The effects of soil moisture stress on water utilization, seed yield components, and grain and baking quality of selected spring wheat accessions
by James Reed Bunker

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
Available soil moisture can have major effects on wheat yield and quality. Screening for drought resistance is important in dryland wheat production. However, soil moisture determinations over time can produce massive quantities of data. Consequently, the ETPROBE software package was developed to calculate seasonal evapotranspiration (ET) from neutron probe moisture determinations. A line-source irrigation system was used to superimpose a moisture gradient across four spring wheat accessions ('Newana', 'Fortuna', MT 7819, and MT 8182) at Manhattan and Huntley, Montana, in 1986 and 1987, respectively. Seed yield, water use efficiency (WUE), kernel weight, plumpness, number, and protein content were determined at both sites. Harvest index was calculated for plots at Manhattan. Wheat samples from each plot were milled, baked, and evaluated for bread loaf volume and texture. Gliadin proteins were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC). Seasonal ET values ranged from 331 to 580 mm at Manhattan and 277 to 485 mm at Huntley. MT 8182 had the highest yield and seed WUE over all moisture regimes at Manhattan. Additionally, MT 8182 had the greatest kernel weight increase at Huntley and the greatest protein percentage decrease with increased ET at both sites. Fortuna had the lowest yield at all moisture regimes and the lowest WUE at the two highest regimes, and was the least responsive accession to increased ET for yield, WUE, kernel number, and protein content at the drier Huntley site. The relative area of a group of late-eluting gliadin peaks (quality gliadin fraction - QGF), expressed as percentage of total gliadin chromatogram area, was positively correlated with increased ET and negatively correlated with loaf volume for Newana and MT 8182 at both sites. Fortuna showed no correlation between increased ET and relative QGF area or between relative QGF area and loaf volume at either site. These results indicate that increased seasonal ET significantly affected protein quality in conjunction with changes in bread baking quality for some spring wheat accessions.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Available soil moisture can have major effects on wheat yield and quality. Screening for drought resistance is important in dryland wheat production. However, soil moisture determinations over time can produce massive quantities of data. Consequently, the ETPROBE software package was developed to calculate seasonal evapotranspiration (ET) from neutron probe moisture determinations. A line-source irrigation system was used to superimpose a moisture gradient across four spring wheat accessions ('Newana', 'Fortuna', MT 7819, and MT 8182) at Manhattan and Huntley, Montana, in 1986 and 1987, respectively. Seed yield, water use efficiency (WUE), kernel weight, plumpness, number, and protein content were determined at both sites. Harvest index was calculated for plots at Manhattan. Wheat samples from each plot were milled, baked, and evaluated for bread loaf volume and texture. Gliadin proteins were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC). Seasonal ET values ranged from 331 to 580 mm at Manhattan and 277 to 485 mm at Huntley. MT 8182 had the highest yield and seed WUE over all moisture regimes at Manhattan. Additionally, MT 8182 had the greatest kernel weight increase at Huntley and the greatest protein percentage decrease with increased ET at both sites. Fortuna had the lowest yield at all moisture regimes and the lowest WUE at the two highest regimes, and was the least responsive accession to increased ET for yield, WUE, kernel number, and protein content at the drier Huntley site. The relative area of a group of late-eluting gliadin peaks (quality gliadin fraction - QGF), expressed as percentage of total gliadin chromatogram area, was positively correlated with increased ET and negatively correlated with loaf volume for Newana and MT 8182 at both sites. Fortuna showed no correlation between increased ET and relative QGF area or between relative QGF area and loaf volume at either site. These results indicate that increased seasonal ET significantly affected protein quality in conjunction with changes in bread baking quality for some spring wheat accessions.

CHAPTER 1

INTRODUCTION

Moisture stress is an important factor limiting worldwide crop production. Moisture availability influences both choice of crop and management techniques. Selection of species and genotypes better adapted to soil moisture stress is a feasible alternative in areas with limited precipitation. Therefore, evaluation of crop responses to available moisture and screening for drought resistance are important.

The line-source sprinkler irrigation system is capable of generating a soil water gradient across genotypes planted in strips perpendicular to the irrigation line. This system, in conjunction with neutron probe soil moisture determinations, provides an excellent means of relating changes in individual crop parameters to soil moisture. However, soil moisture determinations made at frequent time intervals at several depth increments over several treatment combinations result in massive quantities of data. Manipulation and analyses of these data can be cumbersome without a microcomputer software program. Consequently, a microcomputer software package was developed to expedite evaluation of soil moisture data from the field. The ETPROBE program converts raw neutron count data into soil water content for integration with precipitation and irrigation data to calculate soil water depletion, plant available water (PAW), and/or evapotranspiration (ET).

Yield is an important parameter to be examined in field experiments, especially those dealing with crop production improvement such as drought resistance screening. Seasonal ET is used as an indicator of soil water availability. Seed water use efficiency (WUE), which indicates the amount of seed yield per unit of water used (ET), and harvest index (HI), which indicates the proportion of above-ground biomass channeled into seed yield, can be utilized to select plants with optimum yield potentials. These parameters are good indicators of the degree to which yield potential was realized under diverse growing conditions.

Quality is also an important factor in wheat production and utilization. Wheat quality is influenced by kernel weight, size, and protein content. Additionally, kernel number per unit area may affect wheat quality and yield by indirectly affecting kernel weight and size, which ultimately affect relative protein content.

Hard wheat quality is determined by the end-use product which primarily involves bread-making. Bread loaves made from wheat grown over different environments show differences in loaf volume and other baking quality characteristics. However, the effects of growing season soil moisture on specific protein components that influence bread baking quality are not well defined.

The quality and quantity of flour derived from wheat depends on kernel properties and proper grain processing. The protein component of flour, which is directly related to that of the whole grain, is highly important in determining bread quality. One particular characteristic indicative of bread quality is loaf volume, which depends on protein

quantity and quality. The gluten protein fractions, gliadins and glutenins, are closely associated with loaf volume and dough strength, respectively. Each of these fractions contains numerous individual proteins with relatively similar amino acid compositions and functionalities. It is possible that differential relationships among individual proteins comprising the gliadin fraction determine the specific characteristics. Electrophoretic and chromatographic techniques allow separation and comparative analyses of proteins. High-performance liquid chromatography (HPLC) is an analytical tool capable of separating and quantifying individual gliadin protein fractions. This method is extremely sensitive and has a high recovery rate.

The objectives of this study were to 1) develop a software program (ETPROBE) for managing and processing neutron probe soil moisture determination data, 2) evaluate the utility of the line-source irrigation system for multiple screening processes, 3) determine yield, harvest index, and water use relationships of four morphologically diverse hard spring wheat accessions under increasing soil moisture regimes, 4) evaluate the effects of differential seasonal ET on kernel quality characteristics, 5) determine relationships among gliadin protein components influenced by differential field soil moisture, and 6) examine associations between gliadin protein components and bread baking quality.

CHAPTER 2

LITERATURE REVIEW

Crop

Wheat (*Triticum* spp.) is an extremely important food crop throughout the world. Reitz (1967) reported wheat to be the national food staple in 43 countries. Evans et al. (1975) indicated that wheat was cultivated approximately 10,000 years ago in the area of the Fertile Crescent. According to Martin et al. (1976), emmer (an ancestor of common wheat) was cultivated before 7000 B.C. Poehlman (1979) reported that wheat was cultivated in Greece, Persia, Egypt, Europe, and Southeast Asia in prehistoric times, and was brought to the United States by the early colonists.

Wheat is comprised of the genus *Triticum* of the tribe Triticeae in the Poaceae family. The genus was named by Linneaus in 1753. *Triticum* species have been divided into three groups based on chromosome number: diploids (n=7), tetraploids (n=14), and hexaploids (n=21) (Briggle, 1980). Most of the commercially grown wheat is the hexaploid *Triticum aestivum* L. em Thell. (bread or common wheat). Other major species include *T. durum* Desf. (durum wheat, a tetraploid) and *T. compactum* Host (club wheat, a hexaploid; considered by some to be a type of *T. aestivum*).

Line-source Irrigation System

Lack of sufficient soil moisture to ensure optimum crop growth and yield is a worldwide problem. The effects of plant available water (PAW) on specific growth and yield functions have been reported for several crops (Bauder et al., 1978; Black, 1966; Hanks, 1974; Heady and Pesek, 1954; O'Neill et al., 1983; Shimshi et al., 1982; Singh, 1981; Westesen et al., 1987).

The line-source irrigation system described by Hanks et al. (1976) is a field technique capable of providing a uniform water gradient across plots planted perpendicular to the water source. It allows imposition of a water gradient within a relatively small plot area at a single field site, reducing environmental and soil parameter variations inherent with multiple-site studies. Use of collection devices permits monitoring of system uniformity and quantification of water applied in the different regimes within the gradient. O'Neill et al. (1983) stated that the line-source system could be used to screen large numbers of germplasm for drought resistance.

An inherent limitation involves the validity of certain statistical tests with the line-source design. A valid F-test cannot be made for the main effects of irrigation level using analysis of variance (ANOVA), since the irrigation levels are fixed. However, F-tests from ANOVA are valid for randomized treatments and interactions. Hanks et al. (1980) stated that irrigation main effects are generally large enough to be obvious and that statistical analysis of these is not critical. Johnson et al. (1983) described a procedure using multivariate methods that

allows statistical analysis of irrigation main effects when this parameter is critical.

Physiological Effects of Drought Stress

Drought stress affects wheat growth and development by influencing several physiological processes. The plant compensates for decreased plant available water in the soil by creating a lower (more negative) plant water potential. The resulting internal water deficit modifies turgor pressure in the cells. Modification of turgor affects such processes as cell elongation and cell division, which are directly related to leaf expansion and photosynthetic potential (Clarke et al., 1981; Eastham et al., 1984; Turner and Burch, 1983).

Drought stress increases hydrolysis of proteins and results in increased levels of the free amino acids glutamate and proline. Loss of protein may either result from a decrease in RNA synthesis (Shah and Loomis, 1965) or from an increase in the RNA degradation rate (Barnett and Naylor, 1966; Gates and Bonner, 1959; Kramer, 1969; Slayter, 1969).

Plants subjected to drought stress not only show a general reduction in size, but also exhibit leaf structural modifications. Leaf area decreases due to reductions in cell enlargement. Drought-stressed leaves may have increased pubescence, cutinization, and thickness (Kramer, 1969).

Root development is important in processes associated with drought resistance since it is closely related to soil water absorption. In general, plant water stress decreases as the extent of the root system increases (Townley-Smith and Hurd, 1979). However, this does not mean

that yield will be increased with increased root area. Salim et al. (1965) indicated that the extent of cereal crop root growth was highly correlated with soil moisture level. They indicated that root penetration was dependent on the soil depth where water content was above the permanent wilting point (PWP).

Clarke et al. (1981) indicated that plants showed less stress effect on yield when initial soil water was sufficient for plant establishment, since the root system developed prior to anthesis. Passioura (1972) advocated breeding for plants with higher root resistances to limit early season water depletion. However, he also stated that this would not be desirable for wheat grown on low water-holding capacity soils.

Water Stress Measurements

Water stress indicators include leaf water potential, changes in soil water content, and evapotranspiration rate. Plant water potential indicates the ability of a plant to regulate internal water deficits and is dependent on soil water potential. As soil water becomes limiting, the plant has to develop a more negative water potential to maintain water influx. Therefore, it is possible to indirectly evaluate plant water status by measuring soil water status. Gravimetric analysis of soil water content is the standard calibration method. The percentage of water is calculated as the ratio of weight difference between wet and oven-dried soil to the weight of dried soil (Thien, 1983). This ratio is multiplied by soil bulk density to give a gravimetric determination

on a volume basis. Each sampling over time differs spatially over the soil being used in the determination since this method is destructive.

Neutron probe determinations of soil water content have the advantage of allowing measurement of the same sample unit of soil over time. Additionally, this technique allows rapid multiple moisture determinations at different soil profile levels. The neutron emitter-counter is lowered to the desired reading depth through an aluminum or polyvinyl chloride (PVC) access tube placed into the soil. Fast neutrons emitted into the soil collide with soil water hydrogen nuclei and are deflected at a slower rate. A portion of these slow neutrons reach the counter and are registered (Thien, 1983). Neutron counts are translated into moisture units with a calibration equation. Neutron probe determinations were used by Jaradat and Konzak (1983) to study soil water depletion patterns as a potential screening method for wheat drought resistance. They showed a 92% correlation between neutron probe and gravimetric determinations.

Evapotranspiration (ET) accounts for changes in soil water content due to both soil evaporation and plant transpiration. The evaporation component is largely dependent on plant cover and decreases with increased plant canopy growth. Transpiration is dependent on physiological processes involving stomatal aperture, water uptake, and atmospheric demand for plant water. Evapotranspiration can be measured using a variety of lysimeters, atmometers, and pan evaporimeters. Blad (1983) gave an excellent discussion of these measuring techniques.

Various models have been proposed to estimate ET since direct measurement is not always practical. The original Penman (1948) model

was used to estimate evaporation from an open water surface and utilized vapor pressure, air temperature, wind speed, and net radiation. Actual ET over a given time period can also be estimated using the water balance equation cited by Rose (1966):

$$ET = CSM_i + P + I - CSM_t - RO - D$$

where CSM_i is cumulative soil moisture at the beginning of the estimation period, P is precipitation amount, I is the amount of water added by irrigation, CSM_t is cumulative soil moisture at the end of the estimation period, RO is the amount of water lost as runoff, and D is the water lost to drainage. The effects of the last two variables are often considered negligible (Diaz et al., 1983; Garrity et al., 1982).

Yield and Yield Components

Seed yield of wheat and other small grains is a composite of three primary components: spike number per unit area; kernel number per spike; and kernel weight (Singh, 1981). Spike number per unit area is a function of plant number per unit area and spike number per plant (Sebillote, 1980). Spike number per plant is dependent on tiller number, which is inversely related to plant density and buffers seed yield against both overseeding and underseeding (Dewey and Albrechtsen, 1985). Tiller number is determined early in the vegetative phase of growth, and is quite sensitive to drought stress (Kirkham and Kanemasu, 1983).

Kernel number per spike is a function of spikelets per spike and kernels per spikelet. Nicholis and May (1963) suggested that spikelet number per spike is determined by the balance between rate of primordial

initiation and rate of spikelet development. Wardlaw (1971) stated that drought stress may reduce kernel number per spike by decreasing the fertilization rate.

Kernel weight is determined during the grain filling stage after kernel number per unit area has been established (Day, 1981; Kirkham and Kanemasu, 1983). Drought stress during the post-anthesis period can reduce kernel weight by limiting photosynthate availability, reducing translocation, and shortening the grain-filling duration (Clarke et al., 1981; Wardlaw, 1967). Lower kernel weight markedly decreases yield since kernel number per unit area can no longer compensate for yield reduction during the post-anthesis period (Aspinall, 1965). Kernel plumpness is strongly associated with kernel weight.

Kirkham and Kanemasu (1983) indicated that kernel weight and kernel number per spike have the greatest effect on grain yield under drought stress. Conversely, spike number per unit area has the greatest effect on yield when water is adequate. Singh (1981) reported kernel number per unit area to be the most important yield-limiting factor. Shanahan et al. (1985) reported a highly significant positive linear correlation between kernel number per unit area and total yield of winter wheat. Keim and Kronstad (1981) found that final number of spikes per unit area determined winter wheat yield under drought stress, with increased spike number under conditions of low plant water.

Water use efficiency (WUE) and harvest index (HI) are important factors directly related to yield potential. WUE describes the efficiency with which a plant produces economic yield per unit of used water (Turner and Burch, 1983). A high WUE value in wheat and other

cereals indicates a greater efficiency at producing biomass with a given amount of water. Relations between dry matter production and water use are theoretically based on plant transpiration. However, transpiration is difficult to separate from soil water evaporation in the field, so ET is commonly used to calculate crop WUE (Garrity et al., 1982). Harvest index (HI) refers to the proportion of above-ground biomass that has been channeled into grain production.

Wheat Quality

Protein is one of the most important flour components affecting baking quality. Protein content, expressed as percentage by weight, has been shown to be inversely related to grain yield (Loffler et al., 1985; McNeal et al., 1968; McNeal et al., 1972; Terman et al., 1969). However, Loffler et al. (1985) reported that 'Len' wheat exhibited an exception to this relationship.

The inverse relationship between grain yield and protein content has been attributed to dilution of protein nitrogen with a high ratio of carbohydrates in the kernels. McNeal et al. (1972) reported that eight spring wheat crosses with genetically-controlled high or low grain protein contents absorbed similar amounts of nitrogen from the soil and translocated equal amounts of nitrogen to the grain. They concluded that grain protein percentages were entirely dependent on the amount of carbohydrate translocated to the grain, which was influenced by the number of carbohydrate sinks (kernels).

