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ASSESSMENT OF SOYBEAN (*Glycine max* (L.) Merr.) WATER STRESS; LIPO-CHITOOLIGOSACCHARIDES APPLICATION AND SPECTRAL RESPONSE

by

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Soybean response to water stress and a signal molecule

DEDICATION

This thesis is dedicated to my parents, sister and two brothers:
Moncef Atti, Fatima Djelassi Atti, Kamilia Atti Ghouila, Nidam Atti, and Anis Atti.
All were of great moral support to me. Their love and encouragement
have always given me the courage to follow my dreams.
In part, this work is their achievement.

ABSTRACT

M.Sc. Sarra Atti Agricultural and Biosystems Engineering

Although chronic drought is frequent in many areas, not much research has been conducted to assess the impact of *chronic* water stress on crop production. Most existing chemical treatments, used to mitigate yield losses, are effective for reducing water stress but fail to increase photosynthesis. Research on the effects of Lipo-chitooligosaccharides (LCOs) application has been very active. This study was conducted to improve knowledge of the impact of chronic soil water deficit and to test a novel technique of water management consisting of LCO spray application. It also aimed at evaluating changes in canopy reflectance due to water stress and LCO spray. A greenhouse experiment was conducted on soybean (*Glycine max* (L.) Merr.). The individual impact of water stress during reproductive development on soybean growth pattern was investigated in a completely randomised block design (CRD) with 6 blocks of three moisture levels (medium stress - W2, severe stress - W1, and a well watered control - W3). The interaction between water stress and LCO treament was evaluated in a CRD with 6 blocks of factorial combinations of the same 3 moisture levels by 2 LCO levels (treated with LCO - L1, and a non-treated control - L0). A solution of LCO Nod *Bj* V (141.6 µg/L) was used. Water stress during reproductive development resulted in an important decrease of plant physiological activity, vegetative growth, and productivity, and accelerated plant senescence at both water stress levels. Water deficit increased leaf reflectance in the visible and decreased it in the infrared ranges of the spectrum at both imposed stress levels. Severe water stress negatively affected leaf chlorophyll content, but moderate chronic water stress had no significant impact. Foliar application of LCO affected overall plant physiological activity, increased flower and pod numbers. LCO influenced pod induction more than pod enlargement. LCO also accelerated leaf senescence. Thus, LCO could hasten plant physiological maturity. LCO significantly impacted the spectral reflectance signature only at the medium stress level. LCO treatment had the largest positive effect on the growth pattern of soybean at the medium stress level, which is the stress level most commonly observed in standard farm-field conditions. LCO treatment constitutes a potential technology for reducing water deficit effects.

RÉSUMÉ

M.Sc. Sarra Atti Génie agricole et des biosystèmes

Bien que la sécheresse chronique soit fréquente dans de nombreuses régions, les recherches sur l'impact du déficit hydrique chronique sur la production agricole ont été limitées. La plupart des produits chimiques utilisés actuellement pour diminuer les pertes de rendement parviennent a réduire le stress mais n'augmentent pas la photosynthèse chez les plantes. La recherche a activement cherché les effets de l'application de Lipo-chitooligosaccharides (LCO). Cette étude a été conduite afin d'améliorer la connaissance de l'impact du déficit hydrique chronique et de tester une nouvelle technique de gestion de l'eau consistant en une application foliaire de LCO. Le projet visait aussi à évaluer les conséquences du traitement de LCO et du stress hydrique sur la réflectivité spectrale de la canopée de la plante. Une expérimentation fut conduite en serre sur des plantes de soya (*Glycine max* (L.) Merr.). L'-étude des effets individuels du stress hydrique sur la croissance du soya durant sa saison reproductive de développement a été organisé en un dispositif à blocs aléatoires complets (CRD) avec 6 blocks de 3 niveaux de déficit en eau (stress moyen - W2, stress sévère - W1, et un control bien arrosé - W3). L' étude des interactions entre l'application foliaire de LCO et le déficit hydrique a été organisé en un CRD avec 6 blocks des combinaisons factorielles des même trois niveaux de déficit par 2 niveaux de LCO (traité avec LCO – L1, et un control non traité – L0). Une solution de LCO Nod *Bj* V (141.6 µg/L) fut utilisée. Les conséquences du déficit hydrique durant la saison reproductive furent des diminutions de l'activité physiologique, de la croissance végétative, et de la productivité, et une accélération de la sénescence aux 2 niveaux de stress. Le stress hydrique a montré une tendance à augmenter la réflectivité foliaire dans le bande visible du spectre et de la diminuer dans l' infrarouge aux 2 niveaux de déficit. Sous stress sévère, le stress affecta négativement la concentration foliaire en chlorophylle, alors que sous stress moyen, le traitement n'a pas eu d'effet significatif. La pulvérisation foliaire de LCO a influencé l'activité physiologique de la plante, a augmenté le nombre de fleurs et de cosses par plante. Les LCO ont eu plus d'effets sur l'initiation des cosses que sur leur élongation. Les LCO ont accéléré la sénescence des plantes. Ainsi, il apparaît que les LCO peuvent accélérer la maturité physiologique. Le traitement de LCO a eu des effets significatifs sur la réponse spectrale obtenue chez les plantes sous stress moyen.

De manière générale, les effets des LCO furent plus marqués au niveau de stress moyen, qui est le niveau de stress le plus communément observe dans les conditions standard de culture au champ. Le traitement de LCO constitue une nouvelle technique pour réduire les effets néfastes du déficit hydrique.

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Chapter 1.
INTRODUCTION

1.1. Introduction

Soil water deficit is one of the phenomenons that can produce plant drought, (Monneveux and Belhassen, 1996). *Drought* induced by soil water deficit can be defined as a state where a dry soil (due to lack of rain or delayed irrigation) causes a substantial reduction in crop performance in terms of plant survival, economic yield or crop quality (Hall, 2001). Thus, it is important to clarify that soil water deficit is a cause and not a consequence of plant drought. The study of plant water deficit can bring forth information on crop breeding under conditions of drought. A large percentage of the world's crops are exposed to chronic or sporadic periods of climatic drought that might limit crop yield (Boyer, 1982). Water is one of the most yield-limiting factors for fast-growing plants (James, 1993). Water is required for such plant processes as photosynthesis, growth, and transpiration. Evapotranspiration, defined as the total loss of water by the cropped surface (Monneveux and Belhassen, 1996), usually accounts for about 99% of the water used by plants (James, 1993). Water deficit can affect negatively nearly all stages of a plant's life cycle (Mullet and Whitsitt, 1996). The responses of plants to drought vary greatly depending on species and stress severity.

Soybean (*Glycine max* (L.) Merr.) is a widely-produced nitrogen fixing crop. Soybeans fix nitrogen from the air, thereby decreasing the need for fertilizers, reducing production costs and possibly pollution. It is a major source of protein and oil for human and animal consumption. Because of its economic value, its growth characteristics have been widely studied in North-America and Europe. The importance of soybean as a crop is increasing in eastern Canada. In 1993, soybean was tied as the most widely produced crop in Ontario, at 676,000 ha. In Quebec, soybean production has risen from 3,000 ha in 1986 to approximately 140,000 ha in 2001. Soybean is considered drought sensitive (Itani *et al.*, 1992; Mullet and Whitsitt, 1996) and is reported to have a low water use efficiency (Sprent and Sprent, 1990). In recent decades, efforts to cultivate soybean have been extended into areas where soybean growth is more often exposed to severe drought or chronic soil water deficit. An important step in avoiding the detrimental effects of soil

water scarcity, before reaching actual yield reduction, is to assess and quantify the plant's growth patterns. Soybean yields have been related to moisture availability in many studies. Nevertheless, most research was conducted to study catastrophic events, such as severe droughts that can induce profound decreases of plant physiology and productivity. Not much research has been conducted to evaluate the impact of chronic water stress on crop production. It is precisely this long-term low water stress level that actual soybeans production is exposed to in many regions.

Research to mitigate yield losses in soybean crop production by improving water availability or water use efficiency through novel irrigation technologies has been very active. Yet, many of the irrigation techniques are too expensive and labour-intensive. Moreover, they often have had negative impacts on the environment such as salinization and waterlogging of soils. Therefore, simpler, less expensive and environmentally sound methods for soil water deficit control must be introduced. Several chemical treatments such as simazine and silicone have been used in various countries to reduce plant transpiration in arid and semi-arid areas such as California (Cheema and Uppal, 1980; Waggoner *et al*, 1981; Yadav and Pandey, 1997). These chemicals are effective for reducing water stress but fail to increase photosynthesis.

There has been considerable effort to enhance photosynthesis in crop plants that are sensitive to water deficit. Makela et al. (1999) reported enhanced photosynthesis under drought stress in tomato and turnip rape by foliar application of glycine-betanine at very low concentrations. Also, there has been much research on relationships between soybean growth and the rhizobia-legume symbiosis. Several North-American and European plant laboratories are studying Lipo-chitooligosaccharides (LCOs), also called Nod factors. LCOs are bacteria-to-plant signal molecules produced by rhizobia bacteria during the formation of the rhizobia-legume N_2 –fixing symbiosis. There has been much research on relationships between soybean growth and the rhizobia-legume symbiosis. LCOs have been shown to invoke a number of physiological changes in the host plant, which enhance photosynthesis and nitrogen accumulation. Soybean has been shown to react well to LCO treatment. However, the effects of LCO application on soybean plants under water deficit have not been studied.

Remote sensing has become a very dynamic field of research and innovation for crop water management, especially in the area of water use efficiency. Remote sensing may offer producers the potential for straightforward determination of crop water status, with little effort and time on their part. Timely assessment can be achieved by using remotely sensed data such as vegetation spectral reflectance. The study of changes of spectral reflectance may identify plant stress long before any visible observation is possible. Research on the impact of water stress on plant canopy reflectance has been very active in the last decade. However, the effects of LCO application on soybean canopy reflectance have not been studied yet.

1.2. Objectives

The general objectives of this thesis were to improve knowledge of the impact of chronic soil water deficit on soybean and to test a novel technique of water management consisting of LCO application. The specific objectives were:

1) To assess the impact of chronic water stress during reproductive growth on soybean development pattern and productivity and to evaluate changes in canopy reflectance due to the stress imposed,
2) To determine the effects of foliar application of LCO on the growth pattern and productivity of soybean plants under water stress and analyse the potential increase of soybean drought resistance and productivity by LCO treatment, and
3) To evaluate changes in canopy reflectance response due to LCO spray.

1.3. Organization of the thesis

This thesis consists of 7 chapters. Chapter 1 introduces the subject of the project and states the objectives of the study. Chapter 2 presents a concise review of the relevant and pertinent literature on the subject. Chapter 3 reports on the responses of soybean to chronic water stress during reproductive growth under greenhouse conditions. Chapter 4 describes the effects of foliar application of LCO on the growth pattern of soybean plants under water stress under greenhouse conditions. Chapter 5 presents a general discussion. Chapter 6 presents the general conclusions. Chapter 7 proposes suggestions for future research.

Chapter 2.
LITERATURE REVIEW

2.1 Plant water requirements

2.1.1 The plant-soil-atmosphere system

The water content of a physiologically active plant varies from 60 to 95% of the fresh weight of tissues and organs (James, 1993; Monneveux and Belhassen, 1996). Water is required for photosynthesis, growth, and transpiration. Plants extract water from the soil to replenish water lost by transpiration. Water flows from roots to leaves and is transpirated through stomata and the cuticle, which cools the transpiring organs. The water potential gradient from soil/xylem to the growing zone allows water movement into growing zones. Therefore, a small change in soil water potential changes the rate of water loss from leaf surfaces and can lead to growth inhibition (Mullet and Whitsitt, 1996). When the plant is unable to remove any more water from the soil, it wilts permanently.

If soil water is not limiting and the stomata are fully open, ambient conditions control the rate of transpiration. For instance, the stomata of most plants are open during the day and closed at night. However, when there is soil-water stress, the transpiration rate will be controlled by the plant's physiology. Thus, the temperature and humidity of the air, surrounding the plant, become important factors affecting transpiration in field or greenhouse conditions. Increasing ambient air humidity will result in a reduced rate of transpiration. The advantage of greenhouse experimentation over field experiments is that relative humidity, wind speed and temperature are more easily controlled and can be artificially set to any desired level (Monneveux and Belhassen, 1996).

2.1.2 Crop water requirements

2.1.2.1 Crop water requirements: Evapotranspiration

Plants use water primarily for transpiration. The process of *transpiration*, defined as evaporation from a living surface, usually accounts for about 99% of the water used by plants (Monneveux and Belhassen, 1996). Water is also transferred to the atmosphere by

direct evaporation of solid and liquid water from soil. Since these processes each involve evaporation and are not easily separated, they are combined and called evapotranspiration (ET) (James, 1993).

2.1.2.2 Estimation of Crop Evapotranspiration

The determination of plant water requirements is essential for irrigation scheduling. ET is known to be the most water-consuming process. Crop ET is commonly used to determine crop water requirements in field or greenhouse conditions. Crop ET has to be calculated as a part of irrigation planning. The empirical methods are the most commonly cited in the literature. They are much simpler and more convenient, and adequate accuracy can be obtained (James, 1993). The pan evaporation method for crop ET (ETc) estimation is used most frequently. It requires less time and effort to apply, while maintaining accuracy. Measuring the loss of water from an open-faced pan is a relatively inexpensive and simple way of assessing the evaporative capability of the atmosphere. It is used in the field and also in the greenhouse. The frequency of pan evaporation (Ep) measurements normally ranges from hourly to weekly. However, daily observations are most common and suitable. They are many different types of evaporation pans in use. The U.S class A pan is widely used. The following equation is used to estimate daily ETc from Ep:

$$\mathbf{ETc = Kp * Ep * Kc}$$

Kp = pan coefficient, and **Kc** = crop coefficient

Kp accounts for various parameters such as pan type, shape and colour and air humidity (James, 1993). FAO has established tables of data giving the Kp for various conditions and the Kc for different crops (Crop evapotranspiration: guidelines for computing crop water requirements, FAO, 1998). Kc depends on crop growth stages and air humidity.

2.2 Perception of water stress by plants

Plant growth (increase in size) is very sensitive to water status. Dehydration is one of the most common environmental stresses to which plants are exposed. In many regions

it is a serious limitation to agricultural development (McKersie and Leshem, 1994). There is hardly a physiological process in plants that is not impaired by water deficit (Bartels *et al.*, 1996). Under water deficit conditions, a slow down of biological activities is quickly observed at many levels of organisation: metabolism, growth, and turgidity (Monneveux and Belhassen, 1996). Plant responses to stress can be analysed at the whole plant level, at the organ or at the cell level.

Depletion of the soil water reserve causes a variety of responses. Timescales can range from a few minutes (wilting, stomatal closure), to weeks (change in leaf growth, senescence) or months (decrease in total biomass or yield) (Tardieu, 1996). After defining water stress, this chapter will present some of the main plant responses to water deficit, and some of the integrated processes that plants use to control water stress.

2.2.1 A discussion of plant water stress

Drought is said to occur when dry soil (due to lack of rain or delayed irrigation) causes a substantial reduction in crop performance. This can be in terms of either plant survival, economic yield or crop quality. *Water deficit stress* is a drought-induced stress (Hall, 2001). The concept of water deficit stress is difficult to characterize in a quantitative way. *Water Stress* can be defined either by considering, a) water status at the boundaries of the plant (soil, air), or by b) plant water status (leaf or root water status). These approaches to a definition are far from equivalent. The first involves only environmental variables. The second depends on internal control loops of the plant (Tardieu, 1996).

It is reported that only excessive water deficits can cause catastrophic events such as severe cell dehydration (Cornic and Briantis, 1991; Jones and Sutherland, 1991) . Cells of plants under mild and protracted water deficit do not experience water stress. Under a large range of environmental conditions, water stress at the whole-plant level is not necessarily reflected at the single-cell level. Several regulatory processes at the whole-plant level allow these cells to maintain homeostasis.

Defining water stress by plant water status is only possible and accurate when plants are under near-catastrophic conditions. Under water deficits compatible with agricultural situations, the plant crop plant will generally exercise tight control of plant

water status (limiting, for instance, decreases in leaf water potential). Thus, "*water stress*" should be defined by water status at plant boundaries (Tardieu, 1996).

2.2.2 Plant responses to drought: analysis at the whole-plant level

Crop responses to drought stress are mediated by processes of response to water deficit at various levels of plant organization. The following paragraphs will focus on the whole plant level analysis. The effects of and plant responses to drought at the whole plant and crop level are more complex because they reflect the integration of stress effects and responses at all underlying levels of organization over space and time. Scientific methods can reasonably explore the effects of drought by investigating singular facets or unidimentional planes of the whole system. With insufficient information on the subject, the creation of an integrated thesis may be at times overly simplistic and at other times speculative (Blum, 1996). Still, research must strive for an eventual integration if practical application of this knowledge is sought. The following is a concise review of some of the major aspects of crop response to drought stress at the whole plant level.

2.2.2.1 Phenology

Phenology and its modifications by drought stress affect plant production under drought stress through various pathways (Blum, 1996). Water-use is affected by growth duration, which determines water-requirements and the probability of exposure to stress, both of which decrease in early flowering genotypes. Water-use is affected by phenology also by way of leaf area, which is larger in later flowering genotypes even after the difference in growth duration is accounted for (Blum and Arkin, 1984). Blum (1996) reported that phenology has a powerful effect on plant growth, response and productivity under drought stress. Drought often delays developmental events because of the inhibition of growth due to water deficit. For instance, a delaying effect on flowering has often been reported (King and Evans, 1977; Donatelli *et al.*, 1992). However, a careful review of literature suggests that flowering may or may not be delayed depending on the species and the level of stress. Mild stress caused advanced flowering (Angus and Moncur, 1977), while severe stress caused delayed flowering in wheat (Angus and Moncur, 1977; Dwyer and Stewart, 1987). Water stress of a given duration and intensity was found to be more

or less damaging depending on the plant developmental state (Oosterhuis and Cartwright, 1983). For instance, water stress was less damaging when applied earlier than when applied later during the tillering stage of wheat (Blum *et al.*, 1990). The state of plant growth before stress has a major effect on plant behaviour during stress, not only in terms of its development features but also in terms of its carbon status (Blum, 1996).

2.2.2.2 Leaf area

Water loss at the plant level largely depends upon the size of the evaporating areas (leaves, stems) (Monneveux and Belhassen, 1996). As leaf area is determined by phenology, stem morphology, rates of leaf emergence and potential leaf size, any effect of drought on these factors will modify leaf area. Water loss at the plant level largely depends upon the size of the evaporating areas such as the leaves and stems (Monneveux and Belhassen, 1996). The effective light-intercepting leaf area on a single stem is reduced by drought by way of reduced cell expansion, reduced cell division, leaf rolling, Para-heliotropism, death of apical parts of leaves and death of whole leaves (Blum, 1996). It is also worth mentioning that a severe reduction in stomatal conductance is generally observed when leaves approach wilting, such as when cereal leaves roll (Blum, 1996).

2.2.2.3 The formation of yield

As drought stress impairs the growth of organs and their final size, a reduction in final yield can be expected. Since it has not been established how exactly water deficit causes growth reduction, it has not been possible yet to explain how yield components are reduced by drought stress. It is important to point out that most research dealing with yield reduction under drought stress has been largely of a descriptive nature. Blum (1996) explained that this is simply because the disciplines concerned with crops and yield are not equipped to investigate lower levels of plant organisation where explanations may reside. He further explains that disciplines involved with lower levels of plant organization are generally not interested in yield.

Yield is formed by the creation of a sink and its subsequent filling by the source. Drought stress affects yield by depressing both sink and source, depending on the timing and the severity of stress with respect to plant phenology (Blum, 1996). It is generally

agreed that when drought stress is sensed by the plant at any stage when the plant is still in the reproductive stage, its response is to decrease the reproductive demand for carbon by a reduction in the number or size of sinks (Blum, 1996). Therefore, tillers degenerate, flowers drop, pollen dies and ovules abort under stress (Casal, 1998; Blum, 1996).

2.2.3 Integrated processes avoiding plant water stress

Partial maintenance of plant water status under water deficit is allowed by plant regulatory processes. All the controls tend to reduce transpiration (stomatal closure), reduce leaf growth or leaf senescence, or to increase water uptake (maintenance of root growth or increase in root/shoot ratio) (Tardieu, 1996).

2.2.3.1 Stomatal closure

Water deficit often leads to decreases in stomatal aperture. Decreased stomatal conductance is reported to be an adaptive morphologic mechanism that can modulate water losses by transpiration. The decrease in stomatal conductance has been associated with reduction in stomatal transpiration (Monneveux and Belhassen, 1996; De Souza et al., 1997). In response to water stress, stomatal closure can occur rapidly; it takes less than 1 min in some plants (Monneveux and Belhassen, 1996). Stomatal closure simultaneously reduces transpiration and limits the flow of water from roots to leaves. This, in turn, reduces the movement of nitrate and other compounds from roots to leaves and thus reduces nitrogen availability and synthesis of amino acids. Since CO_2 enters the leaf through the stomata, stomatal closure will also result in a decreased photosynthetic rate, causing a reduction in growth, yield, and quality (James, 1993).

2.2.3.2 Control of leaf and root growth

Plant transpiration essentially depends on leaf area. Evaporative demand can be controlled by a decrease in leaf growth, which is usually the first symptom of mild water deficit (Boyer, 1970; Hsiao, 1973; Saab and Sharp, 1989). Leaf area is affected either by reduction of individual leaf growth, or by reduction in the number of leaves. Plasticity in leaf area is an important means by which a drought-stressed crop maintains control over

water-use (Blum, 1996). Before flowering, the reduction in leaf area index and intercepted radiation under stress are largely a result of impaired leaf expansion and changes in leaf display. After flowering, this reduction is mainly a result of progressive leaf senescence. Reduction in leaf expansion rate occurs before any reduction in photosynthesis per unit of leaf area (Saab and Sharp, 1989). It is widely reported that the number of leaves can vary substantially. The number of branches can also be reduced, as suggested by observations of tillering in forage grass species or in wheat.

2.2.3.3 Leaf senescence

Moderate water deficits can induce considerable reduction in green leaf area (Wolfe *et al.*, 1988; Hall, 1993) as a consequence of senescence. Increased senescence rate observed in plants subjected to water deficit in the field resembles "natural" senescence that involves a plant genetic programme: it occurs at relatively moderate leaf water potentials, beginning in older leaves located in the lowest layer of the canopy (Wolfe *et al.*, 1988). As a consequence, drought-induced senescence is considered as a whole-plant mechanism which reduces leaf area in the presence of water stress, in order to: (i) reduce transpiration and the difference in potential between roots and leaves and (ii) remobilise assimilates to seeds or other growing organs.

2.3 Importance of soybean crop

2.3.1 Soybean crop production

Soybean (*Glycine max* (L.) Merr.) belongs to the family Leguminosae, which has a worldwide distribution. The genus *Glycine* has trifoliate leaves. Flowers are inserted singly at each node of the raceme. There are two stem growth habits and floral initiation patterns in soybean. Indeterminate varieties have a terminal bud that continues vegetative activity during most of the growing season, whereas determinate varieties cease vegetative activity at flowering (Allen and Allen, 1981). In eastern Canada, indeterminate soybean varieties are most commonly cultivated because they are more appropriate to the short season.

Soybean growth characteristics have been extensively studied, because of the crop's economic importance. It is the world's most widely produced nitrogen fixing crop. Soybean, in symbiosis with *Bradyrhizobium japonicum*, can fix nitrogen from the air, therefore decreasing the need for fertilizers, reducing production costs and pollution. Moreover, soybean plays an important role in the supplement of protein for livestock and is also one of the major oilseed crops.

In Canada, soybean production is expanding. In Quebec, soybean production has risen from 3,000 ha in 1986 to approximately 140,000 ha in 2001. Production in eastern Canada has expanded largely due to the introduction of early cultivars, creating a demand for comprehensive agronomic techniques for optimum production. Agronomic research on the best soybean production systems has been conducted in Ontario and Quebec.

2.3.2 Soybean water use

The water use efficiency of legumes is generally low and they are, as a group, quite sensitive to drought stress (Sprent and Sprent, 1990; Itani *et al.*, 1992; Mullet and Whitsitt, 1996). Soybean is more sensitive to soil and weather conditions than pea and peanut (Hall, 2001). Mullet and Whitsitt (1996) reported that mild water deficits that cause a reduction in plant turgor, or 10-15% decreases in plants water content, resulting in large changes in growth and metabolism. However, this only caused plant death if these conditions persisted for long periods of time. Soybean seedlings that have lost more than 30% relative water content, show reduced survival. Common bean and soybean are considered to waste available soil water under severe drought conditions. In comparison, cowpea and green gram are known to survive for a longer period of time under drought conditions, due to a better water use efficiency (compared with soybean and common bean) (Itani *et al.*, 1992). Some stages of the plant's life cycle are less susceptible to large reductions in water content. This occurs especially during seed development, where a programmed reduction in water content can occur without loss in viability (Mullet and Whitsitt, 1996).

Soybean requires water to grow. Unstressed, soybean stomatal transpiration represents approximately 90% of total water losses (Monneveux and Belhassen, 1996). Water lost by transpiration during the day frequently exceeds that absorbed by roots,

which creates a diurnal rhythm of internal water stress (Slatyer, 1967). This stress can occur when water is available in the soil but it is, of course, enhanced by soil water deficit and by hot, dry winds, which increase the evaporative demand on the plant.

2.3.3 Research on the relationships between LCOs and soybean development

There has been much research on relationships between soybean growth and the rhizobia-legume symbioses (Long, 1989; Scheres *et al,* 1990; Boone *et al,* 1999).

2.3.3.1 What are the LCOs ?

Lipo-chitooligosaccaride (LCO) molecules were only discovered in 1991. Therefore, the research on LCOs is recent and dynamic. LCOs are bacteria-to-plant signal molecules essential for the establishment of rhizobia-legume symbioses. Known as specific nodulation signal molecules, LCOs also share some common traits with phytohormones. Like most phytohormones, LCOs are active over a very wide range of concentrations. However, to date, the mechanism of the hormone-like activity of LCOs has not been well investigated. Bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Azorhizobium*, collectively known as rhizobia, form specialized organs called nodules on the roots and sometimes stems of legumes and fix atmospheric nitrogen within these structures. Nodule formation is a highly specialized process that requires cross "talk" between the bacteria and the host plant.

In general, the interaction is a two-step process. The first is the release of the plant-to-bacteria signal molecules, usually specific flavanoids, by the host plants. The second step is the release of bacteria-to-plant signal molecules, which are LCOs, also known as Nod factors (Long, 1989; Scheres *et al,* 1990; Kondorosi, 1991; Boone *et al,* 1999).

The structure of LCOs from different rhizobia has been determined as an oligomer of three to five β-1,4 linked molecules of N-acetyl-D-glucosamine with additional substitutions on the terminal sugar residues (Denarie and Cullimore, 1993). Some strains of rhizobia synthesize a large number of LCOs. The LCOs produced by *B. japonicum* are pentameric molecules with C18:1, C16:1, and C16:0 fatty acid chains at the non-reducing end and 2-0-methyfucose at the reducing end of the chitin backbone (Carlson *et al,* 1993).

2.3.3.2 The effects of LCO on soybean development

It has been shown that soybean responds well to LCO treatment. Therefore, research on the effects of LCO on soybean development has recently been very active.

2.3.3.2.1 Effects of LCO on soybean, at the molecular level

LCOs stimulate the expression of host nodulin genes essential for infection thread formation (Horvath *et al.*, 1993; Pichon *et al.*, 1993, Minami *et al.*, 1996). LCOs have also been shown to activate defense-related enzymes (Inui *et al.*, 1997). Further, a number of studies have shown that the structure of LCOs appears to modulate the host specificity of rhizobia-legume interactions (Spaink *et al.*, 1991; Schultze *et al.*, 1992). This indicates that LCOs are mitogenic and morphogenic agents and may produce the same effect as the direct application of cytokinins (BAP, 2iP or kinetin) or inhibitors of auxin transport (Relic *et al.*, 1993). It is known that the perturbation of auxin-cytokinin balance provokes large modifications in plant growth and development.

Moreover, it has been found that the addition of various LCOs to soybean roots rapidly induced the ENOD40 expression (Minami *et al.*, 1996) and it was postulated that ENOD40 can function in plants as a cytoplasmic RNA to control phytohormone balance (Crespi *et al.*, 1994). Therefore, the external application of LCO can modify the control of the balance of phytohormones (e.g auxin-cytokinin ratio) and provoke large changes in plant growth and development.

2.3.3.2.2 Effects of LCO on the growth of soybean roots

LCOs are reported to induce root hair deformation (Spaink *et al.*, 1991), ontogeny of compete nodule structures (Fisher and Long, 1992; Denarie and Cullimore, 1993), and cortical cell division (Sanjuan *et al.*, 1992; Schulaman *et al.*, 1997). It was reported that LCO from different rhizobia are able to directly initiate the formation of new organs, nodules, on the roots of leguminous plants (Perret *et al.*, 2000). This suggests that LCOs have an ability to initiate plant morphogenesis.

Soulamanov et al. (2002) conducted greenhouse experiments to evaluate the effect of LCO on the growth of soybean and corn roots. The roots of 3-day-old seedlings

of soybean and corn were immersed in aerated water solutions containing 10^{-7}, 10^{-9}, and 10^{-11} M LCO. After a 7-day exposure to LCO, the corn shoot biomass and height were by 7-11% and 7%, respectively, greater than the control. At 10^{-9} M and 10^{-7} M LCO, the soybean root biomass was 7-16% larger and roots were 34-44% longer than in control. Soybean shoot biomass was not affected by LCO treatments but there was a 9% stimulation of shoot height at 10^{-7} M LCO. Therefore, the application of LCO stimulates biomass accumulation and changes in plant structure and morphology for soybean and corn roots. Prithiviraj et al. (2000) suggested that LCO is not only active as a nodulation signal molecule but can have growth promoting effect. The LCO-induced increase in plant growth could be caused by the "hormone- like" effect of LCO.

2.3.3.2.3 Effects of LCOs on plant photosynthesis

Research on LCOs effects on plant photosynthesis has been widely conducted for several years. Several authors have shown that LCOs invoke a number of physiological changes in host plant nodulation and nitrogen accumulation (Spaink *et al.*, 1991; Denarie and Cullimore, 1993; Minami *et al.*, 1996).

Several experiments, conducted in D. L. Smith's laboratory (Macdonald Campus of McGill University, QC, Canada) have demonstrated that LCO treatment (Nod BjV ($C_{18:1}$,MeFuc) isolated from *B. japonicum,* strain 532C) enhances germination and early growth of various crop plants (Prithiviraj *et al.*, 2000; Souleimanov *et al.*, 2002). Nod BjV ($C_{18:1}$,MeFuc) increases the photosynthetic rates and productivity under greenhouse and field conditions for a number of crop plants, such as soybean, corn (*Zea mays*), melon (*Cucumis melo*) and potato (*Solanum tuberosum*). Prithiviraj et al. (2000) showed that LCO treatment enhanced the photosynthetic rates of all the plants tested, including both legumes and non-legumes. However, the responses varied with species and LCO concentration used. The days for maximum increase and the most effective concentration of LCO differed among the species. In general, a 10-20% increase in photosynthesis was common. Under greenhouse conditions, soybean (cultivar OAC Bayfield) showed the largest increase in photosynthesis due to LCO spray. Under field conditions, LCO spray treatment of soybean resulted in increased branch number, leaf area, pod number, plant dry matter and grain yield. LCO application enhanced soybean grain yield by 33-44%. In

general, the photosynthetic responses of soybean in the field were similar to those observed under greenhouse conditions. However, Soulamanov et al. (2002) reported that the LCO effect on the field grown plants was less pronounced and required higher concentrations for better effects. This might be related to the greater environmental variability, and increased likelihood of some other stresses imposing limitations under field conditions.

Soulamanov et al. (2002) pointed out that LCO application resulted in increased stomatal aperture without any increase in leaf internal CO_2 concentration. Soulamanov et al. (2002) proposed that there was an increase in CO_2 uptake by chloroplasts inside the leaf, which lead to increased stomata opening. Because the stomata of the C_3 plants (soybean, rice, melon, canola) were more opened, there were concomitant increases in transpiration for the leaves of LCO-treated plants. These results were similar to those observed for glycinębetanine application (Makela *et al.*, 1999). The link between stomatal aperture and photosynthesis rate would seem to apply in the C_3 plants tested. However, Prithiviraj et al. (2000) suggested that, in the case of LCO application, the increase in stomatal aperture was the result of greater photosynthetic CO_2 uptake by the chloroplasts, and not the primary cause of increased photosynthesis rate.

