PLANT-WATER RELATIONSHIPS FOR SEVERAL COMMON BEAN GENOTYPES (*Phaseolus vulgaris* L.) WITH AND WITHOUT DROUGHT STRESS CONDITIONS

By

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DEDICATION

TO my wife Gloria Milena, She is an infinity source of love and softness, and to our son Pablo, who come to us as a Good blessing

Let your acts be a guide unto all mankind, for the professions of most men, be they high or low, differ from their conduct. It is through your deeds that ye can distinguish yourselves from others.
(Baha'u'llah, Gleanings from the Writings of Baha'u'llah, p. 305)

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Chapter 1

Introduction

The common bean (*Phaseolus vulgaris* L.) is the second most important commercial legume crop after soybean (Sing et al. 1999). The common bean is considered as the centerpiece of the daily diet for more than 300 millon of the world's people. It is considered to be the "perfect food", due at its protein, fiber and mineral content (Beebe and McClafferty, 2006). *P. vulgaris* L is currently widely planted in South, Central and North America, Africa, Asia and in the Caribbean, including Puerto Rico.

The common bean is planted in Puerto Rico and in the Caribbean during October to April, when mean air temperatures are mild and do not induce temperature stress. However, during this period, the rainfall is low and supplemental irrigation is often necessary. The supply of water, therefore, constitutes one of the major constraints to common bean production in the Caribbean. Drought stress is an endemic problem throughout the world and the common bean production under water limiting conditions is common (e.g., Muñoz-Perea et al., 2006; Singh, 2007). Common bean is known as a plant that is susceptible to water deficits, especially in pre-flowering and reproductive periods, producing considerable impact on seed yield.

For this reason, the evaluation, selection and creation of new genotypes with drought tolerant characteristics is an active area of research, and the study of the plant-water relationships associated with these new genotypes, including the determination of water requirements, is necessary.

The primary objectives of this research were to: i) Evaluate the drought response for a local variety or genotype and new genotypes with drought tolerance, ii) Estimate the water requirements for two common bean genotypes including the local variety, ii) Evaluate a low cost-method to estimated water requirements in common bean, and that can be utilized with

other short-season-crops, and iii) evaluate drought-stress detection indices with the selected genotypes.

The results of this study are presented in six chapters; each chapter is more or less a complete study containing methods, results and discussion sections. In some cases, for convenience, another chapter is referred to if a methodology was previously presented.

Evaluation of methodologies to estimate water consumption by micro-meteorogical methods were applied, and indices to detect and evaluated drought stress were also studied. The genotypes in this study were: SER 16, SER 21, SEN 3, SEN 21 and BAT 477, which are germplasm released by CIAT (Centro Intenacional de Agricultura Tropical, Colombia) and 'Morales' the most widely planted variety in Puerto Rico.

Chapter 2 presents a non-destructive method for leaflet area estimation for four of these common bean genotypes. The leaf area is an important variable that was used in the other chapters (3, 4 and 5) as a primary variable in the drought-stress response and evapotranspiration estimation. Chapter 3 presents an evaluation of response to drought stress by these common bean genotypes under field and greenhouse environments. Drought stress response was evaluated in terms of stomatal resistance, leaf temperature, relative water content and leaf area. In Chapter 4, crop water requirements were estimated in terms of the crop evapotranspiration and development of crop coefficient curves, under drought stress and non-drought stress conditions, for two genotypes. Chapter 4 presents correlations between the crop coefficients with physiological parameters. Chapter 5 evaluates several methods for estimating surface resistance, which is the most critical variable in terms of calculation in the Penman-Monteith model, currently the most widely recommended micro-meteorological method for estimating crop evapotranspiration. Chapter 6 describes a study in which canopy temperature, measured by infrared thermometers, was applied for the derivation of the crop water stress index. Critical values of this index were related to yield. Chapter 7 estimates the water use efficiency, transpiration efficiency and yield index for the selected common bean genotypes, under field and greenhouse environments.

REFERENCES

Beebe, S., and B. McClafferty. 2006. Biofortified Bean. Centro Agronomico Tropical (CIAT)-Cali, Colombia. 2p. In: www.harvestplus.org/pdfs/bean.pdf.

Muños-Perea., C.G., H. Terán., R.G. Allen., J.L. Wright., D.T. Westermann., S.P. Singh. 2006. Selection for drought resistance in dry bean landraces and cultivars. *Crop Sci*.46:2111-2120.

Singh, P.S., H. Terán., C.G. Muñoz., J.C. Takegami, 1999. Two cycles of recurrent selection for seed yield in common bean. *Crop Sci.* 39:391–397.

Singh, S.P. 2007. Drought resistance in the race durango dry bean landraces and cultivars. *Agron.J*. 99:1219-1225.

Chapter 2

Development of Linear Models for Non-Destructive Leaflet Area Estimation in Common Bean (*Phaseolus vulgaris* L.) Using Direct Leaflet Measurements

ABSTRACT

Plant leaf area is an important physiological trait used in plant physiology, agroclimatology, soil and crop science studies. Direct, non-destructive methods for estimating leaf area have been shown to be effective and also allow for repeated plant sampling. The objective of this study was to evaluate direct, non-destructive leaflet measurements as predictors of actual leaflet area (LA), test previously developed models, and develop genotype-specific linear models for leaflet area estimation in common bean (*Phaseolus vulgaris* L.). Four common bean genotypes were evaluated, BAT 477, 'Morales', SER 16, and SER 21, under greenhouse conditions, for development of appropriate mathematical models for leaflet area estimation. The greenhouse-derived models were applied and evaluated under field conditions using two years of data. Previously developed models were tested and were found to overestimate or underestimate leaflet area. Leaflet measurements included maximum leaflet width (W) and maximum leaflet length (L), which were used to calculate a third variable, LxW. The measurements with the highest values for the coefficient of determination (R^2) were W or LxW for BAT 477, SER 16, and Morales (0.97, 0.95, and 0.95, respectively), and LxW for SER 21 (R^2=0.96). The linear models developed were shown to be effective and robust for predicting leaflet area under both greenhouse and field conditions, and during both vegetative and reproductive stages of plant development.

Key words: Canopy area, legume, leaf length, leaf width, leaf area.

Abbreviations: W, maximum leaflet width; L, maximum leaflet length; LA, leaflet area; RMS, residual mean squares

INTRODUCTION

Leaf area affects light interception, gas exchange, evapotranspiration, and growth rate in plants. Leaf area is often used as an important component in crop modeling (e.g., van Oijen et al., 2005; Wallach et al., 2001), as an indicator of crop growth and productivity (Kandiannan et al., 2002), and as a key variable in plant interaction with the atmosphere (Brenner et al., 1995). Although several models are available for leaflet area estimation in bean, these general models have not been compared to genotype-specific models, and triofoliolate and leaflet morphology can vary significantly in *P. vulgaris*.

LA can be determined either directly or indirectly and using destructive or non-destructive methods (Brenner et al., 1995). To directly determine the area of individual leaves, leaf area meters or leaf imaging are used (Marshall, 1968; Yang and Alley, 2005), while indirect estimation, e.g. multiband vegetation imaging, plant canopy analysis, and hemispherical photography, is based on factors correlated with leaf area (Strachan et al., 2005). For destructive LA measurements, plants are harvested, leaves are separated, and leaf area is measured to obtain the leaf area per plant. Alternatively, using non-destructive methods, plants are left intact and leaf area is estimated based on calculations from combinations of leaf length and width measurements (Wiersma and Bailey, 1975; Wilhem et al., 2000; Gamper, 2005). Non-destructive methods offer the advantage that repeated sampling of the same plant can be conducted over time, which is especially important when studying genetically segregating populations (De Swart et al., 2004), or plant development. Measuring linear dimensions of leaves is an established and successful method for the direct, non-destructive estimation of leaf area (Bange et al., 2000, Lu et al., 2004) and has been used extensively in crop plants, including soybean (Wiersma and Bailey, 1975), sunflower (Bange et al., 2000), black pepper (Kandiannan et al., 2002), common bean (Bhatt and Chanda, 2003), grape (Williams and Martinson, 2003), *Capsicum* (De Swart et al., 2004), taro (Lu et al., 2004), sugar beet (Tsialtas and Maslaris, 2005), corn (Yang and Alley, 2005) and white clover (Gamper, 2005). The accuracy of the estimations, however, is dependent on the variation in leaf shape within a single plant, within genotypes, or among genotypes in a species (De Swart et al., 2004).

For a number of species the relationship between leaf dimensions and leaf area has been sufficiently consistent to allow for the development of mathematical models for leaf area estimation based on leaf measurements. De Swart et al. (2004) developed several methods to estimate leaf area in *Capsicum*, and found that LA= 0.690 x (LxW) was the best model. This model was not dependent on plant age and/or genotype, and thus could be used for leaf area estimation of different genotypes and of plants at all growth stages. Tsialtas and Maslaris (2005) determined a linear correlation between maximum leaf width and leaf area in sugar beet. Kandiannan et al. (2002) developed allometric models to measure leaf area of individual leaves in five genotypes of black pepper using leaf length (L). The models used were of the type $A=aL^b$, where a and b were constants. Bange et al. (2000) found that the most appropriate model for the relationship between linear dimensions and area of an individual leaf in sunflower included both the length and width dimensions, while, by using only one dimension, it was possible to estimate LA with considerable time savings (Wiersma and Bailey, 1975).

In common bean, Cintra de Jesus et al. (2001) mentioned an empirical model developed by Iamauti (1995), for measuring leaf area. Bhatt and Chanda (2003) found a linear correlation between leaflet area and the product of length and width (LxW) and the sum of length and width (L+W) in *P. vulgaris*. The use of leaf area models in common bean was found to reduce sampling effort and cost (Bhatt and Chanda, 2003) and is especially helpful in studies where the leaf area is correlated with other field variables. Leaflet area can subsequently be used for morphological studies or for the estimation of total plant leaf area. However, based on our experience, variability between cultivars or genotypes results in under or over-estimation of leaflet area with these general models and may require the development of more specific models.

The objectives for this study were to first, develop non-destructive, genotype-specific leaflet area estimation models; second, evaluate and compare the previously developed general models with these specific models; and third, test the robustness of the genotype-specific models across divergent environments, stages of plant development, and plant densities.

MATERIALS AND METHODS

Greenhouse experiment. The experiment was conducted at the USDA-ARS Tropical Agricultural Research Station in Mayagüez, Puerto Rico. The greenhouse experiment was planted 23 September 2005 and the average daily temperature was 29.4/24.3ºC (day/night) during the period from planting to harvest. Four common bean (*P. vulgaris* L.) genotypes were planted, including 'Morales', BAT 477, SER 21 and SER 16. Morales is a small white variety (Beaver and Miklas, 1999) while BAT 477, SER 16, and SER 21 are germplasm releases from CIAT (Cali, Colombia). Morales, SER 21 and SER 16 have a type II and BAT 477 has a type III plant architecture. Each genotype was planted in 24 round Pods (15 cm x 11 cm) and Pods were arranged in a randomized complete block design (RCBD) with four replicates. Sunshine mix #1 (Sun Gro Horticulture, Vancouver, British Colombia) was used as the potting mix, two seeds were planted per pot, the plants were fertilized with Osmocote (14-14-14, N-P-K; Marysville, OH), and the plants were thinned to one plant per pot one week after planting. Leaflet samples (one leaflet from each trifoliolate) were collected during vegetative (V3, three nodes in the main stem including the primary leaf node) and reproductive (R4, pods 3 inches long, seeds not discernible) growth stages. Twenty randomly selected individual leaflets from 20 plants were selected for measurement during each sampling.

Field experiments. Two field experiments where carried out at the University of Puerto Rico Agricultural Experiment Station at Juana Diaz, Puerto Rico (18^{o}01'N and 66^{o}22'W, 21 masl). These experiments were planted on 3 February 2006 and 17 January 2007. The average daily temperature in 2006 was 28.8/19.7ºC (day/night), and in 2007 was 27.2/22.9^{o}C during the period from planting to harvest. The plants received 472 mm of water through drip irrigation and rainfall during 2006 and 433 mm during 2007. Fertilizer (16-4-4, NPK) was applied at a rate of 560 lb per hectare and weeds were controlled through cultivation and herbicide application. Two genotypes with similar architecture and phenology were sown in 2006, Morales (13.5 plants m^{-2}) and SER 16 (6.5 plants m^{-2}). During 2007, SER 21 and BAT 477 were planted, in addition to Morales and SER 16, at a plant density of 8.5 plants m^{-2}. Both experiments were arranged in an RCBD, with four replications in 2006 and five replications in 2007. Leaflets were collected on a single day during the vegetative (V) and reproductive (R) growth stages. Twenty five plants of

each genotype and 20 leaflets per plant were randomly selected for measurement at each growth stage.

Leaflet area determination. In the greenhouse trial, actual leaflet area was determined using the ImageJ (version 1.24) program (Rasband, 1997). ImageJ is a public domain image analysis program that can be used to determine areas from images using one or more known measurements and has been used in similar studies (e.g. Gamper, 2005). The image program was first tested and found to be accurate using images with known areas. For actual leaflet area determination, the individual leaf image was captured using a digital camera and the individual leaflet area was determined using ImageJ (Fig. 2.1). For direct leaflet area measurements, maximum width (W, in cm) and length (L, in cm) measurements of each leaflet were measured using a ruler. Length was measured as the distance between the base and the apex of the leaflet and width was measured perpendicular to the length axis at the position on the leaflet yielding the greatest width. Each measurement was fit to a simple linear regression model and correlation coefficients were estimated. In the field model validation experiment, actual leaflet area was determined using graph paper and maximum W and L data was collected using a ruler.

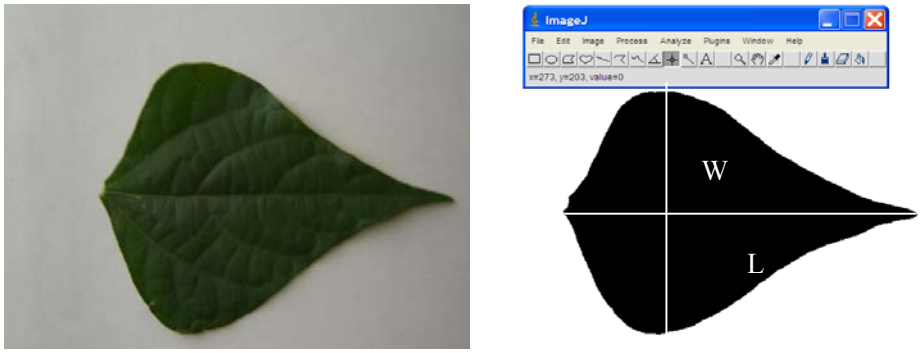

Figure 2.1.Leaflet area analysis using ImageJ. **A**, Digital image of leaflet; **B**, Linear dimensions used.

Model development and testing of previous models. The leaflet area data were fit to single and multiple linear models, and the coefficients of determination (R^2) and the residual mean squares (RMS) were calculated using ANOVA (analysis of variance) to evaluate the model's precision. Model selection and step-wise regression were used to determine the appropriate number of predictors to estimate leaflet area using the coefficient of multiple determination (R^2) and the number of leaflet measurement predictors (K). Two previously developed general models were also evaluated. The Iamauti (1995) model, LA = 2.1371 x ($W^{1.9642}$)-2.7013, where W is the maximum width of the central leaflet of each leaf (cm), developed in Minas Gerais, Brazil, for the common bean cultivar Carioca and the Bhatt and Chanda (2003) model, LA = 11.98 + 0.06 LxW, where L is the leaflet length and W the leaflet width, developed in Gujarat, India. These models were used to estimate leaflet area in the four genotypes and the results compared with the actual leaflet area and the area determined using the genotype-specific models. The accuracy of LA estimation for all of the models were evaluated using ANOVA values of RMSE, slope and R^2, and the Tukey test. All statistical analyses were completed using INFOSTAT Statistical program version 3-University of Cordoba (Argentina).

RESULTS AND DISCUSSION

Accurate and precise models are needed for the estimation of leaflet area in common bean. Initial application of general common bean models did not yield accurate estimations, therefore, genotype specific models were developed and tested in this study and found to be effective and robust.

Genotype-specific model development. In this study, genotype-specific models were developed and high and positive correlations ($R^2 > 0.87$) were observed between individual leaflet area (LA) and linear leaflet dimensions (W, L, and LxW) for the four genotypes tested (Table 2.1). The highest correlation determined based on the coefficient of determination (R^2) and RMS was observed between LA and W ($R^2 > 0.94$), and LA and LxW ($R^2 > 0.95$). Leaflet width and LxW gave identical R^2 values for BAT 477, SER 16, and Morales (0.97, 0.95, and 0.95, respectively), however, LxW was found to have a higher R^2 and lower RMS for SER 21 (R^2=0.96). Leaflet length as a single LA predictor exhibited higher RMS and lower R^2 values as compared with W and LxW, and thus is not as accurate a predictor of leaflet area.

Table 2.1. Results of simple linear regression of leaflet width (W), length (L), and length x width (LxW), with actual leaflet area for four greenhouse grown common bean genotypes.

Genotype	*Slope*	*Intercept*	*R^2†*	*RMS‡*	*p value*
W					
SER 16	9.35	-20.32	0.95	12.21	<0.001
SER 21	7.80	-15.99	0.94	8.52	<0.001
BAT 477	10.73	-29.19	0.97	25.2	<0.001
Morales	7.80	-14.59	0.95	6.90	<0.001
L					
SER 16	6.09	-23.03	0.87	32.70	<0.001
SER 21	5.93	-20.41	0.92	12.16	<0.001
BAT 477	9.10	-42.64	0.87	93.55	<0.001
Morales	5.57	-16.05	0.91	13.16	<0.001
LxW					
SER 16	0.56	1.46	0.95	14.24	<0.001
SER 21	0.53	2.28	0.96	5.08	<0.001
BAT 477	0.62	-0.12	0.97	19.23	<0.001
Morales	0.54	3.04	0.95	6.89	<0.001

† R^2 is the determination coefficient.
‡ RMS is the residual mean square.

High correlations between leaf measurements and leaf area have been found previously in bean and other crops. Bhatt and Chanda (2003) found an R^2 value of 0.74 for W, 0.67 for L, and 0.76 for LxW (p=0.01) for unspecified bean genotypes. Cintra de Jesus et al., (2001) found an R^2 value of 0.97 for the relationship between the leaf area index (LAI) and the central leaflet width (W) in the common bean variety 'Carioca'. In models developed by Williams and Martinson (2003), R^2 values of >0.90 were found between leaf area and L, W, LxW, W^2, and L^2 in grape. Thus, leaf measurements are good estimators of leaf area and often only single predictors are needed for leaf area estimation.

Separate linear models were developed for the relationship between leaflet area and W, L, and LxW for each of the four common bean genotypes. The allometric measures W, L, and LxW were used to fit multiple linear regression models and the simplest model explaining the largest amount of the variance was selected based on an all-subsets-regression procedure through analysis of the relationship between the coefficient of multiple determination (R^2) and the number of individual leaflet measurement parameters (K) (Fig. 2.2). Single predictors were found to explain almost all of the variance and yielded the simplest models. For BAT 477 (Fig. 2.2a), Morales (Fig. 2.2b), and SER 16 (Fig. 2.2d), W or LxW were selected, while LxW was selected for SER 21 (Fig. 2.2c). Although two predictors yielded slightly higher R^2 values using the stepwise procedure (Table 2.2), a simple model, with W as the single predictor (Table 2.1), was sufficient for accurate, efficient and precise leaflet area prediction across genotypes. Thus, in this study, W was selected as the single predictor for leaflet area estimation.

Table 2.2. Step-wise regression results for leaflet area estimation using two predictors for four greenhouse grown common bean genotypes.

Genotype	***Intercept***	***Regression coefficient (W)***	***Regression coefficient (LxW)***	***$R^{2\dagger}$***
BAT 477	-10.72	3.79	0.40	0.98
Morales	-6.04	3.93	0.28	0.96
SER 21	-1.80	1.67	0.42	0.97
SER 16	-11.78	5.50	0.24	0.96

† R^2 is multiple determination coefficient.

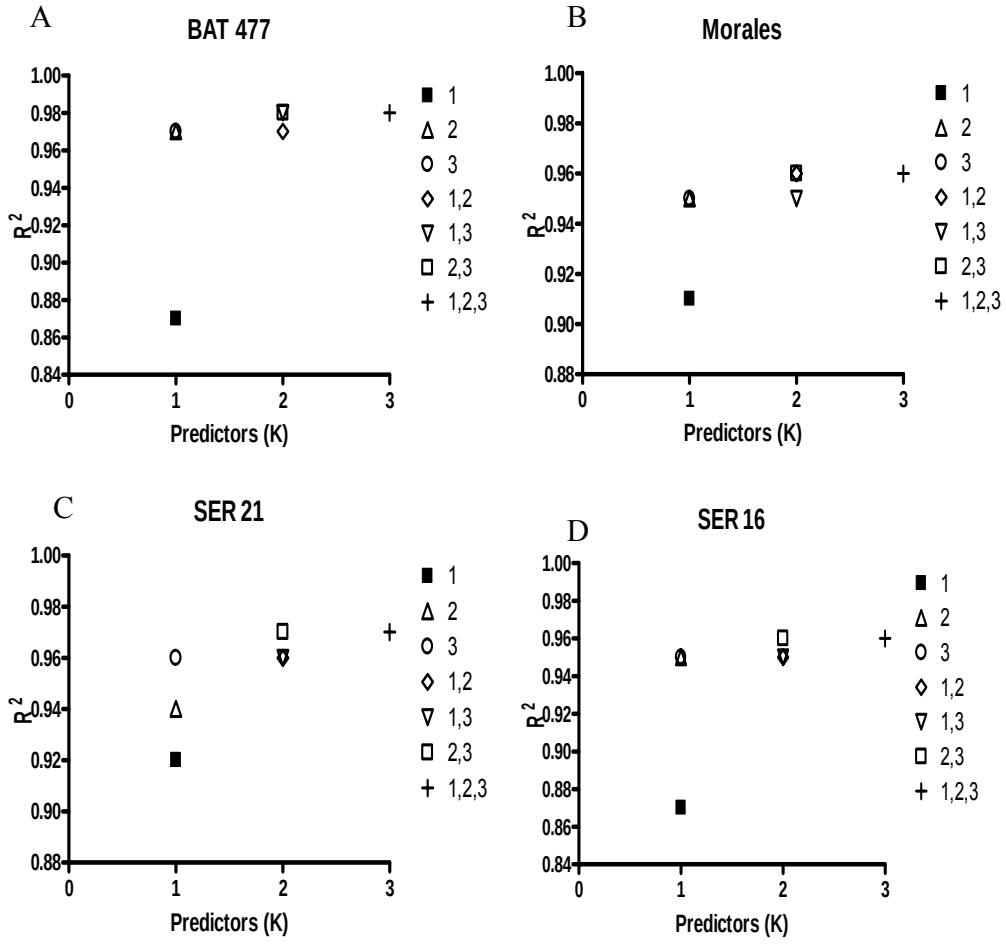

Figure 2.2. Model selection using the plot of the coefficient of multiple determination (R^2) by the number of leaflet measurement predictors (K) for four (**A**, BAT 477; **B**, Morales; **C**, SER 21; and **D**, SER 16) greenhouse grown genotypes.: (1. L; 2. W; 3. LxW; 1,2. L and W; 1,3. L and LxW; 2,3. LxW and W; 1,2,3. L, W and LxW)**.**

Model validation and comparison with previously developed models. The genotype-specific models, developed based on greenhouse data, were then validated with data from field grown plants in 2006 and 2007. No significant differences were found between actual leaflet area and estimated leaflet area from field data using the W model developed from the greenhouse study (Table 2.3). The genotype-specific greenhouse derived models were therefore effective at estimation of leaflet area at both vegetative and reproductive phenological stages, under both the greenhouse and field conditions, and at different plant densities. Under these variable conditions,

no significant differences were observed using W as a single predictor, while using L as a single predictor, significant differences were observed for BAT 477 and SER 21.

The genotype-specific models were also compared with the previously published models (Iamauti, 1995; and Bhatt and Chanda, 2003). Our results indicted that Iamauti's model over-estimated leaflet area in all four genotypes in this study. Bhatt and Chanda's model, on the other hand, under-estimated leaflet area in most cases, however, estimates for Morales and SER16 were not significantly different from actual leaflet area in 2006 (Table 2.3). Therefore, genotype-specific models may be necessary for effective leaflet area estimation in bean. Additional study is needed to determine if race, seed-size, or market class-specific models may yield consistent results given possible similarities in leaflet morphology within these groups of germplasm.

The applicability of the models may be limited to the specific condititions and genotypes associated with this study, and therefore, use of the models under other conditions should be done so with caution.

Table 2.3. Comparison of actual and estimated leaflet area in the field for four genotypes, two years, and two developmental stages using genotype-specific leaflet area models and two previously developed general models.

Method of calculation	Single leaflet area† Cm^2			
	V3‡, 2006			
	Morales	**SER 16**	**SER 21**	**BAT 477**
Actual	17.6 ab (+/-3.42)	22.9 ab (+/-3.3)	nd	nd
W	21.1 b (+/-3.8)	27.7 b (+/-6/4)	nd	nd
L	18.5 ab (+/-4.0)	25.6 ab (+/-5.5)	nd	nd
Iamautti¶	39.9 c (+/-9.1)	58.3 c (+/-20.6)	nd	nd
Bhatt and Chanda§	13.7 a (+/-0.4)	14.4 a (+/-0.22)	nd	nd
	R3, 2006			
Actual	35.0 ab (+/-9.5)	33.8 a (+/-9.5)	nd	nd
W	35.6 b (+/-9.4)	35.3 a (+/-7.8)	nd	nd
L	31.5 ab (+/-8.1)	33.4 a (+/-7.3)	nd	nd
Iamautti¶	84.6 c (+/-31.1)	82.8 c (+/-26.2)	nd	nd
Bhatt and Chanda§	15.5 a (+/-1.2)	15.6 b (+/-1.1)	nd	nd
	V4, 2007			
Actual	19.0 a (+/-5.7)	19.8 a (+/-6.9)	20.5 a (+/-6.3)	24.5 a (+/-7.9)
W	20.7 a (+/-6.5)	21.8 a (+/-6.8)	19.4 a (+/-6.8)	29.1 a (+/-8.1)
L	19.8 a (+/-7.9)	19.0 a (+/-7.3)	15.9 b (+/-7.0)	17.1 b (+/-7.6)
Iamautti¶	42.1 c (+/-13.9)	42.2 c (+/-14.6)	48.5 c (+/-16.7)	58.6 c (+/-19.4)
Bhatt and Chanda§	13.9 b (+/-0.6)	13.9 b (+/-0.5)	13.8 b (+/-0.6)	14.1 b (+/-0.7)
	R6, 2007			
Actual	22.2 a (+/-8.0)	26.4 a (+/-5.4)	22.6 ab (+/-4.8)	28.9 ab (+/-7.2)
W	25.9 a (+/-6.8)	32.2 ab (+/-5.9)	21.3 ab (+/-4.3)	35.7 b (+/-10.0)
L	19.5 a (+/-6.4)	24.7 b (+/-7.4)	18.4 b (+/-4.2)	20.7 a (+/-10.2)
Iamautti¶	53.5 b (+/-22.3)	61.4c (+/-13.5)	57.4 c (+/-15.6)	72.3 c (+/- 22.4)
Bhatt and Chanda§	14.3 c (+/-0.6)	14.5 d (+/-0.80)	14.2 d (+/-0.75)	14.3 d (+/-0.71)

† Different letters denote significant differences, Tukey test ($P<0.05$).
‡ Measured in vegetative and reproductive phases. The values in parentheses represent the standard deviation**.** ¶LA=2.1371 x $(L^{1.9642})$-2.7013 (Iamautti, 1995); § LA = 11.98 +0.06LxW (Bhatt and Chanda, 2003).
nd. No data.
(V3 "Three nodes on the main steam including the primary leaf node", V4 "Four nodes on the main steam including the primary leaf node", R3 "Pods ½-long at first blossom position" and R6 "Seed at least ¼ inch over long axis")

Model robustness. As mentioned, the genotype-specific model based on W as a single predictor of leaflet area was tested across two distinct environments, over two years, at different plant densities, and at different phenological stages and was found to be robust, never yielding results significantly different from the actual leaflet area (Table 2.3). Significant variability was found within genotypes across years in leaflet area (Table 2.4). Thus, there were significant differences both between and within genotypes in leaflet size, yet the models accurately estimated leaflet area based on W. The largest significant differences in leaflet size based on LA were between

BAT 477 (architectural type III) with the other three genotypes (architectural type II) in both the greenhouse and field trials (Table 2.4). Significant variation was also observed in the greenhouse trial between SER 21 (small leaves) and the other three genotypes. Leaflet area also changed with phenology, from vegetative (V) to reproductive (R) growth phases for Morales and SER 16 in the field and greenhouse, and was due to changes in both length and width (data not shown). Notwithstanding this variability, R^2 values of >0.89 were found in a regression analysis of estimated versus actual LA using combined field data from 2006 and 2007 (Fig. 2.3). The variation in leaflet size due to variable environmental conditions did not affect the accuracy of leaflet area estimation, indicating that the genotype-specific models are robust using W as a unique predictor.

Although these models can be used to estimate area from individual leaflet measurements during vegetative and reproductive stages, more precise models may be developed based on developmental stage since morphological variation was also observed in the field evaluation. Linear dimensions were shown to be reliable parameters for generating leaflet area estimates, indicating that the relationship between leaflet width and leaflet area is fairly stable across environmental conditions. Genotype-specific models appear to be more accurate than general models in common bean for leaflet area estimation, however other groupings (such as by common bean race, seed size or market class) may also be effective. Using estimated leaflet area, total leaf area can thus be easily calculated by multiplying by the total number of leaflets.

Table 2.4. Comparison of mean leaflet area of four greenhouse grown genotypes across three environments†.

Genotype	Adjusted means for leaf area (cm^2) ††		
	Greenhouse, 2005	**Field, 2006**	**Field, 2007**
SER 21	**31.06 a**	**nd**	**21.2 a**
Morales	**33.19 b**	**26.3 a**	**20.6 a**
SER 16	**35.10 bc**	**28.3 a**	**22.0 a**
BAT 477	**36.21 c**	**Nd**	**26.0 b**

† These adjusted means are averages of actual leaflet area collected during the vegetative (V3) and reproductive (R3) growing stages.

†† Different letters denote significant differences using Tukey test ($P<0.05$) for adjusted means in a covariance analysis.

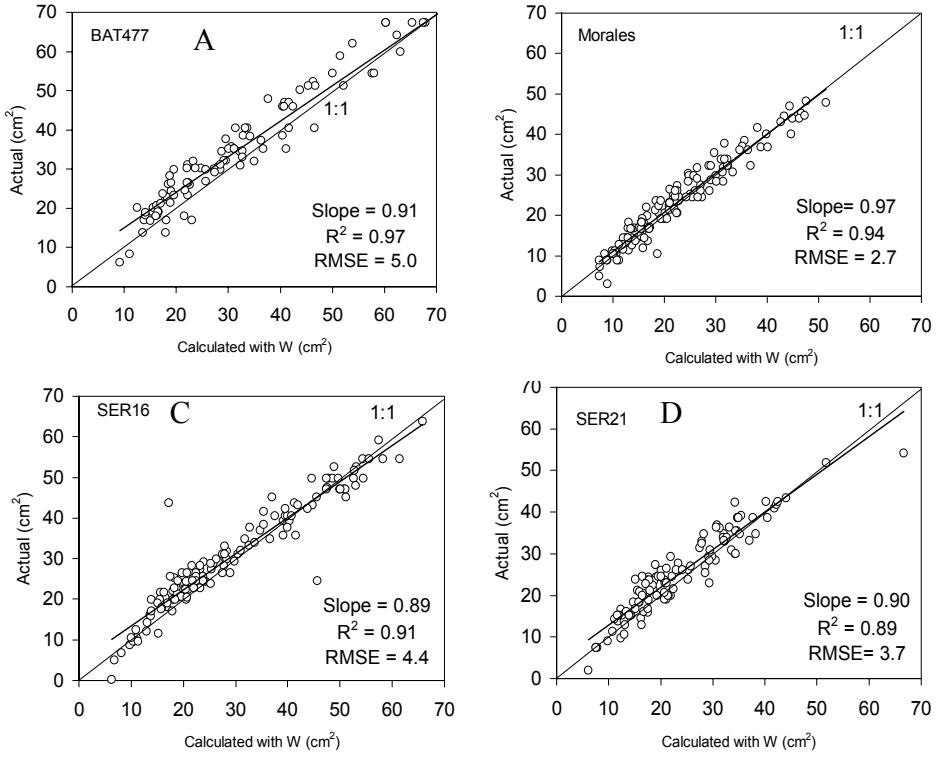

Figure 2.3. Model validation using regression analysis on combined data from the 2006-2007 field trials with W as a single predictor (**A**, BAT 477; **B**, Morales; **C**, SER 16; and **D**, SER 21).2006 included SER 16 and Morales and 2007 all the four.

CONCLUSIONS

This study has shown the effectiveness of individual leaflet measurements for the estimation of individual leaflet area in four genotypes using a direct, non-destructive technique allowing for multiple sampling at different phenological time points. The results indicate that a single predictor (leaflet width) is sufficient for single leaflet area estimation, that genotype-specific leaflet area models for four genotypes were robust across varied greenhouse and field conditions and across growth stages, and that genotype specific models may often be necessary in common bean.

REFERENCES

Bange, M.P., L.G. Hammer, P.S. Milroy, and G.K. Rickert. 2000. Improving estimates of individual leaf area of sunflower. *Agron. J*. 92:761–765.

Beaver, J.S., and P.N. Miklas. 1999. Registration of Morales small white bean. *Crop. Sci*. 39:1257.

Bhatt, M., and S.V. Chanda. 2003. Prediction of leaf area in *Phaseolus vulgaris* by non-destructive method. *Bulg. J. Plant Physiol.* 29:96-100.

Brenner, A.J., M. Cueto Romero, J. Garcia Haro, M.A. Gilabert, L.D. Incoll, J Martinez Fernández, E. Porter, F.I Pugnaire, and M.T. Younis. 1995. A comparison of direct and indirect methods for measuring leaf and surface areas of individual bushes. *Plant Cell Environ*. 18:1332-1340.

Cintra de Jesus, W., F.X. Ribeiro do Vale Jr., C.R. Resende, and C.L. Costa. 2001. Comparison of two methods for estimating leaf area index on common bean. *Agron. J*. 93:989-991.

De Swart, A.M., R. Groenwold, H.J. Kanne, P. Stam, L.F.M. Marcelis, and R.E. Voorrips. 2004. Non-destructive estimation of leaf area for different plant ages and accessions of *Capsicum annuum* L. *J. Hortic. Sci. Biotechnol*. 79:764–770.

Gamper, H. 2005. Nondestructive estimates of leaf area in white clover using predictive formulae. *Crop Sci*. 45:2552-2556.

Kandiannan, K., C. Kailasam., K.K. Chandaragiri, and N. Sankaran. 2002. Allometric model for leaf area estimation in black pepper (*Piper nigrum* L.). *J. Agron. & Crop Sci*. 188:138-140.

Infostat, 2003. InfosStat, version 3. User Manual. Infostat Group, FCA, Universidad Nacional de Cordoba, Argetina. http//infostat.com.ar

Lu, H.Y., C.T. Lu, M.L. Wei, and L.F. Chan. 2004. Comparison of different models for nondestructive leaf area estimation in Taro. *Agron. J.* 96:448–453.

Marshall, J.K. 1968. Methods for leaf area measurement of large and small leaf samples. *Photosynthetica* 2(1):41-47.

Rasband, W.S. 1997-2006. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/,

Strachan, L.B., D.W. Stewart, and A. Pattey. 2005. Determination of leaf area index in agricultural systems. p. 179-198. *In.* J.L. Hatfield and J.M. Baker (ed.) Micrometeorology in agricultural systems. Monograph 47. ASA, CSSA, SSSA, Madison, WI, USA.

Tsialtas, J., and N. Maslaris. 2005. Leaf area estimation in a sugar beet cultivar by linear models. *Photosynthetica* 43:477-479.

Van Oijen, M., M. Hoglind, M.H. Hanslin, and C. Caldwell. 2005. Process-based modeling of timothy regrowth. *Agron J*. 97:1295-1303.

Wallach, D., B. Goffinet, J.E. Bergez, P. Debaeke, D. Leenhardt, and J.N. Aubertot. 2001. Parameter estimation for crop models: A new approach and application to a corn model. *Agron. J.* 93:757-766.

Wilhelm, W.W., K. Ruwe, and M.R. Schlemmer. 2000. Comparison of three leaf area index meters in a corn canopy. *Crop Sci.* 40:1179-1183.

Williams, L., E.T. Martinson. 2003. Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. *Scientia Horticulturae* 98:493–498.

Wiersma, J,V and T.B. Bailey. 1975. Estimation of leaflet, trifoliolate, and total leaf areas of soybean. *Agron. J.* 67:26-30.

Yang, J., and M. Alley. 2005. A mechanistic model for describing corn plant leaf area distribution. *Agron. J.* 97:41-48.

Chapter 3

Physiological Response of Different Common Bean Genotypes (*Phaseolus vulgaris* L.) to Drought Stress

ABSTRACT

Abiotic stress is an important limiting factor in common bean (*Phaseolus vulgaris* L.) production. Currently a number of common bean genotypes with drought tolerance are available, and it is necessary to evaluate which physiological factors are related to drought stress. The goal of this research was to measure physiological parameters related to drought, including stomatal resistance (r_L), leaf temperature (T_L), relative water content (RWC) and leaf area (LA). These parameters/indices were evaluated in different genotypes of common bean in greenhouse and field environments. Six genotypes were studied: BAT 477, Morales, SER 16, SER 21, SEN 3 and SEN 21. Three water levels were used: full water supply (without drought stress) using 80% of the daily available water (DAW) during the complete growing season; Stress 1 with 50% of the DAW before flowering and 60% of the DAW after flowering; and Stress 2 with 20% of the DAW before flowering and 40% of the DAW after flowering. In the greenhouse, the drought stress was applied when the second trifoliate were completely expanded during four seasons of data collection, and in the field experiment, drought stress was applied at the start of flowering during two seasons of data collection. The results indicate that the genotypes have different responses to drought stress. The r_L and T_L in non-drought treatments were similar for all genotypes evaluated, but under strong drought conditions, were significantly different. SER 21, SER 16 and SEN 3 genotypes generally showed lower r_L, T_L and high RWC.

Key words: Common bean, drought, stomatal resistance, leaf temperature, leaf area, relative water content.

Abbreviations: r_L, stomatal resistance; T_L, leaf temperature; SFC, substrate field capacity; Ta, air temperature; RWC, relative water content; θv, volumetric moisture content; WSD, water saturation deficit; SE, standard error.

INTRODUCTION

Stomata, in the physiology of plants, is the regulatory system which regulates CO_2 uptake and the release of water-vapor to the atmosphere by transpiration (Turner, 1974; Leopold et al. 1975; Ting, 1982; Yang, 1995). Stomatal control is one of the mechanisms that enables the plant to adapt to stress (Turner and Begg, 1981). Stomatal resistance (r_L) controls the proportion of latent to sensible heat fluxes (Strachan et al. 2005), and for this reason is related to leaf temperature (T_L). Changes in r_L are important for the regulation of water loss by the plant and for controlling the rate of carbon dioxide uptake (Holbrook, 2002).

Monteith et al. (1965) defined r_L as the difference in the concentration of gas between the ends of the pores divided by the rate of diffusion. Wenkert (1983) indicated that r_L is the total resistance from the cell surface to the exterior leaf surface. r_L plays a major role in cases where the soil is dry, or where only a fraction of the soil surrounding to the roots is wet, and evaporation from the soil is negligible, or when the foliage is very dense (Monteith et al. 1965). According to Lemon (1983), the aerodynamic properties of the crop canopy can have a substantial effect on the water-use efficiency of the crop.

Drought stress reduces the transpiration rate, stomatal conductance ($1/r_L$), water potential and its components (osmotic and turgor potentials), and decreases CO_2 assimilation and therefore growth (Pugnaire et al. 1994; Yang, 1995; Mayek et al. 2002; Brevedan and Egli, 2003). Common bean are sensitive to drought and yield is significantly reduced by water deficits (Markhart, 1985; Ramos et al. 1999; Catonguay and Markhart, 1991; Cruz de Carvalho et al. 1998; Costa Franca et al. 2000; Boutra and Sanders, 2001; Lizana et al. 2006). Sixty percent of common bean production in the world is in regions that are subject to water shortage (Lizana et al. 2006).

Authors such as Torrecillas et al. (1995) in tomatoes, Kang et al. (2000) in corn, De Oliveira et al. (2005) in common bean, found that strong drought stress reduced stomatal conductance ($1/r_L$) and transpiration substantially and increased leaf temperature. Changes in relative water content RWC) associated with droughts stress also has been reported (i.e.,

Ramirez-Vallejo and Kelly, 1998; Stayanov, 2005). The RWC represent a useful indicator of the state of the water balance of a plant, The RWC has been used as an approximate index to evaluate plant water status (Baker and Bland, 1994; Blum, 2006), as a screening tool for evaluation of drought tolerance (Larbi and Mekliche, 2005), and to compare genotypic response in bean related with photosynthesis rates (Castonguay and Markhart, 1991) and drought stress (Stayanov, 2005).

Much effort has been devoted throughout the world to develop common bean germplasm with drought tolerance. Careful comparison of this new germplasm under different drought stress environments, and the response of the principal factors associated with drought stress needs to be determined. It is known that different varieties of bean, cultivated in the same geographic area, display distinct responses to prolonged drought stress (Markhart, 1985; Cruz de Carvalho et al. 1998; Costa Franca et al. 2000, Miklas et al 2006). The aim of this research was to evaluate the response of common bean genotypes with different levels of drought tolerance to water deficit, using the stomatal resistance, leaf temperature, and leaf water content.

MATERIALS AND METHODS

The experiments were conducted in two environmental conditions, greenhouse and field, during 2005, 2006 and 2007.

Greenhouse Experiments. The greenhouse experiments were carried out at the USDA-TARS (Tropical Agricultural Research Station) in Mayagüez, Puerto Rico; coordinates 18º 12`22' N, 67º 8' 20`` W at 18 masl. Two experiments were conducted during July-September 2005 and 2006, and two between October-December 2005 and 2006. The basic weather information was recorded during the study in the greenhouse (Table 3.1).

The genotypes used in to greenhouse experiments were: Morales, SER 16, SER 21, and BAT 477, during 2005; and during 2006 two more genotypes were included: SEN 3 and SEN 21. The description of the genotypes used in the study is presented in the Table 3.2.

Each genotype was planted in Pods (15 cm diameter x 11 cm depth) with Sunshine mix #1 (Sun Gro Horticulture, Vancouver, British Colombia) and Osmocote (14-14-14, N-P-K; Marysville, OH), three seeds per pot were sown and when the first trifoliate leaf was observed two were thinned. Three water levels were used with 2 plants per water level and four replications. The pods were arranged in a split-split-plot experimental design, the main plot was the experiment, sub-plot was the water level and the sub-sub-plot was the genotype..

Table 3.1 Average weather conditions in the greenhouse in the 2005 and 2006 experiments.

Weather Variables	***July-September***	***October-December***
	2005	
Air Temperature (ºC)	27.55	26.06
Air Relative humidity (%)	84.29	82.53
Solar radiation ($W.m^2$)	nd	nd
Wind speed ($m.s^{-1}$)	nd	nd
DII[§]	0.47	0.33
	2006	
Air Temperature (ºC)	26.90	26.58
Air Relative humidity (%)	78.75	77.35
Solar radiation ($W.m^2$)	57.90	61.15
Wind speed ($m.s^{-1}$)	0.0088	0.0089
DII	0.63	0.48

[§]Drought intensity index= $1-(Yield_{witht\ drought\ stress}/Yield_{without\ drought\ stress})$

Table 3.2. Description of six common bean genotypes used in the study.

Genotype	*Source*	*Plant Architecture*	*Seed Type*	*Drought Response*
Morales	UPRM-Puerto Rico†	III	Small White	Unknown
BAT 477	CIAT-Colombia‡	II	Cream	Tolerant
SER 21	CIAT-Colombia	II	Small Red	Tolerant
SER 16	CIAT-Colombia	II	Small Red	Tolerant
SEN 3	CIAT-Colombia	II	Black	Tolerant
SEN 21	CIAT-Colombia	II	Black	Tolerant

† Beaver and Miklas (1999); ‡ seed provided by Dr. Steve Beebe.

Maximum water retention capacity for the substrate (substrate field capacity-SFC) was measured after over-watering the substrate and letting it drain for 7, 24 and 48 hours. Twelve Pots were over-watered and covered to avoid evaporation. Volumetric moisture content was measured with a volumetric moisture sensor "theta probe soil moisture sensor" ML2X (Delta-T Devices Ltd.), the SFC was 0.53 m^3m^{-3} (+/- 0.010). Three water regimes were used: 1) No drought stress, using 80% of the daily available water (DAW) during the complete growing season; 2) Drought stress 1, with 50% of the DAW before flowering and 60% of the DAW after flowering; and 3) Drought stress 2, with 20% of the DAW before flowering and 40% of the DAW after flowering. The drought stress treatments were applied starting from when the second trifoliate leaflet was completely open. The water applications were made every day during the morning, and the volumetric moisture content (θ_v) was measured at different growing phases during each season. At no time during the experiments did the soil moisture content reach the permanent wilting point.

Field experiments. The field experiments were carried out in out in the Experimental Station of the University of Puerto Rico-UPR in Juana Diaz, PR, which is located in south central PR, with 18°01'N latitude and 66°22'W longitude, elevation 21 m above mean sea level, within a semi-arid climatic zone (Goyal and Gonzalez, 1989). The field characteristics are described in Chapter 4.

The field experiments were planted on February 15, 2006 and January 17, 2007. The UPR Agricultural Experiment soil is classified as a San Anton Clay Loam with 30% sand, 44% silt, 26% clay, and 1.28% of organic matter, within the first 40 cm, with a 0.30 $cm^3.cm^{-3}$ field capacity and 0.19 $cm^3.cm^{-3}$ wilting point (USDA, 1987). Intermittent drought stress was applied in both years from the beginning of the reproductive phase (R1: One blossom open at any node) to harvest. The drought stress was sufficient to allow the soil to dry to 75% of the field capacity (FC), at which point irrigation was applied. The stress level (1-total water applied with drought/total water applied without drought stress) in 2006 was 18% that correspond to 387.3 mm of the 472.5 mm total applied in the without drought stress treatment, and in 2007 the stress level was 30.3% that correspond to 302.0 mm of the 433.4 mm total applied in the without drought stress treatment. More precise information about the applied irrigation and rainfall data are presented in Chapter 4.