Terman et al. (1969) suggested that hard wheat protein was affected by soil moisture. Smika and Greb (1973) reported high correlation

between grain protein content and available soil moisture at seeding. Fernandez and Laird (1959) reported that grain protein percentage increased as available soil moisture at irrigation decreased. This agrees with the findings of Neidig and Snyder (1924).

The specific effects of soil moisture stress on bread-making characteristics are not fully understood. Flour protein content is highly dependent upon kernel protein content. Finney and Barmore (1948) and Fifield et al. (1950) reported a strong positive linear relationship between flour protein percentage within the range of 8 to 18% and bread loaf volume for a given wheat variety. This relationship differed among varieties, but did not differ greatly among growing sites within a variety.

Flour bread-making characteristics are determined not only by total flour protein percentage, but also by relationships between various protein components. The gluten proteins are particularly important for their roles in bread-making. Historically, gluten was first considered a single protein extractable in alcohol. However, Taddei (1819, cited by Kasarda et al., 1976) reported separation of two gluten components based on differential solubilities in alcohol. Kjeldahl (1896, cited by Osborne, 1907) considered the alcohol-soluble portion of gluten to be a single protein substance, based on an almost constant carbon:nitrogen content of this fraction from several different wheat flours.

Osborne (1907) redefined the four major protein fractions of wheat flour, according to differential solvent solubilities: albumin (soluble in water); globulin (soluble in saline solution such as 10% NaCl); gliadin (soluble in 70 - 90% aqueous alcohols); and glutenin (soluble in

weak acids, bases, and denaturing agents). Although these four fractions are not always distinctive, this system is still convenient to use in separation schemes.

Albumins and globulins are concentrated primarily in the embryo, whereas gliadins and glutenins are found only in the endosperm. The endosperm storage proteins (gliadins and glutenins) constitute gluten and are the primary protein factors that affect bread and dough characteristics. The 'soluble' proteins (albumins and globulins) found in the embryo play a greater role in the nutritive value of wheat, having four to five times as much lysine (by weight) than the gliadins (Kasarda et al., 1976).

Gliadin and glutenin fractions each consist of several proteins of various molecular weights (Woychik et al., 1961). However, each protein in these fractions has a similar bread-making functionality. The glutenin fraction has been identified with gluten and dough strength and extensibility, while the gliadin fraction has been reported to control loaf volume potential (Finney et al., 1982; Hamada, 1982). Finney et al. (1982) indicated that interactions between these two fractions affect the final baking product quality.

Gliadins are primarily single-chain proteins ranging in size from 11.4 to 100 kDal (Beckwith et al., 1965). The majority of the gliadin components appear to have a molecular weight near 36 kDal when measured using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after disulfide bond reduction (Bietz and Wall, 1972).

Glutenins are characterized as multichain protein complexes linked by disulfide bonds (Beckwith and Wall, 1966). They have molecular

weights averaging between 121 and 300 kDal, depending on the extraction procedure (Nielsen et al., 1962; Wu et al., 1967). Bietz and Wall (1972) detected 15 different subunits with molecular weights of 11.6, 18, 27.5, 32.6, 36, 42.2, 44.6, 49.4, 63.4, 71, 79.1, 87.2, 102, 124, and 133 kDal after disulfide bond reduction. Some of these reduced glutenin subunits with molecular weights of 44 kDal are ethanol-soluble, similar to the gliadins (Bietz and Wall, 1975).

Dough properties result from the contributions of various components (starch, protein, lipids, etc.), but it is generally agreed that gluten proteins are of fundamental importance for cohesiveness and elasticity (Kasarda et al., 1971). The viscoelasticity of dough is thought to be due to a complex network of gluten protein molecules. The rheological properties of this network are greatly dependent on the number and strength of molecular cross-links (Bloksma, 1971). Gliadin is cohesive but only slightly elastic, whereas glutenin is both cohesive and elastic.

Hydrogen-bonding is largely responsible for the cohesiveness of these proteins. The hydrogen-bonding potential is contributed by the multiple amide side chains of the gluten proteins (Beckwith et al., 1963; Holme and Briggs, 1959). The amide groups from the glutamines, which comprise approximately 30% of the amino acid residues, may act as both donors and acceptors in hydrogen-bonding. Intermolecular is favored over intramolecular hydrogen-bonding because the high proline content of these proteins hinders helix formation. Reagents capable of readily dissolving gluten act by saturating the hydrogen bond-forming capacity of single chains to prevent formation of cohesive bonds

(Bloksma, 1971). Water molecules perform an important function in hydrogen-bonding by satisfying some of the bonding sites and facilitating the interchange of these bonds among the molecular species (Kasarda et al., 1971). An excess of water will over-saturate the bonding sites and produce a weak dough. Substitution of deuterium oxide (D_2O) for water tends to strengthen the dough, since D_2O forms stronger hydrogen bonds than ordinary water. The fact that this substitution changes the farinograms and extensigrams demonstrates the importance of hydrogen-bonding on the rheological properties of dough (Tkachuk and Hlynka, 1968).

Hydrophobic and other apolar types of bonding probably contribute to the cohesiveness of gluten proteins because of the relative abundance of non-polar side chains. Bloksma (1971) indicated that gluten proteins contain about 30% non-polar amino acid residues. Therefore, numerous weak cross-links caused by Van der Waals forces are expected. However, there is little direct evidence that such bonding affects cohesion (Kasarda et al., 1971). Areas of non-polar character are most likely involved in lipid binding. The non-polar portion of phospholipid molecules may interact with such regions, which would leave their polar groups free on the surface. This would affect the ionic character of the protein molecule surface and increase ionic bonding potential. Pomeranz (1971) emphasized that hydrophobic bonds are not chemical bonds, but are a thermodynamic phenomenon that may play an important role in the early stages of baking. This is based on the fact that all chemical bonds are weakened with increased temperature, but hydrophobic

bond formation is an endothermic process favored by increased temperature up to approximately 60°C.

Ionic bonds may be involved in some protein interactions. However, this contribution appears to be of minor importance since the gluten proteins have few ionizable side chains. Approximately 7.3% of all amino acid residues in gliadin and 9.3% of those in glutenin are charged under normal conditions (Pomeranz, 1971). As pH drops from neutrality, net charge on these particles becomes more positive, and doughs become less extensible (Bennett and Ewart, 1962). This may result from the destruction of salt linkages by increased hydrogen-bonding, an increase in electrostatic repulsive forces, or changes in protein conformation at lowered pH (Kasarda et al., 1971).

The importance of disulfide bonds in the cohesiveness of gluten is indicated by the fact that solubility is enhanced by mercaptans and sulfite (Wren and Nutt, 1967). Chemical reagents that reduce disulfide bonds have long been known to cause rapid breakdown of doughs. Sulfhydryl-disulfide interchange during dough mixing is a logical explanation for the formation of a highly cross-linked protein network in the dough. The sulfhydryl (-SH) groups may come from low molecular weight thiols naturally present in doughs or from cysteine side chains (McDermott et al., 1969). Disulfide formation from the latter involves:

$$2\ Pr\text{-}CH_2\text{-}SH \rightleftharpoons Pr\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}Pr + 2H$$

cysteine cystine

where Pr represents a protein chain molecule. The double arrow indicates a possible reduction of the disulfide bond to form thiol (sulfhydryl) groups; however, this is not a readily-established

equilibrium (Bloksma, 1971). Approximately 1.4% of the amino acid residues in gluten are either cysteine or cystine (Wall, 1964).

Much work on gliadins has been done using gel-electrophoresis. Electrophoregrams show distinct differences between varieties and species in number and mobility of gliadin components (Bushuk and Zillman, 1978; Jones et al., 1982). Woychik et al. (1961) classified four somewhat distinct fractions within the gliadins, according to relative mobility in starch gel electrophoresis using aluminum lactate buffers. These are referred to, in order of decreasing mobility, as alpha-, beta-, gamma-, and omega-gliadins. These mobility groups are not always visibly distinct (depending on genotype). However, for lack of a better system of nomenclature, this terminology has been widely used. Polyacrylamide gel electrophoresis (PAGE) has become widely used to evaluate banding patterns of gliadins (Bushuk and Zillman, 1978; Lookhart et al., 1982).

Compositionally, omega-gliadins differ more from the alpha-, beta-, and gamma-gliadins than the latter three do among themselves. Booth and Ewart (1969) referred to the omega-gliadins as "athins" because of their relative lack of the sulfur-containing amino acids cysteine and methionine. This fraction also contains relatively high levels of phenylalanine (c. 10% of the residues) not found in the other gliadin fractions (Kasarda et al., 1971).

Bietz and Rothfus (1970) showed evidence of some common peptide sequences among the alpha-, beta-, and gamma-gliadins, based on common peptides resulting from enzymatic degradation. For example, an aggregable alpha-gliadin has three to four more basic amino acids (a

lysine, one to two histidines, and an arginine) than one of the gamma-gliadins.

Aggregable alpha-gliadin (A-gliadin) is one of the most highly characterized gliadin fractions. This protein fraction was first isolated and characterized by Bernardin et al. (1967). The microfibrils formed upon aggregation at a pH near neutral dissociate into monomeric subunits in 0.001 *M* HCl. Kasarda et al. (1976) indicated that interactions involved in this completely reversible aggregation were not covalent, but involved hydrogen, ionic, and hydrophobic bonding. Kasarda et al. (1968) estimated approximately one-third of the polypeptide chain length of the monomeric subunits to be in the alpha-helical configuration, about 10% to be in the form of beta-turns, and the remainder to be random structure. Tatham et al. (1985) indicated that the beta-turns are concentrated in specific proline-rich domains.

Electrophoresis separates proteins on the basis of both size and net charge. However, in the presence of SDS, this separation is based only on molecular size, whereas isoelectric focusing (IEF) utilizes differences in ionized amino acids. These techniques are useful in characterizing cereal proteins, but of limited value for preparative isolation (Bietz, 1985).

High-performance liquid chromatography (HPLC) offers the advantages of speed and increased resolution over other chromatographic methods (Burnouf and Bietz, 1987). Types of HPLC include ion-exchange, size-exclusion, and reversed-phase HPLC. Of these, reversed-phase HPLC (RP-HPLC) is most widely used with the cereal proteins. It is possible to

separate and analyze proteins from small quantities of flour (50 mg or less) or from one-half of a seed with this technique (Huebner and Bietz, 1987).

Originally, protein separations were not possible using HPLC because the pore sizes of the columns ranged from 80 to 100 angstroms (Å) and did not allow protein penetration (Pearson et al., 1981). More recently, "wide-pore" or "large-pore" silica-based packings are available with pore sizes of 300 Å or larger that permit passage of large molecules.

RP-HPLC column packings have bonded phases, such as C_{18} or C_8, which interact hydrophobically with amino acids on the exposed protein surface. The proteins bind hydrophobically to the bonded phase of the column under conditions of relatively high polarity, and are selectively eluted by a solvent gradient of increased hydrophobicity (Bietz, 1984a).

A typical HPLC system for protein separation consists of: 1) a gradient former to control relative concentrations of the solvents comprising the mobile phase; 2) a pump to maintain a steady flow rate of the mobile phase; 3) an injector to introduce the sample into the system; 4) a column specific to the application; 5) a detector to quantify the amount of protein eluting from the column at a given time; and 6) an integrator or other device to process and store data from the detector. A column heater is recommended with gliadins to increase resolution by maintaining the column at 70°C (Bietz, 1984b). Proteins may be detected by absorbance at 280 nm (or 254 nm), which monitors primarily tryptophan, phenylalanine, and tyrosine, or at 210 nm, which monitors carbonyl groups usually associated with peptide bonds (Hancock

and Harding, 1984). Bietz et al. (1984a) recommended monitoring at 210 nm, since tryptophan and tyrosine may not be equally distributed among all proteins and since the sensitivity is increased a hundred-fold at this wavelength.

Gliadins in ground wheat grain or flour samples can be extracted with 70% (v/v) ethanol at room temperature (25 mg flour/ml ethanol). Bietz et al. (1984a) indicated that 97% of the gliadins were extracted within 30 minutes, with 90% being extracted within the first 5 minutes. The sample is then centrifuged for 10 to 15 minutes at 15,000 to 30,000 x *g* (Bietz et al., 1984b; Huebner and Bietz, 1987), and the pellet is discarded.

Samples of the supernatant may be injected directly or be further filtered through 0.45 um filters before injection. Sample injection volumes usually range from 10 to 50 ul for analytical columns. Samples may be injected immediately upon preparation or stored at room temperature for at least 28 days without any appreciable change (Bietz et al., 1984a).

The gliadin proteins generally elute from the column in the range of 25 to 50% acetonitrile (ACN). Reproducibility is high with the use of straight solvents [solvent A = 99.9% water + 0.1% trifluoroacetic acid (TFA), solvent B = 99.9% ACN + 0.1% TFA] in the reservoirs (Huebner and Bietz, 1987; Marchylo et al., 1988). Huebner and Bietz (1987) suggested the use of 0.05% TFA in each solvent to reduce the possibility of protein deamidation and give a slightly more level baseline. Solvents are generally "degassed" (deaerated) before use to prevent bubble formation in the system.

Linear gradients of 25 to 50% ACN over 50 to 55 minutes are generally used for gliadins. These combinations give gradient slopes of approximately 0.5% solvent B minute^{-1}. The solvent mixture is pumped through the column at a rate of approximately 1 ml minute^{-1}. Final gradient conditions are often held isocratically for 5 to 10 minutes to allow elution of the most hydrophobic components before being returned to initial gradient conditions. The system is re-equilibrated at initial conditions for at least 10 minutes prior to subsequent injections (Bietz et al., 1984a; Burnouf and Bietz, 1987). The column eluent is monitored for absorption with a selected wavelength (usually 210 nm) at 0.1 to 4.0 amplitude units full scale (AUFS) and recorded on a data recorder or integrator at 10 mV full scale deflection.

RP-HPLC provides a technique of studying gliadins and other cereal proteins which compliments other separation techniques such as electrophoresis, since the techniques use different separation schemes. It is possible to isolate individual components with a preparative column in quantities that permit further characterization such as molecular weight determinations with SDS-PAGE or amino acid analysis (Huebner and Bietz, 1984).

A major reason for studying cereal proteins is to understand their relationship to wheat and flour quality. Damidaux et al. (1978) showed that durum quality could be predicted by the presence or absence of two major mutually-exclusive bands detected with gliadin PAGE. Burnouf and Bietz (1984) identified peaks corresponding to these durum protein bands using HPLC. Bread-making quality has been successfully predicted from protein component molecular weight. Huebner and Bietz (1985) indicated

that the ratio of high-to-low molecular weight gluten subunits was predictive of bread quality. More recently, Huebner and Bietz (1986) showed a good negative correlation between bread baking scores and the relative amount of a specific gliadin fraction present in a given spring wheat variety. The late-eluting protein peaks, which they termed "baking quality gliadin fraction" (BQGF), elute at similar times to the durum wheat gliadins associated with pasta quality. However, they also reported that these components in the bread wheats do not appear as distinct bands using aluminum lactate gel electrophoresis, either because they do not form distinct bands or because they do not stain well.

Results from Bietz and Burnouf (1985) indicated that gluten proteins elute as groups in order of increasing hydrophobicity approximately as follows: 1) albumins and globulins; 2) omega-gliadins; 3) high molecular weight glutenin subunits; 4) alpha- and beta-gliadins; 5) low molecular weight glutenin subunits; and 6) gamma-gliadins. The omega- and gamma-gliadins and low molecular weight glutenin subunits are coded by genes at the complex *Gli*-1 loci. The same general order of elution was indicated by Popineau and Pineau (1987) using purified gliadin fractions.

Environmental Effects on Gliadins

Gel electrophoresis of gliadins has been advocated for varietal identification. Banding patterns of these proteins on gels (in the presence of SDS) appear to be unique for each wheat variety. Lee and Ronalds (1967) concluded that environment may have only a minor effect

on gliadin bands, and that this aspect is predominately under genetic control.

Use of gliadins for varietal identification is based on the fact that no qualitative differences appear between electrophoregrams of a wheat genotype grown under different environmental conditions (Lee and Ronalds, 1967; Zillman and Bushuk, 1979). However, research on quantitative differences among gliadin proteins due to environmental variability is lacking.

CHAPTER 3

ETPROBE: COMPUTERIZED SYSTEM FOR PROCESSING OF NEUTRON PROBE SOIL MOISTURE DATA

The neutron probe provides a rapid, effective method for determining soil moisture (MacKerron and Jefferies, 1987). A major constraint associated with the use of a neutron probe is the processing of massive amounts of data. A "user-friendly" microcomputer software program can provide a practical means of organizing and analyzing large data files (Hulsman, 1985). The ideal software package should allow both summarization and interpretation of data.