The above review on LCO properties suggests that LCOs can be used to increase soybean productivity and also indicates the potential utility of LCO application with a wide range of crops. Several experiments, conducted in D. L. Smith's laboratory (Macdonald Campus of McGill University, QC, Canada) have demonstrated the increase in photosynthesis due to LCO treatment (Nod BjV ($C_{18:1}$,MeFuc) isolated from *B. japonicum,* strain 532C) was always accompanied with increases in stomatal aperture that led to increases in transpiration for the leaves of LCO treated plants. Thus, it seems that foliar application of LCOs could be a way to mitigate drought stress by enhancing water use efficiency. Such treatment should stimulate photosynthesis, which will increase plant productivity under stress.

2.4 Impact of water stress on soybean development

2.4.1 Impact of water stress on soybean growth at the whole plant level

As it was mentioned before, the effects of and plant response to drought at the whole plant and crop level is complex to analyse. Yet, it allows a sense of the overall effects of water stress on soybean development.

2.4.1.1 Impact of water stress on soybean leaf area

It was shown in the previous sections that, under water stress conditions, the limiting factor for dry matter production is leaf expansion ability (Frederick *et al.*, 1991; Wang *et al.*, 1995). Rapid leaf enlargement is important because leaves are the major light intercepting and photosynthesizing organ of the soybean. Shibles and Weber (1965) found leaf area and growth rate of soybeans to be nearly proportional. Therefore, early moisture stress reduces growth rate by inhibiting leaf enlargement. Field-grown soybean plants, which had been water-stressed for approximately 60 days, had a smaller leaf area than non-stressed plants.

2.4.1.2 Impact of water stress on soybean phenology

As noted in the previous section, water stress modifies the phenology of plants, and thus affects the yield components. Desclaux and Roumet (1996) studied the impact of various periods of water stress on organ appearance rate and on the durations of the main vegetative and reproductive periods of greenhouse grown soybean plants (Weber and Spot cultivars). The soybean plants were subjected to drought stress during a main developmental stage: i.e., vegetative, flowering, pod lengthening or seed filling. Desclaux and Roumet (1996) reported that, under stress, the duration of the main reproductive phase was increased on the main stem but reduced on the branches for cultivar Weber (indeterminate); whereas the opposite response was noted for Spot (determinate cultivar). They concluded that the cultivars differed mostly in the phenologic aspect of their development, as they differed with respect to their strategies of partitioning between the main stem and branches.

Desclaux and Roumet (1996) also showed that drought stress seemed to trigger a signal that caused an early switch of plant development from vegetative to reproductive. Appearance of nodes was delayed, resulting in a small number of nodes produced, whereas flower and pod appearance were hastened. They concluded that each reproductive phase was shorter under stress, mainly because of the appearance of new organs that prevented the emergence of organs belonging to the earlier ontogenetic phases. They reported that the seed-filling stage and the final stage in seed abortion began earlier in stressed plants and the duration of the maturation period was significantly reduced by stress during seed filling, leading to accelerated senescence. In this respect, De Souza et al. (1997) also reported that water stress during seed filling could reduce soybean yield by accelerating leaf senescence and shortening the seed filling period. Furthermore, the extent of the yield reduction depends also on other factors such as the stage of development and the duration of stress.

2.4.1.3 Effect of water stress on soybean canopy architecture

Some adaptive mechanisms are reported to modulate the importance of stomatal or residual transpiration, and therefore limit water stress effects (Wang *et al.*, 1993; Monneveux and Belhassen, 1996). Research showed that leaf movement is related to intercepted radiation and leaf temperature as an adaptive mechanism under water-stress conditions (Wang *et al.*, 1993; Wang *et al.*, 1994). The effective light-intercepting leaf area on a single stem is reduced by drought by several ways, such as leaf rolling and para-heliotropism (Blum, 1996).

2.4.1.3.1 Leaf rolling and wilting

Depletion of the soil water reserve causes wilting in a large range of plants. Leaf rolling, more specific to grass plants, is induced by the loss of turgor of bulliform cells. The induced-reduction of light interception and leaf heating can considerably reduce transpiration (Monneveux and Belhassen, 1996), and therefore mitigate the negative effects of water deficit. Leaf rolling is commonly observed in grasses under water stress (Wang *et al.*, 1993; Wang *et al.*, 1994).

2.4.1.3.2 Heliotropic leaf movement

For heliotropic plants such as soybean, architectural changes occur as the leaves respond to the movement of the sun during the day (Ehleringer and Forseth, 1980; Beggs, 1980; Kimes and Kirchner, 1983). Diaheliotropic movements maintain the leaf surface perpendicular to the direction of incident radiation, maximizing solar energy interception. Paraheliotropic movements result in a leaf orientation parallel to incident radiation. Both diaheliotropic and paraheliotropic movements have been commonly observed in soybean leaves (Travis and Reed, 1983; Moran *et al.*, 1989; Wang *et al.*, 1993; Wang *et al.*, 1994; Isoda and Wang, 2001).

Travis and Reed (1983) reported that leaf cupping, a paraheliotropic movement in which leaflets converge, was associated with sun avoidance. Moran et al. (1989) showed that both heliotropic movements were directly affected by crop water status. Moran et al (1989) observed that, under well-watered conditions, plant leaves tended to track the sun throughout the day through diaheliotropic movements, in both azimuthal and zenithal directions, whereas leaf cupping and paraheliotropic behavior were more pronounced in the stressed leaves. In the stressed plants, diurnal tracking ability diminished as the day progressed and the canopy showed a more vertical profile due to leaflet cupping. This corresponded with minimum leaf water potentials. However, in the afternoon, the plants recovered their sun-tracking ability even though the water potential remained relatively low. Moran et al. (1989) concluded that paraheliotropic movement represents a mechanism permitting stressed plants to avoid solar radiation during midday hours, which was also observed by Travis and Reed (1983). Similarly, Wang et al. (1993) reported that the leaves of water stressed soybean had very active paraheliotropic leaf movement at both diurnal and seasonal scales, as compared with non-stressed leaves, which were diaheliotropic most of the time.

Paraheliotropism has been reported to be drought related, as it was shown to reduce leaf temperature and water loss (Travis and Reed, 1983; Moran *et al.*, 1989; Wang *et al.*, 1993; Wang *et al.*, 1994; Isoda and Wang, 2001). Wang et al. (1993; 1994) studied the leaf movement of soybean under water deficit conditions in terms of canopy structure and leaf temperature. They investigated two soybean cultivars with different leaf movements, Heinong 33 (inactive), and Zhengzhuta 2 (active). They found that the leaves

of the water stressed plots of Zhengzhuta 2 actively moved to be parallel to the sun's rays, whereas the leaves of water stressed plots of Heinong 33 showed signs of wilting during the day. In Zhengzhuta 2, the leaf temperature of the terminal leaflet of the upper layer of plants in the water stressed plots was lower than the air temperature during most of the day. Whereas plants in water stressed plots of Heinong 33 had rather high leaf temperatures, as compared with air temperature. They concluded that soybean cultivars without active leaf movement are more affected by water stress. They also concluded that changes with time of leaf movement correspond to changes in the temperatures of the leaves. Isoda and Wang (2001) studied leaf movement in terms of radiation interception, leaf temperature and transpiration under water stressed conditions. They found that paraheliotropic leaf movement reduced water loss by 71% in the water stressed soybean plants. They reported that paraheliotropic leaf movement decreased radiation interception. They also showed that both leaf movement and transpiration adjust leaf temperature. Therefore, paraheliotropism has been associated with water stress in soybean. It is reported to be a visible morphological response of soybean to water deficit.

2.4.2 Impact of water deficit on soybean physiology

2.4.2.1 Changes in transpiration and stomatal conductance

It is widely reported that one of the major effects of water stress on soybean is decreased stomatal conductance, and that transpiration rate, stomatal conductance and photosynthetic rate are closely related (Shimshi and Ephrat, 1975; Grantz, 1990; Lu *et al.*, 1994; Reynolds *et al.*, 1994; Lee *et al.*, 1994; Tourneux and Peltier, 1995; Blum, 1996; Tardieu *et al.*, 1996; De Souza *et al.*, 1997). Lee et al. (1994) showed that transpiration rate and stomatal conductance rapidly decreased more rapidly with the decreasing of soil moisture than the photosynthetic rate. De Souza et al. (1997) reported that water stress decreased soybean stomatal conductance. Stomatal conductance in the control treatment was >1 cm/s during much of the seed filling period in both experiments they conducted. Stomatal conductance in the severe stress treatment declined rapidly. Stomatal conductance for the moderate stress treatment remained between the well-watered and severe stress treatments. As the plants approached maturity (growth stage R8) and the

leaves were turning yellow, additional declines in stomatal conductance was reported. Moreover, a severe reduction in stomatal conductance is generally observed when leaves are approaching wilting (Blum, 1996).

2.4.2.2 Changes in photosynthesis

The effect of drought stress on photosynthesis has been widely studied (Boyer, 1970; Chaves, 1992; Havaux, 1992; Wang *et al.*, 1995; Allen *et al.*, 1994; Chernyad'ev, 1997; Berkowitz, 1998; Lawlor *et al.*, 1998). Water stress reduces the production of chlorophyll and plant growth (Osborne *et al.*, 2002). The effects of water stress on photosynthesis are related to its effects on leaf area and root growth, and on the stomatal control of transpiration. Reduced photosynthesis is associated with a decrease in the supply of assimilates to the plant, during both vegetative and reproductive development of soybean plants. Boyer (1970) showed that photosynthesis was much less sensitive to moisture stress than was leaf enlargement. When moisture stress is sufficient to limit photosynthesis, nitrogen fixation is also reduced, since photosynthesis, transpiration, and nitrogen fixation are correlated (Huang *et al.*, 1975). El-Kheir et al. (1994) showed that decreasing the available soil moisture content reduced the content of photosynthetic pigments (chlorophyll a and b and carotenoids) in soybean leaves. More generally, water stress reduces the total chlorophyll content (Chernyad'ev, 1997; Osborne *et al.*, 2002), changes the chlorophyll a/b ratio, modifies chloroplast ultra structure, inhibits light and dark photosynthetic reactions, and decreases the efficiency of photosynthetic CO_2 assimilation (Chernyad'ev, 1997). A low photosynthetic rate has been associated with low leaf water potential (Hirasawa *et al.*, 1994).

2.4.3 Impact of water stress on important agronomic characteristics of soybean

Variations in the ambient conditions, at any time of the growing season, may cause a stress that will affect agronomic characteristics of soybean plants. Not all mechanisms of water stress are understood, but the resulting effect on the plant agronomic factors, such as yield, are known.

2.4.3.1 Impact of water stress on soybean yield and yield components

2.4.3.1.1 Impact of water stress on soybean yield components

Seed yield of soybean is produced on both the main stem and branches originating from main stem nodes (Board, 1987). However, most branch vegetative growth does not occur until between growth stage R1 (initial flowering on main stem) and initial seed fill (Egli *et al.*, 1985; Board and Settimi, 1986). Unfavorable growing conditions reduce soybean seed yield primarily by reducing branch growth and branch seed yield per plant (Ramseur *et al.*, 1984b; Board *et al.*, 1990; Frederick *et al.*, 1998; Linkemer *et al.*, 1998). These results indicate that branch seed yield of soybean is dependent on the amount of branch vegetative growth that occurs during the flowering and pod formation stages of development.

Less is known about the effects of drought stress on both soybean branch vegetative growth and branch seed yield, and how drought stress affects the distribution of seed yield between the main stem and branches. Desclaux and Roumet (1996) studied the impact of various periods of water stress on the soybean plant phenology. They found that, under stress, the duration of the main reproductive phase (flowering, pod lengthening or seed filling) was increased on the main stem but reduced on the branches. James et al. (2001) found that water stress occurring between initial flowering and seed fill decreases total seed yield primarily by reducing branch vegetative growth, which reduces branch seed number and branch seed yield. The stress treatment had no effect on main stem seed yield. Therefore, drought stress between initial flowering and seed fill has a greater impact on branch seed yield than maimstem seed yield due to the negative effects of stress on branch growth, which occurs mostly between initial flowering and seed fill.

2.4.3.1.2 Effect of water stress during soybean vegetative development

Moisture stress is an important determinant of crop yield in many environments. Soybean is no exception because plant height, number of nodes, stem diameter, number of flowers, percentage pod set, number of seeds, and seed weight are positively related to soil moisture. Soybean yields have been related to moisture availability in many

experiments (Doss *et al.*, 1974; Ashley and Ethridge, 1978; Korte *et al.*, 1983b). Any period of stress, regardless of length, would be expected to cause changes in the plant, which reduce metabolic activity, and hence, lower seed yield (Hicks, 1978). Even though the soybean plant can withstand a short period of drought, a longer stress period will greatly affect soybean yield. However, dry matter production and yield ability varies greatly among soybean cultivars (Doss *et al.*, 1974; Frederick *et al.*, 1991; Wang *et al.*, 1995).

Moisture deficiency during the vegetative stages of soybean development reduces the rate of plant growth, but, usually, does not affect soybean seed yield. The effects on vegetative growth are reflected in smaller leaves, reduced stem diameter, and reduced plant height (Newark, 1991, E-Kheir *et al.*, 1994). Newark (1991) showed that stem growth in soybean subjected to limited water is inhibited first by a physical limitation followed in a few hours by metabolic changes that reduce the extensibility of cell walls. This inhibition is maintained as long as water deficit is maintained. Many reports have noted that there was less effect of water stress on seed yield before the reproductive stages as compared to after (Doss *et al.*, 1974; Sojka *et al.*, 1977; Ashley and Ethridge, 1978; Martin *et al.*, 1979; Heatherly, 1983; Ramseur *et al.*, 1984a; Hirasawa *et al.*, 1994).

2.4.3.1.3 Effect of water stress during flowering and early pod development

The negative effects of water stress are particularly important during flowering, pod set and pod filling. Moisture stress during the flowering period is widely associated with increased abortion of flowers and young pods. Irrigation, beginning at flowering, is reported to be as effective in increasing yield, as is irrigation throughout the growing season. In soybean, most reproductive abortion occurs at an early stage of embryo development. Soybean plants produce an abundance of floral buds, but a large proportion of the ovaries abort prior to developing into mature pods (Kato, 1964; Brevedan *et al.*, 1978; Wiebold *et al.*, 1981). A deficient water supply during this crucial period is reported to induce a major increase of the rate of abortion (Kato, 1964; Westgate and Peterson, 1993). It has been widely shown that long-term water deficit during flowering and early pod set decreases soybean yield (Shaw and Laing, 1966; Doss *et al.*, 1974; Sionit and Kramer, 1977; Westgate and Peterson, 1993; Kokubun *et al.*, 2001).

Water deficit yield loss is due primarily to a decrease in pod number per plant (Shaw and Laing, 1966; Doss *et al.*, 1974; Westgate and Peterson, 1993; Kokubun *et al.*, 2001). It has been proven that the overall decrease in pod number is due to an increase in flower and pod abortion (Shaw and Laing, 1966; Westgate and Peterson, 1993; Kokubun *et al.*, 2001). It was reported that water stress imposed during flowering reduces photosynthesis and the amount of photosynthetic assimilates allocated to floral organs, and might thereby increase the rate of abortion (Raper and Kramer, 1987). Westgate and Peterson (1993) verified that plant water deficits have a direct effect on the water status and development of reproductive structures in soybean, from the bud stage to early pod formation. Westgate and Peterson (1993) clearly showed that water deficits (low plant water potentials) imposed when flowers reached anthesis, pod set, or early pod expansion inhibited flower development, by decreasing the percentage of flowers that initiated pod expansion and maintained pod development. Therefore, Westgate and Peterson (1993) asserted that water deficit decreases pod set by inhibiting pod expansion and metabolism. A brief water deficit during flowering decreased pod set on terminal racemes of soybean plants by as much as 70%. Kokubun et al. (2001) found similar results, water caused by restriction of watering for 3 days during the preanthesis stage significantly increased the abortion of flowers.

2.4.3.1.4 Impact of water stress during pod/seed filling

It has been proven that moisture stress causes maximum reduction in seed yield if it occurs during the pod-filling stage (Shaw and Laing, 1966; Doss *et al.*, 1974; Sullivan and Brun, 1975; Meckel *et al.*, 1984; Smiciklas *et al.*, 1989; DeSouza *et al.*, 1997). The pod filling (or seed filling) period is always reported to be the most critical for water stress. Doss et al. (1974) concluded from a 3-year study of soybean (cv. Bragg) that stress during any part of the season reduced yields, but greatest reductions occurred when moisture was limiting during the pod-filling stage. Water stress during flowering and early pod development caused more flower and pod abortion, whereas seed size was reduced by water stress during the later stages of pod filling.

Individual seed growth rate is related to assimilate supply (Egli *et al.*, 1985; 1989). It has been reported that water stress during pod/seed filling reduces yield by

reducing seed size rather than reducing seed growth rate, reflecting a decrease of seed dry matter accumulation (Whitt, 1954; Doss *et al,* 1974; Ashley and Ethridge, 1978; Whigham and Minor, 1978; Korte *et al.*, 1983a; Meckel *et al.*, 1984; Smiciklas *et al.*, 1989; Vieira *et al.*, 1992; DeSouza *et al.*, 1997). Several authors reported that seed growth rate is relatively insensitive to plant water stress (Meckel *et al.*, 1984; Westgate *et al.*, 1989; DeSouza *et al.*, 1997). These reductions in seed size have been associated with earlier maturity (Ashley and Ethridge, 1978; DeSouza *et al.*, 1997) and a shorter seed-filling period (Whigham and Minor, 1978; Meckel *et al.*, 1984; Smiciklas *et al.*, 1989; DeSouza *et al.*, 1997). It has been also shown that the shorter seed filling period results from accelerated leaf senescence (Sionit and Kramer, 1977; Whigham and Minor, 1978; Egli and Crafts-Brandner, 1996; DeSouza *et al.*, 1997). However, De Souza et al. (1997) reported the effect of water stress during seed filling depended on the cultivar. They concluded that in this case, the only visible and accurate effect of the water stress was acceleration of senescence.

2.4.3.2 Impact of water stress on leaf senescence

Leaf senescence in soybean, characterized by declines in leaf N, chlorophyll, and photosynthesis, begins early in seed filling and is usually complete by physiological maturity (Wittenbach *et al.*, 1980; Egli and Crafts-Brandner, 1996). Changes in leaf chlorophyll and N are symptomatic of leaf senescence (Thomas and Stoddart, 1980; Egli and Crafts-Brandner, 1996).

Water deficit accelerates senescence, which is caused by the accelerated decline in leaf photosynthetic activity (Chernyad'ev, 1997; DeSouza *et al.*, 1997). De Souza et al. (1997) found that the effect of moisture stress during seed filling on leaf senescence was consistent with the effect on stomatal conductance, yield, seed fill duration, and seed size. The acceleration of leaf senescence was associated with a shorter seed-filling period, which was a major cause of lower yields (Sionit and Kramer, 1977; Egli and Crafts-Brandner, 1996; DeSouza *et al.*, 1997). However, De Souza et al. (1997) also reported that the medium stress treatment (watering at 60% of the water required to bring well-watered pots to yield capacity) reduced stomatal conductance, but had no effect on leaf

senescence. They suggested that there might be a threshold stress level that must be exceeded to trigger accelerated senescence.

2.5 Plant spectral reflectance and assessment of plant stress

The previous sections showed that water stress could inhibit plant growth and reduce leaf chlorophyll content, resulting in important yield losses in soybean production. An important step in alleviating the deleterious effect of soil water scarcity before reaching actual yield reduction is to assess and quantify the plant's growth pattern. Remote sensing may offer producers the potential for straightforward determination of crop water status, with little effort and time on their part. Timely assessment can be achieved by using remotely sensed data such as vegetation spectral reflectance.

2.5.1 Remote sensing technology

Remote sensing has become a very dynamic field of research and innovation for crop water management, especially in the area of water use efficiency. One dynamic field of research has been to develop reliable remote sensing and crop health relationships. Remote sensing techniques, in particular infrared reflectance, can provide an instantaneous, non-destructive, and quantitative assessment of the crop's ability to intercept radiation and photosynthesise (Ma *et al.*, 1996). Increased scientific understanding of spectral responses of crops is increasing the potential for using remote sensing to detect plant stress (Osborne *et al.*, 2002). Recent research has focused on determining the appropriate wavelength or wavelength combinations to characterize crop health status. More particularly, researchers attempting to detect vegetation stress from remotely sensed data have focused on chlorophyll concentration, because it influences the reflectance of vegetation and tends to correlate with vegetation health and stress (Barton C.V.M., 2001).

2.5.2 Leaf reflectance: effects of water content and pigments on leaf spectrum

2.5.2.1 Leaf reactions to solar radiation

There are three leaf reactions to radiation: reflectance, absorptance, and transmittance. Spectral reflectance occurs at the leaf cuticle, and diffuse reflectance from light scattering occurs mainly within leaf mesophyll, which contains water. Leaf pigments absorb a part of the incident solar radiation, the photons with relatively short wavelength (visible light) are used for photosynthesis and photochemical reactions, whereas the photons with longer wavelength (near infrared) are related to heating, evaporation, and transpiration. Finally, the radiation transmitted is the light neither absorbed nor reflected. The potential rate of plant development and biomass accumulation under non-stress conditions depends on the amount of radiation absorption and the efficiency of utilizing the absorbed solar energy to drive photosynthetic processes that produce biomass materials (Wang *et al.*, 2000).

2.5.2.2 Leaf reflectance

The spectral reflectance of a leaf is the radiance reflected from the leaf expressed as a percentage of incident radiance through a range of wavelengths (Carter, 1991). It has been shown that absorptions by water and pigments determine to a large extent the reflectance spectrum of a leaf (Allen *et al.*, 1969; Woolley, 1971; Tucker and Garrat, 1977; Gates, 1980; Carter, 1991).

2.5.2.3 Effects of water content and pigments on leaf spectrum

Reddy et al. (2001) reported that primarily plant pigments, chlorophyll and carotenoids, influence leaf spectral reflectance. Absorption of radiation by pigments in the leaf is known to typically decrease reflectance (Carter, 1991). Carter (1991) reported that chlorophyll and accessory pigments absorb strongly between 400 and 700 nm (especially near 670 nm), therefore reflectance is typically low (and even lower near 670 nm) in the 400-700-nm visible spectral range. However, the absorptivity of chlorophyll is relatively low near 550 to 620 nm, and 700 to 710 nm, therefore diffuse reflectance will be higher at these wavelengths (Hoff and Amesz, 1991). Reflectance in the visible red range (600 to 700 nm) has been used to estimate leaf cholophyll and carotenoid levels

and, by extension, the photosynthetic capability of crops (Benedict and Swidler, 1961; Thomas and Oerther, 1972; Filella *et al.*, 1995).

Absorption of radiation by water in the leaf is commonly reported to decrease reflectance (Carter, 1991). Absorption by water is known to be relatively weak between approximately 700 and 1,300 nm (Curcio and Petty, 1951). Leaves generally do not contain other substances that absorb strongly between 700 and 1,300 nm (Gates *et al.*, 1965; Gates, 1980). Thus, reflectance in the 700 to 1,300 nm range is relatively high. However, water absorbs strongly at wavelengths from approximately 1,300 to 2,500 nm (Curcio and Petty, 1951), resulting in decreased reflectance in the 1,300 to 2,500 nm spectrum. Furthermore, it is reported that throughout the visible spectrum, the absorptivity of water is much weaker than in the infrared, as indicated by its high transmittance (Woolley, 1971). Therefore, leaf reflectance in the visible range is not strongly related to water absorption and leaf water content. However, researchers have given little attention to the influence of water content on reflectance at wavelengths where the absorptivity of water is weak. In this respect, Carter (1991) studied in several species the influence of water content on absorption by other substances in the leaf, such as pigments. He found that when water is lost from the leaf, absorption decreases and reflectance tends to increase in the 1,300-2,500 nm range but also in the 400-1,300 nm range. This indicates that the sensitivity of reflectance to water content was not only important in the water absorption bands, but also between 400 and 720 nm, indicating effects of water content on absorption by pigments. Carter (1991) concluded that leaf water content influences leaf reflectance in the visible spectrum, although this effect is secondary when compared to the effect of water content on leaf reflectance in the 1,300-2,500-nm range.

2.5.2.4 The effect of leaf internal structure on leaf reflectance spectrum

It has been shown that leaf reflectance is also influenced by leaf internal structure such as cell size, shape, number and distribution and leaf thickness (Gausman et al., 1969; Gausman *et al.*, 1977; Gates, 1977; Aldakheel and Danson, 1997). The radiative process by which leaf structure may influence reflectance is reported to be wavelength-independent (Sinclair *et al.*, 1973). Gausman et al. (1969) reported that wavelength-

independent effects on reflectance may tend to be masked by absorption in the 400-700 nm and 1,300-2,500 nm ranges. Therefore, Gausman et al. (1969) concluded that the effects of leaf tissue structures on reflectance might be most observable and should be determined in the near-infrared spectrum (700-1,300 nm range). Kumar and Silva (1973) proposed that the amount of reflectance in the near infrared was determined by the optical properties of the leaf tissues: their cellular structure and the air-cell wall-protoplasm-chloroplast interfaces. These anatomical characteristics are affected by environmental factors such as soil water status (Gausman *et al.*, 1969; Thomas *et al.*, 1971; Blackmer *et al.*, 1994). However, it has recently been shown that leaf reflectance is less affected by leaf internal structure than it is by radiative properties of water and pigments that were analysed above (Carter, 1991). Carter (1991) concluded that leaf reflectance is more sensitive to differences in leaf absorption than to variations in leaf structure.

2.5.3 Responses of leaf spectral reflectance to plant stress

The study of changes of spectral reflectance may identify plant stress long before any visible observation. Leaf reflectance responses to environmental conditions that inhibit growth generally involve increased reflectance in the visible (380-760 nm, Rosotti, 1983) and infrared (760-2,500 nm, Rosotti, 1983) spectra and/or decreased reflectance in the near infrared (760-1,300 nm range). Such reflectance variations have been reported in responses to biological agents (Ahern, 1988; Carter, 1993) as well as physicochemical agents (Schwaller *et al.*, 1983; Carter, 1993; Wang *et al.*, 2000). Carter (1993) studied leaf spectral reflectance responses to different plant stress so as to determine whether leaf reflectance responses may differ according to the agent of stress and species. He studied six vascular species and eight stress agents such as herbicide, dehydration, and senescence. Carter (1993) reported that reflectance at visible wavelengths increased consistently in stressed leaves for all species and stress agents. He also reported that infrared reflectance was comparatively unresponsive to stress. Carter (1993) concluded that increased reflectance in the visible spectrum is the most consistent leaf reflectance response to plant stress whereas infrared reflectance responds consistently only when stress has developed sufficiently to cause severe leaf dehydration.

Therefore, it appears that visible rather than infrared reflectance is the most reliable indicator of plant stress because it is responsive to stress regardless of stress agent or species. The constancy of increased visible reflectance as a response to any stress supports the view that plant physiological responses to stress are similar regardless of the cause of the stress (Chapin, 1991). However, changes in the infrared reflectance are more related to water stress as will be shown in the following sections.

2.5.4 Effects of water stress on the spectral reflectance of leaves

2.5.4.1 Effect of water stress on leaf spectral reflectance in the 400-720 nm range

Water stress can affect visible leaf reflectance in two ways. The first way is related to the effects of water on overall plant physiology, which was largely covered in previous sections, where it was shown that water stress could have an impact on the leaf chlorophyll content. Thus, the visible leaf reflectance is related to the chlorophyll absorptivity in the visible region (Hoff and Amesz, 1991). Any variation in chlorophyll content will therefore induce a variation of leaf visible reflectance (Hoff and Amesz, 1991). Carter (1993) reported that with low absorptivity even small decreases in chlorophyll content (due to plant stress) could result in significantly decreased absorption and increased reflectance in the visible spectrum. Thus, Carter (1993) reported that differences in the reflectance of stressed leaves could be explained mainly by stress-induced decreases in chlorophyll content.

The second way is related to the influence of leaf water content on the absorption by pigments. Carter (1991) reported that decreased leaf water content reduces absorption by pigments, and therefore increases leaf reflectance in the visible spectrum. Water stress may have an impact on the efficiency of the absorption of photosynthetically active radiation.

Water stress is widely reported to decrease spectral absorption and increase reflectance in the visible part of the spectrum (400-760 nm) (Parker, 1952; Woolley, 1971; Bowman, 1989; Hunt and Rock, 1989, Carter, 1993; Wang *et al.*, 2000). Carter (1993) reported that visible reflectance increased in stressed leaves for several species and stress agents. Carter (1993) found that visible reflectance was most sensitive to stress in

the 535-640 nm and 685-700 nm wavelength ranges, more particularly in the green portion of the spectrum, near 550 nm, and red portion of the spectrum, near 710 nm. Notably, increased reflectance near 710 nm represents the often reported "blue-shift"; i.e., the shift toward shorter wavelengths of the red-infrared transition curve that occurs in stressed plants when reflectance is plotted vs. wavelength (Horler *et al.*, 1983; Rock *et al.*, 1988; Cibula and Crater, 1992). Osborne et al. (2002) suggested that reflectance in the red spectrum range was a good indicator of water stress.

2.5.4.2 Effect of water stress on leaf spectral reflectance in the infrared spectrum

Whereas little reflectance difference generally occurs in the visible spectrum in response to leaf water deficit, it has been reported that large reflectance differences occurred throughout the infrared spectrum (Carter, 1991). Reflectance sensitivity to water content in the infrared spectrum has been related to the effects of water status on the specific structures of leaf tissues and their resulting optical properties (Gausman *et al.*, 1969; Thomas *et al.*, 1971; Kumar and Silva; 1973; Carter, 1991; Blackmer *et al.*, 1994). Near infrared reflectance is affected by internal leaf structure, whereas the mid infrared is more affected by water contents. The observations of the stress-induced variations of leaf reflectance in the infrared have not been similar among authors.

2.5.4.2.1 Effect of water stress on leaf reflectance in the near infrared: 720-1,300-nm

Curcio and Petty (1951) reported that absorption by water is relatively weak in the near infrared (700-1,300 nm). Leaf reflectance is relatively high in this spectrum range and variations of water or pigment contents will have little impact on leaf reflectance. However, Kumar and Silva (1973) showed that the amount of reflectance in the near-infrared was more determined by the optical properties of the leaf tissues: their cellular structure and the air-cell wall-protoplasm-chloroplast interfaces. In this respect, Gausman et al. (1969) had reported that the effects of leaf tissue structures on reflectance was more observable in the near-infrared spectrum, because of the weakness of the absorption effects in this spectral range. Leaf tissues can be affected by environmental factors such as soil water status (Gausman *et al.*, 1969; Thomas *et al.*, 1971; Carter, 1991; Blackmer *et al.*, 1994). Carter (1991) found that structural changes that occurred with leaf dehydration

might have increased leaf transmittance, resulting in occasional reflectance decreases in the 720-1,300 nm spectral range. Therefore, reflectance in the near infrared has been widely considered to be a function of leaf tissue structures rather than a function of water content. Nervertheless, leaf internal structure has been shown to have little effect on reflectance as compared with absorption effects by water and pigments (Carter, 1991).
In response to water stress, some authors reported appreciable increases in leaf reflectance in the 720-1,300 nm spectral range (Woolley, 1971; Rock *et al.*, 1988; Bowman, 1989; Carter, 1991). Other authors found decreased reflectance in the same spectral range (Moran *et al.*, 1989; Wang and Shannon, 1999; Osborne *et al.*, 2002).

2.5.4.2.2 Effect of water stress on leaf reflectance in the mid-infrared: 1,300-2,500- nm

Several authors (Carter, 1991; Carter *et al.*, 1992; Carter, 1993; Osborne *et al.*, 2002) showed that the greatest differences in leaf reflectance in response to water stress occurred beyond 1,300 nm, mostly in the water absorption bands near 1,450, 1,950, and 2,500 nm. These wavelengths are similar to wavelengths used by past researchers to detect water stress (Moran *et al.*, 1989; Carter, 1991). Water stress is reported to decrease absorption and increase reflectance in the 1,300-2,500 nm range (Bowman, 1989; Carter, 1991; Carter *et al.*, 1992; Carter, 1993). Reflectance in the mid-infrared has been widely considered to be a function of leaf thickness and water content (Lillesand and Kriefer, 1987; Osborne et al., 2002). In this spectral range, water stress affects leaf reflectance directly by inducing leaf dehydration and reducing absorption of radiation by water. In this respect, Carter (1991) reported that the peaks of reflectance observed in the water bands were characteristic of decreased absorption by leaf internal water. Osborne et al. (2002) suggested that the midinfrared reflectance was a good indicator of water stress. Carter et al. (1992) proposed that reflectance in the water absorption bands would be expected to increase in any leaf following stress-induced damage as a result of dehydration.

