# CARBON ISOTOPE DISCRIMINATION AND WATER-USE EFFICIENCY IN FIELD-GROWN WHEAT

By

JIANFENG CAI

Bachelor of Science
Zhejiang Agricultural University
Hangzhou, China
1982

Master of Science
University of Wisconsin-Madison
Madison, Wisconsin
1990

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1992

CARBON ISOTOPE DISCRIMINATION AND WATER-USE

EFFICIENCY IN FIELD-GROWN WHEAT

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. Brett Carver and Dr. Bjorn Martin for initiating this research project, and for their invaluable assistance and encouragement throughout my graduate program. Many thanks also go to Dr. Edward Smith for serving on my graduate committee, and for his advice in preparing this thesis.

A special thanks is extended to Dr. James Ehleringer of University of Utah for conducting carbon isotope composition analysis. The excellent technical assistance of Wayne Whitemore is also appreciated. I wish to thank Norma Ruiz-Torres and Roger Weyhrich for their wonderful cooperation and assistance.

I wish to thank the Department of Agronomy at Oklahoma State University for providing a research assistantship and for use of research facilities.

I am truly grateful to my wife, Huifen, my parents and my brother for encouraging and supporting me throughout this experience.

TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# CHAPTER I

# INTRODUCTION

Over half the world's land surface is arid or exposed to periodic drought conditions. Productivity in such agricultural situations is largely determined by soil moisture availability (Fisher and Turner, 1978; Boyer, 1982). Agricultural analyses of dry matter production and crop water use have frequently focused on the ratio of these two parameters, with the notion that increasing this ratio would be beneficial in water-limited habitats.

Substantial variation in water-use efficiency (WUE = total biomass production/water use) exists at both the intraspecific and interspecific levels (Stanhill, 1985). However, breeding for increased water-use efficiency in crops has been limited by the lack of screening criteria and methods that can be used to select desirable genotypes from large plant populations under field conditions. Direct methods of measuring WUE require a careful accounting of the amount of water used and are usually conducted in pot experiments. Realistically, these measurements are not well suited for screening germplasms and conducting genetic studies, nor are they fully representative of field-grown plants. Moreover, measurement of rates of photosynthesis and transpiration of individual leaves or canopies by a portable gas-exchange system yields instantaneous water-use efficiency ($WUE_i$) of the leaf at a specific time only. The relationship between such a point measurement and long-term

plant performance is uncertain (Martin and Thorstenson, 1988). Indirect and accurate measurements of WUE in field-grown plants are urgently needed to improve our understanding of plant-water relations and to provide selection criteria in crop improvement programs.

Carbon isotope discrimination ($\Delta$) of plant tissue has been linked to water-use efficiency via the intercellular $CO_2$ concentration in $C_3$ plant species, and may be useful for improving WUE in crops (Farquhar et al., 1989; Condon et al., 1990). Because this approach allows for more extensive and integrative sampling than with traditional approaches, it offers promise as a new tool for exploring long-standing questions on water use and crop productivity. Reliable estimation of water-use efficiency based on $\Delta$ has been clearly demonstrated for plants growing under artificial conditions in isolated stands. However, the relationship between carbon isotope discrimination and water-use efficiency of wheat cultivars must be evaluated under natural crop production conditions ("closed" canopy) before this technology can be adapted for practical wheat improvement programs.

# CHAPTER II

# THEORETICAL RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION AND WATER-USE EFFICIENCY

## Genetic Variation of Water-Use Efficiency

At the single leaf level, the instantaneous water-use efficiency ($WUE_i$) is defined as:

$$WUE_i = \frac{A}{E} = \frac{g_c(C_a - C_i)}{g_w(e_i - e_a)} = \frac{C_a(1 - \frac{C_i}{C_a})}{1.6(e_i - e_a)} \qquad [1]$$

where $A$ and $E$ are rates of photosynthesis and transpiration, respectively; $C_a$ and $C_i$ are partial concentrations of $CO_2$ in ambient air and in intercellular air spaces; $g_c$ and $g_w$ are conductances to diffusion of $CO_2$ and water vapor; $e_i$ and $e_a$ are intercellular and atmospheric vapor concentrations; the factor, 1.6, is the ratio of diffusivities of water vapor and $CO_2$ in air.

From Eq. [1], improved $WUE_i$ may result either from increased efficiency of photosynthetic processes associated with the mesophyll, or from decreased leaf diffusive conductance, primarily stomatal conductance. $WUE_i$ increases if chloroplast photosynthesis become more efficient because $A$ increases independently of $E$. Also reduced stomatal

aperture will cause increased $WUE_i$ because photosynthesis rate is reduced proportionally less than transpiration rate (Cowan, 1982).

During whole plant growth, other losses of carbon and water occur, which are unrelated to a single leaf in the light and are therefore not included in Eq. [1]. A proportion, $\phi_c$, of carbon that is fixed during the day is respired from the shoot at night or from non-photosynthetic organs such as roots during both day and night. $\phi_w$ is the uncontrolled water loss (cuticular, stem, petiole, etc.) as a proportion of daytime stomatal transpiration. At the whole-plant level, WUE is:

$$WUE = \frac{(1-\phi_c)C_a(1-\frac{C_i}{C_a})}{1.6(e_i-e_a)(1+\phi_w)} \qquad [2]$$

## Carbon Isotope Discrimination of $C_3$ Plants

Atmospheric carbon dioxide contains approximately 1.1% of the non-radioactive isotope, $^{13}C$, and 98.9% of $^{12}C$. During photosynthesis, plants discriminate against $^{13}C$ because of small differences in chemical and physical properties imparted by the difference in mass (O'Leary, 1988).

### Measurement of carbon isotope discrimination

Farquhar and Richards (1984) defined carbon isotope discrimination against the heavier isotope $^{13}C$, $\Delta$, as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \qquad [3]$$

where $\delta_a$ is the carbon isotope composition of air (approximately -8 x $10^{-3}$) and $\delta_p$ is the carbon isotope composition of a plant sample, respectively.

Carbon isotope discrimination during photosynthetic $CO_2$ uptake

The carbon isotopic composition of leaves is a manifestation of the isotope fractionation which occurs during the $CO_2$ uptake process, including diffusion, dissolution and carboxylation. The $C_i/C_a$ ratio will differ among plants because of variation in stomatal conductance and the mesophyll capacity for photosynthesis. Indirect screening for $C_i/C_a$ can be made by determining $\Delta$ values of leaves or other plant parts, because $C_i/C_a$ and $\Delta$ are related as follows:

$$\Delta = a - d + (b - a)\frac{C_i}{C_a} \qquad [4]$$

where *a* is the discrimination occurring due to diffusion in air (4.4 x $10^{-3}$); *d* accounts for discrimination due to dissolution of $CO_2$ in the water in the cell wall, liquid-phase diffusion, and respiration (1 - 3 x $10^{-3}$); and *b* is the net discrimination caused by carboxylation (discrimination by Rubisco, approximately 27 x $10^{-3}$). Measurement of $\Delta$, therefore, provides a long-term, productivity-weighted estimate of $C_i/C_a$ since carbon is being continually fixed by the leaf. Carbon isotope discrimination could be useful as a long-term indicator of plant metabolism.