The objective of the preliminary research effort was to develop a software program to facilitate the management of field data involving soil moisture stress. ETPROBE is a menu-driven software program with routines for: 1) creating and appending to input data files; 2) performing user-selected computations; 3) generating reports; and 4) copying selected data fields to files for integration with other software. The program's "user-friendly" attributes include good internal documentation, extensive error trapping, and a non-technical user's manual. The program was designed for use by non-programmers, and has both commercial and research applications.

The neutron probe has been used for determination of soil moisture depletion and infiltration patterns of both cropped and non-cropped areas, plant growth modelling, irrigation studies, and evaluation of

plant water competition (Anonymous, 1970). ETPROBE is highly applicable in these types of studies, especially for comparative analyses of irrigated and non-irrigated treatments. The program is especially valuable in studies involving large numbers of depth increments or in large-scale field evaluations of germplasm drought tolerance or avoidance. Commercial applications include use in irrigation scheduling and monitoring, and in yield predictions based on plant growth models involving soil moisture. Program data interpretation may include translation of raw probe readings into estimates of evapotranspiration (ET) and maximum depth of moisture depletion. Depth of moisture depletion can be extrapolated as crop rooting depth if drainage and runoff are negligible.

Specifications

ETPROBE was developed on an IBM PC XT[1] with 640K RAM, a 10 mByte hard disk, and the MS-DOS 3.1 operating system. It was written for dBASE III+ version 1.0 and is compatible with dBASE III version 1.1. A minimum of 352K RAM is required for operation, inclusive of 256K for dBASE III+ and 96K for the VDISK (RAM-resident disk) used to run program files.

The program is resident on one double-sided, double density (DSDD) 5-1/4" floppy diskette. A hard disk drive is desirable with large data files. Reserve disk space should be available, since the software generates several files per run. For example, a file containing 2240

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[1] Mention of a specific brand, trade, or chemical name does not imply endorsement of that product over others of a similar nature or function.

records (c. 44K) requires total disk storage space of approximately 440K when all options are utilized. Results from small data files (less than 1000 datapoints) will fit on a floppy diskette. A printer is recommended for report generation.

Operation

Inputs required to run the program are:

1) slope and intercept of the calibration equation for the neutron probe;
2) precipitation amount (expressed as equivalent depth) between successive probe reading dates (mm);
3) amount of irrigation applied per irrigation treatment level between successive probe reading dates (mm), if irrigation is a factor;
4) average daily standard count for the neutron probe;
5) increment between each reading depth (cm);
6) soil permanent wilting point (PWP, % by volume) for each depth increment (if the plant available water option is selected);
7) a date code indicating status of each probe reading date with respect to irrigations (1 = within 24 hours following an irrigation, 0 = all other time periods); and
8) a properly configured data file containing raw neutron counts. A menu option is provided with the software to generate the correctly-configured data file.

Program outputs are:

1) neutron probe count ratio (probe reading/standard count), which is the x value in the probe calibration equation;
2) percent soil moisture by volume, which is the y value in the probe calibration equation;
3) soil water amount (cm) which equals percent soil moisture times the soil depth increment;
4) cumulative soil water amount over all depth increments calculated per access tube at each reading date;
5) soil water depleted between successive probe reading dates at each depth increment (cm);
6) soil water depleted over all depth increments for each time period (cm);
7) plant available water for each reading depth level at each probe reading date (cm), calculated as the difference between current soil water depth equivalent and permanent wilting point expressed as a soil water depth;
8) mean plant available water for each treatment combination over all replications at each depth level for each time period (cm);
9) ET (mm) for each plot or subplot between reading dates, calculated using the water balance equation, as cited by Tanner (1967), assuming drainage and runoff to be negligible (Separate values are not calculated for reading dates immediately following an irrigation.);

10) cumulative ET (mm) over time; and

11) total seasonal ET (mm).

The user has the option to choose calculation sets, depending on desired outputs. The 'soil water' option generates outputs 1-4; 'depletion' generates outputs 5-6; 'plant available water' generates outputs 7-8; and 'evapotranspiration' generates outputs 9-11. Options may be chosen in any combination per run, providing the 'soil water' option is first. The program also permits option selection at different times on the same data set.

ETPROBE creates and utilizes several data and calculation files per run. These files contain calculations, user-supplied data, and index values for the applications contained in the program. The following filename conventions are used:

'd:' designates the user-specified disk drive where files are stored. All files created within a run are allocated to the same drive designation.

'#' refers to an upper-case letter corresponding to the user-specified batch number (1 = "A", 2 = "B", etc.). All files created by the same run utilize the same letter.

'.ext' refers to the filename extension allowed by MS-DOS, which should be unique for each application or user.

The program initially creates a file called d:ETDATAF#.ext to contain raw and calculated data. Consequently, all data pertinent to a run, with exception of calculated ET values, are found in the d:ETDATAF#.ext file corresponding to the run. ET calculations are located in d:ETFILER#.ext. Different batch and extension designations

are necessary for each run or files from previous runs with the same designations will be overwritten. Files required for program operation are:

d:ETPROBE.PRG - main menu program;

d:ETPROGRM.PRG - main subprogram file;

d:REPORTR2.PRG - subprogram to generate reports;

d:REPTFMT1.FMT - format file for first report form;

d:REPTFMT2.FMT - format file for second report form;

d:CREATOR2.PRG - subprogram to set up and enter original data;

d:NEWMODEL.DBF - structure for file d:ETDATAF#.ext; and

d:DATACOPY.PRG - subprogram to export data into ASCII files.

The subprogram d:CREATOR2.PRG allows the user to input the name of the file to be created or appended to, following standard format of MS-DOS filenames, then calls for data input. A correction option is provided. Data entry can be interrupted at any time and resumed later.

A variety of reports can be generated from the main menu. A summary report may be generated, which is automatically sent to the printer. This report contains the data entered during the run (irrigation amounts applied, number of reading depths, etc.). Report options are as follows:

Report 1 - lists index variables, neutron probe ratios, percent soil moistures (by volume), soil water amounts (cm) per depth increment, and cumulative soil water amounts (cm) over all depth levels in the profile.

Report 2 - lists index variables, cumulative soil water amounts (cm), soil water depletion (cm) between readings at each depth level for each access tube, and cumulative soil water depletion for the entire profile over time.

Report 3 - lists index variables, amounts of irrigation and precipitation (mm), plant available water per sampling depth by replication, and mean plant available water per depth level over all replications.

Report 4 - lists index variables by plot or subplot, ET values (mm) per plot or subplot between reading dates, cumulative ET (mm) up to each reading date, and total seasonal ET (mm).

An option also exists to selectively export data fields to a delimited ASCII file for further integration with other programs such as statistical or graphics packages.

Applications

Examples of ETPROBE applications are cumulative ET (Fig. 1), maximum rooting depth based on soil moisture depletion (Fig. 2), and soil profile water content over the growing season (Fig. 3). These figures were based on neutron probe data gathered as part of a line-source sprinkler irrigation study on spring wheat in 1986 at Manhattan, Montana. Data shown are for Newana spring wheat under four irrigation regimes ranging from dryland (rain-fed only, 284 mm) to high irrigation (513 mm total water applied). Graphs were plotted using the Plot-It[1] software package.

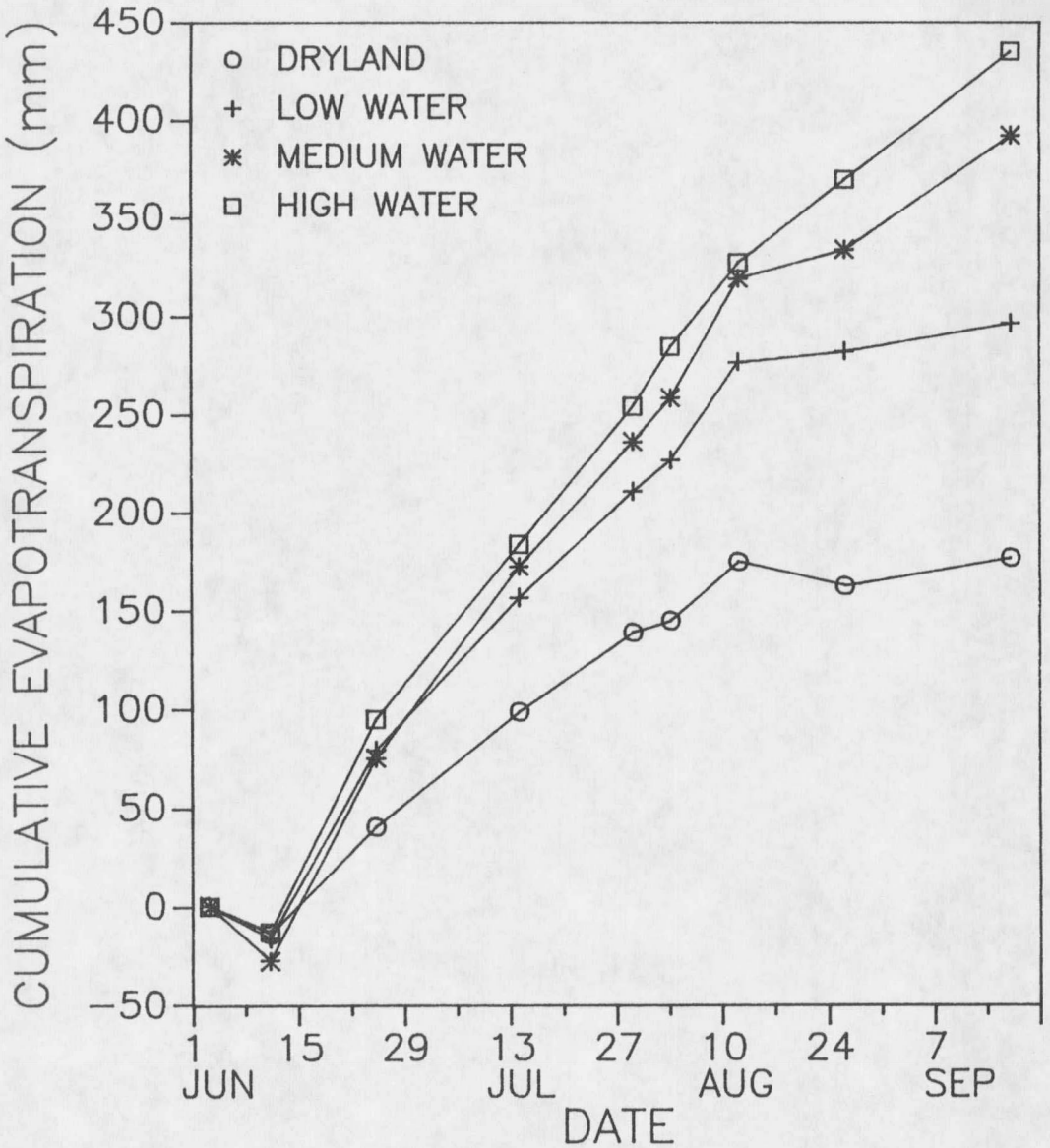

Figure 1. Cumulative evapotranspiration (mm) for Newana spring wheat grown under a line-source irrigation system at Manhattan, Montana, in 1986. Dryland, low, medium, and high irrigation treatments had total seasonal water applications of 284, 401, 477, and 513 mm, respectively. Negative values on 10 June resulted from heavy rainfall after planting.

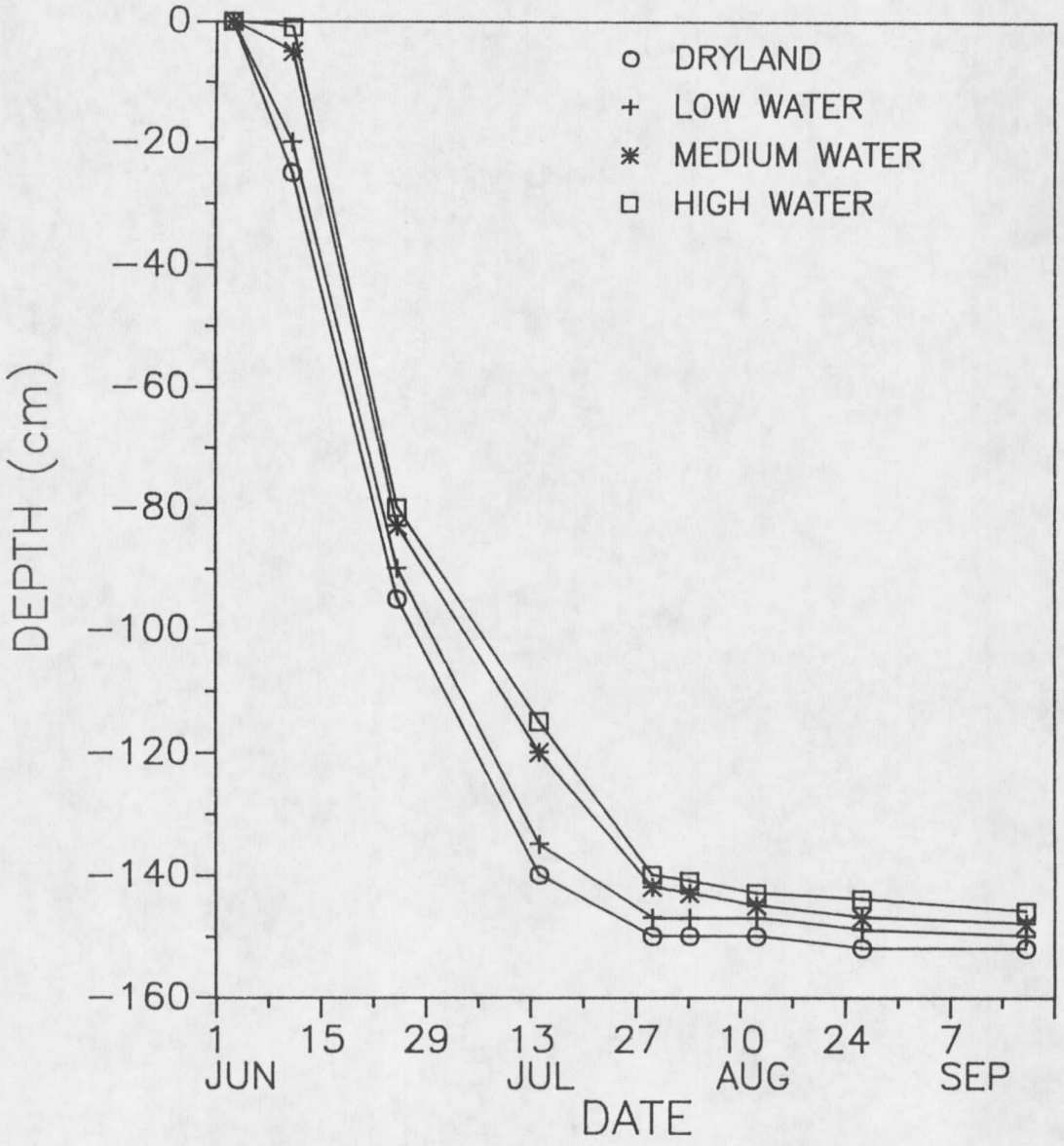

Figure 2. Estimated depth of maximum root penetration (cm) over time, based on soil water depletion data from Newana spring wheat grown under a line-source irrigation system at Manhattan, Montana, in 1986. Depth values indicate depth from soil surface. Drainage and runoff were negligible.