2.5.5 Effects of various factors on reflectance measurements

2.5.5.1 Effect of physical factors on reflectance measurements

A number of physical factors can affect reflectance measurements. When the crop does not cover the entire soil surface, reflectance measured from a certain height above ground level will represent the reflectance of the canopy and the soil surface, rather than just the crop itself. Daughtry et al. (1982) showed that for a 37% soil cover, overall reflectance was close to threefold greater on light-colored soils than on darker soils. The area scanned must be consistently representative of the canopy coverage. Daughtry et al. (1982) showed that the coefficient of variation of reflectance measurements over a soybean crop presenting 71% soil coverage decreased exponentially as sensor height increased. They suggested, therefore, that the sensor's height should be high enough to allow accurate determination of crop reflectance.

2.5.5.2 Importance of canopy architecture in canopy reflectance

2.5.5.2.1 Leaf adaptative mechanisms

Some macro- or micromorphologic adaptative mechanisms can modulate the importance of cuticle reflectance. Pilosity of leaves has an important role in reducing gas exchanges and in reducing the leaf heating by increasing the leaf reflectance (Monneveux and Belhassen, 1996). Pilosity influences leaf reflectance by acting on the thickness of the limit layer. Moreover, the colour of the transpirative organs related to the Chl a/Chl b ratio and the presence of pigments (anthocyans, carotenoids) has an effect on the proportion of the incident light reflected by the leaf and consequently on the leaf heating (Monneveux and Belhassen, 1996).

2.5.5.2.2 Effects of canopy architecture on reflectance measurements

Several authors (Beggs, 1980; Kimes and Kirchner, 1983; Moran *et al.*, 1989) warned that as leaf angle distributions are not constant, it should not be assumed that canopy architecture is constant in canopy reflectance studies on neither diurnal nor seasonal scales. They suggested that stress-induced architectural differences in plant canopies

should be accounted for when using remotely gathered spectral data. Moran et al. (1989) showed that plant canopies are relatively planophile (leaves horizontal) when unstressed, and erectophile (leaves vertical) when stressed. They explained that erectophile and planophile canopies have corresponding differences in canopy reflectance. They stated that erectophile canopies tend to trap reflected radiation within the canopy, whereas, for planophile canopies, more reflected radiation escapes the canopy and reaches the radiometer sensor. Moreover, they indicated that a change from erectophile to planophile canopy also results in a change in the proportion of visible and infrared (IR) reflectance from the canopy. This can have a large effect on spectral vegetation indices, such as IR/red. For instance, Jackson and Printer (1986) established that a nadir-pointing sensor can receive 20 to 30% more total radiation from a planophile than an erectophile canopy. Therefore, it appears important to consider canopy architecture changes when analysing spectral reflectance changes of plants under water stress.

Preface to Chapter 3.

Chapter 3 is comprised of a manuscript that has been co-authored by R. Bonnell, D.L. Smith and myself. The paper was prepared for submission in 2002 to the Canadian Water Resources Journal. All literature cited in this chapter are listed in the reference section at the end of the thesis. All tables, figures, and photos are presented at the end of the chapter.

Chapter 3 describes a greenhouse experiment carried out to study exclusively the effects of chronic water stress on the growth pattern of soybean. The objectives of the experiment were to assess the impact of chronic water stress during reproductive development of soybean plants, to evaluate the variability of response to water deficits among the consecutive reproductive stages of development, and to detect changes in soybean canopy spectral reflectance.

Chapter 3.

RESPONSE OF AN INDETERMINATE SOYBEAN (*Glycine max* (L.) Merr.) TO CHRONIC SOIL WATER DEFICIT UNDER GREENHOUSE CONDITIONS DURING REPRODUCTIVE DEVELOPMENT

ABSTRACT

A greenhouse experiment was conducted during the summer of 2001 to (i) assess the impact of chronic water stress during reproductive development on soybean vegetative growth, (ii) determine the effects of water stress on soybean reproductive growth and productivity, and (iii) evaluate changes in canopy optical reflectance due to chronic water deficit. Soybean plants (cultivar OAC Bayfield) were grown in 10-L soil-filled pots. Plants, all well fertilized, were grown under three moisture regimes: daily watering at 100% (control W3), 50% (medium stress W2) and 25% (severe stress W1) of soybean evapotranspiration (ETc). These three water levels were organized in a randomised complete block design with 6 blocks. A Li-Cor Model-6400 portable photosynthetic meter was used to measure physiologic growth related variables such as the photosynthetic rate. Plant height and other common morphologic growth variables were measured regularly. Reflectance of the whole plant canopy was measured with a portable spectrophotometer, from 300 to 2,500 nm wavelengths. The physiologic results indicated that stomatal conductance, photosynthetic and transpiration rates were considerably reduced at both water stress levels. Moisture deficit resulted in a sharp decrease of the rate of plant vegetative growth at both stress levels. Total canopy dry matter, measured at harvest, dropped by 24.6 and 35.0 %, at the medium and severe stress levels, respectively, compared to the controls. The decrease in total leaf area, measured at harvest, was even greater, as it dropped by 52.7 and 74.5 % at the medium and severe stress levels, respectively. Water stress also induced significant yield losses (yield index = Pod Dry Weight) at both water deficit levels; 23% yield losses for W2 and 36% for W1. Water stress impacted the spectral reflectance signature of the soybean leaves at both water stress levels. Water deficit increased leaf reflectance in the visible and decreased it in the infrared ranges of the spectrum at both stress levels.

Key words: water stress, chronic, evapotranspiration, soybean, spectral reflectance.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a large-seeded grain legume crop. Its growth characteristics have been widely studied because of its economic importance. Soybean is reported to have a low water use efficiency, i.e. it uses a lot of water units per unit of dry matter produced (Sprent and Sprent, 1990). Even though the soybean plant can withstand a short period of drought, a long stress period will greatly affect soybean yield. In general, soybeans are considered a drought sensitive crop (Itani *et al.,* 1992; Mullet and Whitsitt, 1996). Thus, moisture stress is an important determinant of soybean crop yield since plant height, number of nodes, stem diameter, number of flowers, percentage of pod set, number of seeds, and seed weight are positively related to soil moisture.

Determinate types of soybean varieties are more common in the U.S. where there is more soybean production and research. Therefore, in recent decades, most research on soybean production and water stress has been carried out on determinate varieties. Yet, with regard to water deficit studies, it is important to note that moisture deficit can affect vegetative growth of indeterminate varieties during vegetative and reproductive stages because indeterminate soybeans continue to grow vegetatively after the onset of flowering. This means that studies of water stress during the reproductive development of indeterminate types can assess the effects of moisture deficit on both vegetative and reproductive development, whereas the same studies conducted on determinate varieties will only address effects on reproductive development.

During the last few decades researchers have worked to assess and understand the impact of drought on soybeans. The effects of water stress on soybean growth and productivity have been extensively studied at locations around the world. Soybean yields have been related to moisture availability in many studies (Doss *et al.*, 1974; Ashley and Ethridge, 1978; Korte *et al.*, 1983b). Any period of stress, regardless of length, would be expected to reduce metabolic activity, and hence, lower soybean seed yield (Hicks, 1978). Yet, it is widely reported that moisture deficiency during the vegetative stages of soybean development reduces the rate of plant growth, but, does not affect soybean seed yield (Ramseur *et al.*, 1984a; Newark, 1991; E-Kheir *et al.*, 1994; Hirasawa *et al.*, 1994). The negative effects of water stress are widely reported to be particularly important during

reproductive development: flowering, pod set and pod filling (Sionit and Kramer, 1977; Shaw and Laing, 1966; Doss *et al.*, 1974; Hirasawa *et al.*, 1994).

Remote sensing has become a very dynamic field of research and innovation for crop water management, especially in the area of water use efficiency. Timely assessment can be achieved by using vegetation spectral reflectance. Research on the impact of water stress of plant canopy reflectance has been very active in recent decades. Leaf reflectance responses to water deficit conditions have been shown to increase reflectance in the visible spectral (380-760-nm) range and decrease reflectance in the near infrared (760-1,300-nm) range. Water stress is reported to increase reflectance in the mid-infrared (1,300-2,500-nm) range (Carter, 1991; Carter *et al.*, 1992; Carter, 1993). Osborne et al. (2002) suggested that the mid-infrared reflectance was a good indicator of plant water stress.

It is interesting to note that most stress research focuses on catastrophic events, such as severe droughts that last only short periods of time and affect, therefore, only one or two stages of development. Yet, in the field, chronic drought is frequent, and chronic water stress is considered the norm in some areas; cropping systems in these areas are adapted to chronic water limitation. Thus, inasmuch as this is viewed as the norm, chronic low level water stress has been less researched than the extreme condition. However, as researchers, we know that an important step in alleviating the potential harmful effects of chronic soil water deficit, before reaching actual yield reduction, is to assess and quantify the plant's growth responses to these conditions. Therefore, in this research we have attempted to evaluate the impact of long-term chronic water stress on consecutives stages of development for an indeterminate soybean variety, including changes in spectral reflectance that may identify soybean water stress long before any visible observation.

MATERIALS AND METHODS

Growing conditions

The influence of different deficient irrigation regimes on the growth and productivity of soybean plants was studied during the summer of 2001 under greenhouse conditions. The advantage of a greenhouse experiment over field experiments is that relative humidity; wind speed and temperature are more easily controlled and can be artificially set to any desired

level (Monneveux and Belhassen, 1996), making Crop Evapotranspiration (ETc) easier to estimate by pan evaporation. An experiment was set up in the research greenhouse of the Macdonald Campus of McGill University at Sainte Anne-de-Bellevue, Québec. The greenhouse experiment was organized in a randomized complete block design with 6 blocks and three water stress levels (severe stress - W1, medium stress - W2, and a well watered control - W3). This design resulted in a total of 18 experimental units.

The 10-litre polyethylene pots had an upper diameter of 26 cm and a base diameter of 21 cm. Paper was placed in the bottom of each pot prior to the addition of rooting medium to prevent lose of soil material via the drainage holes at the base of the pots. All pots were placed on a bench that was 90 cm off the concrete floor of the growth chamber.

In each of the 6 blocks, the plants were staggered in two rows (see Figure 3.1). Plants were spaced 30 cm (1 ft.) apart both within and between rows. Natural lighting was supplemented with overhead lighting so as to have 16 h of daylight and 8 h darkness. The overhead lighting was provided by 400 W high-pressure sodium bulbs (P.L. Light Systems, Canada). The daytime temperature was maintained between 20 and 25°C, the night-time temperature between 18 and 22°C and relative humidity was maintained at 40±2% throughout the growing season, apart from a few days when the outside air temperature was very high (see Figure 3.2).

Soil and fertilizer characteristics

The soil consisted of a sandy loam topsoil from a local field. Fourteen kg of damp soil were used to fill each pot. While filling the pots, the soil was manually compacted until its bulk density was 1.3 T/m^3. This was done to establish a growth environment closer to field conditions, which explains the importance of pot volume, the soil origin and the compaction. The morning of the day of seeding, the soil was wetted for an easier seeding. All plants were watered until soil saturation (i.e. water dripping at the base of the pot) at 28 Days After Planting (DAP). Thus, soil water content was at field capacity at 29 DAP after 24 hours of free drainage. The water treatment started at 30 DAP.

Total Nitrogen in the soil was found to be 791.3 mg/kg (1028 mg/L). The make of the fertilization solution was based on the nutrient status of the base topsoil. A complete nutrient solution was prepared. It was made of 106 mg/L N, 62 mg/L P_2O_5, 156 mg/L

K_2O, 93 mg/L Ca, 48 mg/L Mg, 64 mg/L S, 3.8 mg/L Fe, 0.46 mg/L B, 0.05 mg/L Cu, 0.81 mg/L Mn, 0.09 mg/L Zn, and 0.03 mg/L Mo. This corresponds namely to a complete fertilizer of 20-12-30 N-P_2O_5-K_20 with appropriate minor and micro-nutrients. This fertilizer was included in the water used to irrigate the plants before and during the water stress treatment so as to prevent any nutrient deficiency that would interfere with the water stress treatment.

Growth conditions

Soybean (cultivar OAC Bayfield) seeds were purchased from Agrocentre BELCAN, QC, Canada. OAC Bayfield is an indeterminate variety. The seeds were planted on the 15th of May 2001 (referred to as 0 DAP, Days After Planting). The seeds were not inoculated with *Bradyrhizobium japonicum*, and the roots were not nodulated. Thus, no supplemental source of nitrogen was available to the plant. Three seeds were sown in the centre of each pot. Full emergence occurred by 6 DAP. At the 1-leaf stage, the seedlings were thinned to one per pot and any damaged seedlings replaced with extras from other pots. All soybean plants were watered and fertilized as required at frequent intervals throughout their vegetative development, which continued until the onset of flowering at 29 DAP, so as to have healthy and uniform plants at the beginning of the water stress treatment. At the end of the vegetative growth stage V8 and beginning of growth stage R1 (Table 3.2), just prior to the development of flower clusters, treatment plants were arranged randomly within blocks such that each block contained one experimental unit of each treatment. The water stress treatment was then applied.

Water stress treatment

Crop Evapotranspiration (ETc) was assumed to represent crop total water requirements. As the objective of the project was to establish water stress conditions at the time of flowering, which is one of the most drought sensitive stages of development (Doss *et al.*, 1974; Hirasawa *et al.*, 1994), the water stress treatment was initiated at 30 DAP during beginning bloom (Table 3.2).

Soybean plants were grown at two water stress levels W1 and W2, corresponding respectively to the irrigation of the plants by 25 and 50 % of ETc. The other third of the

plants were non-stressed and were maintained at 100% ETc; these plants acted as the control (level W3). The water used for irrigation consisted of the nutrient solution described above. As a part of the severe conditions during the summer of 2001 (hot and dry), the outside temperature was very high. The temperature inside the greenhouse reached more than 30°C at 32, 33, 40, 41, 42, 43, and 54 DAP (Figure 3.2), and the W1 plants appeared to be near death. Permanent wilting is a common reaction to excessive soil-water deficit. It should be avoided in any research study on the impact of water stress so as to keep the plant alive and allow collection of useful data. Therefore, all water levels were increased by 25% at 41 DAP, leading to levels of 31, 62 and 125% ETc. The W1 plants continued to show signs of extreme water deficit stress and at 51 DAP the W1 level was increased to 40%. This water stress treatment was maintained until harvest at 76 DAP, during the late grain filling stage of soybean development (growth stage R6, Table 3.2) before physiologic maturity.

Estimation of ETc, irrigation procedure and scheduling

Irrigation amounts were based on the estimation of Crop Evapotranspiration (ETc) (James, 1993; Crop evapotranspiration: guidelines for computing crop water requirements, FAO, 1998). Estimation of Crop ET by measurement of pan evaporation is a relatively inexpensive and simple way of assessing plant water requirements. It requires less time and effort to apply while maintaining accuracy (James, 1993). A *class A* evaporation pan (Crop evapotranspiration: guidelines for computing crop water requirements, FAO, 1998) was installed at the same elevation as the soybean pots on the greenhouse bench (90 cm off the concrete floor) (see Photo 3.2) to allow estimation of soybean ETc (James, 1993; FAO Crop Water Requirements, 1998). Crop total water requirements were assumed to be equal to a fraction of pan evaporation (measured daily) (James, 1993; Crop evapotranspiration: guidelines for computing crop water requirements, FAO, 1998).

As the objective was to establish a steady *chronic* water stress, estimation of ETc and irrigation of the plants were done daily. The pan was filled at 09:00 am each day and the amount of water required to refill the pan was used to estimate cumulative water evaporation during the previous 24 h (referred later as Vp, volume of water added to the pan). The procedure was to add to the pan as much water as needed to reach the tip of a steel needle

situated in the middle of the pan that indicated the height of water in the pan. ETc was then estimated using a simplified equation (see Equation 3.1 below). The predetermined fraction of the ETc was then carefully added to the soil of each pot. A summary of the pan evaporation and ETc values for the whole period of stress is presented in Table 3.1.

The following is the equation and coefficients given by the FAO guidelines for irrigation scheduling, for the estimation of daily ETc.

$$ETc = (Vp / Ps) * Kp * Ss * Kc \quad (cm^3) \qquad \text{(Equation 3.1)}$$

$$= (Vp / 11.310) * 0.8 * 511 * 1.0$$

Where

Ps = pan surface area (cm^2)

Ss = area of soybean pot surface (cm^2)

Vp = volume of water added to pan (cm^3)

Kp = pan coefficient = 0.8

Kc = crop coefficient = 1.0*

* ETc varies with Kc, which depends on crop growth stages and air humidity (Crop evapotranspiration: guidelines for computing crop water requirements, FAO, 1998). However, to simplify the procedure, we chose to use an average value for Kc throughout the reproductive growth of soybean.

Data collection

In order to obtain base-line data, physiological measurements were made 29 and 30 DAP; that is, before initiation of any water deficit. The third or fourth nodal expanded leaves from the bottom were used for measuring physiological variables. Photosynthetic rate (Pr), transpiration rate (Tr) and stomatal resistance (Rs) were measured using a portable photosynthesis system (Li-Cor, Inc., Lincoln, Nebraska, USA) between 10:00 am and 2:00

pm. Use of this machine is shown in Photo 3.1. Tr, Rs, but mostly Pr, are direct indicators of plant health. Thus, variation in these parameters will indicate plant stress. Height, number of leaves and primary branches were recorded 2 times per week. Data on number of flowers and pods were collected every 2 weeks. Nitrogen deficiency is one consequence of water stress and this can adversely impact crop development and yield (El-Kheir *et al.*, 1994; Chernyad'ev, 1997; Osborne *et al.*, 2002). The SPAD chlorophyll meter was found to be a reliable, quick, and non-destructive tool used for directly measuring leaf chlorophyll content (Costa *et al.*, 2001). Because most of the plant nitrogen is contained in the chlorophyll molecules, the measure of leaf chlorophyll content is a good indicator of plant nitrogen status (Costa *et al.*, 2001). Therefore, leaf chlorophyll content was measured each week with a SPAD chlorophyll meter (Minolta Corp., Ramsey, NJ, USA). The SPAD produces point measurements on a single leaf. To achieve better estimates of plant nitrogen, 3 SPAD readings were made on the same leaf and the average value was recorded. Percent variation in morphologic and physiologic variables were calculated. Furthermore, spectral reflectance of the whole plant canopy was measured with a portable spectrophotometer, with 300 to 2500 μm wavelength capability (Analytical Spectral Devices Inc., Co, USA). Use of this instrument is shown in Photo 3.2. Spectral data was collected at 36 DAP, during flowering (Table 3.2), because it is reported to be one the most critical period for water stress (Shaw and Laing, 1966; Westgate and Paterson, 1993; Kokubun *et al.*, 2001). Reflectance was measured at the top of the plant, in order to obtain the reflectance of the whole plant canopy, which is reported to be a better indicator than leaf reflectance (Daughtry *et al.*, 1982).

Plants were harvested on the 76th Day After Planting, at plant physiological maturity corresponding to 45 days of water stress treatment. Soil moisture content was determined using the gravimetric method. Plant shoots were dried in an oven at 90°C for 48 h, and their dry weights were recorded. Data on final numbers of branches and pods, plant height, and total leaf area were collected.

Statistical data analyses

The statistical data analyses were performed using SAS Release 8 for Windows (SAS Institute, 1999) and CoStat Release 6.1 for Windows (CoHort Software, 2001). Normality of the data was assessed using the Proc UNIVARIATE procedure in SAS on the raw data. The

CoStat procedure used for the ANOVA was GLM (general linear model). Protected ANOVA least significant difference tests (LSD) were used to assess the differences among treatment means (Steel and Torrie, 1980). Descriptive statistical data analysis (mean ± standard error) was employed for some variables. Correlation analyses were carried out to assess relationships among variables.

RESULTS AND DISCUSSION

Chronic water stress establishment

At 29 DAP (the day before soil moisture deficit treatments were commenced), the overall average soybean plant photosynthetic rate (Pr) was 18.23 μmol/m²/s (standard error, SE = 0.38); the average stomatal conductance (Rs) was 0.51 mol/m²/s (SE = 0.01); the average transpiration rate (Tr) was 11. 67 mol/m²/s (SE = 0.28). The plants had a mean height of 41 cm (SE = 0.65), and an average of 2.30 primary branches (SE = 0.13) and 5.6 fully expanded leaves on the stem (SE = 0.11). These physiological and morphological data illustrate that the plants were growing well. This also demonstrates that the plants used were both physiologically and developmentally uniform at the outset of the experiment.

The data show that plant water stress, i.e. changes in soybean physiology and vegetative growth, was quickly established and present throughout the duration of the W1 and W2 treatments, and that the watering treatments were successful in establishing two gradual levels of stress (Figures 3.2, 3.3, 3.4, and 3.5; Photo 3.3). Plant water stress was noticeable 3 days after soil moisture deficit treatments were commenced (33 DAP or 1 DOS, *the first* Day Of *plant* Stress, Table 3.1) (Figures 3.2, 3.3, 3.4, and 3.5). Soil moisture deficit began to affect ($p<0.05$) soybean physiology by 1 DOS (Figures 3.2, 3.3, 3.4, and 3.5). Water stress began to affect ($p<0.05$) total leaf number by 4 DOS (Table 3.4), and plant height by 5 DOS (Figure 3.5). Soil water content, measured at harvest, was different among the three water availability treatments (Figure 3.6).

The summer of 2001 was very warm. On 1, 8, 10, 11, 14, and 23 DOS, air temperature was so high that all plants, including the controls, were under acute water stress, as shown by the sharp decrease of their physiological activities (Figures 3.2, 3.3,

3.4). The weather conditions fluctuated widely during most the experiment, resulting in a large variation for daily pan evaporation and soybean ET (Table 3.1).

Impact of water stress on soybean physiology

Pr, Rs and Tr in the well watered treatment were > 6 μmol/m²/s, > 0.1 mol/m²/s, and > 2 mol/m²/s, respectively, during most of the early stress period (from 0 to 15 DOS). The analysis of stomatal conductance (Rs), transpiration and photosynthetic rates (Tr and Pr, respectively) demonstrates that water stress negatively affected ($p<0.05$) soybean physiology for all physiological variables measured throughout the water treatment period (Figures 3.2, 3.3, 3.4). The impact of moisture deficit was, as expected, greater at the severe stress W1 than at the medium stress W2, for all variables analysed.

For the medium stress treatment, Pr, Rs and Tr remained between the control and severe stress treatments, although there was little difference between the W2 and W1 treatments from 5 to 9 DOS (Figures 3.2, 3.3, 3.4). Pr, Rs and Tr in the well watered treatment remained > 5 μmol/m²/s, > 0.05 mol/m²/s, and > 1 mol/m²/s, respectively, during most of the stress period. Whereas Pr, Rs and Tr in the stressed plants were < 5 μmol/m²/s, < 0.03 mol/m²/s, and < 1 mol/m²/s, respectively, during the same period (Figures 3.2, 3.3, 3.4). At 1 DOS, Pr dropped by 67.4 and 78.4 %, Rs by 89 and 92 %, and Tr by 75.2 and 85.4 %, for W2 and W1, respectively, compared to the controls. The proportional reductions were greater for transpiration rate and stomatal conductance than photosynthetic rate, throughout the water stress period, which confirms the observations of Lee et al. (1994), Blum (1996), and De Souza et al. (1997), who found that transpiration rate and stomatal conductance decreased more rapidly than photosynthetic rate, as soil moisture declined. It also appears that stomatal conductance was most negatively affected by the stress treatment, which suggests that the major effect of water stress is a decrease in stomatal conductance. This confirms the observations of Boyer (1970), Lu et al (1994), Lee et al. (1994), and Reynolds et al. (1994), who developed the idea of a central role for stomatal conductance as a modulator of soybean photosynthesis and transpiration under water stress.

Pr, Rs and Tr were all positively correlated ($p < 0.001$) for all moisture regimes (Table 3.3). Similar correlations are reported (Huang *et al.*, 1975; Grantz, 1990; Reynolds *et al.*, 1994; Blum, 1996). At all moisture regimes, Rs was more closely correlated

with Tr than with Pr. The reduction in Rs is reported to be an adaptative morphologic mechanism that can modulate water losses by transpiration, and is associated with reductions in stomatal transpiration (Monneveux and Belhassen, 1996; De Souza *et al.*, 1997). For all water treatments, Pr is more strongly correlated with Tr than with Rs. The decrease in photosynthetic rate is generally ascribed to the effect of water stress on the stomatal control of transpiration water loss (Lee *et al.*, 1994; Blum, 1996).

At the severe stress level, Pr, Rs and Tr were all positively correlated with air temperature (Tair) ($p < 0.05$) (Table 3.3). Tr and Tair were highly correlated ($p < 0.01$). Thus, under severe moisture deficit stress, plant physiological activity varied with air temperature, mainly because Tr was dependent on Tair. There were no correlations of Tair with Pr, Rs and Tr for the well watered and medium stress levels (Table 3.3). These data suggest that the severely stressed plants could no longer exercise effective homeostatic control of leaf gas exchange.

These data indicate that water stress affected plant physiological activity on the first day of noticeable stress (1 DOS) at both water deficit levels. Moreover, they indicate that, from a physiological point of view, two different stress levels were established by 1 DOS.

Impact of water stress on soybean morphology

Because indeterminate soybean varieties continue to grow vegetatively during reproductive growth, water stress treatment affected several morphological growth variables. Decreases in plant height (measured from the base to the tip of the plant stem), number of fully developed leaves on the stem, and, to a lesser extent, number of primary branches, were morphologic effects of water stress that were visually detectable (Photo 3.3).

Soil moisture deficit strongly ($p < 0.001$) reduced leaf number (Table 3.4). Leaf number declined detectably ($p < 0.05$) by 4 and 9 DOS, at the severe and medium stress levels, respectively (Table 3.5). Thus, the effect on leaf number was first observable at the severe stress level, and then at the medium level. At the severe stress level, leaf induction rate was reduced by as much as 88 %, as compared to the control. Water stress completely stopped the induction of new leaves at the severe stress level. The severely stressed plants had 7 leaves from 4 to 24 DOS, whereas the controls had 7 leaves at 4 DOS and 13 leaves at 24 DOS. At the medium stress level, water deficit decreased leaf induction rate by 53 %. The

difference in mean leaf number between the severe and medium stress was detectable by 12 DOS (Table 3.5). For these indeterminate plants, soil moisture deficit did not affect leaf size at either stress levels. Normally, moisture stress on determinate soybean during the vegetative stages of development is reflected in smaller leaves (Newark, 1991; E-Kheir *et al.*, 1994).

Water stress decreased ($p < 0.001$) plant height (Table 3.6), which has also been observed for determinate soybeans by Newark (1991) and E-Kheir et al. (1994). Figure 3.5 indicates that the well watered plants showed a classic sigmoid increase in height throughout the period of stress, whereas, at both stress levels, the rate of increase in height was greatly reduced. From 0 to 23 DOS, plant height at the severe stress level W1 increased from 41 to 57 cm, whereas, for the same period, plant height at the control level increased from 41 to 142 cm. Thus, at the W1 level, the reduction in height growth rate was as high as 84 %. Water stress decreased plant height detectably by 9 DOS for both stress levels (Figure 3.5). By 9 DOS, water deficit decreased plant height by 28 and 33 %, at the medium and severe stress levels, respectively. At 16 DOS, plant height, for the control, medium, and severe stress levels, were 127, 68 and 56 cm, respectively, which corresponded to decreases in plant height of 47 and 56 %, at the medium and severe stress levels, respectively. Mean height was less for the severe than medium stress levels ($p < 0.05$) by 16 DOS (Figure 3.5).

Water stress affected ($p = 0.0507$) the number of branches per plant (Table 3.6, Figure 3.7), reducing the mean number of branches by 33 and 28 %, at the medium and severe stress levels, respectively, compared to the controls (Figure 3.7).

The decrease in plant growth rate during the vegetative stage suggests that soil moisture deficit during vegetative growth might have induced an early switch of plant development from vegetative to reproductive at both stress levels. This could be a function of the indeterminate type of the soybeans used. Indeed, indeterminate soybean varieties likely maintained some control of their growth pattern, and ability to compensate for drought induced reductions, and thus limit yield losses. Yet, at the severe stress, Pr, Rs and Tr were all positively correlated with air temperature. This suggests that the severely stressed plants could not control their development independently of environmental conditions, while control and moderately stressed plants could.

These data show that there is a clear and negative effect of water stress on the morphology and development of indeterminate soybean plants, and that this begins very rapidly (4 DOS). Water stress reduced strongly the rates of plant vegetative growth for all variables measured. The proportional reductions were greater for plant height and leaf number than for number of branches. These data also show that, differences between the two applied stress levels could be detected as early as 12 DOS.

Effect of water stress on canopy architecture

Photos 3.3 and 3.4 illustrate the effects of water deficit stress on plant development, and also show that leaf rolling, and wilting were observable at W1 and W2 stress levels. At the medium stress level, plant leaves showed paraheliotropic behavior. Leaf rolling, wilting and paraheliotropism resulted in a vertical profile for the canopy of water stressed plants, whereas the controls showed a more horizontal profile, because their leaves continued to track the sun through diaheliotropic movements. Leaf rolling is commonly observed in water stressed soybean plants (Wang *et al.*, 1993; Wang *et al.*, 1994; Monneveux and Belhassen, 1996). Paraheliotropic movements of leaves have also been commonly reported in water stressed soybean leaves (Travis and Reed, 1983; Smith *et al.*, 1988; Moran *et al.*, 1989; Wang *et al.*, 1994). Moran et al. (1989) reported that plant canopy is relatively planophile (leaves horizontal) when unstressed, and erectophile (leaves vertical) when stressed. Yet, the present results show that paraheliotropism was not observable at the severe stress level, which suggests that these plants were so negatively affected by water stress that they could not use leaf movement to modulate the effective light-intercepting leaf area, and therefore could not control leaf heating and transpiration water loss. Thus, we suggest that there might be a threshold stress level beyond which leaf movement cannot be activated.

Impact of water stress on soybean leaf chlorophyll content

SPAD meter readings indicate leaf chlorophyll content and plant nitrogen status (Costa *et al.*, 2001). Plant nitrogen content is an indicator of plant health in its particular growth conditions. Water stress negatively affected ($p < 0.05$) leaf chlorophyll content (Table 3.7). Water deficit reduced leaf chlorophyll content at the severe stress level by 12 DOS (Table 3.8). Severe water stress decreased leaf chlorophyll contents of 8 and 11

%, measured at 12 and 19 DOS, respectively. There was no statistically detectable decline at the medium stress level, although there was a numerical reduction in leaf chlorophyll content at this stress level (Table 3.8). It has already been shown that nitrogen deficiency can occur because of water stress (El-Kheir *et al.*, 1994; Chernyad'ev, 1997; Osborne *et al.*, 2002). El-Kheir et al. (1997) showed that decreasing the available soil moisture content reduces total chlorophyll content in soybean leaves. Thus, in the severely stressed plants, the effects of water stress on the photosynthetic rate might have been due to the reduction of leaf chlorophyll content. These data suggest that water stress may have reduced the efficiency of photosynthetic CO_2 assimilation, as observed by Chernyad'ev (1997).

Impact of water stress on flower and pod development

Water stress had a strong negative effect ($p < 0.001$) on the total number of fully open flowers per plant (Table 3.9). This confirms the findings of Shaw and Laing (1966), Dusek et al. (1971), Westgate and Paterson (1993), and Kokubun et al. (2001), who showed that flowering is one the most critical period for long-term water deficit. Flower induction started on 1 DOS (Table 3.2). At 14 DOS, at the end of flowering (Table 3.2), water deficit reduced the number of flowers by 58.8 and 79.4 %, at the medium and severe stress levels, respectively, compared to the controls (Figure 3.8). The previous paragraphs showed that water stress inhibited plant vegetative growth. Thus, the decrease in flower number is mainly due to the reduction of the number of nodes on the main stem. At the same time, a deficient water supply during flowering is reported to induce a major increase of the rate of flower induction (Kato, 1964; Westgate and Peterson, 1993). Therefore, the overall decrease of flower number was likely due to the decrease of node number but also to the increase of flower abortion.

