PROCEEDINGS

OF THE

26th ANNUAL MEETING

July 29 to August 4, 1990

Mayaguez, Puerto Rico

Published by:

Caribbean Food Crops Society

with the cooperation of the USDA-ARS-TARS

Mayaguez, Puerto Rico

BANANA PLANTLETS AS A TOOL IN STRESS PHYSIOLOGY

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ABSTRACT

A major constraint in physiological studies of the Musaceae is the large size of plants and their component parts. Basic growth measurements of field plants are often difficult and become even more so under growth control environments. Physiological measurements such as leaf photosynthesis (Pn), stomatal conductance (Cs), leaf transpiration (Tr) and leaf water potential (LWP) can also be problematic particularly when measurements require whole leaf or other plant parts. The present studies were part of a Caribbean Basin Agric. (CBAG)-USDA project to investigate the influence of container temperature on growth and physiology of selected fruit and ornamental species. Banana was one of the fruit test species and tissue cultured plantlets were utilized as a convenient form for studying root-zone temperature (RZT) and irrigation volume (IRV) effects on growth and physiology. Because of the convenient size of plantlets, a whole range of studies were done and described to include growth, carbohydrate (CHO) analysis, root electrolyte studies, Pn, Cs, Tr and leaf chlorophyll analysis. All physiological measurements were done simultaneously using a portable gas exchange system. A brief review of the methods and resulting data are described.

INTRODUCTION

The technique of tissue culture has traditionally been used mainly to produce disease-free plants, as a method of rapid propagation and for evaluation of biological stress factors. The usage of plantlets to study physiological stress represents a unique and convenient method and in this study stress factors were rootzone temperature (RZT) and soil moisture, the latter imposed by the technique of varying irrigation volume to container plants. Although banana was the focus species of the study, the techniques employed may well apply to any of the Musaceae group including plantain, cooking banana and Heliconia. These are all large-sized plants under field conditions and are quite difficult to work with when recording physiological measurements. While the overall objective of this investigation was to identify the effects of RZT and water deficits on container-grown plants the specific objectives were to: 1) Demonstrate the suitability and convenience of using banana plantlets in physiological studies and 2) Demonstrate methods of monitoring leaf photosynthesis, leaf conductance, leaf water potential and leaf carbohydrate analysis and to relate these

findings to practical applications. The first three measurements were done simultaneously by a portable gas analyzer using whole leaves and non-destructive methods. For carbohydrate analyses, leaves were detached but these were the identical leaves from which the first three measurements were taken so giving better chances for correlation. Experiments were conducted under both greenhouse and growth room environments.

Plant Materials and General Cultural Procedures

Ten- to 12-cm tall tissue-cultured stage III 'Grande Naine' banana plantlets were imported through the postal services from a commercial nursery in Florida. Plants were initially hardened in an intermittent mist (6 secs min^{-1}) under 80% light exclusion for one week, then moved to 40% light exclusion for another week. They were then transplanted to white 4-cm diameter x 21 cm tall conical containers (150 cm^3) using Metro-Mix 300 growth medium (W.R. Grace and Co., Cambridge, MA). Plants were then moved to the experimental greenhouse or growth room where they were watered to container capacity daily and allowed to acclimatize for one week prior to the initiation of each of the following experiments.

Experiment 1

Experiment 1 was initiated in an air-conditioned greenhouse in which maximum photosynthetic photon flux density (PPFD) ranged from 700 to 800 umol m^{-2} s^{-1}, and 25 to 30°C day and 18 to 21°C night temperatures were maintained. Plants were watered daily with 40±8 ml (W3), 20±4 ml (W2) or 10±2 ml (W1) per container. These irrigation volumes (W) provided 85% to 100%, 65% to 75%, and 53% to 60% container capacity (CC), respectively. Water was applied automatically through drip tubes by a battery-operated controller (Water Watch Corp. Seattle, WA). One, two and four 1-mm drip tubes/container constituted the W1, W2 and W3 irrigation treatments, respectively (Fig. 1).