The volumetric moisture content was measured with a profile probe type PR2 sensor (Delta-T Devices, Ltd.). Two access tubes were installed in each main plot at 20 cm and 40 cm depths, and the irrigation was applied two times per week, using a drip irrigation system. Each main plot was divided into six sub-plots which consisted of each genotype, two sub-plots (each with 10 rows) for SER 16 and three for Morales in 2006, and three for each in 2007. The plant density was 13.5 $plant.m^{-2}$ for Morales and 6.5 $plants.m^{-2}$ for SER 16. The other agronomic practices related to the crop were similar in the whole experiment and carried out at the same time. Additionally, in 2007, SEN 21, SEN 3, SER 21 and BAT 477 were planted, arranged in a complete randomized block design with five replications (8.5 $plants.m^{-2}$), the purpose of evaluating differences in r_L and T_L in the field at 13:00-14:00 hours, with and without drought stress.

Stomatal resistance and leaf temperature measurements. There are several methods to study stomatal resistance (r_L). One of the most widely used methods for measuring pore activity is through the use of a porometer (Anda and Löke, 2002). The r_L was measured with a Porometer type AP4-UM-3 (Delta-T Devices Ltd) in 2005 and a Porometer model SC-1 (Decagon Devices, Inc.) in 2006. The T_L was measured using an infrared thermometer gun MX4-TD +/-1^oC (Raytek). The r_L and T_L were measured on a single leaf near the top of the canopy structure (a

fully open leaves) during the vegetative and reproductive growing phases. In the greenhouse, during July-September and October-December 2005, these variables were measured from 7:00 to 18:00 at two hour intervals. During July-September and October-December 2006, measurements were made only between 13:00-14:00 hours, since this time interval was observed to be the most critical measuring response to drought stress. During this time interval leaf rolling, due to a reduction in turgidity, a principal drought stress sign, was observed. In the field, the r_L and T_L were measured at different times during the day as well.

Figure 3.1. Equipment used in the greenhouse and field studies: **A**. Leaf porometer; **B**. Infrared leaf temperature sensor; **C**. Volumetric moisture sensor in greenhouse and **D** in field.

Leaf area. The leaf area (LA) was measured using a non-destructive method for each genotype, developed previously (Chapter 2). At different phenological phases, the maximum single leaf width (W) was measured for each genotype at each water level treatment.

Relative water content. To measure the RWC, leaves were collected for each plot between 13:00 to 14:00, weighed immediately to obtain the fresh weight (FW), and then transported to the laboratory on ice. In the laboratory, the leaves were immersed in de-ionized water overnight (16h) in darkness to minimize physiological activity, and reweighed to obtain turgid weight (TW). The leaves were then oven dried at 72ºC for 48 h and reweighed (dry weighed, DW) (Turner, 1981), and the relative water content was computed RWC=(FW-DW)/(TW-DW)x100.

Analysis of variance, normality and variance homogeneity tests were made. Means were separated with Tukey and LSD multiple range test $P<0.05$, using the INFOSTAT Statistical program version 3-University of Cordoba (Argentina).

RESULTS

Greenhouse experiments.

Stomatal resistance. The mean r_L was strongly influenced by water level, genotype, and time of day (Fig 3.2 and 3.3). Statistical differences were founded for r_L between genotypes and water levels. The lowest r_L was observed between 8:00 to 11:00 hours and during 15:00 to 16:00 hours. Differences in r_L between July-Sep 06 and Oct-Dec 05 were associated with differences in air temperature and volumetric moisture content (θ_v) (Tables 3.1 and 3.4), the high θ_v during Oct-Dec 05, can be explained as follows: during this growing season, the air temperature was low and water demand by plant reduced, compared with July-Sep 05 season, and for this reason for the second year (2006), the water applications were adjusted to keep the θ_v values low, and to increase the drought-stress, due at the atmospheric demand during this period and less water is used in transpiration, based in the observation during the same period October-December 2005.

The r_L in this study for all genotypes increased at the end of the day. With respect to this behavior, Monteith et al. (1965) has stated that equilibrium between water supplied from roots with the loss by transpiration seems to break down at this moment of the day, so that the resistance rapidly increases, due to the increasing moisture stress and decreasing light intensity, that act together to close the stomata. In addition water only was applied in the AM, thus increasing drought stress during the day.

The largest differences in r_L were observed between 13:00 to 14:00 hours, and among all genotypes at 16:00 as well and water levels. The r_L stress 1 (moderate stress) was not significantly different between water level treatments in the Oct-Dec 05 experiment, results that are associated with the relativity high θ_v mentioned earlier.

During the experiments, July-Sep 06 and Oct-Dec 06, the r_L and T_L were measured only in the interval of 13:00-14:00 hours in order to compare the magnitudes of drought stress between genotypes (Table 3.3 and 3.5). The interaction of experiment x genotype x water level was statistically significant ($p<0001$***; Table 3.7). The genotypes with the lowest r_L across experiments were SER 21 and SER 16. During the experiments where the stress 2 resulted greater drought stress (July-Sep06 and Oct-Dec06), the genotypes with the lowest r_L were SER 21 and SEN 3.

The r_L values measured at 13:00 varied between 73.19 $s.m^{-1}$ without drought stress in SER 21, to 6703.25 $s.m^{-1}$ with stress 2 in Morales. The genotype SER 16 presented the highest variability across experiments under stress 2, and SER 21 the lowest variability (Table 3.3). Two experiments, (July-Sep05 and Oct-Dec06) with different drought treatments (stress 2), The genotype SER 21 present the lowest r_L.

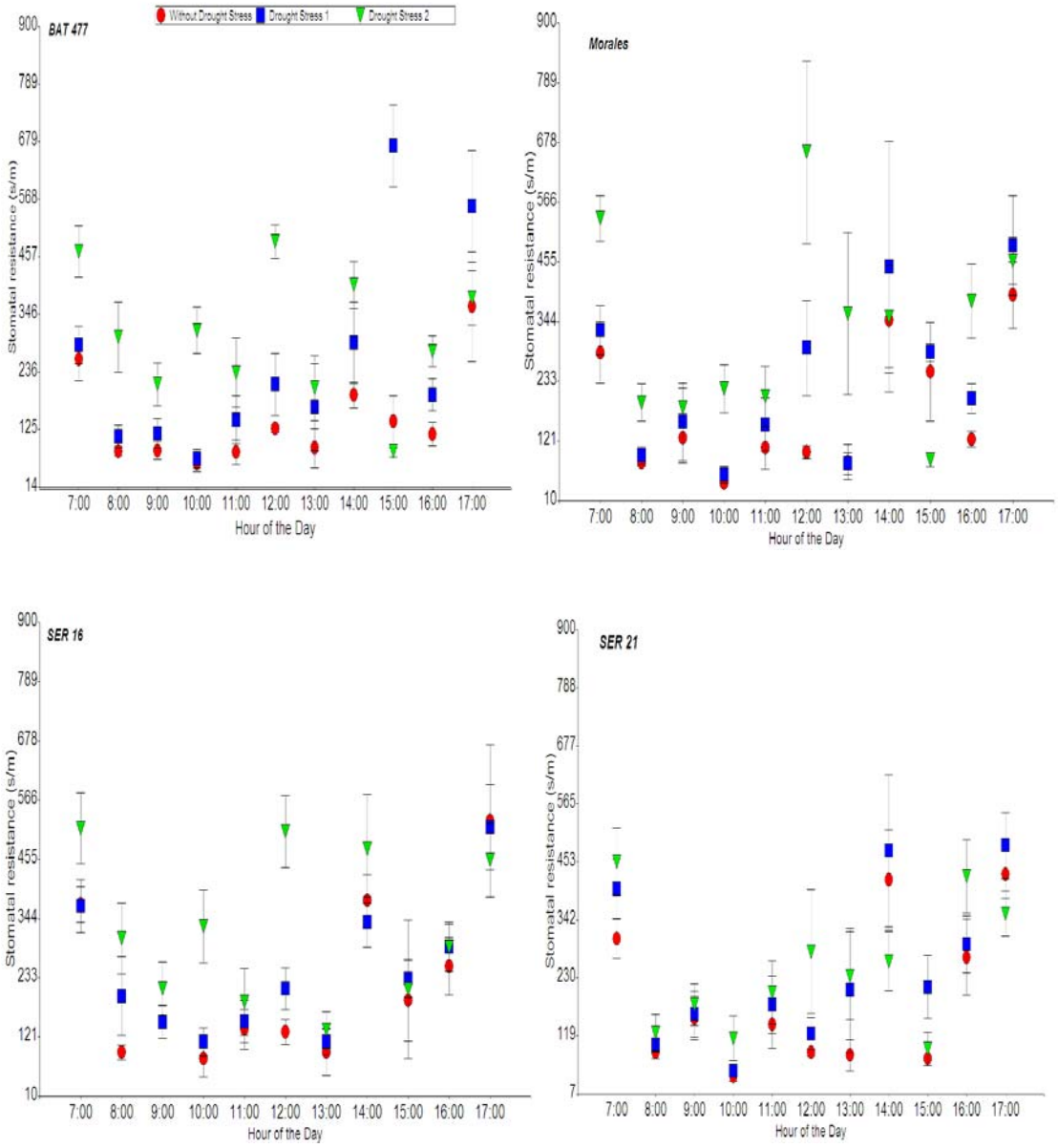

Figure 3.2. Mean stomatal resistance (r_L) during the day and +/-S.E. for four common bean genotypes, average of 5 days: BAT 477, Morales, SER 16 and SER 21, without drought stress, Stress 1 and Stress 2. Greenhouse environment, July-Sep05 experiment.

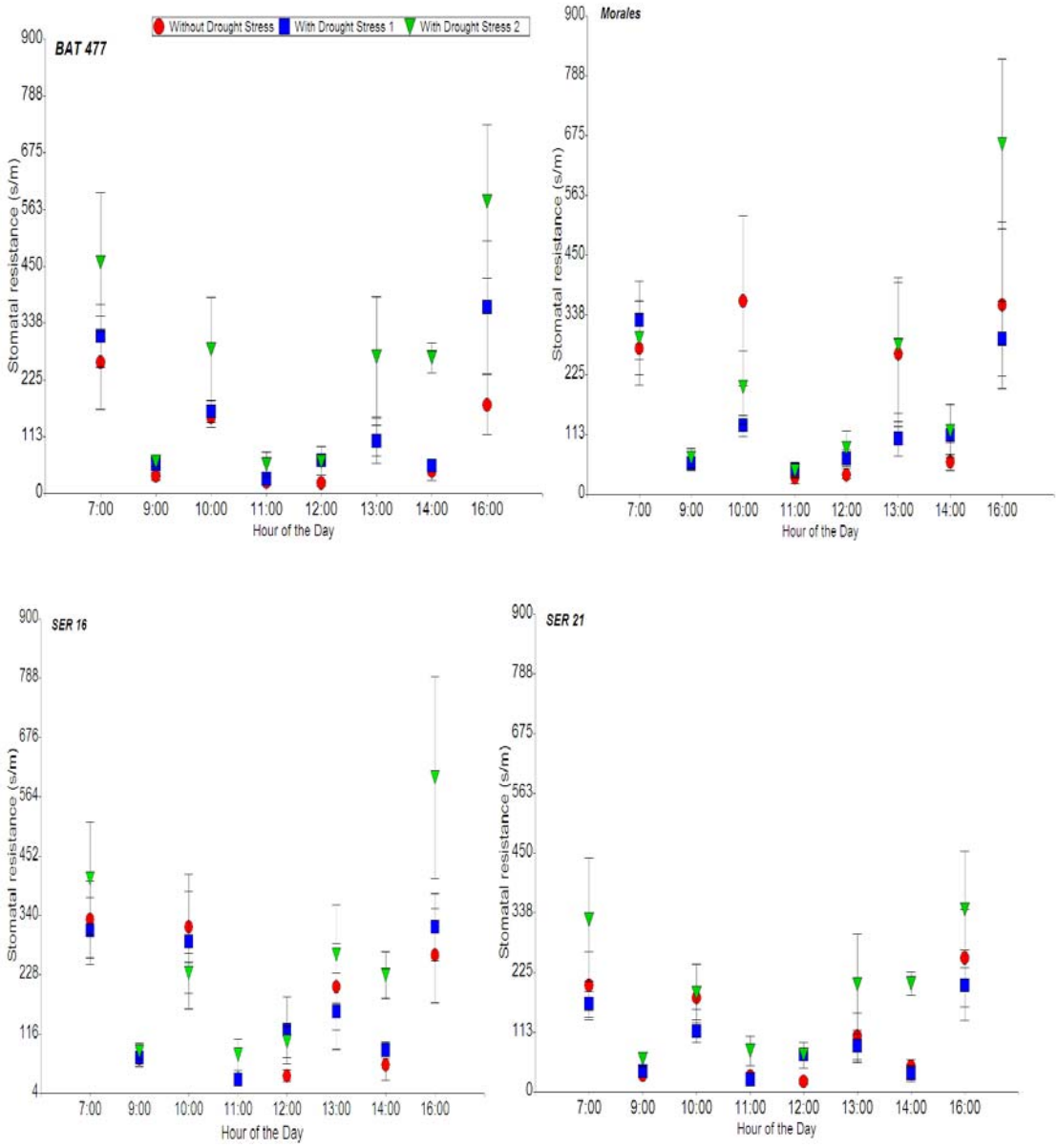

Figure 3.3. Mean stomatal resistance (r_L) during the day and +/-S.E. for four common bean genotypes average of 5 days: BAT 477, Morales, SER 16 and SER 21, without drought stress, Stress 1 and Stress 2. Greenhouse environment, Oct-Dec05 experiment.

Table 3.3. Mean stomatal resistance (r_L) for six common bean genotypes under the greenhouse environment during four experiments, measured between 13:00 to 14:00 hours.

		Experiments			
Water Level	Genotype	July—SepØ5	July—SepØ6	Oct—DecØ5	Oct—DecØ6
		$s.m^{-1}$			
Without Drought Stress	BAT477	93.56 ab	722.75 abcd	104.29 ab	401.86 abcd
With Stress 1	BAT477	176.19 ab	1740.75 cd	104.41 ab	208.40 ab
With Stress 2	BAT477	217.53 ab	4549.25 f	271.02 abc	3916.18 f
Without Drought Stress	Morales	74.58 ab	238.00 ab	263.17 abc	194.57 ab
With Stress 1	Morales	87.38 ab	340.00 abcd	104.43 ab	364.11 abcd
With Stress 2	Morales	371.42 abc	6703.25 g	280.43 abc	1994.04 ef
Without Drought Stress	SEN21		308.75 abcd		116.29 ab
With Stress 1	SEN21		1326.75 bcd		129.11 ab
With Stress 2	SEN21		4421.25 f		1325.32 bcd
Without Drought Stress	SEN3		262.50 abc		219.86 ab
With Stress 1	SEN3		501.50 abcd		395.11 abcd
With Stress 2	SEN3		2148.75 ef		2347.61 e
Without Drought Stress	SER16	87.98 ab	271.00 abc	204.88 ab	76.29 ab
With Stress 1	SER16	120.20 ab	1082.03 abcd	159.23 ab	717.40 abcd
With Stress 2	SER16	147.22 ab	4131.50 f	266.67 abc	1547.04 cd
Without Drought Stress	SER21	73.19 a	500.75 abcd	105.10 ab	74.14 ab
With Stress 1	SER21	214.77 ab	1338.00 bcd	86.37 ab	294.54 abc
With Stress 2	SER21	247.08 ab	1236.00 abcd	203.25 ab	729.04 abcd

Different letters indicate significance at 0.05 level, (LSD test).

Table 3.4. Mean volumetric moisture content (θ_v), during four greenhouse experiments.

Water Level	Experiments			
	July—SepØ5	Oct—DecØ5	July—SepØ6	Oct—DecØ6
	$cm^3.cm^{-3}$ †			
Without Drought Stress	0.51 (+/-0.04)	0.59 (+/-0.12)	0.43 (+/-0.12)	0.35 (+/-0.12)
With Stress 1	0.38 (+/-0.05)	0.43 (+/-0.12)	0.32 (+/-0.12)	0.20 (+/-0.12)
With Stress 2	0.29 (+/-0.07)	0.36 (+/-0.10)	0.21 (+/-0.10)	0.13 (+/-0.10)

† The Substrate Field Capacity = 0.53 $cm^3.cm^{-3}$ (+/- 0.010)
Parenthesis values is standard deviation.

Leaf temperature. The T_L was affected by drought intensity and hour of the day, having a reverse tendency during the day as compared to r_L (Fig 3.4 and 3.5). Differences were observed between genotypes and water levels at 12:00, 13:00 and 14:00 hours. The interaction of the experiment x water level was statistically significant (Table 3.7). The averages across experiments indicate that T_L measured between 13:00 to 14:00 hours increased with increasing drought-stress, particularly in stress 2 (strong stress). Under stress 2 as compared to non-stress, T_L increased, varying from 1.12 ºC in SER 21 to 2.48 ºC in BAT 477 (Table 3.5). Similar to r_L, the largest T_L under stress 2 was reached during the July-Sep 05 and July-Sep 06 experiments, where T_L for the drought stressed treatments were over 30 ^oC. In these experiments, Morales and BAT 477 had the highest T_L, and SER 21 the lowest (Table 3.5). Leaf temperature changes in BAT 477 and Morales were most sensitive under strong drought stress. The least sensitive genotypes were SEN 21, SER 16 and SER 21. The low increase in T_L for SER 21 in stress 2 conditions are in agreement with the low observed r_L across experiments.

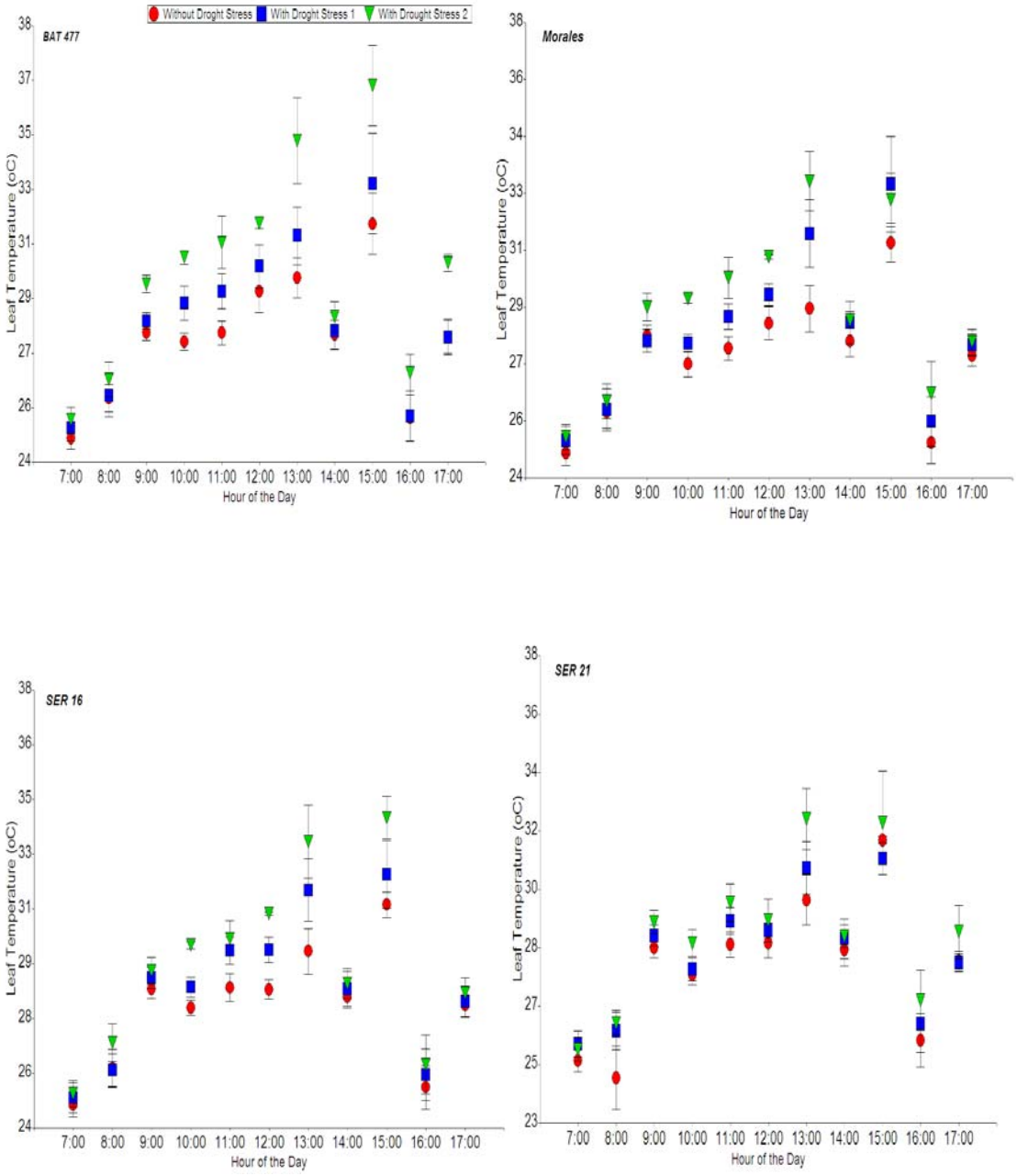

Figure 3.4. Mean leaf temperature (T_L) during the day and +/-S.E. for four common bean genotypes: BAT 477, Morales, SER 16 and SER 21, without drought stress, Stress 1 and Stress 2. Greenhouse environment, July-Sep05 experiment.

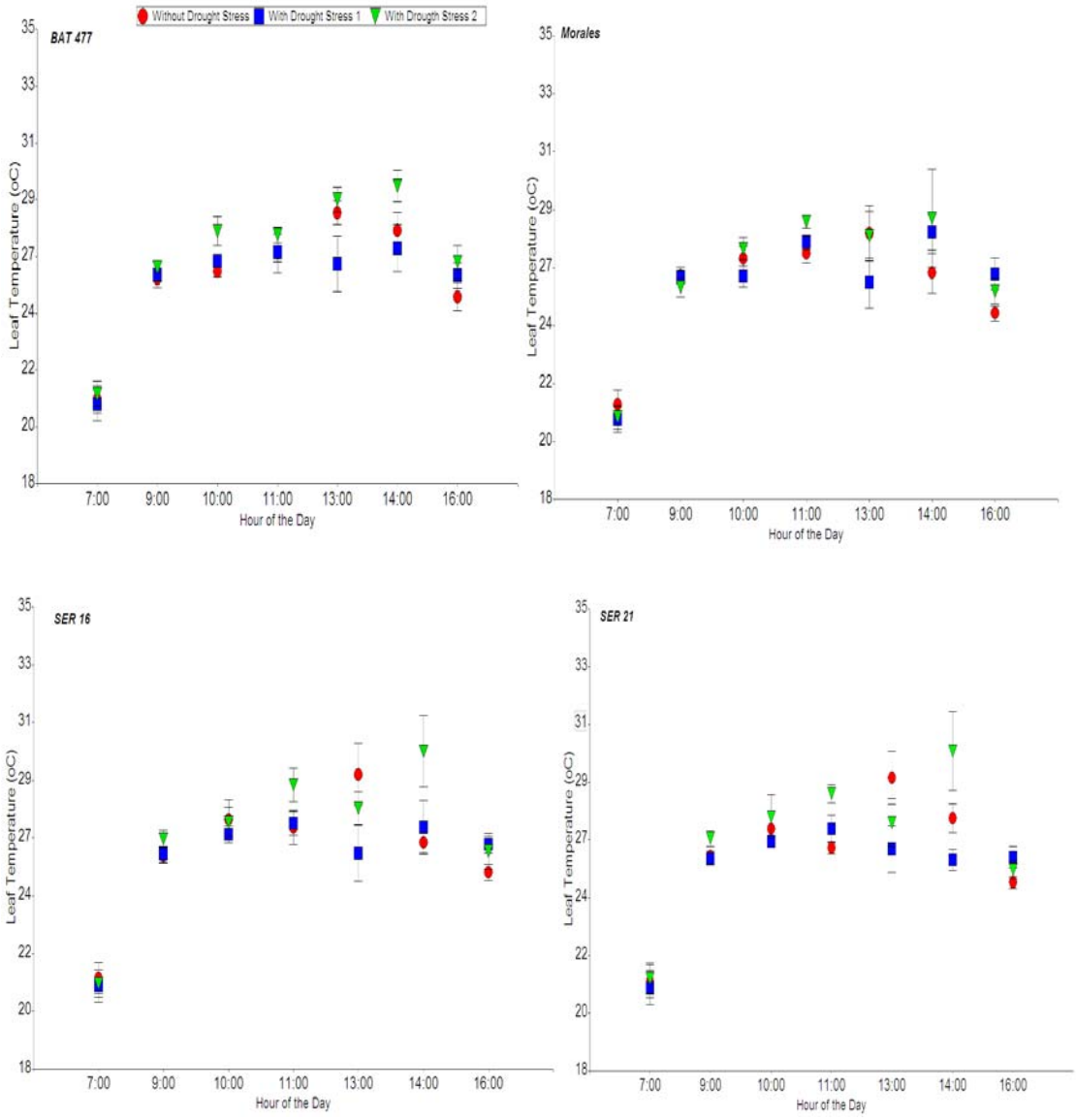

Figure 3.5. Mean leaf temperature (T_L) during the day and +/-S.E. for four common bean genotypes: BAT 477, Morales, SER 16 and SER 21, without drought stress, Stress 1 and Stress 2. Greenhouse environment, Oct-Dec05 experiment.

Table 3.5. Mean leaf temperature (T_L) for six common bean genotypes under greenhouse environment during four experiments, measured between 13:00 to 14:00 hours.

		Experiments			
Water Level	Genotype	July—SepO5	July—SepO6	Oct—DecO5	Oct—DecO6
		°C			
Without Drought Stress	BAT477	29.97 efghi	30.47 fghi	27.93 abcd	27.39 abc
With Stress 1	BAT477	31.36 hijkl	31.02 hijk	25.77 a	27.84 abcd
With Stress 2	BAT477	34.36 n	32.77 lmn	28.63 bcdef	29.93 efgh
Without Drought Stress	Morales	29.11 defg	29.66 defg	27.75 abcd	27.04 ab
With Stress 1	Morales	31.30 hijkl	30.71 ghij	26.10 a	27.45 abc
With Stress 2	Morales	33.00 lmn	32.96 lmn	27.55 abcd	28.75 bcdef
Without Drought Stress	SEN21		29.76 efgh		27.84 abcd
With Stress 1	SEN21		30.88 ghij		27.39 abc
With Stress 2	SEN21		32.50 klm		28.87 cdef
Without Drought Stress	SEN3		30.07 fghi		27.89 abcd
With Stress 1	SEN3		30.32 fghi		27.72 abcd
With Stress 2	SEN3		32.74 lmn		30.13 fghi
Without Drought Stress	SER16	29.69 defgh	29.75 efgh	28.84 bcdef	27.68 abcd
With Stress 1	SER16	31.75 ijklm	30.27 fghi	26.10 a	27.46 abc
With Stress 2	SER16	33.22 mn	32.28 klm	27.59 abcd	29.62 defgh
Without Drought Stress	SER21	30.04 efghi	29.33 cdef	29.43 cdefg	27.42 abc
With Stress 1	SER21	30.96 ghijk	30.05 fghi	26.66 ab	27.89 abcd
With Stress 2	SER21	32.55 klmn	31.75 jklm	27.14 abc	29.26 cdefg

Different letters indicate significance at 0.05 level, (LSD test).

Relative water content. The water level x genotype interaction was statistically significant ($p<0.0414$; Table 3.7). The SER 21 genotype in V4 and R3 phases, under stress 2 conditions, produced the largest RWC values (Table 3.6).

The SER 21 genotype across growing phases exhibited at 22% average water saturation deficit-(WSD=100-RWC; Turner, 1981), followed by SEN 21 with 24.9%, and Morales with 25.5%. SER 16, BAT 477 and SEN 3 exhibited larger average water saturation deficits of 28.8%,

28.5% and 28.6 % respectively. These results indicate the tendency of SER 21 to have a higher RWC under stress conditions. The differences in the RWC between vegetative and reproductive growing phases are associated with fact that the in the vegetative phase the water treatments applied were lower in the reproductive phase.

Table 3.6. Mean relative water content (RWC) for six common bean genotypes under the greenhouse environment during two experiments (July-Sep06 and Oct-Dec06), measured at 13:00 hours.

Water Level	Genotype	V4†	R3
		RWC	
		%	
Without Drought Stress	BAT477	72.27 bc	80.36 defg
With Stress 1	BAT477	71.97 bc	74.69 abc
With Stress 2	BAT477	70.06 abc	72.91 ab
Without Drought Stress	Morales	70.83 bc	83.36 fg
With Stress 1	Morales	72.00 bc	71.24 a
With Stress 2	Morales	73.51 c	75.56 abcd
Without Drought Stress	SEN21	72.33 bc	82.44 efg
With Stress 1	SEN21	71.57 bc	78.47 cdeg
With Stress 2	SEN21	68.29 abc	81.98 efg
Without Drought Stress	SEN3	66.80 abc	76.46 abcd
With Stress 1	SEN3	69.18 abc	77.09 bcde
With Stress 2	SEN3	67.07 abc	75.76 abcd
Without Drought Stress	SER16	70.19 abc	79.54 cdefg
With Stress 1	SER16	68.95 abc	80.01 cdefg
With Stress 2	SER16	64.37 a	78.02 bcdef
Without Drought Stress	SER21	73.80 c	83.43 g
With Stress 1	SER21	73.55 c	82.01 efg
With Stress 2	SER21	73.15 c	82.79 fg

Different letters indicate significance at 0.05 level, (LSD test).
† V3 is Three nodes on the main steem including the primary leaf node, and R4 is Pods 3 inch long-seeds not disernible.

Leaf area. The long-term effects of drought stress include a reduction in leaf growth (Tardieu, 2005). In this study, water stress decreased the leaf area (LA) in four common bean genotypes, reductions being observed after the R3 growth phase (Fig 3.6), (R3: Pods 1 inch long at first blossom position). Highly significant differences were observed in genotypes ($p<0.0001$, Table 3.7), with leaf area reductions of 3% for SER 16, 28% for BAT 477, 35% for Morales and 37% for SER 21 with respect to the without drought stress treatments.

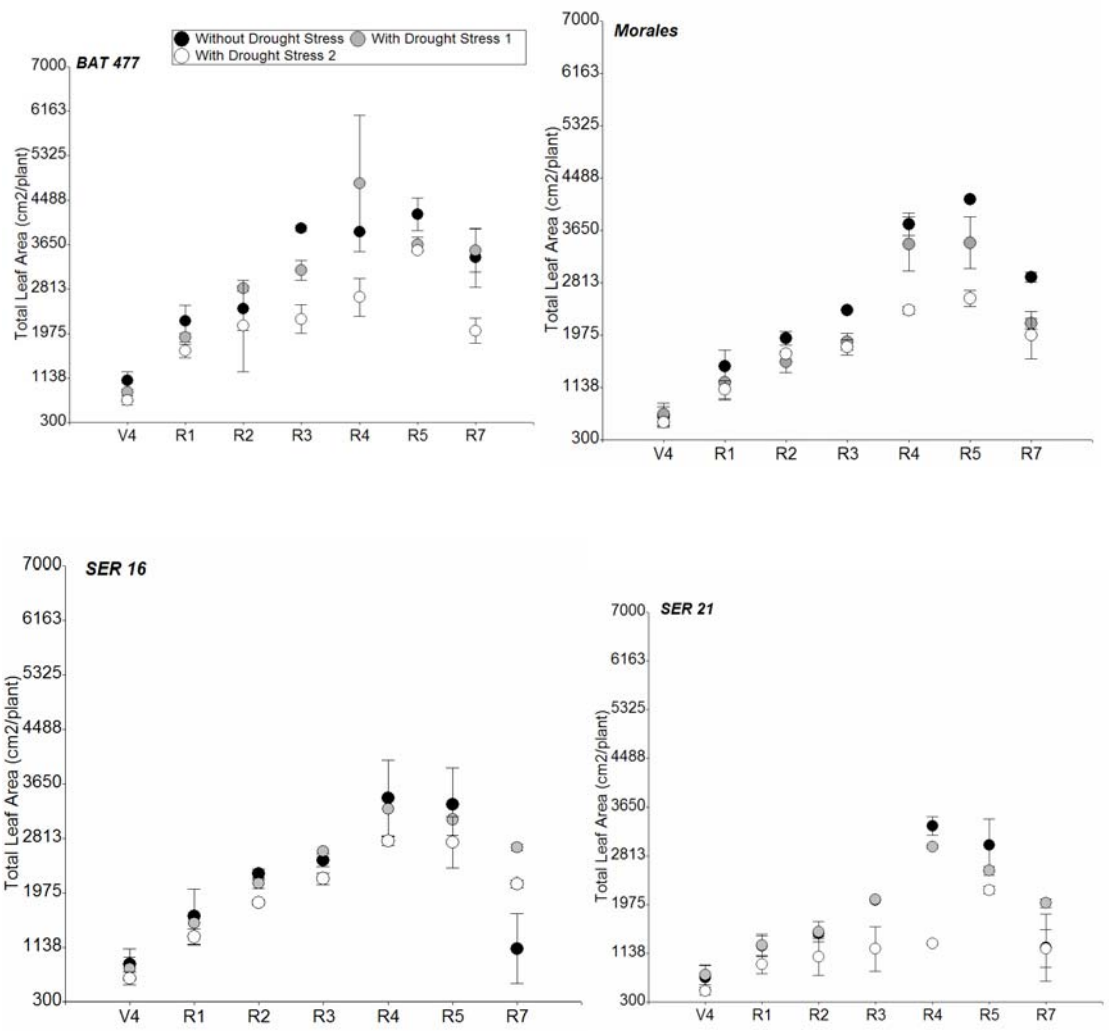

Figure 3.6. Mean total leaf area (LA) and +/-S.E. for four common bean genotypes. BAT 477, Morales, SER 16 and SER 21, under non-drought stress, stress 1 and stress 2. Mean values for three experiments, July-Sep05, July-Sep06 and Oct-Dec06 (Greenhouse environment).

Table 3.7. Mean squares for stomatal resistance (r_L), leaf temperature (T_L), yield components, leaf area (LA) and relative water content (RWC) for six common bean genotypes grown under the greenhouse environment during 2005 and 2006.

Source of variation	df	r_L s.m^{-1}	df	T_L ºC	df	Yield Seed.Plant^{-1}	df	Biomass g.plant^{-1}	df	Leaf Area cm^2	df	RWC† %
Experiment (E)	3	47115574.0 ***	3	718.0 ***	3	1191.7 ***	2	3106.3 *	2	7879716.2 **	1	150.2
Error 1	8	389953.1	12	6.6	11	16.4	9	374.4	3	99448.0	5	500.1
Water Level (WL)	2	58320796.4 ***	2	501.4 ***	2	1100.4 ***	2	1270.6 ***	2	5717367.7 **	2	328.5
WLxE	6	20308129.2 ***	6	33.9 ***	6	140.7 ***	4	171.8 **	4	897640.3	2	407.4
Error 2	16	357729.0	24	5.7	22	13.3	18	27.6	6	406411.2	10	136.6
Genotype (G)	5	2568720.9 *	5	7.1	5	351.9 ***	5	259.9 ***	3	5407250.9 ***	5	320.2 ***
ExG	12	2352640.0 *	12	6.9	12	419.3 ***	8	72.5 ***	6	68401.7	5	30.8
WLxG	10	2116256.3 *	10	1.9	10	27.4 ***	10	55.0 ***	6	571841.9 *	10	101.3 *
ExWLxG	22	2923907.5 ***	24	1.7	22	26.0 ***	16	50.9 *	12	366777.5	10	48.2
Error 3	314	1114494.7	895	4.6	361	8.3	309	17.6	27	258348.4	201	52.1

*, **, and *** implies at P<0.05, p<0.01 and p<0.001,respectively.
† Measured at R3 growing phase.
df is degrees of freedom

Field Experiments.

Stomatal resistance and leaf temperature. Under field conditions the genotypic response in terms of r_L under strong stress (2007), were similar to the greenhouse observations. In 2006, the drought stress was low (18% less water that the without-stress treatment) and measured r_L values were not significantly different between water treatments. However, when the stress increased in 2007 (30.3% less water that the without-stress treatment), the differences between genotypes (Morales and SER16) were evident (Fig 3.7). Morales r_L reached nearly three times the levels of SER16 at 13:00 hours.

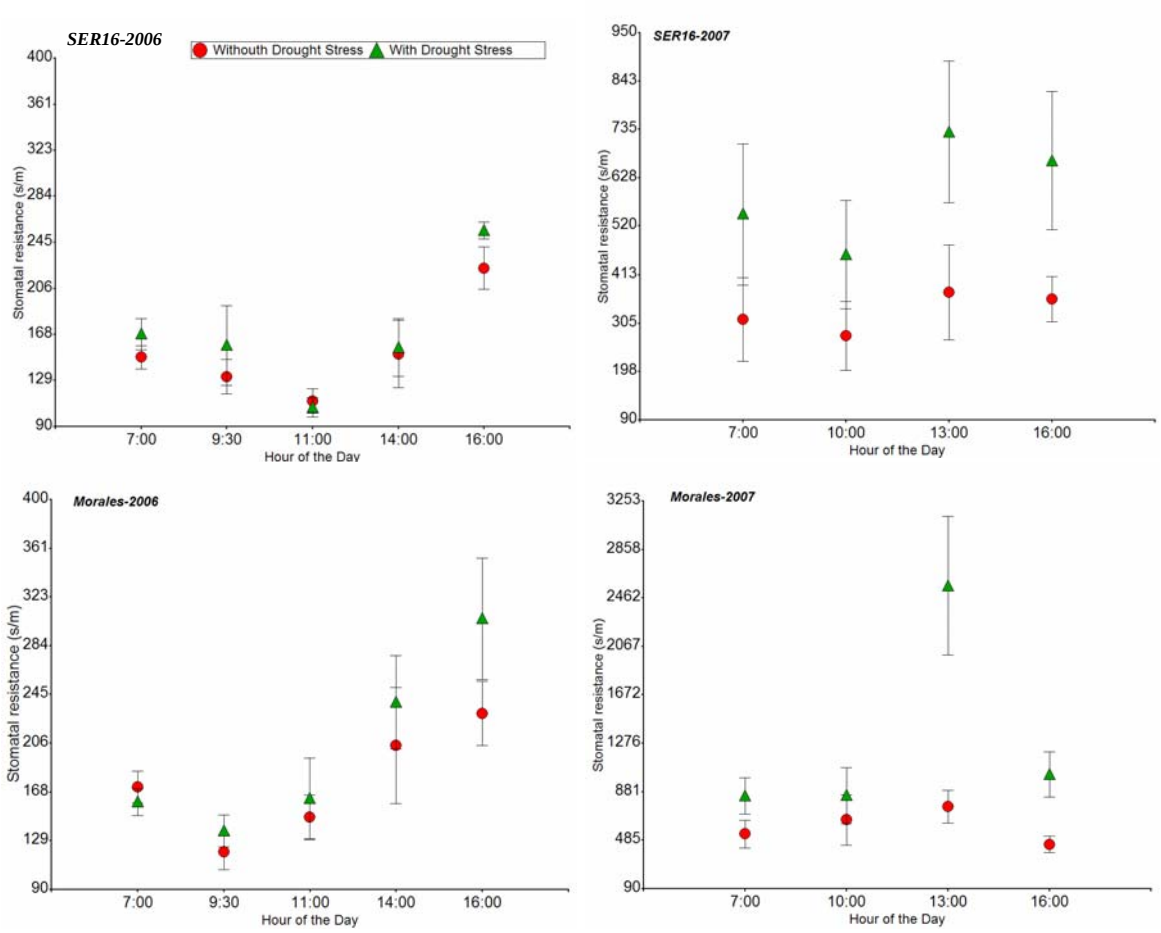

Figure 3.7. Mean stomatal resistance (r_L) during the day and +/-S.E. for two common bean genotypes: Morales, and SER 16, without drought stress, and with drought stress, in the field environment, 2006 and 2007. Measurements were made during the whole growing onces per week.

Additionally, in the field in 2007, small plots were planted with five replications including SER 21, SEN 21, SEN 3 and BAT 477 with and without drought stress. The r_L and T_L were measured in vegetative and reproductive phenological phases, at 13:00 hour.

Differences were estimated as follows: $r_{L\ with\ drought\ stress}$- $r_{L without\ drought\ stress}$ and $T_{L\ with\ drought\ stress}$- $T_{L\ without\ drought\ stress}$. Morales showed the largest differences in r_L and T_L with: 1971.34 $s.m^{-1}$ and 4.86ºC respectively; whereas the smallest differences were observed for SER 16 with 408.00 $s.m^{-1}$, and 3.07ºC, and SEN 21 with 160.33 $s.m^{-1}$ and 4.02ºC, respectively (Table 3.8). All genotypes showed a significant response to drought stress for T_L, while only Morales for r_L,

which indicated that in field conditions all the drought tolerant genotypes, showed non statistical differences, these results indicated that the Morales's stomates are more sensitive to drought stress.

Table 3.8. Stomatal resistance (r_L) and leaf temperature (T_L) measured at 13:00 hour under field conditions (January-March 07).

		Field Experiment 2007†			
Water Level	Genotype	r_L		T_L	
		$s.m^{-1}$		ºC	
Without Drought Stress	BAT477	269.00	a	28.93	a
With Stress	BAT477	1410.83	ab	33.58	bc
Without Drought Stress	Morales	481.33	a	29.18	a
With Stress	Morales	2452.67	b	34.04	c
Without Drought Stress	SEN 21	210.00	a	28.36	a
With Stress	SEN 21	370.33	a	32.38	bc
Without Drought Stress	SEN 3	343.33	a	28.58	a
With Stress	SEN 3	1515.17	ab	31.89	b
Without Drought Stress	SER 16	332.67	a	28.46	a
With Stress	SER 16	740.67	a	31.53	b
Without Drought Stress	SER 21	176.00	a	27.92	a
With Stress	SER 21	1225.67	ab	31.94	b

Differents letter indicate significance at 0.05 level, (LSD test).
† Drought stress applied in R1 phenological phase

Leaf area. Under field conditions, with 30.3% of water reduction relative to without drought stress (2007), the leaf area index (LAI) decreased 50% in Morales and 40% in SER16 (Fig 3.8). The low LAI in SER 16 was associated with low plant density compared with Morales.

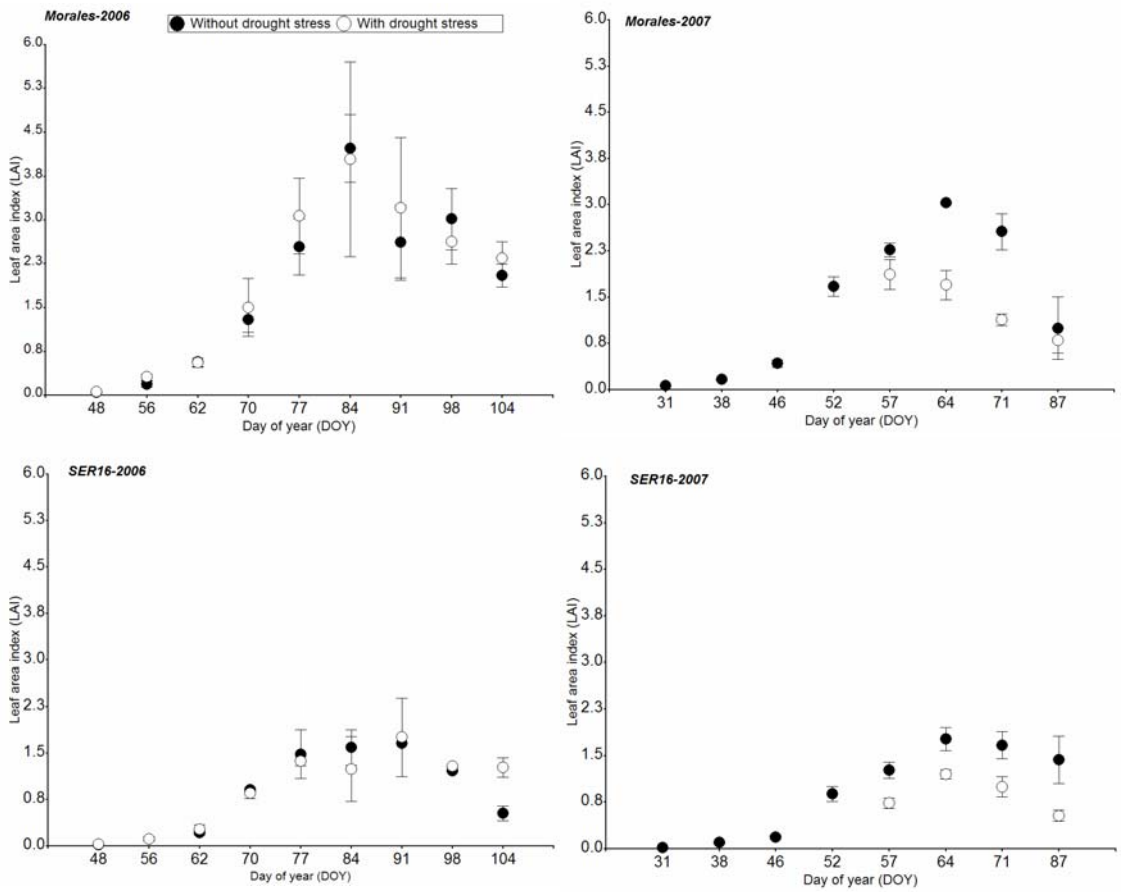

Figure 3.8. Leaf area index (LAI), +/-S.E. for two common bean genotypes in field experiments (2006-2007).

DISCUSSION

The variation in the gas flux into and out of the stomates is a function of different factors: radiation, vapor pressure deficit, air and leaf temperature, CO_2 concentration, leaf water potential and water level in the soil (Troughton and Slatyer, 1969; Turner and Begg, 1981; De Oliverira et al. 2005). It is difficult to separate the effects of individual environmental parameters since all are related to some extent. Strong light is often accompanied by high temperature, which affects water status. Stomatal opening is the result of a balance of different processes, depending on the environment (Ting, 1982). Our results indicate that stomatal resistance responds to drought stress, but parallel to drought stress, are other factors, for example genotypic variability. Stomatal resistance can be observed to have a lower value in the morning (Fig 3.2, 3.3), this behavior also implies that maximum gas exchange occurs between 8:00 and 11:00, but with different orders of magnitude depending on the stress level and genotype. These results indicate sensitivity to other parameter, such as light saturation, VPD, leaf, root and stem ABA concentrations or others. Ribeiro et al. (2004) report in genotypes 'Carioca', 'Ouro Negro' and 'Guarambe', maximal rates of photosynthesis and lower r_L around 8:45 am and maintained until 11:45.

The increase in r_L at noon could be associated with increased in VPD and the closing of the stomates to maintain leaf water potential. The decrease in turgor causes stomatal closure and this mechanism is likely to operate in air at low humidity, when direct water loss from guard cells is too rapid to be balanced by water movement into the guard cells from adjacent epidermal cells (Locy, 2002). The r_L is not the only factor associated with drought stress and reduction in dry matter accumulation (e.g. Reddy et al.2004). It is clear that the conservation of water through stomatal closure, or other mechanisms, reduces photosynthesis, and thus crop growth is limited (White and Izquierdo, 1991). But the degree of stomatal closure depends on the level of drought stress and genotype, as observed in the results section.