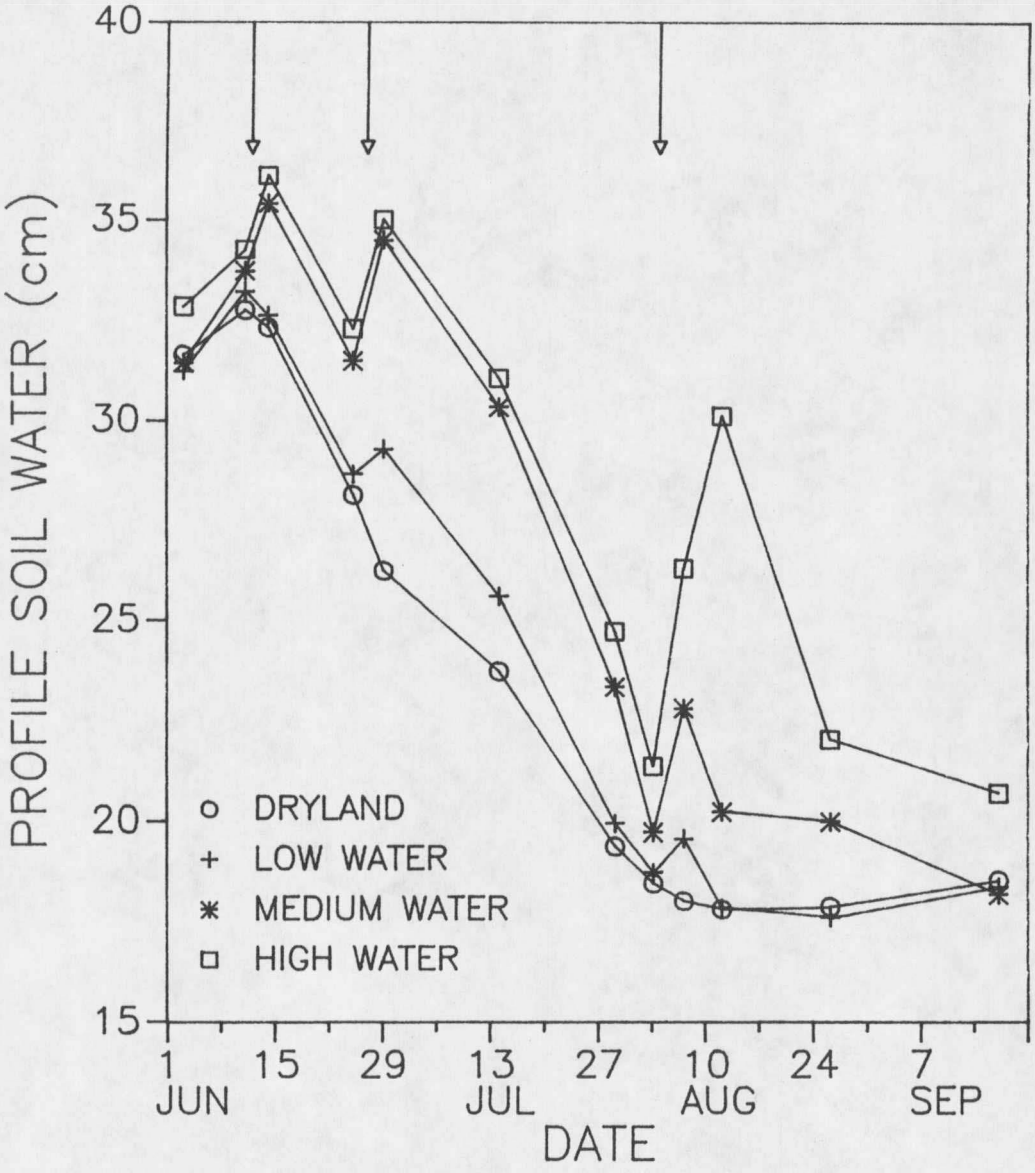

Figure 3. Soil profile water content (cm) for Newana spring wheat grown under a line-source irrigation system at Manhattan, Montana, in 1986. Arrows indicate irrigation dates. Initial soil moisture determination was made at planting and final determination was at harvest.

CHAPTER 4

EVAPOTRANSPIRATION EFFECTS ON SELECTED SPRING WHEAT ACCESSIONS

Moisture stress is one of the primary factors limiting crop production. Few places in the world have optimal moisture throughout the entire crop growing season. Selection and development of crop accessions that better tolerate moisture stress are important, since manipulation of the growing environment is not always possible. Consequently, it is important to evaluate germplasm under diverse soil moisture conditions.

Production input parameters are often confounded due to differential site comparisons with limited control of environmental factors. Relative responses of individual genetic lines to differential soil moisture can be effectively evaluated utilizing a line-source irrigation system to superimpose a controlled soil moisture gradient across plots at a single field site (Hanks et al., 1976). The line-source irrigation system has been used by Garrity et al. (1982) and O'Neill et al. (1983) on grain sorghum, Bauder et al. (1978) on alfalfa, Cruz and O'Toole (1984) on dryland rice, and Westesen et al. (1987) on fababean to evaluate specific yield functions under differential soil moisture conditions. Hang and Miller (1983) used the line-source system to evaluate developmental differences of two wheats under high-frequency irrigation. O'Neill et al. (1983) stated that the line-source system

can be used as a screening tool to evaluate drought resistance of large numbers of germplasm.

Linear relationships have been shown between plant dry matter production and transpiration (Arkley, 1963). However, transpiration is difficult to quantify in the field because of soil moisture evaporation. Consequently, evapotranspiration (ET) has been commonly utilized in moisture stress studies (Garrity et al., 1982).

Water use efficiency (WUE) and harvest index (HI) have been commonly used to assess germplasm potential under diverse environmental conditions. Water use efficiency indicates the ability of a plant to produce a quantity of dry matter per unit of water used. Plants with a higher WUE are often more efficient in utilizing plant available water to produce dry matter. Harvest index is the ratio of seed produced to total above-ground biomass. A high HI indicates that a plant is efficient in channeling a large percentage of total biomass potential into seed production.

Seed yield and protein content are extremely important in commercial wheat production. Seed yield is influenced by various kernel characteristics. Shanahan et al. (1985) reported a highly significant positive correlation between winter wheat kernel number per square meter and total yield. Kirkham and Kanemasu (1983) indicated that kernel weight was an important yield-limiting factor under drought stress. High protein content (often reported as a percentage by weight) is nutritionally and functionally important in wheat production. Loffler and Busch (1982) examined protein percentage as a selection parameter for high protein germplasms.

The objectives of this study were to 1) evaluate the relationships of ET to seed yield, seed WUE, and HI of selected spring wheat accessions, 2) evaluate ET effects on seed protein and kernel characteristics of these spring wheat accessions grown under diverse soil moisture regimes, and 3) determine the utility of a line-source irrigation system in the evaluation of drought resistance.

Materials and Methods

Field Experiments

Field experiments were conducted at Manhattan, Montana, in 1986 and at Huntley, Montana, in 1987. The Manhattan experiment was on a Manhattan sandy loam (coarse-loamy, mixed, typic Calciborolls) and the soil type at Huntley was Fort Collins silty clay loam (fine-loamy, mixed, mesic Ustollic Haplargids). Nitrogen fertilizer based on soil samples taken at depths of 0-30, 30-60, and 60-120 cm was uniformly applied to all plots at a rate sufficient for maximum yield under the high irrigation regime.

Four spring wheat accessions ('Newana', 'Fortuna', MT 7819, and MT 8182) were randomized within four replications at both sites under a line-source irrigation system. Four irrigation regimes (zero, low, medium, and high) were evaluated at Manhattan, whereas Huntley had five irrigation levels (zero, low, medium, medium-high, and high). Seeds were planted 3.8 cm deep at 3.25 g m^{-1} on 15 May 1986 and 9 April 1987 at Manhattan and Huntley, respectively. Rows were placed perpendicular to the irrigation line with 30.5 cm spacing. A 3.7 m border was planted

around each experimental area. Growing season precipitation and air temperature (maximum and minimum) were measured daily at each site.

Weeds were controlled by early-season application of bromoxynil[1] (4-cyano-2,6-dibromophenol) and MCPA (2-methyl-4-chlorophenoxyacetic acid), followed by hand weeding as necessary. Carbofuran[1] (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was applied approximately two weeks prior to harvest to control grasshoppers at Huntley.

Irrigation main plots consisted of 12 rows 3.7 m long at Manhattan and 8 rows 4.0 m long at Huntley. Decreasing water regimes were superimposed perpendicular to the irrigation source with a line-source irrigation system similar to the one described by Hanks et al. (1976). The system utilized Model 25 sprinkler heads with 4 mm nozzles (Rain Bird Sprinkler Mfg. Co., Glendora, California)[1]. Sprinkler pipe diameter was 5 cm at Manhattan and 7.5 cm at Huntley. Sprinkler heads on 2.5 x 90 cm risers were spaced 4.6 m and 6.1 m apart at Manhattan and Huntley, respectively. Wetting radii of 11 m and 15.8 m were obtained at Manhattan and Huntley, respectively. Irrigations were applied when wind speed was judged to be less than 2.3 m s^{-1} to minimize drift.

The soil permanent wilting point was determined with the pressure plate extraction method and gravimetric analysis was utilized to determine field capacity. Plant available water that could be stored in the rooting zone at field capacity was 15 cm. Evaporation was measured daily using a number one pan, as described by Sims and Jackson (1971), located 5.5 m from the line-source. The pan was initially filled to a depth of 15 cm to approximate plant available water in the soil profile. Irrigations were applied when half the initial water evaporated. A pan

coefficient of one, 50% plant available water depletion, and a 140 cm rooting depth were used to schedule irrigations.

Irrigations were applied 27, 40, and 80 days after planting (DAP) at Manhattan, and 14, 22, 29, 60, 67, 83, and 92 DAP at Huntley. Applied water was measured in each moisture regime using catch cans placed at plant canopy height.

Soil moisture was monitored over the growing season at the Manhattan site using a neutron probe (Campbell 503DR Hydroprobe, Campbell Pacific, Pacheco, California)[1]. Polyvinyl chloride (PVC) access tubes were placed in the center of each moisture regime to a depth of 1.52 m. Readings were taken at 20 cm depth intervals to 140 cm. Soil moisture was monitored at two-week intervals throughout the growing season, as well as immediately prior to and 24 hours after irrigation, at Manhattan. Soil moisture at Huntley was determined at planting and harvest both gravimetrically and with the neutron probe.

Soil moisture at planting and harvest for each location were used to calculate seasonal ET from the water balance equation, described by Rose (1966): ET = change in soil water + precipitation + irrigation - runoff - deep drainage. Runoff was minimized by intermittent sprinkler operation, and deep drainage was negligible. Calculation of seasonal ET was performed using the software package ETPROBE described in Chapter 3.

Seed Sample Preparation

A 1.2 x 2.4 m area in the center of each plot was hand-harvested 14 September 1986 at Manhattan, and an area 1.5 x 3.4 m was combine-harvested 28 July 1987 at Huntley. Seed samples were cleaned

and weighed for yield determinations. Seed water use efficiency (WUE) was based on seasonal ET using the equation: WUE = seed yield (kg ha^{-1})/seasonal ET (cm). Harvest index (HI) was calculated for the Manhattan site using the equation: HI = seed yield/total above-ground biomass. HI was not determined at Huntley due to machine-harvesting.

Kernel weight (grams 1000 $kernels^{-1}$) was determined for each sample. Kernels were sized by shaking 100 g of seed on a sieve shaker for 3 minutes. Kernels were separated into three size classes of plump (diameter>2.92 mm), medium (diameter between 2.92 and 2.24 mm), and small (diameter<2.24 mm) based on sieve screen pore diameters. Component classes were weighed and values recorded as percentages. A subsample (10 g) from each plot was ground on a Udy Cyclone[1] mill (1 mm screen) and protein content was determined with near-infrared reflectance (NIR) spectroscopy (Technicon InfraAnalyzer 400, Technicon Instruments Corporation, Tarrytown, New York)[1]. Kernel number per unit area was calculated with the following formula: kernels per unit area = seed yield per unit area/kernel weight.

Data Analysis

Data for each parameter were averaged over replications at each site. Yield and kernel quality parameter means from both sites were used to establish simple correlation coefficients. These coefficients were evaluated for significance according to Snedecor and Cochran (1972). Means were regressed to determine the effects of increased ET on seed yield, WUE, HI, kernel weight, percent plump kernels, kernel number per square meter, and protein content. Significance of quadratic

term (curvilinear) contribution to the regression model and of differences between regression response curves among accessions were tested using the general linear test approach to calculate F-tests, as described by Neter and Wasserman (1974). Differences in regression slopes among accessions were evaluated for each parameter with increased ET using the t-test procedure described by Lund (1987).

Results and Discussion

Environmental Site Conditions

Maximum temperatures for the Manhattan site in 1986 ranged from 5 to 36°C and minimum temperatures ranged from -3 to 16°C (Fig. 4). The Huntley site had daily temperature maximums ranging from 7 to 37°C and minimums from -6 to 19°C. However, early season minimum temperatures at Manhattan were consistently higher than those at Huntley because of the difference in planting dates. Additionally, pre-harvest maximum and minimum temperatures declined at Manhattan but not at Huntley. Fifty percent of the total seasonal precipitation occurred by 5 July (57 DAP) at Manhattan and 16 June (68 DAP) at Huntley (Fig. 4). Total growing season precipitations were 252 mm and 201 mm for Manhattan and Huntley, respectively (Table 1). Initial soil moisture at Manhattan was nearly twice the amount at Huntley. Seasonal ET was highest at Manhattan, possibly as a result of the later planting date, higher early season minimum temperatures, and longer growth season (123 days as opposed to 110 days for Huntley).

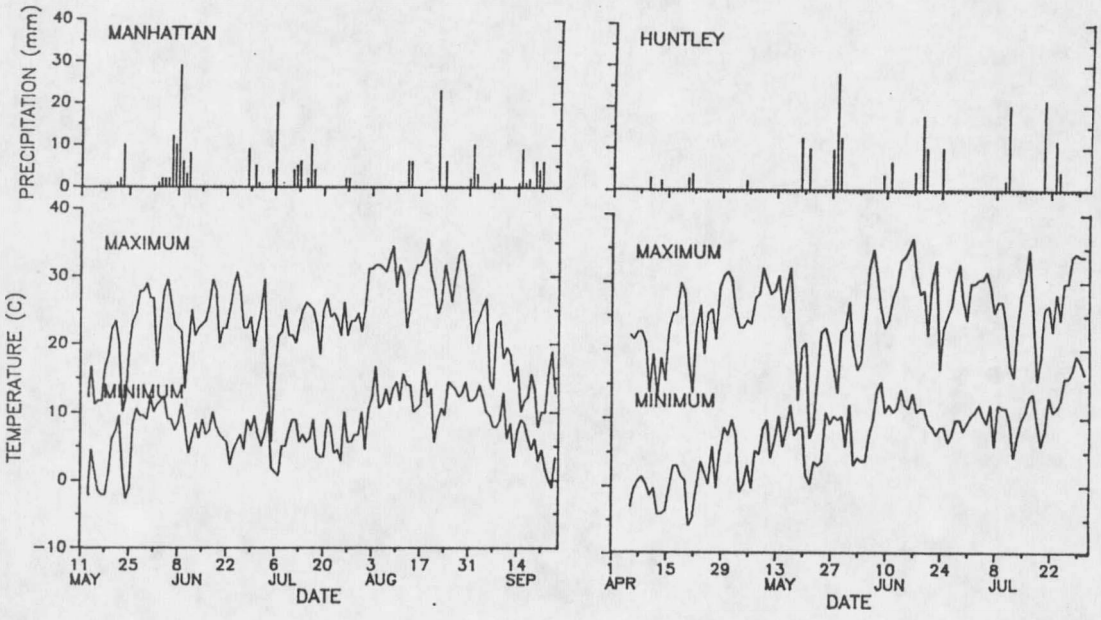

Figure 4. Maximum and minimum daily temperatures (°C) and precipitation (mm) for the growing season at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

Yield and Water Use

Seed yield of all accessions was higher at Manhattan than Huntley at all comparable moisture regimes (Fig. 5). Regression of seed yield on increased ET at Manhattan was significantly different ($P<0.05$) among all accession comparisons except between Newana and MT 7819. MT 8182 had the highest and Fortuna had the lowest yields across all moisture regimes. The regression slope of Fortuna for seed yield on increased ET differed from the other accessions at Huntley ($P<0.05$). These data indicate that Fortuna seed yield was less responsive to changes in field soil moisture than the other accessions under the drier environmental conditions at Huntley.

Table 1. Water budgets for four irrigation regimes at Manhattan, Montana, in 1986 and five irrigation regimes at Huntley, Montana, in 1987.

Irrigation Regime	Initial Soil Moisture	Precipitation	Irrigation	Residual Soil Moisture	Seasonal ET
			mm		
MANHATTAN[a]					
Zero	303[b]	252	32	256	331
Low	303	252	149	247	457
Medium	303	252	225	232	548
High	303	252	261	236	580
HUNTLEY[c]					
Zero	180	201	29	133	277
Low	180	201	75	139	317
Medium	180	201	149	152	378
Medium high	180	201	220	165	436
High	180	201	282	178	485

[a] 140 cm soil profile.
[b] Values were averaged over accessions for each regime, since accession differences were non-significant ($P>0.05$).
[c] 120 cm soil profile.

Seed WUE of all accessions was higher at Manhattan than at Huntley at all moisture levels (Fig. 6). Differential curvilinear regression of WUE on increased ET occurred between sites. The low irrigation regime at Manhattan consistently had the lowest seed WUE for all accessions. Regression equations of WUE on increased ET differed ($P<0.05$) among all accessions, except between Newana and MT 7819, at Manhattan. MT 8182, which was the highest yielding accession, had the highest and Fortuna had the lowest WUE ($P<0.05$). WUE of both Newana and MT 7819 were similar at Manhattan. WUE at Huntley showed a rapid increase between the zero and medium irrigation regimes and either increased at a decreasing rate or stabilized at the higher moisture regimes.