After 14 days of irrigation treatments, diurnal measurements of leaf photosynthesis (PS), transpiration (TR) and leaf conductance (CS) were made using a portable photosynthesis system (Model LI-6000, LI-COR, Inc., Lincoln, NE). Measurements were initiated at 0800 hr on a cloudless day and taken every 2 hr until 1600 hr. A 1-liter cuvette chamber was used for measurements and the mean of eight consecutive 30-sec observations on each leaf constituted a measurement. A zero check of the analyzer was performed between treatments within each replicate. Simultaneous measurements of LWP were made using a pressure chamber (PMS Instrument Model 600, Santa Barbara, CA) as described by Barrett and Nell (1986). The third most recently expanded leaf in banana was selected for measurements of both gas exchange and LWP. This leaf has been shown to be the most resp nsive and the youngest with fully developed stomata (1959). Six plants per treatment were sampled for all measurements.

Experimental design was a randomized complete block with 21 plants per water treatment within each of three blocks. Six replicate plants were randomly selected at each sampling time. Water use efficiency (WUE) was calculated from PS/TR. Means and standard errors were calculated and plotted against time of day.

Experiment 2

In order to further monitor physiological responses of water stress and correlate these with visual plant symptoms, water was withheld from banana plants for 14 days beginning one week after transplanting. This drying cycle study was conducted simultaneously with Expt. 1 in the same greenhouse and similar plants and cultural practices were used. Midday LWP and CS measurements were recorded on five plants at 2-day intervals using the procedures described in Expt. 1. A completely randomized design was used in this experiment and means and standard errors were calculated. There were five replicate plants per treatment for each sampling date.

Experiment 3

In order to investigate physiological responses to water stress under more precisely controlled environmental conditions, Expt. 1 was repeated in a 3.0 m by 7.6 m walk-in growth room. Irradiance of 1100 umol m^{-2} s^{-1} PPFD, as measured by a quantum radiometer (LI-COR Model LI-185A, LI-COR Inc., Lincoln, NE), was supplied at plant canopy level by 1000 W phosphor-coated metal-arc HID bulbs (GTE Sylvania Corp., Manchester NH) from 0600 to 1830 hr. Air temperatures of 28°C day and 21°C night and a relative humidity of 65 to 70% were maintained. Three irrigation volume treatments were applied as in Expt. 1 in a randomized complete block design with three blocks and 21 plants per water treatment per block. On the fifteenth day after water stress treatments, diurnal measurements were initiated at 0800 hr and continued at 2 hr intervals until 1800 hr. Leaf sampling methods and measurement procedures for PS, CS, TR and LWP were employed as in Expt. 1.

High root-zone temperatures (RZT) have been shown to reduce plant growth (Ingram, 1981; Ingram et al., 1986) and affect many physiological processes (Gosselin et al., 1984; Guinn et al., 1968; Itai et al., 1973). Tropical and subtropical plant species are often thought to be heat tolerant, but soil temperatures as high as 52°C have been recorded in the tropics, and temperatures in this range can be lethal for some tropical (Ingram et al., 1986) and subtropical crops (Ingram et al., 1984). An air temperature of 33°C was reported as being optimum for growth and dry weight partitioning in banana (Musa spp. AAA) (Turner et al., 1983) and mineral composition was highly influenced by temperatures from 18 to 33°C (Turner et al., 1985). However, there are no reports on RZT effects on growth and physiology of container-grown banana. It is essential

to identify growth and physiological effects of high RZT before control measures can be developed to alleviate such effects. The following studies investigated the short-term effects of RZT on container-grown 'Grande Naine' banana under greenhouse and growth room conditions.