The interaction between experiments, water level, genotype in r_L, indicated that the six common bean genotypes are different in the response to drought stress, and that the effect of

drought depends on the severity, frequency, intensity and environmental conditions. Muñoz-Perea et al. (2006) report similar effects in other dry bean genotypes related with yield and biomass, under field conditions.

Without drought stress condition, the r_L for the six genotypes were not statistically different, whereas under strong stress (stress 2) statistically significant differences were observed. SER 21 and SER 16 were the least sensitive to water stress in both environment, a result which is consistent with results of a study by Cruz de Carvalho et al. (1998) in *P. vulgaris* cv Carioca and *V. unguiculta* cultivars, who observed that one of the characteristics of the drought tolerant genotypes, was that r_L decreased more slowly than the non-tolerant genotype. In our case, similar comparisons could be made to determine degree of drought tolerance. Trejo and Davis (1991), who measured the stomatal conductance (g_s) at noon, in two common bean genotypes `*Cacahuate-72*`and `*Michoacan-12A3*`found no differences in the g_s under well watered conditions,however, under drought stress conditions, the g_s for `*Cacahuate-72*` decreased earlier than in `*Michoacan-12A3*`.

Costa Franca et al. (2000) reported on four bean cultivars that, as substrate water content decreased below 30%, g_s ($1/r_L$) decreased linearly to its minimum value. When the moisture content was kept between 30% to 50% (high hydration conditions) no significant differences were observed between cultivars. This is similar to what we observed during the Oct-Dec05 experiment, in which θ_v under stress 2 only reached 36% (Table 3.4).

Comparing four dry bean genotypes (BAT 477; TLP-19 drought resistant; *Rio Tibaqi* and *Pinto,* drought susceptible) Mayek et al. (2002) found that BAT 477 showed the highest transpiration rate and the lowest stomatal resistance. In this study, SER 21 and SER 16 showed the lowest r_L across experiments, and `Morales` was similar to BAT 477 with higher r_L under greenhouse and field environment. Cruz de Carvalho et al. (1998) indicated that early stomatal responses to substrate water depletion were not triggered by changes in leaf water content, and therefore root-zone water content alone could not be used as an indicator of drought in these legumes (*P. vulgaris* and *V. unguiculta*).

The differences in r_L among genotypes could be associated with differences in *abscissic acid,* (ABA) levels in leaves or xylem sap concentration and/or the differences in hydraulic signals related to the soil water content. In common bean, changes in the stomatal aperture is sensitive to small changes in ABA, due to its high levels compared with non-legumes (Trejo and Davies, 1991). Differences in ABA concentrations exist between genotypes and influence the stomatal sensitivity to the root-source chemical signals (Serraj et al. 2005). On the other hand, the pH or the ionic composition of the xylem sap a change with soil water deficit and influence stomatal aperture (e.g. Netting, 2000).

The principal components related to plant response to water deficit include: leaf area to intercepted radiation, the rate of net photosynthesis converted into dry matter and distribution of assimilates (Turner and Begg, 1981). Our results indicate that a combination between stomatal control and leaf area distribution were an appropriate combination in the response or SER 21 to drought stress. The SER 21 genotype had low leaf area and low r_L under strong stress conditions in the greenhouse environment, which potentially indicate good source-sink characteristics, reflected in high harvest index (HI) and WUE in all trials (see Chapter 7). This is contrary to BAT477, that has higher leaf area and lower yield component characteristics. BAT 477's high leaf area likely induces fast water loss, and increasing r_L .

Relations between stomatal conductance and dry matter accumulation rates in bean have been documented by Cruz de Carvalho et al. (1998), Costa Franca et al. (2000) and Lizana et al. (2006). Ramirez-Vallejo and Kelly (1998) reported a significant correlation between stomatal conductance and yield index in common bean.

Stomatal resistance will be limiting when the vapor pressure deficit increases with temperature especially in C_3 plants (Pastenes and Horton, 1996). Tardieu (2005) explained that when the stomates partially close, thereby decreasing transpiration, the leaf water potential increases, i.e. leaves become more hydrated, and allow the leaves to maintain their water status in a narrow range, and this could be related to observed differences in RWC responses in the six genotypes. In our case, the RWC or WSD were good indicators for evaluating genotypic differences under drought stress, but not to evaluate differences between drought stress and non

drought stress. In a study by Peng and Weyers (1994) in *Commelina communis* L., they also reported no consistent pattern in the g_s and RWC.

In a study of drought tolerance by Ramos et al. (1999) in *P. vulgaris* cultivar EMGOPA-20,1 no alterations in relative water content or leaf area were observed when the leaf water potential was reduced to -0.78 MPa, with leaves showing no visible signs of wilt. On the other hand, in this study, plants at 30% of soil field capacity showed a sharp decrease in stomatal conductance values, two to three times lower than those of well-watered plants. Stayanov (2005), in different genotypes of common bean ('*Plovdiv* 10', cv. '*Dobrudjanskiran'* and '*Prelom'*), reported differences among genotypes in RWC under drought stress conditions in the first trifoliate leafs. Ramirez-Vallejo and Kelly (1998) found a negative association of RWC with yield and biomass in bean, and suggested that high RWC is the result of lower stomatal conductance (in our case, high r_L), and affirmed that these water relations have implications on water conservation and plant survival under stress, and that their response may negatively impact yield.

With respect to the leaf area, continuous drought stress accelerates leaf senescence and reduces leaf area, in common bean (Turner and Begg, 1981; Costa Franca et al. 2000; Navea et al. 2002; Brevedan and Egli, 2003). Nielsen and Nelson (1998), observed significant LAI reductions in black bean (*P. vulgaris* L) under drought stress in the vegetative stage, but other treatments lost more leaf area at the end of the growing season (late drought stress). Markhart (1985) found significant reductions in the leaf area under drought conditions at 23 days after planting for two bean species (*P. vulgaris and P. acutifolius*). The high GM, HI and WUE in SER 21 could be related with its inherent lower LA and its quick response to drought in terms of LA reduction, follow by SER 16 (See Chapter 7). In the case of BAT 477, its high LA could be influenced by the low GM, HI and WUE under strong stress (Chapter 7), due to the plant expending more energy in respiration process, and lose more rapidly the limited water. Morales was more efficient in relation to LA, GM, HI and WUE compared with drought tolerant genotypes like SEN21 and BAT 477. Also the high LA in BAT 477 could be related with high T_L under strong stress, due to the large area of transpiring surfaces the make that water loss a

faster, and to the stomata's need to close more rapidly, with subsequently increases in the r_L and T_L and reduction in the RWC.

Since maintenance and adjustment of stomatal openings are active processes dependent on plant metabolism, it is expected that temperature plays a direct role. Temperature effects on the above processes will directly affect rate and degree of stomatal opening (Ting, 1982). Troughton and Slatyer (1969) observed a linear relationship between leaf temperature and mesophyll resistance in cotton. Pastenes and Horton (1996) reported increases in transpiration rate in bean with temperature increase (from 20 ^oC to 35 ^oC), but this increase depended on the water status of the leaf compared with the air.

Response of the stomates to humidity (VPD) in bean was reported by Castonguay and Markhart (1992). In our study, the r_L was inversely correlated with VPD under non-drought stress for BAT 477, SER 21 and SER 16 (-0.41*; p_{value} =0.02, -0.35*; p_{value} =0.04, -0.40*; p_{value} =0.01), but under drought stress 2 only SER 16 was inversely correlated (-0.43*; p_{value} =0.01). These results mean that the SER 16 keeps responding to the atmospheric water demand under drought stress. This lack of relation r_L- VPD under drought condition by the other genotypes, could be associated with the stomatal sensitivity to humidity, partially closing in dry air as a way to optimize stomatal function with respect to control of transpiration in relation to photosynthesis uptake of carbon dioxide, and to maintain high leaf water potentials (Hall, 2004). Stomates of some species respond directly to the VPD of the atmosphere surrounding the leaf , but not all species respond directly, but all respond to leaf water potential (Turner and Begg, 1981; Brodribb and Holbrook, 2003), or to `chemical signals`. Trejo and Davies (1991) reported that stomates in *P. vulgaris* L were affected by soil drying without any significant change in the plant water potential (Ψ_w) and reported that changes in the stomatal aperture were associated with changes in ABA concentration. This indicates that there are multiple signals and mechanisms that regulate stomatal behavior (Thompson et al. 1997).

The differences in the response of drought between genotypes, indicates that mechanisms controlling drought tolerance are different, i.e., i) improved water uptake, ii) efficient water conduction, iii) restriction of transpiration, iv) water storage and v) desiccation tolerance

(Larcher, 2001; Tardieu, 2005). The ability to keep growing during drought stress has been attributed to non-stomatal factors. For example, the capacity of the chloroplast to fix CO_2 under drought stress. (e.g., Yordanov et al. 2001). Based on these results, we can hypothesize that SER 16 and SER 21 have a high desiccation tolerance represented in the RWC, improved water uptake represented in the WUE and HI (Chapter 7), and/or high capability to fix CO_2 by non-stomatal factors. More research in necessary to clarify the mechanism in these genotypes.

Root architecture characteristics as a tolerance factor during long-term drought was not considered in this study, due to the limited root grown in the greenhouse environment.

CONCLUSIONS

Stomatal resistance is a sensitive physiological measurement of plant stress; this variable depends on genotype, microclimatic conditions, and substrate moisture content. In this research, differences in r_L between common bean genotypes was observed. The differences in r_L, T_L, LA, and RWC, indicates that the six genotypes evaluated in this study have differences in drought stress response. Generally, genotypes with lower r_L show also the lower T_L, and higher RWC.

For similar root depth conditions (greenhouse experiment), the genotypes with the lower r_L, T_L, and higher RWC under drought stress were SER21, SER 16 and SEN3. A similar tendency was founded under field conditions, but SER 16 showed lower T_L and r_L than SER 21 under drought stress.

REFERENCES

Anda, A. and Löke, Zs, 2002. Stomatal resistance investigations in maize. *Proceedings of the 7th Hungrian Congress of Plant Physiology*.46(3-4):181-183.

Baker, J.M., and W.L. Bland. 1994. Biological measurements. In: John F. Griffiths, ed. *Handbook of Agricultural Meteorology*, pp.88-92. Oxford University Press.

Beaver, J.S., P.N. Miklas, 1999. Registration "Morales" small white bean. *Crop Sci.* 39:1257.

Blum, A. 2006. Towards standard assays of drought resistance in crop plants. In: www.plantstress.com. 9p.

Bountra, T.; F.E. Sanders, 2001: Influence of water stress on grain yield and vegetative growth of two cultivars of bean (*Phaseolus vulgris* L.). J. *Agron and Crop Sci*. 187, 251-257.

Brevedan, R.E., D.B. Egli, 2003. Short periods of drought stress during seed filling, leaf senescence, and yield of soybean. *Crop Sci*. 43:2083–2088

Brodribb, J.T and N.M. Holbrook. 2003. Stomatal clousure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol*. 132:2166-2172.

Castonguay, Y., A.H. Markhart, 1991. Saturated rates of photosyntesis in drought stressed leaves of common bean and tepary bean. *Crop.Sci*.31:1605-1611.

Costa Franca, G.M., P.T.A. Thin., C. Pimentel., R. R.O. Pereira, F.Y. Zuily., D. Laffray, 2000. Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. *Environ and Experimental Botany*. 43:227-237.

Cruz de Carvalho, M.H., D. Laffray, P. Louguet, 1998. Comparison of the physiological responses of *Phaseolus vulgaris* and *Vigna unguiculata* cultivars when submitted to drought conditions. *Environ and Experimental Botany*. 40:197-207.

De Oliveira, A., E.J Fernandes., de T .J.D Rodrigues, 2005. Conductancia estomatica como indicador de estresse hidrico em Feijao. Eng. Agríc., Jaboticabal .**25**:86-95.

Goyal, M.R, and E.A. Gonzalez. 1989. Datos climatológicos de las subestaciones experimentales agrícolas de Puerto Rico. Universidad de Puerto Rico-Recinto de Mayagüez/Colegio de Ciencias Agrícolas/Estación Experimental Agrícola. Publicación 88-70, proyecto C-411. 87p.

Hall, E. A. 2004. Comparative Ecophysiology of cowpea, common bean, and peanut. University of California, Riverside. Riverside, California, U.S.A. 55p. Copyright by Marcel Dekker, Inc.

Holbrook, N. M, 2002. Water balance of plants. In: Plant Physiology. Third Edition by. Taiz, L and Zeigrt, E. Sinauer Associates, Inc., Publishers. Sunderland, Massachusetts, pp. 47-65.

InfoStat, 2003. InfoStat, version 3. Manual de Usurario. Grupo InfoStat, FCA, Universidad Nacional de Cordoba,. Argentina. http//www.infostat.com.ar

Kang, S., W.Shi., and J. Zhang. 2000. An improved water-use efficiency for maize grown under regulated deficit irrigation. *Field Crop Res*. 67:207-214.

Larcher, W. 2001. Physiological plant ecology: Ecophysiology and Stress physiology of functional groups. 4th edition. Springer. 513p.

Larbi, A., and A. Mekliche, 2005. Relative water content (RWC) and leaf senescence as screening tool for drought tolerance in wheat. Institut National Agronomique Paris-Grignon, Chaire d'Agriculture Comparée, France. 4p. In http://ressources.ciheam.org/om/pdf/a60/04600064.pdf.

Lemon, E. 1983. Canopy gas exchange: An Overview In: *Limitations to efficient water use in crop production.* Edited by Howard M. Taylor, Wayne R. Jordan and Thomas R. Sinclair. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America:195-202.

Leopold, A.C., E.P. Kriedemann, 1974. Plant growth and development. McGraw-Hill.545p.

Lizana, C., M. Wentworth, J.P. Martinez, D. Villegas, R. Meneses, E.H. Murchie, C. Pastenes, B. Lecari, P. Vernieri, P. Horton and M. Pinto. 2006. Differential adaptation of two varieties of common bean to abiotic stress. *J. Exp. Bot.* 57(3):685-697.

Locy, D. R. 2002. Stress physiology. In: Plant Physiology. Third Edition by. Taiz, L and Zeigrt, E. Sinauer Associates, Inc., Publishers. Sunderland, Massachusetts, pp. 591-623.

Mayek, P.N., E.R. García., E.C. López., G.J.A. Acosta., J. Simpon, 2002. Water relations, histophatology, and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. *Physiol and Molecular Plant Patho*. 60:185-195.

Markhart, H. A. 1985. Comparative water relations of *Phaseolus vulgaris* L. and *Phaseolus acutifolius* Gray. *Plant Physiol*. **77**:113-117.

Miklas, P.N., J.D. Kelly, S.E. Beebe and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica*. 147:105-131.

Monteith, J.L., G. Szeicz., P.E. Waggoner, 1965. The measurement and control of stomatal resistance in the field. Rothamsted Experimental Station, Harpenden, Herts. 11p.

Muños-Perea, C.G., H. Terán., R.G. Allen., J.L. Wright., D.T. Westermann., and S.P Singh. 2006. Selection fro drought resistance in dry bean landraces and cultivars. *Crop. Sci.* 46:2111-2120.

Navea, C, T.Terrazas, A.Delgado-Salinas and P. Ramirez-Vallejo. 2002. Foliar response of wild and domesticated *Phaseolus vulgaris* L. to water stress. *Genetic Resources and Crop Evolution.* 49(2): 125-132.

Netting, A.G. 2000. pH, abscissic acid and the integration of metabolism in plants under stress and non-stressed conditions: Cellular resposes to stress and their implications for the plant water relations. *J. Exp. Bot*. 51:147-158.

Nielsen, C.D., O. N. Nelson, 1998. Black bean sensitivity to drought stress at various growth stages. *Crop Sci.* 38:422–427.

Pastenes, C., and P. Horton. 1996. Effect of high temperature on photosynthesis in benas, II. CO_2 Assimilation and Metabolte Contents. *Plant.Physiol.* 112:1253-1260.

Peng, Z-Y, and J. Weyers. 1994. Stomatal sensitivity to abscisic acid following water deficit stress. *J. Exp. Bot*. 45:835-845

Pugnaire, F.I., Serrano, E. L., Pardos, J, 1994. Constraints by water stress on plant growth. In: *Handbook of plant and crop stress.* Edited by Mohammad Pessarakli, University of Arizona, Tucson, Arizona, pp: 247-259.

Ramirez-Vallejo, P and J.D. Kelly. 1998. Trait related to drought stress resistance in common bean. *Euphytica.* 99:127-136.

Ramos, M.L.G., A.J.Gordon., F.R. Minchin., J.L. Sprent, R. Parson, 1999. Effect of drought stress on nodule physiology and biochemistry of drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). *Annals of Botany.* 83:57-63.

Reddy, A.R, Chaitanya, V.K, Vivekanandan, M. 2004. Drought-induced responses of photosynthesis antioxidant metabolism in higher plants. *J. Plant Physiol.* 161:1189-1202.

Ribeiro, V.R., M.G, dos Santos, G.M.Souza, E.C. Machado., R.F de Oliveira., L.R. Angelocci., and C. Pimentel. 2004. Environmetal effects on photosynthetic capacity of bean genotypes. *Pesq. agropec. bras*.39(7):615-623.

Serraj, R., R. Wakrim., S. Wahbi., H. Tahi., and B. Aganchich. 2005. Comparative effects of partial root drying (PRD) and regulated deficit irrigation (RDI) on water relations and eater use efficiency in common bean (*Phaseolus vulgaris* L.). *Agric.Ecosy and Envirom*. 106:275-287.

Strachan, I.B., D.E.Stewart., E. Pattiey, 2005. Determination of leaf area index in agricultural systems. In: *Micrometerology in Agricultural Systems*, Agronomy Monograph No 47.American Society of Agronomy, Inc/Crop Science Society of America, Inc/Soil Science Society of America, Inc:179-198.

Stayanov, Z.Z. 2005. Effects of water stress on leaf water relations of young bean plants. *Central European J. Agricul.* 6(1):5-14.

Tardieu, F. 2005. Plant tolerance to water deficit: physical limits and possibilities for progress. C.R. *Geoscience* 337: 57-67.

Thompson, D.S., S.Wilkinson, M.A. Bacon, and W.J. Davies. 1997. Multiple signals and mechanisms that regulated the leaf growth and stomatal behavior during the water deficit. *Physiol. Plantarum.* 100:303-313.

Ting, P.I. 1982. Plant physiology. Addison-Wesley publishing company. 642p.

Torrecillas, A., C. Guillaumeb., J.J.Alarcón., and Ma C. Ruiz-Sanchez.1995. Water relations of two tomato species under water stress and recovery. *Plant Sci*. 105:169-176.

Trejo, C.L., W.J. Davies. 1991. Drought-induced closure of *Phaseolus vulgaris* stomata precedes leaf water deficit and any increase in xylem ABA concentration. *J. Exp. Bot*. 42:1507-1516.

Troughton, J.H., R.O. Slatyer. 1969. Plant water status, leaf temperature, and the calculated mesophyll resistance to carbon dioxide of cotton leaves. *Aust.J.biol.Sci*.22:815-827.

Turner, C.N 1974. Stomatal behavior and water status of maize, sorghum, and tobacco under field conditions. *Plant Physiol*. 53: 360-365.

Turner, C.N. 1981. Techniques and experimental approach for the measurement of plant water status. *Plant and Soil*. 58: 339-366.

Turner, C.N and J.E, Begg. 1981. Plant-water relations and adaptation to stress. *Plant and Soil*. 58: 97-131.

Wenkert, W. 1983. Water transport and balance within the plant: An overview. In: *Limitations to efficient water use in crop production*. Edited by Howard M. Taylor, Wayne R. Jordan and Thomas R. Sinclair. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America:137-159.

White, J.W and J. Izquierdo. 1991. Physiology of yield potential and stress tolerance. In: A van Schoonhoven, and O. Voysest, eds. Common Bean, Research for Crops Improvement, pp.287-443.

Yang, X. 1995.Greenhouse micrometeorology and estimation of heat and water vapor fluxes. *J.agric.Engn.Res*.61:227:238.

Yordanov, I., T.Tsonev., V.Velikova., K. Georgieva., P. Ivanov., N. Tsenov., and T.Petrova. 2001. Changes in CO2 assimilation, transpiration and stomatal resistance of different wheat cultivars experiencing drought under field conditions. *Bulg, J. Plant Physiol.* (3-4):20-33.

Chapter 4

Evapotranspiration and Crop Coefficients for Two Common Bean (*Phaseolus vulgaris L.*) Genotypes With and Without Drought Stress

ABSTRACT

The product of the single crop coefficient (K_c) or the dual crop coefficients (K_{cb} and K_e) and the reference evapotranspiration (ET_o) is a widely used method for crop evapotranspiration estimation (ET_c), recommended by the Food and Agriculture Organization (FAO) of the United Nations in their Irrigation and Drainage Papers 24 and 56. ET_c, K_c, K_{cb} and K_e were measured for two new common bean genotypes, during two growing seasons (2006 and 2007) in southern Puerto Rico, at the University of Puerto Rico Experiment Station at Fortuna, during the driest months of the year (January-April). The genotypes (*P. vulgaris* L.) planted were: Morales, commonly grown in Puerto Rico; and SER 16, which is drought tolerant and was developed at the Centro Internacional de Agricultura Tropical (CIAT) (Colombia); both with a type II plant architecture. Drought stress was applied for both genotypes at flowering through to maduration. ET_c was both measured with drainage lysimeters and estimated using the generalized Penman-Montetith (PM) method with variable aerodynamic (r_a) and surface resistance (r_s). Additionally, an automatic weather station was placed in a nearby (well irrigated grass) to estimate ET_o using the PM-reference model. The linearized K_c for the initial, mid, and end stages of growth for Morales were: $K_{c\ ini}$ =0.25; $K_{c\ mid}$ =0.90 and $K_{c\ end}$ = 0.50, and for SER 16 were: $K_{c\ ini}$ =0.22; K_c mid =0.80 and $K_{c\ end}$ = 0.30. 'Morales' was more adversely affected by drought stress than SER 16. Also the K_c for both genotypes were correlated with the fraction of the soil covered by vegetation (f_c) and cumulative grown degree days (CGDD). The stress coefficient (K_s) maintained a value of 1.0 when the root zone depletion (D_r) was less than 10 mm within 0-20 cm measured soil surface and less than 15 mm within 0-40 cm of top soil surface. The total average ET_c for Morales without drought stress was: 211 mm in the lysimeters and 172.2 mm using the PM method during 2006, and 215 mm in the lysimeters and 190 mm using the PM method in 2007. The total average ET_c for SER 16 without drought stress was 142 mm in the lysimeters and 147 mm using the PM method in 2006, and 152.5 mm in the lysimeter and 166.3 mm using the PM method in 2007. The r_s was a determinant variable in the ET_c estimation under drought stress in both genotypes.

Key words: Evapotranspiration, common bean, crop coefficient, drought stress.

Abbreviations: ET_c, crop evapotranspiration; ET_o, reference evapotranspiration; K_c, crop coefficient; K_{cb}, transpiration coefficient; K_e, evaporation coefficient; K_s, drought stress coefficient; D_r, root zone depletion; f_c, fraction of the soil covered by vegetation; CDGG, cumulative degree days.

INTRODUCTION

The common bean is one of the most important proteins directly consumed, for more than 300 million of the world's people is the centerpiece of their daily diet (Bebe and McClafferty, 2006). During 2001-03, dry bean farm cash receipts averaged $446 million—ninth among U.S. vegetables. Averaging 6.8 pounds per person during 2001-03 (USDA, 2005). In the Caribbean (Cuba, Haiti and Dominican Republic) the area planted to bean is 157 hax10^{-3}, with a production equal to 141 MTx10^{-3} (Broughton et al., 2003). In Puerto Rico, the green-shelled bean production during the period from 2000 to 2003 averaged 11,696 quintales/year (1,169 tons/year), with an increase related to the release of the genotype `Morales`, which is currently the most popular white-seed bean variety in Puerto Rico (Beaver, 2006). The variety 'Morales' has been widely accepted in Puerto Rico for its good yield characteristics, and resistance to bean common mosaic virus (BCMV), and bean rust races prevalent in Puerto Rico (Beaver and Miklas, 1999).

In 2002, the total irrigated area in Puerto Rico was 15,782 ha (NASS, 2002). Because irrigated agriculture consumes such large quantities of water, it is necessary to improve our estimates of water application rates. This need is especially important on small islands where utilization of water supplies by urban and industrial sectors continues to increase.

In Puerto Rico, research has focused on irrigation systems and water use. Harmsen (2003) reviewed evapotranspiration studies in Puerto Rico conducted during the previous fifty years. The review revealed that crop coefficients for bean have never been determined in Puerto Rico, and the studies related with direct water consumption carried out on the island were in sugar cane, grass spp., plantain and rice using the water balance method. Since Harmsen`s review, studies have focused on irrigation rates as a function of pan evaporation (e.g., Goenaga et al. 2004).

Goyal and Gonzalez (1988) estimated water requirements for green bean and other crops using the Blaney-Criddle reference ET method. Recently, Harmsen et al. (2004) re-calculated and made corrections to pan evaporation coefficients in Puerto Rico used to estimate reference

evapotranspiration (ET_o). Harmsen and González (2005), also developed a computer program for estimating crop evapotranspiration in Puerto Rico (PRET).

One of the most critical steps in irrigation scheduling is the quantification of the crop water requirement. One way of estimating this is by multiplying the reference evapotranspiration by the crop coefficient. The resulting crop evapotranspiration estimate is equivalent to the crop water requirement. The FAO has provided the methodology for estimating the crop water requirement, described in their Drainage and Irrigation Papers, numbers 24 and 56 (Doorenbos and Pruitt, 1977 and Allen et al. 1998, respectively). In those documents they introduce and describe in detail the following coefficients: crop coefficient (K_c), basal crop coefficients (K_{cb} and K_e) and stress coefficient (K_s).

The FAO approach for estimating crop water requirements has been applied throughout the world. Sheng Li et al. (2005) estimated crop water requirements and identified timing and magnitude of water deficits for corn (*Zea mays* L.), soybean (*Glycine max* L.) and sorghum (*Sorghum bicolor* L.). Villalobos et al. (2004) used direct application of the Penman-Monteith equation to calculate crop ET_c in two commercial crops of garlic (*Allium sativum* L.) grown in Córdoba-Spain. Lin Li et al. (2003) measured K_c and evapotranspiration using a gravimetric Lysimeter and the Penman-Monteith methods in wheat and maize under the semi-arid conditions of Northen China.

The ET_c and crop coefficients for bean differ owing to genotype, developmental stage, plant density, stress intensity, and agronomic practices (i.e., Barros and Hanks, 1993, Calvache et al. 1997, Madeiros et al. 2001, Muñoz-Perea et al. 2007). Consequently, it necessary to evaluate the ET_c and crop coefficients for local conditions and local varieties, dominant crop management practices, and for the influence of drought stress. Due to the variation in crop development rates between locations and years, thermal-based indices have been used to relate crop coefficient curves more directly to phenological development (e.g., Hunsaker, 1999; Madeiros et al. 2001).

The objectives of this research were: *i*) Estimate the evapotranspiration rates for two common bean genotypes, with and without drought stress *ii*) Derive the crop coefficients, *iii*)

Derive the crop stress coefficient and *iv*) Relate the crop coefficient with easily measurable indices.

MATERIALS AND METHODS

Location. This research was conducted at the Agricultural Experiment Station of the University of Puerto Rico at Juana Diaz, PR, located in south central PR (18^{o}01'N latitude and 66^{o}22'W longitude, elevation 21 m above mean sea level), which has been classified as a semi-arid climatic zone (Goyal and Gonzalez, 1989).

Average annual rainfall is 33 inches (838 mm) and the average rainfall during the months of January, February and March are only 0.78, 0.72 and 0.86 inches, respectively (or 19.8mm, 18.3mm and 21.8mm respectively), (USDA, 1979). The annual average, minimum and maximum air temperature are: 26.22ºC, 21.33ºC, 31.05^{o}C, respectively. The daily average minimum and maximum reference evapotranspiration are 4.3, 3.4 and 5.5 mm/day (Harmsen, et al. 2002). The dominant soil is San Anton Clay Loam. Tables 4.1 and 4.2 summarize the principal agronomic practices and soil physical characteristics.

Table 4.1. Agronomic and management practices during the two years of field experiments.

Parameter	***Unit***	***2006***	***2007***
Sowing	DOY†	33	17
Emergence	DOY	38	23
Plant density	plants.m^{-2}	13.6 (Morales)	13.2 (Morales)
		6.4 (SER16)	6.0 (SER16)
Fertilization	lb.ha^{-1}	560, NPK-16-4-4 (DOY 62)	560, NPK (16-4-4) (DOY 52)
Irrigation system		Drip	Drip
Harvest	DOY	110-111	101-102
Growing period	Days	75	78

†Day of year.

Table 4.2. Soil physical characteristics.

Soil physical information	Depth (cm)			
	0-20	20-40	40-60	60-80
Bulk density (g.cm^{-3})	1.35	1.56	1.61	1.61
Sand (%)	30.25	30.08	20.94	24.23
Silt (%)	44.28	43.79	26.74	19.27
Clay (%)	25.47	26.13	52.32	56.50
FC[1] (m^3.m^{-3})	0.30	0.31	0.35	0.35
WP[2] (m^3.m^{-3})	0.18	0.20	0.21	0.21
TAW[3] (mm)	24	22	28	28

Source: UDSA (1987); [1] Field Capacity (Moisture content at 0.33 bar); [2] Wilting Point (Moisture content at > 15 bar); [3]Total available water = 1000(FC-WP)Z_t.; Zr. Rooting depth (m)

Experimental procedure. This research was carried out during the early months of the year during 2006 and 2007. During the dry period (January to April), the growing environment in southern Puerto Rico and the soil moisture content can usually be controlled by irrigation.

The lysimeters were installed in June of 2005, and the field was planted with beans during July-October. During 2006 and 2007, two common bean genotypes that exhibiting differing responses to drought stress were planted: Morales, the most widely grown small white bean in Puerto Rico, bred for yield and disease resistance, and drought susceptible (Beaver and Miklas, 1999); the second, SER 16, with red seed color, bred by CIAT-Colombia (Dr. Streve Beebe) for drought tolerance. Both genotypes have a type II growth habit. The seed density of planting was 14.0 plant m^{-2} for Morales and 6.5 plant m^{-2} for SER 16 (the differences in plant density between genotypes was due to insufficient seed supplies of SER 16). Fertilizer (16-4-4, NPK) was applied at a rate of 560 lb per hectare and weeds were controlled through cultivation and herbicide application.

The experimentwas arranged in to a randomized complete block design, (Fig 4.1). The site was selected for soil uniformity.

The irrigation practices.

Prior to imposing the drought stress, irrigation was applied at rates to keep the moisture content at field capacity, with irrigation applied two times per week using a drip irrigation system. The moisture content was monitored before and after each irrigation with volumetric moisture content readings (θ_v) using a Profile probe type PR2 sensor (Delta-T Devices, Ltd.). Two soil probe access tubes per treatment were placed at 0-20 cm and 20-40 cm depths.

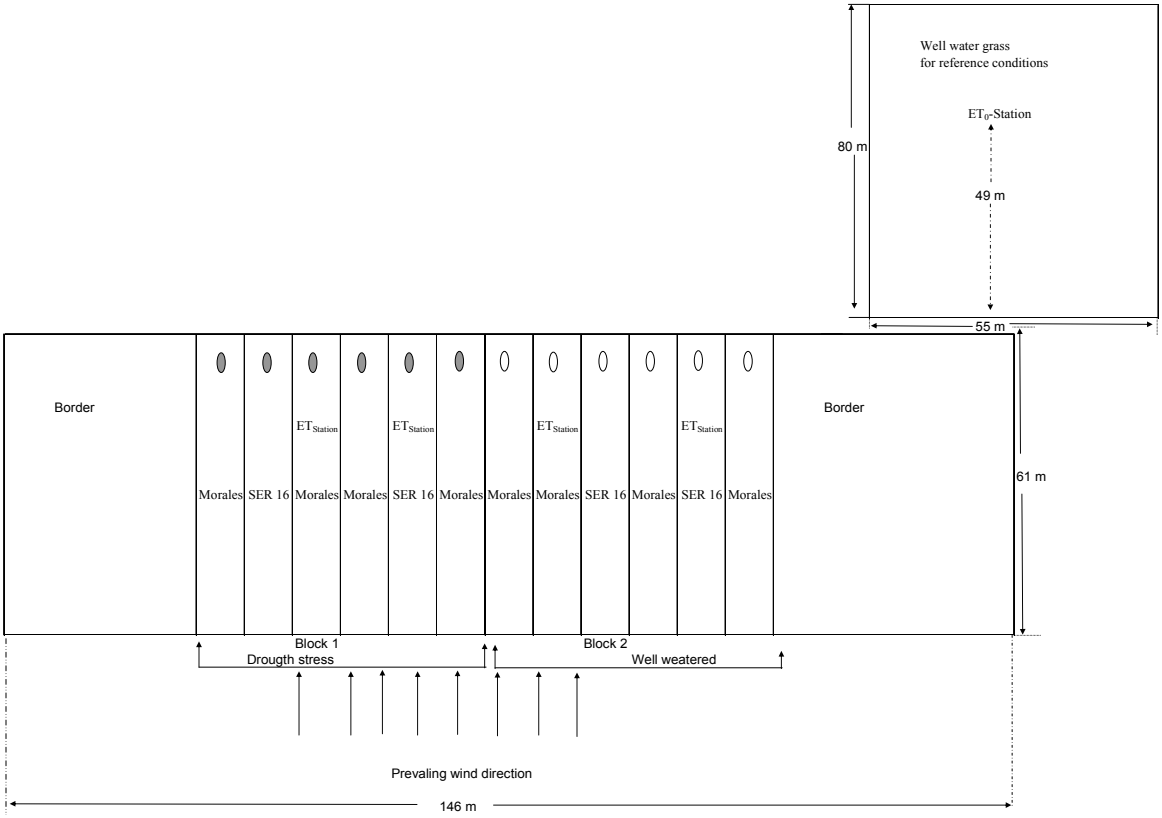

Figure 4.1. Experimental plot distribution, evapotranspiration station and lysimeter location. The circles are the lysimeters, arrange for 2006 experiment.

The volumetric moisture content (θ_v) at field capacity (FC) was measured with a profile probe type PR2 sensor (Delta-T Devices, Ltd.). An area of 1 m^2 and 50 cm was selected for the field capacity test. Two access tubes were install (0-20 cm and 20-40 cm), the area was saturated, and covered with black polyethylene plastic (Fig.4.2A), after three days of free drainage the θ_v was measured and the reading was assumed to be the moisture content at field capacity. Additionally, undisturbed core samples were taken to calibrate the sensor readings (Fig.4.2B).

Figure 4.2. Soil physical parameters measured in field. **A.** Field capacity test, at two soil depths: 0-20 cm and 20-40 cm. **B**. Undisturbed core sample collection.

The drought stress was applied at the beginning of the reproductive phenological stage known as R1 (one blossom open at any node). The drought stress plot received a water equivalent to 25% of total available water (TAW= FC-WP), corresponding with the drought stress level (DSL) (Table 4.3). The irrigation rates applied and rainfall registered during the experiment are listed in Table 4.4.

Table 4.3. Field capacity measured directly in the field with the profile probe type PR2 sensor (Delta-T Devices Ltd).

Depth	FC††	WP	DSL†
(cm)		$m^3.m^{-3}$	
0 – 20	0.38	0.18	0.23
20 – 40	0.31	0.20	0.23

†. DSL: Drought Stress level, that corresponds with the 25% of the TAW.

††. FC: Volumetric moisture content at field capacity measured with the Delta-T Profile probe.

Table 4.4. Irrigation dates and volumes of the various treatments. Juana Diaz- PR during 2006 and 2007.

Date	Growing State	Without drought stress	With drought stress	
		Irrigation (mm)		Rainfall (mm)
		2006		
14 February	V_1	21.0	19.4	3.1
17 February	V_2	18.8	19.9	7.1
22 February	V_3	30.9	31.6	2.7
25 February	V_4	3.4	3.5	0.0
27 February	V_5	12.4	12.1	0.0
3 March	V_8	19.5	20.0	0.0
11 March	R_1*	15.3	0.0	56.1
14 March	R_2	24.1	6.4	0.0
16 March	R_2	0.0	5.1	34.0
25 March	R_4	22.3	0.0	37.3
29 March	R_5	32.8	16.6	2.6
8 April	R_8	8.4	0.0	106.2
11 April	R_9	14.5	3.6	0.0
Total		**223.4**	**138.2**	**249.1**
Water deficit level			**18.0 %**	
		2007		
24 January	V_1	9.71	8.21	0.0
31 January	V_2	21.9	15.3	0.0
1 February	V_2	0.0	22.8	0.0
5 February	V_3	25.0	25.7	0.0
7 February	V_3	26.0	22.5	0.0
13 February	V_4	40.3	14.2	0.0
15 February	V_5	27.3	29.1	0.0
21 February	V_6	24.7	21.2	1.5
24 February	R_1*	10.8	0.0	0.0
26 February	R_2	12.7	0.0	0.0
1 March	R_3	29.9	10.1	0.0
5 March	R_4	34.2	22.5	0.0
6 March	R_4	0.0	9.3	0.0
9 March	R_5	60.2	19.6	0.0
12 March	R_6	27.3	13.2	0.0
15 March	R_6	31.9	0.00	0.7
20 March	R_7	15.4	15.4	0.4
23 March	R_8	14.5	8.6	19.7
28 March	R_8	0.0	0.0	13.9
30 March	R_9	0.0	0.0	17.5
Total		**379.7**	**248.3**	**53.7**
Water deficit level			**30.3%**	

*. Drought stress beginning.

V1: Completely unfolded leaves at the primary leaf node; V2: First node above primary leaf node; V3: Three nodes on the main stem including the primary leaf node. Secondary branching begins to show from branch of V1; Vn n nodes on the main stem including the primary leaf node; R1 One blossom open at any node; R2: Pods at ½-long at the first blossom position. R3: Pods at 1 inch long at first blossom position; R4: Pods 2 inches long at first blossom position; R5: Pods 3 plus inches long, seeds discernible by feel; R6: Pods 4.5 inch long spurs (maximum length). Seeds at least ¼ inch long axis; R7: Oldest pods have fully developed green seeds. Other parts of plant will have full-length Pods with seeds near same size; R8: Leaves yellowing over half of plant, very few small new pod/blossom developing, small pods may be drying. Points of maximum production has been reached; R9: Mature, at least 80% of the pods showing yellow and mostly ripe. (NDSU, 2003)

Crop evapotranspiration. Crop evapotranspiration (ET_c) was measured by two methods. The *Water Balance Method*, used drainage type lysimeters. The drainage lysimeter has been used successfully in evapotranspiration studies [e.g., Pereira and Adaixo (1991); Brian and Boman (1991); Karam et al. (2005)], and can provide satisfactory estimates of water use over 3 and 4-day intervals (Caspari et al. 1993); where the evapotranspiration is given by:

$$ET_c = P + I - RO - DP + (\Delta S) \qquad (4.1)$$

where ET_c is the crop evapotranspiration, P is precipitation, I is irrigation, RO is surface runoff, DP is deep percolation below the root zone, and ΔS is the change in root zone moisture storage, (all units are in mm). The change ΔS was converted to equivalent depth of water in mm by multiplying the lysimeter moisture contents by the conversion factor 0.22 $m^2.mm^{-1}$, and RO and DP measured were converted to equivalent depth of water in mm by dividing by lysimeter conversion factor of 0.22 $L.mm^{-1}$. Twelve drainage lysimeters were installed in the experimental field in 2005, and planted two with SER 16 and four with Morales in 2006 and three with each one per water level in 2007 (Figure 4.3).

The soil into the lysimeter was encased in round polyethylene containers with an exposed soil surface of 0.22-m^2 and 0.8-m depth. The containers were sufficiently deep to accommodate

the plant roots. The lysimeters were located within plots measuring 7-m wide by 61-m long, with the long dimension oriented in the direction of the prevailing wind (Figure 4.1).

In order to achieve similar conditions inside and outside the lysimeters, the following procedure was followed for each lysimeter: i) Soil was removed from the location of the lysimeter in 0.25-m (12 inch) depth intervals. The soil from each depth interval was stockpiled separately; ii) The polyethylene containers were placed in the hole; iii) A 20-cm layer of gravel was placed in the bottom of the polyethylene tank and a 1.25 inch (30 cm) PVC tube was placed in the botton to remove the percolated water during operation (Figure 4.3 C); iv) the stock piled soil was placed in the container in the reverse order that the soil was excavated. Each layer was carefully compacted until the original 0.25-m layer thicknesses were achieved. After the container was full, the surface runoff collector and the access tube to measure the volumetric moisture content were installed (Figure 4.3 B). The runoff collector consists of a small tank (0.20 m deep) connected to the lysimeter with a plastic gutter (Figure 4.3).

Daily rainfall was measured within each lysimeter with a manual rain gauge (Figure 4.3 B) and compared with an automated tipping bucket rain gauge (WatchDog^{TM}-Spectrum Technology, Inc) located within the reference conditions area (Fig. 4.1). The irrigation was measured using a cumulative electronic digital flow meter (GPI, Inc., Fig. 4.4C), and was recorded manually at the beginning and end of each irrigation event every three or four days. Two flow meters were placed on the irrigation supply lines, one on the well-watered treatment supply line and the second on the drought stress treatment water supply line.

Runoff and depth percolation from each lysimeter were collected periodically (every three or four days). Water from RO and DP was removed from the collection containers periodically by means of a small vacuum pump (Shurflu-4UN26, 12V, 4.5GPM).

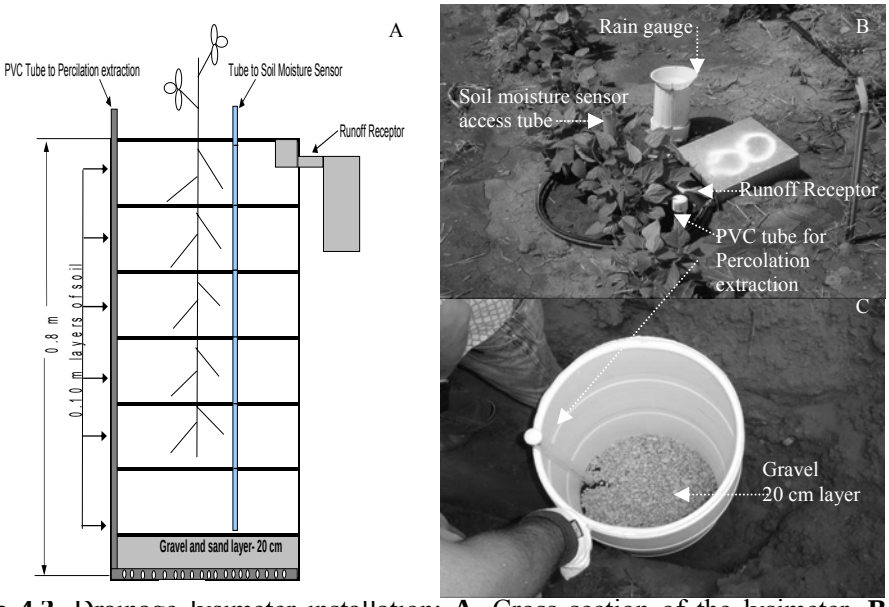

Figure 4.3. Drainage lysimeter installation: **A.** Cross section of the lysimeter, **B** runoff collector, soil moisture sensor, rain gauge, and depth percolation collector's overview, **C**. drainage system to depth percolation measurement.

The depth of water in the soil profile was related to the soil moisture content as follows:

$$S_i = \Sigma (\theta_{v,i0cm} Z_{0cm} + \theta_{v,i10cm} Z_{10cm} + \ldots . \theta_{v,i60cm} Z_{60cm}) \quad (4.2)$$

where S_i is the depth of soil water on day i [mm], $\theta_{v,i}$ is the volumetric soil moisture content on day i and Z is the thickness of the soil layer. Volumetric soil moisture was measured using a profile probe type PR2 sensor (Delta-T Devices Ltd) and measurements were obtained for each 10 cm depth interval (Figure 4.3A).

The crop evapotranspiration was also derived, using a second method from meteorological and crop data by means of the *Penman-Monteith* model (equation 4.3), with direct measurement of canopy and aerodynamic resistances during the whole growing season. For this purpose four automatic weather stations were located within the experimental plots as follows: genotype Morales without drought stress genotype SER 16 with drought-stress,

genotype SER 16 without drought stressd, genotype Morales with drought-stress. Each weather station was equipped with: Kipp & Zonen B.V. net radiometer (spectral range 0.2-100μm), wind direction and wind speed with wind sensor-Met one 034B-L at 2.2 m; air temperature and relative humidity with HMP45C temperature and relative humidity probe at 2.0 m; soil temperature with TCAV averaging soil thermocouple probe at 0.08 m and 0.02 m depth, soil heat flux using soil heat flux plates at 0.06m depth; and a volumetric soil moisture content with a CS616 water content reflectometers at 0.15 m depth. Six data points per minutes were collected by each sensor and stored in a CR10X data logger (Campbell scientific, Inc.-Fig. 4.4B).

The *Penman-Monteith* model described by Monteith and Unsworth, (1990); Allen at al.(1998); and Kjelgaard and Stockle, (2001) was used to calculate the latent heat flux (λE), which was then divided by the latent heat of vaporization (λ) to obtain ET_c.

$$\lambda E = \frac{\Delta(R_n - G) + \rho_a C_p \dfrac{VPD}{r_a}}{\Delta + \gamma\left(1 + \dfrac{r_s}{r_a}\right)} \qquad (4.3)$$

Where: λE is Latent heat flux (Wm^{-2}), R_n is net radiation (Wm^{-2}), G is soil heat flux (Wm^{-2}), VPD is vapor pressure deficit (kPa), Δ is slope of saturation vapor pressure curve (kPa $\circ C^{-1}$) at air temperature, ρ_a is density of air (Kgm^{-3}), Cp is specific heat of air ($J\ Kg^{-1}\circ C^{-1}$), γ is psychometric constant (kPa $\circ C^{-1}$), *VPD* vapor pressure deficit (kPa), r_a is the aerodynamic resistance ($s\ m^{-1}$), and r_s canopy resistance to vapor transport ($s\ m^{-1}$).

The density of air was estimated with the equation 4.10, the virtual temperature (T_{kv}) with the equation 4.5, the C_p was with the equation 4.9, Δ , and λ with the equations 4.8, and 4.7.