Water stress reduced ($p < 0.001$) the number of pods per plant (Table 3.9). This is because water deficit decreased the number of flowers. By 19 DOS, at the beginning of pod development (Table 3.2), water deficit reduced pod number by 67.3 and 92.7 %, at the medium and severe stress levels, respectively, compared to the controls (Figure 3.9). It has been shown that the overall decrease in pod number is due to an increase of pod abortion rather than a reduction of pod induction (Shaw and Laing, 1966; Westgate and Peterson, 1993; Kokubun *et al.*, 2001). Thus, it is likely that the decrease in young pod number was

due to an increase of the abortion of young pods. Yet, the number of pods was measured at 19 DOS, just at the beginning of pod induction (Table 3.2) and the percentages decrease was already very high. This suggests that water stress might have also reduced pod initiation. At 26 DOS, during pod lengthening (Table 3.2), water deficit reduced pod number by 39.5 and 81.6 %, at the medium and severe stress levels, respectively (Figure 3.9). At 26 DOS, both water stress levels largely reduced the number of pods for each pod size category (Figure 3.10). However, the proportional reductions were greater for pods with lengths > 4 cm (Figure 3.10). These data show that water deficit affected negatively pod enlargement at both stress levels.

Overall, these data suggest that the decrease in pod number due to water stress is not only due to a reduction in flower number, but also to the decrease of pod induction, an increase in young pod abortion and the inhibition of pod enlargement.

Impact of water stress on plant canopy reflectance

The spectral reflectance of a leaf is the radiance reflected from the leaf expressed as a percentage of incident radiance (Carter, 1991). The replicate-averaged (6 replicates) reflectance data obtained at 4 DOS for the three moisture regimes are presented in Figure 3.13. Following is a visual analysis of canopy reflectance, regardless of water treatments.

Low reflectance in the visible (400-700-nm spectral range), extremely high reflectance in the near-infrared (NIR, 700-1,300-nm spectral range), and high reflectance in the mid-infrared (MIR, 1,300-2,500-nm spectral range) wavebands are observed for the three water treatments (Figure 3.13). In the visible spectrum, spectral reflectance is higher near 550 nm, and lower near 670 nm. All these features are typically reported for vegetation reflectance curves (Curcio and Petty, 1951; Guyot, 1990; Carter, 1991). Carter (1991) reported that chlorophyll and accessory pigments absorb strongly in the visible spectral range (especially near 670 nm), therefore reflectance is typically low (and even lower near 670 nm). The absorptivity of chlorophyll is relatively low from 550 to 620 nm, and 700 to 710 nm, therefore reflectance will be higher at these wavelengths (Hoff and Amesz, 1991). Absorption by water and pigments is known to be relatively low in the NIR (Curcio and Petty, 1951). Leaves generally do not contain other substances that absorb strongly in the NIR (Gates, 1980). Thus, reflectance in the 700 to 1,300 nm range is relatively high.

However, water absorbs strongly in the MIR (Curcio and Petty, 1951), resulting in decreased reflectance in the 1,300 to 2,500 nm spectrum. Following is a comparative analysis of canopy reflectance in response to water deficit, depending on the moisture regime.

Water deficit increased canopy reflectance in the visible range of the spectrum at both stress levels, W1 and W2, compared to the controls, W3 (Figure 3.13). This supports the findings of Woolley (1971), Bowman (1989), Carter (1993), and Wang et al. (2000). Carter (1991) showed that the increase of visible reflectance is due to a decrease of visible radiation absorption by leaf pigments. The photons with short wavelength (visible light) adsorbed by leaf pigments are used for photosynthesis and photochemical reactions (Wang *et al.*, 2000). Thus, by decreasing the amount of radiation absorption of visible energy, water stress reduces the photosynthetic processes that produce biomass materials. Therefore, in the present experiment, the increase of visible reflectance suggests that by 4 DOS, water stress showed a tendency to negatively affect photosynthesis. This is confirmed by the results presented in the above paragraph titled "*Impact of water stress on soybean physiology*" where it is reported that water stress significantly reduced photosynthetic rate as early as 1 DOS. The greatest proportional increase was from 650 to 700 nm (red spectrum) at both stress levels. This supports the observations of Carter (1993) and Osborne et al. (2002) who suggested that reflectance in the red spectrum was a good indicator of water stress. Carter (1991) also demonstrated that the reduction of absorption by pigments of visible radiation is due to the decrease of both chlorophyll and leaf water contents.

In the present experiment, there was no increase ($p > 0.05$) in visible reflectance at 4 DOS at both stress levels. Thus, at 4 DOS, water stress did not affect chlorophyll content. This is consistent with the results presented in the above paragraph titled "*Impact of water stress on soybean leaf chlorophyll content* " where it is found that a water stress effect on chlorophyll content was evident only by 12 DOS. Water deficit also decreased spectral reflectance near 550 nm (green spectrum) at both stress levels. This confirms the findings of Carter (1993) who found that visible reflectance is very sensitive to stress in the 535-640 nm (more particularly near 550 nm) wavelength ranges.

The greatest differences in canopy reflectance occurred in the NIR. Water deficit decreased spectral reflectance in the NIR at both stress levels (Figure 3.13). This supports the findings of Moran et al. (1989), Wang and Shannon (1999), and Osborne et al. (2002).

The photons with long wavelength (near infrared) adsorbed by leaf pigments are related to heating, evaporation, and transpiration (Wang *et al.*, 2000). Thus, by increasing the absorption of infrared radiation, water stress might affect leaf heating and transpiration. Therefore, in the present experiment, the decrease of infrared reflectance suggests that by 4 DOS, water stress affected plant heating and transpiration. This confirms the results presented in the above paragraph titled "*Impact of water stress on soybean physiology*" where it is reported that water stress significantly reduced stomatal conductance and transpiration rate as early as 1 DOS. Also, leaf tissues can be affected by environmental factors such as soil water status (Gausman *et al.*, 1969; Thomas *et al.*, 1971; Carter, 1991; Blackmer *et al.*, 1994). Although leaf internal structure has been shown to have little effect on reflectance as compared with absorption effects by water and pigments (Carter, 1991), reflectance sensitivity to water content in the NIR has been related to the effects of water stress on leaf internal tissue structure and optical properties, rather than to the variations of water and pigment contents (Gausman *et al.*, 1969; Thomas *et al.*, 1971; Kumar and Silva; 1973; Carter, 1991; Blackmer *et al.*, 1994). In the present experiment, there was no decrease of NIR reflectance ($p > 0.05$) at both stress levels. Thus, by 4 DOS, water stress did not affect leaf tissue structure.

There was little by way of reflectance differences in the MIR (Figure 3.13) at both stress levels, whereas it is reported that the greatest differences in leaf reflectance in response to water stress occurred beyond 1,300 nm (Carter, 1991; Carter *et al.*, 1992; Carter, 1993; Osborne *et al.*, 2002). Water deficit decreased canopy spectral reflectance in the MIR (Figure 3.13), whereas it is reported to increase reflectance (Bowman, 1989; Carter, 1991; Carter *et al.*, 1992; Carter, 1993). Reflectance in the MIR has been considered to be a function of leaf thickness and water content (Lillesand and Kriefer, 1987; Osborne et al., 2002). In this spectral range, water stress affects leaf reflectance directly by inducing leaf dehydration and reducing absorption of radiation by leaf internal water (Carter, 1991). In the present experiment, the variation of MIR reflectance was not significant ($p > 0.05$) at both stress levels. This indicates that, by 4 DOS, water stress did not result in leaf dehydratation.

The response curves of the stressed plants were not of the same magnitude as the controls. This suggests that water stress influenced the spectral features of soybean canopy. The radiance data were taken at 4 DOS, thus plant water stress was just being established.

This explains why the differences between water treatments were not yet significant. It is likely that, later in the stress period, the differences in leaf reflectance were significant. Unfortunately, due to restrictions in instrument availability, no other reflectance data were taken. The analysis of these spectral data also showed that, by 4 DOS, water stress did not affect plant nitrogen status or leaf structure.

Impact of water stress on leaf area, canopy and pod dry weight at harvest

Water stress reduced ($p < 0.001$) plant leaf area (LA) (measured at harvest time 76 DAP -44 DOS) (Table 3.10). The comparison of mean LA ($LSD_{0.05}$ test) shows that total LA decreased by 52.7 and 74.5 %, at the medium and severe stress levels, respectively (Figure 3.11). Water stress had a strong negative effect on leaf area. This should be a function of the indeterminate nature of this soybean cultivar, which explains the large vegetative growth rate of the control plants. Figure 3.11 also shows that LA was still actively photosynthetic, for all water treatments, at the end of the experiment, indicating that the experiment was ended before plant physiological maturity (growth stage R7). The mean percentage of green LA was lower (numerically) for water stressed plants than the controls. Water deficit decreased the ratio of photosynthetic LA by 7 and 10 %, at the medium and severe stress levels, respectively, compared to the controls. This suggests that water stress might have accelerated leaf senescence, which confirms the observations of De Souza et al (1997) who showed that water stress accelerated the decline in soybean leaf photosynthetic activity.

Soil moisture deficit decreased ($p < 0.001$) total canopy dry weight (pod + stem + branch dry weight, TCDW) and pod dry weight (PDW) (Table 3.10). In this report, PDW is used as a yield index. TCDW and PDW were 24.6 and 23.0 %, and 35.0 and 35.4 % less than the control, at the medium and severe stress levels, respectively (Figure 3.12). Thus, at both stress levels, the decrease in TCDW and yield were important, providing a clear verification of the impact of water stress throughout the treatment period. For this experiment, the overall yield reduction was likely primarily due to a decrease in pod number during flowering and early pod development. This verifies the observations of Doss et al. (1974), Westgate and Peterson (1993), and Kokubun et al. (2001) who showed that long-term soil water deficit during flowering and early pod set decreased pod yield of soybean.

Water deficit did not affect the ratio of pod dry weight to total canopy dry weight (Table 3.10). Moreover, there were no detectable differences of this ratio among the water availability treatments ($LSD_{0.05}$ test). The constancy of the pod dry weight ratio (ratio = 0.355) between treatments suggests that, at both stress levels, soybean plants might have managed to maintain some control of their growth pattern and, thus, limit yield decrease.

SUMMARY AND CONCLUSIONS

The data demonstrate that chronic soil water deficit was quickly established and present throughout the duration of the water stress treatment, and that the watering treatments were successful in establishing two different levels of plant water stress W1 and W2. The results indicate that, from the physiological point of view, two different stress levels were significantly established by 1 DOS, but from the morphologic point of view, they were significantly established only by 12 DOS. The reflectance data showed that no difference between W1 and W2 was detectable by 4 DOS.

The drought conditions imposed on the soybean plants resulted in an important decrease in plant physiology and productivity at both water stress levels, W1 and W2, in comparison to the control, W3. This provides a clear verification of the impact of water stress throughout the treatment. The reflectance data suggest that water stress influenced the spectral features of soybean canopy, as it increased spectral reflectance in the visible and decreased spectral response in the infrared spectrum.

Water stress negatively affected soybean physiology for all physiological variables measured, decreased plant area, reduced leaf chlorophyll content, and had a visible impact on plant canopy architecture. The experiment shows that stomatal conductance was most negatively affected by the stress treatment, which suggests that, at the physiological level, the major effect of chronic water stress is a decrease in stomatal conductance. The results show that paraheliotropism behaviour was observable at the medium stress level, but not at the severe stress. Thus, there might be a threshold stress level beyond which leaf movement cannot be activated, preventing control of stomatal transpiration through canopy movement.

The data show a sharp decrease of the rate of plant vegetative growth at both stress levels for all variables measured. Chronic water stress inhibited the appearance of nodes on the main stem. This inhibition led to a large reduction of flower and pod

numbers. The decrease of the rate of plant vegetative growth suggests that soil moisture deficit during reproductive growth might have induced an early switch of plant development from vegetative to reproductive at both stress levels.

This experiment indicates that moderate chronic water stress does not affect leaf chlorophyll content, but that severe chronic water stress decreases leaf chlorophyll content. This shows that decreasing the available soil moisture content to less than 40 % of plant water requirements significantly reduces plant nitrogen status.

The decrease in yield was significant at both stress levels, W1 and W2. Overall yield reduction was primarily due to the decreases of flower and pod numbers. Water deficit, at W1 and W2, did not affect the ratio of pod dry weight to total canopy dry weight. The constancy of the pod dry weight ratio suggests that the proportional allocation of dry matter to reproductive structures was maintained and, in spite of the fact that the stress was imposed during the reproductive period, vegetative and reproductive structures might have been equally affected. In part this could be a function of the indeterminate nature of the soybean cultivar.

Table 3.1. Daily pan evaporation and soybean evapotranspiration (ETc) from the day water stress was commenced (30 DAP, the 30th Day after Planting) to the day before harvest (75 DAP, 43 DOS).

* The first day of plant water stress as measured by decrease of physiological parameters (33 DAP) is referred to as 1 DOS.

*The day soil moisture deficit was commenced (30 DAP) is referred to as –2 DOS, 3 days before plant water stress impact was measured.

* Soybean ETc as calculated from Pan evaporation (Equation 3.1).

Day of month	Day of Stress (DOS)	Day after Planting (DAP)	Pan evaporation (mm)	ETc* (mm)
15-06	-2	30	12.4	9.9
17-06	0	32	8.9	7.1
18-06	1	33	4.4	3.5
19-06	2	34	4.7	3.8
20-06	3	35	5.8	4.7
21-06	4	36	3.4	2.7
22-06	5	37	7.5	6.0
23-06	6	38	2.4	1.9
24-06	7	39	4.6	3.7
25-06	8	40	4.5	3.6
26-06	9	41	3.6	2.9
27-06	10	42	6.1	4.9
28-06	11	43	5.0	4.0
29-06	12	44	4.4	3.5
30-06	13	45	3.6	2.9
01-07	14	46	3.6	2.9
02-07	15	47	5.7	4.6
03-07	16	48	1.7	1.4
04-07	17	49	3.6	2.9
05-07	18	50	5.7	4.6
06-07	19	51	3.4	2.7
08-07	21	53	2.2	1.8
09-07	22	54	2.4	1.9
10-07	23	55	3.4	2.7
11-07	24	56	3.4	2.7
12-07	25	57	3.1	2.5
13-07	26	58	2.4	1.9
18-07	31	63	1.4	1.1
19-07	32	64	3.9	3.1
20-07	33	65	5.1	4.1
21-07	34	66	5.1	4.1
22-07	35	67	5.1	4.1
23-07	36	68	6.1	4.9
24-07	37	69	5.4	4.3
25-07	38	70	4.5	3.6
26-07	39	71	4.6	3.7
27-06	40	72	1.1	0.9
28-07	41	73	5.9	4.7
29-07	42	74	5.1	4.1
30-07	43	75	5.1	4.1

Table 3.2. Description of reproductive stages of development of soybean.

Stage no.	Abbreviated stage title	Description*	Starting day	
			DAP	DOS
V8	8th-node	8 nodes on the main stem and 7 fully developed leaves	29	/
R1	Beginning bloom	1 open flower at any node on the main stem	33	1
R2	Full bloom	Open flower at one of the 2 uppermost nodes on the main stem	36	4
R3	Beginning pod	Pod 5 mm long at 1 of the 4 uppermost nodes on the main stem	51	19
R4	Full pod	Pod 2 cm long at 1 of the 4 uppermost nodes on the main stem	55	23
R5	Beginning seed	Seed 3 mm long in a pod at one of the 4 uppermost nodes on the main stem	66	34
R6	Full seed	Pod containing a green seed that fills the pod cavity at 1 of the 4 uppermost nodes	74	42

* Description of stages from Fehr and Caviness (1977).

DAP: Days After Planting

DOS: Days of plant Stress

Table 3.3. Pearson's simple linear correlation coefficients between stomatal conductance (Rs), photosynthetic rate (Pr), transpiration rate (Tr), and air temperature (Tair) for the three moisture regimes, severe stress (W1), medium stress (W2), and control (well watered, W3).

	Control			W2			W1		
	Pr	Rs	Tr	Pr	Rs	Tr	Pr	Rs	Tr
Rs	0,89***			0,92***			0,96***		
Tr	0,92***	0,93***		0,95***	0,97***		0,96***	0,99***	
Tair	0,14ns	0,20ns	0,32ns	0,25ns	0,25ns	0,34ns	0,52*	0,50*	0,60**

n = 20 observations.
*, **, and *** = significant at the 0.1, 0.05, and 0.01 levels, respectively.
ns = not significant.

Table 3.4. ANOVA table for the effect of water stress on the number of leaves (L) per soybean plant at "x" days of stress.

Source of Variation	Degree of Freedom	*Pr* > F*				
		L4	L9	L12	L19	L24
Block	5	.2207	.1107	.1107	.8634	.4651
Water stress	2	.0235	.0001	.0000	.0000	.0000
Error	10					

* If $Pr \leq 0.05$, then there was a significant effect.

L stands for leave; L4 means number of leaves at 4 day of stress.

Table 3.5. Mean number of leaves per plant for the control, medium stress (W2) and severe stress (W1) at five days of stress.

Day of Stress	LSD value	Mean Leaf Number*		
		Control	W2	W1
4 DOS	.56	7.6 A	7.3 AB	6.8 B
9 DOS	.56	8.8 A	7.3 B	7.0 B
12 DOS	.56	11.2 A	8.7 B	7.0 C
19 DOS	.93	12.7 A	9.7 B	7.5 C
24 DOS	.73	13.3 A	10.0 B	7.5 C

* Mean of 6 observations. In each row, means followed by the same letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

Table 3.6. ANOVA table for the effect of water stress on soybean plant height (H) at "x" days of stress, and the effect of water stress on the number of branches per plant (BR) at harvest on the 44th Day of Stress.

Source of Variation	Degree of Freedom	*Pr* > F*					
		H9	H12	H16	H19	H23	BR
Block	5	.4823	.5958	.4501	.5477	.6034	.5964
Water stress	2	.0001	.0000	.0000	.0000	.0000	.0507
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

H stands for height; H9 means plant height at 9 day of stress.

Table 3.7. ANOVA table for the effect of water stress on soybean leaf chlorophyll content (CL) at "x" day of stress.

Source of Variation	Degree of Freedom	*Pr* > F*				
		CL9	CL10	CL12	CL19	CL23
Block	5	.8814	.8538	.3137	.2127	.5831
Water stress	2	.2084	.1218	.0165	.0067	.0031
Error	10					

* If $Pr \leq 0.05$, then there was a significant effect.

CL stands for chlorophyll content; CL9 means chlorophyll content at 9 day of stress.

Table 3.8. Mean Spad value of soybean leaves for the control, medium stress (W2) and severe stress (W1) at six days of stress.

Day of Stress	LSD value	Mean Spad Value*		
		Control	W2	W1
9 DOS	2.77	40.42 A	40.03 A	38.18 A
10 DOS	2.45	39.27 A	38.13 AB	36.75 B
12 DOS	2.25	42.55 A	41.75 A	39.10 B
16 DOS	2.76	41.78 A	41.46 AB	38.95 B
19 DOS	2.60	43.47 A	41.45 A	38.65 B
23 DOS	2.92	43.13 A	38.08 B	37.90 B

* Mean of 6 observations. In each row, means followed by the same letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

Table 3.9. ANOVA table for the effect of water stress on:

- the total number of open flowers per soybean plant.(F) at 14 day of stress (DOS)
- the total number of pods (all sizes) per plant on 19 (P1), and 26 DOS (P2)
- the number of pods per plant on 26 DOS with lengths:
  < 1cm (P2A), > 1cm and < 4cm (P2B), and > 4cm (P2C)

Source of Variation	Degree of Freedom	*Pr* > F*					
		F	P1	P2	P2A	P2B	P2C
Block	5	.2869	.7024	.2184	.3247	.2219	.2472
Water stress	2	.0001	.0058	.0000	.0010	.0001	.0000
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

Table 3.10. ANOVA table for the effect of water stress on:

- soybean total leaf area (TLA), photosynthetic leaf area (PLA)
- the ratio of PLA to TLA (RLA)
- total canopy dry weight (TDW), pod dry weight (PDW)
- and the ratio of PDW to TDW (RDW)

Source of Variation	Degree of Freedom	*Pr* > F*					
		TLA	PLA	RLA	TDW	PDW	RDW
Block	5	.3253	.0902	.1528	.3898	.2634	.5528
Water stress	2	.0000	.0000	.1721	.0000	.0000	.1844
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

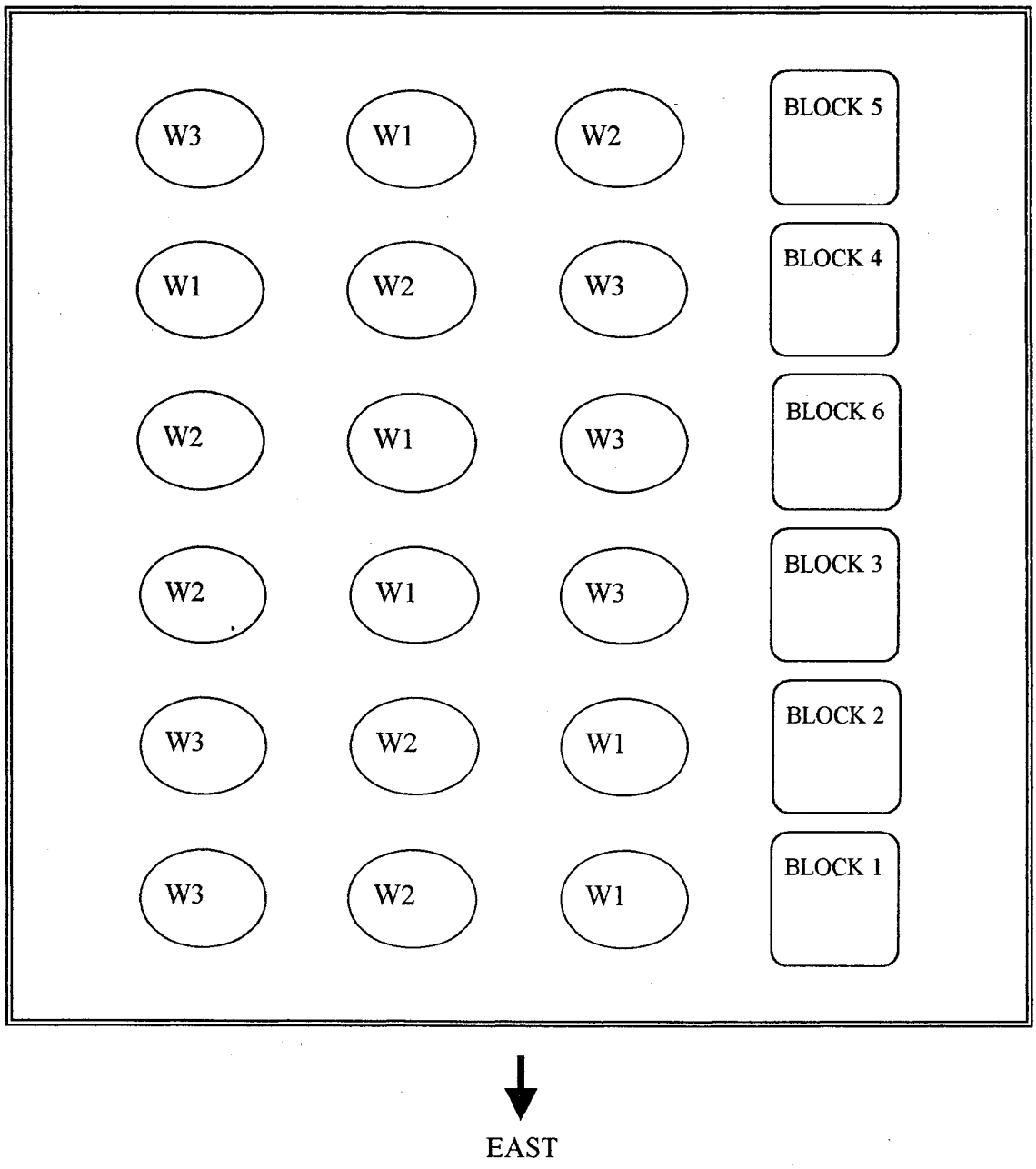


Figure 3.1. Layout of the plants in 6 blocks on the bench in the greenhouse chamber.

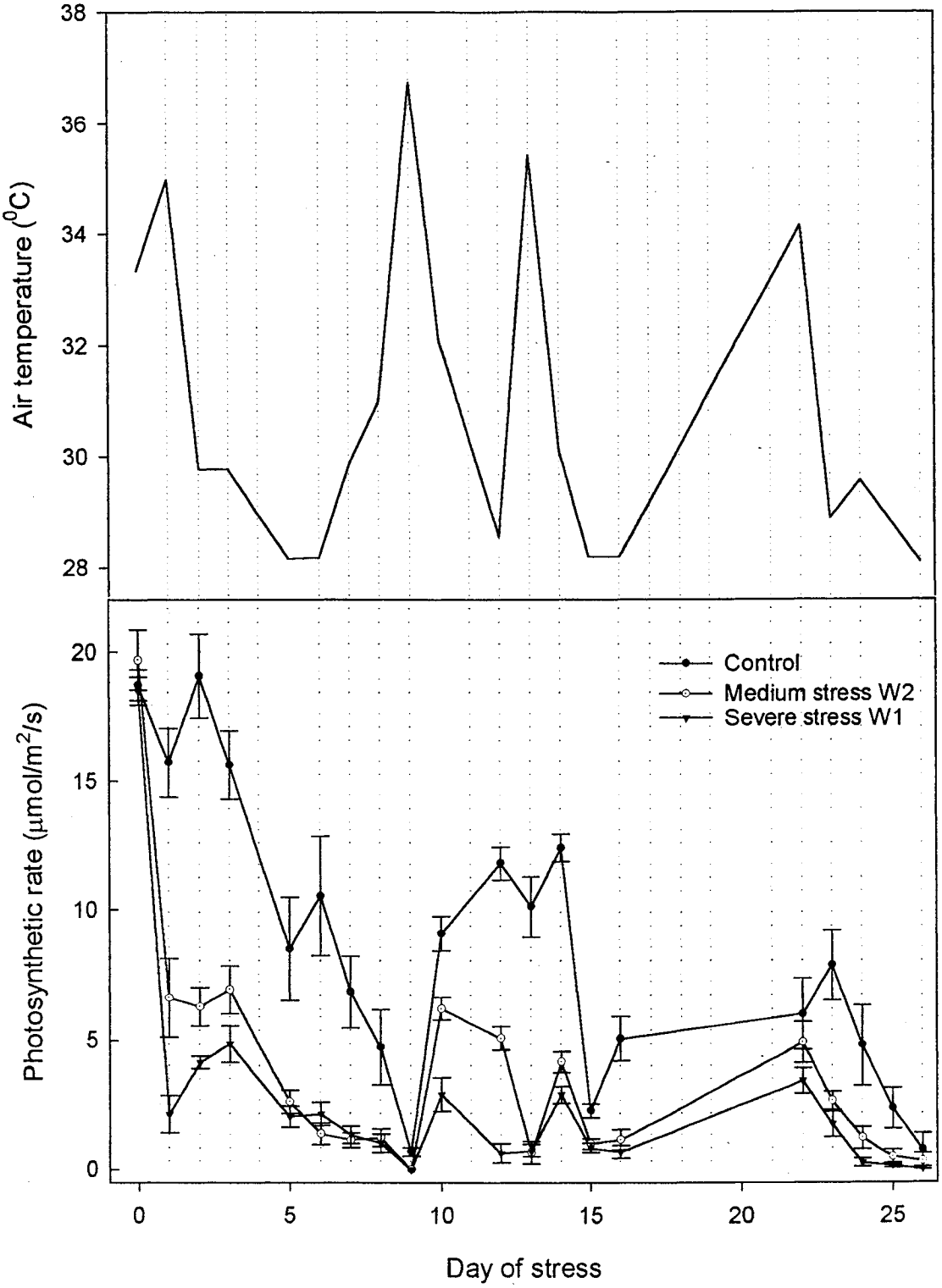


Figure 3.2. Effect of soil moisture stress on photosynthetic rate. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Each point is the mean of 6 observations. Bars represent ± 1 standard error of the mean.

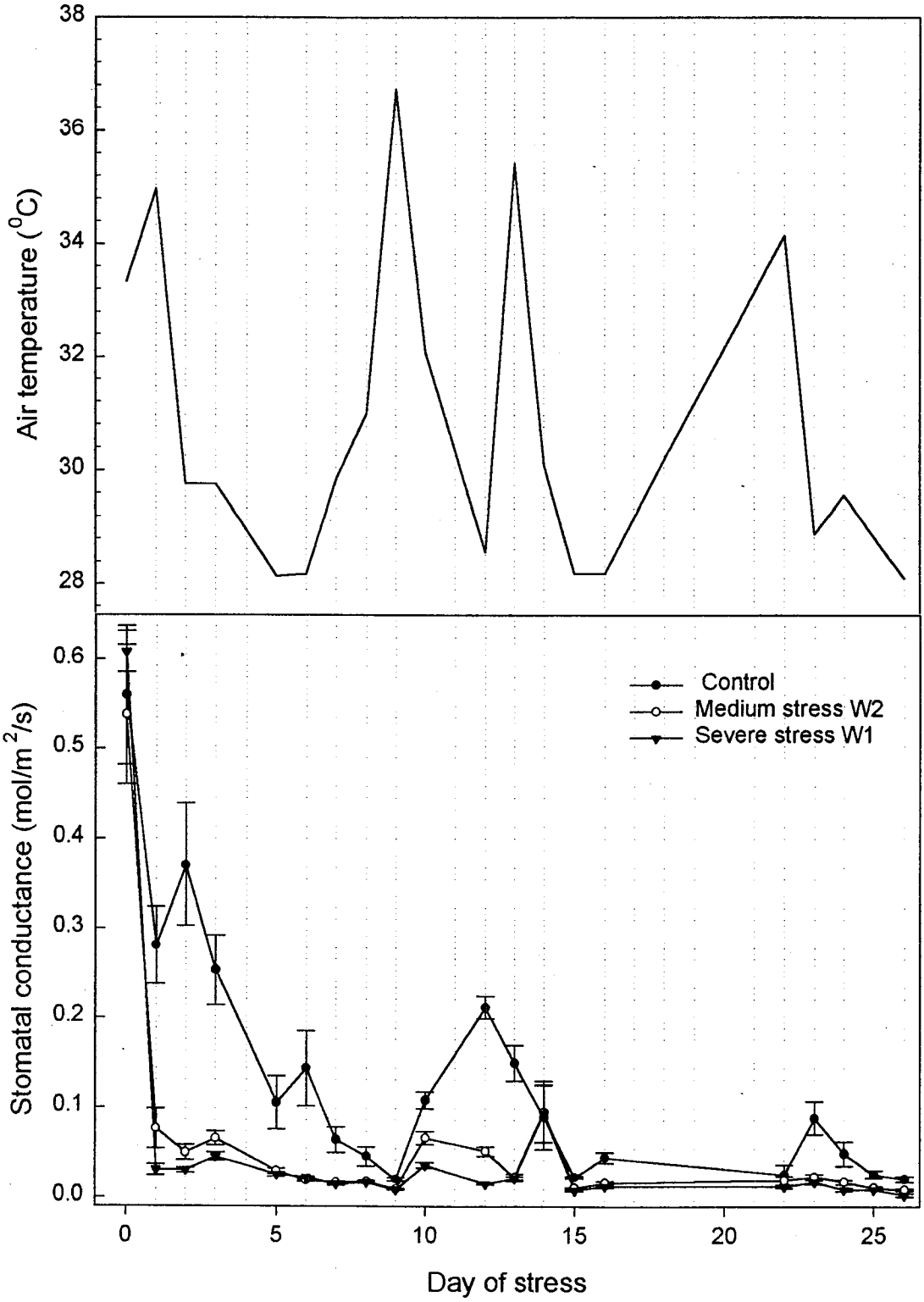


Figure 3.3. Effect of soil moisture stress on stomatal conductance. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Each point is the mean of 6 observations. Bars represent ± 1 standard error of the mean.