Experiment 4

Recently transplanted banana plantlets were watered daily to container capacity and allowed to acclimatize for one week in a greenhouse as described in Expt.1. RZT treatments were established within styrofoam-lined wooden air bath boxes (1 m x 1 m x 20 cm) in which plant containers were firmly inserted to within 2 cm from their upper rims (Fig. 2). Four equally spaced 100 watt aluminum foil-covered incandescent light bulbs provided convective heating of the enclosed root systems. Proper distribution of heat and aeration within the boxes were ensured by two fans (IMC Magnetics, Roch. N.H) per box. The light bulbs in each box were controlled by a pre-set thermostat and the temperatures in each box were verified daily with a thermocouple thermometer (TH 65, Wescor, Inc., Logan, UT). Electrical power to the temperature boxes was controlled by Intermatic Time *ontrols (Intermatic Inc. Spring Grove, Ill), providing daily RZT treatments from 1000 to 1600 hr. Boxes were placed on 1 m high benches and an automatic drip irrigation system provided daily watering of all plants to container capacity. RZT treatments established were 28 ± 1^{o}, 33 ± 1^{o}, 38 ± 1^{o} and 43241^{o}C. Each temperature box contained 12 banana plants and there were three replicate boxes for each treatment temperature arranged in a randomized complete block design. Means and standard errors were calculated for the 12 replicate plants measured at each sampling time.

After 14 days of RZT treatments, diurnal measurements of PS, CS, TR and LWP were made using a portable photosynthesis system as described in Expt.1.

Experiment 5

In order to further monitor the physiological responses of 'Grande Naine' banana to RZT under more precisely controlled environmental conditions, Expt. 4 was repeated in a 3.0-m by 7.6-m walk-in growth chamber.

Each conical container with a transplant was suspended through a tightly fitting styrofoam ring within a specially constructed root heating tube (RHT). Each RHT was 22.5 cm high and constructed from 7.5 cm diameter metal pipe wrapped with 60 watt 120 vac heating tape (Smith-Gates Corp., Farmington, CT) and 1.25 cm thick foam insulation. The RHTs were connected to solid state electronic controllers which maintained preset treatment temperatures by a thermistor feedback mechanism. Each controller maintained treatment temperatures in 16 tubes with

Figure 1. Sistem of irrigation volumes for imposing water stress.

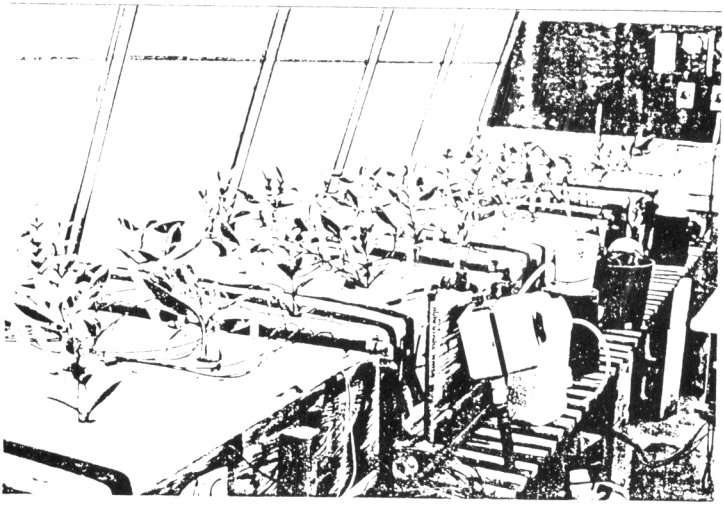

Figure 2. Air bath boxes for imposing RZT treatments.

four tubes at each of four specified temperatures. Treatment temperatures were set and maintained at 28±0.3°, 33±0.3°, 38±0.3, and 43±0.3°C. Similar leaf number and sampling methods were used and the same physiological measurements were recorded as in experiment 4 after 14 days of root-zone temperature treatments.

Research on water and temperature stress effects has tended to concentrate on these stresses separately with little emphasis on defining or separating the characteristics of the two stress factors and elucidating whether high temperature induces a water stress effect. Water stress has been shown to affect growth (5,13), yield and WUE (Bhattacharyya et al. 1984; Bhattacharyya et al., 1985; Ghavami, 1974) and physiological responses (Chen, 1971; Shmueli, 1953) in banana (Musa spp. AAA). Relatively fewer studies have been reported on temperature stress effects (Ingram, et al., 1986; Turner, et al., 1983; Turner et al., 1985). An air temperature of 33°C was reported as being optimum for growth and dry weight partitioning in Cavendish banana (Turner et al., 1983). Investigations on banana involving both stress factors simultaneously have not been reported.