.

$$P = 101.3\left[\frac{293 - 0.0065z}{293}\right]^{5.26} \qquad (4.4)$$

$$T_{kv} = 1.01(T + 273) \qquad (4.5)$$

$$\gamma = 0.665x10^{-3}P \quad (4.6)$$

$$\lambda = 2{,}502.3\text{-}2.308\text{T} \quad (4.7)$$

$$\Delta = \frac{40978\left[0.6108\exp\left(\frac{17.17T}{T+237.3}\right)\right]}{(T+237.3)^2} \quad (4.8)$$

$$C_p = \frac{\gamma.\varepsilon.\lambda}{P} \quad (4.9)$$

$$\rho_a = \frac{P}{T_{kv}.R} \quad (4.10)$$

where z is the elevation in m. The VPD is the difference between saturated and actual vapor pressure deficit (e_s-e_a), e_s estimated using equation 4.11 and e_a using the relationship between relative humidity (RH) and the saturated vapor pressure (equation 4.12). P is the atmospheric pressure (kPa), and R is the gas constant (0.287 $kJ.Kg^{-1}.K^{-1}$).:

$$e_s = 0.6108\exp\left[\frac{17.27T}{T+237.3}\right] \quad (4.11)$$

$$e_a = \frac{e_s RH}{100} \quad (4.12)$$

The soil heat flux was estimated using the soil heat flux plates, soil thermocouples and soil moisture sensor readings as follow:

$$G = FX + S \quad (4.13)$$

where FX is measured soil-heat flux (Wm^{-2}), at a depth of 6 cm below the soil surface, and S is the storage as soil heat (Wm^{-2}) and was calculate using the equation 4.14.

$$S = \left[\frac{\Delta Ts}{\Delta t}\right] d\rho_b \left(C_s + (WC_w)\right) \quad (4.14)$$

where ΔT is the soil temperature gradient at the two depths, Δt is the time interval between measurements (10 seconds), d is the depth to the soil-heat-flux plates (0.08m), ρ_b is bulk density of the dry soil ($kg.m^{-3}$), C_s is the specific heat of the dry soil (840 J/Kg◦C), W is water content of the soil (kg of water/ Kg of soil) and C_w is specific heat of water (4,190 J/Kg◦C).

The aerodynamic resistance (r_a) is the resistance to the transport of heat and water vapor from the evaporating surface into air above the canopy and was estimated with the Perrier equation (Allen et al. 1998, and Alves et al. 1998).

$$r_a = \frac{Ln\left[\frac{(Z_m - d)}{Z_{om}}\right] Ln\left[\frac{(Z_h - d)}{Z_{oh}}\right]}{K^2 u_z} \qquad (4.15)$$

where Z_m is the height of wind measurements [m], z_h is the height of the humidity measurements [m], d is the zero displacement height [m], Z_{om} is the roughness length governing momentum transfer of heat and vapor [m] is 0.123h, Z_{oh} is roughness length governing transfer of heat and vapor [m] is $0.1Z_{om}$, K is the von Karman`s constant [0.41], u_z is the horizontal wind speed ($m.s^{-1}$) at height z and h is the canopy height (m). The canopy height was measured for each genotype one time per week, and polynomial models were developed to estimate daily values of h as a function of the day of the year (DOY). The r_a was calculated at one minute time intervals.

The canopy resistance (r_s) describes the resistance of vapor flow through a transpiring crop and evaporation from the soil surface, which depends on climatic factors and available soil water. Bulk surface resistance was calculated using the equation (4.16) proposed by Szeicz and Long (1969) and recommended by Allen et al. (1998-FAO-56):

$$r_s = \frac{r_L}{LAI_{active}} \qquad (4.16)$$

where LAI_{active} is the active leaf area index (m^2 -leaf area / m^{-2} -soil surface), equal to 0.5 times the leaf are index, and r_L is the stomatal resistance ($m.s^{-1}$) which is the total resistance from cell surfaces to the exterior leaf surfaces (Wenkert, 1983) and is one of the most sensitive elements in

the evapotranspiration under drought stress conditions. The r_L was measured several times during the day from 7:00 to 17:00 in order to obtain a reasonable average value for each phenological growing phase, for each genotype and water level. Two leaf porometers were used: an AP4-UM-3 (Delta-T Devices Ltd) during 2005 and a model SC-1 (Decagon Devices, Inc. Fig 4.4D) during 2006; reading were made once per week.

The LAI was estimated using a non-destructive method described previously in the Chapter 1, which estimates the leaf area (LA) in cm^2 using the maximum single leaf width (W) in cm. The models used were: LA = 9.35(W)-20.32 for SER16, and LA = 7.80(W)-14.59 for SER16, and then according to the plant density the LAI was estimated on a weekly basis.

Reference evapotranspiration. The reference evapotranspiration (ET_o) corresponds to the evapotranspiration from a reference crop (e.g., alfalfa or grass) under reference conditions. It is common to use a hypothetical grass reference, with a constant canopy height, canopy resistance and albedo, under well-watered conditions (Allen et al., 1998). For this research, one automatic weather station (WatchDog-900ET, Spectrum Technologies, Inc) was placed within a field planted with a reference crop (grass-*Panicum maximum* and *Clitoria termatea* L.*)* with enough fetch and sufficient water supply during the research period, and adjacent to the experimental area (Figure 4.1, and 4.4 A). The canopy height was maintained close to 0.15 cm throughout the growing seasons. The automatic weather station measured basic weather information including: solar radiation, temperature, humidity, wind speed and direction every 10 minutes. ET_o was calculated using the Penman-Monteith equation recommending by FAO-56 equations (Allen et al. 1998) and standardized by the American Society of Civil Engineer-ASCE (Walter et al. 2002).

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{C_n}{T + 273} U_2 (e_s - e_a)}{\Delta + \gamma(1 + C_d U_2)} \qquad (4.17)$$

where ET_o is the standardized reference crop evapotranspiration for short (ET_{os}) or tall (ET_{rs}) surfaces (mm d^{-1} for daily time steps or mm h^{-1} for hourly time steps); R_n is net radiation at the

crop surface ($MJm^{-2}d^{-1}$ or $MJm^{-2}h^{-1}$), G is soil heat flux density ($MJm^{-2}d^{-1}$ or $MJm^{-2}h^{-1}$), T is mean daily temperature at 1.5 to 2.5 m height (ºC), U_2 is wind speed at 2 m height ($m\ s^{-1}$), e_s is saturation vapor pressure (kPa), e_a is actual vapor pressure at 1.5 to 2.5 m height (kPa), Δ is the slope of the saturation vapor pressure-temperature curve (kPa $ºC^{-1}$), γ is the psychrometric constant (kPa $ºC^{-1}$), C_n is the numerator constant that changes with the reference type and calculation time step, and C_d is the denominator constant that changes with the reference type and calculation time step. C_n incorporates the effect of the aerodynamic roughness of the surface (i.e., reference type), while C_d incorporates the effects of bulk surface resistance and aerodynamic roughness of the surface (Walter et al. 2002). Values of C_n and C_d are presented in Table 4.5.

The R_n was calculated using the general energy balance equation (4.18), where R_{ns} is the incoming net shortwave radiation equation 4.19 and R_{nl} is the outgoing net longwave radiation, equation 4.20.

$$R_n = R_{ns} - R_{nl} \tag{4.18}$$

$$R_{ns} = (1-\alpha)R_s \tag{4.19}$$

$$R_{nl} = \sigma\left[\frac{T_{\max},K^4 + T_{\min},K^4}{2}\right]\left(0.34 - 0.14\sqrt{e_a}\right)\left(1.35\frac{R_s}{R_{so}} - 0.35\right) \tag{4.20}$$

where R_s is the solar radiation ($MJ.m^{-2}.day^{-1}$) which was measured with a silicon pyranometer sensor installed int the WatchDog-900ET station, α is the albedo (= 0.23) for grass reference conditions, T_{max},K and T_{max},K are the maximum and minimum air temperatures, σ is the Stefan-Boltzman constant ($4.903x10^{-9}$ $MJK^{-4}m^{-2}day^{-1}$) and R_{so} is the clear-sky radiation and was calculated as a function of altitude (z) and extraterrestrial radiation (R_a) equation 4.21.

$$R_{so} = \left(0.75 + 2x10^{-5}z\right)R_a \tag{4.21}$$

Table 4.5. Values of Cn and Cd in the Reference Evapotranspiration equation.

Calculation time Step	Short Reference ET_{os} [0.12m]		Tall Reference ET_{rs} [0.50m]		Units for ET_{os} and ET_{rs}	Units for Rn, G
	Cn	Cd	Cn	Cd		
Daily	900	0.34	1600	0.38	$mm.d^{-1}$	$MJm^{-2}d^{-1}$
Hourly during daytime	37	0.24	66	0.25	$mm.h^{-1}$	$MJm^{-2}h^{-1}$
Hourly durin nigtime	37	0.96	66	1.7	$mm.h^{-1}$	$MJm^{-2}h^{-1}$

When the ET_o is derived from weather data, it is necessary to verify the quality and integrity of the data. Allen (1996) proposed the difference between daily minimum air temperature (T_{min}) with daily average dew point temperature (T_{dew}) as a reference parameter. In this study if the difference T_{min}- T_{dew} was greater than 3°C, the conditions were considered to be "non-reference" (Jia et al. 2005), and ET_o was not calculated. Dewpoint temperature was estimated as a e_a function, with the Tetens equation (4.22).

$$T_{dew} = \frac{237.3 Ln\left[\frac{e_a}{4.584}\right]}{12.27 - Ln\left[\frac{e_a}{4.584}\right]} \qquad (4.22)$$

Where: the e_a is in mmHg.

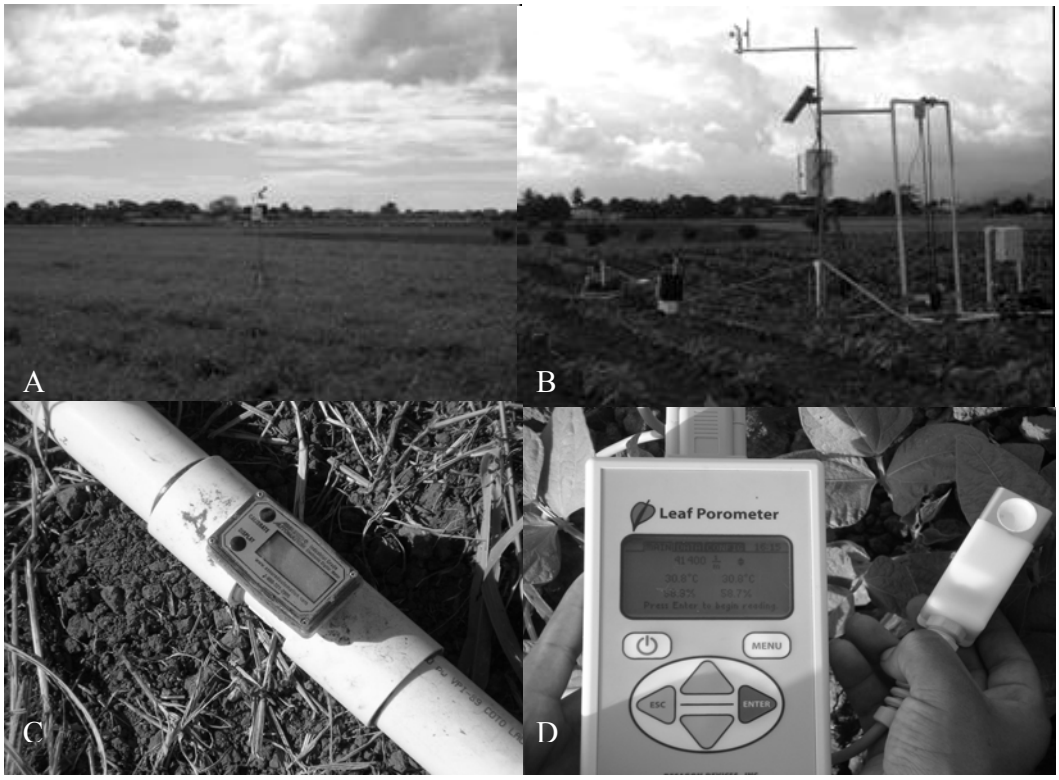

Figure 4.4 A. Automatic weather station to estimate the reference evapotranspiration, **B**. ET station equipment and drainage lysimeter for crop evapotranspiration estimation, **C**. Flow meter in the principal irrigation lines and **D**. Leaf porometer equipment.

Single crop coefficient. The crop coefficient (K_c) accounts for the effects of characteristics that distinguish the field crop from the reference crop (Allen et al. 1998), is a commonly used approach for estimation of consumptive use of water by irrigation, represents the ET under a high level of management and with little or no water or other stresses (Allen et al. 2005), and is equal to the ratio of the crop evapotranspiration to the reference evapotranspiration.

$$K_c = \frac{ET_c}{ET_o} \tag{4.23}$$

The crop effects can be combined into one single coefficient (equation 4.23), or it can be split into two factors describing evaporation from the soil and transpiration from the leaves. As

soil evaporation fluctuates daily as a result of rainfall or irrigation, the single crop coefficient expresses only the time-averaged (multi-day) effects of crop evapotranspiration. In determining crop coefficients for a crop season, four stages of crop growth are normally considered (FAO-56. Allen et al. 1998), which depend on phenological stages, and can be described by a K_c-curve that includes the variation of the coefficient during the whole growing season (Appendix A). The K_c curve is comprised of four straight line segments that represent the initial period ($K_{c\ ini}$), the development period ($K_{c\ dev}$), the midseason period ($K_{c\ mid}$) and the late season period ($K_{c\ end}$). $K_{c\ ini}$ represents the period until approximately 10% of the ground is covered by vegetation (f_c), $K_{c\ mid}$ defines the value for K_c during the peak period for the crop, which is normally when the crop is at "effective full cover", considered to be at the initiation of flowering (R1) in this research, and $K_{c\ end}$ has a sloping line that connects the end of the midseason period with the harvest date (Allen et al. 1998 and Allen et al. 2005).

The cover fraction (f_c) is a function of: vegetation type, ground cover, plant density, canopy architecture, and environmental stresses like drought. In this study weekly f_c measurements were collected for each genotype and water condition.

Dual crop coefficients: The dual crop coefficients are the basal crop coefficient (K_{bc}) and soil evaporation coefficient (K_e). The coefficients K_{bc} and K_e relate the potential plant transpiration and soil evaporation, respectively, to the crop evapotranspiration.

Measurement of K_e and K_{cb} were made using the FAO-56 approach (Allen et al.1998), as follows:

$$ET_c = (K_{cb}+K_e)\ ET_o \qquad (4.24)$$

$$K_{cb} = (ET_c/ET_o)-K_e \qquad (4.25)$$

The soil evaporation coefficient (K_e), was estimated as a function of field surface wetted by irrigation (f_{ew}) and K_c, equation 4.26, and the f_{ew} was, estimated as a minimum value between

the fraction of the soil that is exposed to sunlight and air ventilation and serves as a source of soil evaporation ($1-f_{c-}$; Appendix C), and the fraction of soil surface wetted by irrigation or precipitation (f_w), equations 4.27a and 4.27b, which were measured twice per week.

$$K_e = f_{ew} K_c \quad (4.26)$$

$$f_{ew} = \min (1-f_c; f_w) \quad (4.27a)$$

and for drip irrigation:

$$f_{ew} = \min [(1-f_c); (1-0.67f_c)(f_w)] \quad (4.27b)$$

If the water source was drip irrigation the f_w was estimated as a cover crop fraction (equation 4.28), and on days with rain was equal to 1.0.

$$f_w = 1 - \frac{2}{3} f_c \quad (4.28)$$

The crop stress factor. The crop stress factor (K_s) is an important coefficient because it helps to distinguish which crops are sensitive to water deficit conditions (Roygard et al. 2002). The crop stress factor is a function of the average soil moisture content or matric potential in a soil layer. It can usually be estimated by empirical formulas based in soil water content or relative soil water. The K_s was determined throughout the crop season for each study plot. The crop stress factor, as described by Allen et al. (1998), has a value between 0 and 1. A value of 1 indicates stress-free conditions (e.g., water is readily available for plant use), whereas a value of zero indicates no available water for plant use. As normally applied, the crop stress factor is equal to 1 until the depletion of water reaches some critical depletion. For example, for dry bean the critical value could be between 45-50% of total available water (Allen et al. 1998). Total available water is defined as the field capacity moisture content minus the wilting point moisture content (TAW, equation 4.30). After depletion exceeds the critical value, the crop stress factor drops linearly until reaching zero at the wilting point moisture content.

The K_s was estimated according to FAO-56 methodology by Allen et al. (1998), equation 4.29.

$$Ks = \frac{TAW - Dr}{TAW - RAW} = \frac{TAW - Dr}{(1 - p)TAW} \quad (4.29)$$

where TAW is total available water referring to the capacity of a soil to retain water for plant use (mm), D_r is the root zone depletion (mm), RAW is the readily available soil water in the root zone (mm) equation 4.29, p is the fraction of TAW that the crop can extract from the root zone without suffering water stress.

$$TAW = 1000(\theta_{FC} - \theta_{WP})Z_t \quad (4.30)$$

where θ_{FC} is the water content at field capacity ($m^3.m^{-3}$), θ_{WP} is the water content at wilting point ($m^3.m^{-3}$), and Z_t is the rooting depth (m). RAW can be estimated as follows:

$$RAW = pTAW \quad (4.31)$$

where p is the average fraction of total available soil water (TAW) that can be depleted from the root zone before moisture stress (reduction in ET) occurs. In this study p was estimated according to equation 4.32.

$$p = 0.45+0.004 \text{ x } (5-ET_c) \quad (4.32)$$

RESULTS AND DISCUSSION

Evapotranspiration. Weather conditions prevailing during the two years are shown in Table 4.6, and are compared with the long-term record presented by Goyal and González (1989) and Harmsen et al. (2004). Figure 4.6 shows the seasonal variation in daily climatic elements for the Fortuna-Experiment Station, Juan Diaz, PR. The growing period from February to April in 2006 was cooler and rainier than compared with the same period in 2007, but the solar radiation was higher. 2007 was warmer than the long-term average, with higher values of T_{min}, T_{max} and T_{mean}. In 2006, eighteen (18) rainfall events were recorded, wetter than the long-term average. Rainfalls totals greater than 5.0 mm were register on 8 days (DOY`s: 48= 7.1 mm; 63 = 23.2 mm; 64=32.4 mm; 75=34.0 mm, 78=35.0 mm, 89=61.2 mm; 93=37.5 mm and 96 = 7.2 mm). During 2007, thirteen (13) rainfalls were recorded during the experiment, but rainfall on just 3 day was greater than 5.0 mm (DOY`s: 80 = 17.4mm; 86 = 8.8mm, and 88 = 8.8mm). The drought stress treatment in 2006 were was started on DOY 70 (March 11) and 2007 on DOY 55 (February 24).

Six days in 2006 and ten days in 2007 were determined to be "non-reference" conditions for ET_0 estimation, where the T_{min}-$T_{average\ dew}$ >3.0ºC (Fig 4.5), which is indicated a lack of well-watered conditions. These days were corrected using methodology presented by Allen et al. (1998).

Table 4.6. Mean daily weather conditions during the experiment at the Fortuna Experimental Station (Juana Diaz, PR), measured under reference conditions and compared with long-run means (1960-1987).

	Jan	***Feb***	***Mar***	***Apr***
	2006			
Min. Air temperature (^oC)	nd‡	18.2	19.5	20.5
Max. Air temperature (^oC)	nd	30.7	29.4	30.1
Mean. Air temperature (^oC)	nd	24.7	24.2	24.8
Solar Radiation ($W.m^{-2}$)	nd	200.6	224.2	243.2
Relative humidity (%)	nd	65.0	72.6	74.7
Wind speed ($m.s^{-1}$)	nd	2.2	3.1	2.9
Rainfall (mm)	nd	13.5	191.2	57.7
	2007			
Min. Air temperature (^oC)	19.9	20.3	21.3	17.8
Max. Air temperature (^oC)	29.5	29.9	30.2	30.6
Mean. Air temperature (^oC)	24.7	25.0	25.3	23.9
Solar Radiation ($W.m^{-2}$)	181.6	181.2	184.8	254.7
Relative humidity (%)	66.7	66.9	66.5	57.5
Wind speed ($m.s^{-1}$)	2.9	2. 8	3.1	2.8
Rainfall (mm)	1.8	1.4	46.9	0
	(1960-1987)†			
Min. Air temperature (^oC)	22.6	18.6	18.9	19. 8
Max. Air temperature (^oC)	29.7	29.7	30.2	30.6
Mean. Air temperature (^oC)	24.1	24.2	24.6	25.2
Solar Radiation ($W.m^{-2}$)	nd	nd	nd	nd
Relative humidity (%)	nd	nd	nd	nd
Win direction (Deg)	nd	nd	nd	nd
Wind speed ($m.s^{-1}$)	nd	nd	nd	nd
Rainfall (mm)	23. 8	20.0	32.5	53.34
Reference Evapotranspiration (mm)¶	104	107	139	147

† Goyal and Gonzalez, (1989); ‡ No data.; ¶. Harmsen et al. (2004).

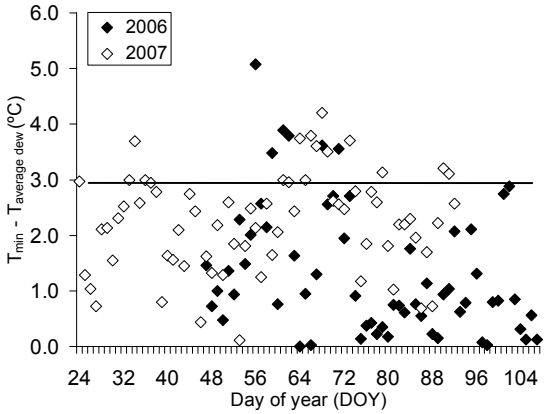

Figure 4.5. Difference in minimum temperature and daily average dew temperature, for reference evaluation in the ET_o estimation during 2006 and 2007.

When the daily mean value of surface sensible heat flux (H) is negative, Berengena and Gavilán (2005) reported that the advection intensity could be quantified using the evapotranspiration fraction ET/R_n, when $H < 0$ and $ET/R_n > 1.0$. In this study, all the crop and references evapotranspiration (ET_c and ET_o) estimations with the P-M model registered $ET/R_n < 1.0$ in both years, and when the reference correction was made, small changes in the ET_o were observed (0.1 $mm.day^{-1}$), Berengena and Gavilán (2005) reported that the P-M reference evapotranspiration can give appropriate estimates of ET_o even under strong advective conditions.

The ET_o rate for 2006 varied between 2.4 to 6.5 $mm.day^{-1}$ with a mean of 4.3 $mm.day^{-1}$. In 2007 the ET_o rate varied between 2.2 to 6.3 $mm.day^{-1}$ with a mean of 4.0 $mm.day^{-1}$. The lower value for 2007 may be attributable to the lower solar radiation. Harmsen et al. (2004) estimated the long-term ET_o rates for January, February, March and April as 3.4, 3.8, 4.5 and 4.9 $mm.day^{-1}$ respectively, with a mean of 4.1 mm.

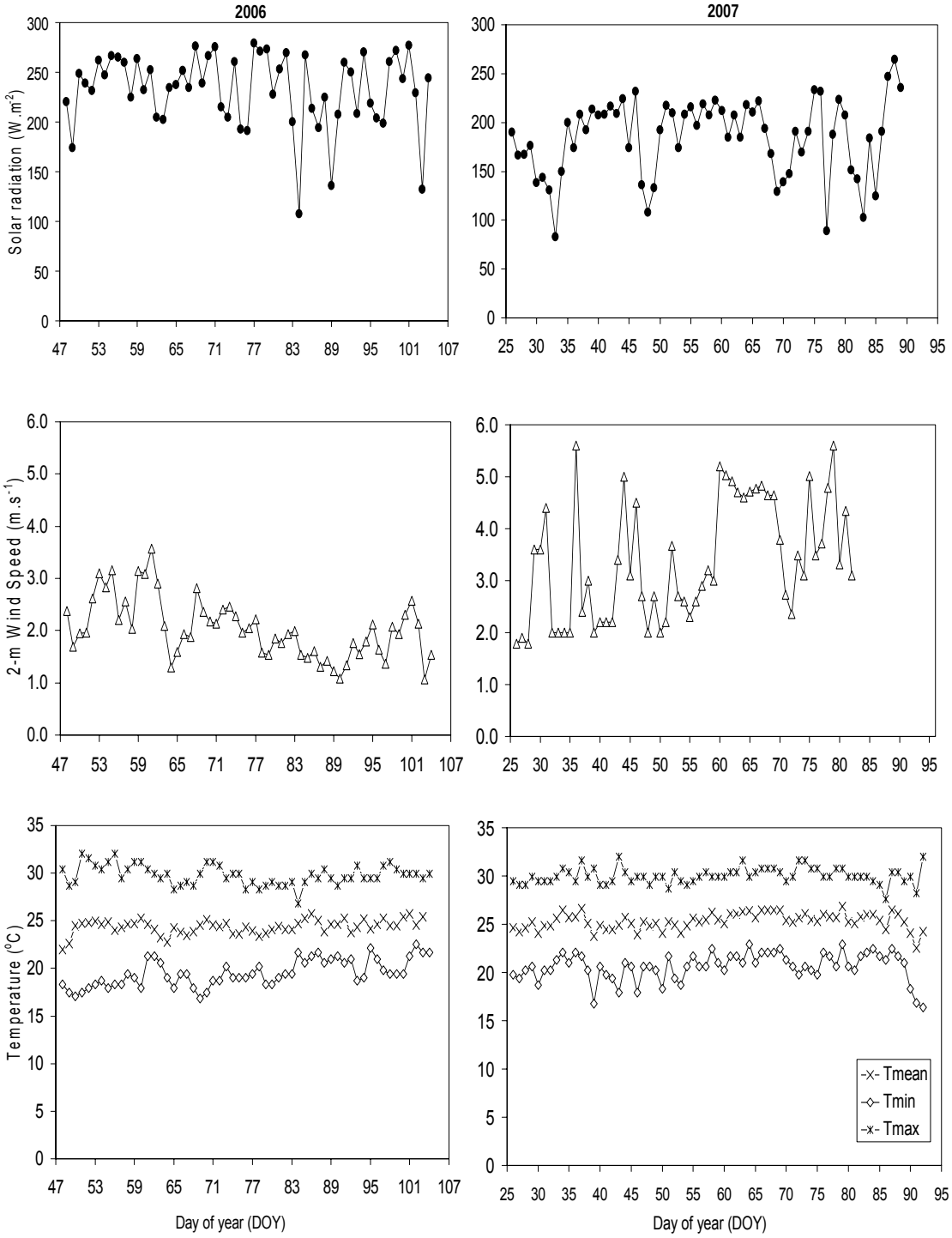

Figure 4.6. Daily climatic parameters for the 2006 and 2007 seasons at the Fortuna Experiment Station-Juan Diaz,PR.

The ET_c measured by the drainage lysimeters for Morales without drought stress totaled 211 mm in 2006 and 215 mm in 2007, compared with 172.2 mm and 190.0 mm respectively using the P-M model. The ET_c measured by the drainage lysimeters for SER 16 totaled 142.0 mm in 2006, and 152.5 mm in 2007 and with PM-Model 147.2 and 166.3 mm respectively (Table 4.7). The lower ET_c values for SER 16 were associated with the lower plant density, compared with Morales. The water requirements for dry bean for a 90 to 100-day season ranges from 350 to 500 mm depending upon the soil, climate and cultivar (Allen et al. 2000 in Muñoz-Perea et al. 2007). For a 122-day season, Calvache et al. (1997) reported a crop water requirement of 447 mm for dry bean.

The low seasonal crop evapotranspiration values in this study are associated with: short crop season (75 and 78 days in 2006-2007 respectively), low plant density, climatic factors (low evaporative demand), and the irrigation system (drip), that dismiss the soil evaporation. Muñoz-Perea et al. (2007) reported genotypic differences in ET_c of 318 mm for NW63 and 457 mm for Othello under well water conditions, and 270 mm for Othello to 338 mm for Common Pinto under drought stress in Kimberly-Idaho conditions.

During the 2006 growing season, SER 16 without drought stress reached maturity earlier than the stressed treatment, which induced the high ET_c rates at the end of the season. Adams et al. (1985) reported that dry bean required 25 to 30 mm of water per week (3.6 to 4.3 $mm.day^{-1}$); and the dry bean water use rates increased from 1.3 to 6.3 $mm.day^{-1}$, during pod development (NDSU, 1997). In this study, the ET_c increased from 0.7 $mm.day^{-1}$ in vegetative growing phase to 5.1 $mm.day^{-1}$ during pod filling for Morales in 2006 without drought stress, and 0.6 $mm.day^{-1}$ to 4.6 $mm.day^{-1}$ in 2007. For SER 16, ET_c increased from 0.4 $mm.day^{-1}$ in the vegetative growing phase to 5.1 $mm.day^{-1}$ in pod filling phase in 2006, and from 0.3 $mm.day^{-1}$ to 6.7 in 2007 (Fig. 4.7).

Table 4.7. Cumulated ET_c from V2 to R9 phenological phases for two common bean genotypes, measured by water balance methods (Lysimeter) and energy balance method (Generalized Penman-Monteith).

Year	Genotype	Without drought stress		With drought stress		Reference Evapotranspiration
		Lysimetry	P-M	Lysimetry	P-M	
		mm				
2006	Morales	211.0 (5.6)†	172.2	167.3 (20.2)	154.8	256
2007	Morales	215‡	190.0	140 (26.6)	151.8	263
2006	SER16	142.0 (5.9)	147.2	100.0 (6.8)	157.6	256
2007	SER16	152.5 (0.7)	166.3	107.2 (37.7)	137.1	263

† Parenthesis values indicated 1-SD; ‡ the other two lysimeters has a plant establishing problems.

The differences in ET_c ($ET_{c\ without\ drought\ stress} - ET_{c\ with\ drought\ stress}$) from the beginning of drought stress are presented in Fig. 4.8. During 2007, the drought stress was greater, and the difference in ET_c was greater, with 40.2 mm for Morales, and 33.5 mm for SER 16 during R1 to R9, compared with 12.3 mm for Morales and 7.3 mm for SER 16 during 2006 for the same stage of plant development. The most critical differences were observed during: R2, R4 and R6 in 2006 and R2, R3, R4, R5, R6 and R9 stages of plant development in 2007. The common bean is most sensitive to drought stress during the pre-flowering and reproductive stages (e.g., Calvache et al. 1997; Muños-Perea et al. 2007).

The intermittent drought stress from the R1 to R9 growth stages induced seed-yield reduction for Morales of 33% in 2006 and 76% in 2007, and for SER 16 of 29% in 2006 and 67% in 2007, for small plots (2.0 m long, harvested at 6.0 $g.kg^{-1}$ of seed moisture). In the larges plots the yield reduction was exactly the same that in the small plot for both years for Morales, and for SER 16 was 33% in 2006 and 73% in 2007 (Table 7.5, chapter 7).

Without drought stress, the cumulative ET_c during vegetative growth (V1 to R1- DOY 46 to 65) in 2006, was 30 mm for Morales and 21.1 mm for SER 16. During the same growth period in 2007 (DOY 28 to 54) the cumulative ET_c was: 74.8 mm for Morales and 61.7 mm for SER 16.

The cumulative ET_c during the reproductive growth stage (R1 to R8- DOY 66 to 97) in 2006, was 118.2 mm for Morales and 108.7 mm for SER 16. During the same growth period in 2007 cumulative ET_c was 103.3 mm for Morales and 92.1 mm for SER 16. During seed maturity to harvest (DOY 98 to 104), the cumulative ET in 2006, was 26.3 mm for Morales and 17.4 mm for SER 16, and for the same growth period in 2007 was 12.0 mm for Morales and 12.5 mm for SER 16.

The larger ET_c rates were reached for both genotypes after pod initiation (R3), and maximum leaf area index (LAI) was registered at the R4 growth stage: Morales 4.2 $m^2.m^{-2}$ in 2006, and 3.0 $m^2.m^{-2}$ in 2007; SER 16 1.70 $m^2.m^{-2}$ in 2006 and 1.8 $m^2.m^{-2}$ in 2007. The differences in LAI are directly associated with the plant densities used in both genotypes (Table 4.1).

The low ET_c rates at the beginning of the growing season, DOYs 40 to 62 in 2006 and 28 to 46 in 2007, were associated with high surface resistances (r_s) as shown in Table 4.8. Changes in r_s are also associated directly with stomatal resistance (r_L) and leaf area index (LAI). The low drought stress during 2006 did not generate significant changes in LAI and r_s; however larger differences in LAI, r_L and subsequently r_s were observed in 2007 (Table 4.8), which suggest that r_s is one of the most sensitive parameters controlling ET during drought stress.

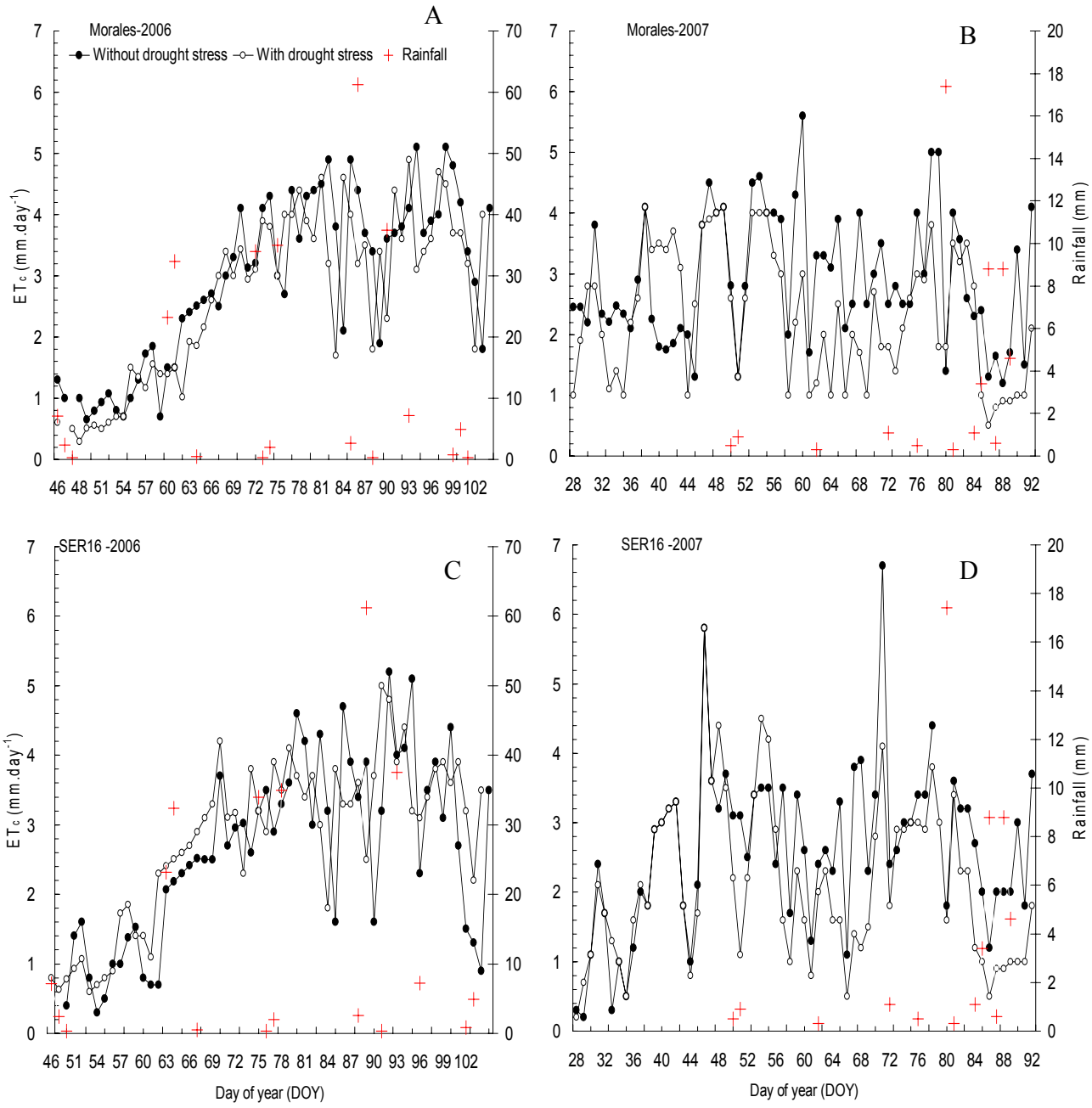

Figure 4.7. Daily crop evapotranspiration rates for two common bean genotypes, with and without drought stress during two growing seasons: **A**. Morales-2006, **B**. Morales-2007, **C**. SER 16-2006, and **D**. SER 16-2007.

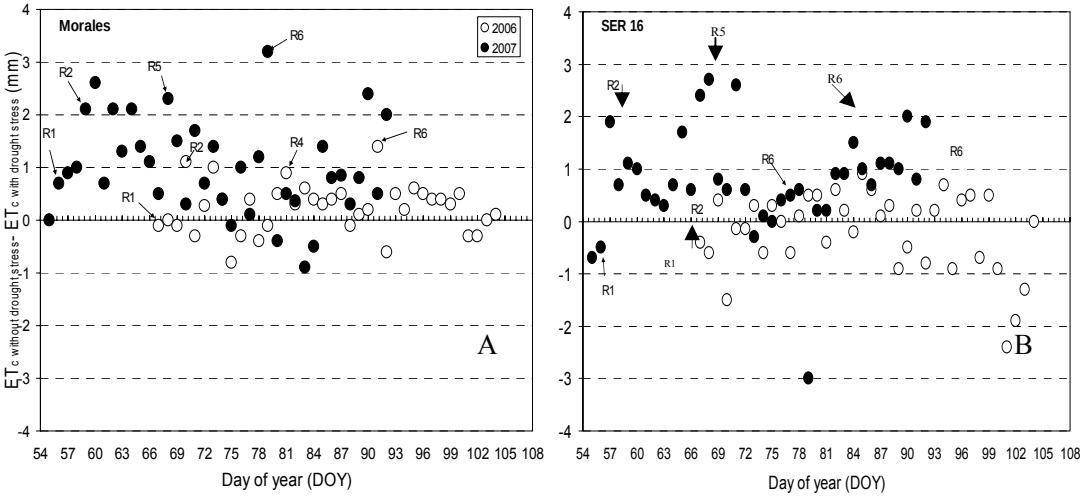

Figure 4.8. Daily crop evapotranspiration differences with and without drought stress for two common bean genotypes, during two growing seasons, for R1 to harvest. 2006-2007: **A**. Morales, **B**. SER 16.

The decreasing ET_c during the DOYs: 58, 60, 61, 64 and 66 in 2007 for both water levels, are associated with low aerodynamic resistance (r_a), and high surface resistance (r_s). The mean r_a values were 53 sm^{-1} for SER 16 and 54 sm^{-1} for Morales without drought stress, as compared with mean r_s values equal to 220 sm^{-1} for SER 16 and 200 sm^{-1} for Morales. The decreasing ET_c is associated with high r_s values for the same period (DOY 57 to DOY 64, Table 4.8). The low values of r_a were directly related to the high wind velocities registered during that period between 10:00 am to 3:00 pm (range: 5.0 to 8.0 ms^{-1}), which likely induced stomatal closure. The wind speed and r_a have an influence on the transpiration and have been reported by several authors (e.g., Davies et al. 1978; Bailey and Davies, 1980; Dixon and Grace, 1984; Smith, 1984). Decreases or increases in the r_L depends on the plant species (Dixon and Grace, 1984). Davies et al. (1978) found that stomates closed markedly, resulting in increasing r_L and subsequently increasing r_s, with abrupt increases in wind speed in prostrate plants. This inverse relationship between r_L and r_a will be further explored in Chapter 5.

Table 4.8. Surface resistance (r_s) distribution for two common bean genotypes, during two growing seasons, with and without drought stress conditions. The drought stress was applied in R1.

Water Level	Genotype	Phenologic phase	DOY‡	DAP†	r_s	S.E¶	LAI	Phenologic phase	DOY	DAP	r_s	S.E	LAI
2006					—— $s.m^{-1}$ ——		$m^2.m^{-2}$	2007			—— $s.m^{-1}$ ——		$m^2.m^{-2}$
Without drought stress	Morales	V2	48	16	3944.3	(651.2)	0.05	V2	31	14	1518.2	(144.3)	0.10
		V4	56	24	1738.3	(527.7)	0.20	V3	38	21	649.0	(69.9)	0.20
		V6	62	30	596.2	(69.7)	0.60	V5	46	29	637.7	(177.6)	0.43
		R1	70	39	259.6	(53.6)	1.30	V6	52	35	191.7	(26.1)	1.68
		R3	77	46	132.4	(20.2)	2.60	R2	57	40	435.2	(18.9)	2.27
		R4	84	53	79.6	(13.2)	4.22	R4	64	47	644.0	(6.1)	3.03
		R6	91	60	128.5	(30.5)	2.60	R6	71	54	217.1	(25.4)	2.57
		R8	98	67	111.5	(31.5)	3.00	R8	87	70	758.0	(473.8)	1.00
		R9	104	73	164.1	(16.8)	2.10						
Without drought stress	SER16	V2	48	16	21020.9	(21899.9)	0.04	V2	31	14	9100.0	(472.3)	0.02
		V4	56	24	2624.4	(298.2)	0.12	V3	38	21	1308.0	(236.5)	0.10
		V6	62	30	1431.8	(211.5)	0.21	V5	46	29	992.5	(402.7)	0.20
		R1	70	39	336.0	(6.8)	0.91	V6	52	35	230.3	(32.6)	1.00
		R3	77	46	206.1	(59.5)	1.50	R2	57	40	439.4	52.9)	1.27
		R4	84	53	192.2	(35.6)	1.60	R4	64	47	519.4	(51.0)	1.77
		R6	91	60	184.2	(1.5)	1.70	R6	71	54	258.2	(38.15)	1.67
		R8	98	67	252.1	(4.5)	1.20	R8	87	70	388.8	(160.0)	1.43
		R9	104	73	581.8	(140.0)	0.50						
With drought stress	Morales	R1	70	39	221.2	(74.7)	1.50	R2	57	40	769.0	(101.5)	2.00
		R3	77	46	108.1	(26.3)	3.10	R4	64	47	1767.0	(410.1)	2.00
		R4	84	53	82.2	(83.5)	4.00	R6	71	54	795.3	(61.7)	1.13
		R6	91	60	103.5	(59.5)	3.20	R8	87	70	4800.0	(996.8)	0.80
		R8	98	67	126.2	(21.5)	2.60						
		R9	104	73	141.9	(18.2)	2.30						
With drought stress	SER16	R1	70	39	374.2	(36.0)	0.90	R2	57	40	957.7	(99.5)	0.73
		R3	77	46	233.2	(12.5)	1.40	R4	64	47	1150.0	(73.8)	1.20
		R4	84	53	257.7	(132.4)	1.20	R6	71	54	375.1	(86.3)	1.00
		R6	91	60	182.5	(76.3)	1.80	R8	87	70	4194.1	(539.7)	0.53
		R8	98	67	248.2	(4.4)	1.30						
		R9	104	73	251.9	(32.7)	1.30						

‡ Day od the year; † Days after planting; ¶ Standard error.

Crop coefficients. The crop coefficient curves are shown in Fig. 4.9, the largest differences in the K_c between drought stress (open circles) and without drought stress (close circles) were observed during 2007 (Fig 4.9 B and D) for Morales and also for SER16. The K_c difference between water levels were more pronounced in Morales than in SER16 in both years.

The linearized crop coefficients (K_c) are shown in Table 4.9. SER 16 did not show differences across years, the reduction in the K_c during the mid season in 2007 was associated with low leaf area index during that year compared with the first, and differences in r_a and r_s. Also an additional stress by high wind conditions during the mid season reduced the ET_c in 2007, and Morales was more susceptible. The K_c values presented in this study are lower than those reported by the Irrigation and Drainage Paper-FAO 56 (Allen et al. 1998). The large row spacing and differences in plant density (low LAI), and irrigation system (drip) help to explain lower K_c values obtained in this study.

In Table 4.10, K_c measured in intervals of 3 and 4 days by the drainage lysimeter are listed. The $K_{c\ mid}$ are similar to those in the Table 4.9 estimated with the PM model. A mean value for the reproductive phase (R1 to R9) was 0.9 in 2006 and 1.0 in 2007 for Morales without drought stress and decreased to 0.8 in 2006 and to 0.7 in 2007. For the genotype SER 16, the average K_c during R1 to R9 was 0.7 in 2006 and 0.6 in 2007 without drought stress; the K_c decreased from 0.6 in 2006 to 0.3 in 2007 with drought stress.

Table 4.9. Length of common bean growth stages and crop coefficients (K_c),without drought stress. Estimated with Penman-Monteith general model, for Juana Diaz, PR.

Genotype	Year	Bean Growth stage	Length of Stage days	Crop coefficient K_c
Morales	2006	Initial	21	0.25
		Crop development	13	0.25 to 0.90
		Mid season	32	0.90
		Late season	6	0.90 to 0.50
	2007	Initial	18	0.50
		Crop development	19	0.50 to 0.80
		Mid season	35	0.80
		Late season	6	0.80 to 0.30
SER 16	2006	Initial	21	0.22
		Crop development	13	0.22 to 0.82
		Mid season	32	0.82
		Late season	6	0.80 to 0.30
	2007	Initial	18	0.24
		Crop development	19	0.24 to 0.80
		Mid season	35	0.80
		Late season	6	0.80 to 0.30

‡.Crops were grown during the period of January to April, which is considered to be be the driest time of the year; drip irrigation was used; site elevation 28 m asml, row spacing was 90 cm; plant densities for Morales and SER 16 were 13.2 plants per m^2 and 6.4 plants per m^2, respectively; registered wind velocities were greater during 2007 than during 2006.

Table 4.10. Crop coefficients (K_c) for two common bean genotypes without and with drought stress, measured in drainage lysimeters, each value is an average of three lysimeters.