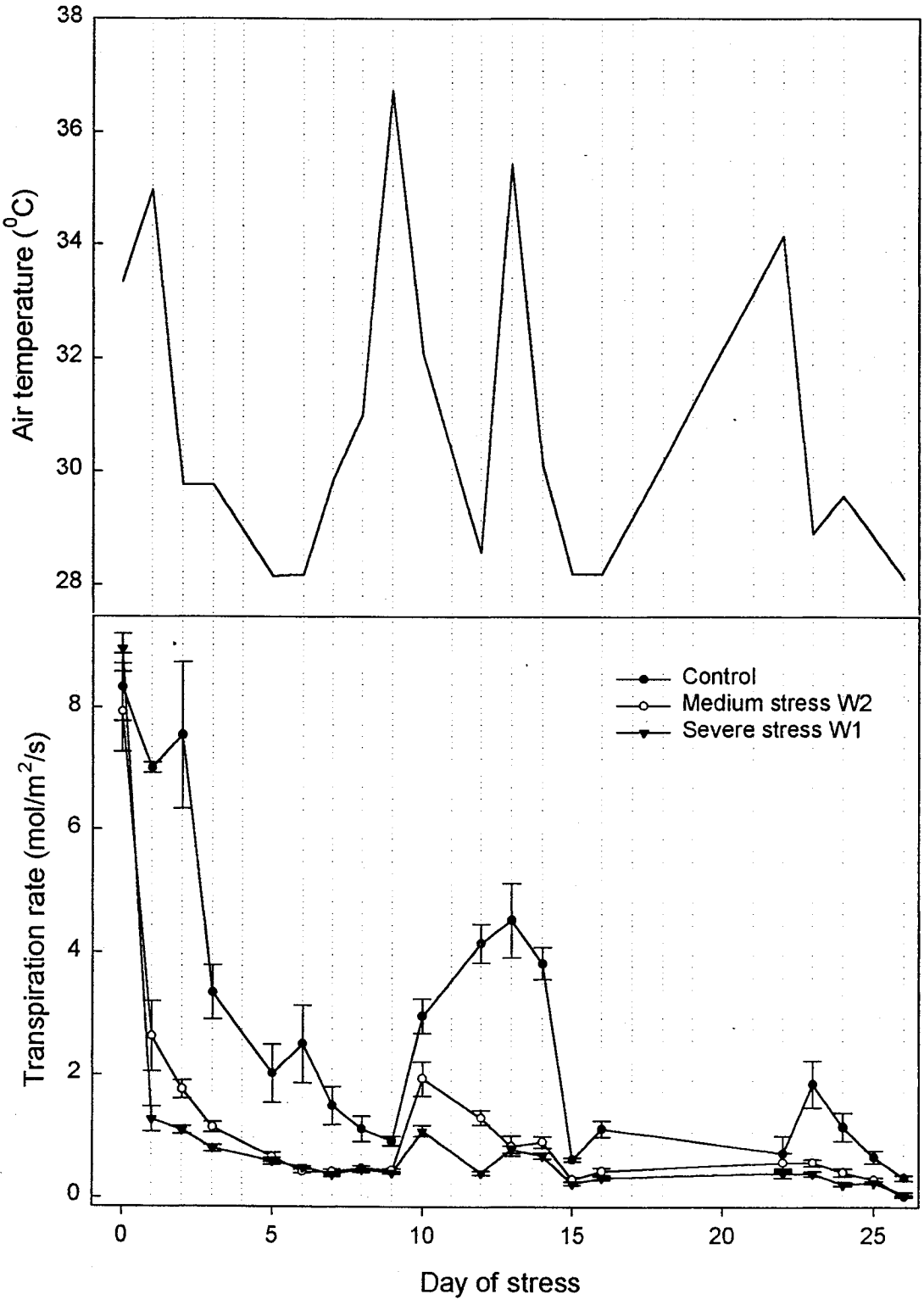


Figure 3.4. Effect of soil moisture stress on transpiration rate. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Each point is the mean of 6 observations. Bars represent ± 1 standard error of the mean.

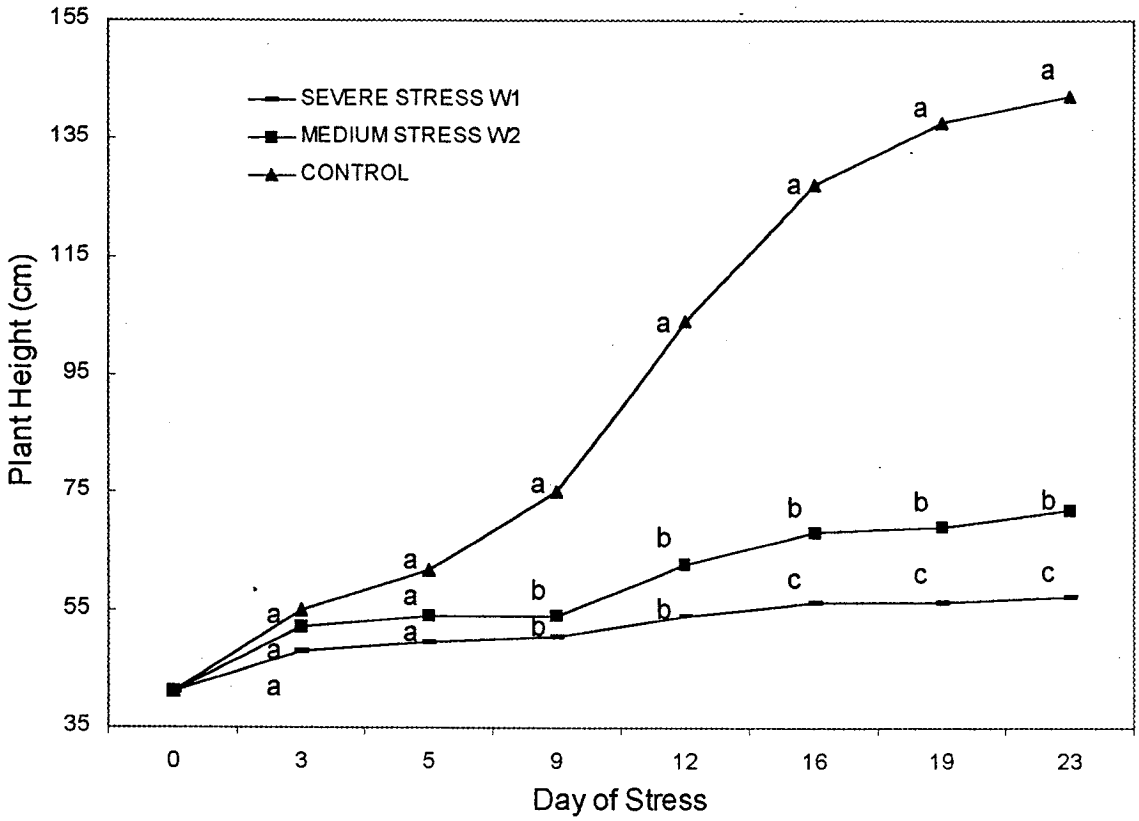


Figure 3.5. Effect of soil moisture stress on plant height of soybean plants throughout the stress period. Each point is the mean of 6 observations. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Within the same day, mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

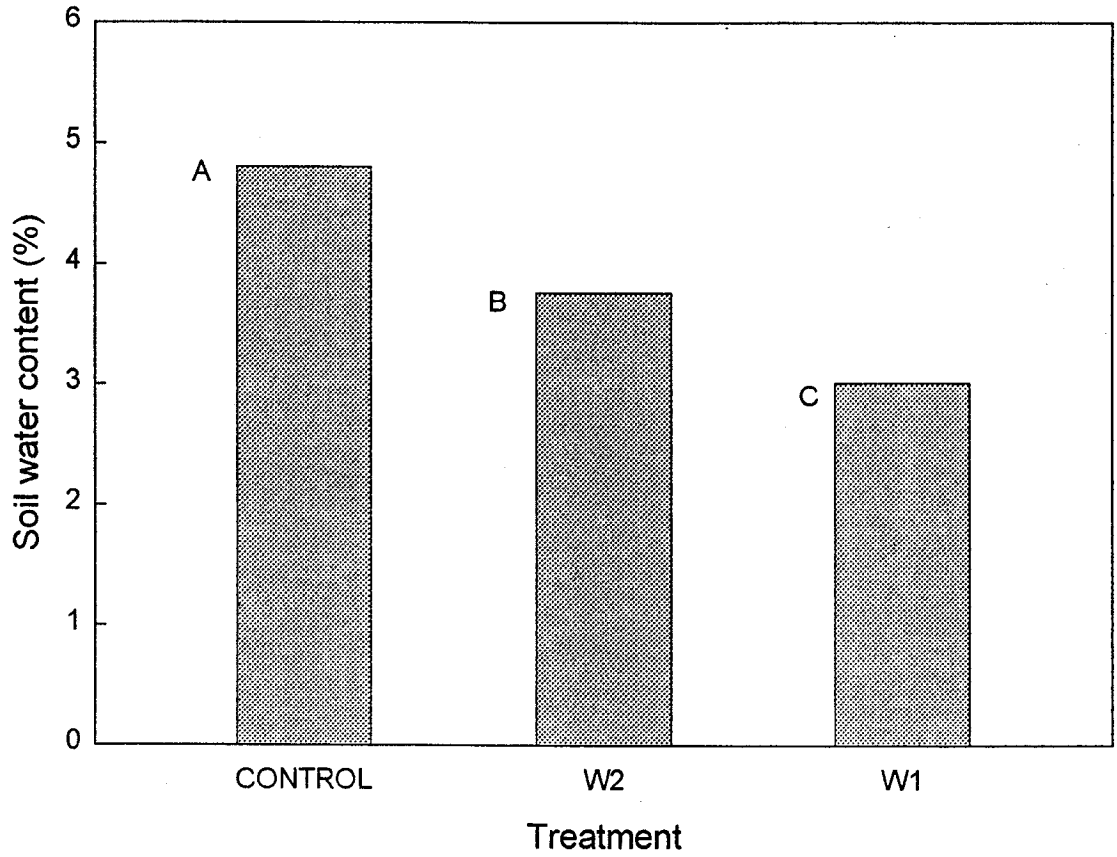


Figure 3.6. Soil water content (i.e. percentage of water in the soil by weight) measured at harvest after 44 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

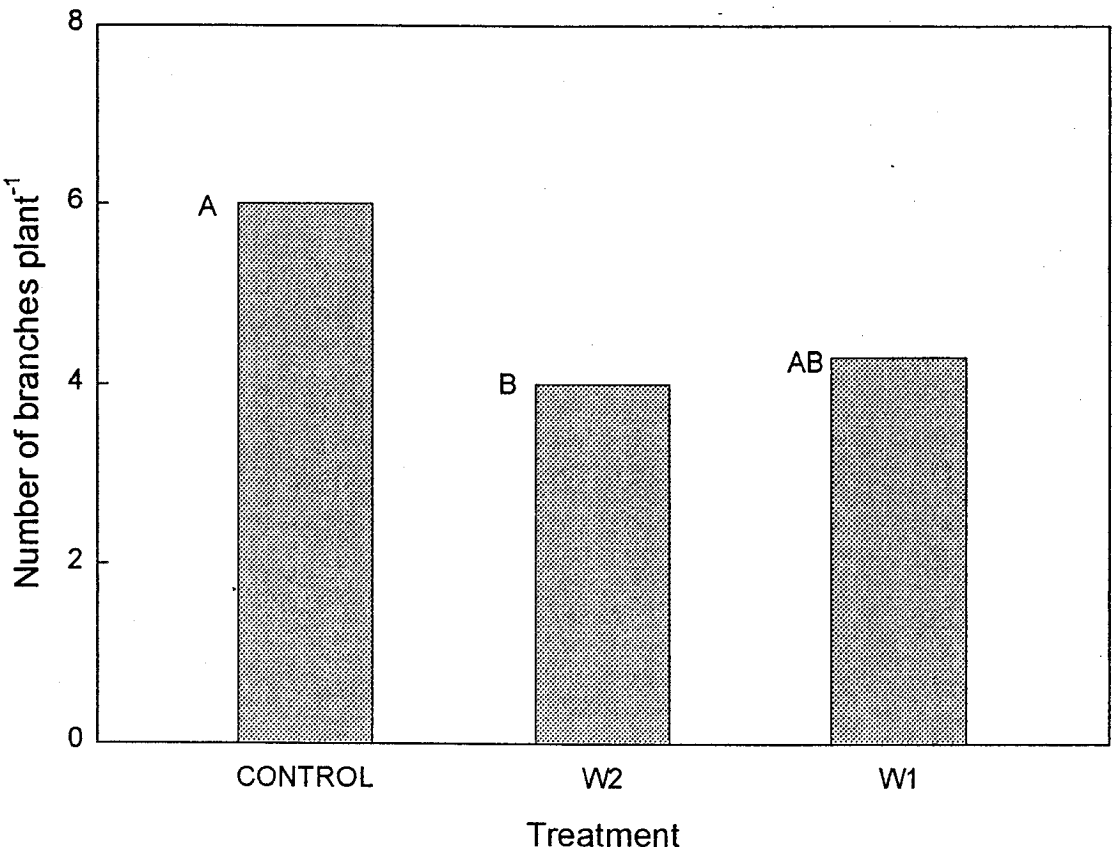


Figure 3.7. Effect of soil moisture deficit on the total number of branches per plant, measured at harvest after 44 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

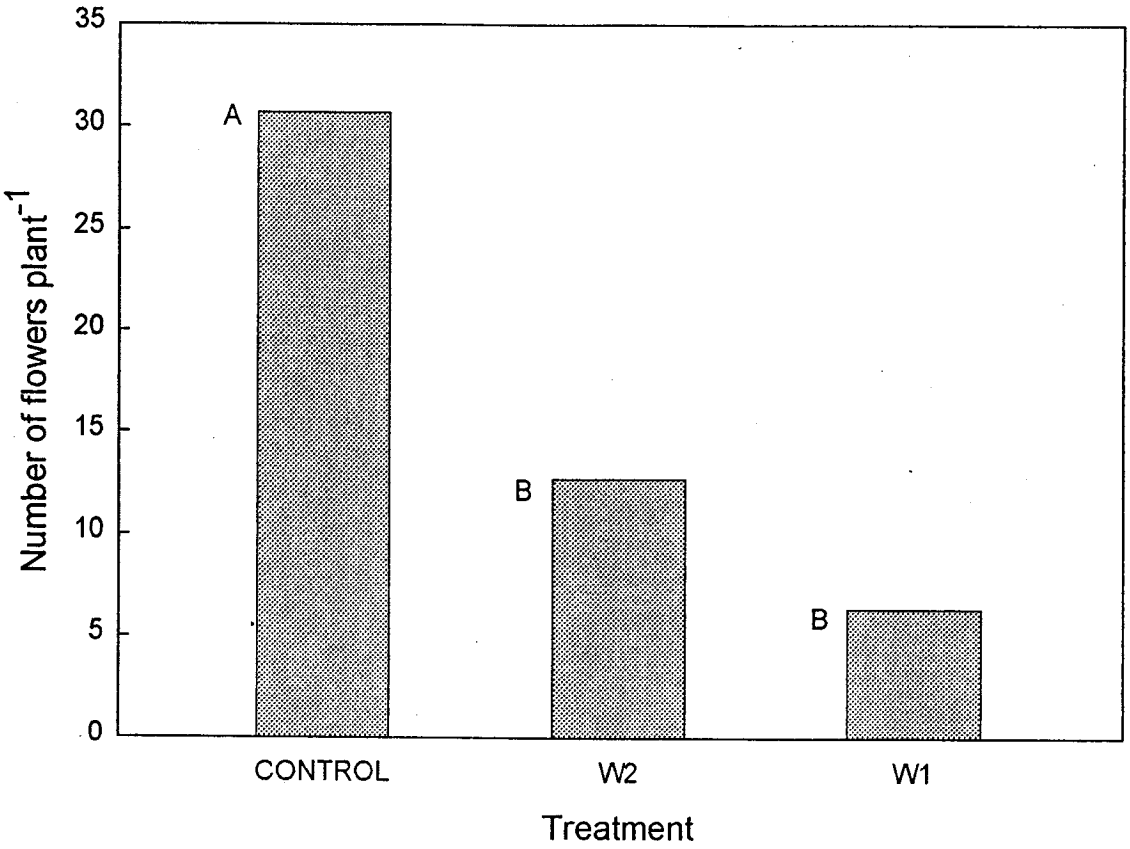


Figure 3.8. Effect of soil moisture deficit on the total number of fully opened soybean flowers per plant, measured after 14 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

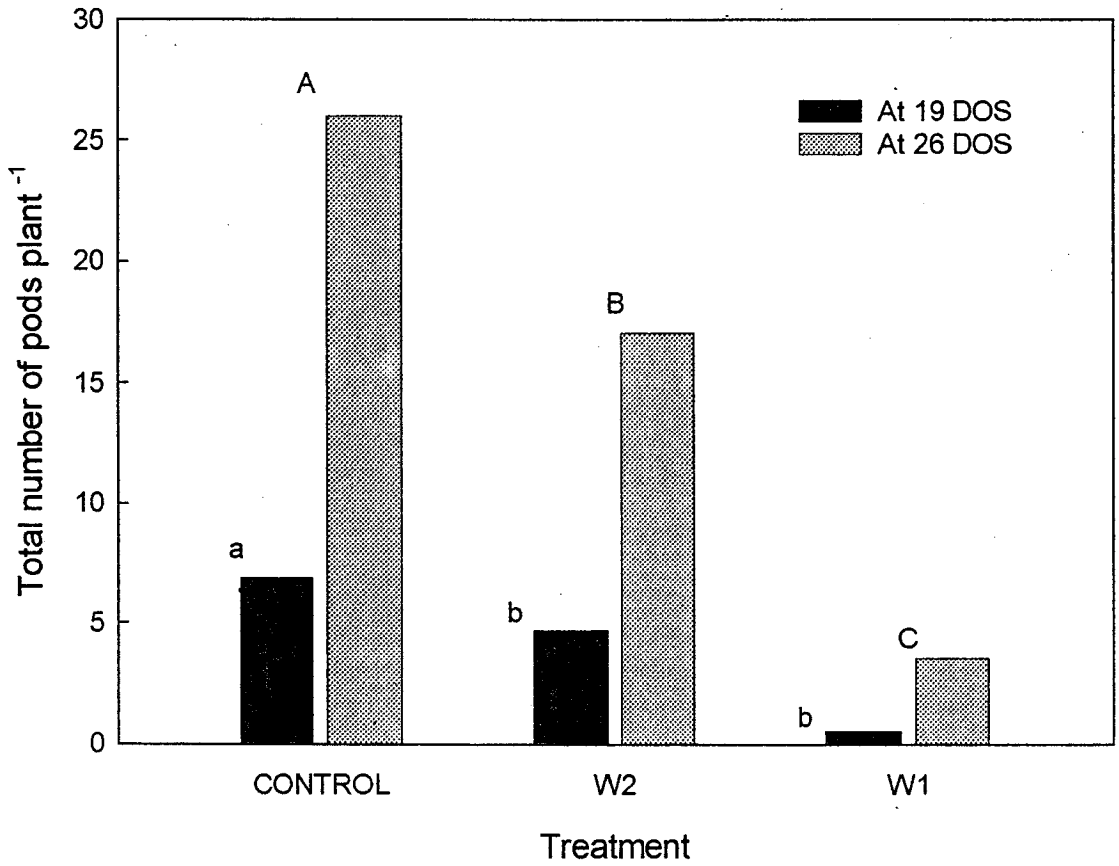


Figure 3.9. Effect of soil moisture deficit on the total number of pods (all sizes) per plant after 19 and 26 days of stress (DOS). Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter* are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

* Letters in lower case refer to the comparison of pods after 19 days of stress.
* Letters in upper case refer to the comparison of pods after 26 days of stress.

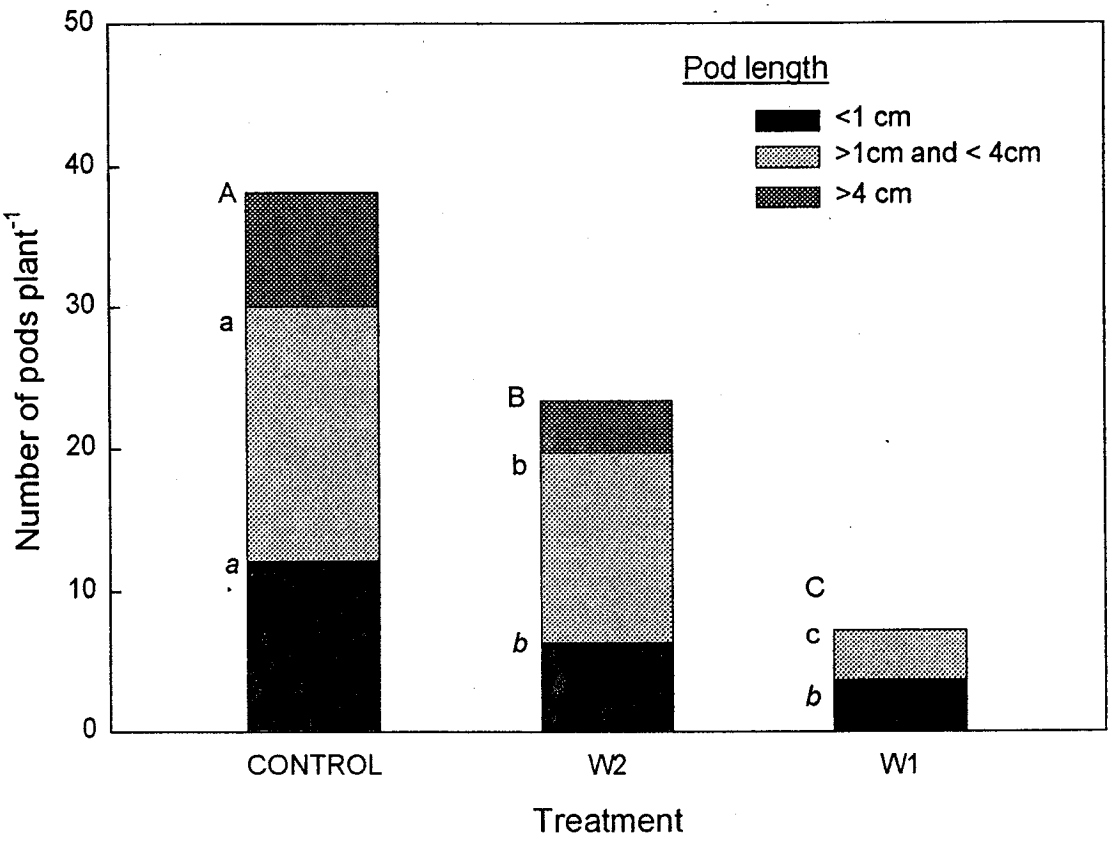


Figure 3.10. Effect of soil moisture deficit on pod repartition by size after 26 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter* are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

* Letters in italics refer to the comparison of pods with lengths < 1 cm.
* Letters in lower case refer to the comparison of pods with lengths > 1cm and < 4 cm.
* Letters in upper case refer to the comparison of pods with lengths > 4cm.

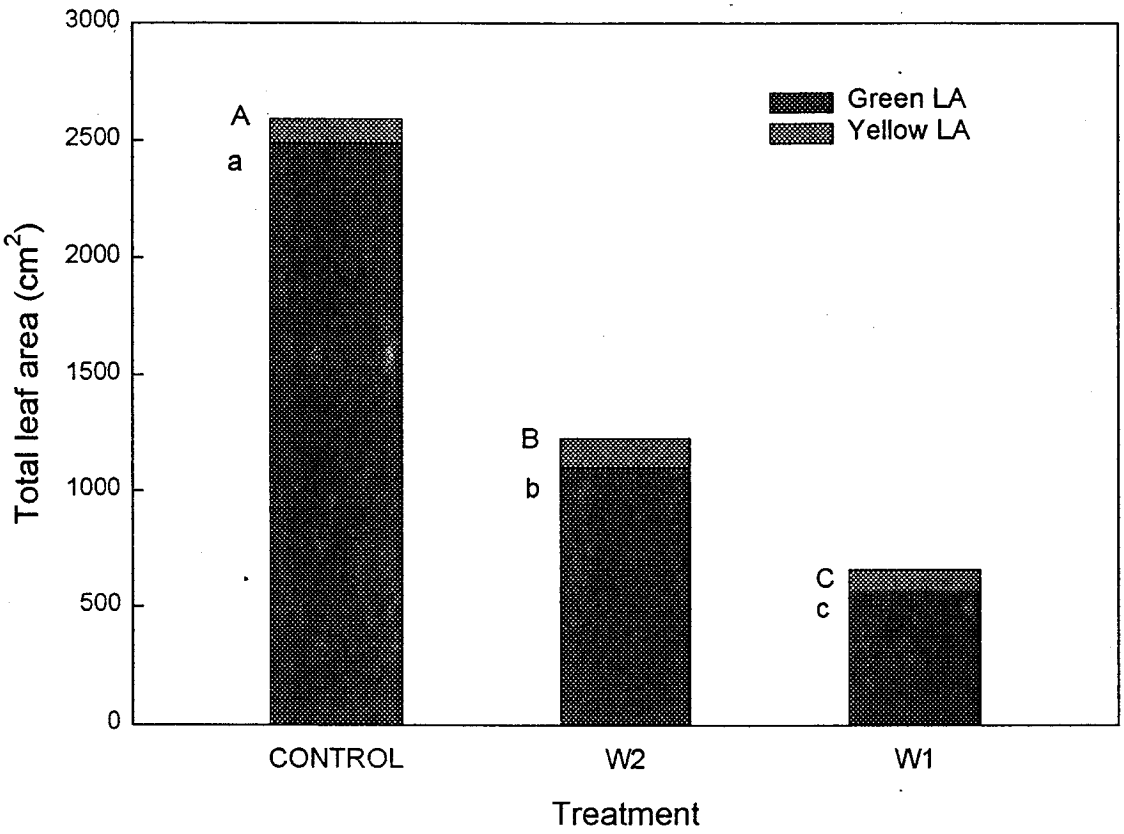


Figure 3.11. Effect of soil moisture deficit on total photosynthetic leaf area (Green LA) and senescent leaf area (Yellow LA) measured the day of harvest after 44 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter* are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

* Letters in upper case refer to the comparison between treatments of senescent leaf area.
* Letters in lower case refer to the comparison between treatments of Green leaf area.

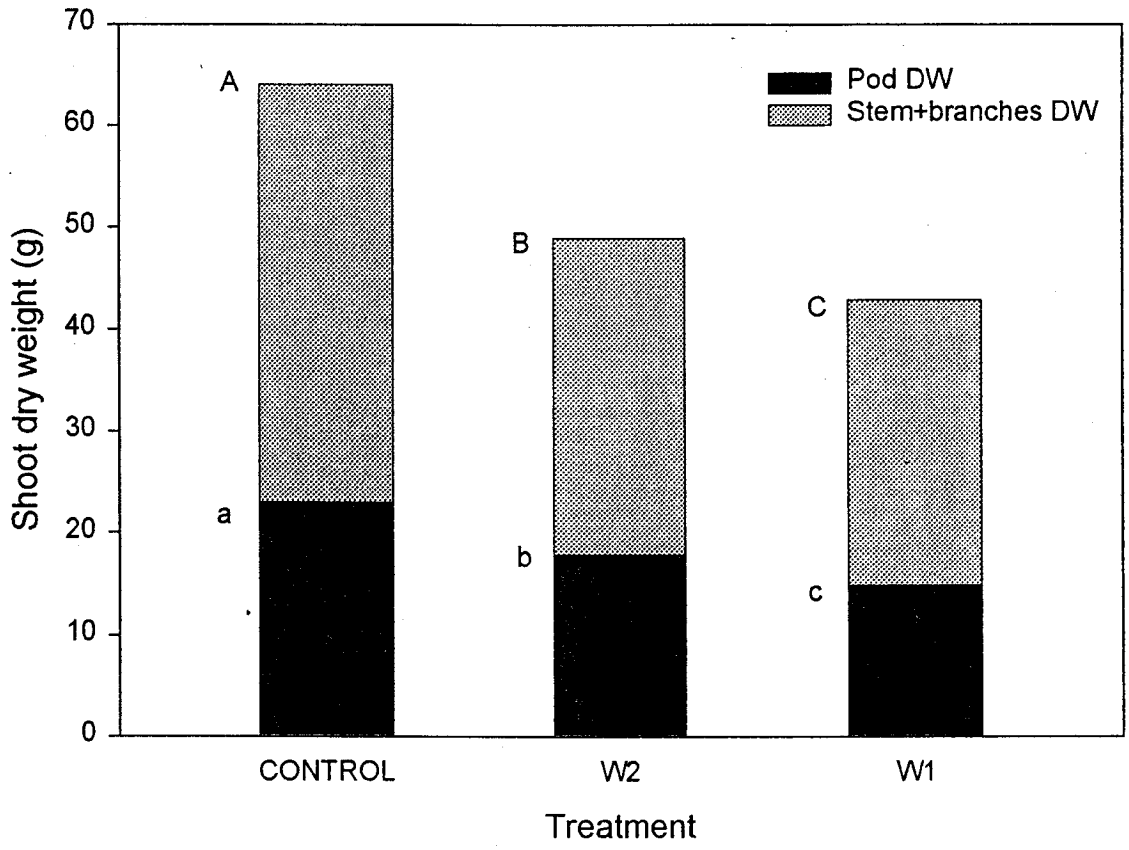


Figure 3.12. Effect of soil moisture deficit on shoot dry weight, divided by pod dry weight (Pod DW) and canopy dry weight (Stem + Branches DW) measured after harvest. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Mean values (n = 6) followed by a common letter* are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

* Letters in upper case refer to the comparison of Stem + Branches DW.

* Letters in lower case refer to the comparison of Pod DW.

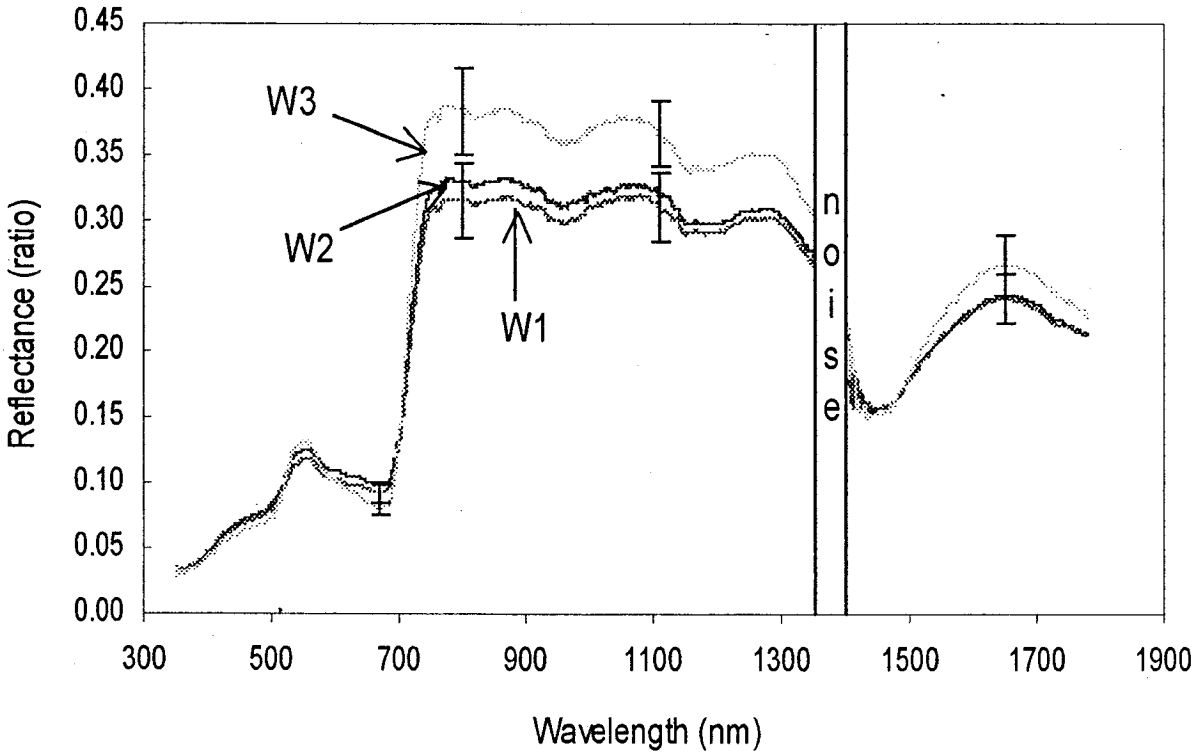


Figure 3.13. Effect of soil moisture deficit on soybean spectral reflectance after 4 days of stress. Control (or W3) is well-watered, W2 is medium stress, and W1 is severe stress. Spectral response curve for entire plant canopy. Each curve is the mean of 6 observations. Bars show ± 1 standard error of the mean, for W3 and W1 only (for clarity). Spectral data are not reported from 1,350 to 1,400 nm because of the presence of excessive noise.