The following experiments investigated the effects of four RZTs and two irrigation volumes on growth, physiology and carbohydrate status of 'Grande Naine' banana under greenhouse and growth room conditions.

Plant Materials and General Cultural Procedures.

Similar banana plantlet as used in the preceding experiments were transplanted to 6.5-cm diameter x 25.0-cm tall conical containers (555 cm^3) using Metro-Mix 300 growth medium. Plants were then moved to the experimental greenhouse or growth room where they were watered to container capacity daily and allowed to acclimatize for one week prior to the initiation of each experiment.

Experiment 6

Two irrigation volumes and four RZT treatments were factorially combined in a split plot design experiment. Plants were watered daily with 50±5 ml (W1) and 100±10 ml (W2) per container (555 cm^3). These volumes represented 70% to 60% and 100% to 85% container capacity (CC), respectively. Water was applied automatically through drip tubes by a battery-operated controller (Water Watch Corp. Seattle, WA). The W1 and W2 irrigation treatments were applied by one and two 6-cm dramm irrigation rings/container, respectively.

RZTs of 28±1°, 33±1°, 38±1° and 43±1°C were established in air-bath boxes as described in Expt 4. Each temperature box contained 12 banana plants to which the irrigation treatments W1 and W2 were randomly assigned.

Temperature boxes were replicated three times. RZT treatments were applied daily from 1000 to 1600 hr and irrigation was supplied at 2200 hr so that the imposition of temperature and irrigation treatments did not overlap. Plants were fertilized weekly with the appropriate volume of water and concentrations of 20N-8.8K-16.6P fertilizer (Peters 20-20-20, W.R. Grace and Co., Cambridge, MA) to apply 250 mg N/container. Fertilizer was applied with a hand operated injector (Chem-trol Inc, Kansas City, KS).

Weekly measurements of plant height, leaf area, leaf number and stem diameter were made. Plant height was considered the distance from the soil surface to the leaf apex. Stem diameter was measured at pot rim level and leaf area of the third newest leaf was calculated from 0.65 of leaf length multiplied by leaf width (Simmonds, 1959). Leaf numbers were derived from the number of whole green functional leaves and emerging leaves were assigned a value of 0.25, 0.50 or 0.75 depending on the fraction of the leaf lamina fully exposed.

Ten weeks after the initiation of the experiment, PS, CS and TR were recorded as in Expt. 1. Simultaneous measurements of LWP were made using the pressure chamber technique.

After physiological measurements, leaf and root samples were immediately taken for carbohydrate analysis. The third newest leaf was sampled. A 5 to 7 g composite sample of carefully washed roots was used for root carbohydrate analysis. Plants were then separated into leaves, stems and roots before drying for at least 48 hr in a forced-air oven at 70^{o}C prior to recording dry weights. Ethanol-soluble sugars and starch were determined according to the procedure used by Stamps (1984).

The experiment was arranged in a split plot design with three blocks in which RZT were main plots and irrigation treatments represented subplots. Single degree-of-freedom orthogonal comparisons between treatment means were performed and partitioning of interactions for linear, quadratic and cubic models for final growth, and carbohydrate data were made using the General Linear Model procedure of the Statistical Analysis System (1983).

Experiment 7

In order to further monitor the physiological responses of banana to RZT and irrigation treatments under more precisely controlled environmental conditions, Expt. 6 was repeated in a walk-in growth room as described in Expt. 3. Irrigation and RZT treatments were the same as for Expt. 6 but root heat tube (RHT) containers as described in Expt 5. were used. Similar plant materials and cultural procedures were used as for Expt. 1.