Water Level	Genotype	DAP†	Phenologic phase	K_c	S.E¶	DAP	Phenologic phase	K_c	S.E
2006 Trial						2007 Trial			
Without drought stress	Morales	16	V2	0.9	(0.22)	19	V3	0.6	(0.09)
	Morales	21	V3	1.0	(0.22)	26	V4	0.9	(0.13)
	Morales	24	V4	1.4	(0.30)	29	V5	1.7	(0.39)
	Morales	28	V7	0.6	(0.15)	35	V6	0.8	(0.10)
	Morales	30	V8	0.2	(0.02)	38	R1	1.0	(0.09)
	Morales	35	R1	1.0	(0.75)	40	R2	0.8	(0.02)
	Morales	41	R2	0.8	(0.15)	43	R3	0.6	(0.06)
	Morales	52	R4	0.9	(0.30)	47	R4	0.9	(0.05)
	Morales	56	R5	0.9	(0.20)	51	R5	0.8	(0.02)
	Morales	59	R6			54	R6	1.6	(0.19)
	Morales	63	R7	1.3	(0.05)	62	R7	1.4	(0.35)
	Morales	66	R8	0.4	(0.13)	70	R8	1.2	(0.28)
	Morales	72	R9	0.6	(0.14)	75	R9	0.2	(0.05)
		Average‡		**0.9**				**1.0**	
With drought stress	Morales	16	V2	0.6	(0.09)	19	V3	0.8	(0.16)
	Morales	21	V3	0.9	(0.17)	26	V4	1.0	(0.34)
	Morales	24	V4	1.3	(0.22)	29	V5	0.3	(0.01)
	Morales	28	V7	0.5	(0.10)	35	V6	1.3	(0.28)
	Morales	35	R1	1.7	(0.01)	38	R1	0.9	(0.10)
	Morales	41	R2	0.2	(0.01)	40	R2	0.1	0.03
	Morales	45	R3	1.4	(0.01)	43	R3		
	Morales	52	R4	0.8	(0.19)	47	R4	0.3	(0.06)
	Morales	56	R5	0.2	(0.01)	51	R5	0.8	(0.13)
	Morales	59	R6		(0.01)	54	R6	0.8	(0.11)
	Morales	63	R7	1.3	(0.24)	62	R7	0.9	(0.33)
	Morales	66	R8	0.4	(0.06)	70	R8	0.7	(0.16)
	Morales	72	R9	0.8	(0.15)	75	R9	0.0	
		Average		**0.8**				**0.7**	
Without drought stress	SER16	16	V2	0.6	(0.05)	19	V3	0.5	(0.08)
	SER16	21	V3	0.6	(0.05)	26	V4	0.5	(0.06)
	SER16	24	V4	`		29	V5	1.0	(0.20)
	SER16	28	V7	0.3	(0.01)	35	V6	0.6	(0.13)
	SER16	35	R1	1.3	(0.30)	38	R1	0.7	(0.09)
	SER16	41	R2	0.4	(0.02)	40	R2	0.4	(0.05)
	SER16	45	R3	1.5	(0.05)	43	R3	0.3	(0.04)
	SER16	52	R4	0.9	(0.10)	47	R4	0.5	(0.06)
	SER16	56	R5	0.5		51	R5	0.6	(0.07)
	SER16	59	R6			54	R6	1.1	(0.18)
	SER16	63	R7	0.8	(0.05)	62	R7	0.7	(0.14)
	SER16	66	R8	0.3	(0.10)	70	R8	0.6	(0.07)
	SER16	72	R9	0.4	(0.13)	75	R9	§	(0.02)
		Average		**0.7**				**0.6**	
With drought stress	SER16	16	V2	0.2	(0.02)	19	V3	0.5	(0.11)
	SER16	21	V3	0.6	(0.15)	26	V4	0.4	(0.12)
	SER16	24	V4	0.7	(0.20)	29	V5	0.3	(0.06)
	SER16	28	V7	0.3	(0.05)	35	V6	0.6	(0.06)
	SER16	35	R1	1.1	(0.10)	38	R1	0.4	(0.08)
	SER16	41	R2	0.1	(0.01)	40	R2	0.1	(0.09)
	SER16	45	R3	1.1	(0.10)	43	R3		
	SER16	52	R4	0.4	(0.19)	47	R4	0.1	(0.02)
	SER16	56	R5	0.2		51	R5	0.4	(0.05)
	SER16	59	R6			54	R6	0.3	(0.03)
	SER16	63	R7	0.8	(0.15)	62	R7	0.7	(0.05)
	SER16	66	R8	0.3	(0.05)	70	R8	0.3	(0.10)
	SER16	72	R9	0.4	(0.10)	75	R9	§	(0.01)
		Average		**0.6**				**0.3**	

† Days after planting; ¶ Standard error; ‡ Average corresponded since R1 to R9.

§ Plants completely dry; The spaces in white, correspond with strong rainfall events, where the Kc, could no be successfully measured.

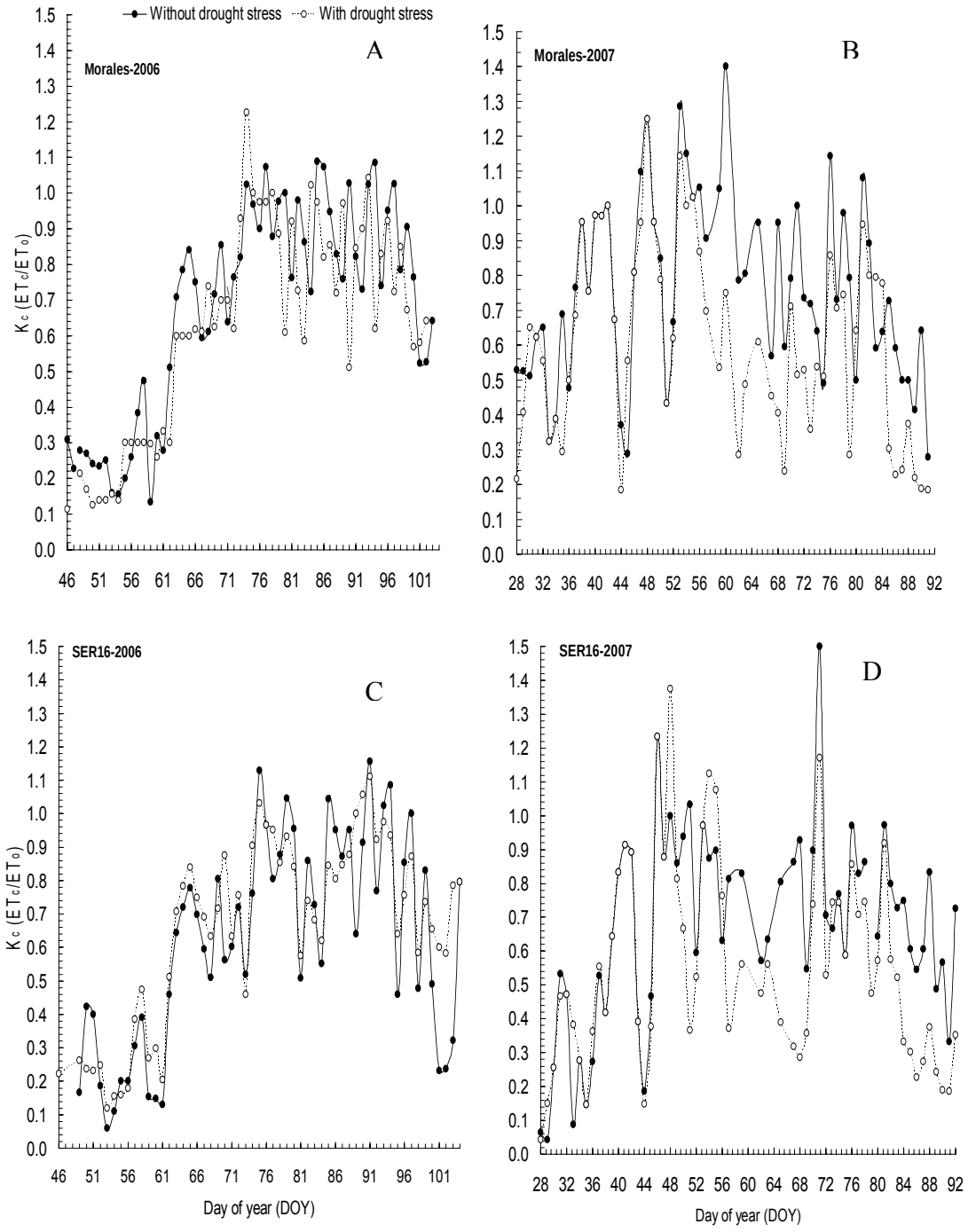

Figure 4.9. Daily crop coefficients (K_c), for two common bean genotypes, with and without drought stress during two growing seasons: **A**. Morales-2006, **B**. Morales-2007, **C**. SER 16-2006, and **D**. SER 16-2007.

The variation in crop development rates between location and year have been expressed as correlations between crop coefficients and indices such as the thermal base index, ground cover, days after emergence or planting, and growth rate (i.e., Wright and Jensen, 1978; Hunsaker et al. 1999; Brown et al . 2001; Nasab et al. 2004; Hanson et al. 2004), and in bean cv., '*Carioca*' by (Madeiros et al. 2001; and Madeiros et al. 2005). In this study, the K_c was correlated with the fraction covered by vegetation (f_c) calculated as the ratio between plant canopy diameter and row spacing, and with the cumulative growing degree days (Fig. 4.10), calculated as follows:

$$CGDD = \frac{\left[T_{\max} + T_{\min}\right]}{2} - T_b \qquad (4.33)$$

where T_{max} is the maximum daily temperature, T_{min} is the minimum daily temperature and T_b is the base temperature = 10 ^{o}C.

The plant density adopted in the present study induce differences in the seasonal trend in f_c (equation 4.35 and 4.37), but not in CGDD (equations 4.34 and 4.36).

The K_c curve as related to CGDD and f_c (Fig. 4.10), were fit in a second degree polynomial equation for each genotype. For Morales with 13.6 plants.m^{-2}, the equations were:

$$K_c = -3x10^{-6} CGDD^2 + 0.0033 CGDD - 0.053; R^2 = 0.76; p < 0.0001 \qquad (4.34)$$

$$K_c = -1.4019\, f_c^{\,2} + 2.5652\, f_c - 0.2449; R^2 = 0.70; p < 0.0003 \qquad (4.35)$$

For SER 16, with 6.4 plants.m^{-2} the equations were:

$$K_c = -3x10^{-6} CGDD^2 + 0.0034 CGDD - 0.0515; R^2 = 0.60; p < 0.0001 \qquad (4.36)$$

$$K_c = -0.6726\, f_c^{\,2} + 1.90086\, f_c - 0.2560; R^2 = 0.60; p < 0.0032 \qquad (4.37)$$

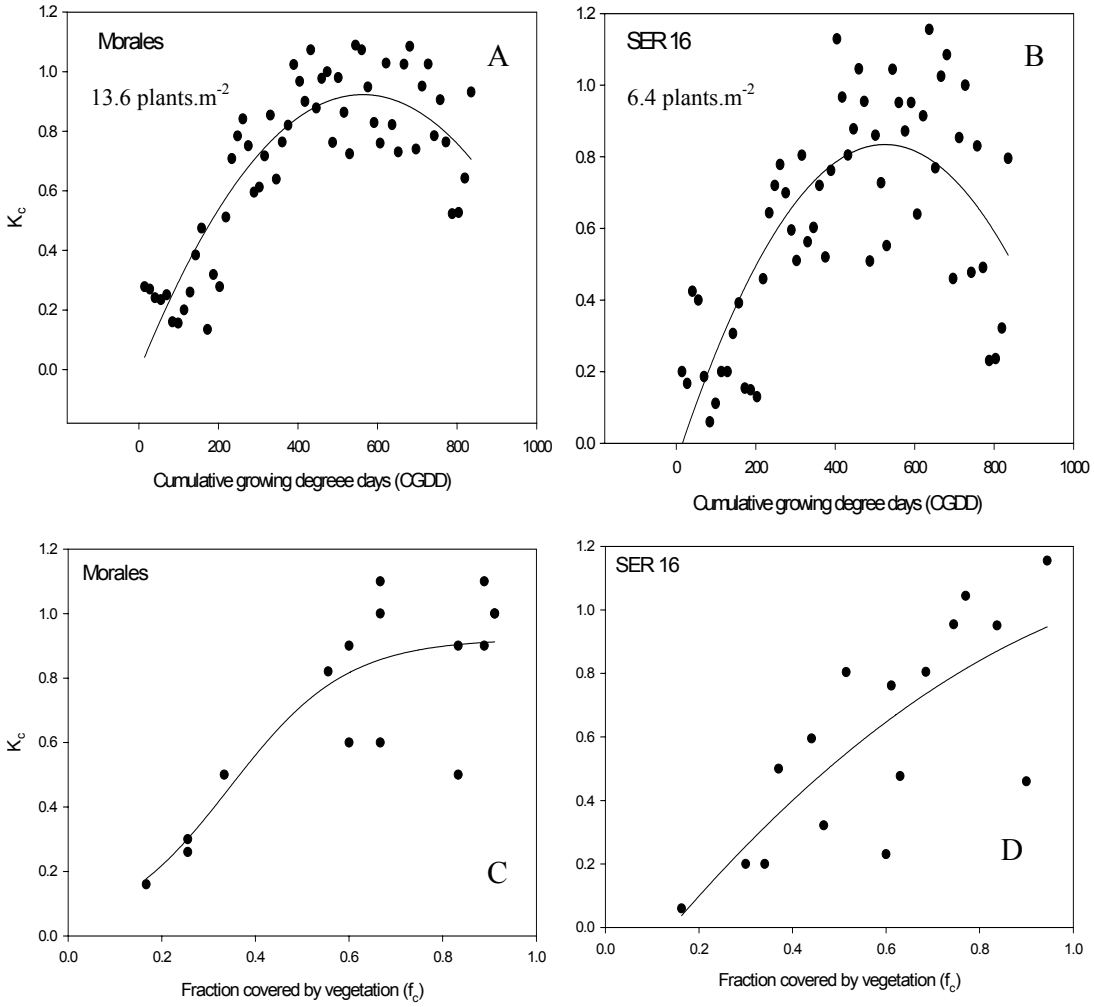

Figure 4.10. Crop coefficients (K_c) as a related to cumulative growing degree days (CGDD) and fraction covered by vegetation (f_c) for: **A**. Morales CGDD vs k_c , **B**. SER 16 CGDD vs k_c , **C.** Morales f_c vs k_c , **D**. SER 16 f_c vs k_c . The curves were fit from V1 to R9.

The accumulated observed and simulated evapotranspiration values were 166.5 mm and 166.3 mm for Morales at 13.6 plants/m, and 143.7 mm and 146.3 mm for SER 16 at 6.4 plants/m, respectively. The comparison between observed and simulated values are presented in the figure 4.11, where the relative errors of 0.17 % for Morales 1.7% for SER 16 (models shown in the equations 4.35 and 4.37).

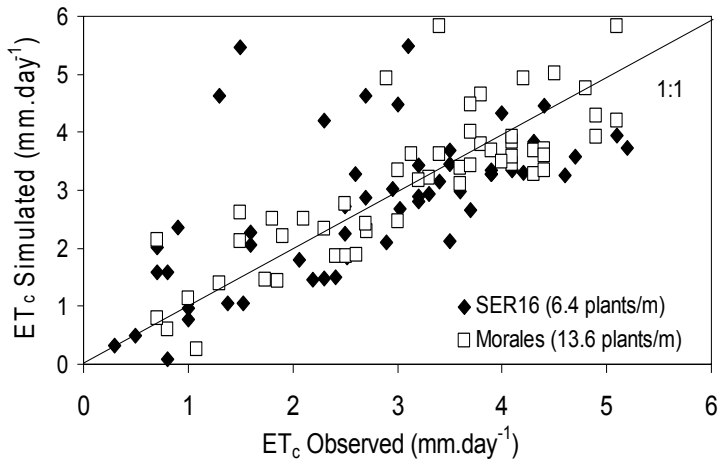

Figure 4.11. Observed and simulated evapotranspiration from K_c models described in the equations 4.35 and 4.37 and reference ET estimated with the PM-model, and the observed ET estimated with the generalized PM-model for bean in 2006 at Juana Diaz, PR.

Dual crop coefficients. The single crop coefficient (K_c) was separated into two coefficients, which represent the crop and soil participation in the evapotranspiration process, and which are used to predict the effects of specific wetting events on the K_c (Allen et al. 1998). The dual crop coefficients are especially useful in the case where the soil surface layer is dry, but the average soil water content in the root zone is adequate to sustain full plant transpiration (Allen et al. 2005). The dual crop coefficients include the basal coefficient or transpiration coefficient (K_{cb}) and the soil evaporation coefficient (K_e).

Figures 4.12 and 4.13 show the K_{cb} distribution during the growing season for water treatment, genotype, and year. The upper limited in K_{cb} in the mid season was 0.85 for SER 16 and 0.91 for Morales without drought stress. The initial K_{cb} values are lower than 0.15 for both genotypes and growing seasons, which are close to the reported values of the FAO (Allen et al. 1998), for dry bean. The mid and end values in this study were lower than the reported values.

The change from vegetative to reproductive states of development, is associated with an increase in the transpiration, due to the abrupt increase in the $K_{cb,}$ observed in DOY 74 (Fig.

4.12), and DOY 53 (Fig. 4.13), that is directly associated with the increase in leaf area and transpiration surface.

During the mid season in 2006, the drought stress treatment K_{cb} values reached 0.4 for Morales (DOY's 81, 83,84 and 91; Fig. 4.12 D) which corresponded with the R4 to R6 stages of development, and 0.30 for SER 16 (DOY 73) corresponding with R2. Morales exhibited a higher frequency of low K_{cb} values than SER 16 in the drought stressed treatments.

The low K_{cb} measured in 2007, during the mid season for both genotypes and water levels (Fig 4.13), indicated low transpiration rates in an important stage of development, possible due to a "physiological stress" associated with high wind speeds and low irrigation rates, that influenced a both water levels, and which induced high r_L as was discuss in the ET_c results. SER 16, responded similarly for both water level treatments Figures 4.13 C and D.

The larger difference between K_c and K_{cb} in 2006 vs 2007, can be explained in 2006 due to the water supplied in the experiment was higher than in 2007, associated with several rainfall events that keep the soil wetted longer times, and also due to higher wind speed in 2007 than 2006, that dismiss the transpiration rates.

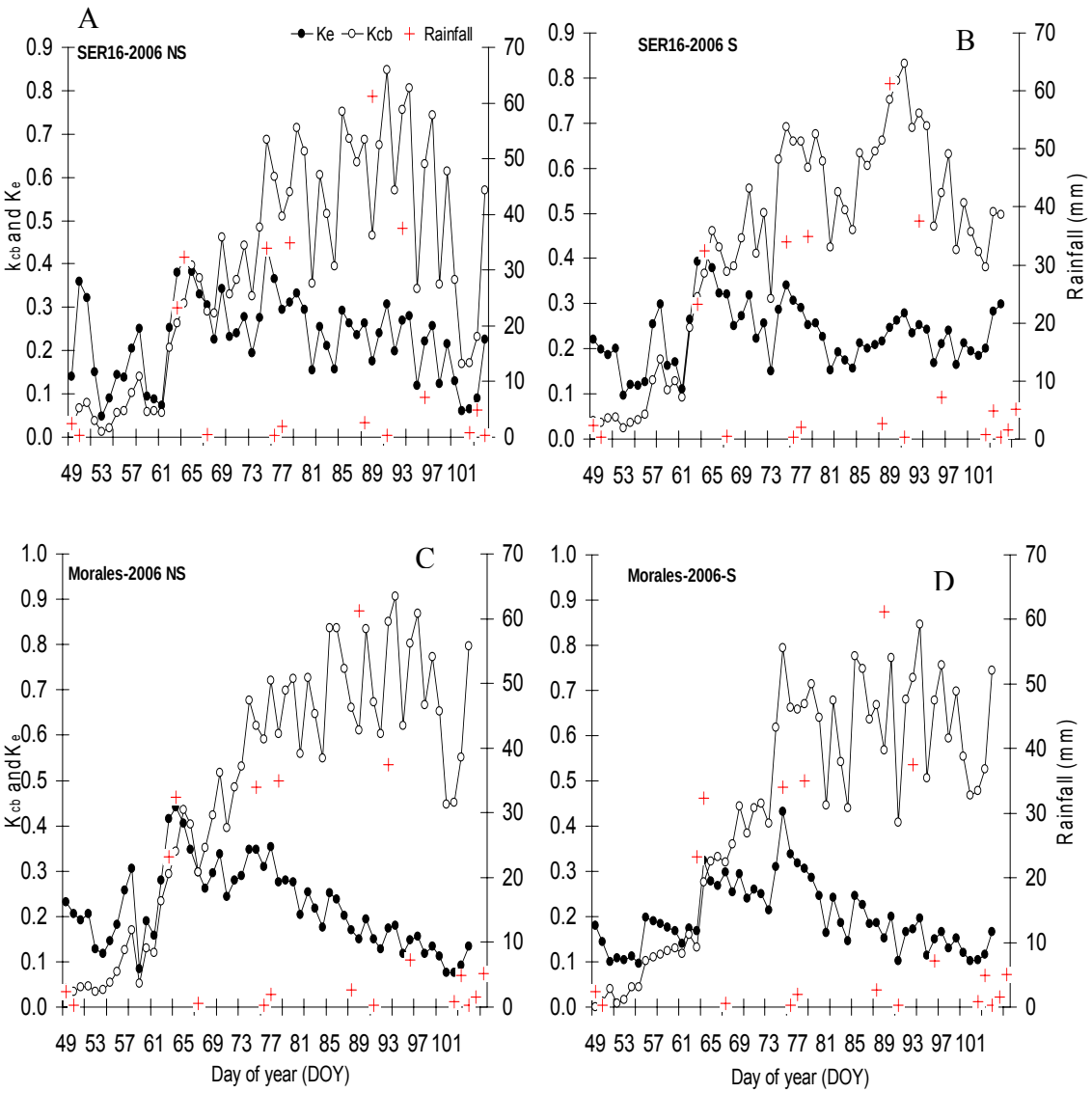

Figure 4.12. Basal crop coefficients (K_{cb}) and soil evaporation coefficient (K_e) for two common bean genotypes-2006: **A**. SER 16 without stress, **B.** SER 16 with drought stress, **C.** Morales without drought stress and **D**. Morales with drought stress.

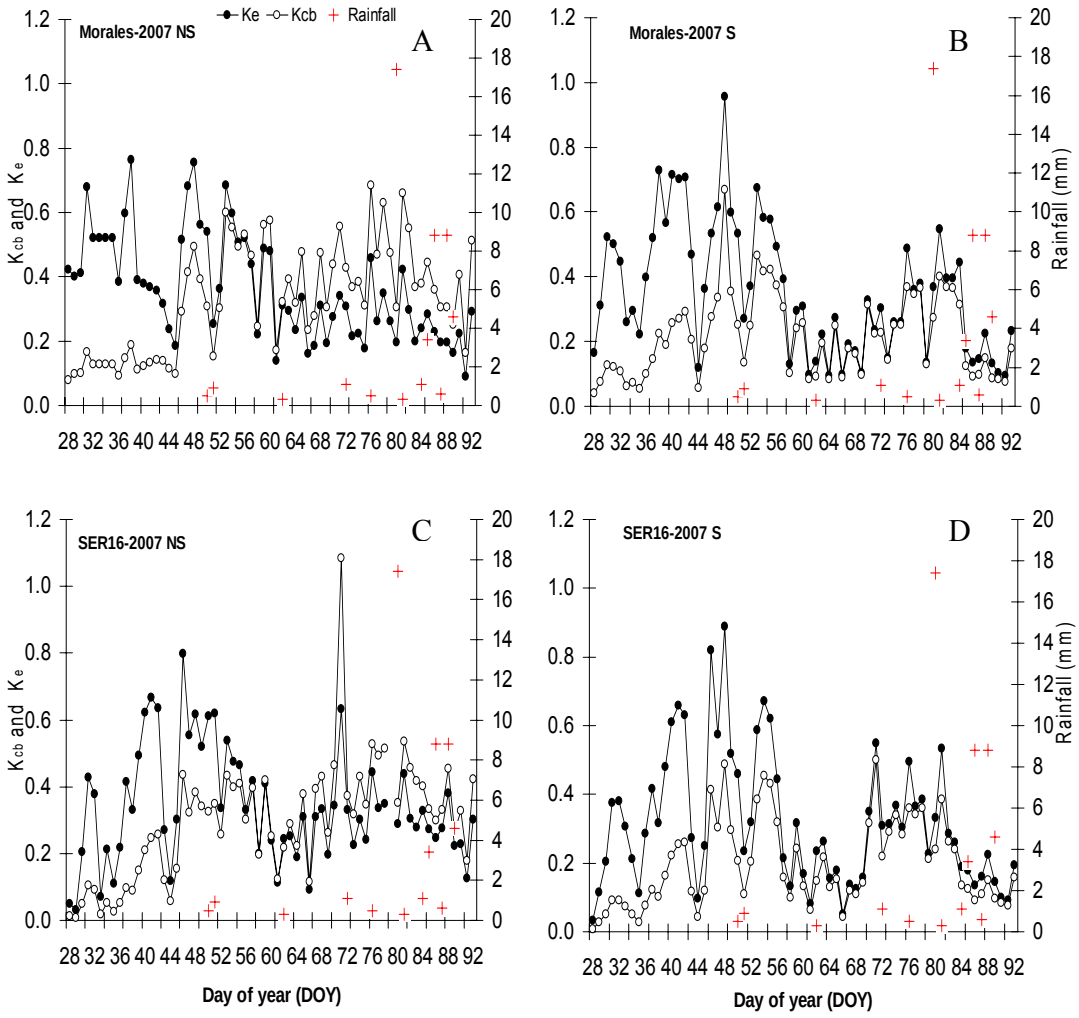

Figure 4.13. Basal crop coefficients (K_{cb}) and soil evaporation coefficient (K_e) for two common bean genotypes-2007: **A**. Morales without drought stress, **B**. Morales with drought stress, **C**. SER 16 without drought stress and **D**. SER 16 with drought stress.

The ET_c was adjusted to account for water stress by using the water stress coefficient (K_s). This coefficient is related to the root zone depletion (D_r), calculated using the water balance equation:

$$D_{r,i} = D_{r,i-1} - (P - RO)_i - I_i + ET_{c,i} + DP_i \quad (4.38)$$

where $D_{r,i}$ is the root zone depletion at the end of the day i; $D_{r,i-1}$ is water content in the root zone at the end of the previous day, i-1; $(P\text{-}RO)_i$ is the difference between precipitation and runoff on the day i; I_i is the irrigation depth on the day i; $ET_{c,i}$ is the crop evapotranspiration on day i and DP_i is the water loss out of the root zone by deep percolation on day i, all the units are in mm.

The root zone depletion associated with a K_s = 1.0 (i.e., no water stress), was up to 10 mm for a root depth between 0 to 20 cm, and up to 15 mm for a root depth of 0 to 40 cm (Fig. 4.14). 50% of the transpiration reduction was reached for D_r = 22 mm and 25 mm in Morales and SER 16, respectively. Transpiration ceased completely (K_s = 0) when Dr = 37 mm and 46 mm, respectively for Morales and SER 16.

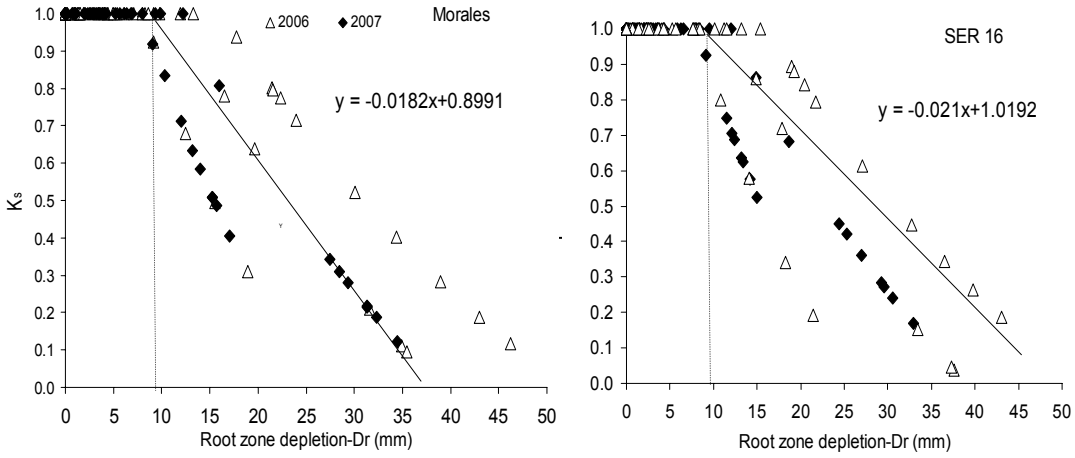

Figure 4.14. Water stress coefficients (K_s) for two common bean genotypes: **A.** Morales **B.** Morales

CONCLUSIONS

In this study, crop evapotranspiration was estimated with the generalized Penmna-Monteith model and drainage lysimeters for two common bean genotypes, with and without drought stress. The maximum ET rates for both genotypes were reached at the beginning of the reproductive phase to seed maturity, and were equivalent to 67% of the total ET for Morales and 73% for SER 16, in 2006, and 54% and 55%, respectively, in 2007. One of the causes for the reduction in ET in 2007 was associated with an increase in surface resistance due to windy and drought stress conditions. The increasing surface resistance was also related to an observed decrease in the transpiration coefficient (K_{cb}).

The $K_{c\ mind}$ values for the well watered treatment were lower than 1.0 for both genotypes, measured by lysimeters and the PM-model, indicating relatively low water requirements for both genotypes. Both genotypes exhibited a K_c reduction during drought stress of similar magnitude. The K_c for non-limited soil water conditions was well correlated with the cumulative degree day (CGDD) and with the fraction covered by vegetation (f_c) for both genotypes with different plant densityies.

The largest differences in the ET estimations between the lysimeter and the PM-model were observed in the beginning of the crop season, which was particularly associated with low LAI, increasing r_s, and a decrease in ET. The change in ET rates associated with drought stress were variable between genotypes: Morales ET in 2006 was reduced by 10% with the PM model, as compared with 0.0 % by SER 16. The change in ET due to drought stress for 2007 was 20% for Morales and 18% for SER 16. Note that the two genotypes should not be compared due to differences in the plant density.

The intermittent drought stress applied from floral differentiation to harvest was stronger during 2007 than 2006, with a subsequent effect on yield components (see Chapter 7). The genotype Morales exhibited the highest reduction in evapotranspiration during critical drought stress periods (R1, R2, R5 and R6).

Values of surface resistance as a function of stomatal resistance and LAI were also derived in this study, as well as values of the crop stress coefficient (K_s), and critical values of root zone depletion were estimated as a K_s function, for both genotypes.

The crop coefficients (K_c) derived in this study, are specific to the genotypes considered and the agronomic practices used, including the irrigation system. Additionally, it is important to consider that the plant density is a critical component in the K_c estimation, and it is suggested that adjustments be made to the K_c based on the fraction of the soil covered by vegetation (f_c). The specific wind conditions present during the study can have a considerable effect on the derived crop coefficients, and therefore, caution should be exercised when applying these coefficients under wind conditions which vary from those in this study.

REFERENCES

Adams, M.W., D.P. Coyne., J.H.C. Davis., P.H. Graham, and C.A. Francis. 1985. Comon bean (*Phaseolus vulgaris* L.) In: Grain Legume Crops, Edit R.J. Sumerfield and E.H. Roberts. Collin ed:433-476.

Allen, R. G. 1996. Assessing integrity of weather data for reference evapotranspiration estimation. *J. Irrig. Drain. Eng*. 122(2):97–106.

Allen G.R, L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. Food and Agricultural Organization of the United Nations (FAO). Publication No. 56. Rome. 300p.

Allen, G.R., L.S. Pereira, M. Smith, D. Raes, and J.L Wright. 2005. FAO-56 dual crop coefficient method for estimating evaporation from soil and application extensions. *J.Irrig. Drain. Eng.* 131(1):2-13.

Alves, I., Perrier, A.; Pereira, L. S. 1998. Aerodynamic and Surface Resistente of Complete Cover Crops: How Good is the "Big Leaf"?. *Trans. ASAE*. 41(2):345-351.

Anda, A. and Löke, Zs, 2002. Stomatal resistance investigations in maize. Proceedings of the 7th Hungrian Congress of Plant Physiology. 46(3-4):181-183.

Barros, L.C.G., and J.R. Hanks. 1993. Evapotranspiration and yield of bean as affected by mulch and Irrigation. *Agron. J*. 85(3): 692-697

Beebe, S., and B. McClafferty. 2006. Biofortified Bean. Centro Agronomico Tropical (CIAT)-Cali, Colombia. 2p. In: www.harvestplus.org/pdfs/**bean**.pdf.

Beaver, J.S., P.N. Miklas, 1999. Registration "Morales" small white bean. *Crop Sci*. 39:1257.

Beaver, J.S. 2006. FY05 Report of the leaders of the basic grain commodity group. In.: Agricultural Experiment Station, University of Puerto Rico: http://eea.uprm.edu/decano/bienvenida.aspx.

Berengena, J and P. Gavilan. 2005. Reference evapotranspiration in a highly advective semiararid environment. *J.Irri and Drain. Eng*. 132(2):147-163

Brian J, J.B. 1991. Alfalfa ET measurements with drainage lysimeters. In: *Lysimeter For Evapotranspiration and Enviromental Measurements* Edited by: Richard G. Allen, Terry A. Howell, William O. Pruitt, Ivan A. Walter and Marvin E. Jenser: 264-271.

Broughton, W.J., G. Hernandez., M. Blair., S. Beebe., P. Gepts., and J. Vanderleyden. 2003. Bean (*Phaseolus* spp.)-model food legumes. *Plant and Soil*. 252: 55-128.

Brown, P.A., C.F. Mancino., M.H Joung., T.L. Thompson., P.J Wierenga, and D.M. Kopec. 2001. Penman Monteith crop coefficients for use with desert turf system. *Crop. Sci*. 41:1197-1206.

Calvache, M., Reichardt, K., Bacchi. O.O.S., and Dourado-Neto, D. 1997. Deficit irrigation at different growth stages of the common bean (*Phaseolus vulgaris* L.,cv.IMBABELLO). *Sci. agri. Piracicaba.* 54:1-16.

Caspari, H.W, S.R. Green, W.R.N. Edwards. 1993. Transpiration of well-watered and water-stressed Asian pear tress as determined by lysimetry, heat-pulse, and estimated by a Penman-Monteith model. *Agric. For. Meteorol.* 67:13-27.

Davis, W.J., K. GiLL, and G. Halliday. 1978. The influence of wind on the behaviour of stomata of photosynthetic stems of *Cyticus scoparious* (L.) Link. *Annals of Botany*. 42:1149-1154.

Dixon, M and J. Grace. 1984. Effect of wind on the transpiration of young trees. *Annal of Botany*. 53: 811-819.

Doorenbos, J. and W.O. Pruitt. 1977. Guidelines for predicting crop water requirements. Food and Agricultural Organization of the United Nations (FAO). Publication No. 24. Rome. 300p.

Food and Agriculture Organization. (FAO). 2002 Food Outlook, No. 2.in: www,fao.org.

Fritschen, J.L., and L.C. Fritschen. 2005. Bowen ratio energy balance method. In: *Micrometeorology in Agricultural Systems*, Agronomy Monogaph no, 47. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 677 S. Soe Rd., Madison, W153711, USA: 397-405.

Goenaga, R., Rivera, E., Almodovar, C. 2004. Yield of papaya irrigated with fractions of Class A pan evaporation in a semiarid environment. *J. Agric.Univ.P.R.*88 (1-2):1:10.

Goyal, M.R. and E.A. González. 1988d. Water requirements for vegetable production in Puerto Rico. ASAE Symposium on Irrigation and Drainage, July. 8p.

Goyal, M.R, and.E.A Gonzalez. 1989. Datos climatológicos de las subestaciones experimentales agrícolas de Puerto Rico. Universidad de Puerto Rico-Recinto de Mayagüez/Colegio de Ciencias Agrícolas/Estación Experimental Agrícola. Publicación 88-70, proyecto C-411. 87p.

Hanson, R.B., and D.M. May. 2004. Crop coefficients for drip-irrigated processing tomatoes. In: ASAE/CSAE Annual International Meeting. Fairmont Chateau Laurier, The Westing, Government Centre, Ottawa, Ontario, Canada.12p.

Harmsen, E.W. 2003. Fifty years of crop evapotranspiration studies in Puerto Rico. *J. Soil Water Conserv*. 58(4):214-223.

Harmsen, E.W, A. González-Pérez, and A. Winter. 2004. Re-evaluation of pan evaporation coefficients at seven location in Puerto Rico. *J.Agric.Univ.P.R.* 88(3-4):109-122.

Harmsen, E.W., A. González-Pérez. 2005. Computer program for estimating crop evapotranspiration in Puerto Rico. *J.Agric.Univ.P.R.* 89(1-2):107-113.

Hunsaker, D.J. 1999. Basal crop coefficients and water use for early maturity cotton. *Trans. ASAE*. 42(2):927-936.

Jia, X., E.C. Martin, and D.C. Slack, F. 2005 Temperature adjustment for reference evapotranspiration calculation in Central Arizona. *J. Irrig. Drain. Eng.* 130(5):384–390.

Karam, F, R. Masaad, T. Sfeir, O. Mounzer, and Y. Rouphael. 2005. Evapotranspiration and seed yield of field grown soybean under deficit irrigation conditions. *Agri. Water. Manage.* 75:226-244.

Kjelgaard, J.F.; and Stokes, C.O.2001. Evaluating Surface Resistance for Estimating Corn and Potato Evapotranspiration with the Penman-Monteith Model. *Trans. ASAE*. 44(4):797-805.

Lin Li, Y., Yuan Cui, J., Hui Zhang, T., and Liu Zhao, H. 2003. Measurement of evapotranspiration of irrigated spring wheat and maize in a semi-arid region of north China. *Agric. Water. Manage* .61(1): 1-12.

Madeiros, G.A., F.B. Arruda., E. Sakai., and M. Fujiwara. 2001.The influence of the crop canopy on evapotranspiration and crop coefficients of bean (*Phaseolus vulgaris* L.). *Agric. Water. Manage*. 49:211-234.

Madeiros, G.A., F.B. Arruda., E. Sakai. 2004. Crop coefficient for irrigated bean derived using three reference evaporation methods. *Agric. Water. Manage.* 135:135-143.

Monteith, J.L, and M.H. Unsworth.1990. Principles of environmental physics. Chapman and Hall, Inc.292p.

Muñoz-Perea, C.G., Allen, G.R., D.T. Westermann., J.L. Wring, and S.P. Singh. 2007. Water used efficiency among dry bean landraces and cultivars in drought-stressed and non-stressed environments. *Euphytica*. 155:392-402.

National Agricultural Statistic Services, of the United States Department of Agriculture. (NASS). 2002.

Nasab, B.S., H.A. Kashkuli., and M.R. Khaledian. 2004. Estimation of crop coefficients for sugarcane (ratoon) in Haft Tappeh of Iran. In: ASAE/CSAE Annual International Meeting. Fairmont Chateau Laurier, The Westing, Government Centre, Ottawa, Ontario, Canada.10p.

North Dakota State University (NDSU). 1997. Dry bean production guide. Publication series No. A-1133.In: http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1133-5.htm

North Dakota State University (NDSU). 2003. Dry bean production guide. Publication series No. A-1133.120p.

Pereira, L.S, and M.S. Adaixo. 1991. Lysimeter-based evapotranspiration research in Portugal. In: *Lysimeter For Evapotranspiration and Enviromental Measurements* Edited by: Richard G. Allen, Terry A. Howell, William O. Pruitt, Ivan A. Walter and Marvin E. Jenser: 142-150.

Roygard K,F.R.; Alley, M.M.; and Khosla, R. 2002. No-Till Corn Yield and Water Balance in the Mid-Atlantic Coastal Plain. *Agron. J.* 94:612-623.

Rosenberg, J. N, B.L. Blad, and S.B, Verma. 1983. Microclimate; The Biological Environment. A Wiley-Interscience Publication, Jhon Wiley & Sons. Edt. 495p.

Sheng Li, Q., Villardson, S.L., Deng, W., Jun Li, X, and Jiang.L.C. 2005. Crop water deficit estimation and irrigation scheduling in western Jilin province, Northeast China. *Agric. Water. Manage.*71(1):47-60.5

Smith, K.W. 1980. Importance of aerodynamic resistance to water use efficiency in three conifers under field conditions. *Plant Physiol*. 65:132-135.

Szeicz, G, and I.F. Long. 1969. Surface resistance of crop canopies. *Water Resour. Res*. 5:622-633.

U.S.Department of Agriculture- (USDA). 1987. Primary characterization data of San Anton soil, Guanica country, Puerto Rico. Soil Conservation Service, National Soil Survey laboratory, Lincoln, Nebraska.

U.S.Department of Agriculture- (USDA). 2005.Economic research services. Dry bean trade.In: http://www.ers.usda.gov/Briefing/DryBean/Trade.htm

Villalobos, F.J., Testi, L.; Rizzalli, R.; and Orgaz, F. 2004. Evapotranspiration and crop coefficients of irrigated garlic [*Allium sativum* L.] in a semi-arid climate. Agric. Water. Manage. 64(3): 233-249.

Wenkert, W. 1983. Water transport and balance within the plant: An overview. In: *Limitations to Efficient Water Use in Crop Production.* Edited by: Howard M. Taylor, Wayne R. Jordan and Thomas R. Sinclair. Published by: ASA-CSSA-SSSA:137-172.

Wright, J.L., and M.E. Jensen. 1978.Development and evaluation of evapotranspiration models for irrigation scheduling. *Trans ASAE.* 21(1):88-96.

Zhang, Y.; Yu, Q.: Liu, C., Jiang, J., and Zhang, X. 2004. Estimation of water wheat evapotranspiration under water stress with two semiempirical approaches. *Agron J.* 96:159-168.

Chapter 5

Surface Resistance Derived From Micrometeorological Data and Crop Measurements Under Variable Leaf Area Index and Soil Moisture in Common Bean (*Phaseolus vulgaris* L.)

ABSTRACT

After rainfall, evapotranspiration (ET) is often the second largest component of the hydrological cycle. It is a important variable in the fields of climatology, hydrology, environmental and agricultural sciences. The Penman-Monteith model (PM) is a useful "one-step" method for ET estimation, if surface resistance (r_s-ms^{-1}) estimates can be derived. This study had as its objective to evaluate different methods for r_s estimation and the accuracy in the resulting ET estimates in common bean (*P. vulgaris* L.). The experiment was conducted at the Fortuna Agricultural Experiment Station at Juana Diaz, PR. Four automated weather stations were placed in plots planted with two genotypes of common bean (*Phaseolus vulgaris* L.). Net radiation, soil heat flux, soil temperature soil moisture, air temperature, relative humidity, wind speed and direction were recorded at ten second intervals. Each weather station had an elevator system that moved the air temperature and relative humidity sensor between two vertical positions over the crop canopy every two minutes during a complete day. The r_s was derived by stomatal resistance (r_L) and leaf area index (LAI) measurements (PM-1), and by direct micrometeorological variables as follows: inverse of the general PM-model (PM-2), as a function of the soil moisture (PM-3), and as a latent heat flux-λE (PM-4 and ETstation). The results indicate that PM-1 under-estimated r_s at low LAI, and that r_s and r_L are influenced inversely by the aerodynamic resistance (r_a), which affected the precision of the PM-2 and ET station estimation especially under windy and dry conditions, but not the PM-3 and PM-4.

Key words. Evapotranspiration, combination-method, surface resistance.

Abbreviations: r_L, stomatal resistance; r_s, Surface resistance; r_a, aerodynamic resistance, ET, evapotranspiration; PM, Penman-Monteith.

INTRODUCTION

Evapotranspiration (ET) is a widely studied variable throughout the world, due to its applicability in various disciplines, such as hydrology, climatology, and agricultural science. An accurate estimation of evapotranspiration is necessary for appropriate agricultural water management. The most precise method for estimating ET is the mass balance method using weighing lysimeters, but the principal disadvantages are its cost and immobility. Evapotranspiration may also be estimated based on micrometeorological methods, which have been used with good precision in many countries and with different vegetation covers. The generalized Penman-Monteith model (PM) for estimating ET has been recommended by the United Nations Food and Agriculture Organization (FAO) as the sole meteorological method that should be used in the world. However, one of its limitations is obtaining an estimate of the surface resistance (r_s), which is a required input for the method, and the lack of tables with effective r_s values for different crops, as are available for the evapotranspiration crop coefficient (Shuttleworth, 2006).

The Drainage and Irrigation Paper-FAO56 (Allen et al. 1998), recommends the Szeicz and Long (1969) method for calculation of r_s, in which an average of r_L for different positions within the crop canopy, weighted by LAI or $LAI_{effective}$ is used. This method seems to give good results only in very rough surfaces, like forest and partial cover crops with a dry soil (i.e Monteith, 1987). Alves et al. (1998) concluded that r_s of dense crops cannot be obtained by simply averaging stomatal resistance (r_L) because the vapor pressure deficit (VPD) which is the "driving force" is not constant within the canopy. Alves and Pereira (2000) said "The PM model can be used to predict ET if accurate methodologies are available for determining the r_s that take into account the energy partitioning". These conditions include if the canopy is sparse, if the evaporating surface is completely covering the soil, and if the soil is wet or dry.

In addition to the lack of r_s values for crops, questions have been raised relative to the appropriateness of using the PM model for partial or sparse canopies because the source/sink fluxes may occur at significantly distances (Kjelgaard et al. 1994; Farahami and Bausch, 1995; Ortega-Farias et al. 2006). Adequate parameterization of the surface resistance makes the PM

model a good tool for ET estimation (i.e., Rana et al. 1997; Alves and Pereira, 2000; Ortega-Farias et al. 2004).

Thus, Rana et al. (1997) used micrometeorological parameters to estimate the r_s for use in the PM model to estimate the ET in sorghum and sunflower under well and deficit water conditions. Ortega-Farias (2004 and 2006) successfully estimated the r_s using micrometeorological and soil moisture parameters used in the P-M model to estimate ET for soybean and tomato crops. Tomilson (1994) found good agreement using r_s estimated with the PM inverse model based on ET derived from the Bowen ratio method in grass. Kjelgaard et al. (1994) observed good performance of the PM model in ET estimation at twenty-minute intervals in corn, where r_s was calculated with the Szeicz and Long (1969) method (equation 5.3). Latter Kjelgaard and Stockle (2001) used PM models with r_s estimated using the equation 5.3 with adjustments based on crop height, solar radiation, and vapor pressure deficit, and obtained good results for potato (low crop), but relatively poor results with corn (tall crop).

Blad and Rosenberg (1976) applied a resistance model that depended on the canopy energy balance, and surface and air temperatures. The method did not give good ET estimations in alfalfa when the soil became too dry. Rana et al. (1997) who estimated the r_s with micrometeorological variables obtained good results in the estimation of ET in sunflower and sorghum when the crops were not stressed, or during senescence. However, when the crops were stressed (weak or strong stress) the model did not perform as well. On the other hand, Ortega-Faria et al. (2004 and 2006), who used micrometeorological variables and a normalized soil water factor to estimate r_s and then to estimated ET, obtained good ET results under water stress and non-stress conditions in soybean and tomato, as compared with a Bowen ratio energy balance system.

There is a need to evaluate the existing methods to determining r_s under variable canopies and soil moisture conditions in common bean, and to apply it to “one step” ET calculation. Therefore, in this work we compare ET estimates using the “one-step” or generalized PM model, using r_s as a function of r_L measured and derived with micrometerological and soil moisture data.

Theoretical background. The evapotranspiration from a crop canopy as expressed by the generalized Penman-Monteith (PM) equation has been presented by Allen et al. (1998) in the following equation:

$$\lambda E = \frac{\Delta(Rn - G) + \rho_a C_p \dfrac{VPD}{r_a}}{\Delta + \gamma\left(1 + \dfrac{r_s}{r_a}\right)} \qquad (5.1)$$

where λE is Latent heat flux [Wm^{-2}], Rn is net radiation [Wm^{-2}], G is soil heat flux [Wm^{-2}], VPD is vapor pressure deficit [kPa], Δ is the slope of saturation vapor pressure curve [kPa $^{\circ}C^{-1}$] at air temperature, ρ_a is the density of air [Kgm^{-3}], C_p is specific heat of air [J $Kg^{-1}{}^{\circ}C^{-1}$], γ is the psychometric constant [kPa $^{\circ}C^{-1}$], *VPD* is the vapor pressure deficit, r_a is the aerodynamic resistant [s m^{-1}], r_s is the surface resistance to vapor transport [s m^{-1}]. The crop evapotranspiration was estimated by dividing λE by λ. Equation 5.1 is referred to as the "one step" method because it does not rely on the use of a crop coefficient.

