1514). The mean grain Cd concentration (0.063 +/- 0.002 ppm) was lower than the F_1 DT471/Nile individual and within one standard error of the DT471 mean (Table 11). The F_2 Nile/DT471 values ranged from a minimum of 0.032 ppm to a maximum of 0.101 ppm. Four plants had grain Cd concentrations over 0.090 ppm, the maximum

concentration of the F_2 DT471/DT637 population. The fact that no Nile parent check plants survived to maturity hampers the drawing of conclusions. Penner et al. (1994) though, did not observe their RAPD marker (linked to HCGA) on Nile, Biodur or several DT breeding lines derived from Hercules. Thus it is possible that Nile and the Hercules lines may have the same LCGA allele.

TABLE 13. Means, T-tests, Wicoxan Rank Sum Test, and Range Values of Cd Concentrations of DT471/DT624 F_3 Families Homozygous for Gliadins, Glutenins or Tyrosinase Activity

Potential Marker	Parental Classif-ication	Marker Category	Number of Homozygous F_3 Families	Grain Cd Means (ppm)	P-Value	Range (ppm)
Gliadin	DT471	β2	34	0.093[a]	0.515	0.039-0.152
	DT624	β1	42	0.088[a]		0.042-0.162
Glutenin	DT471	LMW-2$^-$	17	0.095	0.136	0.049-0.152[b]
	DT624	LMW-2	29	0.084		0.042-0.166[b]
Tyrosinase	DT471	Low	34	0.093[a]	0.471	0.042-0.181
	DT624	High	42	0.099[a]		0.039-0.165

[a] Means not significantly different at 0.05 level of probability according to t-test

[b] Distribution of both groups are not significantly different at 0.05 level of probability according to Wilcoxan Rank Sum test

4.3 Molecular Markers

4.3.1 Gliadins

From the cross DT471/DT624, 79 lines were electrophoretically evaluated for β gliadins. The 34 families

containing the β2 gliadin and the 42 families with the β1 gliadin were samples from normal populations (prob<W 0.0684, 0.1344 respectively). The remaining families were heterozygous for the two gliadins.

The exact two-sample t-test (Table 13) indicated the grain Cd means of the two groups were not significantly different (Prob>T=0.5153). For this population the t-test supports the hypothesis of no linkage between the genes controlling grain Cd accumulation and the β gliadins. This was further supported by a preliminary screening of the HCGA and LCGA families. Of the 18 highest HCGA families, 9 had the β1 gliadin and 9 had β2 and of the 18 lowest LCGA families, 6 had β1 and 12 had β2.

4.3.2 Low Molecular Weight Glutenins

F_3 families of the cross DT471/DT624 were classified for LMW-2 type using a monoclonal antibody. The amount of coloured product from each DT624 and/or DT471 protein extract which bound to MAb ***Gli*** 45-8 varied slightly. Normally most, if not all, of the DT624 seeds had higher OD readings than the DT471 seeds. The F_3 families were then scored as belonging, or not belonging, to the high OD range (based on DT624 seeds). Two of the eight plates of seeds tested had confusing control values; the DT471 and the DT624 OD ranges overlapped. Therefore the results of these two plates, which contained families 7777 to 7792 and families 7809 to 7824, were dropped. The remaining 94 F_3 families were grouped into 17 expressing the LMW-2^- allele,

48 segregating, and 29 expressing the LMW-2 allele. On the basis of five seeds per family 2.93 may have been accidentally classed as low binding, and 2.93 as high binding that were actually segregating families. The observed segregation is a good fit to a 1:2:1 ratio (P= 0.3-0.2) indicating that a single, codominant gene controls this glutenin.

The group with LMW-2$^-$ allele was not a sample from a normal distribution (Pr<W=0.0107) and the frequency distribution is very skewed toward low Cd values. Therefore a nonparametric Wilcoxan Rank Sum test was used to test the difference in Cd levels of the two populations. Based on their Cd levels (Table 13) the LMW-2$^-$ and LMW-2 populations are not significantly different for Cd level (P=0.1360). Hence, there is no apparent association between these two traits.

4.3.3 Tyrosinase Test

The F_3 families of DT471/DT624 segregated 34 low tyrosinase: 59 segregating: 33 high tyrosinase (P= 0.90-0.7). Potentially 1.97 segregating plants may have been misclassified into each of the low and high tyrosinase groups assuming a theoretical 31.5:63:31.5 ratio. The low tyrosinase families and high tyrosinase families are both samples from normally distributed populations (Prob<W= 0.0160, 0.6301 respectively). The F_3 population had a good fit to the 1:2:1 model of a single dominant gene model controlling tyrosinase production.

Mean grain Cd levels for the low tyrosinase and high tyrosinase groups (Table 13) were not significantly different (Prob>T=0.4713). There is no relationship between tyrosinase and Cd levels in this test. The loci for tyrosinase production and grain Cd accumulation are not linked.

4.3.4 Random Amplified Polymorphic DNA

4.3.4.1 Bulk Segregant Progeny Analysis

Two sets of screenings were carried out in connection with the DT471/DT624 cross. PCR reactions were conducted with rapid leaf disc extracted gDNA. First, the parents alone were screened. Out of 173 arbitrary (RAPD) primers which were tested on DT471 and DT624, 118 produced bands in agrose gels. All polymorphisms between the parents were recorded. Next, the parents and two bulks, the low and high grain Cd accumulating families, were screened together with those primers which had produced polymorphisms in the preliminary parental screening. To ensure each individual contributed an equivalent amount of gDNA in creating the bulks, the concentration of gDNA per individual was first estimated with a fluorometer. However, out of 12 primers which produced bands in the parents and the two bulks during the second screening, all showed identical banding patterns in the parents and the bulks. The original polymorphisms which had been observed in the parents were no longer present.

While some of the polymorphisms which were recorded during the first screening may have been incorrectly

identified, other sources of potential problems have been identified. In the original paper by Edwards et al. (1991) it is stated that plant gDNA should be stable at 4°C for over a year. The gDNA is extracted in a buffer that contains EDTA. Normally, EDTA is used to inactivate deoxyribonucleases. However wheat contains two types of enzymes, one of which is activated by EDTA and digests double-stranded DNAs (Jones and Boffey, 1984). Secondly, other researchers also using the fluorometer to estimate DNA concentrations, expressed the belief that the machine was not estimating accurately. Therefore the bulk gDNA PCRs may not have reproduced the conditions of the preliminary parental PCRs. Thirdly, several PCR components were stored at -20°C between uses. Hu et al. (1992) believe repeated freezing and thawing of $MgCl_2$ cause it to precipitate. This would change the actual available amount of $MgCl_2$ during reactions, resulting in mistyping. Lastly, due to environmental variation for Cd concentration, it is possible some families were incorrectly identified as HCGA and LCGA based on information from only one test.

TABLE 14. Suspected RAPD Markers Linked to the Cd Gene Tested on the Five Lowest and Five Highest F_4 Sceptre/Biodur Families

Families Ranked by Increasing Grain Cd Concentrations				
Family #	F_2 Derived F_4 1-87 Ranking	F_2 Derived F_3 1-105 Ranking	Primer OPC-20[a]	Primer OPC-2[a]
GK741	1	3	-	-
GK818	2	4	-	-
GK730	3	6	-	-
GK735	4	1	-	-
GK773	5	9	no bands	+
GK723	83	96	-	-
GK820	84	103	+	-
GK823	85	69[b]	-	+
GK828	86	82[b]	-	+
GK718	87	91	+	+

[a] Detected by temperature sweep gel electrophoresis

[b] These families were classified as LCGA/segregating according to arbitrary divisions of the 1992 frequency distribution

Penner and Bezte (personal communication), using slightly lower Mg concentrations, identified three potentially polymorphic primers in a different HCGA/LCGA cross. Table 14 shows the results of screening the two most tightly linked primers (according to their data) on the extreme lowest and highest Cd accumulating F_4 families. Primer OPC-2 produced polymorphisms between the parents, but similar bands in some of the low and high families. Penner and Bezte later determined this primer was not linked to Cd accumulation in their cross either. The OPC-20 primer was polymorphic between the parents, as was also observed by Penner et al. (1994). The fragment was present in Sceptre, the high parent. However, the high Cd accumulating Sceptre/Biodur progeny did not all have the diagnostic fragment.