## Carbon Isotope Discrimination and Water-Use Efficiency

The relationship between WUE and Δ is not causal but derives from their independent links with $C_i/C_a$. By combining Eq. [2] and Eq. [4], we can relate water-use efficiency to carbon isotope discrimination:

$$WUE = \frac{(1-\phi_c)C_a(b-d-\Delta)}{1.6(e_i-e_a)(b-a)(1+\phi_w)} \qquad [5]$$

If all components in the expression above, except for Δ and WUE, are assumed constant, we can estimate WUE from Δ. Δ could be a selection criterion for greater WUE if it provides an integrative assessment of genotypic variation in leaf $WUE_i$. The validity of Eq. [5] has been demonstrated at the plant level for several $C_3$ species, including barley (Huibick and Farquhar, 1989), peanut (Huibick et al., 1986), wheat (Condon et al., 1990), and tomato (Martin and Thorstenson, 1988). Initial screening has revealed that genotypic variation in Δ is heritable, so measuring Δ holds promise as a valuable tool for plant WUE selection. While empirical results at leaf and pot-grown plant levels are consistent with Eq. [5], problems could result when scaling to large and closed canopies where variation in stomatal conductance may be unimportant when superimposed on the quantitatively smaller canopy boundary layer conductance (Farquhar et al. 1989). The application of Δ as a selection criterion to wheat improvement will depend on verification of this close relationship under field conditions.

# CHAPTER III

# ESTIMATION OF WATER-USE EFFICIENCY OF FIELD-GROWN WHEAT SELECTED FOR CARBON ISOTOPE DISCRIMINATION

## Introduction

Improvement in water-use efficiency (WUE = total biomass production/water use) may lead to increased yield in environments where crop growth is water-limited. Genetic variation in WUE exists among and within crop species (Stanhill, 1985). Breeding cultivars with increased WUE has been limited by the lack of suitable screening criteria. Direct measurement of WUE of pot-grown plants is both time-consuming and labor-intensive, which limits their usefulness in breeding programs. No method has existed to select the most water-use efficient plants from segregating breeding populations in the field.

The carbon isotope discrimination ($\Delta$) of plant tissue has been correlated with WUE in wheat and other $C_3$ crops (Condon et al., 1987; Ismail and Hall, 1992; Martin and Thorstenson, 1988). Plant leaves discriminate against the naturally occurring and heavier isotope of carbon ($^{13}C$) during diffusion and fixation of $CO_2$ in photosynthesis. The extent of the net discrimination is a function of the ratio of the intercellular to atmospheric $CO_2$ concentrations ($C_i/C_a$) (Farquhar et al., 1989). Theory proposed that $\Delta$ measured in plant dry matter should be positively correlated with $C_i/C_a$ ratio, but should be negatively associated with WUE. Variation in $C_i/C_a$ ratio, and therefore $\Delta$ and WUE,

could result from differences in stomatal conductance or from differences in photosynthetic capacity among genotypes.

Considerable genotypic variation for Δ has been demonstrated in wheat (Condon et al., 1987; Ehdaie et al., 1991). In the greenhouse, Δ of wheat dry matter was negatively correlated with WUE and positively correlated with $C_i/C_a$, and variation in $C_i/C_a$ was attributed about equally to variation in leaf conductance and photosynthetic capacity (Condon et al., 1990). Estimates of broad-sense heritability for Δ averaged > 95% in field experiments where common genotypes were grown in numerous environments (Condon and Richards, 1992). Δ was positively associated with aboveground yield and grain yield in field experiments (Ehdaie et al., 1991).

However, a significant correlation has not yet been demonstrated between WUE and Δ of wheat genotypes in field experiments. The relationship between Δ and WUE must be verified under natural crop production conditions before this criterion can be applied to wheat improvement programs. Objectives of this study were to identify variability in yield, gas exchange characters, and Δ of wheat genotypes grown under rainfed conditions in the field and under a rainout shelter; and to define the relationship between Δ and WUE measured under natural crop production conditions ("closed" canopy) vs. that of isolated spaced plants ("open" canopy).

# Materials and Methods

## Experiment One

Experimental materials were 24 winter wheat genotypes of diverse drought tolerances based on grain yield records collected under drought and well-watered conditions at the same location (E.L. Smith, unpublished data). The field experiment was conducted at the Agronomy Research Station at Stillwater, Oklahoma in 1991, where the soil type is a Kirkland silt loam (a fine, mixed, thermic Udertic Paleustoll). Rainfall during the crop season was 410 mm, 43% of which was received in May. Plots were fertilized prior to planting according to soil-test recommendations. Plant materials were grown in a randomized complete block design with four replications in the field. Each plot consisted of four rows (3 m long with 0.3 m row spacing), seeded at a rate common to the southern Great Plains (250 seed $m^{-1}$). Gas exchange measurements were made between 1100 and 1600 h (CST) during anthesis (April 6, 1991) with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). The system was programmed to determine the net $CO_2$ assimilation rate ($A$), transpiration rate ($E$), stomatal conductance ($g$), and intercellular $CO_2$ concentration ($C_i$). Leaf instantaneous water-use efficiency ($WUE_i$) was calculated as the ratio of net $CO_2$ assimilation rate to transpiration rate ($WUE_i = A/E$, µmol $CO_2$/mmol $H_2O$). Measurements were made at an irradiance level above 1000 µmol photons PAR $m^{-2}s^{-1}$ on the youngest, fully-expanded leaf on two plants of each plot. A typical measurement required 45 seconds, during which the leaf-to-air vapor pressure gradient remained relatively constant. Immediately following

gas exchange measurement, the measured leaf section was excised and placed into a chilled test tube sealed with plastic stoppers to avoid water loss. Leaf area (one surface) was determined using a LI-COR LI-3000 area meter. Fresh, turgid (24 h rehydration in distilled water), and dry (70 °C for 72 h) weights were determined for calculation of leaf relative water content (RWC).

At maturity, ten plants were randomly harvested at the soil surface from two interior rows of each plot. Sampled plants were dried at 70 °C for 4 days and then ground to a fine powder using a Thomas-Wiley laboratory mill (Model 4, Arthur U. Thomas Co., PA). A subsample was prepared to measure the stable carbon isotope composition by ratio mass spectrometry following the techniques of Ehleringer et al. (1990). Carbon isotopic discrimination ($\Delta$) was calculated assuming a carbon isotope composition of ambient air relative to the Pee Dee belemnite standard of $-8 \times 10^{-3}$, as described by Farquhar et al.(1989). Two interior rows were harvested by machine to determine aboveground dry matter, grain yield, and harvest index.

Experiment Two

The same set of 24 wheat genotypes was assigned to 3 m single-row plots inside a rainout shelter. Each plot was separated by a common single border row of cultivar TAM W-101. The planting arrangement was a randomized complete-block design with four replications. The rainout shelter was covered with two layers of plastic films after April 1, 1991. Two gas exchange measurements were made on April 4 (jointing stage) and April 27 (grain-filling stage). Drought stress symptoms were

evident throughout the grain-filling stage. Gas exchange of fully-expanded leaves of two plants per plot were measured as described above. At maturity, ten interior plants of each plot were harvested at the soil surface, and then a 0.6 m length of row per plot was hand-harvested. Aboveground dry matter, grain yield, and harvest index were measured. Drought resistance of each genotype was expressed as a function of grain yield obtained inside the rainout shelter relative to grain yield in the field, with adjustment of plot area. Sampled plants were dried and ground for measuring Δ as described above. Means of three replicates were used in regression analysis for Δ values of plants grown inside the rainout shelter vs. in the field.