Relative chlorophyll concentration of leaves used for physiological measurements was recorded using a Spad 501 chlorophyll meter (Minolta Corp. Ramsey, NJ) and expressed as umol m^{-2}.

SUMMARY RESULTS AND IMPLICATIONS

The major objective of this project was to evaluate the effects of increasing RZT and irrigation volume, independently and in interactive studies, on container-grown 'Grande Naine' banana (Musa spp. AAA) started from tissue culture. A portable CO_2 gas analyzer system was used effectively to measure gas exchange processes simultaneously and the pressure chamber technique gave good indications of plant water status. The more controlled conditions of the walk-in growth room and the system of electronically controlled RHTs allowed for a more concise study of banana plantlet responses to RZT treatments than greenhouse conditions and air bath boxes. The successful use of tissue-cultured banana plantlets in these studies indicated that banana micropropagation can indeed be an effective tool for investigating physiological responses to stress factors.

Experiments on the independent effects of irrigation volume indicated conclusively that all physiological parameters in banana were decreased by decreasing irrigation volumes below the W3 (40±8 ml per 150 cm^3 container daily; 85-100% CC) treatment (Fig. 3). This parallels the results of most banana field research where physiological responses were reportedly disrupted by soil moisture levels below 66% available soil moisture (ASM) or 33% depletion of ASM (Bhattacharyya et al., 1984; Ghavami, 1974; Shmueli, 1953)

Although gas exchange processes were reduced with decreasing irrigation levels, midday LWP in plants at W2 (20±4 ml per 150 cm^3 container daily; 65-75% CC) were comparable to those at the W3 level (Fig. 3). The morphological mechanism of lamina leaf folding, although commonly seen in field plants at midday, has never been characterized in terms of leaf water status. In Expt. 2, leaf folding was clearly evident in plants that were water stressed to LWP of -0.51 to -0.65 MPa (Fig. 4).

WUE has generally been quantified on a yield/evapotranspiration basis in banana field experiments (Bhattacharyya et al., 1984; 1985), and though reported as leaf PS/leaf TR in this study, there was some parallel between the two interpretations. Plants in greenhouse Expt. 1 grown under the W2 irrigation regime maintained PS with decreasing TR rates thus increasing photosynthetic WUE over the W3 treated plants. At the W1 irrigation level (10±2 ml per 150 cm^3 container daily; 50 to 60% CC) plants could not effectively maintain any of the measured physiological processes. There was chlorophyll degradation as evidenced by leaf chlorosis and PS was severely reduced in the W1-treated plants. Although not considered