Aerodynamic resistance describes the resistance of heat and water vapor transport from the evaporating surface into the air above the canopy and was estimated with equation 5.2 (Allen et al. 1998, and Alves et al. 1998).

$$r_a = \frac{Ln\left[\dfrac{(Z_m - d)}{Z_{om}}\right] Ln\left[\dfrac{(Z_h - d)}{Z_{oh}}\right]}{k^2 u_z} \qquad (5.2)$$

where Z_m is the height of wind measurements [m], z_h is the height of humidity measurements [m], d is the zero displacement height [m] is 2/3h, Z_{om} is the roughness length governing momentum transfer of heat and vapor [m] is 0.123h, Z_{oh} is the roughness length governing transfer of heat and vapor [m] is $0.1Z_{om}$, k is the von Karman`s constant [0.41] and u_z is the wind speed at height z.

The bulk surface resistance describes the resistance of vapor flow through the transpiring crop and evaporation from the soil surface (Appendix B). The surface resistance involves plant parameters like the stomatal resistance and leaf area index. Szeicz and Long (1969) propose the use of equation 5.3 to estimate the surface resistance and say that it can be used when the evaporation from the soil is negligible, when the surface resistance of a crop may be very close to the compound resistance of all its leaves in parallel. In a full developed canopy, the lower leaves may no be illuminated well enough to open their stomatates, therefore, the effective LAI contributing to transpiration is less than the total leaf area, and for this reason the active LAI = LAI x 0.5 can be used. Equation 5.3 is recommended in the Drainage and Irrigation Paper No. 56 (Allen et al. 1998).

$$r_s = \frac{r_L}{LAIactive} \tag{5.3}$$

where r_s is bulk surface resistance (s m^{-1}), LAI_{active} is 0.5 times the leaf area index (m^2 leaf by m^2 the soil), and r_L is stomatal resistance equal to the average resistance of and individual leaves and well-illuminated leaf (s m^{-1}).

Harmsen et al. (2006) developed a method to estimated resistance factors when one of them (i.e., r_s or r_a) is not available or measured. In this study, this method is referred to as the ET-Station method, and relies on a functional form of the gradient flux equation (5.4) in combination with generalized PM equation (5.1):

$$ET = \left[\frac{\rho_a . c_p}{\gamma . \rho_w}\right] . \frac{\left[\rho_{vL} - \rho_{vH}\right]}{r_a + r_s} \tag{5.4}$$

where ρ_w is the density of water, ρ_v is the water vapor density of the air, and L (down) and H (up) are vertical positions above the ground. All other variables were defined previously. In this study L and H were 0.5 m and 2 m above the ground.

We will now provide the details associated with the application of the ET station methodology. Unlike the VPD, which depends on the difference between the actual and saturated vapor pressures, this method uses only the actual vapor pressures (converted to vapor densities, equation 5.4) . It is important to note that the resistance factors in equation 5.4 are identical to those used in equation 5.1. If it is assumed that equation 5.1 and 5.4 are both valid estimates of ET, then the two equations (gradient flux and generalized Penman-Monteith) can be equated to estimate one of the resistance factors.

Ortega-Farias et al. (2004), evaluated a methodology for calculating the canopy surface resistance ($r_{cv} \approx r_s$) in soybean and tomatoes, which is presented in equation 5.5.

$$r_s = \frac{\rho_a.c_p.VPD}{\Delta.(Rn - G))}.\frac{\theta_{FC} - \theta_{WP}}{\theta_i - \theta_{WP}} \tag{5.5}$$

where θ_{FC} is the volumetric moisture content at field capacity (fraction), θ_{wp} is the volumetric moisture content at wilting point (fraction) and θ_I is a volumetric soil content in the root zone (fraction) measured every day.

Szeicz and Long (1969) describe a profile method to estimated r_s (equation 5.6). This method can be used in the field when the rate of evapotranspiration is measured by lysimeter or calculated from the Bowen ratio, and the temperature, humidity and wind profiles are measured within the boundary layer simultaneously.

$$r_s = \frac{\rho_a.C_p,VPD}{\gamma.\lambda E} \tag{5.6}$$

The inverse of the equation 5.1 could be used to estimate an effective Surface resistance when all the other parameter are known or measured (Monteith, 1995), the inverse of the equation 5.1 is presented in the equation 5.7.

$$r_s = r_a . \frac{\left[\frac{\Delta(R_n - G) + \rho_a C_p \left(\frac{VPD}{r_a} \right)}{\lambda E} - \Delta - \gamma \right]}{\gamma} \qquad (5.7)$$

MATERIALS AND METHODS

This research was carried during 2006 and 2007 at the Experiment Station of the University of Puerto Rico in Juana Diaz-Puerto Rico, which is located in south central Puerto Rico, latitude 18°01'N, longitude 66°22'W longitude, and elevation 21-m above mean sea level, classified as a semi-arid climatic zone (Goyal and Gonzalez, 1989).

The field experiment had a plot size 60 m x 117 m. This area was divided into two plots, one half received a water application rate sufficient to maintain the soil moisture content at field capacity (no drought stress) during the entire growing season, while the second plot was submitted to drought stress at the beginning of the reproductive growth period. Each half (drought and non-drought treatments) were divided into 6 sub-plots of 9 m x 60 m. Two of the sub-plots were planted with common bean genotype 'SER 16' (6.5 plants.m^2) and four were planted with common bean genotype 'Morales' (13.5 plants.m^2) in 2006, and three sub-plots with each genotype were planted in 2007. Part of the neighboring plot was well irrigated grass, and irrigated fruit trees. The crop was irrigated two times per week to maintain the soil moisture near field capacity; the water stress was applied to half of the main plot after flowering differentiation. The water stress consisted of irrigation reduction of 75% of the total soil available water.

Fetch Requirements. The air passing over a surface is affected by the field surface feature (Rosenberg et al. 1983); the minimal fetch requirement was estimated based on the thickness of the internal boundary layer (δ in m) and a roughness parameter (Z_o in m) for each genotype considering the minimal and maximal crop height during the growing season. The δ was calculated using the relation proposed by Monteith and Unsworth (1990).

$$\delta = 0.15.L^{4/5}.Z_o^{1/5} \qquad (5.8)$$

where L is the distance of traverse (fetch) across a uniform surface with roughness Z_o. Z_o for crops is approximately one order of magnitude smaller than the crop height h, and was calculated using the equation 5.9 (Rosenberg et al. 1983).

$$Log_{10}Z0 = 0.997 \log_{10} h - 0.883 \qquad (5.9)$$

The results of the fetch related parameters are summarized in the table 5.1. As a factor of safety a height to fetch of 1:50 to 1:100 is usually considered adequate for studies made over agricultural crop surfaces (Rosenberg et al. 1983, Allen et al. 1998) but may be too conservative and difficult to achieve in practice. Alves et al. (1998) obtained full profile development using a 1:48 fetch relation in Wheat and lettuce. Heilman et al. (1989) found that for Bowen-Ratio estimates a fetch 1:20 was sufficient over grass, and Frithschen and Fritschen (2005) obtained similar results. For this research the height to fetch ratio was 1:32, and in Table 5.1 the minimal and maximal fetch requirement are summarized. They indicated that the data where collected over the minimum fetch requirement.

Table 5.1. Fetch requirement for both genotypes.

Genotype	$h_{c\cdot min}$ (m)	$h_{c\cdot max}$ (m)	$Z_{o\cdot min}$ (m)	$Z_{o\cdot max}$ (m)	L (m)	$\delta_{\cdot min}$ (m)	$\delta_{\cdot max}$ (m)	Ratio height: fetch min	Ratio height: fetch max
SER 16	0.18	0.51	0.024	0.067	46	1.52	1.86	1:30	1:24
Morales	0.17	0.60	0.029	0.079	46	1.93	1.93	1:29	1:23

Data collection and instrumentation. Four Campbell Scientific weather stations were located in the four of the treatments plots:

- genotype 'Morales', non-drought
- genotype 'Morales', drought-stress
- genotype 'SER-16', non-drought stress
- genotype 'SER-16', drought-stress

Each weather station measured net radiation with a Kipp & Zonen B.V. net radiometer (spectral range 0.2-100μm), wind direction and wind speed with Wind sensor-Met one 034B-L, at 1.9 m; soil temperature with TCAV averaging soil thermocouple probe at 0.08 m and 0.02 m depth, soil heat flux using a soil heat flux plates at 0.06 m depth; volumetric soil moisture content with a CS616 water content reflectometer at 0.15 m depth; and air temperature and relative humidity with HMP45C temperature and relative humidity probe, at two height levels (0.50 m and 2.0 m). All sensors were connected to a CR10X data logger (Campbell Scientific, Inc).

An automated elevator device was developed for moving the temperature and relative humidity sensor (Temp/RH) between the two vertical positions. The device consisted of a plastic (PVC) frame with a 12 volt DC motor (1/30 hp) mounted on the base of the frame. One end of a 2-m long chain was attached to a shaft on the motor and the other end to a sprocket at the top of the frame. Waterproof limit switches were located at the top and bottom of the frame to limit the range of vertical movement (Fig. 5.1).

For automating the elevator device, a programmable logic controller (PLC) was used which was composed of "n" inputs and "n" relay outputs. To program the device, a ladder logic was used which is a chronological arrangement of tasks to be accomplished in the automation process. The Temp/RH sensor was connected to the elevator device, which measured relative humidity and temperature in the up position for two minutes then changed to the down position where measurements were taken for two minutes, and the process continued indefinitely until the experiment ended. When the elevator moves to the up position it activates the limit switch which sends an input signal to the PLC. That input tells the program to stop and remain in that position for two minutes. At the same time it activates an output which sends a 5 volt signal to the control port C2 in the CR10X data logger in which a small subroutine is executed. This subroutine assigns a "1" in the results matrix which indicates that the temperature and relative humidity corresponding to the up position. At the end of the two minute period, the elevator moves to the down position and repeats the same process, but in this case sending a 5 volts signal to the data logger in the control port C4, which then assigns a "2" in the results matrix. All information was stored in the weather station data-logger CR-10X (Campbell Scientific, Inc) for later downloading to a personal computer (Harmsen et al. 2006). A Microsoft Excel-spreadsheet-macro was developed to automatically separate the data into up and down positions, produce graphs of RH, air temperature, net radiation, wind speed, and soil temperature as a function of time, calculate the vapor density differences between the up and down positions, and estimate evapotranspiration using the vapor gradient approach (equation 5.4) and the PM equation (equation 5.1).

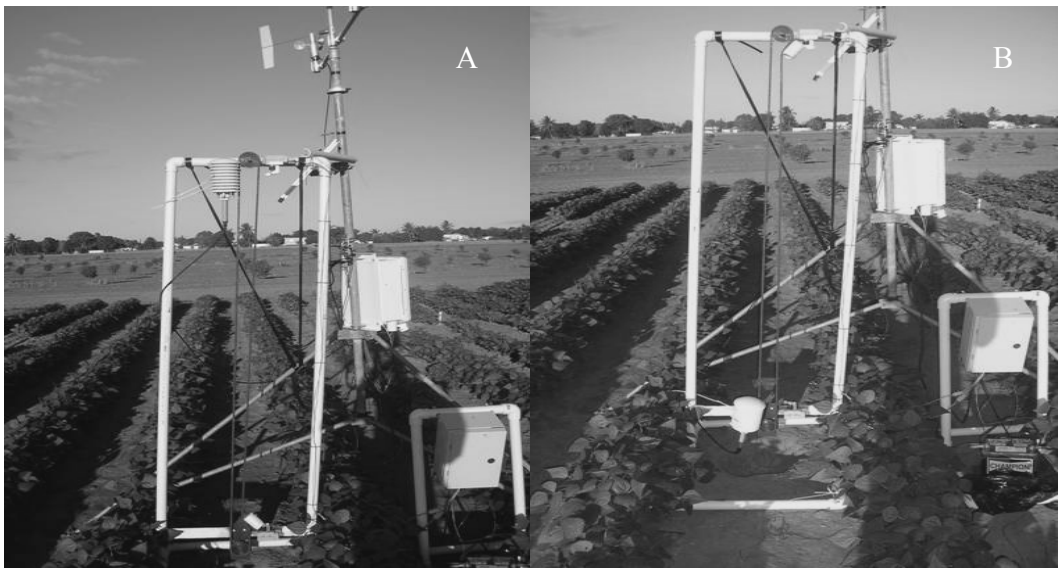

Figure 5.1. Automated elevator device developed for moving the Temp/RH sensor between the two vertical positions. **A.** Temp/RH sensor in up position and **B.** Temp/RH sensor in down position.

The values of λE used in equations 5.6 and 5.7 were estimated using the Bowen-ratio method (equation 5.10). This method combines measurements of certain atmospheric variables (gradients of temperature and vapor concentration) and available energy-net radiation and changes in stored thermal energy (Tanner, 1960; Lloyd, 1992).

$$\lambda E = \frac{(Rn - G)}{(1 + \beta)} \tag{5.10}$$

where λE is latent-heat flux (Wm^2), β is Bowen ratio calculated using equation 5.11. The Bowen ratio is defined as:

$$\beta = \gamma \frac{\Delta T}{\Delta e} \tag{5.11}$$

where γ is psychometric constant, ΔT is difference in air temperature at two heights (◦C) and Δe is the difference in vapor pressure at two heights (kPa).

The hourly Bowen ratio estimates were validated using the Payero et al. (2003) guidelines, where the fluxes with incorrect sign and $\beta \approx -1$ where not considered. Also the Monin-Obukhov stability factor (ζ) was calculated using equation 5.12 (Rosember et al. 1983; Campbell, 1985), flux with ζ negative sing where also exclude.

$$\xi = \frac{(-k.z.g.H)}{\left(\rho_a.C_p.T_a.u*^3\right)} \tag{5.12}$$

where k is von Karman's constant, z is height of wind and air temperature measurements (m), g is the gravitational constant (9.8 $m.s^{-2}$), $H = \beta\lambda E$, T_a is air temperature (^oK), u* is the friction velocity given by Kjelgaard et al. (1994) without the stability correction factor.

$$u* = \frac{k.u_z}{\ln\left(\frac{z-d+Z_{om}}{Z_{om}}\right)} \tag{5.13}$$

The crop height (h) was measured for each genotype each week, and, polynomial model was derived for each genotype and year, from which daily h values were interpolated. The r_a was calculated at one minute time intervals using equation 5.2. The r_L was measured with a Porometer type AP4-UM-3 (Delta-T Devices Ltd) in 2006 and Porometer model SC-1 (Decagon Devices, Inc.) in 2007, one time per week at different time intervals from 7:00 am to 5:00 pm. The leaf area index was measured one time per week using a non-destructive method (Chapter 2).

Undisturbed cores with soil samples were collected periodically to calibrate the moisture sensor readings.

Hourly P-M ET estimates were calculated using four methods to determine r_s and compared to crop measurements. The methods were as follows:

PM-1: r_s was estimated from equation 5.3, called the "Measured method".

PM-2: r_s was estimated from equation 5.7, called the "Inverse method".

PM-3: r_s was measured from equation 5.5, called "Ortega-Faria method"

PM-4: r_s was measured from equation 5.6, called "Szeicz and Long method" and

ET-Station: r_s was estimated from the equations 5.1 and 5.4, called "ET-Station method"

Evaluation of model performance. The performance of the models were evaluated using regression analysis, means, standard deviation (STD), the root mean square error (RMSE), hypothesis test and two model efficiency coefficients. One the Nash and Sutcliffe (R^2) (Prenger et al. 2002), and second the Legates and McCabe modified coefficient (E) (Tolk and Howell 2001).

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(Y_o - Y_m\right)^2}{\sum_{i=1}^{N}\left(Y_o - \overline{Y_m}\right)^2} \tag{14}$$

$$E = 1 - \frac{\sum_{i=1}^{N}\left|Y_o - Y_m\right|}{\sum_{i+1}^{N}\left|Y_o - \overline{Y_m}\right|} \tag{15}$$

where Y_o is the observed data, Y_m is the model measured and $\overline{Y_m}$ is the mean of the measured values.

RESULTS AND DISCUSSION

The crop height distribution (h_c) was affected by year to year variability, drought stress and genotype (Fig. 5.2). The h_c was lower in 2007 for both genotypes, which coincides with r_a variability between genotypes and water levels.

Table 5.2 summarizes the average weather conditions during the study. The differences in air temperature and available energy (R_n-G) in both years are associated with the fact that the experiment in 2007 was planted earlier (January 17) than in 2006 (February 1), and that windier conditions occurred during 2007.

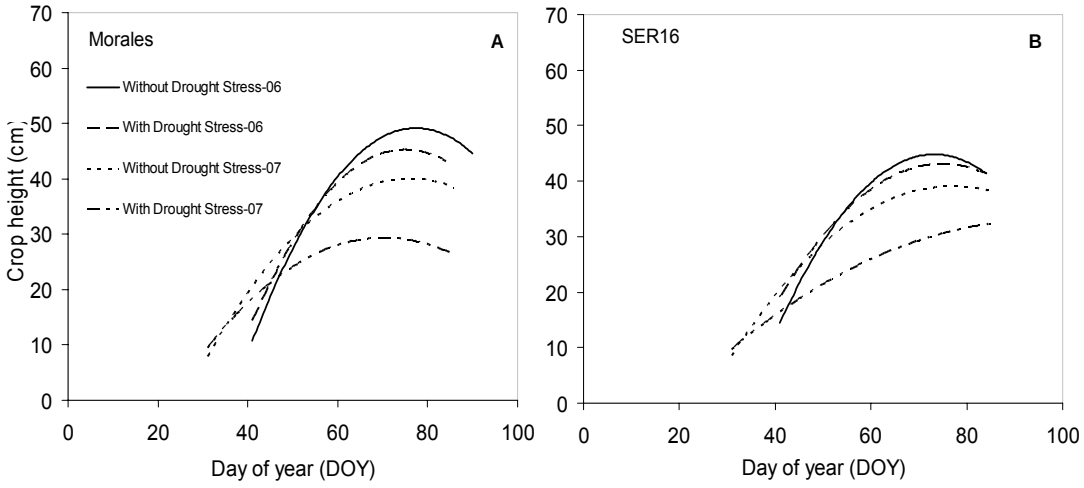

Figure 5.2. Crop height distribution during two years with and without drought stress: **A.** Morales and **B.** SER 16.

Table 5.2. Day-time climatic conditions during the experiment in 2006 and 2007.

Variable	Units	Range of values	Mean value
	2006		
Wind speed (u)	m/s	1.12 to 6.03	3.78
R_n-G	$W.m^{-2}$	0 to 610.7	326.10
Air Temperature (T_a)	^oC	22.5 to 30.0	28.10
Vapor pressuare deficit (VPD)	kPa	0.88 to 0.51	0.83
	2007		
Wind speed (u)	m/s	0.41 to 7.03	4.25
R_n-G	$W.m^{-2}$	0 to 633.0	307.90
Air Temperature (T_a)	^oC	21.7 to 30.4	28.10
Vapor pressuare deficit (VPD)	kPa	0.50 to 0.80	0.79

ET by PM model variable Surface resistance measured v_s ET by Bowen ratio. The daily ET estimation with the PM model with r_s based on r_L and $LAI_{effective}$ gave a good estimation in both common bean genotypes with variable LAI, without and with moderate drought stress (Table 5.3) for both years. This conclusion is based on a t-test of b ($b= ET_{PM}/ET_{Bowen}$), which was determined not to be significantly different from 1. For SER 16 with drought stress (reduced soil moisture conditions and low LAI), the PM model over-estimated ET. In the case of Morales with drought stress, PM under-estimated ET in both years with b = 0.9 in 2006 and 0.7 in 2007. The under-estimation in 2007 was significantly different from 1, and was associated with high r_L during the drought stress, with a mean value of the 1226 $s.m^{-1}$ (1SD = 727 $s.m^{-1}$), as compared with SER16 with a mean r_L value of 584 $s.m^{-1}$ (1SD = 408 $s.m^{-1}$).

A similar situation was observed when the models were compared at hourly intervals. Morales under drought stress in 2007 showed the lowest efficiency coefficients ($R^2_{Nasch\text{-}Sutcliffe}$

and E) and linear coefficients (slope and determination coefficient), with respect to the other models.

Table 5.3. Statistical results for daily evapotranspiration for two common bean genotypes with and without drought stress estimated with Penman-Monteith equation with variable r_s estimated with equation 3 and the Bowen ratio.

	$b(ET_{PM}/ET_{BR})^a$	SEE^b	$T\text{- test}^c$
2006			
Morales-without drought stress	1.0 4.2<LAI>0.6	0.07 $mm.day^{-1}$	T
SER16- without drought stress	1.0 1.7<LAI>0.12	0.07 $mm.day^{-1}$	T
Morales-with drought stress	0.9 4.0<LAI>3.0	0.06 $mm.day^{-1}$	T
SER16-with drought stress	1.4 1.8<LAI>0.9	0.06 $mm.day^{-1}$	F
2007			
Morales-without drought stress	1.0 3.0<LAI>0.5	0.007 $mm.day^{-1}$	T
SER16-without drought stress	0.9 2.0<LAI>0.12	0.011 $mm.day^{-1}$	T
Morales-with drought stress	0.7 2.0<LAI>0. 8	0.080 $mm.day^{-1}$	F
SER16-with drought stress	1.0 2.0<LAI>0. 5	0.005 $mm.day^{-1}$	T

[a] b= ratio between the ET measured using the Penman-Monteith equation (variable r_s) and Bowen ratio.
[b] SEE = standard error of estimate b
[c] T= true hypothesis (b=1), F false hypothesis (b≠1)

ET with r_s measured vs ET with r_s estimated by micrometeorological variables. The models PM-3 and PM-4 were more closely related with the model PM-1, with the higher efficiency coefficients-$R^2_{Nasch\text{-}Sutcliffe}$ and E in both years, with and without drought stress (Tables 5.4 and 5.5). For Morales in 2006 with drought stress (Morales-2006-S; Table 5.4) the PM-3 and PM-4 give efficient coefficient $R^2_{Nasch\text{-}Sutcliffe}$ and E >0.90 and slopes of 0.95 and 1.0 respectively, with LAI ranking between 1.5 and 4.0.

The PM-3 value for Morales without drought stress in 2007, was $R^2_{Nasch\text{-}Sutcliffe}$ = 0.92 and slope = 0.86, with LAI ranking between 0.1 and 3.0 (Tables 5.5). When the drought stress was moderate, the $R^2_{Nasch\text{-}Sutcliffe}$ = 0.99 and slope = 095 with LAI ranking between 1.5 and 4.0 (Tables 5.4). The advantage of PM-3 with respect to PM-2, PM-4 and the ETstation methods is related to the PM-3 model estimated the r_s as a function R_n, G, VPD and the normalized soil water factor.

The PM-2 resulted in the lowest efficiency in ET estimation during both years (Tables 5.4 and 5.5). This situation is closely related with the aerodynamic resistance (r_a), which is included in both models for r_s. In the case of equation 5.7 for r_s, when all the other parameter are constant, if r_a increases then r_s also increases, and a high r_s decreases the ET. This situation can be observed during: DOY 91-2006 (r_a = 492 $s.m^{-1}$, Fig 5.4 A and B), DOY 46-2007 (r_a =489 $s.m^{-1}$, Fig 5.5 A), DOY 71-2007 (r_a = 220 $s.m^{-1}$, LAI= 2.6 m^2,m^{-2} and θ_v=0.22 $m^3.m^{-3}$-graph no-showed). Those results are contrary to observations in this study, as well as those reported by Alves and Pereira (2000), where the r_s was inversely related with r_a (Fig 5.7), which implies that with low r_a (windy conditions), the r_L (and therefore r_s) increases. The Alvers and Pereira (2000), study did not measure the r_L, the r_s was estimated based on micrometeorological parameters.

Disparities in the r_s measured using the PM-inverse (PM-2) arise from: a) imperfect sampling of leaves and the arbitrary method of averaging leaf resistance over the whole canopy, b) from the dependence of r_s on non-stomatal factors such as evaporation from wet soil or stems, or others and c) the complex aerodynamic behavior of canopies (Monteith, 1995).

The ET station also adequately estimated hourly ET for two common bean genotypes at different plant densities, without and with moderate drought stress (e.g., Fig 5.3A and B).

When the drought stress was high, the difference among the models was evident, and especially in the low LAI case for DOY 64-2007 (Fig. 5.5 B) and DOY 79-2007 (Fig 5.6 A and B) where PM-2, PM-4 and ET station were greater than PM-1, and PM- 3 was lowest. This result was associated with the moisture content readings that were made at 15 cm, and the overestimation of r_s.

At low LAI (≤1.0) the differences among models was evident. The ET calculated by PM-1 was lower than that of the PM-3, PM-4 and ETstation (DOY 79-2007,Fig 5.6 B) calcultaion. The differences between models can be associated with the effect of the local sources of sensible heat from nonevaporating surfaces such as dry soil surrounding transpiring plants (Ritchie, 1983).

When the LAI >1.0, and without and moderate drought stress, all the models with the exception of PM-2 were closely related (Fig 5.4 B) with the PM-1. PM-2 was close related with PM-1 and similar that the other models, when the ET rate during the day was low (Fig 5.3 A, and B).

When soil moisture decreased, r_s estimated with PM-3 and PM-4 models were the most closely related to PM-1.

Table 5.4. Statistical parameters for ET estimation based on r_s-measured compared r_s-estimated with micrometeorological variables, for variable LAI water supplied in 2006.

Cases	Statistical parameters	PM-1 Measured ($mm.h^{-1}$)	PM-2 Inverse ($mm.h^{-1}$)	PM-3 Ortega-Faria ($mm.h^{-1}$)	PM-4 Szeicz and Long ($mm.h^{-1}$)	ET-Station ($mm.h^{-1}$)
SER16-2006-NS‡						
	Mean	0.18	0.17	0.24	0.19	0.22
0.2<LAI>1.7	STD	0.18	0.13	0.2	0.18	0.19
	RMSE		0.13	0.09	0.06	0.12
	R^2 (Nasch-Sutcliffe)		0.51	0.78	0.89	0.54
	E		0.45	0.55	0.74	0.53
	Regresions					
	Slope		0.98	0.88	0.93	0.78
	r^2		0.51	0.91	0.90	0.66
	n		843	843	843	843
Morales-2006-NS						
	Mean	0.19	0.18	0.19	0.22	0.25
0.6<LAI>4.2	STD	0.18	0.13	0.19	0.19	0.19
	RMSE		0.12	0.08	0.10	0.15
	R^2 (Nasch-Sutcliffe)		0.55	0.78	0.69	0.30
	E		0.48	0.55	0.54	0.30
	Regresions					
	Slope		1.09	0.82	0.79	0.62
	r^2		0.56	0.83	0.73	0.47
	n		774	774	774	774
SER16-2006-S†						
	Mean	0.29	0.31	0.21	0.34	0.35
0.9<LAI>1.8	STD	0.23	0.21	0.18	0.246	0.24
	RMSE		0.13	0.11	0.08	0.09
	R^2 (Nasch-Sutcliffe)		0.68	0.76	0.86	0.86
	E		0.54	0.58	0.73	0.68
	Regresions					
	Slope		0.9	1.21	0.90	0.91
	r^2		0.7	0.91	0.92	0.94
	n		1144	1144	1144	1144
Morales-2006-S						
	Mean	0.32	0.19	0.32	0.30	0.30
1.5<LAI>4.0	STD	0.25	0.16	0.26	0.25	0.25
	RMSE		0.15	0.02	0.03	0.02
	R^2 (Nasch-Sutcliffe)		0.64	0.99	0.98	0.99
	E		0.43	0.92	0.9	0.93
	Regresions					
	Slope		1.59	0.95	1.00	1.01
	r^2		0.99	0.99	0.99	0.99
	n		460	460	460	460

‡: Withouth drough stress: † With drought stress

Table 5.5. Statistical parameters for the ET estimation based on r_s-measured compared r_s-estimated with micrometeorological variables, for variable LAI water supplied in 2007.

Cases	Statistical parameters	PM-1 Measured ($mm.h^{-1}$)	PM-2 Inverse ($mm.h^{-1}$)	PM-3 Ortega-Faria ($mm.h^{-1}$)	PM-4 Szeicz and Long ($mm.h^{-1}$)	ET-Station ($mm.h^{-1}$)
Morales-2007-NS						
	Mean	0.30	0.21	0.34	0.33	0.39
0.1<LAI>3.0	STD	0.23	0.14	0.26	0.23	0.25
	RMSE		0.14	0.06	0.04	0.13
	R^2 (Nasch-Sutcliffe)		0.61	0.92	0.96	0.69
	E		0.43	0.77	0.85	0.58
	Regresions					
	Slope		1.52	0.86	0.96	0.84
	r^2		0.88	0.97	0.97	0.87
	n		657	657	657	657
SER16-2007-NS‡						
	Mean	0.17	0.24	0.24	0.24	0.30
0.1<LAI>1.8	STD	0.19	0.18	0.19	0.20	0.23
	RMSE		0.10	0.09	0.08	0.15
	R^2 (Nasch-Sutcliffe)		0.67	0.71	0.80	0.18
	E		0.50	0.56	0.62	0.22
	Regresions					
	Slope		0.82	0.81	0.81	0.66
	r^2		0.78	0.84	0.92	0.80
	n		774	774	774	774
SER16-2007-S†						
	Mean	0.22	0.19	0.19	0.23	0.33
0.7<LAI>1.2	STD	0.16	0.11	0.17	0.16	0.20
	RMSE		0.12	0.06	0.02	0.12
	R^2 (Nasch-Sutcliffe)		0.71	0.93	0.99	0.72
	E		0.62	0.85	0.92	0.65
	Regresions					
	Slope		1.07	0.94	1.03	0.79
	r^2		0.51	0.92	0.98	0.83
	n		396	396	396	396
Morales-2007-S						
	Mean	0.10	0.13	0.07	0.16	
0.8<LAI>2.0	STD	0.10	0.09	0.06	0.14	
	RMSE		0.06	0.08	0.08	
	R^2 (Nasch-Sutcliffe)		0.61	0.45	0.43	
	E		0.35	0.34	0.20	
	Regresions					
	Slope		0.96	1.16	0.71	
	r^2		0.69	0.53	0.97	
	n		373	373	373	

‡: Withouth drough stress: † With drought stress

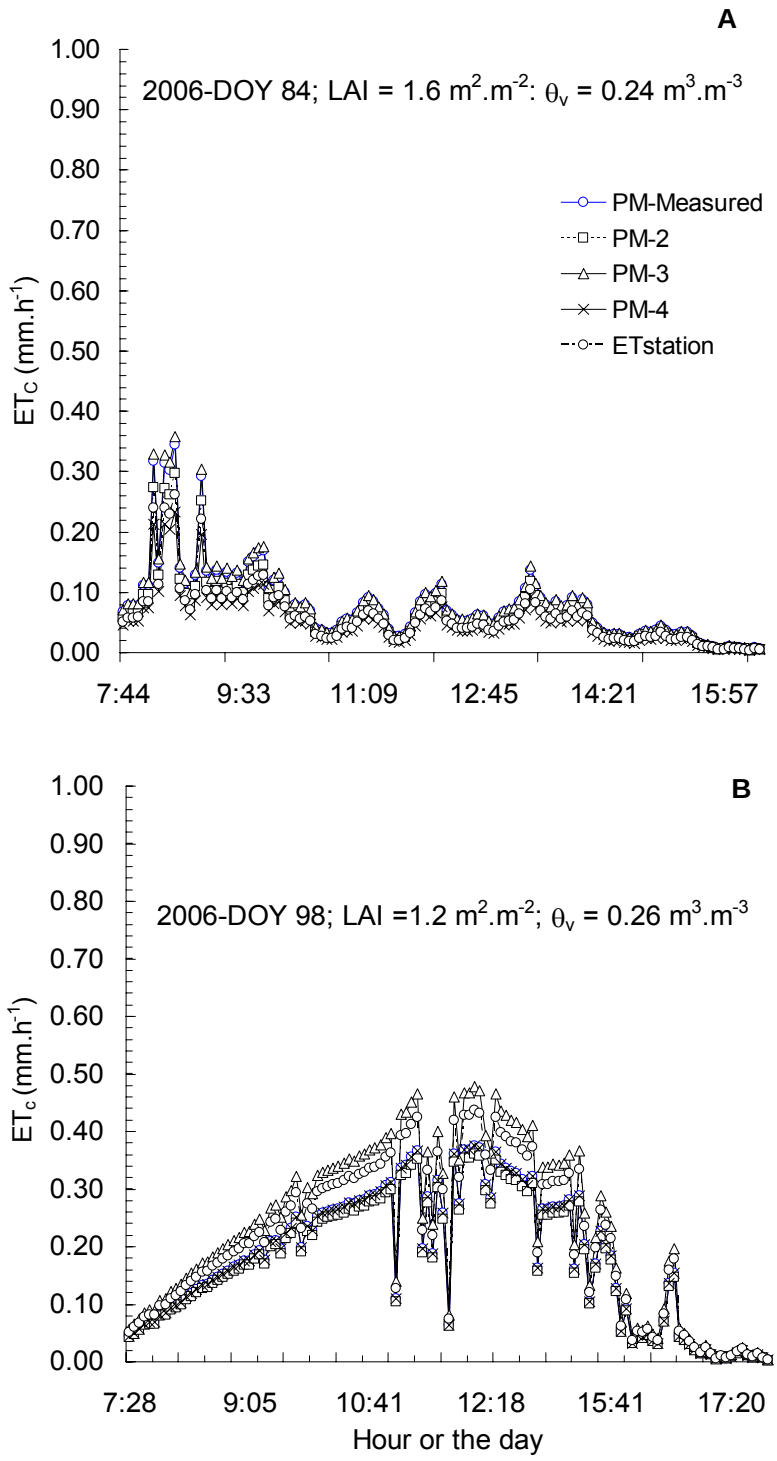

Figure 5.3. ET distribution with different PM-models, with variable LAI and soil moisture (θ_v) in the SER 16 common bean (*P.vulvaris* L) genotype, 2006 trial. For two selected days. **A**. Day or the year 84 and **B**. Day of the year 98.

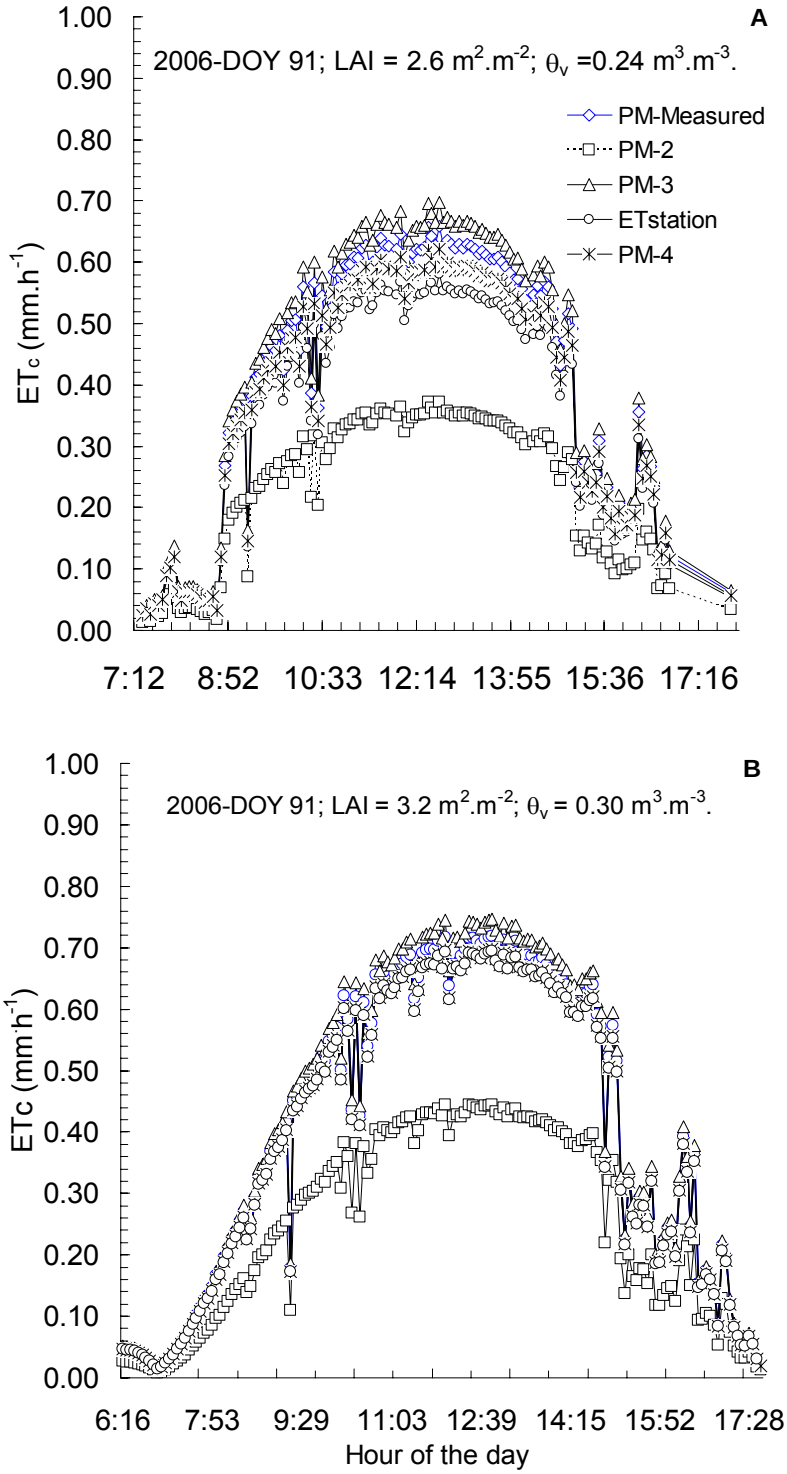

Figure 5.4. ET distribution with different PM-models, with variable LAI and soil moisture (θ_v) in the Morales common bean (*P.vulvaris* L) genotype, 2006 trial. **A.** with drought stress and **B**. without drought stress.

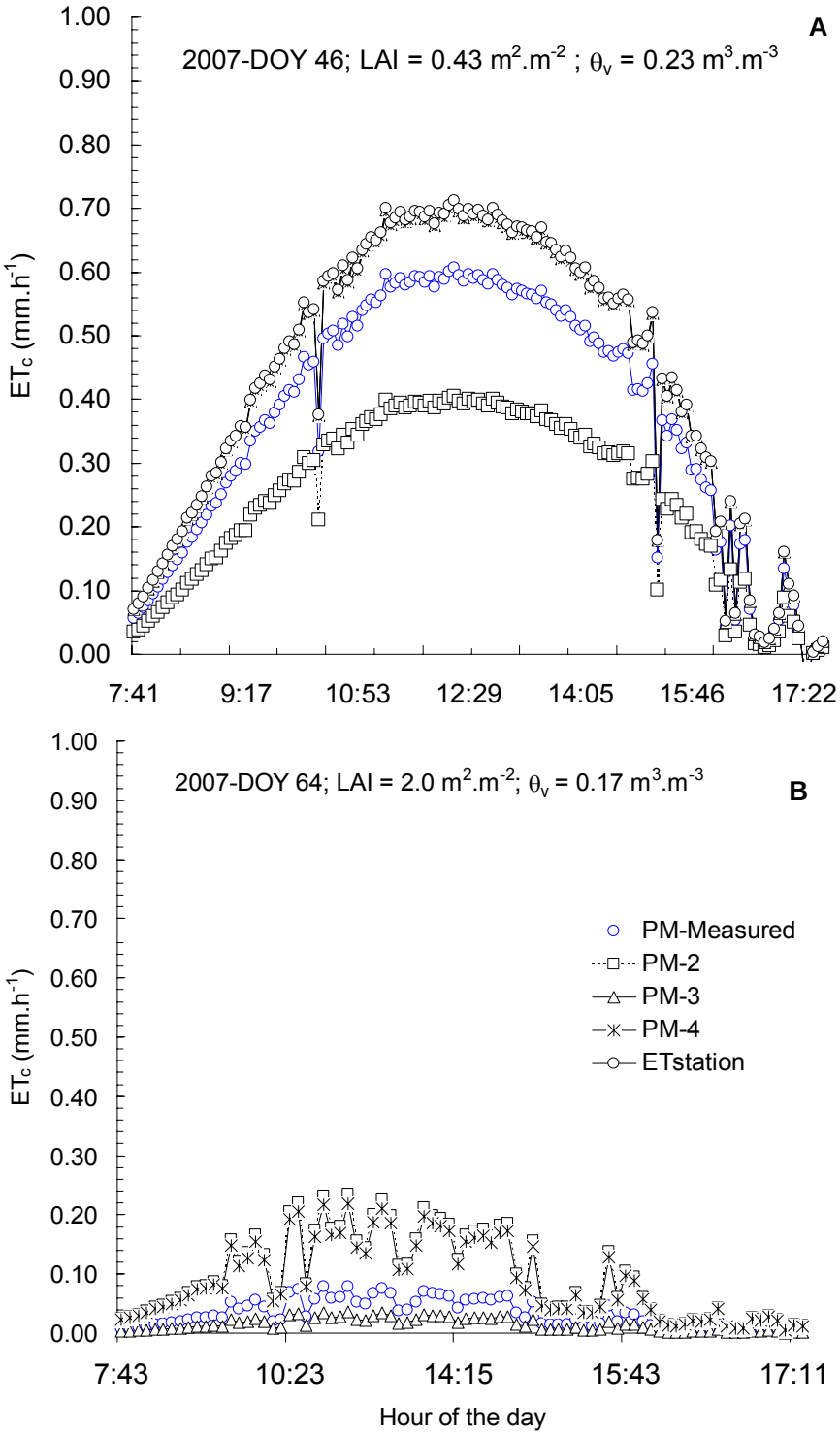

Figure 5.5. ET distribution with different PM-model, with variable LAI and soil moisture (θ_v) in the Morales common bean (*P.vulvaris* L) genotype, 2007 trial. For two selected days. **A**. Day or the year 46 and **B**. Day of the year 64.

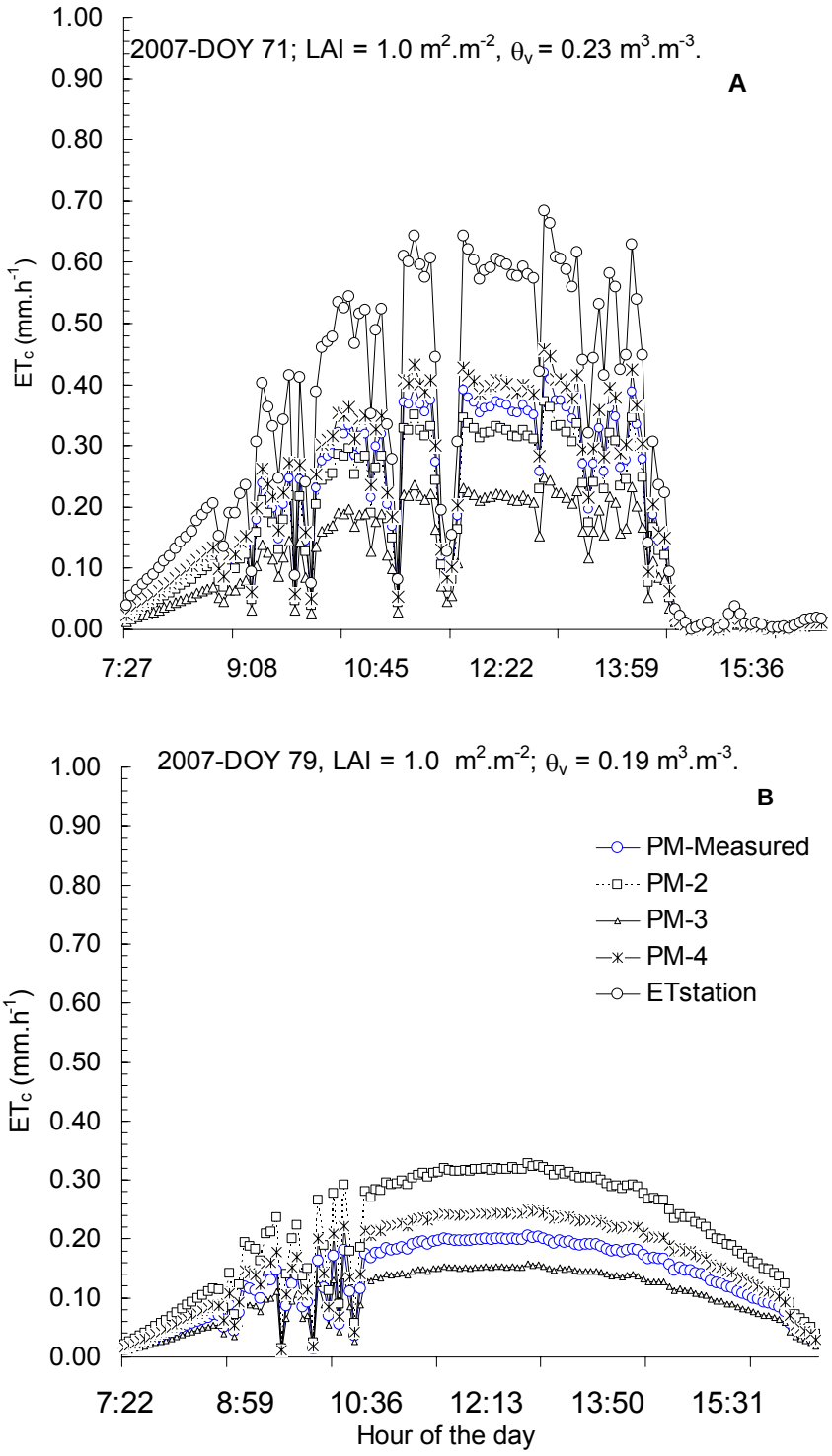

Figure 5.6. ET distribution with different PM-model, with variable LAI and soil moisture (θv) in the SER 16 common bean (*P.vulvaris* L) genotype, 2007 trial. For two selected days. A. Day or the year 71 and B. Day of the year 79.

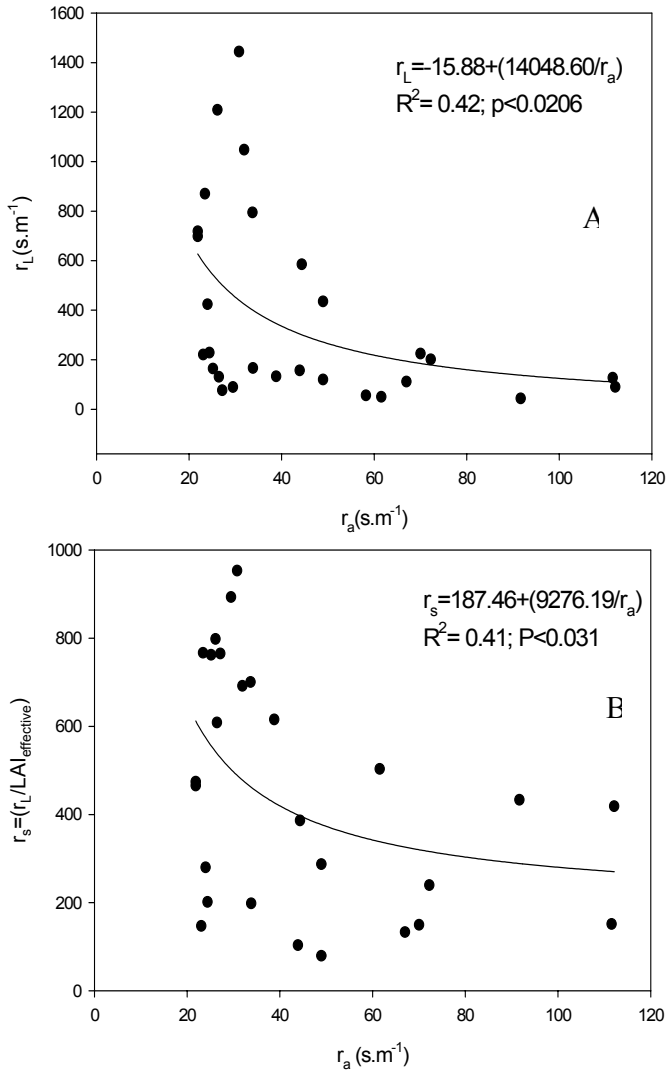

Figure 5.7 Aerodynamic resistance (r_a) as a function of: **A**. Stomatal resistance (r_L) and **B.** Measured surface resistance (r_s).