There are some discrepancies in those families categorized as HCGA. The Cd levels of the families over two generations are recorded in Table 14. Curiously, two of the 1993 HCGA families did not have very high Cd concentrations in 1992. Perhaps the high Cd levels of these families was the result of some environmental influence. Secondly, variation in Cd accumulation of lines has been noted before. Thomas and Harrison (1989) observed that a low Cd lettuce introduction used as a parent in their crosses was very high one in one experiment, higher than the progeny mean, and not significantly different from the high Cd parent.

4.3.4.2 Aneuploids

Grain samples of Langdon(*T. dicoccoides*) substitution lines for all chromosomes except 2B, the original Langdon-16 parent, and the checks Medora and DT471, were grown at Glenlea in 1993 (Appendix 5). The substitution lines were obtained from Dr. L. Joppa, USDA, ARS, Fargo ND. Langdon 16 had a mean Cd concentration of 0.138 ppm, while Medora and DT471 means were 0.148 and 0.077 ppm respectively. The Langdon (*T. dicoccoides*-5B) substitution line had a Cd concentration of 0.056 ppm; values for the other substitution lines ranged from 0.129 to 0.232 ppm. This indicates that chromosome 5B of *T. dicoccoides* has a Cd accumulation locus.

Langdon-16 and Langdon (*T. diccocoides*) substitution lines (except 2B) were grown in a growth cabinet. DNA was obtained by rapid leaf disc extraction with Proteinase K treatment and examined by agrose-electrophoresis for the OCP-20 RAPD marker. The OPC-20 band appeared in all substitutions and the parent. This suggests that the low Cd accumulation derived from chromosome 5B of *T. dicoccoides* is not linked to the OPC-20 marker.

Triticum aestivum cv. Chinese Spring nullisomic-tetrasomic plants were tested with OPC-20 for polymorphisms. All nullisomic-tetrasomics were available except nulli 2A, 4A, or 4B. The methodology was the same as for the substitution lines. No grain Cd data was available but other hexaploid

wheats tested have been low Cd accumulators (Leisle, personal communication; Meyer et al., 1982).

All nullisomic-tetrasomic lines had the OPC-20 band. Perhaps the marker sequence is present in more than one location in the Chinese Spring genome. Brewer, et al. (1969) theorized that in wheat evolution has occurred at the tetraploid and hexaploid levels for identical gene content of the different genomes. They observed 38 nullisomic-tetrasomic wheat strains analyzed for the isozyme patterns of 12 different enzymes. There was no significant variation patterns with 11 of the enzymes. Secondly, during RFLP analysis with genomic DNA clones of *Triticum aestivum* cv. Chinese Spring as probes, Liu and Tsunewaki (1991) found homologous sequences on different chromosomes and at different loci on the same chromosome.

5.0 GENERAL DISCUSSION

The grain Cd concentration of the HCGA and LCGA parents varied over the different environments investigated in this study. For example, the cultivar Sceptre, a HCGA which was grown most widely as a parent and check ranged from a mean value of 0.084 ppm from a bulked row at Glenlea (1993) to 0.339 ppm in replicate two at Morden (1993). There have been many studies on the effects of various soil parameters on Cd availability as were noted in the Section 2.3.2. Penner et al., (1994) reported that durum wheat grain Cd levels are affected by environmental conditions. High precipitation has been observed to cause high Cd concentrations in winter wheat grain (Andersson and Pettersson, 1981). Thomas and Harrison speculated that higher temperatures may have caused a parent lettuce line which had been low Cd accumulating when grown in field plots and under controlled environments to have a relatively high concentration when grown in a greenhouse. Cd uptake in excised barley roots did increase linearly with increasing temperature (Cutler and Rains, 1974). Disease resistant gene expression is also known to be affected by temperature (Dyck and Kerber, 1985).

In the present study the various populations were grown under four different environments: 1992 Glenlea, 1993 Glenlea, 1993 Morden, and 1994 greenhouse. The Morden site has been shown to result in high grain Cd levels and there was considerable difference between replicates in the same test.

There was an unusually large amount of rain, and subsequent abnormally high levels of Fusarium head blight and leaf diseases during the cool 1993 summer. These diseases and the water flooding could be expected to affect grain Cd accumulation.

Despite these diverse and adverse environmental conditions, the relationship for grain Cd levels between LCGA and HCGA parents was fairly constant. Under three environments mean Cd concentration of the LCGA parent Biodur ranged from 46.0% to 50.0% of the mean of the HCGA parent Sceptre. In the 1993 field data DT471 mean Cd concentration was 32.5% of the DT624 value. Parents, repeated four times each in the Morden test, had a wide range of Cd values. Still, the mean Cd concentration of DT627 (LCGA parent) in replicates one and two were 28.9% and 26.1% of DT618 (HCGA parent), respectively. Sceptre was consistently lower than the other HCGA cultivars, which accounts for the higher relative value of the LCGA parent. These results indicate that incorporating these fairly consistent sources of LCGA into acceptable cultivars would significantly lower the grain Cd concentration of Canadian durum wheat.

An attempt was made to determine the inheritance of LCGA in two sources; Biodur, and breeding lines that derive their LCGA trait from Hercules (DT471, DT627). The inheritance of Cd accumulation in durum wheat grains has been drawn from observations of frequency distribution charts, arbitrary

divisions, comparisons of progeny Cd accumulation means to midparental means and by attempting to fit various hypothetical genetic ratios to the populations. Environmental variability hampered the drawing of conclusions in some cases.

Both the Sceptre/Biodur F_3 and DT471/DT624 F_3 populations were shifted towards low Cd accumulation values indicating partial dominance. The Sceptre/Biodur F_3 progeny mean was lower than the midparent mean and data on F_4 lines in 1993 further support this conclusion. However, the DT471/DT624 progeny mean was higher than its midparental mean, possibly due to a single low value for a DT624 bulked row.

Although DT627, like DT471, is derived from Hercules the frequency distribution for the DT618/DT627 F_3 population grown at Morden was quite different from the DT471/DT624 cross. The two replicates of the DT618/DT627 population were also very different from each other. The results suggest a lack of dominance for Cd accumulation with the DT627 cross. It has been noted in the study of disease resistance genes that the same gene in different backgrounds can be expressed differently. Anderson (1966) observed from backcrosses of wheat varieties Bage and Thatcher for leaf rust resistance that one resistant allele was dominant in one background and recessive in another. Dominance behaviour of a crown rust resistance gene of oats also depends on the host background (Harder et al., 1980).

Identifying the LCGA and HCGA accumulating families was difficult because of the continuous phenotypic variation. This presented a problem when attempting to estimate the number of genes controlling grain Cd levels from the F_3 frequency distributions and when making the bulk pools for segregant analysis.

Thomas and Harrison (1989) divided their lettuce populations into low and high Cd groups by the midparental mean, then tried to fit 3:1 genetic ratio to their data. This method presumes complete dominance with the heterozygotes phenotypically identical to the homozygote classes. The division ignores the actual distribution of the data. Also, into what category should lines and/or individuals with Cd concentrations equal to the midparental mean value be should placed? An advantage of using the midparental value is that the checks are affected by relatively the same environmental conditions as the cross being tested. Penner et al. (1994) categorized F8 durum wheat individuals based on their Cd accumulation into two groups to create bulk pools for testing with RAPD markers. They chose 75% below and 120% above the midparental value to differentiate the low and high durum wheat grain Cd accumulating groups. Both methods are based on the midparental value and a potential source of problems is that this mean may be based on the Cd concentrations of only a few rows or plants.