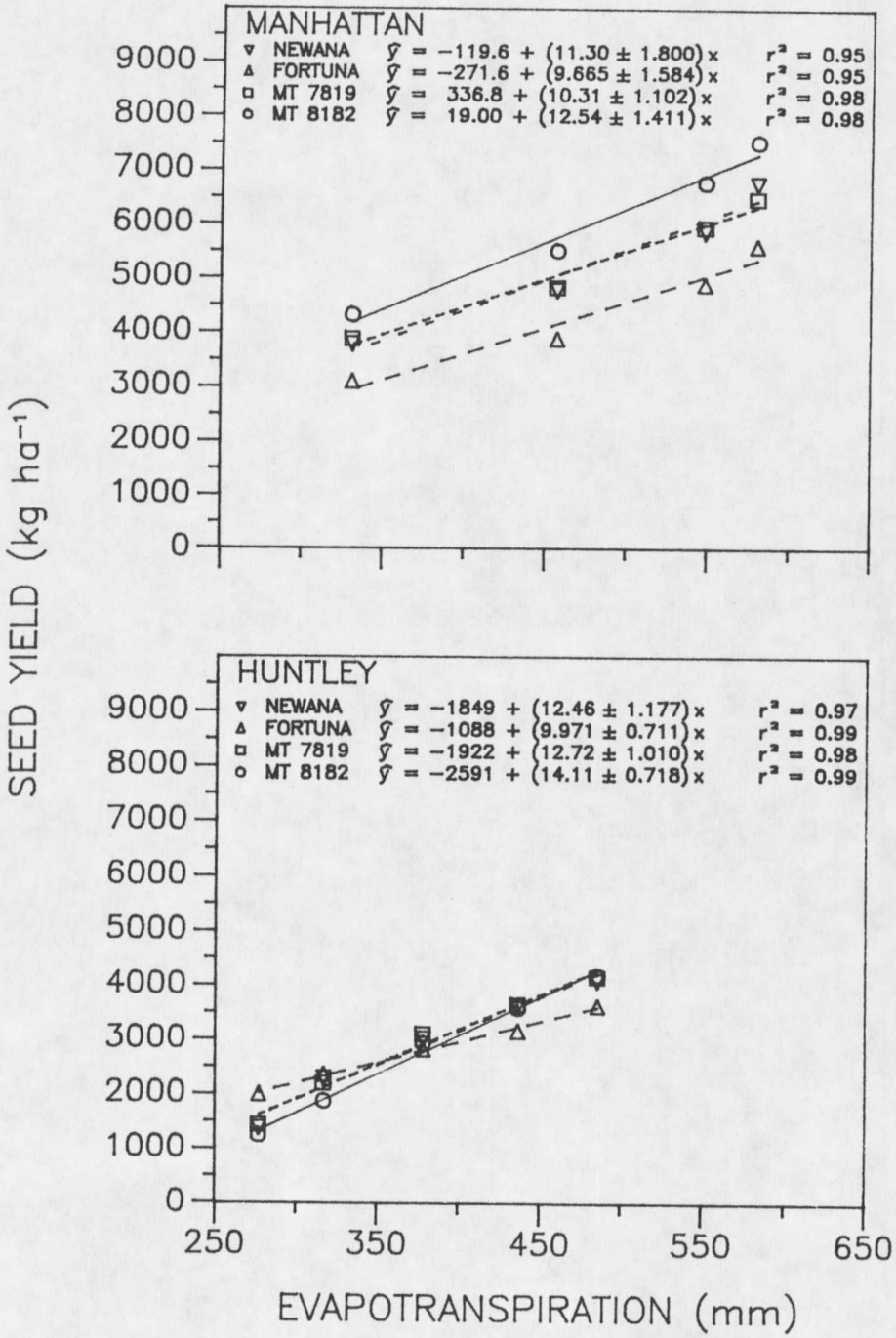

Figure 5. Evapotranspiration effects on seed yield of four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

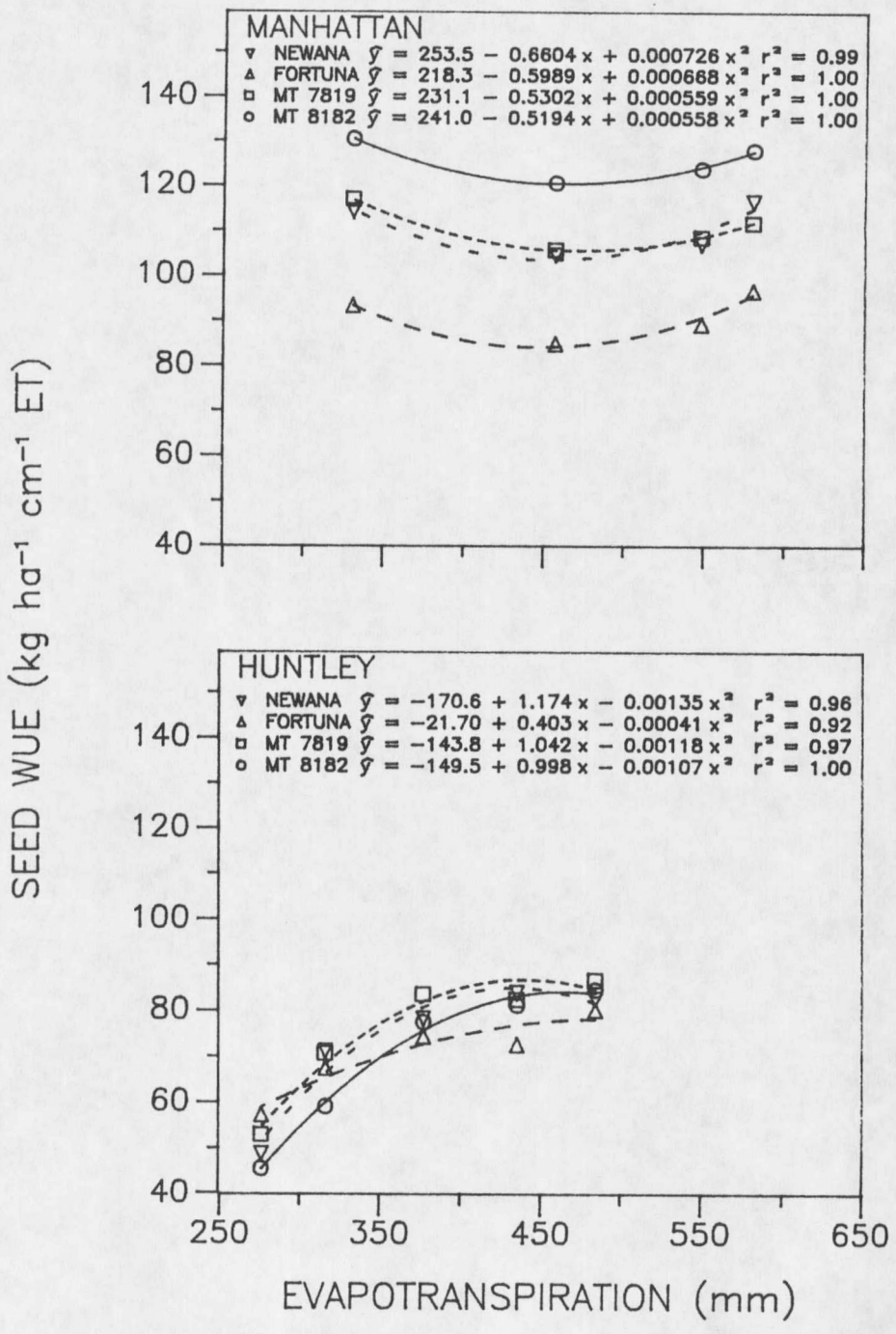

Figure 6. Evapotranspiration effects on seed water use efficiency (WUE) of four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

Regression equations for seed WUE on increased ET differed significantly ($P<0.01$) only between Fortuna and the other accessions at Huntley. The WUE responses to increased ET at Huntley are in agreement with the convex curvilinear response of WUE to increased ET reported by Garrity et al. (1982) for grain sorghum. The differential responses of seed WUE to increased ET between sites may have resulted from climatic-plant growth interactions due to differential planting dates between the two sites.

MT 8182 and MT 7819 had the highest and Newana and Fortuna had the lowest HI in all moisture regimes at Manhattan (Figure 7). Testing for differences in HI response among the four accessions resulted in no distinction between MT 8182 and MT 7819 or between Newana and Fortuna. However, the two groups of regressions (MT 8182 and MT 7819; Newana and Fortuna) were highly different ($P<0.01$). The positive relationship between HI and increased ET is in agreement with the findings of Clarke et al. (1984), who reported that the ratio of spring wheat head yield to biological yield was greater under irrigated than dryland conditions.

Kernel Quality Parameters

Kernel Characteristics. Overall correlations were highly significant ($P<0.01$) for combinations among ET, seed yield, kernel weight, percent plump kernels, and kernels per square meter (Table 2). Both ET and kernels per square meter had extremely high correlations with seed yield ($r>0.90$). The effect of differential ET on seed yield was discussed previously.

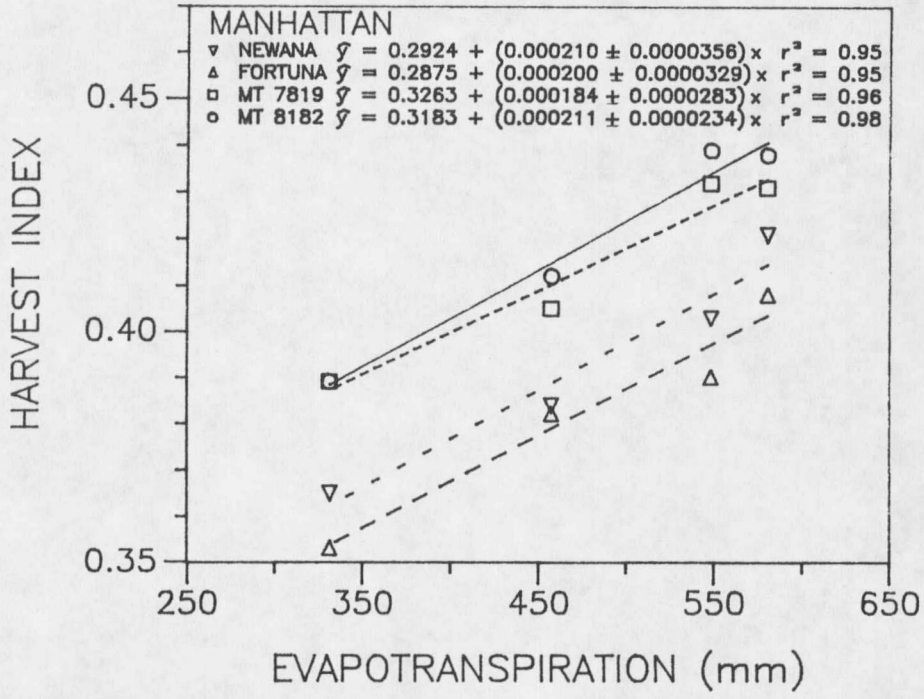

Figure 7. Evapotranspiration effects on harvest index (HI) of four spring wheat accessions grown under a line-source irrigation system at Manhattan, Montana, in 1986.

Kernel weight was less responsive to increased ET at Manhattan than at Huntley for all accessions (Fig. 8). Regression of kernel weight on increased ET at Manhattan differed significantly ($P<0.05$) among all accessions except between Newana and MT 7819. Fortuna had the highest and MT 7819 and Newana had the lowest kernel weights across all moisture regimes. Regression of kernel weight on increased ET differed ($P<0.05$) among all accessions at Huntley. MT 8182 kernel weight had the greatest response (increased slope) to increased ET at this site. Fortuna had the highest kernel weight in the zero and low irrigation regimes and MT 7819 and Newana had the lowest in the higher moisture regimes at Huntley. These data indicate that kernel weight of MT 8182

Table 2. Overall correlation coefficients for seed yield, kernel weight, percent plump kernels, kernels per square meter, protein content, and evapotranspiration (ET) for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

	ET	Seed Yield	Kernel Weight	Percent Plump	KPSM[a]	Protein[b] Content
Seed Yield	.92 **	---				
Kernel Weight	.77 **	.65 **	---			
Percent Plump	.82 **	.79 **	.88 **	---		
KPSM	.82 **	.96 **	.43 **	.67 **	---	
Protein Content	-.51 **	-.35 *	-.52 **	-.65 **	-.29 ns	---

*, ** Significance at P<0.05 and P<0.01, respectively.
ns Non-significance.
[a] Kernels per square meter.
[b] On a 14% moisture basis.

was more sensitive to increased ET than the other accessions under the drier conditions at Huntley, and that Fortuna had the potential for consistently higher seed weight than Newana or MT 7819.

Plump kernel percentage at Manhattan was less responsive to increased ET than at Huntley for all accessions (Fig. 9). Regression of kernel plumpness on increased ET differed (P<0.05) among all accessions at Manhattan except between Newana and MT 8182. Regression slope of Fortuna kernel plumpness on increased ET differed (P<0.05) from the other accessions. Fortuna had the highest plump kernel percentage across all except the highest moisture regime and MT 7819 had the lowest across all regimes at Manhattan. Curvilinear responses of kernel plumpness to increased ET occurred at Huntley. MT 7819 plump percentage had the least response (P<0.05) to increased ET and was the lowest of all accessions in the two higher moisture regimes at this site. Plumpness percentages for MT 7819 were approximately 10% below those of

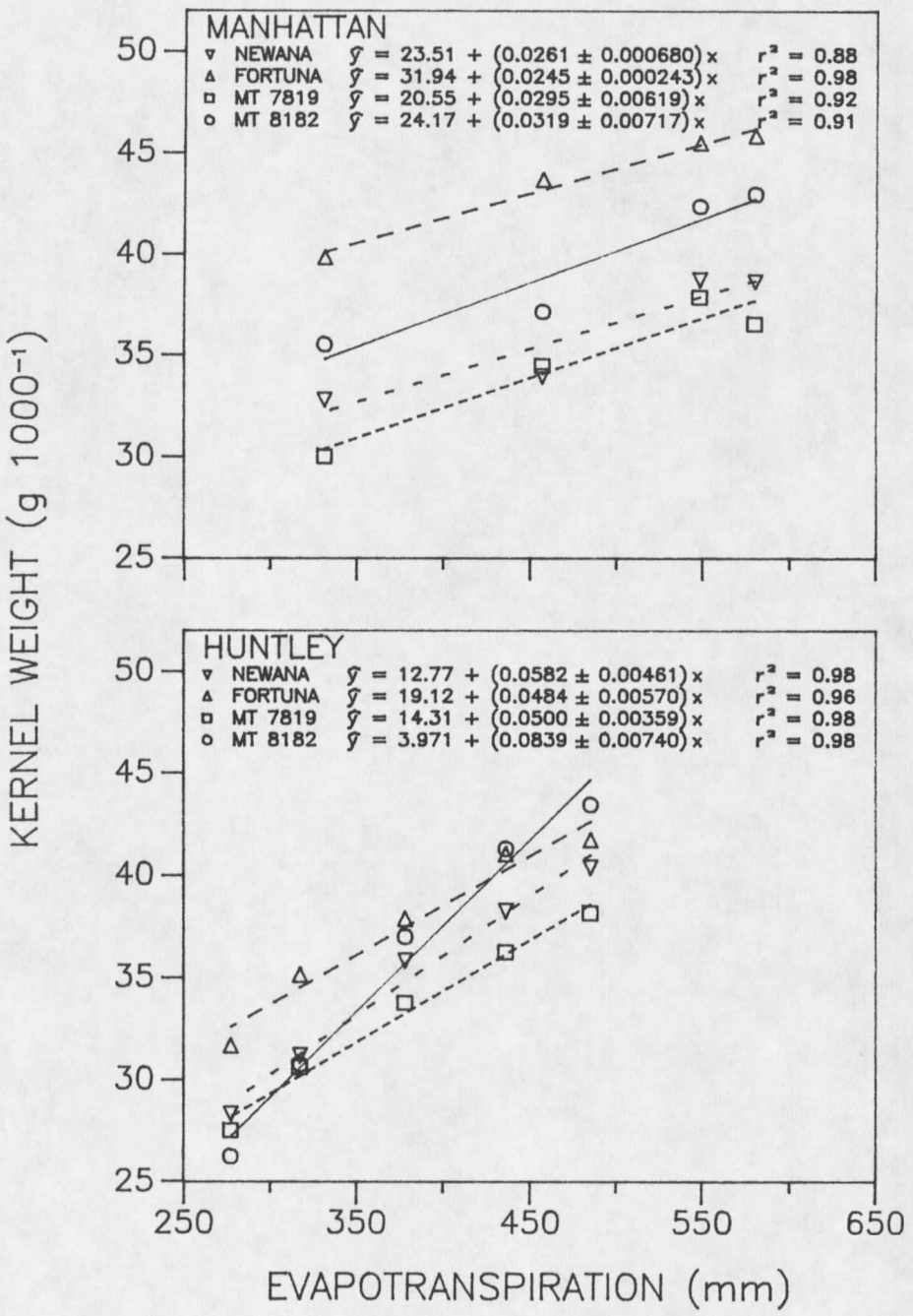

Figure 8. Evapotranspiration effects on kernel weight for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