Experiment Three

Twelve wheat genotypes (Table 1) representing the range of Δ values in 1991 were grown at the Stillwater Agronomy Research Station in 1992. Seeds were sown in flats in the greenhouse on Oct. 2, 1991. Seedlings with two to three leaves were moved into a cold room (about 10 °C) for one week. The plants were transplanted to the field on Nov. 3. The experiment was conducted according to a split-plot design with four replications. Three combinations of canopy density and containerization (containerized plants in open or closed canopy, and non-containerized plants in closed canopy) were assigned to whole plots. Genotypes were assigned to split plots.

Split-plot experimental units in the containerized whole-plot treatments consisted of a tube-in-tube arrangement (outside PVC tube, 12.7 cm diam x 83.8 cm length; inside PVC tube, 10.2 cm diam x 81.3 cm

length). This arrangement facilitated removing and weighing of internal tubes and minimized temperature differences between field soil and tubes. In a whole plot, 12 outside tubes were imbedded 1.2 m apart within two parallel rows with 96 cm row spacing. Each inside tube was sealed at the bottom and filled with about 2 kg of Redi-Earth peat-lite mix (Grace Sierra Co., CA). The inside tube was positioned such that its rim was 1 cm above the rim of the outside tube. The space between the two tubes was sealed at the top with silicone gel. Each tube was topped around the plant with a layer of small white rock to reduce soil evaporation.

One plant of each genotype was planted per tube. In addition, four plantless tubes per treatment were used to correct for soil evaporation and precipitation. In one set of split plots, the row space between tubes was filled with wheat cultivar Chisholm at normal planting density (containerized plants in closed canopy) , while in another set the inter-plot space remained open (containerized plants in open canopy). The third whole-plot (non-containerized plants in closed canopy) was as described above, except that plants were grown without tubes (direct planting in the field). Replications were placed along a north/south line (southern winds predominate).

After transplanting, 0.4 g of water-soluble 20-9-17 N-P-K [20-20-20 oxide form] fertilizer was applied, giving 0.1 g N plant$^{-1}$, which corresponded to 36 kg N ha$^{-1}$ on an area basis (Johnson and Tucker, 1982). During the growing season, each plant was watered 7 times with a total 7.8 kg water. Dead leaves collected during the season were included in the final measurement of total dry matter. The entire plot

area was enclosed within a rainout shelter, and was covered with one layer of plastic film after May 14 to prevent heavy preharvest rain from falling into the tubes. Gas exchange measurements were made on the topmost fully-expanded leaf of each plant from jointing to grain-filling (April 4, April 23, April 28, and May 5, 1992), as described above. Heading day ranged from 193 to 209 days past sowing.

Tubes were weighed at the start of the experiment and again at the end. The weight difference between the start and end, plus the amount of water added, minus the same difference of plantless tubes (correction for precipitation and soil evaporation) represented the season-long water use by each plot. At maturity (June 5), plants were harvested at the soil surface. Roots were washed free of soil. Shoots and roots were separately dried at 70 °C to a constant weight. Weights of roots and shoots were summed to measure the total plant dry matter. The season-long water-use efficiency was determined by dividing the total plant dry matter weight by the total water use. Shoots from three replications were used to prepare samples for carbon isotope discrimination analysis as described above.

Experiment Four

This experiment was conducted in the greenhouse at the Stillwater Agronomy Research Station in 1992, as a supplement to Experiment 3. The greenhouse was well-ventilated and maintained at about 20 °C. Humidity was not controlled. The same set of 12 genotype was sown in flats on Nov. 26, 1991. At the three- to four-leaf stage, seedlings were moved outside for vernalization for 40 days. In the greenhouse, plants were

transplanted into PVC tubes (10.2 cm diam x 40.6 cm length), containing Redi-Earth peat-lite mix. Each tube was sealed at the base and placed on the ground. After transplanting, 0.4 g of water-soluble 20-9-17 N-P-K fertilizer was added to each tube. Every week these tubes were weighed and watered twice. Water loss was replaced so that the soil water content was maintained above 75% of the maximum gravimetric soil water content. Five plantless tubes were used to estimate soil evaporation. Tubes were placed 20 cm apart and arranged in three randomized complete blocks. Placing of tubes within blocks was changed randomly on a weekly basis to reduce effects of non-uniform environment inside the greenhouse. Gas exchange measurements were made on the penultimate leaf on March 21 and the flag leaf on March 25. At maturity, tiller number, aboveground dry matter, grain yield, and root dry weight were recorded. The season-long water-use efficiency was determined. Carbon isotope discrimination of plants was analyzed as described above.

## Results and Discussion

### Experiments One and Two

The 24 wheat genotypes listed in Table 1 are ranked from small to large Δ in the field experiment in 1991. The Δ value varied significantly among genotypes grown in the field, but not in the rainout shelter. Plants in the rainout shelter had higher CVs and LSDs for aboveground yield and Δ. Hall (1990) reported that cowpea plants grown under extreme drought stress had higher CVs for quantitative properties such as grain yield than those grown under well-watered conditions. The mean of Δ value was significantly lower in the rainout shelter, probably

because of lower stomatal conductance, as previously observed in studies with wheat (Condon et al., 1990; Read et al., 1991) and other species (Hall et al., 1992; Martin and Thorstenson, 1988). In the field, Δ had a range of $1.38 \times 10^{-3}$ (Table 1). The highest and lowest values corresponded to $C_i/C_a$ ratios of 0.79 and 0.73 using Eq. [4]. This was within the range 0.7 ± 0.1 typical of $C_3$ plants (Wong et al., 1979). The combined analysis of variance for Δ across environments showed strong effects associated with environments (field vs. rainout shelter) and genotypes (Table 2). The genotype x environment interaction was not significant, indicating that genotype differences in Δ were generally consistent across environments.

The $C_i/C_a$ ratio was positively correlated between environments ($r=0.43^*$). There was significant variation among genotypes for $C_i/C_a$ in the field, with a differential of 0.148. No relationship was observed between Δ and $C_i/C_a$ in the field or in the rainout shelter. Possible reasons for these results may be a poor relationship between the time-integrated parameter Δ and a point measurement of $C_i/C_a$ (and hence a poor relationship between Δ and $WUE_i$) among wheat genotypes, or a poor relationship between $WUE_i$ at the leaf level and $WUE_i$ at the crop canopy level. The latter may result from the increased influence of boundary layer resistance on transpirational water loss from field canopies (Jarvis and McNaughton, 1986; Cowan, 1988).

Significant negative correlations were found between $WUE_i$ and $C_i/C_a$ in the field ($r=-0.69^{**}$) and the rainout shelter ($r=-0.70^{**}$), as predicted by Eq. [1] (Fig. 1); However, no relationship between $WUE_i$ and Δ was found in either environment. Hall (1990) showed that drought-

induced differences in Δ of cowpea were associated with expected differences in gas exchange efficiency (A/g), whereas genotypic differences in Δ were not. In greenhouse studies with wheat, genotypic differences in A/g were small and were not consistent across experiments (Condon et al., 1990).

In the field, a significant genotypic difference was observed for *E*, but not for *A*, and genotypic means for $WUE_i$ were negatively correlated with *E* ($r=-0.66^*$). Thus, genotypic variation in $WUE_i$ was mainly related to differences in *E* under field conditions. In the rainout shelter, $WUE_i$ was positively correlated with *A* during jointing, but the correlation was moderate ($r=0.59^{**}$, Fig. 2). When drought stress was more severe during grain-filling, $WUE_i$ was highly correlated with *A* ($r=0.86^{**}$, Fig. 2), but not with *g*. Genotypic variation in $WUE_i$ was mainly due to differences in photosynthetic rate under more severe drought conditions. McCree (1974) found that stomata of less water-stressed plants tended to be more sensitive to water deficits than plants that encountered greater water stress. Leaf RWC was positively correlated with *A* ($r=0.40^*$), but not with *E*, indicating that leaf water status affected $WUE_i$ mainly through *A*.