The shape of the frequency distribution for the Sceptre/Biodur cross suggested some arbitrary division points. F_4 verification of F_3 lines from that region of the distribution supports the hypothesis of a single partially dominant gene for LCGA. For the Hercules-source (DT471, DT627) crosses, it was not possible to arrive at an estimate of the number of genes.

Heritability estimates were obtained for both sources of low Cd accumulation. The "nearly-narrow" sense heritability estimate from Sceptre/Biodur cross (68.6%) and the broad sense heritability confidence intervals from the DT618/DT627 cross (91.69% to 85.29%) were both quite high. This indicates that it will be possible to make good progress in breeding for low grain Cd concentration using either of these two sources.

It is likely the same locus controlling LCGA in the Biodur and the Hercules source (DT627, DT637) breeding lines. While the F_2 populations were not perfectly bell-shaped, most of the suspected HCGA families from the Biodur reciprocal crosses were shown in F_3 to be LCGA families. One suspected HCGA F_2 individual had no viable seed left to grow to confirm that it was a high accumulator, while another had only two F_3 progeny survive and mature to test for conformation. The conclusion is supported by the recent results of Penner et al. (1994). They applied their OPC-20 RAPD marker, developed using the LCGA cultivar Nile, to various other LCGA and HCGA material. The diagnostic fragment was not present in Nile,

Biodur, or various LCGA lines with Hercules ancestry (including DT627, DT637), but was present in Sceptre and DT648. In the present study the OPC-20 diagnostic fragment was also absent on all LCGA families from the Sceptre/Biodur cross, although HCGA families gave variable results. The latter crosses may have been wrongly classified as high accumulators, or the product of recombination between the marker and the Cd locus.

The Cd locus does not appear to be linked to any of the traits tested in the DT471/DT624 cross: tyrosinase, LMW-2, or the β-gliadins.

Langdon(*T. dicoccoides*) substitution lines were evaluated to determine if any substitutions were lower in grain Cd concentration; Langdon is a HCGA cultivar while the Cd concentration of the *T. dicoccoides* parent is unknown (Leisle, personal communication). The Langdon (*T. dicoccoides*-5B) substitution line was relatively low in grain Cd. This is quite interesting, since mineral stress multi-tolerance is strongly suspected of being controlled by alleles or clusters of similar gene(s) predominantly located on the homoeologous group 5 chromosomes of the tribe *Triticeae* (Manyowa and Miller, 1991). While it is currently unknown if there is any relationship between plant metal tolerance and metal accumulation, exclusion of metals could be one mechanism of tolerance (Zhang and Taylor, 1989). The OPC-20 RAPD primer diagnostic fragment was present in all the Langdon(*T.*

dicoccoides) substitution lines tested, including chromosome 5B, which suggests that the gene for low Cd in *T. dicoccoides* is different from that in durum wheat germplasm. This is not surprising since the relationship is relatively distant. However, the results are not conclusive since it could represent a crossover between the marker and the Cd locus. Confirming if *T. dicoccoides* does have a different gene(s) controlling Cd accumulation, and its mode of action, would be very useful knowledge for a durum wheat breeding program. Although there has been no information on the physiology of grain Cd accumulation of durum wheat published as of yet, two mechanisms individually controlling Cd uptake and Cd translocation appear to exist in lettuce (Thomas and Harrison, 1991). If different mechanisms to control Cd accumulation do exist in wheat combining two such systems might further reduce grain Cd levels.

Finally, the OPC-20 marker was applied to a set of Chinese Spring nullisomic-tetrasomics to determine if there was any polymorphism, possibly as a further clue to the location of the marker and hence to the Cd locus. Since all lines had the band, no further information was obtained.

The present study provided some useful information toward incorporating low grain Cd concentration into commercial cultivars. LCGA cultivars and breeding lines already in the Canadian durum breeding program have been confirmed. Families from crosses involving these cultivars and lines have been

identified through through field studies as LCGA and advanced in the durum wheat breeding program. There are many other areas for further study. The inheritance of grain Cd levels should be defined in several other sources of low Cd. Whether they are allelic should be further verified. Due to the effect of environmental variability, and the complicating factor of the heterozygous class, the number of genes might best be determined by developing random inbred lines. Pertaining to the aneuploid screening, it would be useful to determine the relationship of other low Cd accumulation sources to that in the Langdon(*T. dicoccoides*-5B) substitution line. Lastly, determining the mechanism(s) involved in low grain Cd accumulation could provide information making possible a further reduction in Cd levels in Canadian durum wheat.

6.0 REFERENCES

Ahmad, S., Waheed, S., Mannan, A. Fatima, I. and Qureshi, I.H. 1994. Evaluation of Trace Elements in Wheat and Wheat By-Products. AOAC. 77 (1):11-18.

Allard, R.W. 1960. Genetic Components of Continuous Variation. In "Principles of Plant Breeding." pp. 99-108. John Wiley and Sons, Inc. New York.

Anderson, R.G. 1966. Studies on the Inheritance of Resistance to Leaf Rust of Wheat.In " Proceedings of the Second International Wheat Genetics Symposium." pp. 144-155. Hereditas Suppl. 2.

Andersson, A. and Pettersson, O. 1981. Cadmium in Swedish Winter Wheat. Regional Differences and Their Origin. Swedish J. Agric. Res. 11:49-55.

Antonovic, J., Bradshaw, A.D., and Turner, R.G. 1971. Heavy Metal Tolerance in Plants. Adv. Eco. Res. 7:1-84.

Barinaga, M. 1991. Biotech Nightmare: Does Cetus Own PCR? Science 251:739-251.

Bernard, A., and Lauwerys, R. 1984. Cadmium in Human Population. Experientia. 40:143-152.

Bernier, A.M. and Howes, N.K. 1994. Quantification of Variation in Tyrosinase Activity Among Durum and Common Wheat Cultivars. J. Cereal Science 19:1-3.

Bozzini, A. 1988. Origin, Distribution, and Production of Durum Wheat in the World. In "Durum Chemistry and Technology." (G. Fabriani and C. Lintas eds). pp. 1-16. Am. Assoc. of Cereal Chem. St. Paul, Minnesota.

Brewer, G.J., Sing, C.F. and Sears, E.R. 1969. Studies of Isozyme Patterns in Nullisomic-Tetrasomic Combinations of Hexaploid Wheat. Proc. Natl. Acad. Sci. USA 64:1224-1229.

Brown, K.R., Grant, C.A. and Racz, G.J. 1994. Effect of Nitrogen Source, Rate, and Placement on Cd Bioavailability in Durum Wheat. Canadian Society of Agronomy Abstracts, Annual Meeting July 11-14.

Bushuk, W. and Zillman, R.R. 1978. Wheat Cultivar Identification by Gliadin Electrophoregrams. I. Apparatus, Method and Nomenclature. Can. J. Plant Sci. 58:505-515.

Canada Grains Council, 1994. Canadian Grains Industry Statistical Handbook 93. Winnipeg, Manitoba.

Carrillo, J.M., Vazquez, J.F., and Orellana, J. 1990. Relationship Between Gluten Strenth and Glutenin Proteins in Durum Wheat Cultivars. Pl. Breeding 104:325-333.

Chaney, R.L., Schneiter, A.L., and Brown, S.L. 1993. Effect of Sewage Sludge Cadmium Concentration and Soil pH on Cadmium in Grain of Sunflower, Wheat, Peanut, Oat, and Soybean. Agronomy Abstracts. pp. 27. ASA, CSSA, SSSA Annual Meeting. Nov. 7-12. Ohio.

Choudhary, M., Bailey, L.D. and Grant C.A. 1993. Effect of Zn Application on Cd Concentration in Durum Wheat (*Tritcum turgidum* l. *var durum*) as Affected by Soil Temperature and Growth Stages. Agronomy Abstracts. pp. 27-28. ASA, CSSA, SSSA Annual Meeting. Nov. 7-12. Ohio.