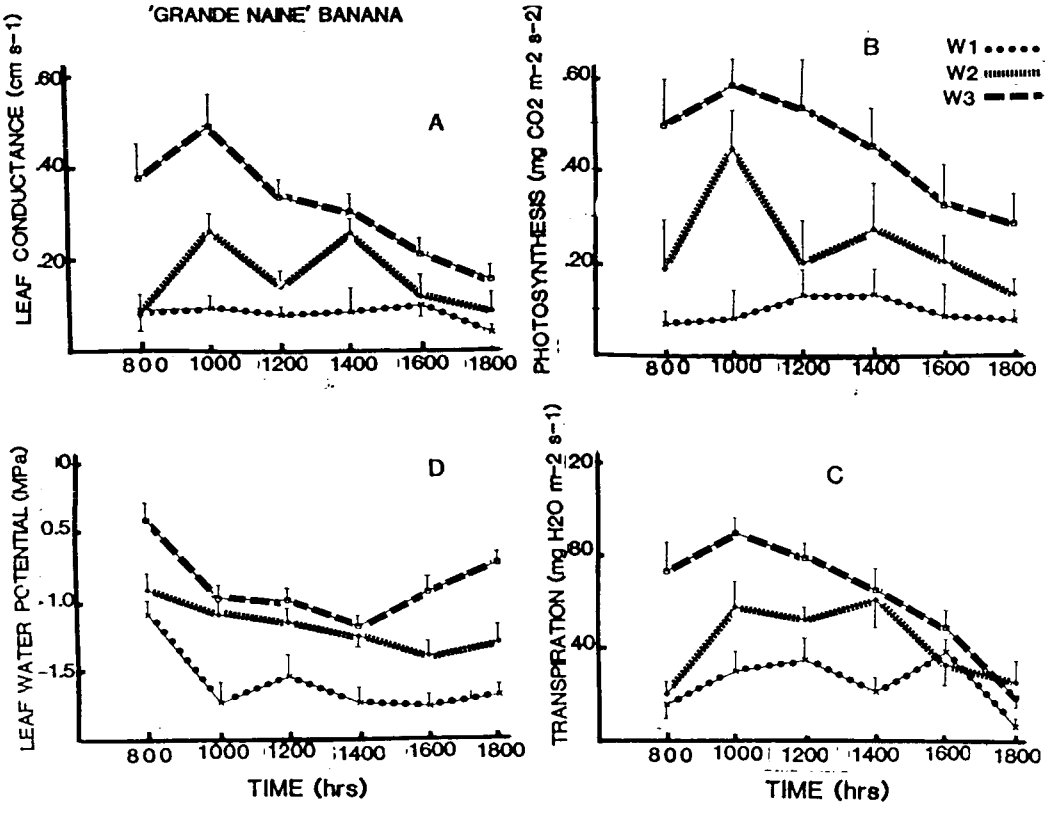

Figure 3. A. Leaf conductance B. Leaf photosynthesis C. Transpiration D. Leaf water potential under growth chamber conditions

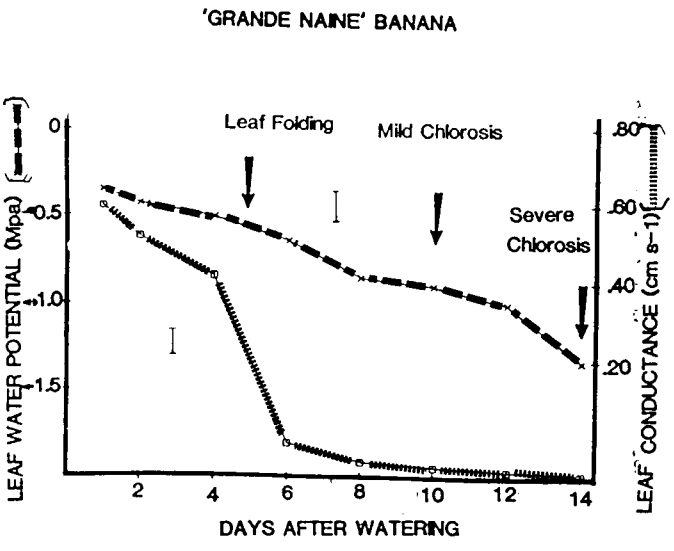

Figure 4. Leaf conductance and leaf water potential during a 14 day drying cycle.

drought tolerant, the banana plant can apparently increase its photosynthetic WUE under declining irrigation.

Under growth room conditions, effects of decreasing irrigation volume on physiological responses were not as drastic as in the greenhouse experiment. At midday hours, plants under the W2 irrigation volume exhibited similar PS, CS and TR as those at the higher irrigation level. This increase in physiological responses of plants at the W2 level was attributed mainly to the effect of increased irradiance in the growth room on stomatal opening and PS.

Plant responses to RZTs of 28°, 33°, 38° and 43°C imposed for 2 weeks were monitored in Expts. 4 and 5. While the 33°C RZT induced maximum rates of gas exchange processes in banana under greenhouse conditions, plants grown in the growth room attained highest PS, CS and TR at the 38°C RZT (Fig. 5). All parameters were reduced by the 43°C RZT under both environmental conditions. Most tissue-cultured banana plants are started in black polyethylene containers and RZTs above 33°C are commonly attained in such containers (Ingram, 1981). Results from these studies, therefore, indicate that nursery plants could be grown in such containers provided that media temperatures are limited to a maximum of 38°C.