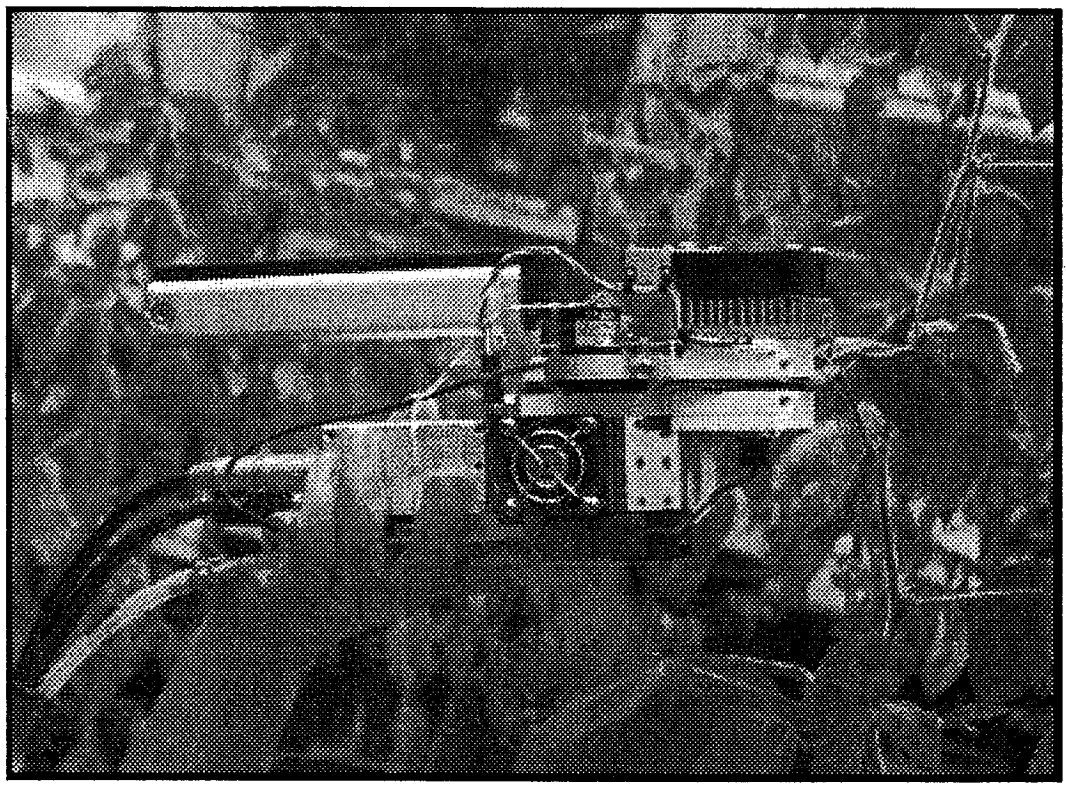


Photo 3.1. Head of the portable photosynthesis system.

Photo 3.2. Use of the spectrophotometer. Pistol of the instrument held over the plant.

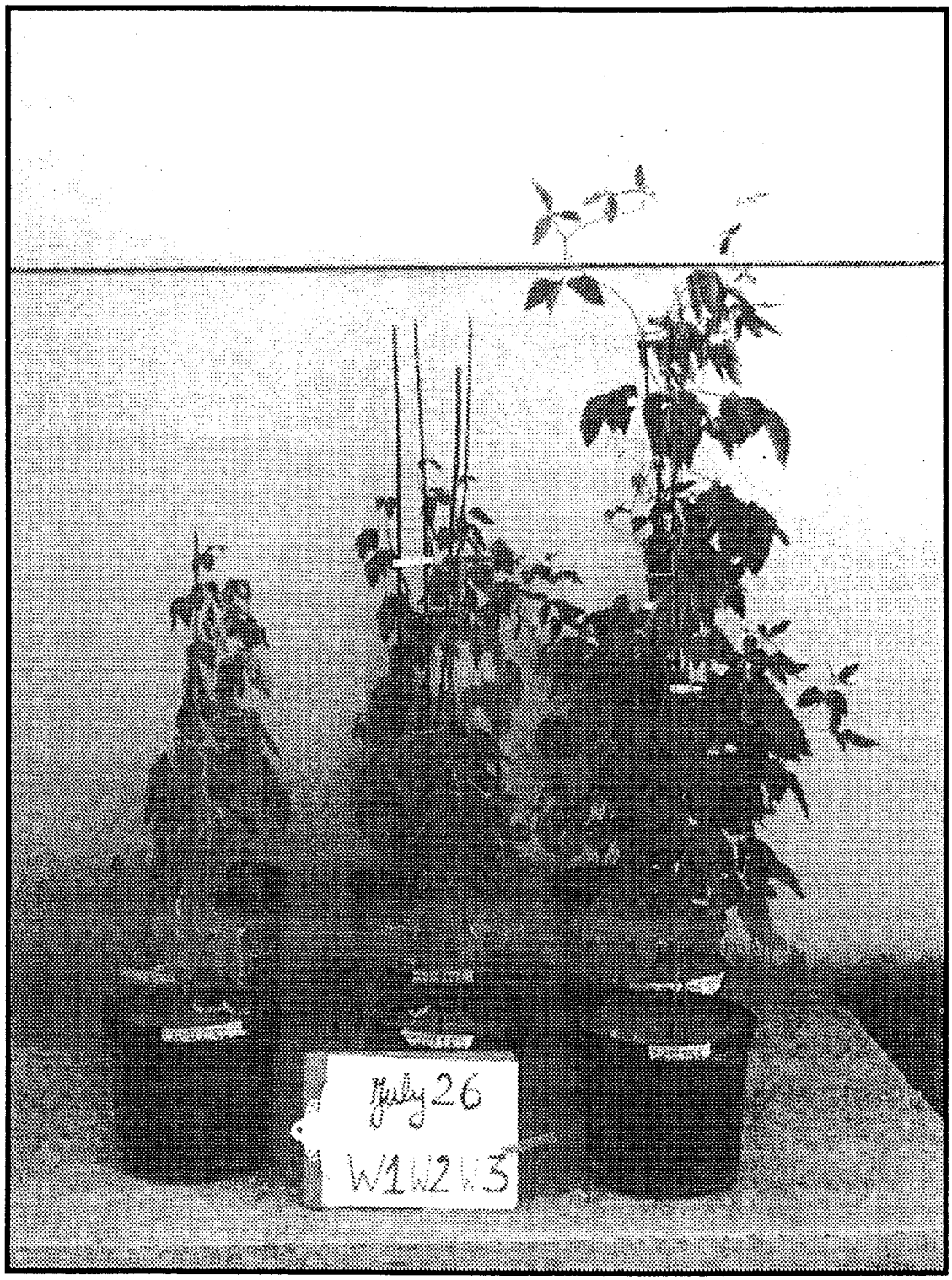


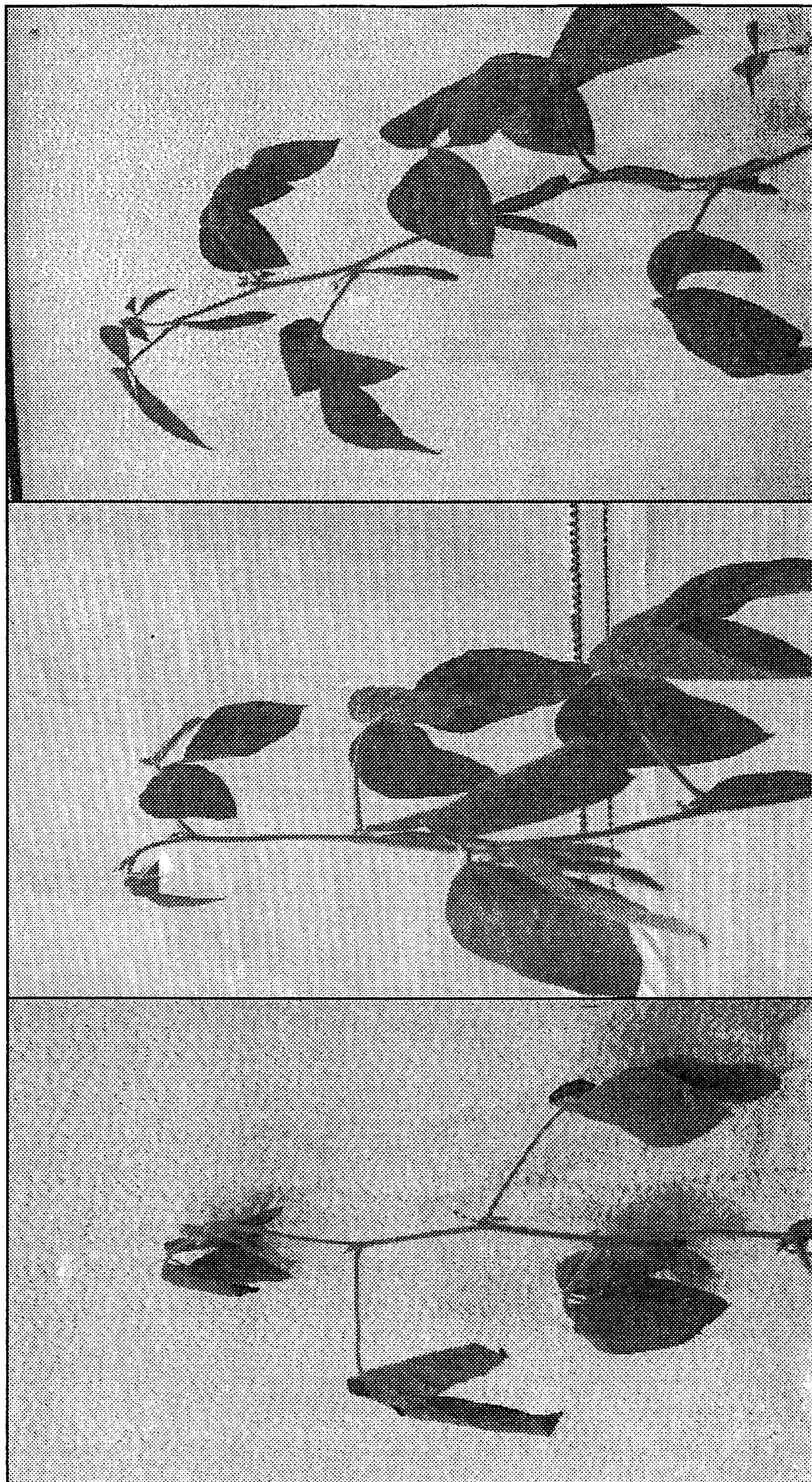
Photo 3.3. Effect of soil water deficit on soybean plant canopy area. Photo taken 71 DAP, after 39 days of stress, for the severe stress (W1), medium stress (W2) and control (W3), left to the right respectively.

Photo 3.4. Effect of soil water deficit on soybean canopy architecture*; the situation in the afternoon of 71 DAP, 39 days of stress for the severe stress (W1), medium stress (W2) and control (W3), from bottom to top, respectively.

* Leaf rolling and wilting are observable on the leaves of W1 and W2 plants and give a vertical profile to the stressed plant canopy.

* Paraheliotropic behavior is observable in the leaves of W2 plants.

* Diaheliotropic behavior maintains the canopy surface of the control plants perpendicular to the direction of incident radiation, maximizing solar energy interception.



Preface to Chapter 4.

Chapter 4 is comprised of a manuscript that has been co-authored by R. Bonnell, D.L. Smith and myself. The paper was prepared for submission in 2002 to the Canadian Water Resources Journal. All literature cited in this chapter are listed in the reference section at the end of the thesis. All tables, figures, and photos are presented at the end of the chapter.

One of the objectives of the thesis was to study interactions between water deficit and LCO application. Knowledge of the individual effects of water stress was necessary for a better understanding of the supplemental effects of the LCO application. Therefore, it was essential to conduct an experiment without the use of LCO in order to study exclusively the effects of chronic water stress on the growth pattern of soybean. This study was presented in chapter 3. In chapter 4, this information will be used for comparisons with soybean, under chronic water stress, grown along with the application of LCOs.

Chapter 4 describes a greenhouse experiment carried out to study the effects of LCOs spray application on the growth pattern of soybean plants under chronic water stress. The objectives of the experiment were to assess the interactions between chronic water stress and LCO treatment during reproductive development of soybean plants (cultivar OAC Bayfield), to determine the specific effects of LCO spay application on the development pattern of indeterminate soybean, and to detect changes in soybean canopy spectral reflectance due to LCO application.

Chapter 4.

RESPONSE OF SOYBEAN (*Glycine max* (L.) Merr.) UNDER CHRONIC SOIL WATER DEFICIT TO LCO APPLICATION UNDER GREENHOUSE CONDITIONS DURING REPRODUCTIVE DEVELOPMENT

ABSTRACT

There has been considerable effort to enhance photosynthesis in crop plants that are sensitive to water deficit. Lipo-chitooligosaccharides (LCOs) are bacteria-to-plant signal molecules essential for the establishment of rhizobia-legume symbioses. LCOs (or Nod factors) are known to invoke a number of physiological changes in the soybean plant. A greenhouse experiment was conducted during the summer of 2001 to (i) assess the interactions between soil moisture deficit and spray application of LCOs during reproductive development of soybean plants, and (ii) evaluate changes in canopy spectral reflectance due to LCO application. The study was aimed principally at analysing the potential impact of LCO spray on the soybean's drought resistance and productivity. Soybean plants (cultivar OAC Bayfield), all well fertilized, were grown under three moisture regimes: daily watering at 100% (control W3), 50 % (medium stress W2) and 25% (severe stress W1) of soybean evapotranspiration (ETc). LCO spray treatment started only after the water stress was established. 50% of plants in each water treatment were sprayed with the LCO solution (plants L1), the other half of the plants (L0) were sprayed with distilled water. A solution of LCO Nod *Bj* V (141.6 μg/L) was used. The experiment was organized in a completely randomised block design with 6 blocks of factorial combinations of the 3 moisture levels by the 2 LCO levels. A Li-Cor Model-6400 portable photosynthetic meter was used to measure physiologic growth related variables. Plant height and other common morphologic growth variables were also measured regularly. Reflectance of the whole plant canopy was measured with portable spectrophotometers, at 300 to 2,500 μm or 300 to 1,300 μm wavelengths. Soil moisture deficit strongly reduced soybean productivity at both water stress levels. Foliar application of LCO affected overall plant physiological activity, increased flower and pod numbers, and accelerated leaf senescence of soybean plants under water stress. LCO impacted the spectral reflectance signature at the medium stress level. LCO treatment had

most positive effects on the growth pattern of soybean at the medium stress level, which is the stress level most commonly observed in standard farm-field conditions.

Key words: water stress, lipo-chitooligosacccharide, Nod factor, soybean, spectral reflectance.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a large-seeded grain legume crop. Its growth characteristics have been widely studied because of its economic importance. Soybean is reported to have a low water use efficiency, i.e. uses a lot of water units per unit of dry matter produced (Sprent and Sprent, 1990). Even though the soybean plant can withstand a short period of drought, a long stress period will greatly affect soybean yield (Hicks and Pendleton, 1969). In general, soybean crop is considered a drought sensitive crop (Itani *et al.*, 1992; Mullet and Whitsitt, 1996). During the last few decades researchers have worked to assess and understand the impact of drought on soybean. The effects of water stress on soybean growth and productivity have been extensively studied at locations around the world. Soybean yields have been related to moisture availability in many studies (Doss et al., 1974; Ashley and Ethridge, 1978; Korte et al., 1983b).

Research to mitigate yield losses in soybean crop production by improving water availability or water use efficiency through novel irrigation technologies has been very active. Yet, many of these techniques are too expensive and labour-intensive for their implementation and maintenance in many areas. Moreover, they often have had negative impacts on the environment such as salinization and waterlogging of soils. Therefore, simpler, less expensive and environmentally sound methods for soil water deficit control must be introduced. Several chemical treatments such as simazine and silicone have been used in various countries to reduce plant transpiration in arid and semi-arid areas such as California (Waggoner *et al*, 1981; Cheema and Uppal, 1980; Carbonnier *et al.*, 1981; Yadav and Pandey, 1997). These chemicals are effective for reducing water stress but fail to increase photosynthesis.

There has been considerable effort to enhance photosynthesis in crop plants that are sensitive to water deficit (Nonomura and Benson, 1992; Lu *et al.,* 1998; Morrison *et*

al., 1999). Makela et al (1999) reported enhanced photosynthesis under drought stress in tomato and turnip rape by foliar application of glycine-betanine at very low concentrations. Also, there has been much research on relationships between soybean growth and the rhizobia-legume symbiosis. Several North-American and European plant laboratories are studying Lipo-chito-oligosaccharides (LCOs). LCOs are bacteria-to-plant signal molecules produced by rhizobia bacteria during the formation of the rhizobia-legume N_2 –fixing symbiosis. There has been much research on relationships between soybean growth and the rhizobia-legume symbiosis.

Bacteria of the genera *Rhizobium, Bradyrhizobium, Sinorhizobium, and Azorhizobium,* collectively known as rhizobia, form specialized organs called nodules on the roots of legumes and fix atmospheric nitrogen within these structures. Nodule formation is a highly specialized process that requires cross "talk" between the bacteria and the host plant. In general, the interaction is a two-step process. The first is the release of plant-to-bacteria signal molecules, usually specific flavanoids, by the host plants. The second step is the release of bacteria-to-plant signal molecules, which are lipo-chitooligossacharides, also called Nod factors (Long, 1989; Kondorosi, 1991; Boone *et al,* 1999). Several authors have shown that LCOs invoke a number of physiological changes in the host plant nodulation and nitrogen accumulation (Spaink *et al.,* 1991; Denarie and Cullimore, 1993; Minami *et al.*, 1996). Several experiments, conducted in D. L. Smith's laboratory (Macdonald Campus of McGill University, Québec, Canada) have demonstrated that LCO treatment enhances germination and early growth of various crop plants (Prithiviraj *et al.,* 2000; Souleimanov *et al.,* 2002). It increases the photosynthetic rates and productivity under greenhouse and field conditions for a number of crop plants such as soybean, corn (*Zea mays*), melon (*Cucumis melo*) and potato (*Solanum tuberosum*). Further, the authors have shown that the application of LCO stimulates biomass accumulation and changes in plant structure and morphology of soybean and corn roots.

Soybean has been shown to react well to LCO treatment. Because it is water sensitive, soybean will also respond strongly to water stress treatment. Thus, LCO treatment could be a means to mitigate drought stress in plants such as soybean by enhancing water use efficiency. However, no research has been conducted yet to study

the response to LCO treatment of soybean grown under water stress conditions. Therefore, it would be advantageous to analyse the effects of LCO application on soybean plants under non-standard conditions of growth. Research on the impact of water stress on plant canopy reflectance has been very active in the last decade. However, the effects of LCO application on soybean canopy reflectance have not been studied yet. Therefore, we have attempted in this paper to evaluate the impact of LCO spray application on the physiology and productivity of soybean plants under soil moisture deficit, including changes in spectral reflectance that may show that LCO treatment can affect spectral features of the soybean leaves.

MATERIALS AND METHODS

All materials and methods used are the same as those reported in Chapter 3, except that, in this chapter, we are dealing with a combination of water and LCO factors, Chapter 3 dealt only with water stress. Thus, refer to Chapter 3 for methodological details. The only important particularity is that the experiment was organized in a completely randomised block design with 6 blocks of factorial combinations of 3 moisture levels (medium stress - W2, severe stress - W1, and a well watered control - W3) by 2 LCO levels (treated with LCO - L1, and a non-treated control - L0). This design resulted in a total of 36 experimental units. In each of the 6 blocks, the plants were staggered in two rows (Figure 4.1). Thus, there were 12 replicates for each moisture level, whereas 6 replicates were used to study the individual effects of water stress in the experiment reported in Chapter 3.

Production extraction and purification of LCOs

The LCOs used for the present research were isolated from *Bradyrhizobium japonicum* (strain 532C) obtained from Liphatech (Milwaukee, USA). This strain is able to infect soybean plants over a large range of soil temperatures. The bacteria were cultured at 28°C in yeast extract mannitol broth in 2 L flasks shaken at 150 rpm. When the end of the exponential growth phase was reached (9-10 days), the biosynthesis of LCO was induced by adding the isoflavanoid genistein at a final concentration of 5 μM.

After 48-96 h of induction with genistein, the LCO was extracted with 0.4 volume of the HPLC-grade1-butanol. The upper butanol layer was collected and rotary-evaporated at 50°C under vacuum. The residue was redissolved in 18% acetonitrile and chromatographed by HPLC using a Vydac C18 reversed phase column (0.46 * 25 cm; 5 μM) (The Separation Group Inc, Hesperia, CA, USA) with a gradient of acetonitrile from 18 to 60%. The chromatographic peak corresponding to LCO was identified by comparing with the retention time of an LCO standard {(Nod *Bj*-V(C18:1), MeFuc)} prepared from *Bradyrhizobium japonicum* strain USDA110 and graciously provided by G. Stacey of the Department of Microbiology of the University of Tennessee, USA. The peak was collected and used in the LCO solution, which was sprayed on the soybean plants during the greenhouse experiment.

LCO treatment

LCO spray treatment started only after plant water stress was established (at 30 DAP) so as to analyze the potential rescuing effect of LCO. At 36 DAP (beginning of full bloom, see Table 3.2), 50% of the plants in each water treatment were randomly marked L1 and sprayed with the LCO solution (141.6 μg/L, 0.02 % Tween 20). The other half of the plants (L0) were sprayed with a control solution (0 μg/L, 0.02% Tween 20) and served as controls. Three other sprays were conducted at 43 (during full bloom, Table 3.2), 53 (during pod induction, Table 3.2), and 61 (during pod enlargement, Table 3.2) DAP. The entire plant canopy (over and under) was sprayed, using an atomizer, with the solution until dripping. Each spraying was carried out in the morning.

Data collection

It has been shown in several studies, conducted in D. L. Smith's laboratory (Macdonald Campus of McGill University at Sainte Anne-de-Bellevue, Québec) that LCO treatment starts to increase plant photosynthesis 24 hours after a spray application. It is, therefore, essential to collect physiologic data from *day2* to *day6* after each LCO spray conducted on *day1*. This method is used to assess precise LCO effects and also to determine different phases of LCO activity. All data collection was always done two hours after watering. Spectral reflectance of the whole plant canopy was collected at 18

(Days of plant Stress), at the end of flowering and after 2 LCO sprays, and at 26 DOS (at the beginning of pod enlargement and after 3 LCO sprays).

RESULTS AND DISCUSSION

Effects of water stress on soybean growth and productivity

The effects of soil water deficit were generally similar to those reported for the water stress experiment in Chapter 3. As before, water stress strongly affected soybean plant physiology (Figures 4.2, 4.3, and 4.4), morphology (Tables 4.2, 4.3, 4.4,and 4.5), and productivity (Tables 4.6 and 4.7) at both water stress levels, W1 and W2, in comparison to the control treatment, W3. Therefore, refer to Chapter 3 for more details on the impact of water stress on soybean growth. However, it is interesting to note that the work contained herein lead to additional results.

Water stress negatively affected ($p < 0.05$) leaf chlorophyll content as early as 10 days after stress (DOS) (Table 4.5), compared to 12 DOS in the water stress experiment of Chapter 3. As reported in Chapter 3, water stress decreased chlorophyll content, which confirms the findings of El-Kheir et al. (1994), Chernyad'ev (1997) and Osborne et al. (2002). Nevertheless, in both experiments, soil moisture deficit decreased leaf chlorophyll content at the severe stress level, but not at the medium stress level ($LSD_{0.05}$, means of 12 observations, data not shown). This indicates that moderate chronic water stress does not affect leaf chlorophyll content, but that severe chronic water stress has negative effect on this variable.

Water stress, in the present experiment, affected ($p < 0.01$) the ratio of photosynthetic leaf area to total leaf area (RLA) (Table 4.7), whereas only a numerical tendency was observed in the water stress experiment reported in Chapter 3. Soil moisture deficit decreased RLA by 10.5 and 13.4 % ($p < 0.05$), at the medium and severe stress levels, respectively ($LSD_{0.05}$, means of 12 observations). This indicates that water stress accelerated leaf senescence. Chernyad'ev (1997) and DeSouza et al. (1997) already reported that water deficit accelerates senescence. Yet, the present experiment showed that chronic water stress accelerates plant senescence at both the medium and severe stress levels, whereas DeSouza et al. (1997) reported that the medium stress treatment (60

% of plant water requirements) had no effect on leaf senescence. Furthermore, Sionit and Kramer (1977), Egli and Crafts-Brandner (1996), and De Souza et al. (1997) showed that accelerated leaf senescence results in a shorter seed filling period. Thus, although the present experiment was ended before the end of the seed filling period, the acceleration of leaf senescence might have shortened the duration of pod filling, and therefore decreased seed size.

Water stress, in the present experiment, affected ($p < 0.01$) the ratio of pod dry weight to total dry weight (RDW) (Table 4.7), whereas there were no detectable differences of this ratio for the water stress experiment of Chapter 3. Soil moisture deficit decreased RDW by 4.3 and 6.2 % ($p < 0.05$), at the medium and severe stress levels, respectively ($LSD_{0.05}$, means of 10 observations). The decrease in RDW demonstrates that the proportional allocation of dry matter to reproductive structures was not maintained.

Effects of LCO on soybean growth and productivity

LCO affected ($p < 0.1$) total leaf number per plant (Table 4.2). By 24 days of stress (after 3 LCO sprays), LCO treatment increased leaf number by 4.3 % ($LSD_{0.05}$, means of 18 observations, Table 4.3). This indicates that LCO had some impact on soybean vegetative development. Yet, LCO did not affect other morphological variables, such as plant height, and number of branches. Also, LCO did not impact leaf chlorophyll content (Tables 4.4 and 4.5).

A numerical analysis shows that, by 14 DOS (after 2 LCO sprays), LCO increased the number of fully open flowers per plant by 13.8 % at the L1 level, compared to the controls L0. This suggests that LCO has a positive impact on flower development.

LCO affected ($p = 0.0903$) the total number of pods per plant (Table 4.6) by 26 DOS (after 3 LCO sprays). LCO treatment increased total pod number by 12.7 % at the L1 level ($LSD_{0.05}$, means of 18 observations). This shows that LCO had a positive impact on pod development. A numerical analysis shows that the proportional increase of pod number at the L1 level was greater for pods with lengths < 1 cm (16.2 %), than for pods with lengths > 1 cm and < 4 cm (14.2 %); LCO did not affect the number of pods with

lengths > 4 cm. Thus, LCO might have more impact on pod induction than pod enlargement.

LCO affected ($p = 0.0904$) the soil moisture content (WC) measured at harvest (76 DAP). LCO treatment increased WC by 14.5 % at the L1 level, compared to the L0 controls ($LSD_{0.05}$, means of 18 observations). This shows that LCO decreased the total amount of water used by the plants. A numerical analysis shows that LCO decreased the photosynthetic leaf area by 5.8 % at the L1 level. LCO also reduced the ratio of photosynthetic leaf area to total leaf area by 5.4 % at the L1 level. This suggests that LCO might have accelerated leaf senescence at the L1 level, which would explain the increase of soil water content at harvest. This indicates that LCO accelerated the decline in soybean leaf photosynthetic activity that occurs as plants approach senescence.

Stomatal conductance, Rs, transpiration rate, Tr, and photosynthetic rate, Pr, were all positively correlated ($p < 0.05$) for all six treatments. It is interesting to note that LCO treatment increased the correlation coefficients between all physiological variables at both stress levels (Table 4.1). At the severe water stress, LCO enhanced the correlation coefficient between Pr and Rs, Tr and Rs, and Pr and Tr, by 18.6, 7.1, and 5.0 %, respectively. At the medium stress, the same three correlations were increased by 21.3, 25.7, and 6.2 %, respectively. At the control level, LCO increased only the correlation between Tr and Rs by 3.4 % (Table 4.1). These data indicate that LCO treatment altered the overall physiology of soybean plants, at all water levels. The proportional increases in the correlation were greater between Pr and Rs, and Tr and Rs, than between Pr and Tr. This shows that the variable most affected by LCO treatment, at all water levels, was stomatal conductance. The proportional increases are greater for the medium stress, indicating that LCO had more impact at the moderate stress.

For all three physiological variables measured (Pr, Tr, and Rs) and for each of the 3 LCO sprays reported, the data show that LCO had little effect on the well watered (W3) and severely stressed (W1) plants (Figures 4.2, 4.3, and 4.4). However, the data indicate that LCO treatment most strongly affected the growth pattern of soybean at the medium stress level. This is of particular interest, as field grown soybean plants would encounter moderate levels of moisture stress during at least a reasonable portion of most summer

growing seasons. Thus, the following paragraphs will focus on LCO impact at the medium stress W2.

Effects of LCO at the medium stress level

For the three measured physiological variables (Pr, Tr, and Rs), the analysis demonstrates that LCO treatment positively affected ($p < 0.05$) soybean physiology. Specific results depend on the variable considered and the number of sprays (*spray 1*: conducted at 4 DOS, *spray 2*: at 11 DOS, *spray 3*: at 21 DOS, *spray 4*: at 29 DOS) (Figures 4.5, 4.6, and 4.7). For spray 4, the impact of LCO was very small for all physiological variables (data not shown). Spray 4 was conducted during pod enlargement. This indicates that LCO may not have much impact on pod enlargement. Sprays 1, 2, and 3, were more effective than spray 4. Sprays 1 and 2 were conducted during flowering, spray 3 was applied during pod induction. This suggests that LCO has more impact on plant physiology during flowering and pod induction than during late stages of pod development.

LCO increased Pr after each LCO spray (Figures 4.5, 4.6, and 4.7). For spray 1, Pr was higher ($p < 0.05$) on *day 2* and *day 3*. Whereas, for sprays 2 and 3, Pr was higher on day 3. For all sprays, Pr was not affected on days 4 and 5, which has been commonly observed in D. L. Smith's laboratory. For spray 1, Rs and Tr were higher on *day 2* and *day 3*. Whereas, for sprays 2 and 3, Rs and Tr were not affected. It is important to note that in cases where there was no LCO effect ($p < 0.05$), there were numerical increases for all physiological variables and for each of the 3 LCO sprays reported (Figures 4.5, 4.6, and 4.7), suggesting that an increase in the level of replication might have resulted in statistical detection of these numerical increases. These data indicate that LCO treatment altered overall plant physiology. They also show that LCO had more effect on Pr, than on Rs and Tr, suggesting that, at the physiological level, the major impact of LCO was an increase of photosynthetic rate.

Numerical analyses show that LCO increased plant height and leaf number. By 19 DOS, LCO enhanced plant height by 3.7 % compared to the controls. By 24 DOS, LCO increased leaf number by 5 % compared to the controls. It seems that LCO might have

some impact on plant vegetative development. However, LCO did not alter number of branches or leaf chlorophyll content.

LCO affected ($p < 0.01$) the total number of open flowers per plant. By 14 DOS, LCO increased flower number by 38 % at the L1 level ($LSD_{0.05}$, means of 6 observations, Figure 4.8). This shows that LCO had a positive impact on flower development.

LCO affected ($p < 0.01$) the total number of pods per plant (Figure 4.10). By 19 DOS (after 2 LCO sprays), LCO treatment increased total pod number by 37.8 % compared to the controls ($LSD_{0.05}$, means of 6 observations, Figure 4.10). Thus, LCO had a positive impact on pod development. A numerical analysis shows that, by 26 DOS (after 3 LCO sprays) the proportional increase of pod number was higher for pods with lengths < 1 cm (+ 23.5 %), than for pods with lengths > 1 cm and < 4 cm (+ 13.1 %); LCO decreased the number of pods with lengths > 4 cm by 13.5 %. This suggests that LCO might have a tendency to impact pod induction more than pod enlargement.

LCO reduced ($p < 0.05$) photosynthetic leaf area by 13 % compared to the controls ($LSD_{0.05}$, means of 6 observations, Figure 4.9). It is interesting to note that LCO also affected ($p = 0.0632$) the ratio of photosynthetic leaf area to total leaf area (RLA). LCO decreased RLA by 7.5 % ($LSD_{0.05}$, means of 6 observations). This indicates that LCO accelerated leaf senescence. A numerical analysis shows that LCO increased soil moisture content (measured at harvest time 76 DAP) by 8.8 %. This suggests that LCO decreased the total amount of water used by the plants and confirms that LCO accelerated the decline in soybean leaf photosynthetic activity as the plants approached senescence.

LCO treatment did not affect canopy dry weight, although, there was a numerical increase in the ratio of pod dry weight to total canopy dry weight (2.4 %) following LCO treatment. Thus, suggests that LCO may have a small positive impact on soybean final yield.

Impact of LCO on canopy reflectance at the medium stress level

LCO application showed a tendency to influence the pattern of the canopy reflectance curve at all water levels (data not shown). Further, the data indicate that LCO treatment affected the soybeans at the medium stress level most strongly. Thus, the following paragraphs will focus on LCO impact on reflectance at the medium stress W2.

The replicate-averaged (6 replicates) reflectance data obtained at 18 and 26 DOS, after 2 and 3 LCO sprays, are presented in Figure 4.11 and 4.12, respectively. For both days and for both LCO treatments, the spectral features observed are typical for vegetation reflectance curves, according to Guyot (1990) and Carter (1991) (refer to Chapter 3 for details on typical spectral curves). For both days, the shape of the response curve was similar for both LCO treated plants (L1) and controls (L0). However, for both sets of data, the amplitude of reflectance response was different at the L1 level throughout most of the spectrum range.