Clarke, J.M., Howes, N.K., J.G. McLeod, and DePauw, R.M. 1993. Selection for Gluten Strength in Three Durum Wheat Crosses. Crop Sci. 33(5): 956-958.

Conn, E.E., Stumpf, P.K., Bruening, G., and Doi, R.H. 1987. Polyacrylamide Gel Electrophoreis of Proteins. In."Outlines of Biochemistry 5/E." pp. 86-89. John Wiley and Sons. Toronto.

Costa, G. and Morel, J.L. 1993. Cadmium Uptake by *Lupinus Albus* (L.):Cadmium Excretion, A Possible Mechanism of Cadmium Tolerance. J. Plant Nutr. 16(10):1921-1929.

Crosby, N.T. 1977. Determination of Metals in Foods. Analyst. 102 (1213): 225- 268.

Cunningham, J.D., Ryan, J.A. and Keeney, D.R. 1975. Phytotoxicity In and Metal Uptake from Soil Treated with Metal-Amended Sewage Sludge. J. Environ. Qual. 4(4):455-460.

Cutler, J.M. and Rains, D.W. 1974. Characterization of Cadmium Uptake by Plant Tissue. Plant Physiol. 54:67-71.

Dabeka, R.W. and McKenzie, A.D. 1992. Total Diet Study of Lead and Cadmium in Food Composites Preliminary Investigations. AOAC. 75(3): 386-395.

Devos, K.M. and Gale, M.D. 1992. The Use of Random Amplified Polymorphic DNA Markers in Wheat. Theor. Appl. Genet. 84:567-572.

Du Cros, D.L., Joppa,L.R. and Wrigley, C.W. 1983. Two-Dimensional Analysis of Gliadin Proteins Associated with Quality in Durum Wheat: Chromosomal Location of Genes for Their Synthesis. Theor. Appl. Genet. 66:297-302.

Dos Santos, J.B., Nienhuis, J., Skroch, P., Tivang, J. and Slocum, M.K. 1994. Comparison of RAPD and RFLP Genetic Markers in Determining Genetic Similarity Among *Brassica Oleracea* L. Genotypes. Theor. Appl. Genet. 87:909-915.

Dudley, J.W. and Moll, R.H. 1969. Interpretation and Use of Estimates of Heritability and Genetic Variances in Plant Breeding. Crop Sci. 9(3): 257-261.

Dweikat, I., Mackenzie, S., Levy, M., and Ohm, H. 1993. Pedigree Assessment Using RAPD-DGGE in Cereal Crop Species. Theor. Appl. Genet. 85:497-505.

Dyck, P.L. and Kerber, E.R. 1985. Resistance of the Race-Specific Type. In "The Cereal Rusts Volume II". (W.R. Bushnell and A.P. Roelfs eds). pp. 469-500. Academic Press, Inc. N.Y.

Edwards, K., Johnstone, C. and Thompson, C. 1991. A Simple and Rapid Method for the Preparation of Plant Genomic DNA for PCR Analysis. Nucleic Acids Research 19(6):1349

Erlich, H.A. 1989. Principles and Applications for DNA Amplification. In "PCR Technology." pp. 11-37. Macmillan Pub. N.Y.

Fairbanks, D.J., Waldrigues, A., Ruas, C.F. Ruas, P.M., Maughan, P.J., Robison, L.R., Andersen, W.R., Riede, C.R., Pauley, C.S., Caetano, L.G. Arantes, O.M.N., Fungaro, M.H.P., Vidotto, M.C. and Jankevicius, S.E. 1993. Efficient Characterization of Biological Diversity Using Field DNA Extraction and Random Amplified Polymorphic DNA Markers. Brazilian J. Gen. 16(1):11-22

Falahi-Ardakani, A., Corey, K.A., and Gouin, F.R. 1988. Influence of pH on Cadmium and Zinc Concentrations of Cucumber Grown in Sewage Sludge. HortSci. 23(6):1015-1017.

Falconer, D.S. 1989. Heritability In "Introduction to Quantitative Genetics" 3rd Ed. pp. 163-186. Longman, Scientific and Technical copublished with John Wiley and Sons, New York.

FAO/WHO. 1989. Evaluation of Certain Food Additives and Contaminants. 33rd Report of the Joint FAO/WHO Expert

Committee on Food Additives. pp. 28-30. WHO Technical Report Series 776.

FAO/WHO. 1991. Consideration of Proposed Draft Guideline Levels for Cadmium and Lead in Food. pp.36. Codex Alimentarius Commission. Nineteenth Session. Rome. 1-10 July.

Feillet, P. 1988. Protein And Enzyme Composition of Durum Wheat. In "Durum Wheat: Chemistry and Technology." (G Fabriani and C. Lintas eds). pp. 93-119. Am. Assoc. Cereal Chem. St. Paul. Minnesota.

Florijn, P.J., Nelemans, J.A. and Van Beusichem, M.L. 1992. The Influence of the Form of Nitrogen Nutrition on Uptake and Distribution of Cadmium in Lettuce Varieties. J. Plant Nutr. 15(11):2405-2416.

Foth, D. 1990. Soil Chemistry. In "Fundamentals of Soil Science" 8th Ed. pp. 164-184. John Wiley & Sons. Toronto.

Gale, M.D., Sharp, P.J., Chao, S. and Law, C.N. 1989. Applications of Genetic Markers in Cytogenetic Manipulation of the Wheat Genomes. Genome 31:137-142.

Gartside, D.W. and McNeilly, T. 1974a. Genetic Studies in Heavy Metal Tolerant Plants I. Genetics of Zinc Tolerance in *Anthoxanthum odoratum*. Heredity 32(3):287-297.

Gartside, D.W. and McNeilly, T. 1974b. The Potential for Evolution of Heavy Metal Tolerance in Plants II. Copper Tolerance in Normal Populations of Different Plant Species. Heredity 32(3):335-348.

Gasser, C.S., Fraley, R.T. 1989. Genetically Engineering Plants for Crop Improvement. Science 244:1293-1299.

Gibson, S. and Somerville, C. 1993. Isolating Plant Genes. Trends in Biotechnology 11:306-313.

Gowalka, E. and Tkachuk, R. 1994. Comparison of Focused Open and Closed Vessel Microwave Digestion Procedures with Conventional Digestion Procedures for Trace Element Analysis of Cereals. Federation of Analytical Chemistry and Spectroscopy Societies, 21st Annual Conference October, 1994 St. Louis Missouri

Goyer, R.A. and Cherian, M.G. 1979. Health Effects of Cadmium. In " National Research Council Canada Associate Committee on Scientific Criteria for Environmental Quality - Effects of

Cadmium in the Canadian Environment" - Subcommittee on Heavy Metals and Certain Other Elements. pp. 94-110. Publication No. NRCC 16743 of the Environmental Secretariat.

Haghiri, F. 1974. Plant Uptake of Cadmium as Influenced by Cation Exchange Capacity, Organic Matter, Zinc, and Soil Temperature. J. Environ. Qual. 3(2):180-183.

Hallenbeck, W.H. 1984. Human Health Affects of Exposure to Cadmium. Experientia. 40:136-142.

Harder, D.E., McKenzie, R.I.H., and Martens, J.W. 1980. Inheritance of Crown Rust Resistance in Three Accessions of *Avena sterilis*. Can. J. Genet. Cytol. 22:27-33.

Heun, M., Murphy, J.P. and Phillips, T.D. 1994. A Comparison of RAPD and Isozymes Analyses for Determining The Genetic Relationships Amoung *Avena sterilis* L. Accessions. Theor. Appl. Genet. 87:689-696.

Hinesly, T.D., Alexander, D.E., Redborg, K.E. and Ziegler, E.L. 1982. Differential Accumulations of Cadmium and Zinc by Corn Hybrids Grown on Soil Amended with Sewage Sludge. Agron. J. 74:469-474.

Howes, N.K., Kovacs, M.I. and Leisle, D. 1989. Screening of Durum Wheats for Pasta-Making Quality With Monoclonal Antibodies for Gliadin 45. Genome 32:1096-1099.