An interesting comparison between RZT and water stress effects was initially observed in the short-term RZT experiments. Plant water status as reflected by LWP generally declined with increasing RZT but plants did not exhibit leaf folding or show any chlorosis as exhibited in the directly water-stressed plants of the irrigation experiments. Some mechanism of conditioning or maintaining turgor under reduced LWP was theorized. In the subsequent long-term experiments, carbohydrate analyses suggested that there may have been osmotic adjustment.

In the final experiments, two irrigation volumes were factorially combined with four RZTs under greenhouse and growth room environments. Some interactions between RZT and irrigation volume occurred that could possibly be exploited in the container production phase of banana. Banana plants exhibited tolerance to increasing RZT up to 38°C with significantly decreased rates in measured physiological responses induced by the 43°C RZT in the greenhouse environment. Under growth room conditions, increased irrigation (150±15 ml per 1200 cm^3 container daily; 90% to 100% CC) significantly increased midday PS in plants grown at a RZT of 33°C. At 38°C RZT, overall PS declined but increased irrigation application had no effect. Stress effects induced by the 43°C RZT were moderated by increased irrigation in the high light condition of the growth room, but increased watering was actually detrimental to plants at the 43°C RZT in the greenhouse experiment. Root injury at the 43°C RZT probably caused reduced root absorptive capacity

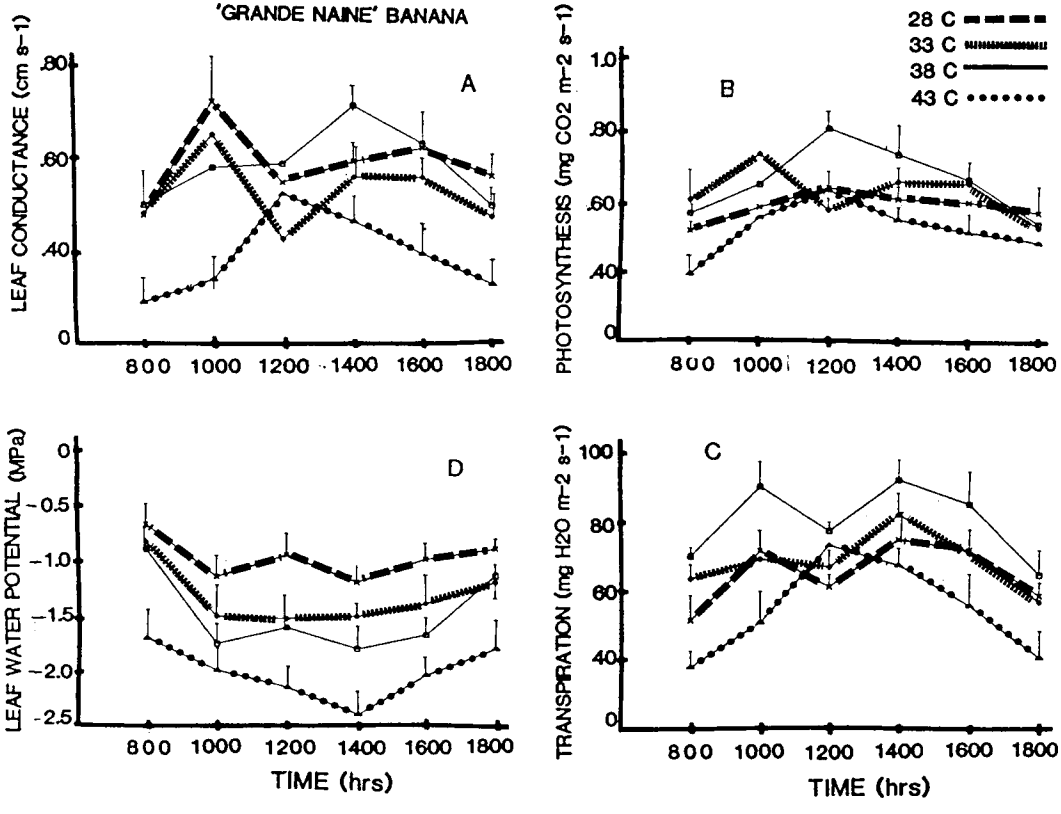

Figure 5. A. Leaf conductance B. Leaf photosynthesis C. Transpiration
D. Leaf water potential under growth chamber conditions

(Rylski et al., 1976.) and this was aggravated by the relatively small container volume in the greenhouse experiment.