For plants in the field, there was significant genotypic variation for biomass and grain yield. A slight positive correlation existed between Δ and biomass in the rainout shelter ($r=0.36$, $P=0.07$). There were positive correlations between biomass and *g* ($r=0.38^*$) and between grain yield and *g* ($r=0.42^*$) during jointing in the rainout shelter. Ehleringer (1990) explained that the increased dry matter production of high Δ lines probably resulted from lower stomatal limitation to

photosynthesis in these lines. Stomata impose a diffusional limitation on the rate of $CO_2$ uptake by a leaf, resulting in different intercellular $CO_2$ concentrations among cultivars. Positive correlations between *g* and Δ in studies with common bean and wheatgrass (Ehleringer, 1990), and with well-watered wheat (Condon et al., 1987) supported the hypothesis that stomata represented a significant barrier to $CO_2$ diffusion. By inference, an increase in stomatal conductance could contribute to increased dry matter production. Δ is a long-term parameter of plant metabolism. In our experiment, most of the dry matter was produced before the drought treatment commenced. No relationship existed between Δ and drought resistance measured by the ratio of grain yield retained in the rainout shelter to grain yield in the field.

Experiment Three

Genotypic variation was observed for Δ, aboveground yield, tiller number, water use, and water-use efficiency (Table 3). Strong environmental treatment effects were associated with Δ, aboveground yield, and tiller number. Genotypic means for Δ were lower in the open-canopy treatment than in the closed-canopy or direct-planting treatment (Table 4). Greater soil evaporation in the open-canopy treatment reduced stomatal aperture and consequently decreased Δ values, as found in a study with field-grown wheat (Ehdaie et al., 1991). Plants in the containerized treatments had higher tiller numbers and aboveground yields than those in the direct-planting treatment. Better soil and moisture conditions at the tillering stage might contribute to that result. However, genotypes exhibited mixed responses of aboveground dry

matter to environmental treatments. While cultivars Abilene, TAM W-101, Pioneer 2157, and TAM 200 in the direct-planting treatment produced the same amount of aboveground yield as in those in closed-canopy and open-canopy treatments, the other genotypes had greatly reduced aboveground dry matter in the direct-planting treatment. The open-canopy treatment resulted in higher WUE than the closed-canopy treatment. Apparently, drier soil conditions in the open-canopy treatment reduced stomatal aperture and increased WUE because photosynthesis rate was reduced proportionally less than transpiration rate.

Genotype x treatment interaction was not significant for Δ, indicating that genetic differences were relatively consistent across three environmental treatments (Table 3). This consistency was also reflected by positive correlations among three environmental treatments (Fig. 3). Lack of genotype x environment interaction was also observed with wheat (Condon et al., 1987), common bean (White et al., 1990), and wheatgrass (Johnson et al., 1990). Condon and Richards (1992) found that genotype x environment interaction for Δ, although often statistically significant, was generally a relative minor component of the variation in Δ. The averaged Δ values across three treatments in 1992 were also positively correlated with the Δ value in the rainout shelter ($r=0.57^*$) and with the Δ value in the field experiment ($r=0.65^*$) in 1991, indicating moderately high repeatability for this parameter between years.

Correlations between Δ and other agronomic characters in three environmental treatments are shown in Table 5. Only in the direct-planting treatment was Δ positively correlated with aboveground yield.

Studies of Δ in relation to dry matter productivity has previously shown variable results. Dry matter productivity and Δ were sometimes positively correlated, as observed by Condon et al. (1987) in field-grown wheat under well-watered conditions. A positive relationship was also observed between grain yield and Δ for both irrigated and drought-stressed wheat (Read et al., 1991). In the greenhouse, Condon et al. (1990) found no relation between aboveground dry matter and Δ for well-watered wheat plants in pots. However, for water-stressed wheat plants, a negative correlation existed between Δ and aboveground dry matter. Ehdaie et al. (1991) showed that Δ was positively correlated with the aboveground yield and grain yield of wheat for dryland field experiments in both years. Condon et al. (1987) indicated importance of stomatal conductance in determining aboveground yield and grain yield.

The ratio of root-to-shoot biomass was positively correlated with Δ in the open-canopy treatment (Table 5). Plants with larger root-to-shoot ratios might have greater requirements for maintenance respiration, resulting in decreased WUE. A negative correlation was observed between the season-long WUE and root-to-shoot ratio in the closed-canopy treatment ($r=-0.63^{*}$). Read et al. (1992) found that the shoot to total dry weight ratio of crested wheatgrass averaged higher for low-Δ clones than high-Δ clones.

The Δ value was negatively correlated with WUE in the closed-canopy treatment (Fig. 4 ), but no correlation was found in the open-canopy treatment. A negative relationship was shown for individual plants in pots or even for small plots in the field (Condon et al., 1990; Ehdaie et al., 1991). Farquhar et al. (1989) suggested that this

relationship might become inconsistent for larger crop areas if the canopy and leaf boundary resistances to heat were very large. In this experiment, the small field area and low leaf area indexes of wheat should not result in low canopy and leaf boundary resistances to heat, and should not change the negative relationship between WUE and Δ value.

No consistent relationships existed between Δ and four measurements of gas exchange characters from jointing to grain-filling, indicating that these point measurements did not correlate well with the time-integrated parameter Δ.

Experiment Four

In the greenhouse, the overall mean and range for Δ of aboveground dry matter were $19.47 \times 10^{-3}$ and $1.17 \times 10^{-3}$, respectively. The Δ values measured in the greenhouse were not correlated with those averaged across three environmental treatments in the field, indicating the environmental difference between the greenhouse and the field influenced Δ rankings. Plants grown in the greenhouse had higher Δ values than in the field. The higher humidity of the air and well-watered status of plants resulted in greater stomatal conductance, which, according to the theory, should result in greater Δ.

Δ value increased slightly with $C_i/C_a$ of flag leaves (r=0.54, P=0.07), which was consistent with Eq. [4]. The expected positive correlation between Δ and $C_i/C_a$ has been tested for various time scales. At the instantaneous scale, Evans et al. (1986) have observed a close agreement between theoretically-expected and observed discrimination values for wheat. Condon et al. (1990) found that Δ of dry matter

increased with $C_i/C_a$ of wheat flag leaves under well-watered greenhouse conditions, and variation in $C_i/C_a$ was divided about equally between variation in stomatal conductance and in mesophyll photosynthetic capacity.

The 12 genotypes varied significantly for aboveground dry matter. A negative correlation existed between aboveground yield and Δ ($r=-0.79^{**}$) (Fig. 5). The amount of water use was positively correlated with aboveground dry matter ($r=0.72^{**}$), root weight ($r=0.90^{**}$), and root-to-shoot ratio ($r=0.73^{**}$). The more water plants used, the more aboveground dry matter was produced, but could not be proportionally transformed into higher grain yield. WUE was positively correlated with grain yield ($r=0.83^{**}$), indicating that higher grain yield in the greenhouse was associated with higher season-long WUE. WUE was negatively correlated with root weight ($r=-0.84^{**}$), indicating that a well-developed root system was actually detrimental to WUE.