Howes, N.K., Skerritt, J.H., and Zawistowski, J. 1991. Selection of Hexaploid Wheats for Gluten Strength Using Monoclonal Antibodies Which Discriminate Gli-B1, Glu-B1 and Glue-B3 Alleles. Am. Assoc. Cereal Chem. 76th Annual Meeting. 36(8): 699.

Howes, N.K., Zawistowski, J. and Leisle, D. 1995. Two Durum Wheat LWM-2 Glutenin Alleles Distinguished by a Monoclonal Antibody Test. (In Press) Plant Breeding.

Hu, C-Y., Allen, M. and Gyllensten, U. 1992. Effect of Freezing of the PCR Buffer on the Amplification Specificity: Allelic Exclusion and Preferential Amplification of Contaminating Molecules. PCR Methods and Applications 2:182-183.

Humphreys, M.O., and Nicholls, M.K. 1984. Relationships Between Tolerance to Heavy Metals in *Agrostis Capillaris* L. (*A. Tenuis* Sibth.). New Phytol. 98:177-190.

Hutchinson, T.C. 1979. Cadmium in Aquatic and Terrestrial Vegetation. In "National Research Council Canada Associate Committee on Scientific Criteria for Environmental Quality Effects of Cadmium in Canadian Environment - Subcommittee on Heavy Metals and Certain Other Elements. pp. 47-67. Publication No. NRCC 16743 of the Environmental Secetariat.

Jackson, P.J. Unkefer, C.J., Doolen, J.A., Watt, K., and Robinson, N.J. 1987. Poly(γ-glutamylcysteinyl)glycine: Its Role in Cadmium Resistance in Plant Cells. Proc. Natl. Acad. Sci. USA. 84:6619-6623.

Jalil, A., Selles, F., and Clarke, J.M. 1993. Effect of Cadmium on the Growth and Nutrient Uptake of Durum Wheat. Agronomy Abstracts. pp. 36. ASA, CSSA, SSSA Annual Meeting. Nov. 7-12. Ohio.

Jarvis, S.C., Jones, L.H.P., and Hopper, M.J. 1976. Cadmium Uptake From Solution By Plants and its Transport From Roots to Shoots. Plant and Soil 44:179-191.

Jeyaprakash, A., Welch, J.W. and Fogel, S. 1991. Multicopy CUP1 Plasmids Enhance Cadmium and Copper Resistance Levels in Yeast. Mol. Gen. Genet. 225:363-368.

Jones, M.C. and Boffey, S.A. 1984. Deoxyribonuclease Activities of Wheat Seedlings. FEBS Letters 174(2); 215-218.

Jones, K.C. and Johnston, A.E. 1989. Cadmium in Cereal Grain and Herbage from Long-term Experimental Plots at Rothamsted, UK. Environ. Pollut. 57:199-216.

Jones, R.L., Hinesly, T.D., Ziegler, E.L. and Tyler, J.J. 1975. Cadmium and Zinc Contents of Corn Leaf and Grain Produced by Sludge-amended Soil. J. Environ. Qual. 4(4):509-514.

Jones, R.W., Taylor, N.W. and Senti, F.R. 1959. Electrophoresis and Fractionation of Wheat Gluten. Arch. Biochem. Biophys. 84:363-376.

Kamizono, A., Nishizawa, M., Teranishi, Y. Murata, K., and Kimura, A. 1989. Identification of a Gene Conferring Resistance to Zinc and Cadmium Ions in the Yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. 219:161-167.

Kaneta, M, Hikichi, H., Endo, S. and Sugiyama, N. 1986. Chemical Form of Cadmium (and Other Heavy Metals) in Rice and Wheat Plants. Envir. Health Perspectives 65:33-37.

Kastori, R., Petrovic, M., and Petrovic, N. 1992. Effect of Excess Lead, Cadmium, Copper, and Zinc on Water Relations in Sunflower. J. Plant Nutr. 15(11):2427-2439.

Kawashima, I., Kennedy, T.D., Chino, M. and Lane, B.G. 1992. Wheat E_c Metallothionein Genes. Like Mammalian Zn^{2+} Metallothionein Genes, Wheat Zn^{2+} Metallothionein Genes are Conspicuously Expressed During Embryogenesis. Eur. J. Biochem. 209:971-976.

Khan, K. 1982. Polyacrylamide Gel Electrophoresis of Wheat Gluten Proteins. Bakers Digest. (October):14-19.

Kim, S.J., Chang, A.C. Page, A.L., and Warneke, J.E. 1988. Relative Concentrations of Cadmium and Zinc in Tissue of Selected Food Plants Grown on Sludge-Treated Soils. J. Environ. Qual. 17(4):568-573.

Knapp, S.J., Stroup, W.W. and Ross, W.M. 1985. Exact Confidence Intervals for Heritability on a Progeny Mean Basis. Crop Sci. 25:192-194.

Ko, H.L., Henry, R.J., Graham, G.C., Fox, G.P., Chadbone, D.A., and Haak, I.C. 1994. Identification of Cereals Using the Polymerase Chain Reaction. J. Cereal Sci. 19:101-106.

Krotz, R.M., Evangelou, B.P. and Wagner, G.J. 1989. Relationships Between Cadmium, Zinc, Cd-Peptide, and Organic Acid in Tobacco Suspension Cells. Plant Physiol. 91:780-787.

Lamkin, W.M.,Miller, B.S., Nelson, S.W., Traylor, D.D., and Lee, M.S. 1981. Polyphenol Oxidase Activities of Hard Red Winter, Soft Red Winter, Hard Red Spring, White Common, Club and Durum Wheat Cultivars. Cereal Chem. 58(1):27-31.

Leisle, D., Kovacs, M.I. and Howes, N. 1985. Inheritance and Linkage Relationships of Gliadin Proteins and Glume Color in Durum Wheat. Can. J. Genet. Cytol. 27:716-721.

Liu, Y.-G., and Tsunewaki, K. 1991. Restriction Fragment Length Polymorphism (RFLP) Analysis in Wheat. II. Linkage Maps of the RFLP Sites in Common Wheat. Jpn. J. Genet. 66 (5):617-633.

Mahoney, R.R. and Ramsay, M. 1992. A Rapid Tyrosinase Test for Detecting Contamination of Durum Wheat. J. Cereal Sci. 15(3):267-270.

Maiti, I.B., Wagner, G.J., Yeargan, R., and Hunt, A.G. 1989. Inheritance and Expression of the Mouse Metallothionein Gene in Tobacco. Impact on Cd Tolerance and Tissue Cd Distribution in Seedlings. Plant Physiol. 91:1020-1024.

Manyowa, N.M., and Miller, T.E. 1991. The Genetics of Tolerance to High Mineral Concentrations in the Tribe *Triticeae*- A Review and Update. Euphytica 57:175-185.

Margoshes, M. and Vallee, B.L. 1957. A Cadmium Protein From Equine Kidney Cortex. Am. Chem. Soc. 79:4813-4814.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, G.S., Coffman, W.R. and Tanksley, S.D. 1988. Molecular Mapping of Rice Chromosomes. Theor. Appl. Genet. 76:815-829.

McLaughlin, M.J., Palmer, L.T., Tiller, K.G., Beech, T.A. and Smart, M.K. 1993. Soil Salinity Causes Elevated Cadmium Concentrations in Field-Grown Potato Tubers. Personal Communication, Unpublished.

McMillin, D.E. 1983. Plant Isozymes: A Historical Perspective. In Isozymes in Plant Genetics and Breeding, Part A. Eds. S.D. Tanksley and T.J. Orton. Elsevier Science Publishers. New York.

Meyer, M.W., Fricke, F.L., Holmgren, G.G.S., Kubota, J., and Chaney, R.L. 1982. Cadmium and Lead in Wheat Grain and Associated Surface Soils of Major Wheat Production Areas of the United States. Agron. Abstr.:34.