The precision in the r_s in this research was directly influenced by the various parameters used as inputs in its estimation. The r_s estimated as a function of R_n, G, VPD and soil moisture (PM-3) was closely related with the measured r_s (PM-1); overestimation (Fig. 5.8B) was related with strong drought stress conditions, where the soil moisture at 15 cm approached the wilting point (WP). The overestimation of r_s in model PM-3 can be partially explained by the fact that the soil moisture was measured only at 15 cm, and the depth of the roots at complete development extended to 35-40 cm, indicating that the drought stress below 15 cm was not strong and the plants had more available water.

The PM-2 model (inverse model) overestimated the r_s (relative to PM-1), particularly when the aerodynamic resistance was high (Fig 5.8A), due to r_a being in the numerator in the inverse model. This situation is not consistent with the measured data presented in Figure 5.7. The r_s by the PM-1 model (measured) was higher when the LAI was low (Fig. 5.8 A, B, C and D), this situation is associated with the LAI being in the denominator. In those cases when the LAI < 2.0, the r_s increases geometrically. For example, when LAI = 0.5 the r_s is four times higher than r_L. In this study, the larger differences among r_s-models were observed when LAI <1.0.

The large differences early in the season; when the LAI <0.5, among the PM-1 model and the others, indicates that during the initial growth stage all the leaves are effective in the transpiration process. This indicates that the use of the $LAI_{effective}$ when LAI <0.5 or 1.0 is not necessary and tends to overestimate the r_s and under-estimate the ET.

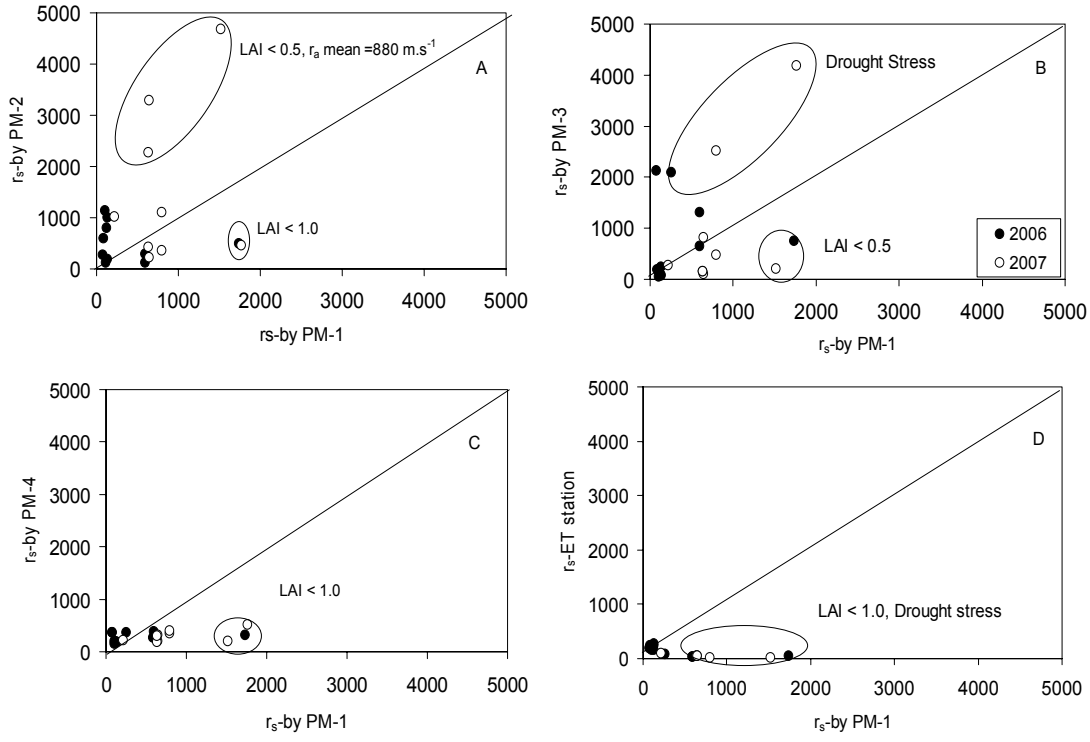

Figure 5.8. Surface resistance (r_s) derived from different methods as a function of the leaf area index (LAI), soil moisture, and aerodynamic resistance (r_a). The axis unit are $s.m^{-1}$.

CONCLUSIONS

This study indicates that crop evapotranspiration (ET_c) in common bean can be estimated in a one-step procedure using the Penman-Monteith model (PM) under drought stress and non-drought stress conditions, if the surface resistance (r_s) is appropriately parameterized. The model proposed in the Drainage and Irrigation Paper-FAO No. 56 (Allen et al. 1998), referred to in this study as the PM-1 model, gave reasonable ET estimates when the LAI was over 1.0, and in the genotypes with drought tolerance when strong drought conditions was present. The model proposed by Ortega-Faria et al. (2004) also provided good estimates of ET, with appropriate soil moisture readings under drought and no drought conditions. The advantage of this model is that the stomatal resistance is not accounted for directly, but the surface resistance is estimated as a function of micrometerological parameters and soil moisture, that are directly related with stomatal control.

The inverse of the PM model does not give a good ET estimation in windy conditions or dry conditions, which directly influences the stomatal resistance. The accuracy in the ET estimation appeared to be related to stomatal control under drought conditions.

The ET station gave good evapotranspiration estimation when LAI was over 1.0, without stress and/or with moderate drought stress.

The principal limitations of this chapter relate to the assumptions inherent in the latent heat flux estimation (λE), which are: i) steady-state conditions exist, ii) the transport is one-dimensional, iii) the surfaces is homogeneous, iv) the eddy diffusivity for heat and water vapor are assumed equal, and v) and that the temperature and humidity profiles were measured under the conditions of sufficient fetch.

REFERENCES

Alves. I., A. Perrier and L.S. Pereira. 1998. Aerodynamic and surface resistance of complete cover crops: How Good is the "Big Leaf"?. *Trans. ASAE*. 41(2):345-351.

Alves. L., and L.S. Pereira. 2000. Modeling Surface resistance from climatic variables?. *Agric. Water. Manage*. 42:371-385.

Allen, G.R.. S,L Pereira., D. Raes., and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. Food and Agricultural Organization of the United Nations (FAO). Publication No. 56. Rome. 300p.

Blad, B.L., and N.J. Rosenberg. 1976. Evaluation of resistance and mass transport evapotranspiration models requiring canopy temperature data. *Agron. J*. 68:764-769.

Cambell, G.S. 1985. Soil physics with basic transport models for soil-plant systems. Developments in soil Science No. 14. Elsevier, ed.150p.

Farahami, H.J., and W.C. Bausch. 1995. Performance of evapotranspiration models for maize-bare soil to close canopy. *Trans. ASAE*. 38 ( ):1049-1059.

Fritschen, J.L., Fritschen, L.C. 2005. Bowen ratio energy balance method. In: *Micrometeorology in Agricultural Systems*, Agronomy Monogaph no, 47. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 677 S. Soe Rd., Madison, W153711, USA: 397-405.

Goyal, M.R and E.A. González. 1989. Datos climatológicos de las subestaciones experimentales agrícolas de Puerto Rico. Universidad de Puerto Rico, Recinto de Mayagüez-Colegio de Ciencias Agrícolas-Estación Agrícola de Río Piedras-Puerto Rico. Publicación 88-70, proyecto C-411.87p.

Harmsen, E. W., V. H. Ramirez Builes, J. E. Gonzalez, M. D. Dukes, and X. Jia. 2006. Estimation of Short-Term Actual Crop Evapotranspiration. Proceedings of the 42nd Annual Meeting of the Caribbean Food Crops Society, Vol. XLII, No. 2. July 9-14, 2006, San Juan, Puerto Rico.

Heilman, J.L., C.L. Brtittin., and C.M.U. Neale. 1989. Fetch requirements fro bowen ratio measurements of latent and sensible heat fluxes. *Agric. For. Meteorol*. 44: 261-273.

Kjelgaard, J.K., C.O. Stockle., J.M, Villar Mir., R.G., Evans and G.S. Campbell. 1994. Evaluation methods to estimate corn evapotranspiration from short-time interval weather data. *Trans.ASAE.*37(6): 1825-1833,

Kjelgaard, J.K., and C.O. Stockle. 2001. Evaluating Surface resistance for estimation corn and potato evapotranspiration with the Penman-Monteith model. *Trans. ASAE*. 44(4): 797-805.

Montheith, J.L., and M.H. Unsworth.1990. Principles of Enviromental physics. 2nd ed., Edward Arnold Publisher. 291p.

Monteith, J.L. 1981. Evaporation and surfaces temperature. *Quart. J. R. Met.Soc*. 107 (451):1-27.

Monteith, J.L. 1995. Accomodation between transpiring vegetation and the convective boundary layer. *J. Hydrol.* 166:251-263.

Ortega-Farias, S.O., A. Olioso., R. Antonioletti and N. Brisson. 2004. Evaluation of the Penman-Monteith model for estimatig soybean evapotranspiration. *Irrig Sci* 23:1-9.

Ortega-Farias, S.O., A. Olioso., S. Fuentes., H. Valdes. 2006. Latent heat flux over a furrow-irrigated tomato crop using Penman-Monteith equation with a variable surfaces canopy resistance. *Agric. Water. Manage.* 82:421-432

Payero, J.O., C.M.U. Neale., J.L.Wright., and R.G. Allen. 2003. Guidelines for validating bowen ratio data. *Trans. ASAE.* 46(4):1051-1060.

Prenger, J.L. R.P. Fynn., and R.C. Hansen. 2002. A comparison of four evapotranspiration models in a greenhouse environmnent. *Trans. ASAE*. 45(6): 1779-1778.

Rana, G., N. Katerji., M. Mastrorilli., and M. El Moujabber. 1997. A model for predicting actual evapotranspiration under soil water stress in a mediterranean region. *Theor. Appl. Climatol*. 56:45-55.

Rosenberg, J. N, B.L. Blad, and S.B, Verma. 1983. Microclimate; The Biological Environment. A Wiley-Interscience Publication, Jhon Wiley & Sons. Edt. 495p.

Ritchie, J.T. 1983. Efficient water use in crop production: Discussion on generality of relation between biomass and evapotranspiration. In: *Limitation to Efficient Water Use in Crop Production,* Edited by:Howard M. Taylor, Wayne R. Jordan, and Thomas R. Sinclair. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America:29-43.

Shuttleworth, W.J. 2006. Towards one-step estimation of crop water requirements. *Tran. ASABE*. 49(4):925-935.

Szeicz, C., and L.F. Long. 1969. Surface resistance of crop canopies. *Water Resour. Res*. 5(8):622-633.

Tanner, C.B. 1960. Energy balance approach to evapotranspiration from crops. *Soil Sci. Soc. Am. Proc*. 24(1):1-9.

Tomlinson, A.S. 1994. Intrumentation, methods and preliminary evaluation of evapotranspiration from grassland in the arid lands ecology reserve, Benton country, Washington, May-October 1990. U.S. Geological Survey, Water-Resources Investigation Report 93-4081. 32p.

Tolk, J.A., and T.A. Howell. 2001. Measured and simulated evapotranspiration of grain sorghum grown with full and limited irrigation in three high plains soils. *Trans. ASAE.* 44(6): 1553-1558.

Chapter 6

Crop Water Stress Index and Yield Components for Common Bean (*Phaseolus vulgaris* L.) Genotypes in Greenhouse and Field Environments

ABSTRACT

The ability to detect and characterize the magnitude of drought stress has been an area of active research during the last three decades. With the development and increased popularity of the infrared thermometer, a thermal stress index has been proposed and applied. One of the most popular and useful is the crop water stress index (CWSI-Idso et al. 1981). The principal objective of this research was to develop baselines for CWSI for four common bean genotypes, and relate the index with yield components and soil available water under field and greenhouse environments. Three years of research (2005-2007) was conducted in two environments (greenhouse and field) in the west and south of Puerto Rico. Three water levels were applied in the greenhouse and two water levels in the field were used in randomized block experiments. Four common bean genotypes were studied: Morales, drought susceptible and BAT477, SER16 and SER 21, drought tolerant. The CWSI was derived for a total of five growing seasons; including two field and three greenhouse experiments. The results indicate differences in drought tolerance between genotypes. The effect of wind induced additional "physiological stress" that was detected by the CWSI. The differences in the CWSI between genotypes were well correlated with the stomatal control, root available water, and yield components.

Key words: Common bean, canopy temperature, crop water stress index, air temperature, drought stress.

Abbreviations: CWSI, Crop water stress index; VPD, vapor pressure deficit; $T_{c,}$ canopy temperature; $T_{a,}$ air temperature.; SD standard deviation.

INTRODUCTION

Common bean is highly susceptible to drought stress or water deficit, and the production of this crop in many places of the world is carried out under drought stress conditions, due to insufficient water supply by rainfall and/or irrigation. Drought stress influences several important plant processes, including plant water potential and stomatal behavior, which have direct effect on gas exchange. Changes in plant water status are directly related to the plant's canopy temperature.

Permanent and intermittent drought stress adversely influence crop yield and growth. Methods for drought stress detection have been developed in a number of crops with different technology. Jones (2004) described methods for drought detection and irrigation scheduling. The most popular methods are the thermal methods, which have been widely used with the aim of detecting drought stress and improving water management (i.e, Tanner, 1963; Idso et al. 1981; Jackson et al. 1981; Howell et al. 1984; Stöckle and Dugas, 1992; Karamanos and Papatheohari, 1999; Wanjura and Upchurch, 2000; Ajayi and Olufayo, 2004). Measuring the canopy temperature by infrared thermometry is a popular technique because it is noninvasive, nondestructive and can be automated (Blom-Zandstra and Metselaar, 2006). One of these methods that has been successfully applied since the 70`s to detected drought stress utilizes the change in canopy temperature with respect to air temperature (Idso et al. 1977; Jackson et al. 1977).

One of the most widely used methods is the crop water stress index (CWSI) proposed by Idso et al. (1981), that relates the difference between canopy and air temperature with the vapor pressures deficit. The index ranges from 0, for non-drought stress conditions, to 1 for maximum drought stress, a condition when water is not available for transpiration. The CWSI generates two baselines: an upper baseline for complete drought stress and a lower baseline for no drought stress, both curves being functions of the vapor pressure deficit. Jackson et al. (1981) have shown that the CWSI is an index of transpiration reduction ($CWSI = 1- E/E_p$), where E is the actual and E_p is the potential evapotranspiration or transpiration.

The CWSI has been increasing used recently due to the availability of the infrared thermometer, satellite thermal imaging and other remote sensing tools, that could be used in the detection of crop water stress at the macro and micro scales.

Indso (1982), determined the baseline for several crops, including bean, where the model was: T_c-T_a= -2.35VPD+2.91. Erdem et al. (2006) reported T_c-T_a= -2.69VPD+3.5309 as a lower baseline for a *P. vulgaris* L., cv., 'Sehirali 90'. The CWSI can be applied in the analysis of the irrigation scheduling. For common bean, this type of application was used in a study by Erdem et al. (2006).

Plant water status is a function of the available water in the soil. Water availability in plant tissues varies by cultivar, and genotype, which is directly related to water potential and stomatal control. This would suggest that baselines are strongly location, species and variety dependent (Gardner et al. 1992; Alderfasi and Nielsen, 2001). More effort is needed in the development of baselines for the CWSI method. The CWSI method has never been applied for bean in Puerto Rico.

The objectives of this work were*: i*) Develop baselines for different common bean with and without drought susceptibility in greenhouse and field environments, *ii*) Estimate the CWSI for common bean genotypes in greenhouse and field environments and relate the CSWI with yield components, and *iii*) Relate the CWSI with available soil water as a tool for crop water management, and detect the variability of these relationships among genotypes with and without drought stress.

MATERIALS AND METHODS

This experiment was conducted during a three year period (2005, 2006 and 2007) and included several growing seasons in the greenhouse and field. All experimental arrangements, drought treatments, and soil and atmospheric conditions are documented in Chapters 3 and 4. In this section, the procedures for the thermal analysis will be described.

The greenhouse trials. The common bean genotypes (*P. vulgaris* L) evaluated in this research in the greenhouse were: Morales (white seed color), the most widely planted small white bean in Puerto Rico, SER 16 and SER 21 (red seed color), SEN 3 and SEN 21 (black seed color), and BAT 477 (cream seed color). BAT 477 has a plant architecture type III and the others are type II. Morales is considered to be drought susceptible, and the other drought tolerant. The experiments where conducted during 2005 and 2006.

All pots were irrigated by hand every day in the morning with set amonunts of water. Three water levels were used: Level 1, full water supply (no drought stress) using 80% of the daily available water (DAW) during the complete growing season; level 2 (stress 1) with 50% of the DAW before flowering and 60% of the DAW after flowering; and level 3 (stress 2) with 20% of the DAW before flowering and 40% of the DAW after flowering. The DAW was defined as the total water required to keep the moisture at substrate field capacity (SFC). A SFC test was previously carried out, to estimate the total daily water needs by each pot (Methodology for SFC was described in Chapter 3) .

The field trials. The bean genotypes (*P. vulgaris* L) evaluated in this research in greenhouse were: Morales and SER 16. The experiments where conducted during 2006 and 2007.

The soil water in the field experiment was monitored two times per week in each main plot with a Profile probe type PR2 sensor (Delta-T Devices, Ltd.), the access tubes were placed at 0-20 cm and 20-40 cm with two per treatment, to determine the timing of the irrigation

applications. The soil water balance was monitored daily to estimate the actual soil moisture (ASM) as follows:

$$ASM_{initial} = TAW\text{-}ET_c \quad (6.1)$$

Then: if $MR + ASM_{initial} < TAW$: $ASM = MR + ASM_{initial}$

Or: if $MR + ASM_{initial} > TAW$: $ASM = TAW$

where TAW is Total available water, ET_c is crop evapotranspiration and MR is the moisture recharge.

The TAW was calculated as follows:

$$TAW = 1000(\theta_{FC} - \theta_{WP})Z_r \quad (6.2)$$

where θ_{FC} and θ_{wp} are the volumetric moisture content at field capacity and the wilting point, respectively, in $m^3.m^{-3}$, and Z_r is the root depth (m). The moisture recharge is estimated from the following equation:

$$MR = ASM+R+I\text{-}RO\text{-}ET_c \quad (6.3)$$

where R is rainfall, I is irrigation, RO is runoff. The RO was measured in twelve drainage lysimeters and ET_C was estimated using the Penman-Monteith "one-step" model, as was described in the chapter 4. In the greenhouse experiment the total water applied per pot, was recorded daily.

The irrigation system used in the field was drip-type irrigation, with two irrigations per week.

To relate CWSI and yield components for both environments, an average value of CWSI estimated at 13:00 hour was used. This was the time of day when the water stress is likely to be highest (Chapter 3) and when the need for irrigation using CWSI should be determined (Irmak et al. 2000).

In the field experiment, the canopy temperatures were recorded on clear sky days, during the day of the year (DOY): 48 to 98 in 2006 and 31 to 71 in 2007. These days included vegetative and reproductive growing stages, similar to the periods evaluated in the greenhouse experiments. The lower (non-stressed) and upper (stressed) baselines (Fig. 6.1) were measured for each common bean genotype at different vapor pressure deficits and canopy temperature levels. Additionally, the lower base-line was estimated for each genotype for both environments from data for clear sky days for the treatments without drought stress.

For both environmental conditions, the leaf temperature was measured at different development stages and at different time interval during the day (7:00 am to 6:00 pm). The canopy temperature (T_c) was measured using an infrared thermometer gun (MX4-TD +/-1^{o}C, Raytek), a spectral range of 8 to 14 μm, and a resolution of 0.1^{o}C. The measuring was made on a single leaf within the upper canopy structure. An automatic weather station (WatchDog-900ET, Spectrum Technologies, Inc.) was installed in the greenhouse and in the field. In addition to the weather station, the air temperature and absolute and relative humidity were measured in the greenhouse with a Hobo-Pro datalogger (Onset Computer Company, Pocassette, Maine). T_c was measured two times per replication in both environments.

The crop water stress index (CWSI) was calculated as follows:

$$CWSI = \frac{\left[dT - dT_{low}\right]}{\left[dT_{up} - dT_{low}\right]} \tag{6.4}$$

where dT is the measured difference between the crop canopy and air temperature; dT_{low} is the measured difference between the canopy temperature for well-watered crop and air temperature (lower baseline), and dT_{up} is the difference between the canopy temperature non-transpiring crop and air temperature (upper baseline).

The data were subjected to the analysis of variance procedure for linear models to determine the relationship between T_c-T_a and VPD, and the relationship between CWSI and yield components, using Infostat statistical program version 3 and SigmaPlot® program version 802,SPSS.

RESULTS AND DISCUSSION

The baselines. Figure 6.1 represent the upper and lower baselines obtained for the genotype Morales in the field environment (6.1 A) and greenhouse (6.2 B). The range of VPDs for the baseline were 0.8 to 3.5 kPa in the field and 0.1 to 3.5 in greenhouse. The baseline developed by Erden et al. (2006) was between 1.1 to 2.7 kPa.

The upper baseline that represent the T_c-T_a for plants that are severely stressed, were selected from the greenhouse and the field from plants under drought stress between 12:00 to 14:00 h. Then the average values of canopy temperature obtained from these plants were related with the average air temperature to obtain the upper baseline values. The values for upper baseline varied from 1.1 to 4.7 ^{o}C, but differed among genotypes. For this study the upper baseline selected for each genotypes were: Morales: 2.8^{o}C (1 SD = 1.5 ^{o}C), BAT 477: 3.1 ^{o}C (1 SD = 1.5 ^{o}C) SER 16: 3.1 ^{o}C (1 SD = 1.7 ^{o}C) and SER 21: 2.9 ^{o}C (1 SD = 1.5 ^{o}C). Erdem et al. (2006) found 2.4 ^{o}C as an upper baseline for bean in field environment (*P. vulgaris* L., cv., Sehirali 90).

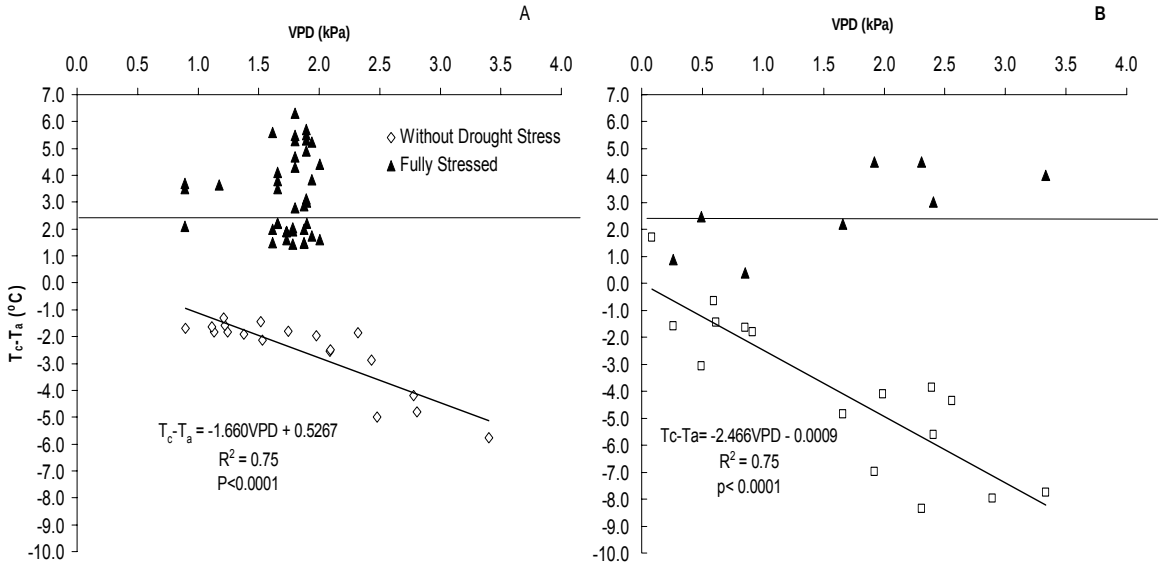

Figure 6.1 Canopy-air temperature differential (T_c-T_a) versus vapor pressure deficit (VPD) for full drought stressed and non- drought stressed common bean genotype *Morales* in **A**. field environment and **B**. Greenhouse environment.

Lower baselines are different among genotypes and environments (Table 6.1). In the greenhouse the slope was over 2.17 and in field was lower. All the lower baseline models were statistically significant, and the determination coefficients (R^2) were greater than 0.68. The correlation between T_c-T_a and VPD is affected by other micrometeorological variables such as clouds or wind, and equipment calibration (Erdem et al 2006). For example, Ajayi and Olufayo (2004) found that a higher correlation was obtained for low wind speeds in sorghum, with T_c-T_a using from -2 to +8 ^oC Also, as will be discussed later, wind speed directly influences surface resistance and induces changes in the canopy temperature, that are not necessarily indicative of drought stress.

Differences in CWSI between genotypes in the field environment were more evident in 2007, where the water deficit with respect to the control (well irrigated) was 30.3% as compared with 18% in 2006. The DOY 57, 64 and 71 during 2007 (Fig 6.2 C and D) clearly showed the difference between genotypes SER 16 and Morales. The lowest CWSI in SER 16 in the 2007 season, could be attributed to a lower average stomatal resistance for those days, including 349, 690, and 187 sm^{-1} respectively, compared with 769, 1747 and 449 sm^{-1} for Morales (data not

shown in the figure). Genotypic variations in CWSI and its relationship with stomatal conductance were also reported for seven winter wheat varieties by Alderfasi and Nilsen (2001).

The CWSI for well irrigated treatments for both genotypes during 2006 were lower than 0.3 (Fig. 6.2 A and B), similarly during 2007 for days with low wind speed.

Table 6.1. Lower baseline functions for four common bean genotypes, in the greenhouse and field environment.

Genotype	Lower baseline function	$R^{2\dagger}$	P-level‡	RMSE§
	Greenhouse			
BAT 477	T_c-T_a=-2.17*VPD+0.12	0.64	0.0002	1.75
Morales	T_c-T_a=-2.47*VPD-0.0044	0.75	0.0001	1.50
SER 16	T_c-T_a=-2.29*VPD+0.17	0.77	0.0001	1.32
SER 21	T_c-T_a=-2.17*VPD-0.74	0.60	0.0001	1.99
	Field			
Morales	T_c-T_a=-1.66*VPD+0.5267	0.68	0.0001	0.68
SER 16	T_c-T_a=-1.33*VPD+0.1442	0.68	0.0001	0.42

†R^2 is the determination coefficient;
‡p-level is the probability level associated with the models and
§RMSE is the model root mean square error.

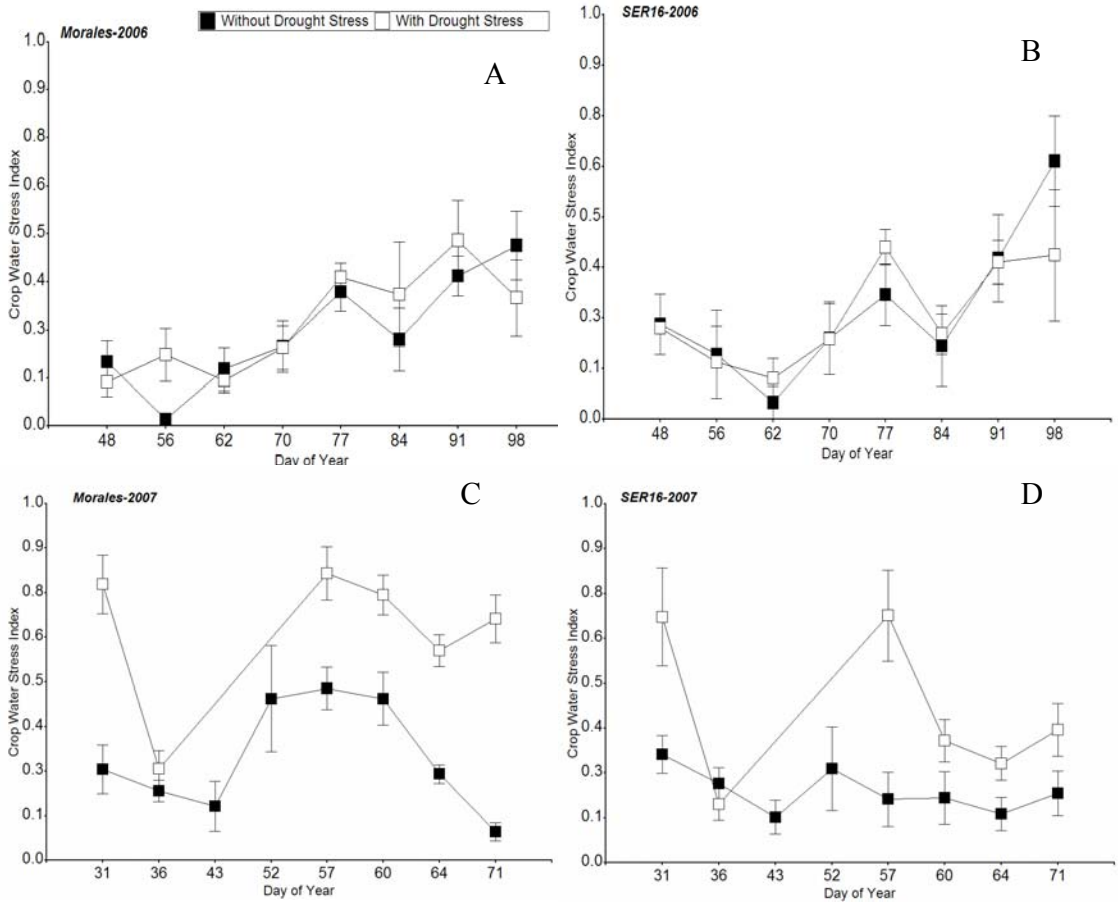

Figure 6.2 Seasonal trend of the crop water stress index (CWSI), for two common bean genotypes (Morales and SER16) and two growing seasons (2006-2007).

The CWSI and yield components. The drought stress treatments in the greenhouse were applied from the vegetative phase to maturity. The T_c was measured at 13:00 hour, when the drought stress was likely to be the highest (and when the maximum stress was in fact detected). The yield reduction in the four common bean genotypes were correlated with the CWSI, but with differences in magnitude (Fig 6.3). For Morales, BAT 477 and SER 21, the linear models were statistically significant, while for SER 16 the relation was not linear (Fig 6.3 D).

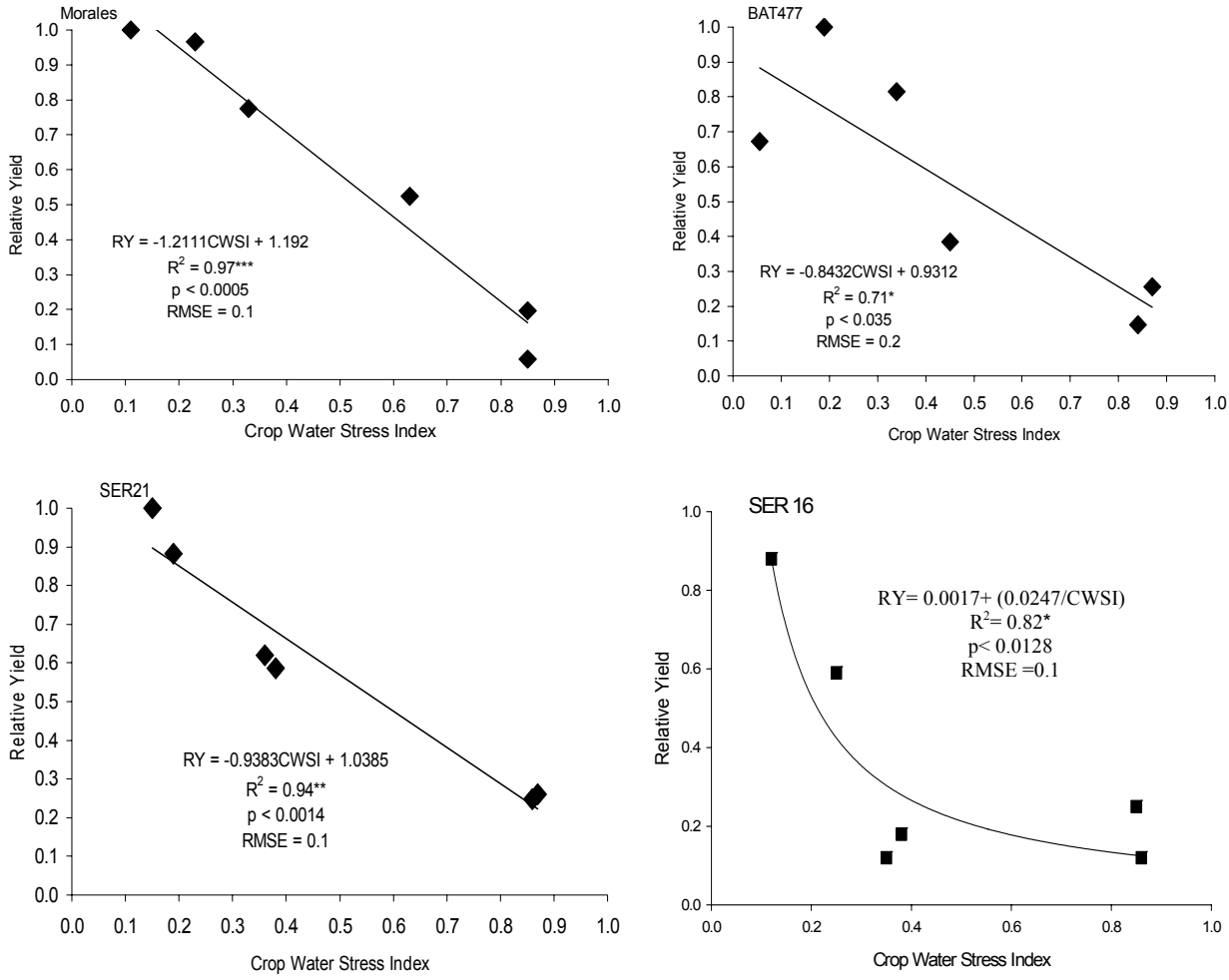

Figure 6.3. Relative yield (RY = $Y_{obs}/Y_{max.WS,}$ Where Y_{obs}: is the yield observed an $Y_{max.WS}$ is the maximum yield observed without drought stress), as related to seasonal mean of crop water stress index (CWSI), under greenhouse environment for four common bean genotypes, during two years and three growing seasons.

The high slope in the genotype Morales relative to the others could be associated directly with the drought susceptibility of this genotype. Ten percent (10%) in yield reduction (RY = 0.9) is associated with CWSI value of 0.04 for BAT 477; 0.12 for SER16, 0.15 for SER 21 and 0.24 for Morales. Erdem et al. (2006), working with bean (cv., *Sehirali* 90), demonstrated under field conditions that an average CWSI value of about 0.07 prior to irrigation produces the maximum

yield. Albuquerque et al. (1998) also working with bean, reported 0.15 as a CWSI limit for water management to avoid significant yield loss.

Similar results were observed in the field-environment, but no functions were fit, owing to the fact that the results were available only for the 2007 trial (Fig 6.4). These results indicate that the most susceptible genotypes were also Morales and BAT 477, which when reaching the same drought stress level produced the lowest seed yields and highest CWSI values (0.96 and 0.95 receptivity). SER 16 showed the lowest CWSI under drought stress (0.82), but had a lower seed yield than SER 21 having a CWSI = 0.92. These results indicate that SER 21 under drought stress has a higher transpiration reduction than SER 16 (between 13:00 to 14:00 h), but can maintain a relatively high seed yield.

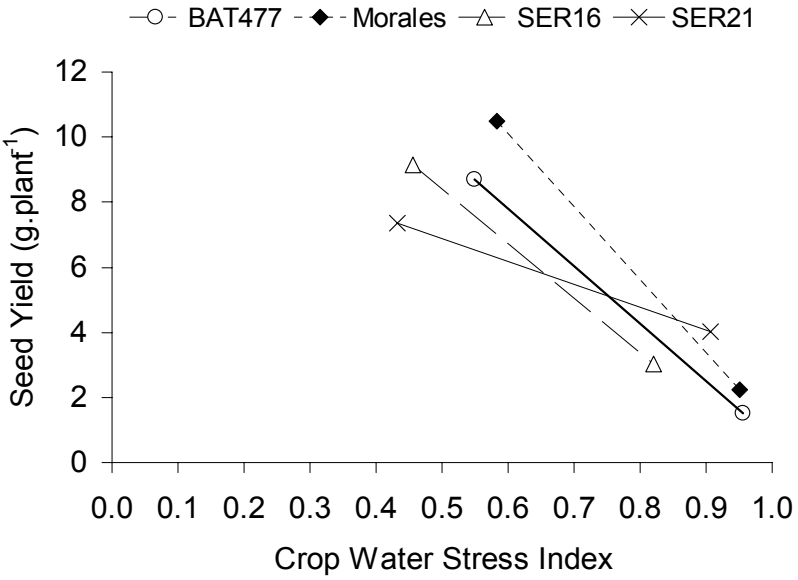

Figure 6.4. Mean yield, as related to seasonal mean of crop water stress index (CWSI), under 2007 field environment for four common bean genotypes.

CWSI and soil moisture in field. The crop water stress index was also well correlated with the water in the root zone. The relationships were fit in linear regression models that showed statistical significance for SER 16 and Morales (Fig. 6.5 A and B). If the water in the root zone is 50% of the total available water (TAW), the CWSI= 0.41 for SER 16 and 0.61 for

Morales and if the water in the root zone is 75% of the TAW, the CWSI = 0.55 for SER 16 and 0.79 for Morales.

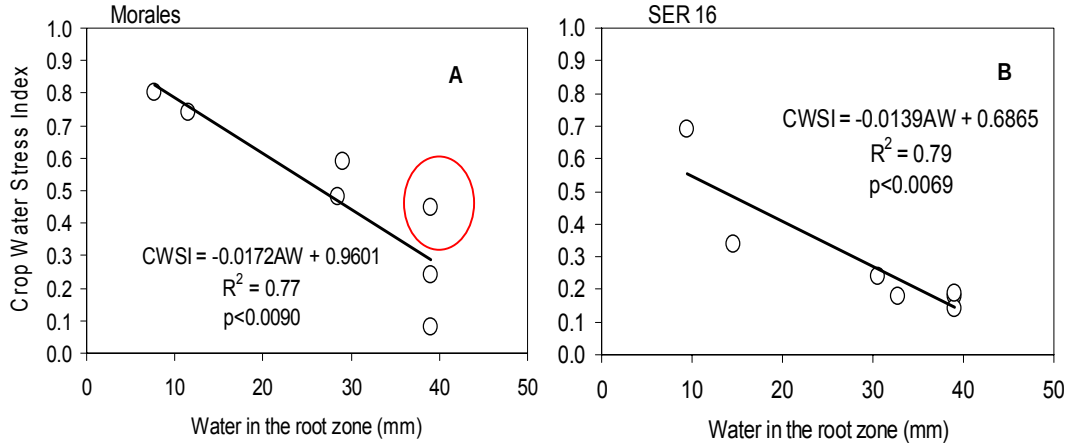

Figure 6.5. Mean crop water stress index as a function of the water in the root zone (AW) for two common bean genotypes: **A**. Morales and **B**. SER 16. The red open circle indicates low r_a (high wind conditions), which induced "physiological stress".

The CWSI is also affected by the aerodynamic and stomatal resistance relationship, and the genotypic stomatal response. In the case of Morales (Fig. 6.5 A), for example, the red open circle indicated low r_a (windy conditions), which increased the CWSI. The red open circle corresponds to March 1 (DOY 60), where the mean wind speed during the canopy temperature reading was 5.8 ms^{-1}, and the mean daily r_a was 29.9 $s.m^{-1}$. The CWSI was 0.45 for Morales and 0.19 for SER 16 during the same day. Stomatal resistance (r_L) measured for the same period was 729 $s.m^{-1}$ (1 SD = 236 $s.m^{-1}$) for Morales and 560 $s.m^{-1}$ (1 SD = 717 $s.m^{-1}$) for SER16.

CONCLUSIONS

The CWSI was computed as a function of direct canopy and air temperature for well irrigated plots of common bean and with drought stressed plots, in greenhouse and field environments in four common bean genotypes with and without drought susceptibility. These results indicate that the CWSI was well correlated with yield components, but varied in magnitude among the different genotypes. The lower baselines derived from the greenhouse were different than those derived from the field, principally due to differences in atmospheric conditions, especially air temperature, and wind speed, with the field having windier and cooler conditions than the greenhouse.

The high wind speeds, induced a physiological stress with increasing stomatal resistance and decreasing aerodynamic resistance. For the genotype Morales, the influence of wind speed was detected by the CWSI, which could indicate that this genotype is more stomatally susceptible under windy conditions than SER 16.

The CWSI was well correlated with the water available in the root zone, indicating that this index is an excellent indicator of the plant-soil water status. The CWSI should, however, be used in combination with an analysis of wind speed and genotypic characteristics. The CWSI was also a good tool to characterize drought stress under greenhouse conditions. The upper and lower baselines developed as a part of the CWSI approach are highly genotype-dependent, and therefore, the applicability of the baselines developed in this study should be verified before they are used with other genotypes or varieties.

REFERENCES

Albuquerque, P.E.P., R.L. Gomide., and A.E. Klar. 1998. Crop water stress index for bean obtained from temperature difference between canopy and air. *Water and Environment*. 1: 189-196.

Aldesfasi, A.A., D.C. Nielsen. 2001. Use of crop water stress index for monitoring water status and scheduling irrigation in wheat. *Agric. Water Manage.* 47:69-75.

Ajayi, A.E., and A.A. Olufayo. 2004. Evaluation of two temperature stress indices to estimate grain sorghum yield and evapotranspiration. *Agron. J*. 96:1282-1287.

Blom-Zandstra, M., and K. Metselaar. 2006. Infrared thermometry for early detection of drought stress in *Chrysanthemum*. *HortScience*. 41(1):136-142.

Erdem, Y., S. Sehirali., T. Erdem., and D. Kanar. 2006. Determination of crop water stress index for irrigation scheduling of bean (*Phaseolus vulgaris* L.). *Turk. J Agri For.* 30:195-202.

Gardner, B.R., Nielsen, D.C., Shock, C.C., 1992. Infrared thermometry and the crop water stress index. II. Sampling procedures and interpretation. *J. Prod. Agric.* 5: 466-475.

Howell, T.A., J.L Hatfield., H. Yamada., and K.R. Davis. 1984. Evaluation of cotton canopy temperature to detect crop water stress. *Trans. ASAE*. 27:84-88.

Idso, S.B., R.D. Jackson., and R.J. Reginato. 1977. Remote sensing of crop yields. *Science*. 196:19-25.

Idso, S.B., R.D. Jackson., P.J Pinter., and J.L Hartield. 1981. Normalizing the stress-degree-day parameter for environmental variability. *Agric. Meteorol*. 24: 45-55.

Idso, S.B., 1982. Non-water-stressed baselines: a key to measuring and interpreting plant water stress. *Agric. Meteorol.*27:59-70.

InfoStat, 2003. InfoStat, version 3. Manual del Usuario. Grupo InfoStat, FCA, Universidad Nacional de Cordoba, Argentina. http//www.infostat.com.ar

Irmak. S, D.Z. Hanan, and R. Bastug. 2000. Determination of crop water stress index for irrigation timing and yield estimation of corn. *Agron. J*. 92:1121-1227.

Jackson, R.D., R.J Reginato and S.B. Idso. 1977. Wheat canopy temperature: A practical tool for evaluating water requirements. *Water. Resour. Res*. 13:651-656.

Jackson, R.D., S.B Idso., R.J Reginato and P.J Pinter Jr. 1981. Canopy temperature as a crop water stress indicator. *Water. Resour. Res*. 17:1133-1138.

Jones, G. H. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. *J. Exp. Botany*. 55(407):2427-2436.

Karamanos, A.J., and A.Y. Papatheohari. 1999. Assessment of drought resistance of crop genotypes by means of the water potential index. *Crop Sci*. 39:1792-1797.

SigmaPlot. 2005. SigmaPlot.802,SPSS. User manual.http//www.sigmaplot.com

Stökle, C.O., and W.A. Dugas.1992. Evaluating canopy temperature-based index for irrigation scheduling. *Irri. Sci*. 13(1):31-37.

Tanner, C.B. 1963. Plant temperatures. *Agron. J*.55:210-211.

Wanjura, D.F., and D.R. Upchurch. 2000. Canopy temperature characterization of corn and cotton water status. *Trans. ASAE*. 43(4):867-875.

Chapter 7

Water Use Efficiency and Transpiration Efficiency for Common Bean Genotypes (*Phaseolus vulgaris* L.)

ABSTRACT

Water-use efficiency (WUE), the ratio of biomass or dry matter (DM) produced per unit of water evapotranspired, has been commonly used as an index to characterize the efficiency in water use. The efficiency in water used is also well described by the transpiration-use efficiency (TUE) expressed as DM/T=k/VPD, where k is a crop dependent constant, and VPD is the day time vapor pressure deficit. In this study, our objectives were i) Estimate the WUE for six common bean genotypes (*P. vulgaris* L) under greenhouse and field environments; ii) Determine k for two common bean genotypes in Juana Diaz, PR. Four trials in the greenhouse and two in the field were conducted during three years (2005, 06 and 07). Three water levels in the greenhouse, and two in the field were imposed. The evapotranspiration was estimated by the general Penman-Monteith (PM) model with variable aerodynamic and surface resistances. Transpiration and soil evaporation were estimated from the ET according with the FAO-56 methodology. Yield analyses were included in the study using the yield index (YI), and the geometric mean (GM) was also estimated as a genotype comparison criteria. The WUE was highly genotype, water level and environment-dependent. The genotypes SER 21, SER 16 and SEN 3 had the highest WUE, and yield components in the field and greenhouse environments. The transpiration efficiency represented by k was also different among genotypes and water levels.

Key words: Common bean, drought, water use efficiency, transpiration efficiency, harvest index.

Abbreviations: YI, yield index; GM, geometric index; WUE, water use efficiency; TE, Transpiration efficiency; HI, Harvest index.