## Summary

This paper is the first reporting the relationship between carbon isotope discrimination and water-use efficiency of wheat grown under field conditions typical of commercial wheat production. We confirmed the negative correlation between Δ and WUE in field-grown wheat under closed-canopy conditions, as previously observed in potted-plant experiments. Significant genotypic differences for Δ existed in a broad sample of hard red winter wheat cultivars, and could likely be improved through breeding.

The Δ value was poorly correlated with instantaneous measurements

of WUE in field, rainout shelter, and greenhouse experiments, indicating that the relationship between such a point measurement and the time-integrated parameter, Δ, was weak.

The extent of shading in a typical southern Great Plains wheat canopy is not great as in denser canopies elsewhere. Rates of leaf transpiration and stomatal conductance to water vapor should be closely linked to the ambient environment. Canopy effects may, therefore, have less influence on the extension of results for individual leaves to plants grown under field conditions. The negative relationship between WUE and Δ should remain. There may not be a direct association between Δ and wheat grain yield because several factors may alter dry matter weight independent of Δ. Our results suggest that breeders could use Δ as an indicator in selection for greater season-long WUE in field-grown wheat.

## LITERATURE CITED


Boyer, J. S. 1982. Plant productivity and environment. Science 218:443-448.

Condon, A.G., R.A. Richards, and G.D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci. 27:996-1001.

Condon, A.G., G.D. Farquhar, and R.A. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat: leaf gas exchange and whole plant studies. Aust. J. Plant Physiol. 17:9-22.

Condon, A.G. and R.A. Richards. 1992. Broad sense heritability and genotype x environment interaction for carbon isotope discrimination in field-grown wheat. Aust. J. Agric. Res. 43:921-934.

Cowan, I.R. 1982. Regulation of water use in relation to carbon gain in higher plants. p 589-613. *In*: O.L. Lang, P.S. Nobel, C.B. Osmond, and H. Ziegler (ed.) Encyclopedia of Plant Physiol. II Water Relations and Carbon Assimilation. New Series. Vol. 12B. Springer-Verlag, New York.

Cowan, I.R. 1988. Stomatal physiology and gas exchange in the field. p. 160-172. *In*: W.L. Steff and O.T. Denmead (ed.) Flow and Transport in the Natural Environment: Advances and Applications. Springer-Verlag, New York.

Ehdaie, B., A.E. Hall, G.D. Farquhar, H.T. Nguyen, and J.G. Waines. 1991. Water-use efficiency and carbon isotope discrimination in wheat. Crop Sci. 31:1282-1288.

Ehleringer, J.R. 1990. Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. Plant Physiol. 93:1422-1425.

Evans, J.R., T.D. Sharkey, J.A. Berry, and G.D. Farquhar. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate $CO_2$ diffusion in leaves of higher plants. Aust. J. Plant Physiol. 13:281-292.

Farquahar, G.D., and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11:539-552.

Farquhar, G.D., K.T. Huibick, A.G. Condon, and R.A. Richards. 1988. Carbon isotope fractionation and plant water-use efficiency. p. 21-40. *In*: P.W. Rundel, J.R. Ehleringer, and K.A. Nagy (ed.) Stable Isotopes in Ecological Research. Springer-Verlag, New York.

Farquhar, G.D., J.R. Ehleringer, and K.T. Huibick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Bol. 40:503-537.

Fisher, R.A., and N.C. Tuner. 1978. Plant productivity in the arid and semiarid zones. Annu. Rev. Plant Physiol. 29:277-317.

Hall, A.E., R.G. Mutters, K.T. Huibick, and G.D. Farquhar. 1990. Genotypic difference in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci. 30;300-305.

Hall, A.E., R.G. Mutters, and G.D. Farquhar. 1992. Genotypic and

drought-induced differences in carbon isotope discrimination and gas exchange of cowpea. Crop Sci. 32:1-6.

Huibick, K.T., G.D. Farquhar, and R. Shorter. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Archis*) germplasm. Aust. J. Plant Physiol. 13:803-816.

Huibick, K.T., and G.D. Farquhar. 1989. Genetic variation of transpiration efficiency among barley genotypes in negatively correlated with carbon isotope discrimination. Plant Cell Environ 12:795-804.

Jarvis, P.G., and K.G. McNaughton. 1986. Stomatal control of transpiration: scaling up from leaf to region. Adv. Ecol. Res. 15:1-49.

Johnson, D.A., K.H. Asay, L.L. Tieszen, J.R. Ehleringer, and P.G. Jefferson. 1990. Carbon isotope discrimination: potential in screening cool-season grasses for water-limited environments. Crop Sci. 30:338-343.

Johnson, G.V., and B.B. Tucker. 1982. OSU soil test calibration. Okla. State Univ. Coop. Ext. Serv. Facts 2225.

Martin, B., and Y.R. Thorstenson. 1988. Stable carbon composition, water use efficiency, and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii*, and the $F_1$ hybrid. Plant Physiol. 88:213-217.

McCree, K.J. 1974. Changes in stomatal response characteristics of grain sorghum produced by water stress during growth. Crop Sci. 14:273-278.

O'Leary, M.H. 1988. Carbon isotope in photosynthesis. BioScience 38:328-

336.

Read, J.J., R.C. Johnson, B.F. Carver, and S.A. Quarrie. 1991. Carbon isotope discrimination, gas exchange, and yield of spring wheat selected for abscisic acid content. Crop Sci. 31:139-146.

Read, J.J., D.A. Johnson, K.H. Asay, and L.L. Tieszen. 1992. Carbon isotope discrimination: relationship to yield, gas exchange, and water-use efficiency in field-grown crested wheatgrass. Crop Sci. 32:168-175.

Stanhil, G. 1986. Water use efficiency. Adv. Agron. 39:53-85.

White, J.W., J.A. Castillo, and J. R. Ehleringer. 1990. Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. Aust. J. Plant Physiol. 17:189-198.

Wong, S.C., I.R. Cowan, and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature (London) 282:424-426.

Table 1. Aboveground yield (AY), grain yield (GY), and carbon isotope discrimination (Δ) of 24 wheat genotypes grown in the field and the rainout shelter in 1991.