The observed interactions could have important implications in the nursery phase of banana production. If growth medium temperatures are maintained at 33°C, increased irrigation would be beneficial. At a RZT of 38°C, overall PS would be reduced and increased irrigation would not alter this effect. Control measures would be essential if medium temperatures approached 43°C, since this RZT was shown to be definitely supraoptimal under both environmental conditions investigated. Irrigation would need to be closely regulated since root injury caused by the 43°C could actually be aggravated by increased irrigation.

RZT stress was characterized morphologically by reduced leaf area in banana (Fig. 6). A distinct decrease in leaf width but not leaf length or plant height was recorded in plants at the 38° and 43°C RZTs. A RZT-induced hormone mediated response associated with the loss of root tips at the high RZTs was postulated. This reducing effect on leaf area was not overcome by increased irrigation volume, indicating another interesting contrast between water and RZT stress effects. The relatively small leaves at higher RZTs had increased chlorophyll concentration but this was not related to increased PS or shoot dry weight.

Carbohydrate analyses revealed significant differences in shoot/root and sugar/starch partitioning patterns. Comparison of the observed carbohydrate status with other physiological parameters suggested a possible role of sugars in allowing plants to maintain turgor under RZT-induced decreases in LWP. More detailed analyses, however, are needed to validate these theories and further interrelate RZT and water stress effects. The increase in stem diameter but not stem dry weight also supported the theory of increased plant turgor as one response to increased RZT.

Interactive effects of RZT and irrigation volumes observed in these studies could therefore have significant applications to the container phase of banana production schemes that convert to the tissue-culture method of propagation.

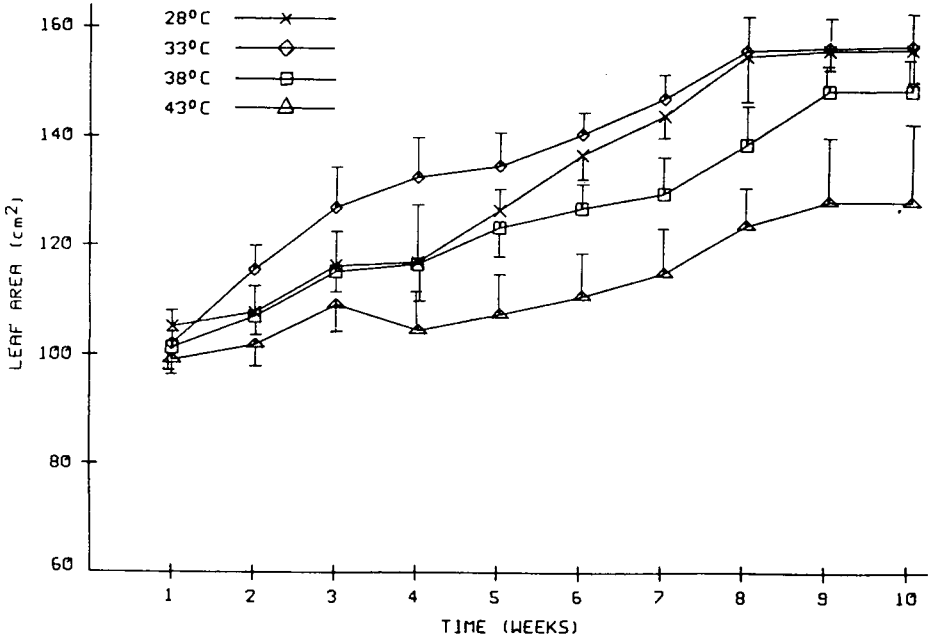

Figure 6. Root zone temperature effects on leaf area in 'Grand Naine' banana.

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