Michelmore, R.W., Paran, I. and Kesseli, R.V. 1991. Identification of Markers Linked to Disease-Resistance Genes by Bulked Segregant Analysis: A Rapid Method to Detect Markers in Specific Genomic Regions by Using Segregation Populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Mitchell, G.A., Bingham, F.T. and Page, A.L. 1978. Yield and Metal Composition of Lettuce and Wheat Grown on Soils Amended with Sewage Sludge Enriched with Cadmium, Copper, Nickel, and Zinc. J. Environ. Qual. 7(2):165-171.

Monte, J.V., McIntyre, C.L. and Gustafson, J.P. 1993. Analysis of Phyogenetic Relationships in the Triticeae Tribe Using RFLPs. Theor. Appl. Genet. 86:649-655.

Nisonoff, A. 1984. Immunological Assays and the Purification of Antibodies. In "Introduction to Molecular Immunology." 2nd

Ed. Sinauer Associates, Inc. Sunderland, Massachusetts, QR 183.6.N57

Oliver, D.P., Hannam, R. Tiller, K.G., Wilhelm, N.S., Merry, R.H. and Cozens, G.D. 1994. The Effects of Zinc Fertilization on Cadmium Concentration in Wheat Grain. J. Environ. Qual. 23:705-711.

Oliver, D.P., Gore, P.J., Moss, H.J., and Tiller, K.G. 1993. Cadmium in Wheat-Grain and Milling Products From Some Australian Flour Mills. Aust. J. Agric. Res. 44:1-11.

Oliver, D.P., Schultz, J.E., Tiller, K.G., and Merry, R.H. 1993. The Effect of Crop Rotations and Tillage Practices on Cadmium Concentration in Wheat Grain. Aust. J. Agric. Res. 44:1221-1234.

Oliver, D.P., Tiller, K.G., Conyers, M.K., Slattery, W.J., Merry, R.H. and Alston, A.M. The Effects of Soil pH on Cadmium Concentration in Wheat Grain Grown in the Field. Personal Communication, unpublished.

Outridge, P.M., Rauser, W.E., and Hutchinson, T.C. 1991. Changes in Metal-Binding Peptides Due to Acclimation to Cadmium Transferred Between Ramets of *Salvinia minima*. Oecologia. 88:109-115.

Payne, P.I., Jackson, E.A. and Holt, L.M. 1984B. The Association Between γ-Gliadin 45 and Gluten Strength in Durum Wheat Varieties: A Direct Causal Effect or the Result of Genetic Linkage? J. Cereal Sci. 2:73-81.

Payne, P.I., Holt, L.M., Jackson, E.A. and Law, C.N. 1984A. Wheat Storage Proteins: Their Genetics and Their Potential for Manipulation by Plant Breeding. Phil. Trans. R. Soc. Lon. B 304:359-371.

Penner, G.A. 1994. RAPD Analysis in Cereals."Methods of Genome Analysis in Plants, Merits and Pitfalls." In press.

Penner, G.A. and Bezte, L.J. 1994. Increased Detection of Polymorphism Among Randomly Amplified Wheat DNA Fragments Using a Modified Temperature Sweep Gel Electrophoresis (TSGE) Technique. Nucleic Acids Res. 22(9):1780-1781.

Penner, G.A., Clarke, J., Bezte, L.J. and Leisle, D. 1994. Identification of RAPD Markers Linked to a Gene Governing Cadmium Uptake in Durum Wheat. In press, Genome.

Penner, G.A., Bush, A., Wise, R., Kim, W., Domier, L., Kasha, K., Laroche, A., Scoles, G., Molnar, S.J., and Fedak, G. 1993. Reproducibility of Random Amplified Polymorphic DNA (RAPD) Analysis Among Laboratories. PCR Methods and Applications 2:341-345.

Petit, C.M. and Van De Geijn, S.C. 1978. In Vivo Measurement of Cadmium (^{115}Cd) Transport and Accumulation in the Stems of Intact Tomato Plants (*lycopersicon esculentum*, Mill.) I. Long Distance Transport and Local Accumulation. Planta 138:137-143.

Pettersson, O. 1977. Differences in Cadmium Uptake Between Plant Species and Cultivars. Swedish J. Agric. Res. 7:21-24.

Pomeranz, Y. 1988. Composition and Functionality of Wheat Flour Components. In "Wheat Chemistry and Technology" 2nd Ed. (Y. Pomeranz ed). pp. 219-370. Amer. Assoc. Cereal Chem. Inc. St. Paul, Minnesota.

Rauser, W.E. 1993. Metal-Binding Peptides in Plants. In "Sulfur Nutrition and Sulfur Assimilation in Higher Plants." (L.J. de Kok ed). pp. 237-249. SPB Academic Publishing. Den Hauge, Nederland.

Rauser, W.E. 1990. Phytochelatins. Annu. Rev. Biochem. 59:61-86.

Rauser, W.E. 1992. γ-Glutamylcysteinyl Isopeptides: Structure, Synthesis and Metal-Binding Complexes. Conference Preceedings, 3rd International Meeting on Metallothionein. Tsukuba, Japan. Dec 8-10.

Salt, D.E. and Wagner, G.J. 1993. Cadmium Transport Across Tonoplast of Vesicles from Oat Roots. J. Bio. Chem. 268(17) 12297-12302.

Statistical Analysis System Institute, Inc. 1991. SAS Languages and Procedures. Usage 2 Version 6 First Edition. Sas Institute Inc., Cary, NC, USA.

Sela, M., Tel-Or, E, Fritz, E. and Huttermann, A. 1988. Localization and Toxic Effects of Cadmium, Copper and Uranium in *Azolla*. Plant Physiol. 88:30-36.

Sims, J.T., and Kline, J.S. 1991. Chemical Fractionation and Plant Uptake of Heavy Metals in Soils Amended with Co-Composted Sewage Sludge. J. Environ. Qual. 20:387-395.

Steffens, J.C., Hunt, D.F., and Williams, B.G. 1986. Accumulation of Non-Protein Metal-Binding Polypeptides (γ-Glutamyl-Cysteinyl)$_n$-Glycine in Selected Cadmium-Resistant Tomato Cells. J. Bio. Chem. 261(30):13879-13882.

Sudhakar, C., Syamalabai, L. and Veeranjaneyulu. 1992. Lead Tolerance of Certain Legume Species Grown on Lead Ore Tailings. Agric. Ecosystems Envir. 41:253-261.

Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W. 1989. RFLP Mapping in Plant Breeding: New Tools for an Old Science. Biotechnology 7:257-264

Thomas, G.M., and Harrison, H.C. 1989. Inheritance of Cadmium Concentration in Lettuce. J. Amer. Soc. Hort. Sci. 114(1):121-125.

Thomas, G.M. and Harrison, H.C. 1991. Genetic Line Effects on Parameters Influencing Cadmium Concentration in Lettuce (*Lactuca sativa* L.). J. Plant Nutr. 14(9):953-962.

Thys, C., Vanthomme, P., Schrevens, E., De Proft, M. 1991. Interactions of Cd with Zn, Cu, Mn, and Fe for Lettuce (*Lactuca sativa* L.) in Hydroponic Culture. Plant, Cell and Environ. 14:713-717.

Tohoyama, H., Tomoyasu, T., Inouhe, M., Joho, M. and Murayama, T. 1992. The Gene for Cadmium Metallothionein from a Cadmium-Resistant Yeast Appears to be Identical to CUP1 in a Copper-Resistant Strain. Curr. Genet. 21:276-279.

Tomsett, A.B. and Thurman, D.A. 1988. Molecular Biology of Metal Tolerances of Plants. Plant, Cell, and Environ. 11:383-394.

Turner, R.G. and Marshall, C. 1972. The Accumulation of Zinc by Subcellular Fractions of Roots of *Agrostis Tenuis* Sibth. In Relation to Zinc Tolerance. New Phytol. 71:671-676.