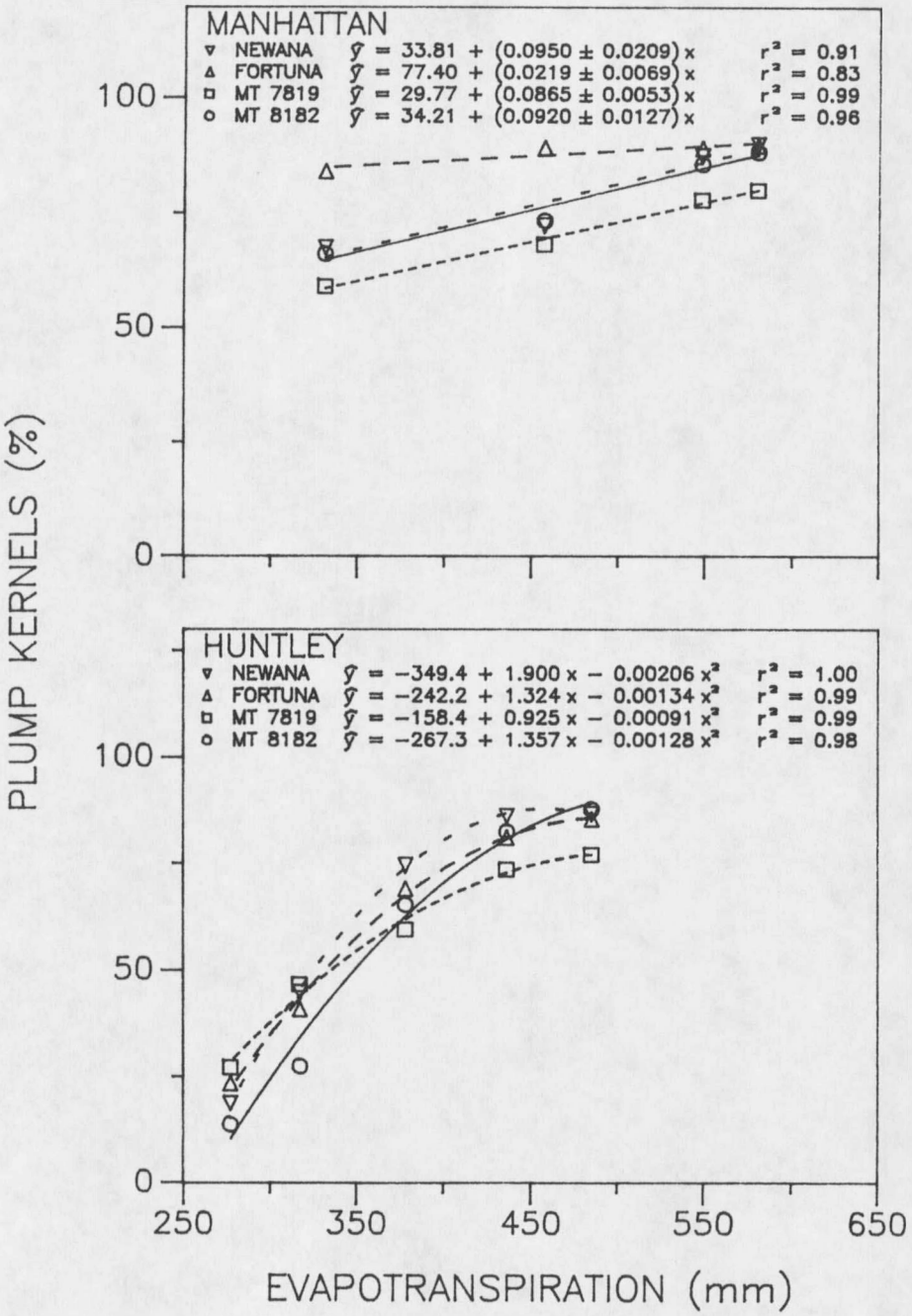

Figure 9. Evapotranspiration effects on percent plump kernels for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

the other accessions in the high moisture regime at both sites. These data suggest a possible genetic limitation associated with kernel plumpness potential for MT 7819 relative to the other accessions.

Kernel number per square meter was higher for all accessions at Manhattan than Huntley at all comparable moisture levels (Fig. 10). Regression of Fortuna kernel number per square meter on increased ET differed significantly ($P<0.05$) from the other accessions at Manhattan. Fortuna also had the lowest number of kernels per square meter across all moisture regimes at this site. At Huntley, Fortuna differed from the other accessions in response (slope) of kernel number per square meter to increased ET ($P<0.05$) and had the lowest kernel number at the higher moisture regimes.

These data suggest that kernel number is inversely related to kernel weight. This is in agreement with the findings of Haugerud and Cantrell (1984) and Knott and Taludkar (1971). Fortuna generally had higher kernel weight and lower kernel number per unit area than the other accessions over all moisture regimes at both sites. Conversely, MT 7819 generally had lower kernel weight and higher kernel number over the moisture regimes at both sites.

Protein Content. Seed protein content had highly significant ($P<0.01$) negative correlations with ET, kernel weight, and percent plump kernels (Table 2). Additionally, protein content correlation (also negative) with seed yield was significant ($P<0.05$), but protein content showed no correlation with kernels per square meter.

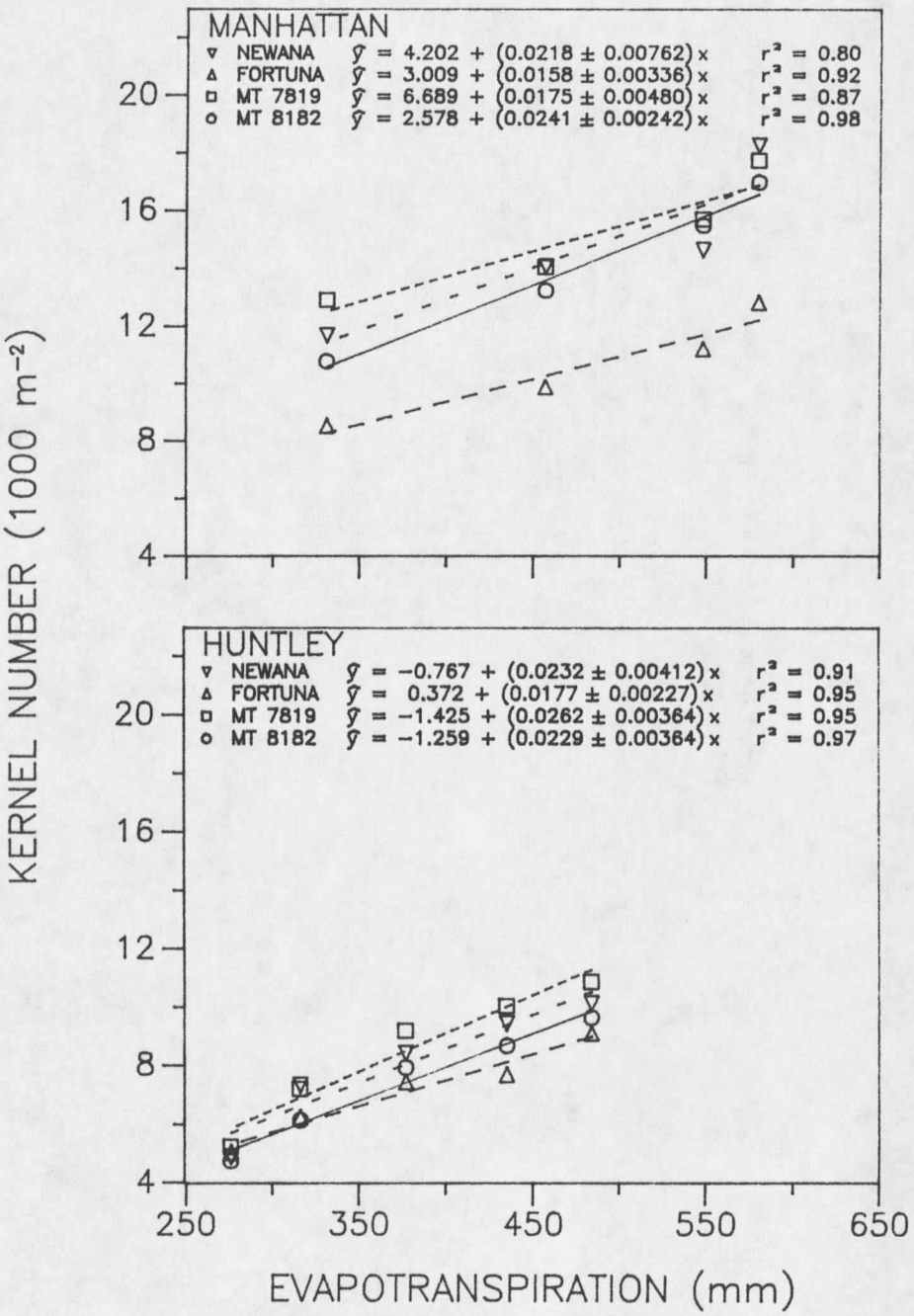

Figure 10. Evapotranspiration effects on kernels per square meter for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

Increased ET had the greatest effect (decreased slope) on MT 8182 seed protein content at both sites (Fig. 11). Fortuna had the least response of seed protein to increased ET at the drier Huntley site. Regression slope of seed protein content on increased ET differed significantly ($P<0.05$) between MT 8182 and the other accessions at Manhattan and among all accessions except Newana and MT 7819 at Huntley ($P<0.05$). MT 8182 had the highest overall protein content (17.7%) of the accessions in the dryland regime at Huntley. MT 7819 had the lowest seed protein of all accessions in all moisture regimes at both sites. These data suggest that seed protein was genotypically limited at a lower level for MT 7819 than for the other accessions in this study.

Conclusions

Accession selection should not always be based on yield maximization with no regard for cost of water inputs. Yield optimization within a given range of water inputs might often be a more viable option. A high-yielding accession selected in the presence of adequate soil moisture is not always the best choice for a cropping system where water may be limiting.

WUE may have relationship to drought resistance. The accessions did not show differences in water use (seasonal ET), therefore WUE was directly related to accession yield. However, care must be taken when relating drought resistance to WUE, since some accessions tolerate drought by maintaining a high plant water status while others do so by maintaining a low plant water status.

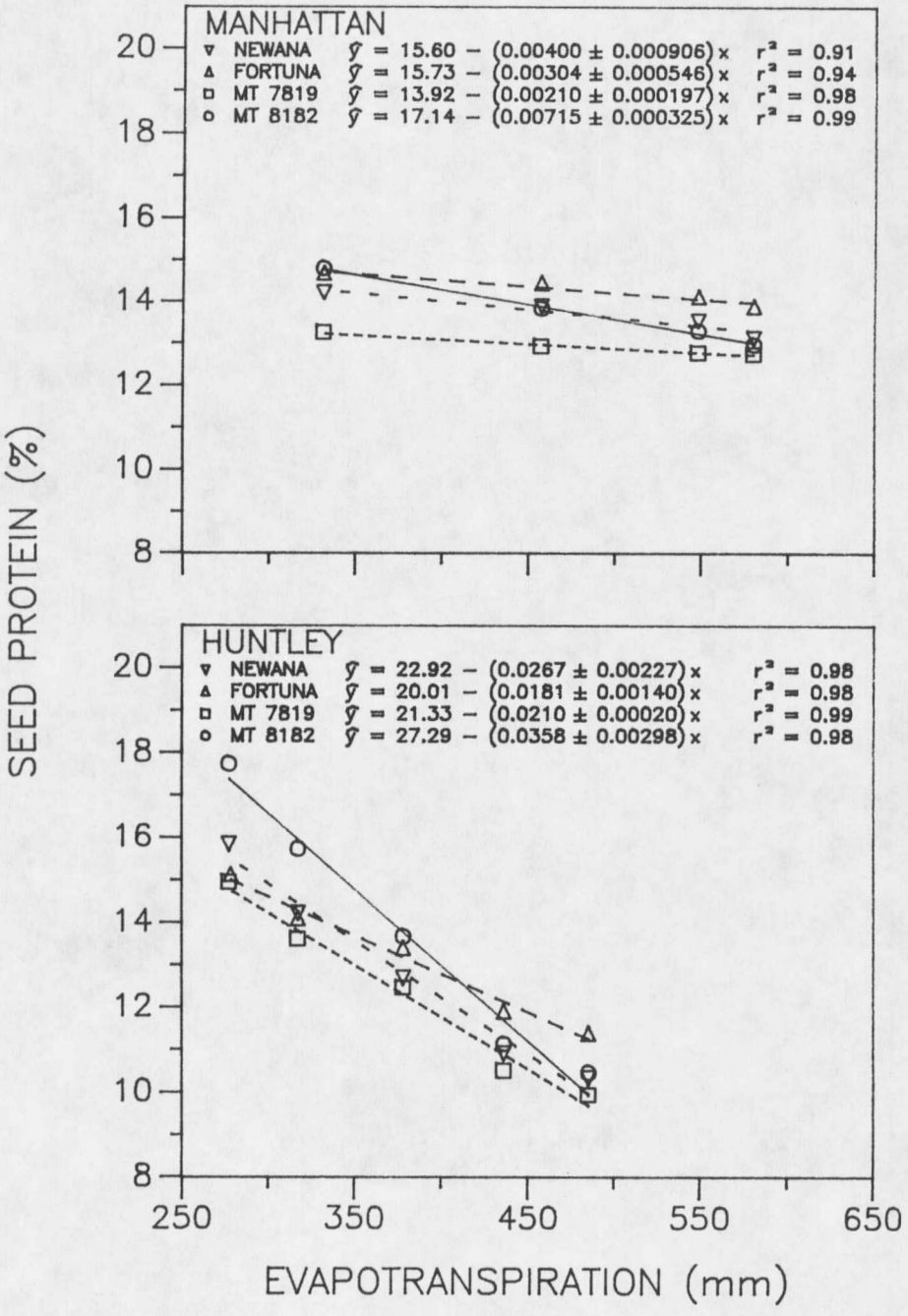

Figure 11. Evapotranspiration effects on seed protein content for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

HI is a function of both grain and total biomass yield. HI differed significantly between MT 7819 and Newana across all moisture regimes at Manhattan, though these accessions did not differ in seed yield at this site. This indicates that Newana had a greater straw yield than MT 7819 across all moisture regimes. Increases in HI associated with increased ET may result from a greater increase in grain production compared to straw (non-grain above-ground biomass) or, conversely, a greater reduction in straw yield compared to grain.

Several important growth factors, such as number of spikes per plant or kernels per spike, have been identified as significant wheat yield inputs. These inputs, as well as wheat quality, are highly dependent on field moisture availability during the crop growing season. Yield tends to have a positive linear relationship with increased ET until soil moisture is no longer the limiting factor. However, the relationship of seed protein content to ET is generally negative.

These data suggest that the effects of increased ET on seed yield and protein content are strongly associated with kernel weight and/or plumpness. This is in agreement with the results indicated by Kirkham and Kanemasu (1983).

Kernel weight and relative kernel plumpness are both good indicators of wheat kernel quality and are strongly associated with each other. Kernel weight has been suggested as the major factor influencing protein content percentages (McNeal et al., 1972). However, relative plumpness had a higher overall correlation with ET, seed yield, kernels per unit area, and protein content than did kernel weight.

Data from different location-years and different sites within a given year are often confounded by variability in temperature, fertility, solar radiation, and other environmental factors. The line-source sprinkler irrigation system is capable of imposing differential plant available water regimes while maintaining local control of other environmental variables. Consequently, a high degree of precision is afforded by this system when evaluating the effect of differential soil moisture stress on plants.

CHAPTER 5

SOIL MOISTURE EFFECTS ON BREAD LOAF QUALITY DETERMINED BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF GLIADINS

Bread-making characteristics of wheat flour are dependent on protein quality. Gliadin and glutenin endosperm storage proteins are important to the bread-making capability of flour. Hamada et al. (1982) reported that the glutenin fraction of the protein solubility classes defined by Osborne (1907) imparted toughness and strength to gluten. Finney et al. (1982) utilized solubility and ultracentrifugation to separate gliadins and glutenins which showed no loss of functionality when reconstituted into doughs. These fractions were interchanged between good and poor baking quality flours to define the functional bread-making role of each class. Their study indicated that loaf volume potential was related to gliadin quality and mixing requirements were gluten quality functions. They also indicated that cultivars which have good quality glutenins also commonly produce good quality gliadins.