At 18 DOS and after 2 LCO sprays, differences in leaf reflectance, in response to LCO treatment, occurred throughout the spectrum range from 350 to 1,050 nm (Figure 4.11). The differences were significant ($p < 0.05$, $LSD_{0.05}$) in the NIR (700 to 1,050 nm), but not in the visible (350 to 700 nm). These data suggest that the influence of LCO on leaf reflectance is effective as early as after 2 LCO sprays. These results also suggest that LCO had more impact on leaf reflectance in the NIR.

At 26 DOS and after 3 LCO sprays, differences in leaf reflectance in response to LCO treatment occurred from 555 to 1,780 nm (Figure 4.12). The differences were significant ($p < 0.05$, $LSD_{0.05}$) from 750 to 980 nm (NIR), and from 1,200 to 1,300 nm (NIR). The differences were not significant ($p > 0.05$) from 550 to 750 nm (visible), from 1,000 to 1,200 nm (NIR), and from 1300 to 1,780 nm (MIR). These data show that LCO effect is not significant throughout the full infrared spectrum.

For both days, the proportional differences in leaf reflectance in response to LCO treatment were greater in the near infrared spectrum (NIR), than in the visible or mid infrared (MIR). LCO increased reflectance in the infrared. The photons with long wavelength (near infrared) adsorbed by leaf pigments are related to heating, evaporation, and transpiration (Wang *et al.*, 2000). Thus, by decreasing the amount of radiation absorption in the infrared, LCO seems to affect leaf heating and transpiration. Therefore, in the present experiment, the increase of infrared reflectance suggests that by 18 DOS, LCO showed a tendency to affect leaf heating and transpiration. This is confirmed by the results presented in the above paragraph titled "*Effects of LCO at the medium stress level*" in which it is reported that LCO tended to increase stomatal conductance and transpiration rate as early as 5 DOS.

These data showed that LCO application affected whole plant canopy reflectance, suggesting that LCO might affect the absorption properties of leaves. LCO might have affected the percentage of absorption of incoming radiation by internal leaf water or leaf pigments. Previous analysis demonstrated that LCO did not affect leaf chlorophyll content, thus LCO could only affect absorption by pigments by influencing its efficiency. LCO might have affected leaf water content or the efficiency of absorption by water. Thus, it is likely that LCO influenced leaf reflectance by changing the rate or efficiency of absorption of radiation by leaf water and pigments. Also, LCO application might have changed leaf internal tissues structure.

It is reported that canopy reflectance measurements are influenced by physical factors such as sensor height (Daughtry *et al.*, 1982), stress-induced architectural differences in plant canopies (Moran *et al.*, 1989), and canopy density. Yet, L1 and L0 plants had the same height and canopy density, and the spectral data presented above were collected from the same height above canopy for both L1 and L0 plants. Moreover, since the plants were at the same level of stress W2, there were no important differences in their canopy architecture. At both levels, L1 and L0, plant canopy tended to be erectophile (leaves vertical) because of water stress (W2). Therefore, the differences in canopy reflectance between L1 and L0 plants were not due to differences in plant height or canopy architecture.

SUMMARY AND CONCLUSIONS

As reported in Chapter 3, soil moisture deficit negatively affected soybean growth and productivity at both water stress levels, W1 and W2, in comparison to the control, W3. The present experiment showed some interesting additional results.

Chapter 4 demonstrates that chronic water stress strongly decreased the ratio of pod dry weight to total canopy dry weight. This demonstrates that water stress reduced the proportional allocation of dry matter to reproductive structures, compared to vegetative structures. It also shows that, at both stress levels, soybean plants could not maintain control of their growth pattern, and thus could not limit yield decrease.

The present experiment shows that chronic water stress strongly accelerated leaf senescence at both stress levels, whereas De Souza et al. (1997) reported that the medium stress treatment (60 % of plant water requirements) had no effect on leaf senescence. Shaw and Laing (1966), Doss et al. (1974), Ashley and Ethridge (1978), and De Souza et al. (1997) reported that an earlier maturity leads to a decrease in seed size. Therefore, although it could not be concluded in Chapter 3, a reduction in seed size could account for and explain the overall drought induced yield loss, as reported by Doss et al. (1974) and De Souza et al. (1997).

Although LCO had some effect on soybean growth for all water treatments, this experiment shows that LCO had limited impact on well watered and severely stressed plants. However, all data indicate that LCO treatment affected the growth pattern of soybean at the medium stress level most strongly. This is the stress level most commonly observed in standard farm-field conditions. The spectral data confirm that LCO had more impact at the medium stress level.

At medium water stress, LCO had a significant effect on overall plant physiology, leaf number, flower and pod numbers, and accelerated leaf senescence. LCO had more impact on plant physiology during flowering and pod initiation, than during pod enlargement. LCO showed a tendency to impact the total number of short pods more than the number of long pods. This indicates that LCO influences pod induction more than later stages of pod development.

For all water treatments, LCO did not affect any soybean plant vegetative development variables. LCO had limited effect on number of branches, plant height, and leaf chlorophyll content. LCO had more impact on reproductive growth. In part this could be a function of the fact that LCO treatment was applied during plant reproductive development. Yet, the soybeans used were indeterminate types, thus, their vegetative growth was still going on after LCO treatment.

At the physiological level, the major impact of LCO was the increase of photosynthetic rate. At the phenological level, two important effects of LCO were the increase in flower and pod numbers. From an agronomic point of view, the major impact of LCO was the acceleration of leaf senescence, which can impact final yield.

LCO impacted the spectral reflectance signature at the medium stress level. This showed that LCO application tends to induce changes of the spectral properties of soybean leaves under water stress, and therefore might influence the crop's ability to intercept radiation and photosynthesise. This fits well with the previous chapter, in which it was shown that LCO treatment altered the overall physiology of the soybean plants.

Table 4.1. Pearson's simple linear correlation coefficients between stomatal conductance (Rs), photosynthetic rate (Pr), and transpiration rate (Tr) for the three moisture regimes, severe stress (W1), medium stress (W2) and control (well watered, W3). L1: with LCO spray; L0: no LCO.

	W3L0		W3L1		W2L0		W2L1		W1L0		W1L1	
	Pr	Rs	Pr	Rs	Pr	Rs	Pr	Rs	Pr	Rs	Pr	Rs
Rs	0.86***		0.86***		0.75***		0.91***		0.59*		0.70**	
Tr	0.89***	0.89***	0.88***	0.92***	0.81***	0.74***	0.86***	0.93***	0.60*	0.57*	0.63**	0.61*

n = 16 observations.
*, **, and *** = significant at the 0.1, 0.05, and 0.01 levels, respectively.
ns = not significant

Table 4.2. ANOVA table for the effect of water stress on the number of leaves (L) per soybean plant at "x" days of stress.

Source of Variation	Degree of Freedom	*Pr* > F*		
		L12	L19	L24
Block	5	.4076	.9165	.9595
Water stress	2	.0000	.0000	.0000
LCO	1	.0991	.0625	.0295
WS * LCO	2	.6307	.1789	.2252
Error	10			

* If $Pr \leq 0.05$, then there was a significant effect.

L stands for leave; L12 means number of leaves at 12 day of stress.

Table 4.3. Mean number of leaves per soybean plant for the LCO treated plants (L1) and the controls (L0) at three days of stress (DOS).

Day of Stress	LSD value	Mean Leaf Number*	
		L0	L1
12 DOS	.33	8.9 A	9.2 A
19 DOS	.47	9.9 A	10.4 A
24 DOS	.39	10.3 B	10.7 A

* Mean of 6 observations. In each row, means followed by the same letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

Table 4.4. ANOVA table for the effects of water stress and LCO treatment on soybean plant height (H) at "x" days of stress, and on the number of branches per plant (BR) at harvest on the 44th Day of Stress.

Source of Variation	Degree of Freedom	*Pr* > F*					
		H9	H12	H16	H19	H23	BR
Block	5	.8807	.8241	.9100	.5482	.5419	.8403
Water stress	2	.0000	.0000	.0000	.0000	.0000	.0012
LCO	1	.2173	.2436	.3007	.5375	.7628	.6912
WS * LCO	2	.4069	.4876	.5272	.6173	.7771	.9998
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

H stands for height; H9 means plant height at 9 day of stress.

Table 4.5. ANOVA table for the effects of water stress and LCO treatment on soybean leaf chlorophyll content (CL) at "x" day of stress.

Source of Variation	Degree of Freedom	*Pr* > F*				
		CL10	CL12	CL16	CL19	CL23
Block	5	.6825	.6292	.8448	.4986	.5553
Water stress (WS)	2	.0526	.0003	.0057	.0004	.0003
LCO	1	.3453	.7635	.5197	.9738	.4648
WS * LCO	2	.3659	.7792	.6800	.4898	.5326
Error	25					

* If $Pr \leq 0.05$, then there was a significant effect.

CL stands for chlorophyll content; CL10 means chlorophyll content at 10 day of stress.

Table 4.6. ANOVA table for the effects of water stress and LCO treatment on:

- the total number of open flowers per soybean plant (F) at 14 day of stress
- the total number of pods (all sizes) per plant on 19 (P1), and 26 DOS (P2)
- the number of pods per plant on 26 DOS with lengths:
  < 1cm (P2A), > 1cm and < 4cm (P2B), and > 4cm (P2C)

Source of Variation	Degree of Freedom	*Pr* > F*					
		F	P1	P2	P2A	P2B	P2C
Block	5	.5773	.7041	.1825	.5800	.5449	.0998
Water stress	2	.0000	.0000	.0000	.0000	.0000	.0000
LCO	1	.2187	.1782	.0903	.2330	.1556	.9227
WS * LCO	2	.1643	.3224	.5642	.6216	.1206	.8342
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

Table 4.7. ANOVA table for the effects of water stress and LCO treatment on:

- soybean total leaf area (TLA), photosynthetic leaf area (PLA)
- the ratio of PLA to TLA (RLA)
- total canopy dry weight (TDW), pod dry weight (PDW)
- and the ratio of PDW to TDW (RDW)

Source of Variation	Degree of Freedom	*Pr* > F*					
		TLA	PLA	RLA	TDW	PDW	RDW
Block	5	.0634	.0598	.1856	.6016	.0063	.1136
Water stress	2	.0000	.0000	.0019	.0000	.0000	.0018
LCO	1	.6184	.3124	.1362	.9973	.8758	.9529
WS * LCO	2	.6184	.6199	.5368	.5091	.6428	.4342
Error	10						

* If $Pr \leq 0.05$, then there was a significant effect.

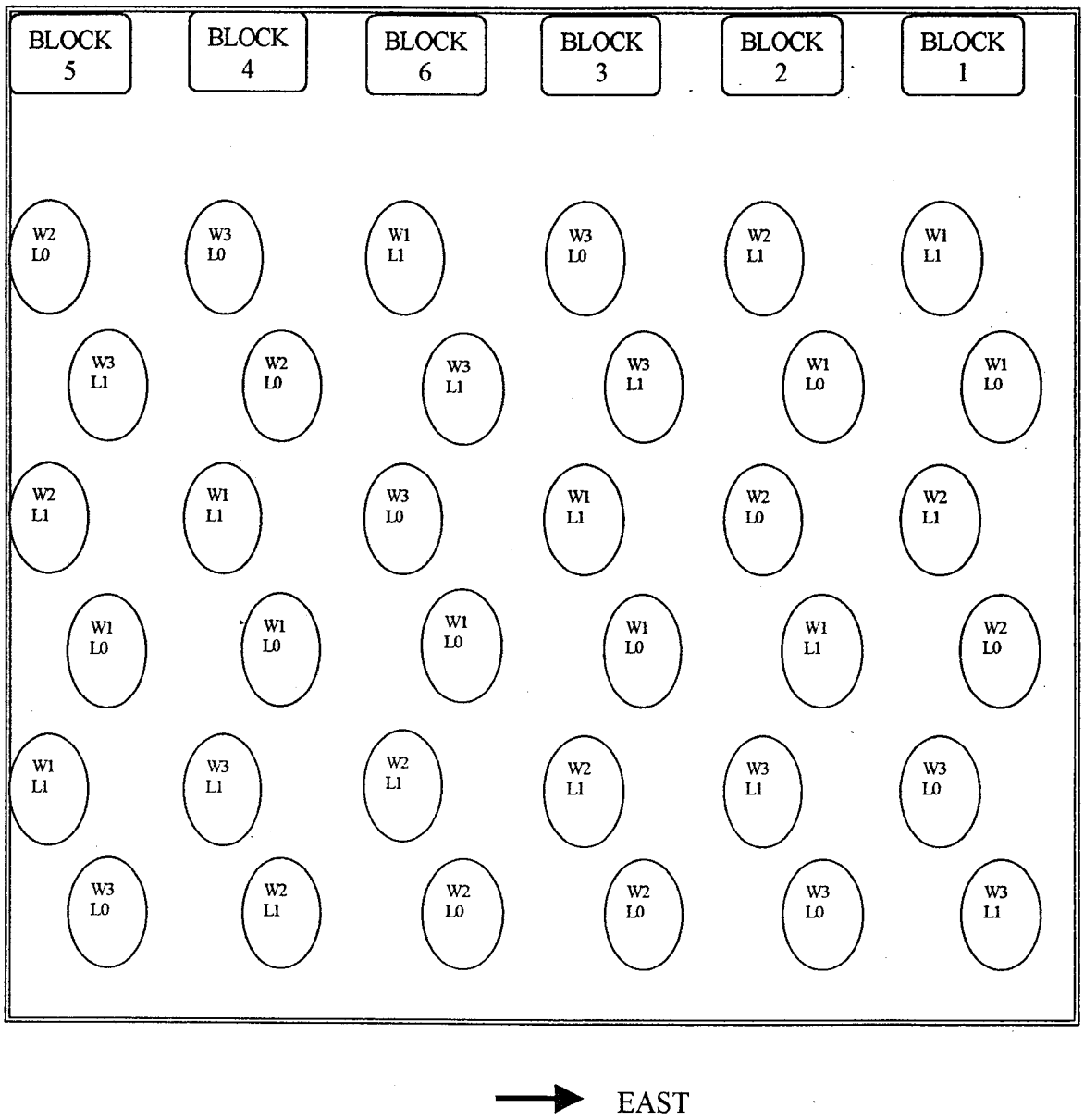


Figure 4.1. Layout of the plants in 6 blocks on the bench in the greenhouse chamber.

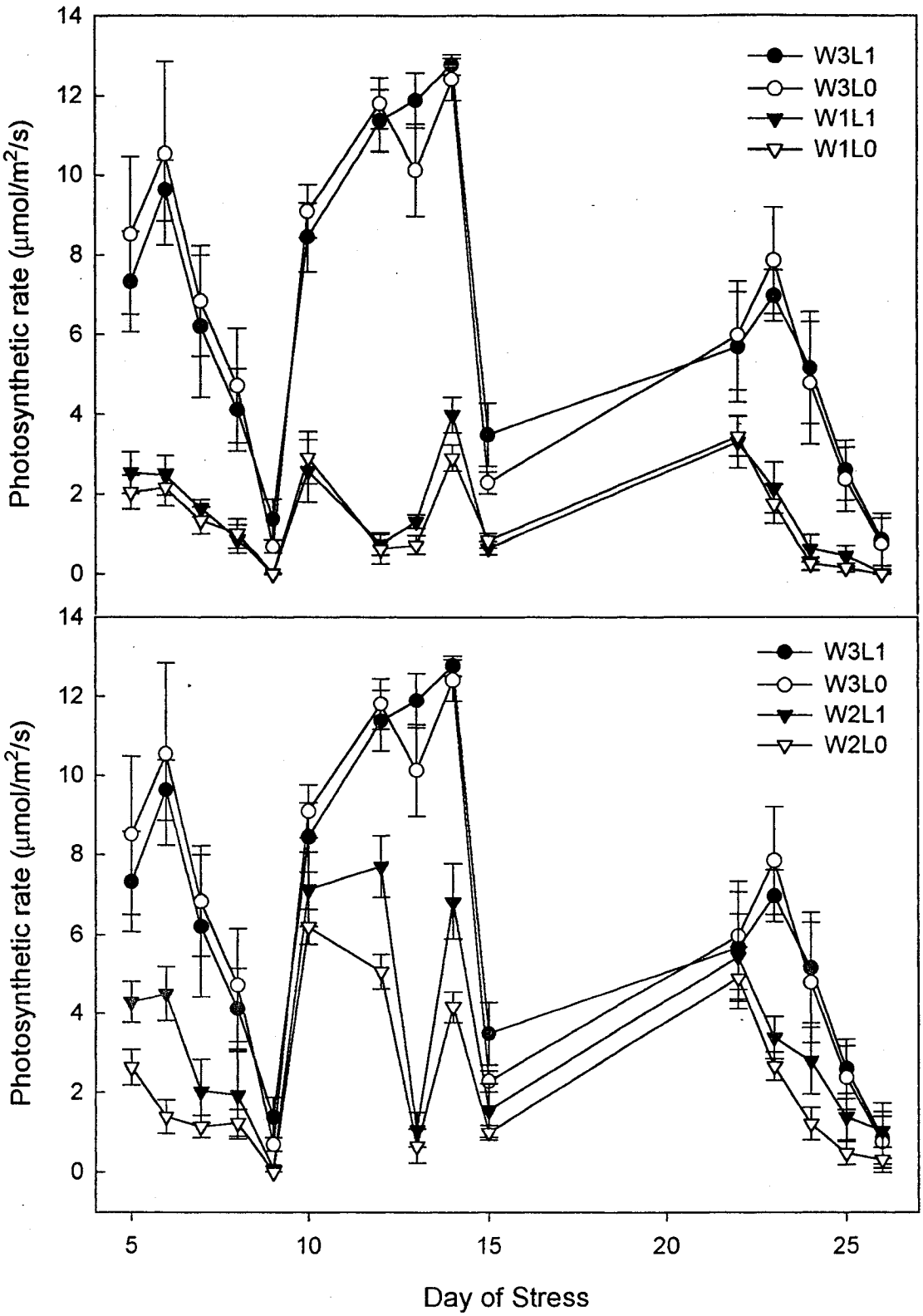


Figure 4.2. Effect of LCO on photosynthetic rate at the severe stress W1 (upper graph), and medium stress W2 (bottom), compared to the control W3. Each point is the mean of 6 observations. Bars represent ± standard error of the mean. L1: with LCO; L0: no LCO.

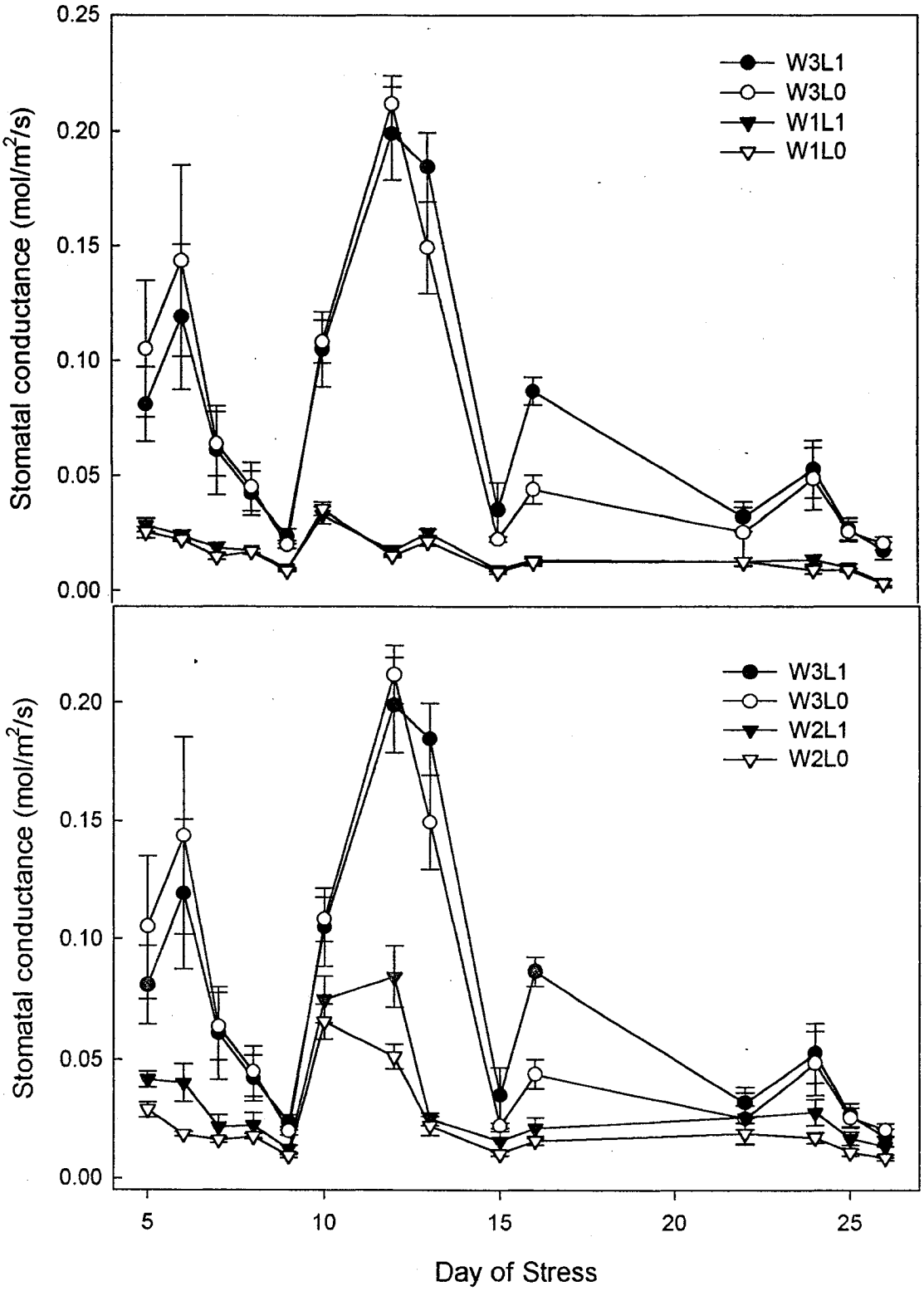


Figure 4.3. Effect of LCO on stomatal conductance at the severe stress W1 (upper graph), and medium stress W2 (bottom), compared to the control W3. Each point is the mean of 6 observations. Bars represent ± 1 std.error of the mean. L1: with LCO; L0: no LCO.

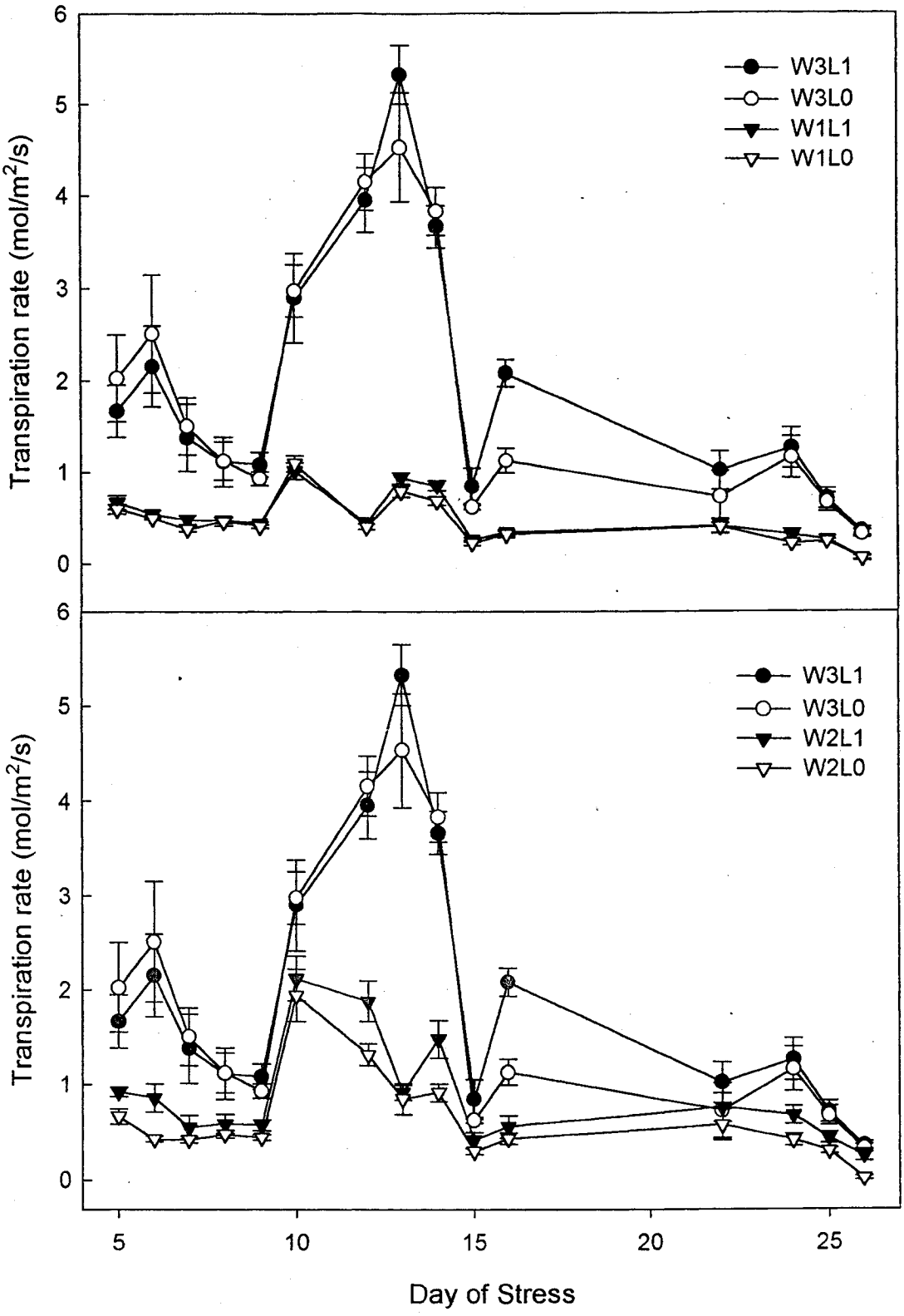


Figure 4.4. Effect of LCO on transpiration rate at the severe stress W1 (upper graph), and medium stress W2 (bottom), compared to the control W3. Each point is the mean of 6 observations. Bars represent ± 1 standard error of the mean. L1: with LCO; L0: no LCO.

Figure 4.5. Effect of LCO on physiological parameters at the medium stress W2. L1: with LCO spray, L0: no LCO. LCO spray was conducted at 4 DOS. Within the same day, points labeled with a common letter are not significantly different at the 5% level of significance ($LSD_{0.05}$).

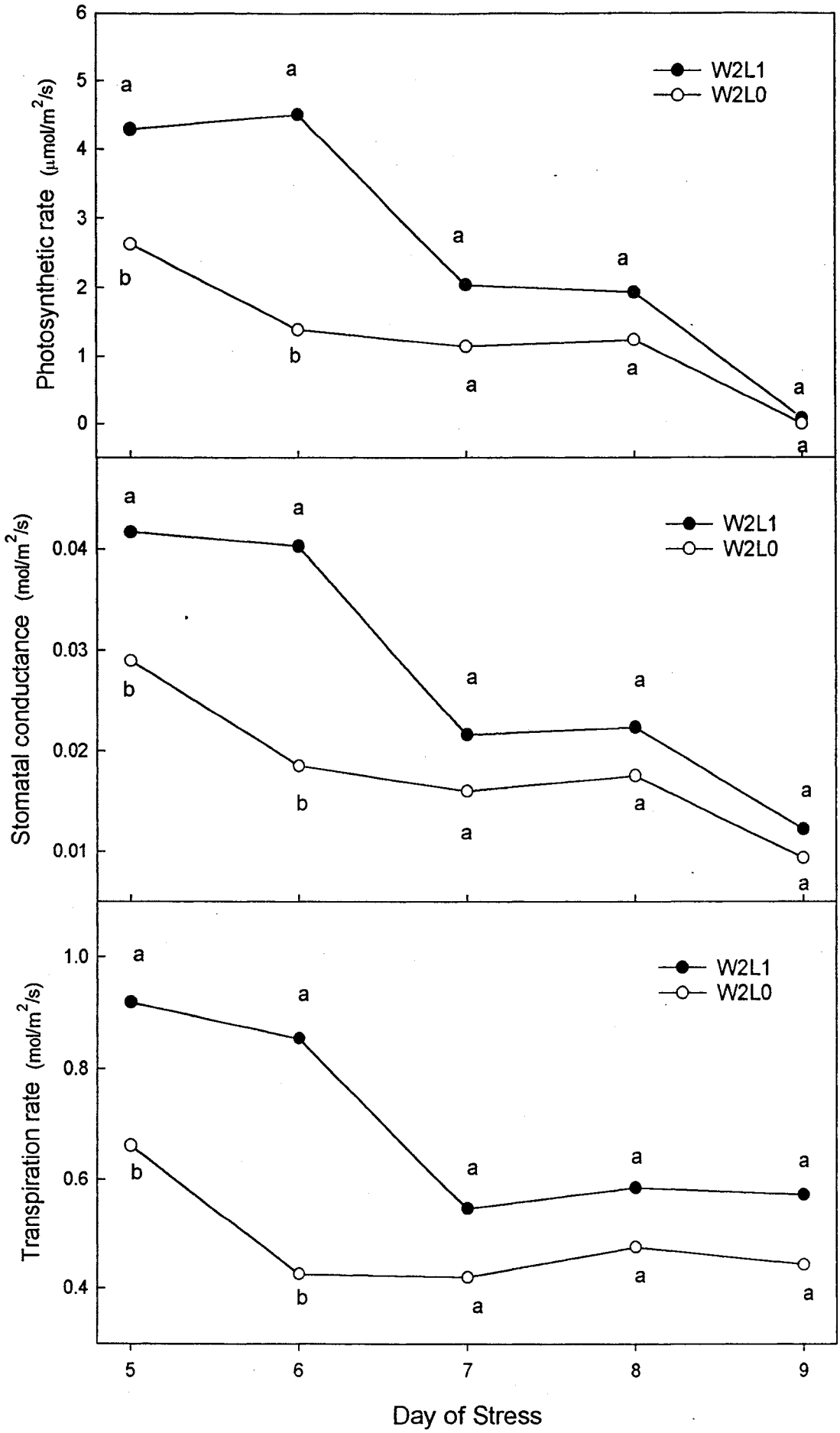

Photosynthetic rate (μmol/m²/s)
Stomatal conductance (mol/m²/s)
Transpiration rate (mol/m²/s)
W2L1
W2L0
Day of Stress
5
6
7
8
9

Figure 4.6. Effect of LCO on physiological parameters at the medium stress W2. L1: with LCO, L0: no LCO. LCO spray was conducted at 11 DOS. Within the same day, points labeled with a common letter are not significantly different at the 5% level of significance ($LSD_{0.05}$).