Van Bruwaene, R., Kirchmann, R., and Impens, R. 1984. Cadmium Contamination in Agriculture and Zootechnology. Experientia. 40: 43-49.

Van Dokkum, W., De Vos, R.H., Muys, T.H. and Wesstra, J.A. 1989. Minerals and Trace Elements in Total Diets in the Netherlands. Brit. J. Nutri. 61:7-15.

Van de Geijn, S.C. and Petit. C.M. 1978. In Vivo Measurement of Cadmium (^{115m}Cd) Transport and Accumulation in the Stems of

Intact Tomato Plants (*lycopersicon esculentum*, Mill.)II. Lateral Migration from the Xylem and Redistribution in the Stem. Planta 138:145-151.

Verkleij, J.A., Koevoets, P., Van'T Riet, J., Bank., R., Nijdam, Y. and Ernst, W.H.O. 1990. Poly(γ-Glutamylcysteinyl)Glycines or Phytochelatins and Their Role in Cadmium Tolerance of *Silene vulgaris*.13:913-921.

Vogeli-Lange, R. and Wagner, G.J. 1990. Subcellular Localization of Cadmium and Cadmium-Binding Peptides in Tobacco Leaves. Implication of a Transport Function for Cadmium-Binding Peptides. Plant Physiol. 92(4):1086-1093.

Wagner, G.J. 1993. Accumulation of Cadmium in Crop Plants and its Consequences to Human Health. Advances in Agron. 51:173-213.

Wall, J.S. 1979. The Role of Wheat Proteins in Determining Baking Quality. In "Recent Advances in Biochemistry of Cereals."(D.L. Laidman and R.G. Wyn Jones eds.) pp. 275-311. Academic Press, N.Y.

Walley, K.A., Khan, M.S.I., and Bradshaw, A.D. 1974. Heredity 32(3); 309-319.

Watson, J.D., Gilman, M., Witkowski,J. and Zoller, M. 1992. Genetic Engineering of Plants. In "Recombinant DNA" 2nd Ed. pp. 273-292. W.H. Freeman and Company, New York.

Waugh, R., and Powell, W. 1992. Using RAPD Markers for Crop Improvement. Trends in Biotechnolgy 10:186-191.

Williams, C.H. and D.J. David. 1972. The Effect of Superphosphate on the Cadmium Content of Soils and Plants. Aust. J. Soil Res. 11:43-56.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers. Nucleic Acids Res. 18:6531-6535.

Winge, D.R., Nielson, K.B., Gray, W.R., and Hamer, D.H. 1985. Yeast Metallothionein. J. Bio. Chem. 260(27):14464-14470.

Woychik, J.H., Boundy, J.A. and Dimler, R.J. 1961. Starch Gel Electrophoresis of Wheat Gluten Proteins with Concentrated Urea. Arch. Biochem. Biophys. 94:477-482.

Wrigley, C.W., Autran, J.C. and Bushak, W. 1982. Identification of Cereal Varieties by Gel Electrophoresis of the Grain Proteins. In "Advances in Cereal Science and Technology." Vol 5. pp. 211-259. Amer. Assoc. Cereal Chem. St. Paul, Minnesota.

Wrigley, C.W. 1982. The Use of Genetics in Understanding Protein Composition and Grain Quality in Wheat. Qual. Plant Foods Hum. Nutr. 31:205-227.

Xue, Qi and Harrison, H.C. 1991. Effect of Soil Zinc, pH, and Cultivar on Cadmium Uptake in Leaf Lettuce (*Lactuca sativa* L. var. *crispa*). Commun. Soil Sci. Plant Anal. 22(9&10):975-991.

Yuran, G.T. and Harrison, H.C. 1986. Effects of Genotype and Sewage Sludge on Cadmium Concentrations in Lettuce Leaf Tissue. J. Amer. Soc. Hort. Sci. 111(4):491-494.

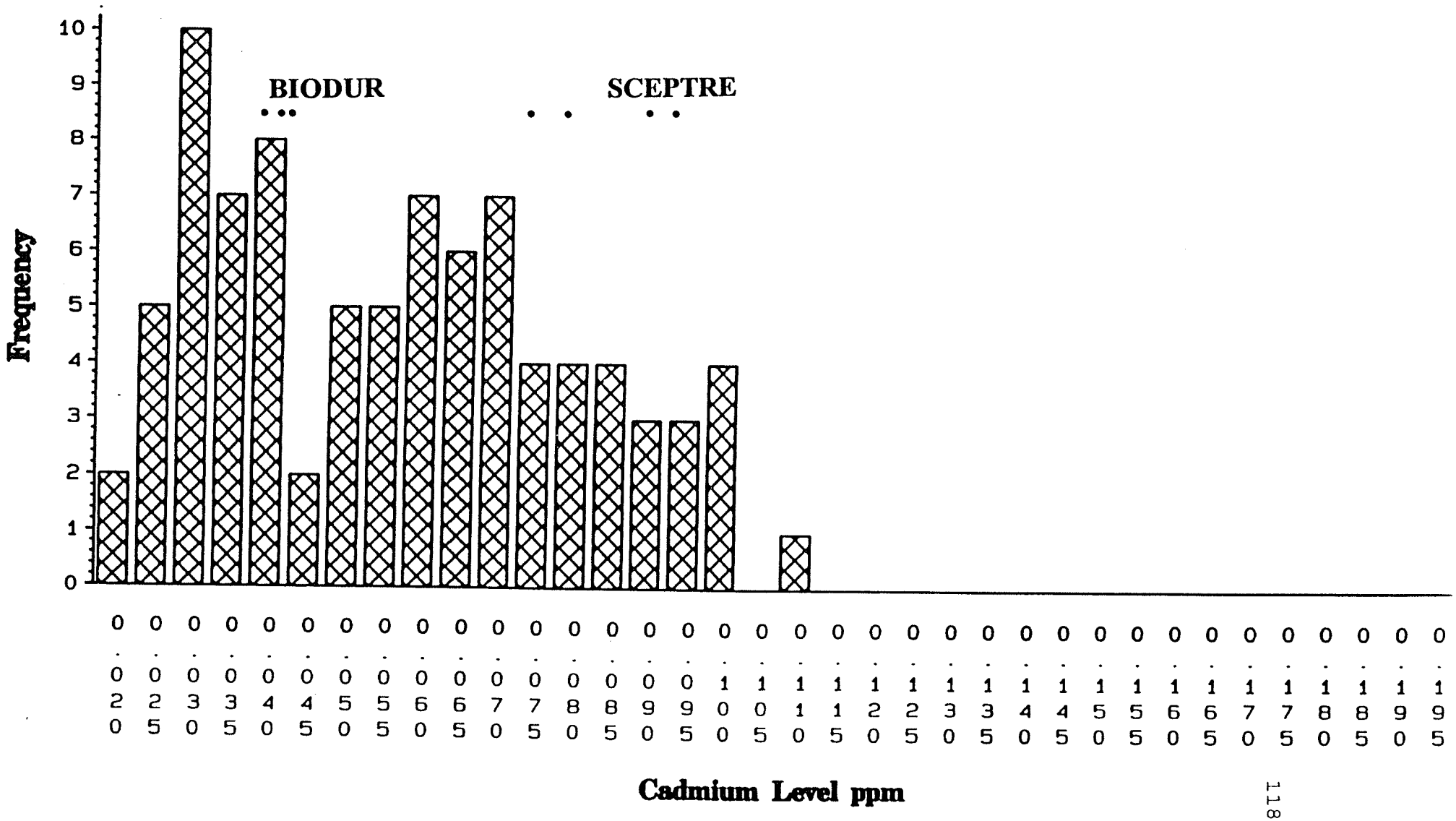
Zeven, A.C. 1972. Identification of Chromosomes Carrying a Locus for a Gene Conditioning of Production of Tyrosinase in Wheat Grains. Wheat Info. Services:3-8.