Damidaux et al. (1978), using aluminum lactate-polyacrylamide gel electrophoresis (AL-PAGE), identified a pair of allelic gliadin bands whose presence or absence was strongly correlated with durum wheat gluten strength. These components of the gamma-mobility gliadins, as classified by Woychik et al. (1961), were designated bands 42 and 45 which corresponded to poor and good quality durum dough characteristics, respectively. Burnouf and Bietz (1984), using reversed-phase high-

performance liquid chromatography (RP-HPLC), identified durum wheat chromatogram peaks which corresponded to PAGE bands 42 and 45. These peaks eluted in the more hydrophobic (late-eluting) region of the chromatogram, and their relative areas gave durum quality rankings similar to those based on the electrophoretic bands. Huebner and Bietz (1986) found a similar correlation between a group of late-eluting gliadin components and bread-making quality in the bread wheats. This group of gliadin components, which they called the baking quality gliadin fraction (BQGF), contained peaks with elution times similar to the quality-related durum wheat protein peaks. Furthermore, they found a high negative correlation between the area under the BQGF peaks, expressed as a percentage of the total gliadin area, and baking score.

Lee and Ronalds (1967) stated that gliadins are not qualitatively affected by environment. They noted that the gliadin banding pattern of a given genotype determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) did not change when the wheat cultivar was grown under several environments. However, PAGE does not lend itself well to quantification of these components, since different proteins have very different dye-binding capacities, which affects densitometric measurements of protein band content (Lawrence et al., 1970). Quantification of gliadin components has improved significantly with the utilization of HPLC. This technique permits quantitation of peptide bonds present in the eluent by measuring absorbance at 210 nm.

The objectives of this study were to 1) determine the effects of differential soil moisture levels on gliadin components of selected hard

spring wheats and 2) identify specific relationships of the more hydrophobic gliadins with loaf volume and crumb score.

Materials and Methods

Four accessions of spring wheat ('Newana', 'Fortuna', MT 7819, and MT 8182) were replicated four times across four irrigation levels at Manhattan, Montana, in 1986 and five irrigation levels at Huntley, Montana, in 1987 using a line-source irrigation system similar to the one described by Hanks et al. (1976). Plots were uniformly fertilized for maximum yield under high irrigation, based on analysis of soil samples from each site. Evapotranspiration (ET) was calculated from soil moisture determinations at planting and harvest.

Irrigation main plots were 3.7 x 3.7 m at Manhattan and 2.4 x 4.0 m at Huntley. A 1.2 x 2.4 m area was hand-harvested from the center of each plot at Manhattan and a 1.5 x 3.4 m area was combine-harvested from each plot at Huntley for protein and bread baking quality analyses. Grain samples from each plot (1300 g) were cleaned, weighed, tempered to 16% moisture [AACC (1983) method 26-10], and milled to approximately 70% flour extraction on a Buhler[1] laboratory flour mill (type MLU-202) according to AACC (1983) method 26-20. Flour extraction rate was calculated for each sample as the ratio of recovered flour weight to the combined weights of all recovered milling fractions.

Flour from each plot was collected individually from the mill for test baking. A subsample of flour was taken from each mill sample for protein determination using NIR spectroscopy (Technicon InfraAnalyzer

400, Technicon Instrument Corporation, Tarrytown, New York)[1] as indicated by AACC (1983) method 39-11, and also for HPLC analysis.

Two replicate test loaves (100 g) were made from each flour sample using the optimized straight dough method [AACC (1983) method 10-10B]. Loaves were characterized by loaf volume and crumb score (rated on a scale of 1 = poor to 10 = very good). Crumb score was visually rated against loaves from 'Mello Judith', a commercial baking flour. Standard loaves were assigned a crumb score value of five.

Means (over field replications) were used for correlation and regression analyses to determine associations among the parameters. Correlation coefficients were tested for significance using the procedure outlined by Snedecor and Cochran (1972). Differences among regression lines were tested using F-values from the general linear test approach outlined by Neter and Wasserman (1974). A t-test was used to determine slope differences (Lund, 1987).

HPLC Sample Preparation

Flour samples (25 mg) from each plot were suspended in 1 ml 70% (v/v) aqueous ethanol with a vortexer. Suspensions were allowed to stand for one hour at room temperature and centrifuged at 15,600 x *g* for 10 minutes. Supernatants were removed and pellets were discarded. Ethanol extractions were analyzed directly by HPLC.

HPLC System and Run Conditions

The HPLC system consisted of a Spectroflow 430 gradient former, a Spectroflow 400 solvent delivery system, and a Spectroflow 757 variable-wavelength absorbance detector (Kratos Analytical Instruments, Ramsey,

New Jersey)[1]. Analyses were performed using a 250 x 4.1 mm Synchropak[1] RP-P (C_{18}) analytical column. Column temperature was maintained at 70°C with an HPLC column water jacket (Alltech Associates, Inc., Deerfield, Illinois)[1] and a VWR 1130 circulating water bath (VWR, Seattle, Washington)[1]. All solvents were HPLC grade. Solvent A was distilled water and 0.1% trifluoroacetic acid (TFA) and solvent B was acetonitrile and 0.1% TFA. A modified linear gradient (25 to 50% solvent B over a 55 minute period with a 15 minute isocratic hold at 39% solvent B concentration) was used with a flow rate of 1.0 ml min^{-1}. An additional five minute isocratic hold at final gradient conditions allowed for lag time between the gradient former and the detector. The gradient was returned to initial conditions over a 3-minute period and allowed to equilibrate for 10 minutes prior to subsequent injections. Total run time between sample injections was 88 minutes. Random blank runs showed no protein carry-over between sample runs. The function of the 15-minute isocratic hold was to achieve spatial (and temporal) separation of the late-eluting gliadin peaks from the other gliadin components. Samples were injected manually using a 20 μl sample loop, which was flushed and filled by injecting four times the loop volume.

Eluents were detected at 210 nm and data were processed and plotted on a Chromatopac C-R3A integrator (Shimadzu Scientific Instruments, Inc., Columbia, Maryland)[1] at 0.2 AUFS/8 mV. Chromatograms were automatically corrected for baseline drift at run-time and stored on an IBM PC-XT[1] computer for replotting. Replotted chromatograms were standardized to the height of the peak eluting at 38 minutes. Total chromatogram area utilized for analyses excluded the area of the solvent

peaks (first six minutes of run). Areas of individual peaks were obtained from the integrator in both relative chromatogram units and as percentages of the total chromatogram area. The area of a group of late-eluting gliadin peaks (referred to as the quality gliadin fraction - QGF) was calculated per chromatogram by summation of peak areas in the fraction. Relative QGF area was calculated as the ratio of the QGF area to total chromatogram area.

The ratios of actual loaf volume to total flour protein percentage and actual loaf volume to QGF percentage allowed comparisons of the relative mean increase in loaf volume associated with a 1% change of each protein type (Hamada et al., 1982). Correlations were obtained for actual loaf volume with loaf volume per total protein percentage and per QGF percentage.

Results and Discussion

Evapotranspiration values ranged from 331 to 580 mm at Manhattan and from 277 to 485 mm at Huntley (Table 3). Manhattan was the wetter of the two sites with nearly twice the initial soil water content and approximately 25% more precipitation than Huntley during the growing season.

MT 8182 had the greatest decrease ($P<0.05$) in flour protein content with increased ET at both sites [14 to 12% and 16 to 10% at Manhattan and Huntley, respectively (Fig. 12)]. Conversely, Fortuna had the least decrease ($P<0.05$) in protein content at Huntley (14 to 11%). Regression slopes for flour protein content on increased ET were significantly different ($P<0.05$) between MT 8182 and the other

Table 3. Mean seasonal evapotranspiration (ET) of four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

Moisture Regime	ET[a]	
	MANHATTAN	HUNTLEY
	mm	
Dryland	331[b]	277
Low	457	317
Medium	548	378
Medium-high	---[c]	436
High	580	485

[a] Seasonal evapotranspiration = $CSM_i + P + I - CSM_f - RO - D$, where CSM_i is initial soil water content, P is precipitation amount, I is irrigation amount, CSM_f is final soil water content, RO is runoff, and D is deep drainage. Runoff and deep drainage were negligible.
[b] Mean values for each irrigation regime were averaged over accessions since ET differences among accessions were not significant ($P>0.05$).
[c] Four irrigation regimes were utilized at the Manhattan site (dryland, low, medium, and high).

accessions at Manhattan and among all accessions, except between Newana and MT 7819, at Huntley. Flour protein showed a strong negative linear correlation ($P<0.01$) with seasonal ET for all accessions at both sites except Newana at Manhattan (Table 4).

Differences among chromatograms from samples grown under soil moisture extremes [dryland (zero) and high irrigation] for each accession at both sites are presented in Fig. 13. The QGF area is shown as a group of peaks eluting between 52 and 62 minutes. As expected, some non-QGF peaks decreased with increased field moisture as QGF area increased. Major peak components had similar elution times among all accessions. However, corresponding individual peak areas differed among accessions. Relative QGF area had significant positive correlations ($P<0.05$) with increased ET for Newana and MT 8182 at both sites and for

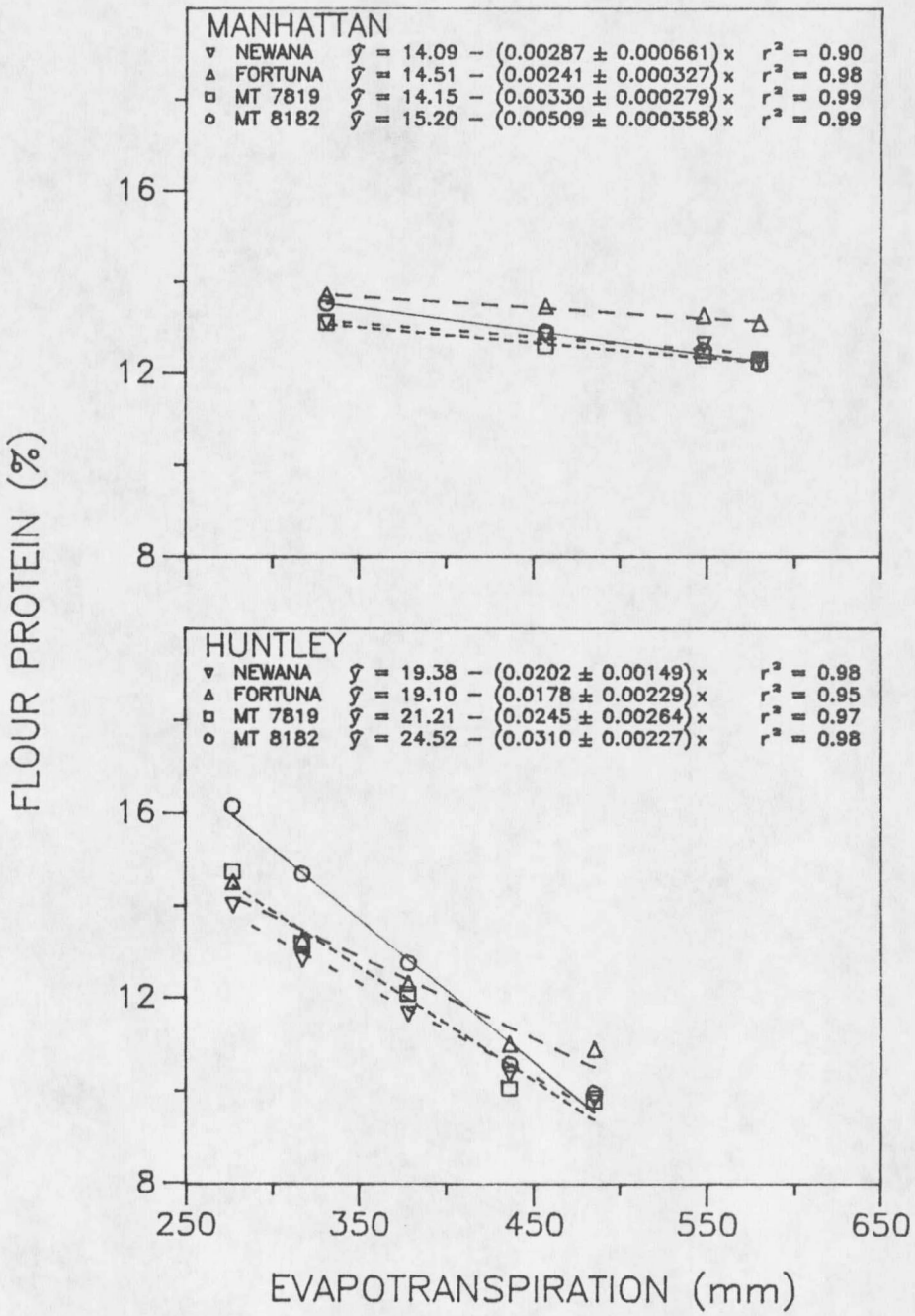

Figure 12. Evapotranspiration effects on flour protein content (%) for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

Table 4. Site-specific correlation coefficients for evapotranspiration (ET), total protein content, relative quality gliadin fraction (QGF) area, loaf volume, and crumb score for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

	Correlations[a]							
	MANHATTAN				HUNTLEY			
Parameters	NEWANA	FORTUNA	MT 7819	MT 8182	NEWANA	FORTUNA	MT 7819	MT 8182
ET and Total Protein (%)	-0.94 NS	-0.99 **	-0.99 **	-0.99 **	-0.98 **	-0.98 **	-0.99 **	-0.99 **
ET and % QGF[b]	0.97 *	0.58 NS	0.79 NS	0.95 *	0.99 **	0.51 NS	0.89 *	0.99 **
ET and Loaf Volume	-0.97 *	-0.83 NS	-0.76 NS	-0.90 NS	-0.96 **	-0.91 *	-0.98 **	-0.98 **
% QGF and Loaf Volume	-0.98 *	-0.93 NS	-0.94 NS	-0.99 **	-0.93 *	-0.58 NS	-0.79 NS	-0.95 *
ET and Crumb Score[c]	-0.74 NS	-0.76 NS	-0.81 NS	-0.87 NS	-0.88 *	-0.93 *	-0.96 *	-0.91 *

*, ** Significance at P<0.05 and P<0.01, respectively.
NS Non-significance (P>0.05).
[a] Of treatment means (n = 4 at Manhattan and n = 5 at Huntley).
[b] Relative QGF area (percentage of total chromatogram area).
[c] Crumb score (1 = poor, 10 = very good).

MT 7819 at Huntley (Table 4). Fortuna relative QGF area was not significantly correlated with increased ET at either site.

Associations between relative QGF area and increased ET were strongest for Newana at Manhattan and MT 8182 at Huntley (Fig. 14). Slopes of relative QGF area regressed on increased ET were significantly different (P<0.05) between Newana and the other accessions at Manhattan, and among all accessions, except between Newana and MT 7819, at Huntley. The response of relative QGF area to increased ET for Newana was similar at both sites, while the slope of the response for Fortuna did not differ from zero (P>0.05) at either site.