| Genotypes | Field | | | Rainout shelter | | |
|---|---|---|---|---|---|---|
| | AY | GY | Δ | AY | GY | Δ |
| | --kg $ha^{-1}$-- | | x $10^3$ | --kg $ha^{-1}$-- | | x $10^3$ |
| Chisholm* | 5410 | 2260 | 17.58 | 4790 | 1650 | 16.61 |
| Collin | 5390 | 2290 | 17.58 | 5040 | 1980 | 16.68 |
| Karl | 5690 | 2120 | 17.65 | 7000 | 2410 | 17.11 |
| 2157* | 5180 | 1890 | 17.70 | 4180 | 1370 | 16.49 |
| TAM W-101* | 6770 | 2920 | 17.84 | 4570 | 1580 | 17.01 |
| Stallion | 5490 | 2270 | 17.86 | 5040 | 1710 | 17.25 |
| Pony* | 4260 | 1790 | 17.90 | 5010 | 1930 | 16.49 |
| Siouxland* | 4750 | 1760 | 17.93 | 5790 | 2110 | 16.82 |
| Quantum 554* | 6270 | 2590 | 17.96 | 5540 | 2080 | 17.34 |
| Cimarron* | 6010 | 2700 | 18.05 | 4380 | 1630 | 16.60 |
| Redland | 7230 | 2850 | 18.26 | 4880 | 1620 | 16.56 |
| 2158 | 6700 | 2780 | 18.29 | 5060 | 1810 | 16.55 |
| Mesa* | 6270 | 2810 | 18.31 | 7000 | 2640 | 16.99 |
| Mustang | 5550 | 2370 | 18.32 | 5010 | 1730 | 17.44 |
| Payne | 6110 | 2560 | 18.36 | 5630 | 1830 | 16.99 |
| Hawk | 5320 | 2140 | 18.42 | 5940 | 2160 | 17.25 |
| TAM 200* | 5600 | 2360 | 18.47 | 5790 | 2100 | 17.48 |
| Century | 5920 | 2480 | 18.54 | 3580 | 1370 | 17.11 |
| Quantum 555 | 6350 | 2840 | 18.55 | 4800 | 1800 | 16.29 |
| Cody* | 5200 | 1930 | 18.57 | 5430 | 1650 | 17.29 |
| Lamar* | 6420 | 2360 | 18.62 | 4970 | 1500 | 16.98 |
| Carson | 5590 | 2050 | 18.62 | 4730 | 1730 | 16.82 |
| TAM 105 | 4440 | 1540 | 18.81 | 2700 | 930 | 16.88 |
| Abilene* | 6420 | 2830 | 18.96 | 6110 | 2220 | 17.53 |
| Mean | 5780 | 2350 | 18.21 | 5120 | 1810 | 16.94 |
| LSD(P=0.05) | 780 | 390 | 0.64 | 2190 | 930 | 1.09 |

* Genotypes selected for experiment in 1992.

Table 2. Analysis of variance for carbon isotope discrimination (Δ) of 24 wheat genotypes grown in the field and the rainout shelter in 1991.

| Source | df | Δ |
|---|---|---|
| Environment (Env.) | 1 | ** |
| Rep./Env. | 4 | NS |
| Genotype (Gen.) | 23 | * |
| Gen. x Env. | 23 | NS |

*,** Significant at P=0.05 and 0.01, respectively. C.V. = 3.08%.

Table 3. Selected mean squares from analysis of variance for carbon isotope discrimination (Δ) and other agronomic traits of 12 wheat genotypes under three environmental treatments in 1992.

| Source | df | Mean squares | | | df | Mean squares | |
|---|---|---|---|---|---|---|---|
| | | Δ | Aboveground yield | Tiller Number | | Water use | WUE |
| Env. | 2 | 17.32** | 1950** | 1645** | 1 | 0.75 | 5.21 |
| Gen. | 11 | 0.86* | 247** | 201** | 11 | 0.25** | 0.48** |
| Env.x Gen. | 22 | 0.20 | 253** | 45** | 11 | 0.06 | 0.29 |
| CV(%) | | 2.11 | 20 | 20 | | 2.6 | 7.5 |

*, ** Significant at P=0.05 and 0.01, respectively.

Table 4. Carbon isotope discrimination (Δ) and other agronomic traits of 12 wheat genotypes under three environmental treatments in 1992.

| Treatment | Δ | Aboveground yield | Tiller Number | Water use | WUE |
|---|---|---|---|---|---|
| | $\times 10^3$ | g $plant^{-1}$ | no. $plant^{-1}$ | kg | g $kg^{-1}$ |
| Open-canopy | 17.29 | 51.5 | 28.3 | 10.6863 | 5.646 |
| Closed-canopy | 18.21 | 48.4 | 24.3 | 10.8626 | 5.180 |
| Direct-planting | 18.64 | 39.3 | 16.8 | | |
| LSD (P=0.05) | 0.24 | 5.2 | 2.3 | NS | NS |

Table 5. Correlation coefficients between carbon isotope discrimination (Δ), dry matter traits, ratio of root to shoot (R/S), and water-use efficiency (WUE) of 12 wheat genotypes in field experiment in 1992.

| Traits | Δ | | |
|---|---|---|---|
| | Closed canopy | Open canopy | Direct planting |
| Total dry matter | -0.42 | 0.06 | -- |
| Shoot dry matter | -0.39 | -0.11 | 0.57* |
| Root dry matter | -0.23 | 0.56 | -- |
| R/S | 0.17 | 0.77** | -- |
| WUE | -0.58* | -0.02 | -- |

*,** Significant at P=0.05 and 0.01, respectively.

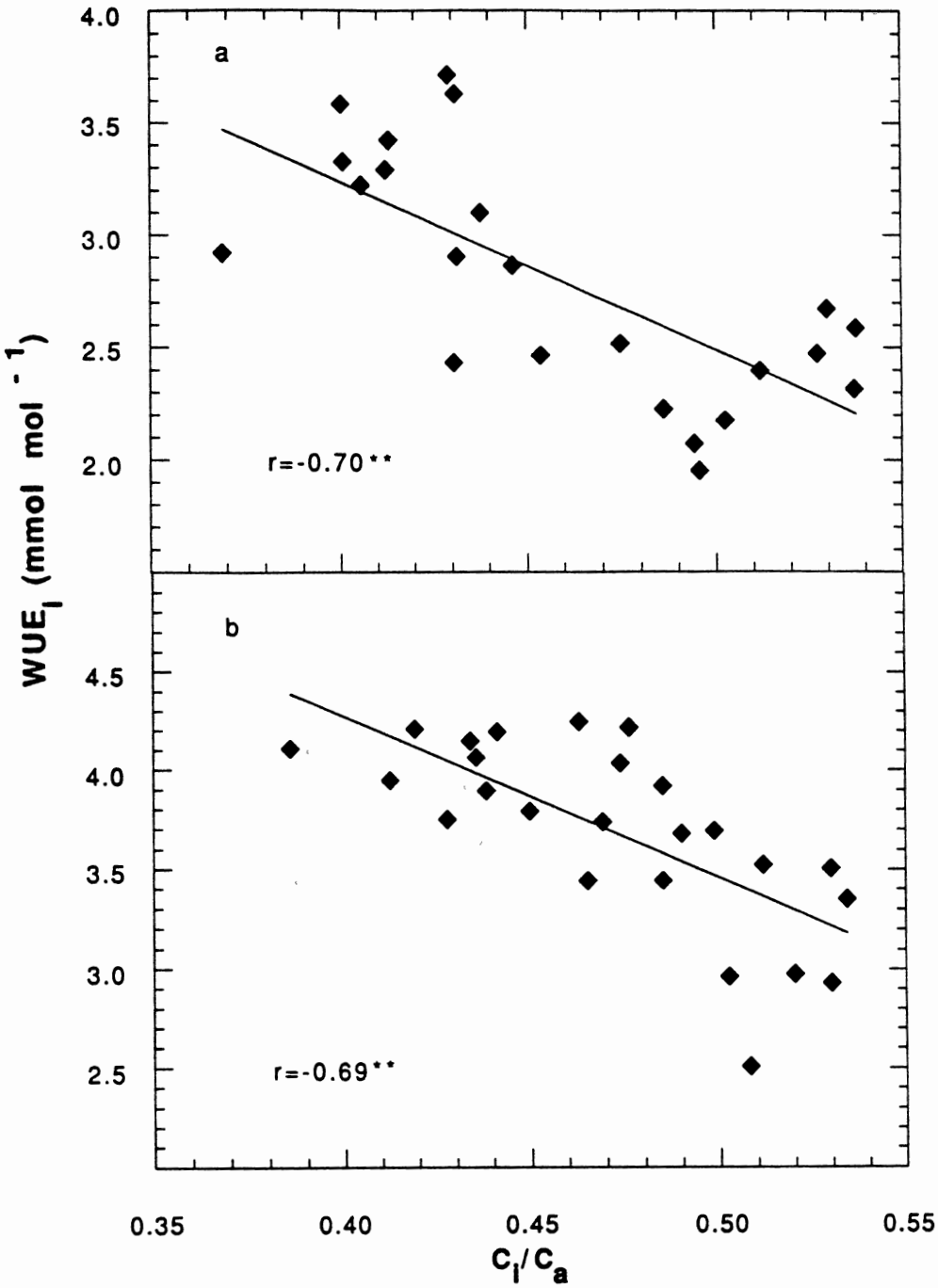


**Fig. 1. Relationship between ratio of intercellular $CO_2$ concentration to ambient $CO_2$ concentration ($C_l/C_a$) and leaf instantaneous water-use efficiency ($WUE_l$) of wheat in the rainout shelter (a) and in the field (b).**