INTRODUCTION

Common bean (*P. vulgaris* L.) is the most important food legume (Broughton et al. 2003), and important source of calories, proteins dietary fiber and minerals (Sing et al. 1999). Annual production of dry and snap bean exceeds 21 millon metric tons, and represents more than half of the world's total food legume production (Micklas et al. 2006). Bean production under drought conditions is very common in many countries of the word, where physiological alteration results in yield reductions (Terán and Singh, 2002). One of the principal alterations are in dry matter accumulation due to its sensitivity to temperature and drought stress (Boutra and Sanders, 2001; Brevedan and Egli, 2003; Porch, 2006), relative growth rate, stomatal conductance, transpiration rate, photosynthesis and abscisic acid synthesis (Lizana et al. 2006), and relative water content (Stayanov, 2005). Drought stress can produce differential effects, for example i) early drought can affect the uppermost soil layer, and is responsible for poor seedling establishment and possible crop failure ii) drought during the vegetative period results in low leaf area index, biomass and grain number and iii) late drought can affect grain filling (Debeake and Aboudrare, 2004).

Its unquestionable that plants need transpiration to produce yield, and that the water supply for transpiration causes major variations in crop yield (Ritchie, 1983). The basic unit of moles of carbon gained in photosynthesis per mole of water used in transpiration is a physiologist definition of WUE; for farmers and agronomists, the unit of production is more likely the yield of harvested product achieved from the water available to the crop through precipitation and/or irrigation (Condon et al. 2004). In this study, WUE is split in two main concepts: The WUE as the ratio of the dry matter yield per unit of evapotranspiration, and the second one is the transpiration efficiency, which is the ratio of the dry matter yield per unit of transpiration.

Knowledge of the biomass production per unit cropped area per unit of water evaporated and transpired provides an excellent tool for genotype evaluation under limited water conditions and under specific local conditions (e.g., Muñoz-Perea et al. 2007). This knowledge, in addition

to the 'efficiency in water use" (see. Tanner and Sinclair, 1983), makes it possible to reduce agricultural water consumption.

On a small island where utilization of water supplies by urban and industrial sectors continues to increase, increasing the agriculture water use efficiency is a constant challenge. The objective of the present work was to evaluate the water use efficiency for common bean genotypes under different drought levels and environments in Puerto Rico.

MATERIALS AND METODS

This research was conducted under two environmental conditions: greenhouse and field, during 2005, 2006 and 2007.

Greenhouse experiments. The greenhouse experiment was carried out at the USDA-TARS (Tropical Agricultural Research Station) in Mayagüez, Puerto Rico; coordinates 18º 12`22' N, 67º 8' 20`` W at 18 masl. Four trials or experiments were conducted during July-September, 2005, and two between October-December 2005 and 2006. Basic weather information was recorded during the study in the greenhouse (Table 3.1-Chapter 3).

The greenhouse experimental arrangements were: During 2005 four common bean genotypes were evaluated as follows: Morales, SER 16, SER 21, and BAT 477. During 2006 two more genotypes were included: SEN 3 and SEN 21. The description of the genotypes used in the study is presented in Table 3.2-Chapter 3.

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Each genotype was planted in pots (15 cm diameter x 11 cm depth) with Sunshine mix #1 (Sun Gro Horticulture, Vancouver, British Colombia) and Osmocote (14-14-14, N-P-K; Marysville, OH), three seeds per pot were sown and when the first trifoliate leaf was observed, two were thinned. Three water levels were used with 2 plants per water level and four replications. Each genotype was planted in 24 round pots. The pots were arranged in a split-split-

plot experimental design, the main plot was experiment, sub-plot was the water level and the sub-sub-plot was the genotype.

Maximum water retention capacity for the substrate (substrate field capacity-SFC) was measured after over-watering the substrate and letting it drain for 7, 24 and 48 hours. Twelve pots were over-watered and covered to avoid evaporation. Volumetric moisture content was measured with a volumetric moisture sensor "theta probe soil moisture sensor" +/-0.01 $m^3.m^{-3}$ ML2X (Delta-T Devices Ltd.), the SFC was 0.53 m^3m^{-3} (+/- 0.010). Three water regimes were used: Without drought stress using 80% of the daily available water (DAW) during the complete growing season; Drought stress 1 with 50% of the DAW before flowering and 60% of the DAW after flowering; and Drought stress 2 with 20% of the DAW before flowering and 40% of the DAW after flowering. The drought stress treatments were applied starting from when the second trifoliate leaflet was completely open. The water applications were made every day during the morning, and the volumetric moisture content (θv) was measured at different growing phases during each season. At no time during the experiments did the soil moisture content reach the terminal drought stress level.

Field experiments. The field experiments were carried at the Experimental Station of the University of Puerto Rico in Juana Diaz, PR, which is located in south central PR, at 18°01'N latitude and 66°22'W longitude, elevation 21 masl, within a semi-arid climatic zone (Goyal and Gonzalez, 1989). The field characteristics are described in Chapter 4.

The field experiments were planted on February 15, 2006 and January 17, 2007. The UPR Agricultural Experiment soil is classified as a San Anton Clay Loam with 30% sand, 44% silt, 26% clay, and 1.28% of organic matter, within the first 40 cm, with 0.30 $cm^3.cm^{-3}$ field capacity and 0.19 $cm^3.cm^{-3}$ wilting point (USDA,1987). One intermittent drought stress level was applied in both years at the beginning of the reproductive phase (R1: One blossom open at any node) to harvest. The drought stress was sufficient to allow the soil to dry to 75% of field capacity (FC), at which point the irrigation was applied. The stress level in 2006 was 18% corresponding to 387.3 mm of water applied as compared to the 472.5 mm total applied under

the non-drought stress treatment, and In 2007, the stress level was 30.3% corresponding to 302.0 mm of water applied as compared to the 433.4 mm total applied under the non-drought stress treatment. More precise information about the applied irrigation and rainfall data are presented in Chapter 4.

The volumetric moisture content was measured with a profile probe type PR2 sensor (Delta-T Devices, Ltd.), two access tubes were install in each main plot at 20 cm and 40 cm depths, and the irrigation was applied two times per week, using a drip irrigation system. Each main plot was divided into six sub-plots which consisted of each genotype, two sub-plots (each with 10 rows) for SER 16 and three for Morales in 2006, and three for each one in 2007. The plant density was 13.5 plant.m^{-2} for Morales and 6.5 plants.m^{-2} for SER 16. The other agronomic practices related to the crop were similar in the whole experiment and carried out at the same time. Additionally, in 2007, SEN 21, SEN 3, SER 21 and BAT 477 were planted and arranged in a complete randomized block design with five replications (8.5 plants.m^{-2}) with the purpose of evaluating differences in r_L and T_L in the field at 13:00 hour, with and without drought stress.

Yield components. Yield index (YI), and geometric means (GM), have been used successfully in bean to evaluate response of different genotypes to a biotic stress (e.g. Ramírez-Vallejo and Kelly, 1998; Porch, 2006). To apply these indices once the plant reached harvest maturity, leaves were removed, and stems and pods were collected and dried in an oven at 24^0C for 48 hours. Dry weights for pods with seeds and stems were obtained and yield (seeds per plant) determined. The YI and GM were computed as: $YI = (X_{ns}/X_s)$, and $GM = (X_{ns}*X_s)^{1/2}$, where X_{ns} is mean yield without drought stress and X_s is mean yield under drought stress. Additionally, the harvest index (HI) was estimated as the ratio of grain yield to net above-ground biomass (Howell et al. 1998; Hammer and Broad, 2003).

Water use efficiency. The water use efficiency (WUE) has been defined as the ratio of the mass of CO_2 fixed per unit of mass of H_2O transpired. In this study the WUE is defined as the aerial crop biomass divided by the volume of water transpired and *evaporated* in association with the production of that biomass (Keller and Seckler, 2006). The WUE in the greenhouse trials was estimated as the ratio of grain yield per plant per total mass of water applied, and in the field

trials was estimated as the ratio of the field yield per unit of evapotranspiration (Howell et al. 1998).

The transpiration efficiency (TE) was defined by the Bierhuizen and Slatyer Method (Tanner and Sinclair, 1983), which is the crop above-ground (aerial) biomass (DM, dry matter of stems, leaves, and fruit) divided by the volume of water transpired during the accumulation of that biomass, and is represented by:

$$\frac{DM}{T} = \frac{k}{\overline{VPD_d}} \tag{7.1}$$

Equation 7.1 indicates that the correlation between DM and T is dependent on k, which is a species-dependent water-use constant, and atmospheric vapor pressure deficit (VPD), which defines the moisture content of the atmosphere (Sinclair, 1998). In this study, k was estimated from field data using a mean daytime vapor pressure deficit similar to Howell et al. (1998), and T was estimated using the FAO-56 methodology (Allen et al.1998), where crop potential transpiration is assumed to be approximately equal to the basal crop evapotranspiration coefficient (ET_{cb}) multiplied by the reference evapotranspiration, ET_o. T and K_{cb} were estimated daily for each genotype and water level treatment. A full description of ET_o, ET_c and K_{cb} estimations are presented in Chapter 4.

$$T \cong ET_{cb} = K_{cb}\, ET_O \tag{7.2}$$

DM and T are expressed in $kg.m^{-2}$ and k in Pa.

The crop evapotranspiration was estimated by the Penman-Monteith general model (Monteith and Unsworth, 1990) with variable aerodynamic and surface resistance, and drainage lysimeters. The dual crop coefficients were derived using the methodology described in the Drainage and Irrigation Paper-FAO-56 (Allen et al. 1998). A complete description of theses estimations is presented in the Chapter 4.

Analysis of variance, normality and variance homogeneity tests were made. Means were separated with Tukey and LSD multiple range test $P<0.05$, using the Infostat version 3 statistical program.

RESULTS AND DISCUSSION

Yield Indices. In the greenhouse experiments, the yield index was significantly different between genotypes (Table 7.1). Under stress 1 (moderate stress), Morales produced the highest yield index and SEN 21 the lowest. Under stress 2 conditions (strong stress), SEN 21 and SER 16 produced the lowest values, with 0.24 respectively, and SER 21, Morales and BAT 477 the higher values.

The GM of seed yield has been accepted as the best predictor of bean genotype performance under stress and non-stress environments (e.g. Schneider et al. 1997, Ramirez-Vallejo and Kelly,1998; Smith, 2004; 1998; Porch, 2006). The GM for BAT 477, Morales, SEN 21, and SER 16 under stress 2 were not significantly different. The genotypes with high GM under stress 2 conditions were SEN 3 and SER 21, that could be indicating the good performance of this genotypes under drought stress.and non-stress conditions. Under field conditions, in 2006 the GM for SER 16 was higher than Morales and in 2006 slightly less, but the yield reduction was highest in Morales in both years (Table 7.4).

Water use efficiency (WUE) and harvest index (HI). The experiment x water level x genotype interaction was significantly different ($p<0.001$) in the WUE and HI in the greenhouse experiments (Tables 7.2 and 7.3). The most efficient genotypes in terms of water use in the greenhouse experiments were SER 21, SEN 3, and SER 16 (Table 7.2). Morales and BAT 477 were less efficient. The experiment with the most severe drought stress was July-Sep05, where the lowest WUE and HI were observed. During this period the, genotype SER 21 and SER 16 showed the highest WUE and HI coefficients (Tables 7.2 and 7.3). No statistical differences were observed between Morales and BAT477 during the three experiments, except under the water stress 2, for WUE. For HI, statistical differences were observed in the experiment in

Oct-Dec06 with 0.32 for Morales and 0.21 for BAT 477 in stress 1. Morales during this experiment was not statistically different from SER 21 for both coefficients. The genotype SER 21 was the only genotype that increased in WUE during the three experiments, compared with the other genotypes, which indicated the good performance of this genotype across the experiments.

For harvest index (HI) in the greenhouse drought stress treatments, SER 21 (ranged from 0.25 to 0.54) and SEN 3 (0.38 to 0.51) outperformed the other genotypes, e.g. BAT 477 (0.15 to 0.34) and Morales (0.04 to 0.40). The harvest index results were consistent for those found in the field with SER 16 and Morales. In the field under non-stress conditions, HI for SER 16 ranged from 0.24 to 0.37 and for Morales from 0.16 to 0.31. Under drought stress in 2006, HI was 0.31 for Morales and 0.37 for SER 16, and in 2007, HI was 0.16 for Morales and 0.24 for SER 16.

The lowest WUE values were observed in the July-Sep05 experiment, and may be directly related with the high mean air temperature observed during that period (27.6 ^{o}C), compared with 26.0 ^{o}C in July-Sep06 and 26.58 ^{o}C in Oct-Dec06.

In the greenhouse, SER 21 and SEN 3 (only tested in two experiments) showed consistently high WUE when compared to the other genotypes tested (Table 7,2). In the greenhouse environment, where root growth is limited to the area of the pot, there are few mechanisms of drought stress escape (such as deep taproots). Thus, effective control of transpiration and water use efficiency may play a critical role in the greenhouse environment.

Under field conditions the drought stress was more severe in 2007 (30.3%, relative to the well irrigated treatment) than in 2006 (18.0%, relative to the well irrigated treatment), causing an average reduction in seed yield of 76% in Morales and 67% in SER 16, as compared to 33% for Morales and 29% for SER 16 in 2006. The lower stress during 2006 was associated with high rainfall events registered during the stress treatments.

The severity in the drought stress in 2007 may be due to higher air temperatures during the pre-flowering and pod filling period, where the mean air temperature was 25.15^{o}C in 2007

compared with 24.45^{o}C in the same period on 2006, additionally during pod-filling several days exhibited windy conditions that imposed an additional 'Physiological stress" (Discussed in the Chapters 4, 5 and 6). Drought stress in both years for Morales resulted in a reduction in biomass, seed yield and HI with the most marked effect registered in 2007 (Table 7.5). SER 16 showed a higher HI in both years relative to the non-stress treatment, but a reduction in seed yield and biomass were also observed. Reductions in HI for different genotypes due to drought stress have also been reported by Ramirez-Vallejo and Kelly (1998) and Muñoz-Perea et al. (2006).

Favorable climatic conditions in 2006 increased the WUE values for both genotypes (Table 7.4). Muñoz-Perea et al. (2007) reported values of mean WUE in bean, under favorable climate conditions in Kimberly, Idaho, of 8.7 kg.ha^{-1}.mm^{-1} (0.87 kg,m^{-3}) for non-stress conditions and 9.8 kg.ha^{-1}.mm^{-1} (0.98, kg,m^{-3}) for stress conditions. Under unfavorable climatic conditions (strong stress), the same investigators reported WUE values ranging from 0.44 kg.m^{-3} for the genotype Othello to 0.11 kg.m^{-3} for the genotype Common pinto. The WUE varied with plant growth stage and was affected by drought stress (Muños-Perea et al. 2007). They mentioned that the WUE normally ranges from 3 to 6 kg ha^{-1} mm^{-1} (0.3 to 0.6 kg, m^{-3}), which indicates that the WUE values reported in this study, under strong stress, were lower than their normal range, and with non-stress and moderate stress our values were greater than their range (Table 7.5).

The SER 16 and Morales seed yield were no statistically different, indicating yield compensation in SER 16 to the fact that SER 16 had lower plant density that Morales (Table 7.4). These results were similar in both years of experiments.

Table 7.1. Analysis of stress-indices on seed yield for three experiments (July-Sep05, July-Sep06 and Oct-Dec06) under greenhouse environment.

Water Level	Genotype‡	YI†		GM	
With Stress 1	BAT477	0.88	a	6.21	a
With Stress 2	BAT477	0.71	aef	5.11	ad
With Stress 1	Morales	1.45	b	6.08	a
With Stress 2	Morales	0.88	f	4.86	ad
With Stress 1	SEN21	0.57	a	7.33	a
With Stress 2	SEN21	0.24	d	4.73	d
With Stress 1	SEN3	0.85	a	15.76	c
With Stress 2	SEN3	0.52	de	12.31	f
With Stress 1	SER16	0.82	a	10.25	b
With Stress 2	SER16	0.24	d	4.96	d
With Stress 1	SER21	0.94	a	10.40	b
With Stress 2	SER21	0.73	ef	8.61	e

‡ Differents letter indicate significance at 0.05 level, (LSD test).

† YI is the Yield Index= (Xs/Xns), GM is the Geometric mean =$(Xs \times Xns)^{1/2}$.

Where Xs and Xns indicate genotypic yield under drought stress and non- stress conditions respectively.

Table 7.2. Water use efficiency (WUE) among six common bean genotypes growing under greenhouse environment during 2005 and 2006.

Water Level	Genotype	Experiments		
		July—SepO5	July—SepO6	Oct—DecO6
		WUE ($g_{Seed}/L_{Water\ applied}$)		
Without Drought Stress	BAT477	0.16 ghi	0.59 pqrst	0.43 klmnopqr
With Stress 1	BAT477	0.18 ghij	0.64 rstuv	0.59 pqrst
With Stress 2	BAT477	0.17 ghij	0.40 jklmnopq	1.16 abc
Without Drought Stress	Morales	0.06 g	0.43 klmnopqr	0.55 nopqrs
With Stress 1	Morales	0.14 gh	0.62 qrstu	0.83 uvwxy
With Stress 2	Morales	0.06 g	0.52 klmnopqr	1.29 bcd
Without Drought Stress	SEN21		0.57 opqrst	0.79 tuvwx
With Stress 1	SEN21		0.14 gh	0.91 wxyz
With Stress 2	SEN21		0.21 ghijk	0.50 klmnopqr
Without Drought Stress	SEN3		0.84 uvwxy	1.20 abc
With Stress 1	SEN3		1.01 xyza	1.37 cd
With Stress 2	SEN3		1.03 yza	1.33 cd
Without Drought Stress	SER16	0.25 ghijk	0.86 vwxyz	1.07 zab
With Stress 1	SER16	0.33 hijklmn	0.74 stuvw	1.51 c
With Stress 2	SER16	0.28 ghijkl	0.37 ijklmnop	0.35 hijklmno
Without Drought Stress	SER21	0.24 ghijk	0.79 tuvwx	1.01 xyza
With Stress 1	SER21	0.31 hijklm	0.93 wxyz	1.45 c
With Stress 2	SER21	0.51 lmnopqr	1.05 yza	1.37 cd

Differents letter indicate significance at 0.05 level, (LSD test).

Table 7.3 Harvest Index (HI) among six common bean genotypes growing under greenhouse environment during 2005 and 2006.

		Experiments		
Water Level	Genotype	July—SepO5	July—SepO6	Oct—DecO6
		H.I (g. Seed /g. Biomass)		
Without Drought Stress	BAT 477	0.26 nopqr	0.27 nopqr	0.19 jklmn
With Stress 1	BAT 477	0.23 klmnop	0.29 opqrs	0.21 klmno
With Stress 2	BAT 477	0.15 hijk	0.15 hijk	0.34 qrstuvw
Without Drought Stress	Morales	0.16 hijklm	0.24 klmnop	0.24 klmnop
With Stress 1	Morales	0.15 hijk	0.26 mnopq	0.32 pqrtsuv
With Stress 2	Morales	0.04 g	0.18 ijklmn	0.40 tuvwxy
Without Drought Stress	SEN 21	nd	0.36 rstuvwx	0.42 vwxyz
With Stress 1	SEN 21	nd	0.07 gh	0.42 vwxyza
With Stress 2	SEN 21	nd	0.09 ghi	0.21 klmno
Without Drought Stress	SEN 3	nd	0.45 xyzab	0.50 yzab
With Stress 1	SEN 3	nd	0.38 stuvwx	0.51 zab
With Stress 2	SEN 3	nd	0.40 uvwxy	0.39 tuvwx
Without Drought Stress	SER 16	0.39 tuvwx	0.39 tuvwx	0.42 vwxyza
With Stress 1	SER 16	0.31 pqrstu	0.36 rstuvwx	0.51 ab
With Stress 2	SER 16	0.25 mnopq	0.15 hijkl	0.10 ghij
Without Drought Stress	SER 21	0.35 qrstuvw	0.43 wxyza	0.49 xyzab
With Stress 1	SER 21	0.25 lmnopq	0.41 vwxyz	0.54 b
With Stress 2	SER 21	0.30 opqrst	0.42 vwxyz	0.39 stuvwx

Differents letter indicate significance at 0.05 level, (LSD test).

Table 7.4. Mean square for seed yield, number of pods and biomass for SER 16 and Morales growing under field environment during 2006 and 2007.

Source of variation	df	Seed yield g/m2	Pods #/m2	Biomass g/m2
2006				
Water Level (WL)	1	57234.85 ***	27306.67 ***	24182.75 ***
Genotype (G)	1	1253.63	10546.88 **	16757.22 ***
Error	57	60148.86	930.83	993.10
2007				
Water Level (WL)	1	34594.8 ***	44915.42 ***	9611.73 ***
Genotype (G)	1	611.28	9773.8 ***	1683.93 ***
Error	42	290.1	507.74	191.29

*, **, and *** implies at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.
df is degree freedom

Table 7.5. Yield components, harvest index, evapotranspiration and water use efficiency for two common bean genotypes with and without drought stress at Juana Diaz, Puerto Rico in.2006 and 2007.

	Morales				SER16			
	Without drought stress		With drought stress		Without drought stress		With drought stress	
Parameter	2006	2007	2006	2007	2006	2007	2006	2007
Grain yield[Ŧ] (6.0 +/-2.3, g.kg^{-1} moisture),	195.4 +/- 29.7†	85.9 +/- 16.8	131.6 +/- 39.1	21.0 +/- 11.8	202 +/- 23.1	69.1 +/- 25.5	144.4 +/- 32.3	22.6 +/- 6.7
Biomass yield, g.m^{-2}	133.7 +/- 43.0	57.0 +/- 13.8	94.0 +/- 28.7	30.4 +/- 11.4	98.9 +/- 20.5	47.6 +/- 20.8	58.0 +/- 15.1	15.1 +/- 4.6
Plant density, no,m^{2}	13.6 +/- 4.1	13.2 +/- 4.4	14.0 +/- 2.6	14.7 +/- 2.8	6.4 +/- 1.0	6.0 +/- 2.8	6.5 +/- 1.7	6.0 +/- 0.3
HI[¶], kg.kg^{-1}	0.32 +/- 0.04	0.27 +/- 0.03	0.31 +/- 0.04	0.16 +/- 0.04	0.36 +/- 0.02	0.37 +/- 0.02	0.30 +/- 0.04	0.24 +/- 0.04
Pods number, no.m^{-2}	220.5 +/- 0.8	137.7 +/- 25.1	168.9 +/- 41.1	56.0 +/- 14.2	178.9 +/- 23.5	89.7 +/- 29.0	154.2 +/- 22.7	44.3 +/- 9.0
Evapotranspiration, mm	172.2	189.9	154.8	151.8	147.2	166.3	157.6	137.1
WUE[‡], (grain basis), kg.m^{-3}	1.13	0.45	0.85	0.14	1.37	0.42	0.92	0.16
WUE, (dry matter basis), kg.m^{-3}	0.78	0.30	0.61	0.20	0.67	0.29	0.37	0.11
Yield reduction[Ŧ]	0.33	0.76			0.29	0.67		
Yield reduction-Whole plots	0.33	0.76			0.33	0.73		
G.M [§]	160.4	42.5			170.8	39.5		

[Ŧ] Field data represented five row (2.0 m long) and average for three replication.

[†] Values 1 Standard deviation; [¶] Harvest index calculated as the ratio of the grain yield to biomass yield.

[‡] Water use efficiency (WUE) computed as a field yield per unit evepotranspiration.

[§] is the Geometric mean =(Yns x Ys)$^{1/2}$, where Yns and Ys represent genotypic yield without and with drought stress respectively.

Transpiration use efficiency. The estimated k for the field experiments is presented in the Table 7.6. The k results indicate differences between genotypes, water levels and experiments. The k values without drought stress are similar to those of other C_3 groups (Keller and Seckler, 2006; Kemanian et al. 2005). For both years, Morales showed the largest reductions in k, under drought stress conditions, where the difference between $k_{without\ drought\ stress} - K_{with\ drought\ stress}$ were: 2.0 Pa in 2006 and 2.3 Pa in 2006, compared with 1.7 Pa in 2006 and 1.1 Pa in 2007 for SER 16. Genotype variability in k has not been widely reported and studied in common bean.

Table 7.6. Transpiration efficiency constant (k) for two common bean genotypes, with and without drought stress during two year replication.

Experiments	Genotype	Water Level	VPD Mean daytime	k
			Pa	
2006	SER16	Without Drought Stress	1318.3	4.2
	Morales	Without Drought Stress	1347.9	3.8
	SER16	With Drought Stress	1289.3	2.6
	Morales	With Drought Stress	1328.8	1.8
2007	Morales	Without Drought Stress	1451.9	3.6
	SER16	Without Drought Stress	1451.9	2.2
	SER16	With Drought Stress	1464.2	1.0
	Morales	With Drought Stress	1498.7	1.3

CONCLUSIONS

These results indicate that genetic and environmental factors are reflected in the WUE, TE and HI. Under greenhouse conditions and under water limited conditions the genotypes SER 21, SER 16 and SEN 3 responded more favorably. Under field conditions, the genotype SER 16 performed best in terms of the TE, WUE and HI compared with Morales. Under severe drought stress conditions, water use efficiency, transpiration efficiency, and yield components were reduced compared with moderate drought stress conditions, with esception of SER 21 which in several cases increased.

REFERENCES

Allen G.R, L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. Food and Agricultural Organization of the United Nations (FAO). Publication No. 56. Rome. 300p.

Bountra, T.; F.E. Sanders, 2001: Influence of water stress on grain yield and vegetative growth of two cultivars of bean (*Phaseolus vulgris* L.). J. *Agronomy and Crop Science*. 187, 251-257.

Brevedan, R.E., D.B. Egli, 2003. Short periods of drought stress during seed filling, leaf senescence, and yield of soybean. *Crop Sci.* 43:2083–2088

Broughton, W.J., G. Hernandez., M. Blair., S. Beebe., P. Gepts., and J. Vanderleyden. 2003. Beans (*Phaseolus* spp.)-model food legumes. *Plant and Soil*. 252: 55-128.

Condon, A.G., R.A. Richards., G.J. Rebetzke., and G.D. farquhar. 2004. Breeding for high water-use efficiency. *J. Exp. Bot*.55(407):2447-2460.

Debaeke, P. A. Aboundrare. 2004. Adaptation of crop management to water-limited environments. *Europ. J. Agronomy.* 21:433-446.

Goyal, M.R, and A.E. Gonzalez. 1989. Datos climatológicos de las subestaciones experimentales agrícolas de Puerto Rico. Universidad de Puerto Rico-Recinto de Mayagüez/Colegio de Ciencias Agrícolas/Estación Experimental Agrícola. Publicación 88-70, proyecto C-411. 87p.

Hammer, G.L and I.J. Broad. 2003. Genotype and environment effects on dynamics of harvest index during grains filling in sorghum. *Agron. J.* 95:199-206

Howell, T.A, J.A. Tolk, A.D. Schneider, and S.R. Evett. 1998. Evapotranspiration, yield, and water use efficiency of corn hybrids differing in maturity. *Agron. J*. 90:3-9.

InfoStat, 2003. InfoStat, version 3. Manual de Usurario. Grupo InfoStat, FCA, Universidad Nacional de Cordoba,. Argentina. http//www.infostat.com.ar

Kemanian. A.R.., C.O. Stöckle,, and D.R. Huggins. 2005. Transpiration-use efficiency of barley. *Agric For Meteorol*.130:1-11

Keller, A and D. Seckler. 2006. Limits to increasing the productivity of water in crop production. manuscript under review for publication in a special issue of *Irrigation Science*. 20p In: http://www.winrockwater.org/docs/Limits.doc

Lizana, C., M. Wentworth, J.P. Martinez, D. Villegas, R. Meneses, E.H. Murchie, C. Pastenes, B. Lecari, P. Vernieri, P. Horton and M. Pinto. 2006. Differential adaptation of two varieties of common bean to abiotic stress. *J. Exp. Bot*. 57(3):685-697.

Miklas, P.N., J.D. Kelly, S.E. Beebe and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica.* 147:105-131.

Monteith, J.L, and M.H. Unsworth.1990. Principles of environmental physics. Chapman and Hall, Inc.292p.

Muñoz-Perea, C.G., H. Terán., R.G. Allen., J.L. Wright., D.T. Westermann., and S.P Singh. 2006. Selection fro drought resistance in dry bean landraces and cultivars. *Crop. Sci.* 46:2111-2120.

Muñoz-Perea, C.G., R.G. Allen., D.T. Westermann., J.L. Wright., and S.P Singh. 2007. Water use efficiency among dry bean landraces and cultivars in drought-stressed and non-stressed environments. *Euphytica*. 155:393-402.

Porch, T.G. 2006. Application of stress indices for heat tolerance screening of common bean. *J. Agron and Crop Sci*. 192:390-394.

Ramirez-Vallejo, P and J.D. Kelly. 1998. Trait related to drought stress resistance in common bean. *Euphytica*. 99:127-136.

Ritchie, J.T. 1983. Efficient water use in crop production: Discussion on the generality between biomass production and evapotranspiration. In: *Limitations to efficient water use in crop production*. Edited by Howard M. Taylor, Wayne R. Jordan and Thomas R. Sinclair. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America:29-44.

Schneider, K.A., M.E. Brothers and J.D. Kelly. 1997. Marketer-assisted selection to improve drought resistance in common bean. *Crop Sci*.37:37-51.

Singh, P.S., H. Terán., C.G. Muñoz., J.C. Takegami, 1999. Two cycles of recurrent selection for seed yield in common bean. *Crop Sci.* 39:391–397.

Singh, P.S. 2007. Drought resistance in the Durango dry bean landraces and cultivars. *Agron. J*. 99:1219-1225.

Sinclair, T.R. 1998. Limits to crop yield. In: NAS-Colloquium, Plants and Population: is there time?. 5-6 December. Beckman Center of the National Sciences, UC Irvine/ Academy of USDA-ARS, Agronomy Department-University of Florida. 8p. http://www.lsc.psu.edu/nas/Panelists/Sinclair%20Comments.html

Smith, J.R. 2004. Selection protocols for increased yield and stress tolerance in common bean. *J.Agric.Univ.P.R.*88:27-43.

Stayanov, Z.Z. 2005. Effects of water stress on leaf water relations of young bean plants. *Central European J, Agric*. 6(1):5-14.

Tanner, C.B, and T.R. Sinclair. 1983. Efficient water use in crop production: Research or Re-search?. In: Limitations to efficient water use in crop production. Edited by Howard M. Taylor, Wayne R. Jordan and Thomas R. Sinclair. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America:1:27.

Terán, H., P.S. Singh, 2002. Comparison of sources and lines selected for drought resistance in common bean. *Crop Sci*. 42:64–70

U.S.Department of Agriculture- (USDA). 1987. Primary characterization data of San Anton soil, Guanica country, Puerto Rico. Soil Conservation Service, National Soil Survey laboratory, Lincoln, Nebraska.

Chapter 8

SUMMARY

This research presents results related to plant-water relationships under drought and non-drought stress conditions for several common bean (*Phaseolus vulgaris* L.) genotypes, including the local and most-widely planted variety in Puerto Rico, and new genotypes known to be drought tolerant.

The genotypes evaluated were: 'Morales' which is currently the most popular white-seed bean variety in Puerto Rico, and with unknown drought response, BAT 477 cream-seed, which was one of the first genotypes released with drought tolerant characteristics, SER 21 and SER 16 red-seed, and SEN 3 and SEN 21 black-seed, which are germplasm released by CIAT (Centro Intenacional de Agricultura Tropical, Colombia) with drought tolerant characteristics.

The experiments were conducted in a greenhouse environment in Mayagüez, Puerto Rico in the Tropical Agricultural Research Station (TARS) facilities, and a field environment at the University of Puerto Rico, Experiment Station at Fortuna in Juana Diaz, Puerto Rico. During the three year study, a total of eight experiments were conducted, five in the greenhouse and three under field conditions, during 2005, 2006 and 2007.

Under greenhouse conditions, no statistical differences in the stomatal resistance (r_L) and leaf temperature (T_L) were observed among genotypes without drought stress conditions. Statistical differences were observed in both, with moderate and strong drought stress. The genotypes with the lowest increases in r_L and T_L under high drought stress conditions were, in the following order: SER 21 and SEN 3. The genotypes BAT

477 and Morales both exhibited the highest r_L and T_L. Under field conditions, without drought stress, no statistical differences were observed in r_L and T_L among genotypes, however, with drought stress the genotypes BAT 477 and Morales were statistical different from the others and the genotypes SER 16 and SER 21 showed the lowest values of r_L and T_L.

With respect to the water status measured with the relative water content (RWC) in the greenhouse experiments, statistical differences were observed among genotypes. The genotype SER 21 exhibited the highest values and BAT 477 the lowest values, indicating the high capability of SER 21 to conserve water under strong stress conditions.

The poor response of BAT 477 to drought stress in these experiments could be associated with the high leaflet size and high total leaf area. The genotypes with the greatest reduction in the leaf area under drought stress was SER 21, and also this genotype showed the highest water use efficiency (WUE) and harvest index (HI) values.

The genotype Morales can be considered to have some degree of drought tolerance, based on its response under moderate drought stress conditions, in field and greenhouse environments.

Crop coefficient were derived following the methodology proposed by the Irrigation and Drainage Papers (FAO-24 and 56) for two genotypes (SER 16 and Morales) during two years of experiments. The crop coefficient (K_c) derived in this study were lower than those reported by the Irrigation and Drainage Paper No. 56 (Allen et al. 1998) due to several factors, such as: different atmospheric demand, low plant density especially for SER 16, and the irrigation system used (drip) that reduced significantly the soil evaporation. Also the K_c was estimated indirectly measuring the fraction of soil covered by vegetation (f_c) or with the cumulative growing degree days (CGDD).

In addition to drought stress, high wind speed contributed to stress. The genotype most susceptible at high wind conditions was Morales compared with SER 16. This

susceptibility under windy conditions generated an inverse relation between stomatal resistance and the aerodynamic resistance (i.e., with increased wind speed, the aerodynamic resistance decreased while the stomatal resistance increased).

The genotype SER 16 under field conditions with 6 plants.m^{-2} do not show statistical differences in the seed yield with respect to Morales with 13 plants.m^{-2}, but statistical differences were observed in biomass and pods, indicating a yield compensation phenomenon, that is highly desirable under limited water conditions. SER 16 exhibited lower cumulative evapotranspiration rates, and higher WUE and transpiration efficiency values than Morales.

The critical variable in the generalized Penman-Monteith (GPM) methodology is the surfaces resistance (r_s) which is a function of the stomatal resistance (r_L) and the leaf area index (LAI). A disadvantage in applying the GPM method is the necessity to directly measure r_L and LAI, which are difficult and time consuming. In this study, the GPM method with the measured r_s was referred to as "Measured". We also considered other methods for estimating r_s based on the latent heat flux (λE), such as as the "inverse of the GPM model", the vertical gradient "Szeicz and Long method" (Szeicz and Long,1969), the "ET station" (Harmsen et al. 2006), and the micrometeorologically-based method of Ortega-Faria et al. (2004), which depends on net radiation (R_n), vapor pressure deficit (VPD), soil heat flux (G) and the change in soil moisture.

These results indicated that the ET can be estimated directly using the GPM method if the r_s is appropriately parameterized. We found that r_s could be reliably estimated based on the method recommended in the Drainage and Irrigation Paper (FAO-56) when: a) the LAI is greater than 1.0. Conversely, if the LAI is less than 1.0, this indicates all of the leaf area is contributing to transpiration and not just the effective area (i.e., LAI x 0.5). b) In this study, the inverse of the GPM method performed poorly for large values of the aerodynamic resistance (r_a), causing r_s to increase, which is contrary to the physiological response, and subsequently under estimating the ET. c) the Szeicz and Long method and the ET station predicted correctly the r_s with LAI values over 1.0, but

did not work well under high drought stress conditions (Figure 5.8), and d) the Ortega-Farias et al.(2004) method estimated appropriately the r_s with and without drought stress if soil moisture is correctly estimated in the root zone. Under windy and drought stress conditions, the r_s estimation was not appropriate for any of the methods for the genotype Morales, which is stomatally susceptible under windy conditions.

The upper and lower baselines for the crop water stress index application (CWSI-Idso et al. 1981) were developed for these genotypes, indicating genotypic variations in the baselines. The drought tolerant genotypes showed higher upper baselines, and the rate of change (slope) in the lower baseline was also higher in the most drought susceptible genotypes. The upper and lower baselines, in this study were different than those previously reported for common beans, indicating also the environmental and genotypic variability.

The CWSI was well related with the water content in the root zone. When the soil reached the field capacity, the CWSI for both genotypes was between 0.1 and 0.2, which has been previously reported for other common bean genotypes, and also the maximum relative yield under greenhouse conditions corresponded for this range of CWSI. The CWSI also detected the "physiological stress" induced by windy conditions in the genotype Morales under field conditions.

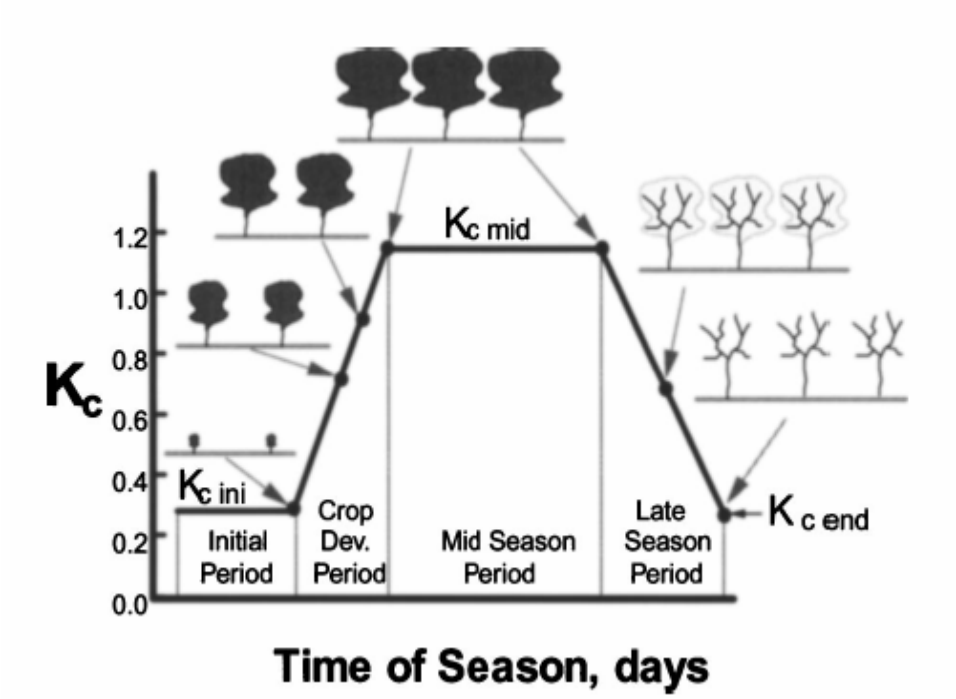
RECOMMENDATIONS

The following recommendations are offered for future research:

- In this study the photosynthetic rate and gas exchange efficiency were not measured in the genotypes. The parameters should be measured in future studies of these genotypes.
- The mechanisms that control drought resistance studied in this research (e.g., stomatal response) could be studied at the cellular level.
- In this research, it was observed that the ET_c using the Penman-Monteith model overestimated when leaf area index (LAI) was less than 1.0. Therefore, it is recommended that for the genotypes studied, when the LAI is less than 1.0, the surface resistance should be calculated as the stomatal resistance divided by the LAI and not the $LAI_{effective}$.
- The crop water stress index is an excellent tool for stress detection and irrigations scheduling; it is recommended that studies be conducted in Puerto Rico to determine the critical crop water stress index values for variety of crops.
- The use of the ET station approach is recommended for estimating crop evapotranspiration and crop coefficients for short crops in Puerto Rico. The advantage of the methodology is that it provides estimates of ET_c, surface resistance, energy balance components and the Bowen ratio. ET_c estimates from the ET station have been shown to compare reasonably well with the eddy covariance system (Harmsen et al., 2006) and is considerably less expensive (approximately 1/7 the cost).

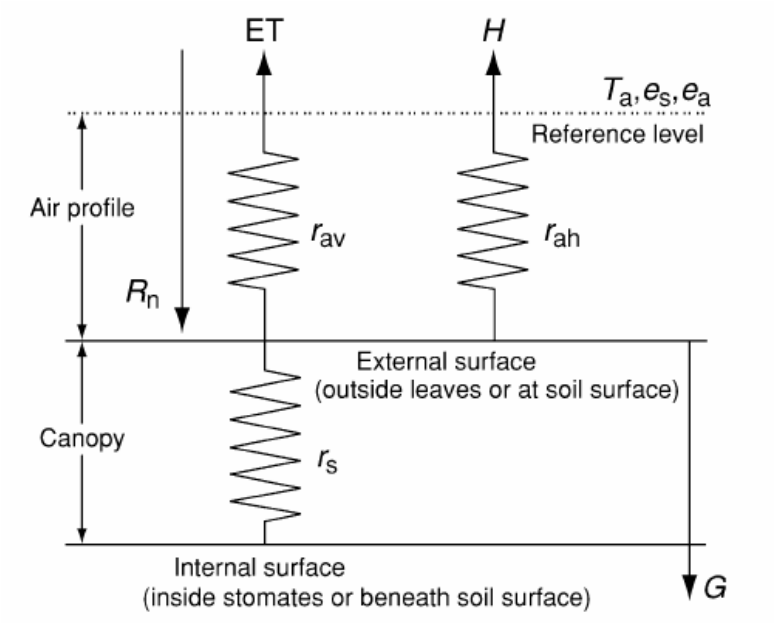
APPENDICES

APPENDIX A



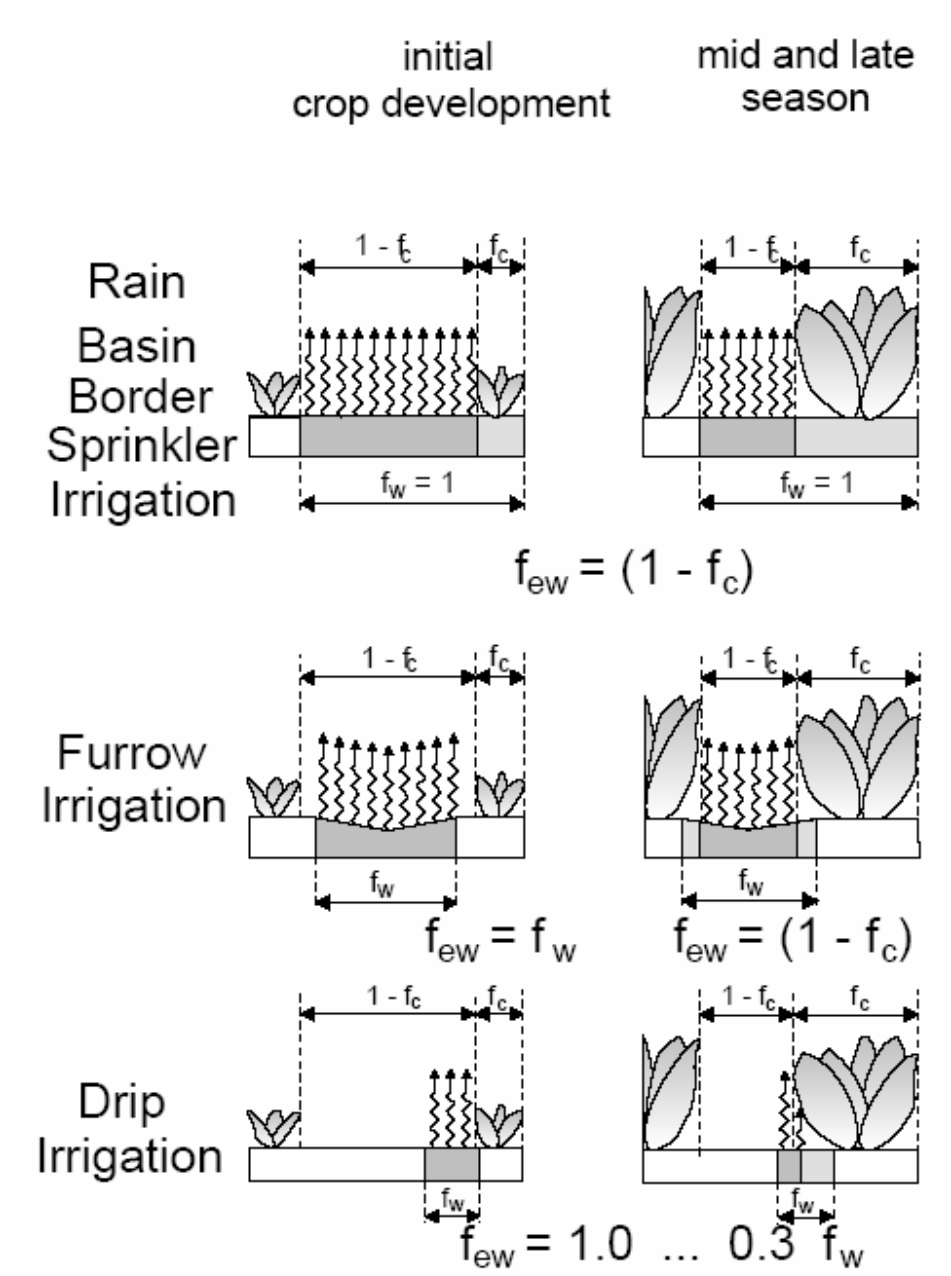
Schematic showing generalized shape of Food and Agricultural Organization (FAO) K_c curve with four crop stages and three K_c or (K_{cb}) values and relative development of vegetation. **Source:** Allen, R.G., Pereira, L.S., Smith, M., Raes, D., and J.L. Wright. 2003. FAO-56 Dual crop coefficient method for estimating evaporation from soil and application extensions. *Journal of Irrigations and Drainage Engineering*.131:2-13.

APPENDIX B



Schematic showing linkage between resistance terms in the Penman–Monteith equation relative to the surface and elevation of temperature and humidity measurements. ET, evapotranspiration; e_s, saturation vapor pressure at mean air temperature; e_a, actual vapor pressure of the air; G, heat exchange from surface to soil; H, heat exchange from surface to air; r_a, aerodynamic resistance; r_s, bulk surface resistance; R_n, net radiation flux at the surface; T_a, air temperature; r_{av}, aerodynamic resistance to vapor transfer; r_{ah}, aerodynamic resistance to heat transfer. **Source:** Allen, R.G.2005. *Penman-Monteith equation* In: Soil in the environment: Edited by: Elsevier, Ltd:180-188.

APPENDIX C



Determination of variable f_{ew} (cross-hatched areas) as a function of the fraction of ground surface coverage (f_c) and the fraction of the surface wetted (f_w). **Source.** Allen G.R, L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. Food and Agricultural Organization of the United Nations (FAO). Publication No. 56. Rome. 300p.

APPENDX D

Moisture calibration curves for the Profile probe type PR2 sensor (Delta-T Devices Ltd) and for the weather stations CS616 water content reflectometer-TDR (Campbell Scientific, Inc). For Fortuna Experiment Stations soil conditions.