Zhang, G. and Taylor, G.J. 1989. Kinetics of Aluminum Uptake by Excised Roots of Aluminum-Tolerant and Aluminum-Sensitive Cultivars of *Triticum aestivum* L. Plant Physiol. 91:1094-1099.

Zillman, R.R. and Bushuk, W. 1979. Wheat Cultivar Identification by Gliadin Electrophoregrams. II. Effects of Environmental and Experimental Factors on the Gliadin Electrophoregram. Can. J. Pl. Sci. 59(2):281-286.

7.0 APPENDICES

Appendix 1. Sceptre/Biodur F_4 Frequency Histogram (Glenlea, 1993)

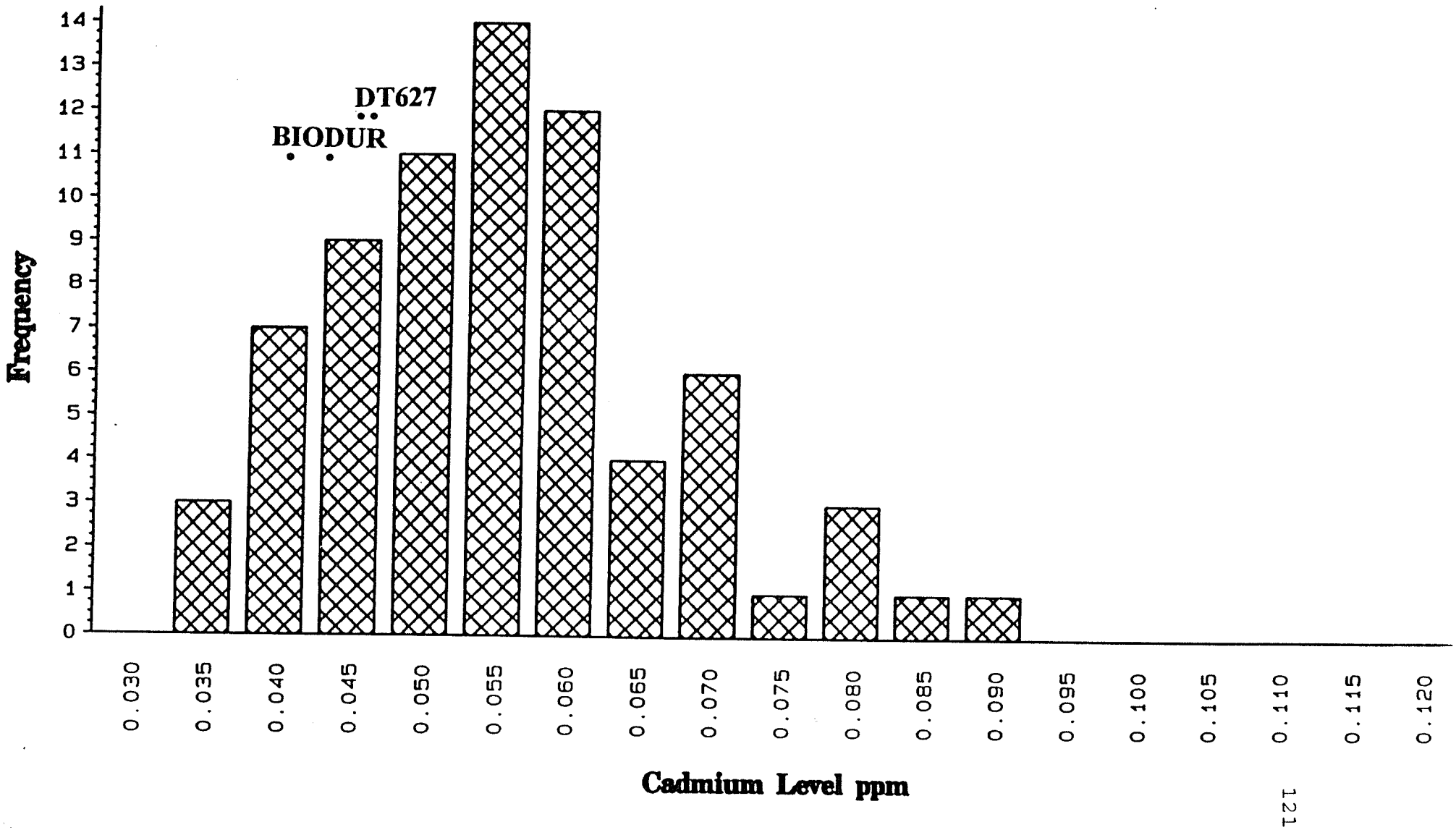

BIODUR
SCEPTRE
Frequency
0
1
2
3
4
5
6
7
8
9
10
0.020
0.025
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
0.105
0.110
0.115
0.120
0.125
0.130
0.135
0.140
0.145
0.150
0.155
0.160
0.165
0.170
0.175
0.180
0.185
0.190
0.195
Cadmium Level ppm

Appendix 2. Cd Concentrations of Check Plants of the Allelism Study and Neighbouring Sceptre/Biodur Check Concentrations

Cd Levels of Single Plants (ppm)[a]			Cd Levels of Bulked Rows (ppm)[a]		
DT627	0.046	0.047	Sceptre	0.074	
DT627	0.047		Sceptre	0.093	
Biodur	0.041		Sceptre	0.079	
Biodur	0.043		Sceptre	0.090	
DT471	0.063	0.065	Medora	0.112	
DT471	0.048	0.049	Medora	0.087	
DT471	0.061	0.052	Biodur	0.041	
DT471	0.097	0.087	Biodur	0.044	
DT471	0.063	0.060	Biodur	0.039	
DT471	0.056	0.054	DT471	0.043	0.048
F_1 DT471/Nile	0.070	0.060	F_1 Sceptre/ Biodur	0.042	
F_1 DT627/Biodur	0.062		F_1 Sceptre/ Biodur	0.052	
F_1 DT627/Biodur	0.068	0.063			

[a] Grain Research Laboratory arbitrarily tested some samples twice. Both Cd values are shown in those cases

Appendix 3. DT627/Biodur F_2 Frequency Histogram (Glenlea, 1992)


Frequency
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
DT627
BIODUR
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
0.105
0.110
0.115
0.120
Cadmium Level ppm

Appendix 4. Allelism Study Single Plant Values (Greenhouse)

Cross/Parent	Single Plant Cd Values				
Sceptre	0.096	0.127	0.158	0.194	-
Biodur	0.039	0.063	0.082	0.106	0.092
DT627	0.085	0.062	0.077	0.096	0.061
DT637	0.076	0.044	0.099	0.059	0.042
5.126	0.098	0.102	0.114	0.114	0.141
5.692	0.090	0.099	0.070	0.082	0.092
5.143	0.080	0.072	0.077	0.082	0.066
5.131	0.077	0.083	0.082	0.074	0.086
5.693	0.073	0.091	0.126	0.101	0.062
5.337	0.085	0.081	0.040	0.058	0.042
12.353	0.065	0.084	0.088	0.080	0.072
12.343	0.062	0.068	0.080	0.065	0.061
12.366	0.063	0.055	0.064	0.068	0.090
12.371	0.042	0.051	0.059	0.038	0.040
12.617	0.115	0.092	-	-	-
12.597	0.064	0.062	-	-	-

5._ identifies a DT627/Biodur F_2 individual
12._ identifies a Biodur/DT637 F_2 individual

Appendix 5. Grain Cd Concencentrations of Langdon 16 and Langdon *T. dicoccoides* (Glenlea 1993)

Substitution Line	Grain Cd Level (ppm)	
Ldn 16	133.8	141.9
LD1A	164.5	
LD2A	135.5	
LD3A	231.9	
LD4A	141.5	
LD5A	129.3	157.5
LD6A	209.3	
LD7A	175.4	
LD1B	206.9	
LD3B	192.0	
LD4B	198.3	
LD5B	59.1	53.1
LD6B	182.4	171.9
LD7B	152.2	
Medora	147.5	149.1
DT471	79.4	75.2

Field trials results from Hybrid Nursery (Dr. Leisle, personal communication).