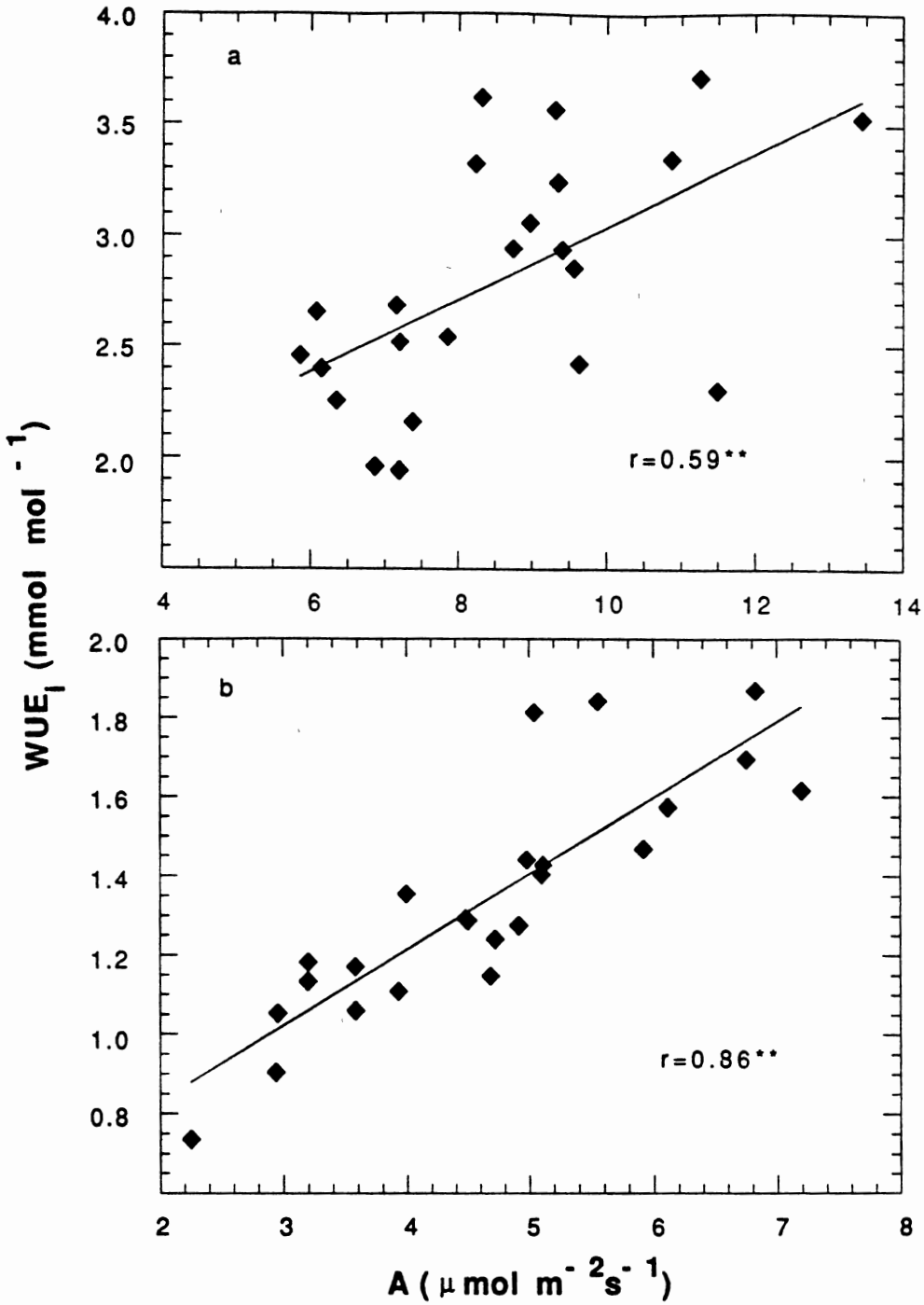


**Fig. 2. Relationship between leaf instantaneous water-use efficiency ($WUE_I$) and photosynthesis rate (A) of wheat during jointing (a) and grain-filling (b) grown under the rainout shelter.**