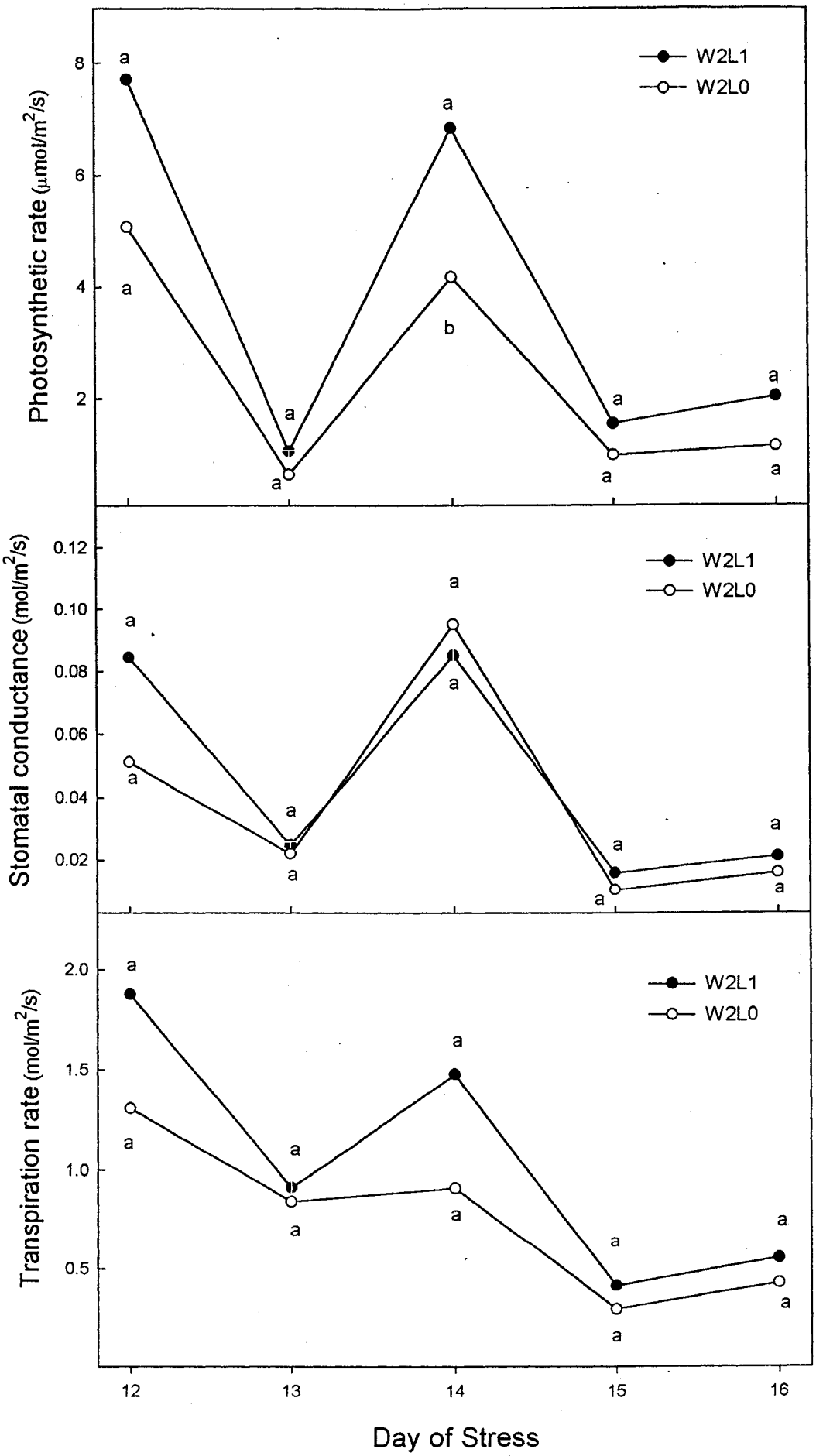
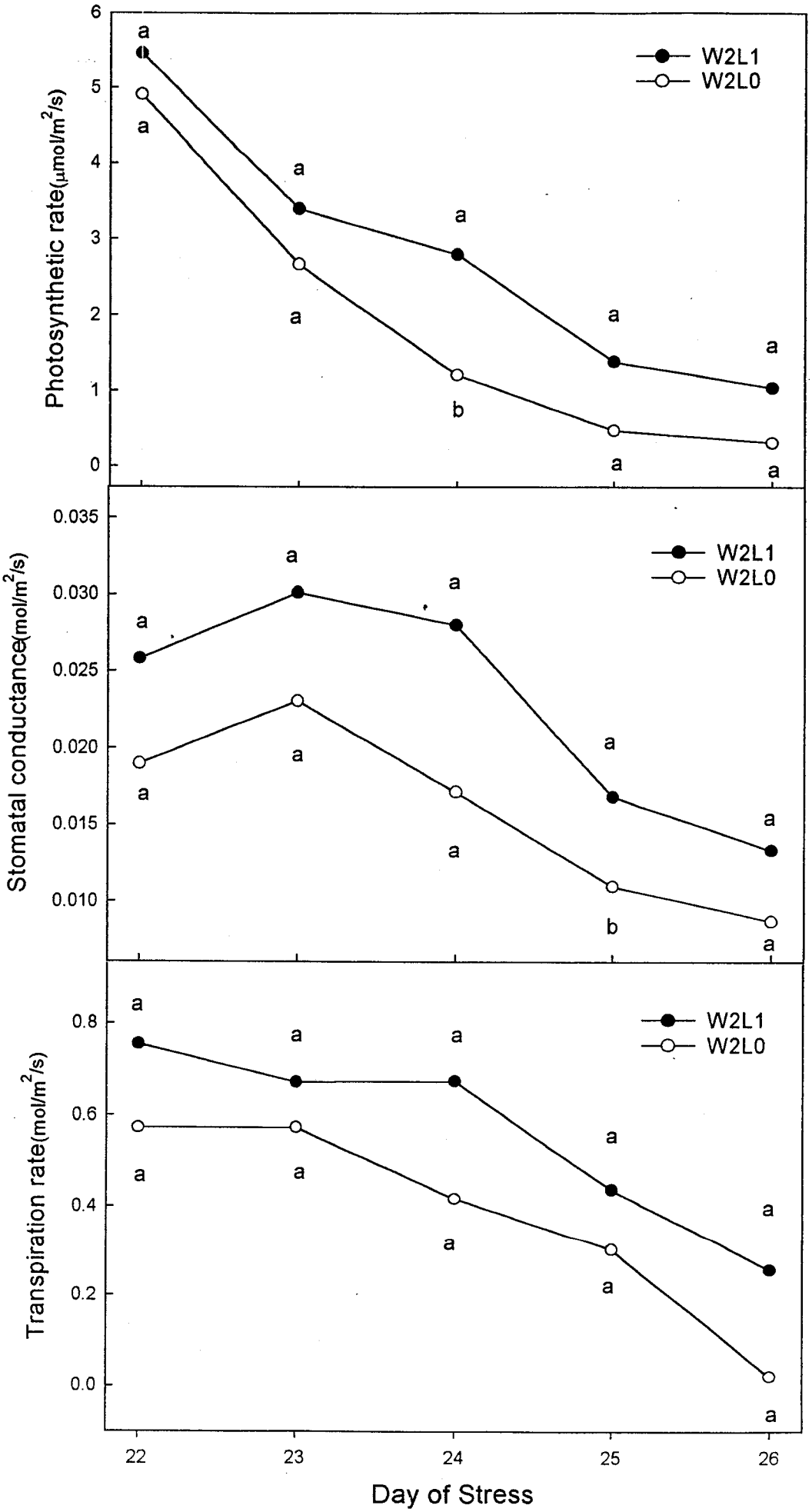

Photosynthetic rate (μmol/m²/s)
8
6
4
2
W2L1
W2L0
a a a a a a a a a a
Stomatal conductance (mol/m²/s)
0.12
0.10
0.08
0.06
0.04
0.02
W2L1
W2L0
a a a a a a a a a a
Transpiration rate (mol/m²/s)
2.0
1.5
1.0
0.5
W2L1
W2L0
a a a a a a a a a a
12
13
14
15
16
Day of Stress

Figure 4.7. Effect of LCO on physiological parameters at the medium stress W2. L1: with LCO spray, L0: no LCO. LCO spray was conducted at 21 DOS. Within the same day, points labeled with a common letter are not significantly different at the 5% level of significance($LSD_{0.05}$).


Photosynthetic rate(μmol/m²/s)
Stomatal conductance(mol/m²/s)
Transpiration rate(mol/m²/s)
W2L1
W2L0
Day of Stress
22
23
24
25
26

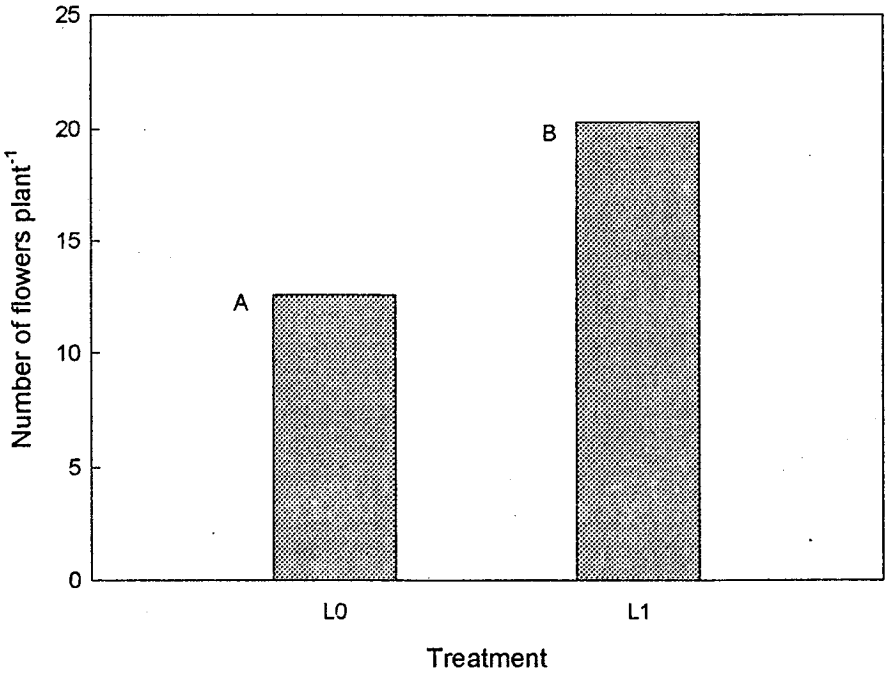


Figure 4.8*. Effect of LCO treatment on the total number of open flowers per plant measured after 14 days of stress, and 2 LCO sprays. L1: with LCO spray, L0: no LCO.

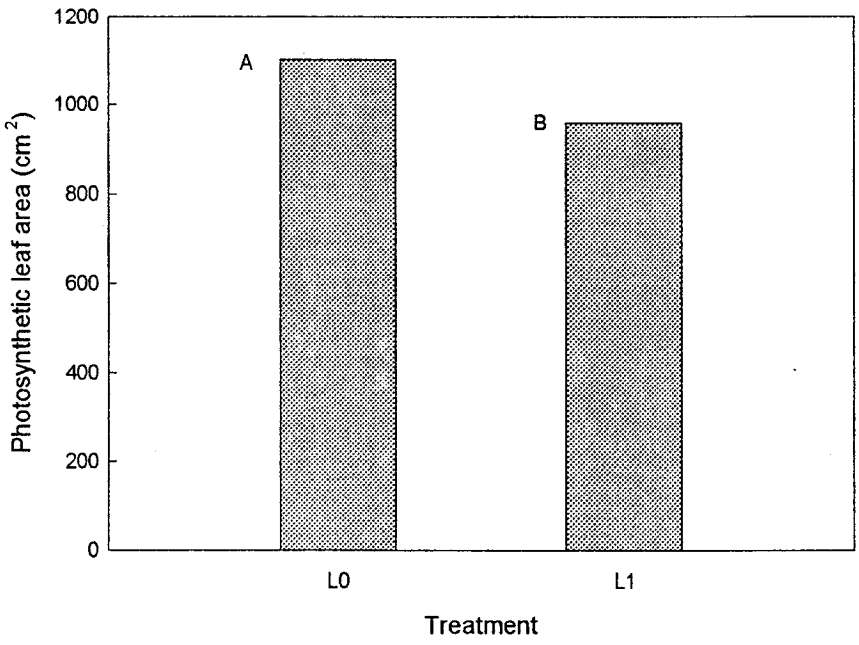


Figure 4.9*. Effect of LCO treatment on photosynthetic leaf area measured at harvest after 4 sprays of LCO. L1: with LCO spray, L0: no LCO.

* Mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

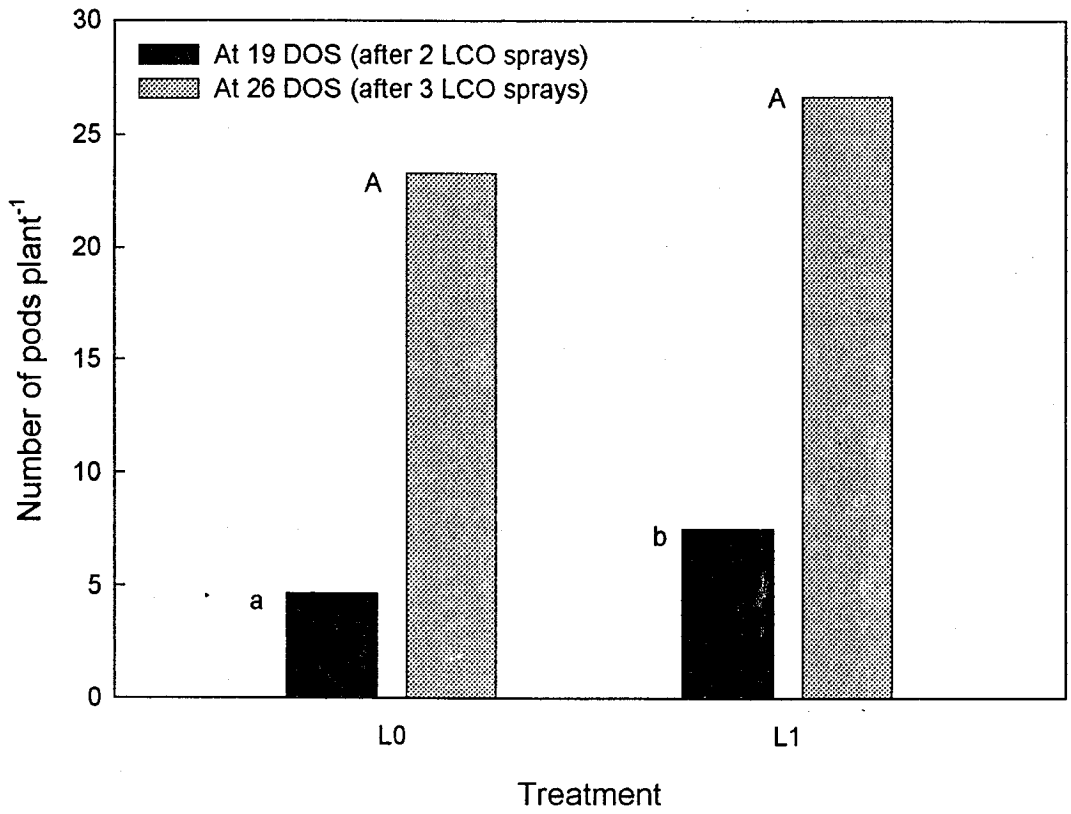


Figure 4.10. Effect of LCO treatment on the total number of pods (all sizes) per plant after 2 and 3 LCO sprays, and 19 and 26 days of stress (DOS), respectively. L1: with LCO spray, L0: no LCO. Mean values (n = 6) followed by a common letter are not significantly different from each other by an ANOVA-protected LSD ($P \leq 0.05$).

* Letters in lower case refer to the comparison of pods at 19 DOS, after 2 LCO sprays.
* Letters in upper case refer to the comparison of pods at 26 DOS, after 3 LCO sprays.

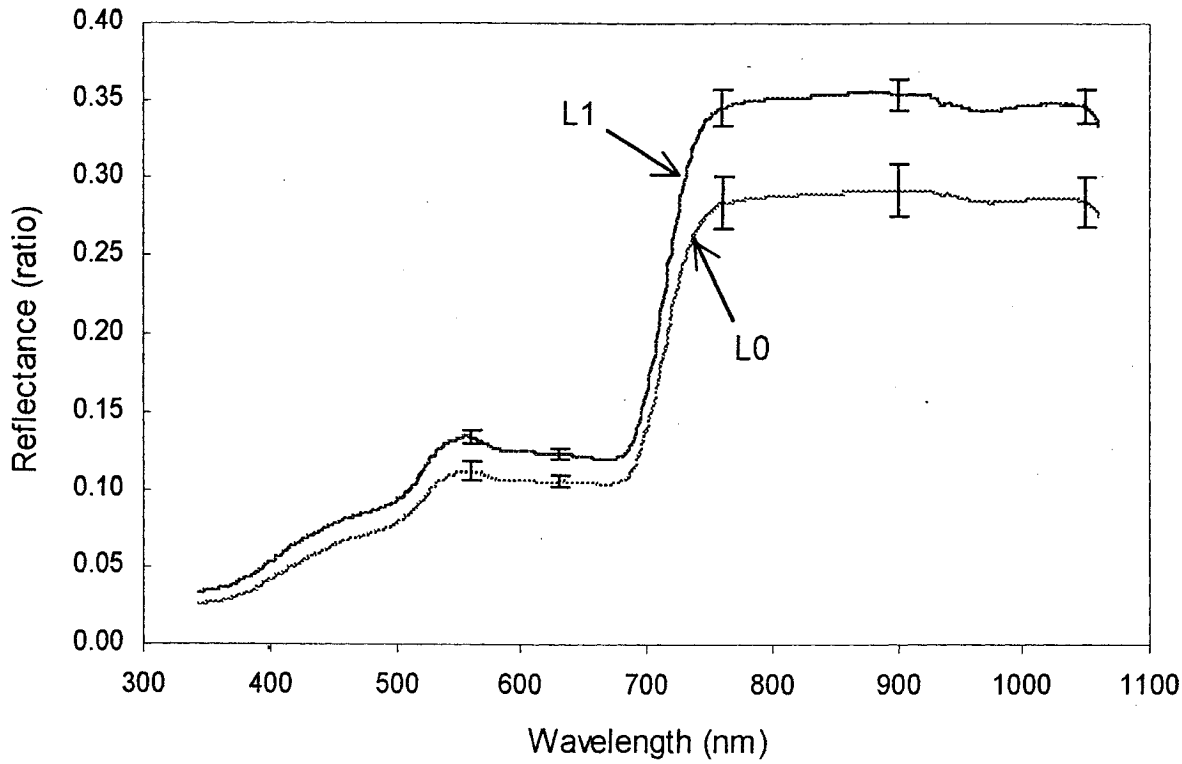


Figure 4.11. Effect of soil moisture deficit on spectral reflectance after 2 LCO sprays (on the 18th Day of Stress) for the medium stress W2. L1: with LCO spray, L0: no LCO. Spectral response curve for entire plant canopy. Each curve is the mean of 6 observations. Bars represent ± 1 standard error of the mean.

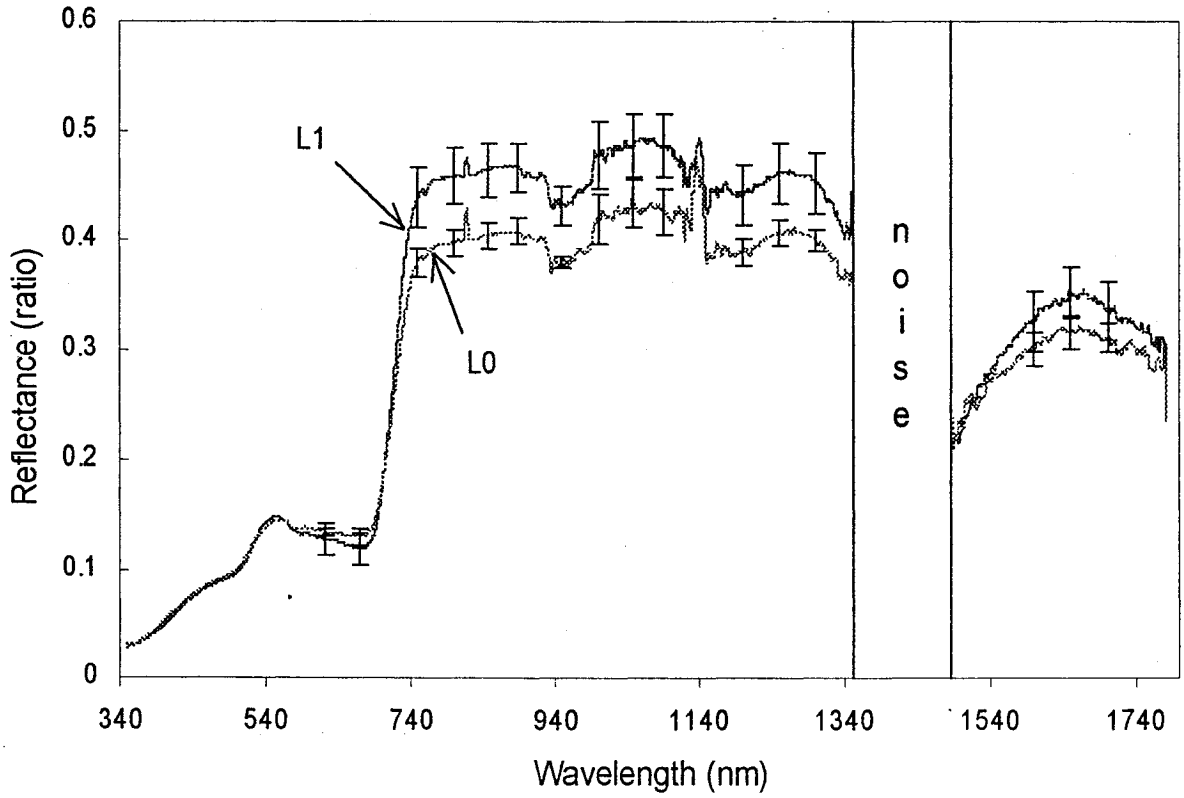


Figure 4.12. Effect of soil moisture deficit on spectral reflectance after 3 LCO sprays (on the 26th Day of Stress) for the medium stress W2. L1: with LCO spray, L0: no LCO. Spectral response curve for entire plant canopy. Each curve is the mean of 6 observations. Bars represent ± 1 standard error of the mean. Spectral data are not reported from 1,350 to 1,500 nm because of presence of excessive noise.

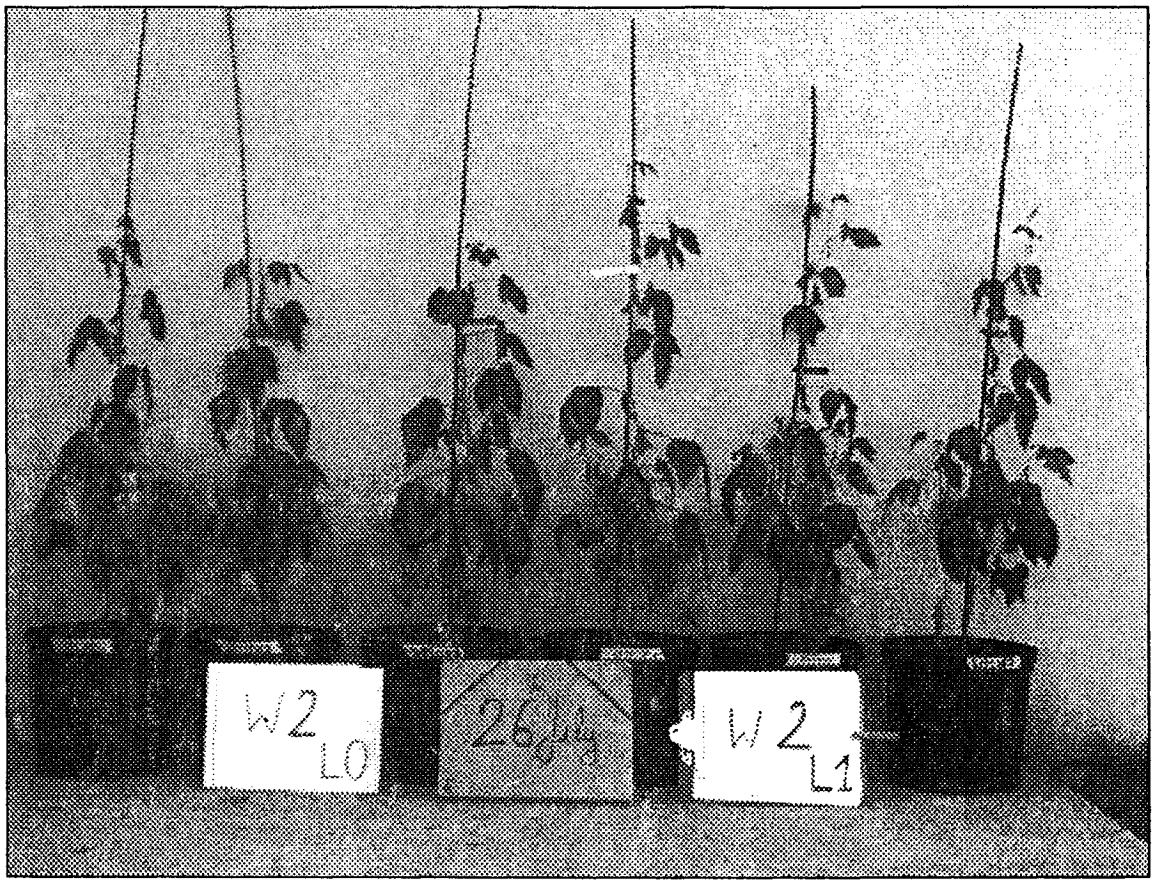


Photo 4.1. LCO treatment increased plant height. Photo taken 71 DAP after 4 LCO sprays. W2L0: medium stress, no LCO. W2L1: medium stress, plus LCO application.

Chapter 5.
GENERAL DISCUSSION

Chapter 3 showed that chronic soil moisture deficit resulted in a sharp decrease of the rate of plant vegetative growth at both stress levels. This suggests that chronic soil moisture deficit during reproductive growth might induce a switch in plant development from vegetative to reproductive in indeterminate soybean varieties. Indeed, these soybean types likely maintain some control of their growth pattern and, thus limit yield losses.

Chapter 4 showed that chronic water stress strongly decreased the ratio of pod dry weight to total canopy dry weight. This indicates that chronic water stress resulted in a relatively greater decrease in pod growth than in shoot growth. Thus, chronic water stress might have affected plant reproductive development more than vegetative development. This could be because the stress treatment was imposed during the reproductive stages of soybean plant development. Or, because the soybean variety were indeterminate types, water stress also strongly affected plant vegetative growth. Therefore, it is likely that the decrease in dry matter pod/shoot ratio was a function of the nature of the water stress treatment rather than the period of application of stress. Chronic soil moisture deficit negatively affects plant growth to an important and continuous extent and results in significant yield losses. The decrease in dry matter pod/shoot ratio could be a result of the poor capacity of soybean to maintain control of its growth pattern under chronic water deficit. Thus, in spite of being an indeterminate type, the soybean variety used showed limited capacity to minimize the negative effects of water stress.

Chapter 4 showed that chronic water stress accelerated plant senescence at *both* stress levels, whereas De Souza et al. (1997) reported that a medium stress treatment (60 % of plant water requirements) had no effect on leaf senescence. The acceleration of plant senescence likely shortened the duration of pod filling, therefore reducing seed size. Thus, under moderate and severe chronic water stress conditions during reproductive development (from R1 to mid-R6 growth stages), the overall yield reduction might not only be due to the decrease of flower and pod number (occurring during flowering and early pod development), but also to a reduction of seed size (occurring during the seed filling period).

Chapters 3 and 4 showed that *severe* chronic water stress (irrigation at less than 40% of plant water requirements) negatively affects leaf chlorophyll content, but that *moderate* chronic water stress (irrigation at more than 50% of plant water requirements) does not affect plant nitrogen status. El-Kheir et al. (1997) showed that decreasing the available soil moisture content significantly reduced total chlorophyll content in soybean leaves. The present experiment suggests there might be a threshold level beyond which leaf chlorophyll content is affected.

Chapters 3 and 4 showed that chronic water stress, at both stress levels, affected:

- Plant physiology: by 3 days after the beginning of the deficit treatments
- Leaf spectral reflectance: by 6 days after
- Plant height and leaf number: by 11 days after
- Leaf chlorophyll content: by 14 days after

Thus, we suggest that chronic soil water deficit could be detected very early by use of a portable photosynthesis system, and quite early with a spectrophotometer system, whereas any visual detection would be only possible after 11 days. A detection of plant nitrogen status decrease is only possible at a later stage. We conclude that the most practical, most effective and least time consuming way to detect plant water stress at an early stage is the measurement of plant canopy spectral reflectance.

Chapter 4 showed that, for all water treatments, LCO affected leaf number but did not affect other plant vegetative variables. LCO had a positive influence on flower and pod numbers. The data also suggested that, for all water treatments, LCO had a tendency to accelerate leaf senescence, as it decreased the total amount of water used by the plants and decreased the leaf area photosynthetic/total ratio measured at harvest time. Yet, LCO had almost no effect on the well-watered and severely stressed plants. The well watered plants were not under water stress, therefore the LCO treatment likely had little impact on their physiology. On the other hand, the severely stressed plants were so water limited that the LCO treatment was ineffective.

However, all data indicate that LCO treatment more strongly affected the growth pattern of soybean at the medium stress level. This is the water stress level most commonly observed in standard farm-field condition. At medium water stress, LCO

significantly affected overall plant physiology, increased flower and pod numbers, and accelerated leaf senescence. LCO had limited effect on number of branches and leaf chlorophyll content. This indicates that LCO affected reproductive growth more than vegetative development. The data showed LCO influences pod induction more than late stages of pod development. This could be a function of the fact that LCO affects primary photosynthetic rate. A spectral reflectance signature of LCO impact was observable at the medium stress level. This shows that LCO does not only affect plant physiology, productivity, and phenology, but also influences leaf optical features.

LCO did not affect canopy dry weight. This might indicate that the effects of LCO were not strong enough, under the conditions of this experiment, to reduce yield loss induced by soil moisture deficit. Yet, LCO altered overall plant physiology. This shows that LCO tends to increase the crop's ability to photosynthesize and produce biomass, indicating that LCO influence positively the heath of soybean crop under water stress. Also, it is interesting to note that all data show LCO had more impact on the development of reproductive structures, than vegetative structures. Moreover, LCO showed a tendency to affect the ratio of pod dry weight to total canopy dry weight. This could in turn increase crop yield under conditions of chronic soil water deficit. LCO also affected leaf senescence. Thus, LCO could hasten plant physiological maturity. This could be considered as a positive impact as it decreases the duration of exposure of the plants to stress conditions. Moreover, early senescence, i.e. early maturation, could be of benefit in regions with a short growing season. Thus, it appears that biomass production was increased by the LCO induced increase in photosynthetic rate, and this was sufficient to offset the shorter duration of photosynthetic activity resulting from the LCO induced early leaf senescence.

LCO treatment constitutes a potential technology for reducing water deficit effects. This opens the possibility of harnessing these signal molecules for improving crop production under conditions of water scarcity, and ultimately augmenting world food production.

Chapter 6.
CONCLUSIONS

Based on the findings reported in this thesis, the following conclusions may be drawn:

1. Soybean responded strongly to chronic soil moisture deficit at both imposed stress levels. Thus, soybean is sensitive to both moderate and severe chronic water stress conditions.

2. Chronic water stress during reproductive development resulted in an important decrease of plant physiological activity, vegetative growth, and productivity at both water stress levels.

3. Chronic water stress during reproductive development strongly decreased the ratio of pod dry weight to total canopy dry weight at both stress levels in the indeterminate soybean genotype used in this work.

4. Chronic water deficit during soybean reproductive development accelerated plant senescence at both stress levels.

5. Para-heliotropism behavior of the leaves was observable at the medium water stress level, but not at the severe water stress level.

6. Chronic water stress affected the spectral features of the soybean leaves. Water deficit increased leaf reflectance in the visible and decreased it in the infrared ranges of the spectrum at both imposed stress levels.

7. Severe chronic water stress negatively affected leaf chlorophyll content, but moderate chronic water stress had no significant impact. Thus, there is likely to be a threshold level beyond which leaf chlorophyll content is affected.

8. LCO {(Nod *Bj*-V(C18:1), MeFuc)} treatment most strongly affected the growth pattern of soybean at the medium water stress level.

9. LCO affected overall plant physiological activity, increased flower and pod numbers, and accelerated leaf senescence.

10. LCO influenced pod induction more than pod enlargement.

11. Under the conditions of this experiment, LCO had limited effects on soybean vegetative development.

12. LCO impacted significantly the spectral reflectance signature obtained only at the medium water stress level. LCO increased leaf reflectance response in the infrared range. LCO influenced leaf interaction with incident radiation.

Chapter 7.
SUGGESTIONS FOR FUTURE RESEARCH

To expand on the work reported here and elucidate the role of LCOs in influencing the growth pattern and productivity of soybean plants under chronic water stress, the following research should be conducted:

1. Determination of the mechanisms by which LCO affects plant physiological activity.

We found that LCO increases stomatal conductance, transpiration and photosynthetic rates. It is important to determine the mechanism by which LCO affects these parameters.

2. Determination of the mechanisms by which LCO affects reproductive development.

This research showed that LCO increases flower and pod numbers. It would be interesting to evaluate the influences of LCO on flower induction and flower abortion rates. The results have suggested that LCO influences pod induction, more than pod enlargement. It would be advantageous to verify this finding.

3. Determination of the optimum frequency, number and period of LCO applications, and concentration for an optimum impact on soybean growth under water stress.

The present study tested the influence of a single concentration (10^{-8} M), four LCO sprays, conducted during early flowering, late flowering, pod initiation, and pod enlargement. The sprays were conducted roughly once every 10 days. Under these conditions, it was found that LCO treatment had limited effects of soybean vegetative development, and did not result in a yield increase. Thus, further research should test different LCO application conditions so as to determine the most effective for increasing soybean productivity under chronic water stress.

Determine whether or not LCO could affect the growth and productivity of soybean under acute water stress.

This study only investigated chronic water deficit. Thus, future research should test the impact of LCO on soybean plants under acute water stress (short period of stress).

Testing the effects of LCO on other crops.

The experiment was conducted only on soybeans. Further research should test the impact of LCO treatment on other crops, legumes and non legumes.

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