Samples grown at Huntley had a wider range of loaf volumes over the evaluated moisture regimes for each accession (Fig. 15). No regression slope differences for loaf volume on increased ET were found between Newana and MT 8182 or between Fortuna and MT 7819 at either

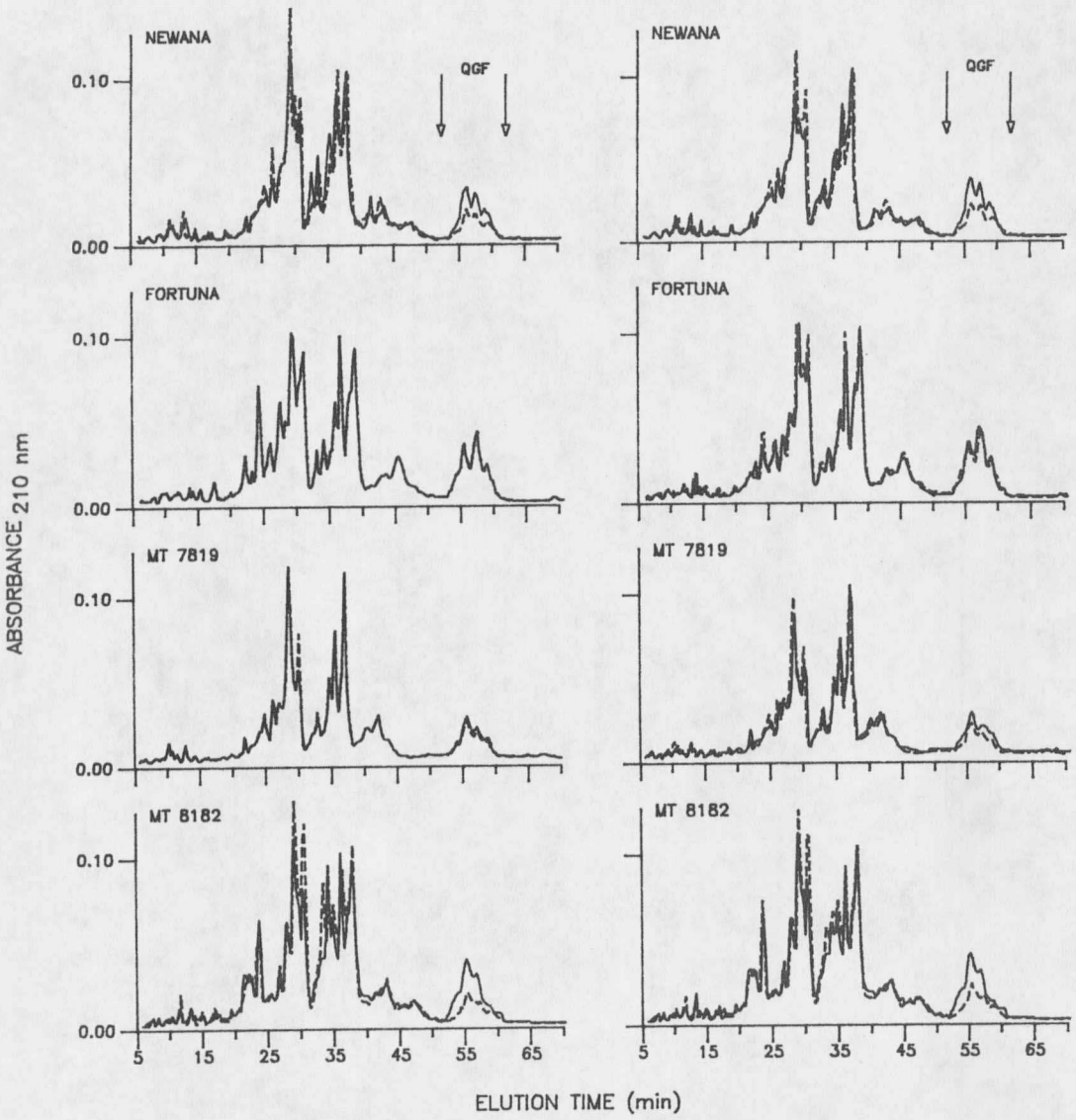

Figure 13. Comparative chromatograms of four spring wheat accessions grown under dryland (- - -) and high irrigation (-----) conditions at Manhattan (left column) and Huntley (right column), Montana, in 1986 and 1987, respectively. Chromatograms were scaled to standardize the height of the peak eluting at 38 minutes. Quality gliadin fraction (QGF) area is designated between arrows at the extreme right of each chromatogram.

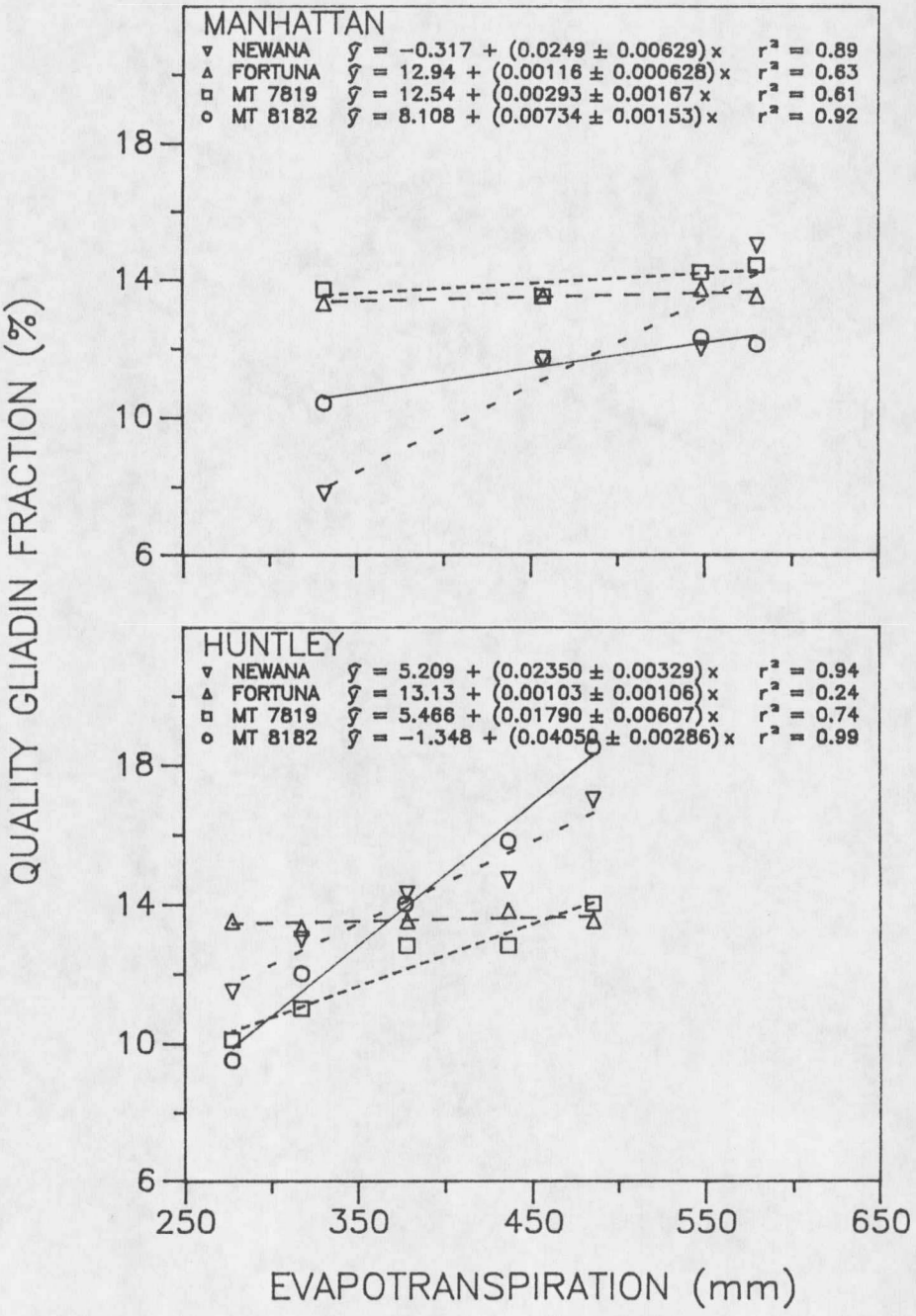

Figure 14. Evapotranspiration effects on relative quality gliadin fraction (QGF) peak area for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

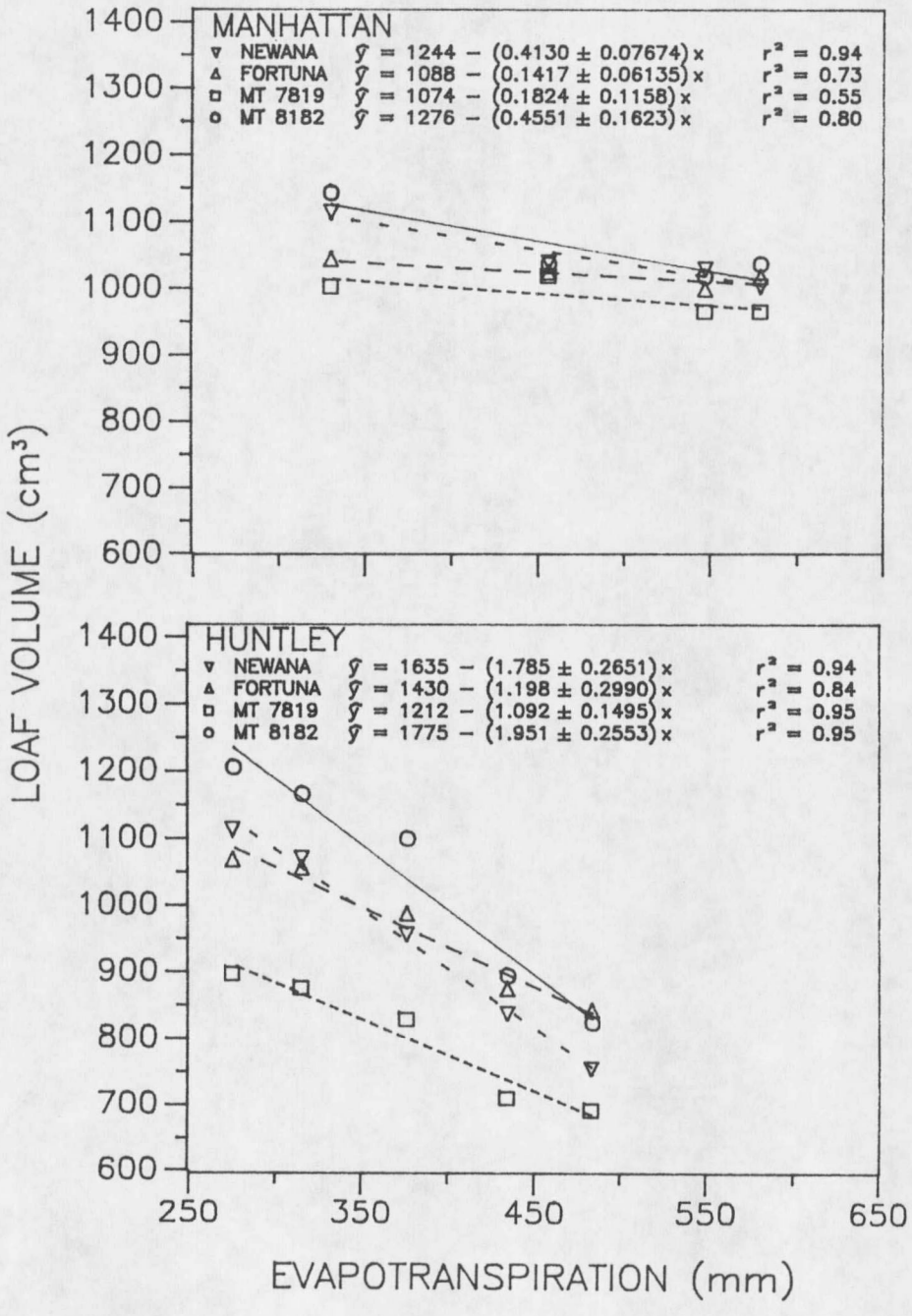

Figure 15. Evapotranspiration effects on loaf volume for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

site. However, significant differences ($P<0.05$) occurred between the groups (Newana and MT 8182; Fortuna and MT 7819) at Huntley. Loaf volume was negatively correlated with increased ET for Newana at Manhattan and for all accessions at Huntley (Table 4). Loaf volume was correlated with relative QGF area (% QGF) for Newana and MT 8182 ($P<0.05$) at both sites. Correlation of crumb score with ET was non-significant for all accessions at Manhattan and significant for all accessions at Huntley ($P<0.05$).

Correlation of actual loaf volume with loaf volume per relative QGF area percentage was highly significant ($P<0.01$) for all accessions at Manhattan and for Newana and Fortuna at Huntley (Table 5). Additionally, correlations were significant ($P<0.05$) for MT 7819 and MT 8182 at Huntley. Correlation of actual loaf volume with loaf volume per total flour protein percentage was not significant for any accession at either site ($P>0.05$). These data suggest that changes in actual loaf volume for all accessions were more directly associated with relative QGF area than with total protein content.

Conclusions

These data suggest that field soil moisture, as indicated by ET, may play a major role in bread loaf volume variability of some wheat accessions through interactions of environment with gliadin components. The strong negative correlation of loaf volume to relative QGF area for Newana and MT 8182 is in agreement with results of other accessions evaluated by Huebner and Bietz (1986). However, Fortuna appears to not

Table 5. Site-specific correlation coefficients for actual loaf volume with loaf volume per relative quality gliadin fraction (QGF) area percentage and with loaf volume per total flour protein percentage for four spring wheat accessions grown under a line-source irrigation system at Manhattan and Huntley, Montana, in 1986 and 1987, respectively.

	MANHATTAN		HUNTLEY	
	Loaf Volume/QGF (cm^3 $percent^{-1}$)	Loaf Volume/Protein (cm^3 $percent^{-1}$)	Loaf Volume/QGF (cm^3 $percent^{-1}$)	Loaf Volume/Protein (cm^3 $percent^{-1}$)
NEWANA	0.99 **	0.91 NS	0.97 **	0.52 NS
FORTUNA	0.99 **	0.18 NS	0.99 **	0.01 NS
MT 7819	0.99 **	0.36 NS	0.88 *	-0.86 NS
MT 8182	0.99 **	0.53 NS	0.94 *	-0.60 NS

*, ** Significance at P<0.05 and P<0.01, respectively.
NS Non-significance (P>0.05).

vary the proportion of QGF with differential ET. Therefore, any variation in baking quality of this accession over environments is due to factors other than percent QGF.

The high correlation between QGF and baking quality may provide a potentially useful and easily measured quality parameter for hard wheats. The apparent stability of QGF percentage in Fortuna may also be a desirable character which, if bred into a broad array of hard wheat cultivars, could reduce the dependence of quality upon dry low-yield environments.

CHAPTER 6

SUMMARY

The line-source irrigation system was effective in producing controlled soil moisture gradients for evaluation of crop response to differential ET. This system allowed for reduction of non-moisture factor effects which can bias crop-soil moisture response studies conducted at different sites. Soil moisture determinations with a neutron probe allowed comparisons of differences between levels of soil water content, cumulative ET, and estimated maximum rooting depth based on depth of water depletion over time without frequent destructive soil sampling.

The computer software program ETPROBE provided an effective means of managing and analyzing the soil moisture data resulting from frequent neutron probe determinations. The program effectively reduced calculation time from weeks to hours by simultaneously calculating ET and several other interrelated moisture parameters.

Seed yield was generally higher at Manhattan for all accessions over all moisture regimes, with MT 8182 and Fortuna producing the highest and lowest seed yields at this site, respectively. Fortuna also showed the least yield response to increased ET at Huntley, which was a more stressful site with a shorter growing season, lower initial soil moisture and seasonal precipitation, and lower minimum temperatures in the early part of the season. Accessions showed similar rankings for

effects of increased ET on seed yield and WUE. However, seed WUE regressed on increased ET showed differential site responses. Accessions at Manhattan had shallow concave response curves, whereas those at Huntley were convex and increased rapidly over the lower moisture regimes for all accessions. HI was higher for MT 8182 and MT 7819 than for Newana and Fortuna across all moisture levels, and increased with increased ET.

Fortuna had the highest kernel weight over all moisture regimes at Manhattan and over all except the highest regime at Huntley. MT 8182 kernel weight had the greatest response to increased ET at Huntley. Fortuna had the highest plump kernel percentage over all except the highest moisture regime at Manhattan, but had the least change in kernel plumpness over increased ET at this site. Conversely, MT 7819 had the lowest level of kernel plumpness over increased ET at Manhattan. Newana had the greatest plumpness response to increased ET at Huntley and MT 7819 had the least. Fortuna had the lowest kernel number per unit area over all moisture regimes at Manhattan and over the three highest regimes at Huntley.

MT 8182 had the greatest response (decrease) of both kernel and flour protein content to increased ET at both sites. Fortuna had the highest protein levels at the higher moisture regimes at both sites. Newana and MT 8182 consistently showed differences in QGF area between moisture regime extremes at both sites, whereas Fortuna consistently showed no difference at either site. Newana relative QGF area response (slope) to increased ET differed from the other accessions at Manhattan and from all except MT 7819 at Huntley. Additionally, Newana had the

greatest response of all accessions for relative QGF area to increased ET at Manhattan and MT 8182 had the greatest response at Huntley. Fortuna and MT 7819 consistently had the least response of relative QGF area to increased ET at both sites. MT 8182 and Newana consistently had the greatest loaf volume response (decrease) to increased ET at both sites, and Fortuna and MT 7819 had the least response. Additionally, MT 7819 had the lowest loaf volumes over all moisture regimes at both sites. Loaf volumes per relative QGF area percentage were correlated with actual loaf volumes, whereas loaf volumes per total flour protein percentage were not.

These data indicate that soil moisture stress affects kernel and baking quality of bread wheats primarily through the influence on kernel weight and plumpness, which affect yield and protein percentage. Additionally, soil moisture stress is highly correlated with relative hydrophobic gliadin content of some spring wheat accessions.

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