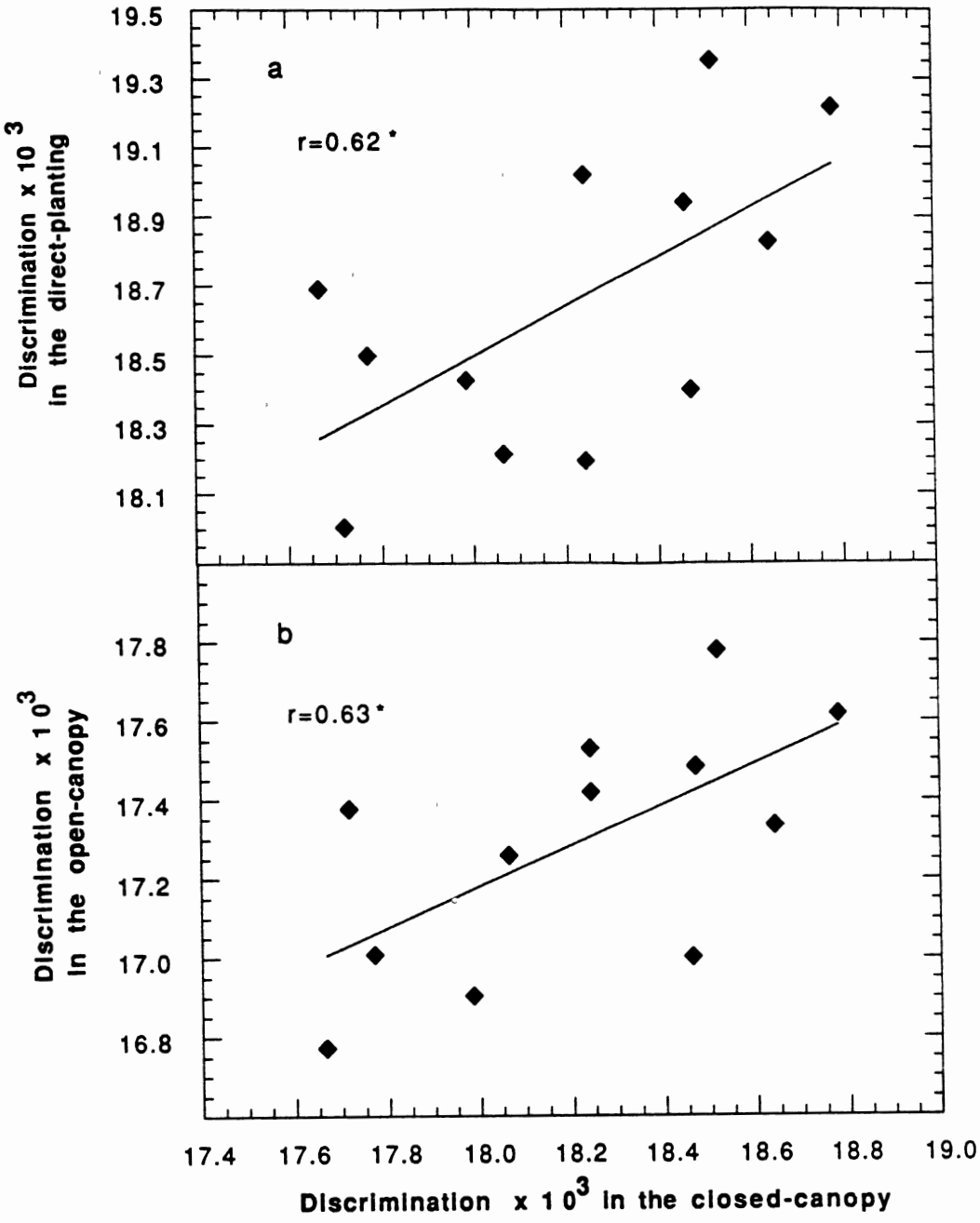


**Fig. 3. Correlations for carbon isotope discrimination of wheat between direct-planting and closed-canopy treatments (a), and between open-canopy and closed-canopy treatments (b).**

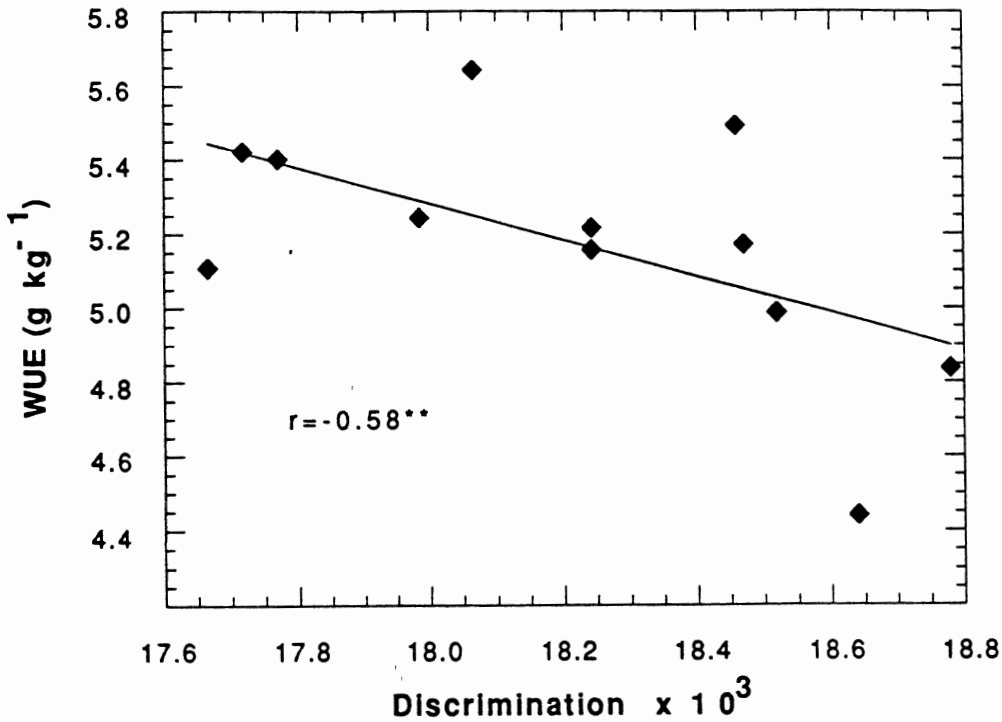


**Fig. 4. Relationship between carbon isotope discrimination and water-use efficiency (WUE) of wheat for the containerized closed-canopy treatment in the field.**

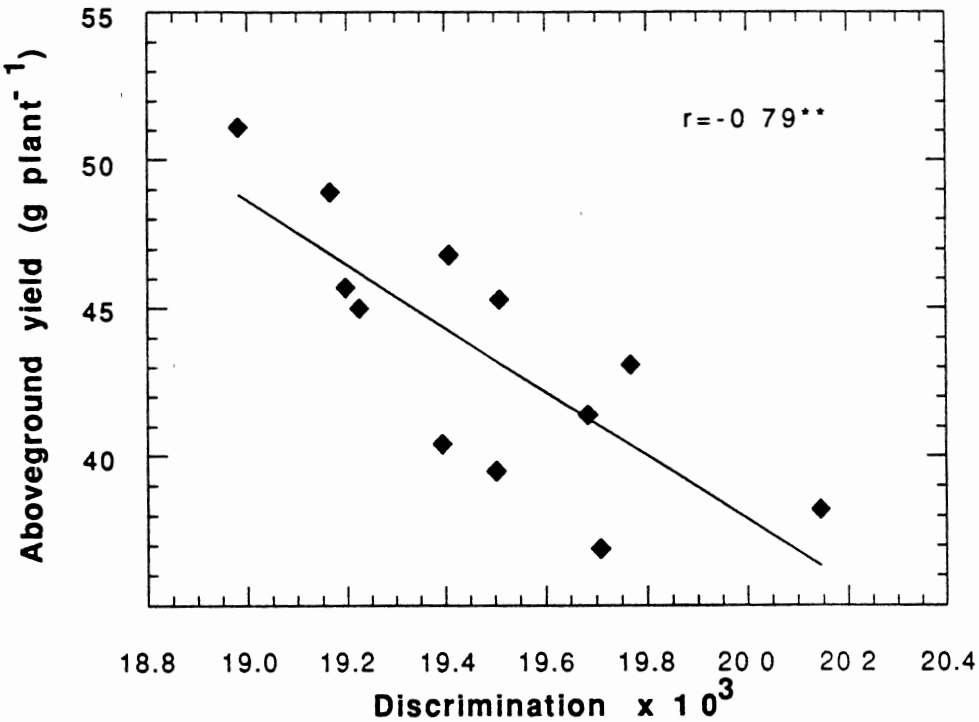


**Fig. 5. Relationship between carbon isotope discrimination and aboveground yield of pot-grown wheat in the greenhouse.**

# VITA

Jianfeng Cai

Candidate for the Degree of

Master of Science

Thesis: CARBON ISOTOPE DISCRIMINATION AND WATER-USE EFFICIENCY IN FIELD-GROWN WHEAT

Major Field: Agronomy

Biographical:

Personal Data: Born in Zhejiang Province, China, January 7, 1963, the son of Daihui Cai and Juanyi Chen.

Education: Graduated for Xinpu High School in 1987; received Bachelor of Science Degree in Agronomy from Zhejiang Agricultural University in China in January 1982; received Master of Science Degree in Agricultural Journalism from University of Wisconsin-Madison in December 1990; completed requirements for the Master of Science Degree in Agronomy at Oklahoma State University in December 1992.

Professional Experience: Junior Researcher, Zhejiang Academy of Agricultural Sciences in China from February 1982 to August 1988; Graduate Research Assistant, Department of Agronomy, Oklahoma State University, January 1991 to December 1992.

Professional Organizations: Chinese Society of Agronomy; American Society of Agronomy; Crop Science Society of America.