IMPACT OF ENVIRONMENTAL STRESS ON REPRODUCTIVE DEVELOPMENT IN SWEET PEPPER (*CAPSICUM ANNUUM* L.)

By

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This thesis is dedicated to the sweet peppers of my life, Alfasha, Fairuz and Farah Suhaila, and my beloved one, Zulkifli Abdul Majid. My deepest appreciation and thanks for all the love, sacrifices, endurance and understanding throughout the years of my yearning for more knowledge. May this be our achievement and the platform to all our ambition.....

.....23:8 Bert

ABSTRACT

This investigation was aimed at determining the impact of environmental stresses such as high temperature, low irradiance and drought on reproductive development in sweet pepper, particularly var. Blue Star. Special attention was given to abscission of the primary and secondary flowers. The role of assimilate accumulation and partitioning and the endogenous growth regulator ethylene in mediating stress effects on flower abscission were investigated. The hypothesis that flower abscission is promoted by these stress factors and that abscission is mediated by increased ethylene production and reduced assimilate partitioning to the flowers was tested.

Imposition of a mean daily temperature (26 °C) from the third true leaf stage accelerated the development of the first primary flowers to anthesis when combined with high irradiance (4.9 MJ m^{-2} d^{-1}). However, abscission was increased by 17% as compared to lower temperature treatments at the same irradiance. The combination of high temperature and low irradiance (2.4 MJ m^{-2} d^{-1}) induced complete abscission of the primary flowers. Although flower abscission was reduced at the lowest temperature examined (14 °C), development of the primary flowers to anthesis was slower than at higher temperatures.

Both varieties, Blue Star and Bell Boy, were able to grow over a wide range of temperatures, as indicated by the large difference (c. 35 °C) between the base and maximum temperatures for growth indicated by a germination trial. In Blue Star, the base (T_b), optimum (T_o) and maximum (T_m) temperatures were 6.0, 27.5 and 41.5 °C respectively, whereas in Bell Boy, the corresponding values were 8.5, 23.0 and 44.0 °C.

Severe water stress imposed progressively after the appearance of the first flower bud promoted the initial development of the primary, but not the secondary flowers to anthesis, but induced early and increased abscission of both primary and secondary flowers shortly afterwards. The high percentage abscission of the primary flowers was partially offset by the lower abscission of secondary flowers. Percentage abscission increased as the severity and duration of water stress increased. However, short exposures to stress did not reduce abscission, or advance anthesis. A more advanced stage of flower development (4.0 mm diameter) proved more susceptible to early abscission than younger flower buds (1.0 mm) when exposed to severe stress. Temporary osmotic adjustment occurred soon after the imposition of water stress, during which osmotic potential decreased sharply from -1.15 to -1.80 MPa, and noticeable reductions in turgor were observed in all treatments between 11 - 22 d after the imposition of stress. Although water stress reduced vegetative growth under low irradiance, complete flower abscission occurred after anthesis.

The advancement of anthesis in stressed plants was associated with a decrease in dry matter accumulation in the leaves and stems. However, at the onset of flower abscission, assimilate accumulation and partitioning were not significantly affected by water stress, and flower abscission was not directly related to any reduction in assimilate production or its distribution within the shoot. Instead, prior to flower abscission in severely stressed plants, ethylene evolution in the flowers increased by 8-fold as compared to unstressed plants, and by 40-fold relative to severely stressed plants measured just before anthesis. The application of the ethylene releasing substance, 2-chloroethylphosphonic acid (CEPA), mimicked the effects of severe water stress, as reflected by a surge in ethylene evolution prior to abscission, followed by increased bud abscission. Sweet pepper flowers were also capable of forming abscission zones at the base of their pedicels in response to elevated ethylene production, whilst mature leaves were apparently incapable of this response. Foliar application of silver thiosulphate (STS) to water stressed plants and STS pre-treatment of plants subsequently sprayed with CEPA blocked the action of elevated ethylene resulting from severe stress or CEPA application in inducing flower abscission.

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IMPACT OF ENVIRONMENTAL STRESS ON REPRODUCTIVE DEVELOPMENT IN SWEET PEPPER (*CAPSICUM ANNUUM* L.)

CONTENTS

Page

Page

Page

LIST OF TABLES

Table **Page**

Table **Page**

LIST OF FIGURES

Figure **Page**

Figure **Page**

Figure **Page**

LIST OF PLATES

ABBREVIATIONS AND SYMBOLS

ACC	1-aminocyclopropane-1-carboxylic acid
Ag	silver
ANOVA	analysis of variance
BB	hybrid variety Bell Boy
BS	hybrid variety Blue Star
BS1	bud stage 1 (diameter 3.5 - 4.5 mm)
BS2	bud stage 2 (diameter 2.0 - 3.0 mm)
BS3	bud stage 3 (diameter 0.5 - 1.5 mm)
BVS	flower bud-visible stage
c.	approximately
°C	degree Celsius
°C d	degree day
cal cm^{-2} d^{-1}	calories per square centimetre per day
CEPA	2-chloroethylphosphonic acid
cf.	compare
Con	constantine
Cu	copper
cv.	cultivar
cvs.	cultivars
cm	centimetre
CO_2	carbon dioxide
d	day
DAT	days after start of treatment
ed.	editor
eds.	editors
e.g.	example
ft-c	foot candle

EFE	ethylene forming enzyme
ETP	evapotranspiration
g	gram
h	hour
HI	high irradiance
HS	high stress
i.e.	that is
IRR	irradiance
kg	kilogram
l	litre
μl	microlitre
$\mu l\ kg^{-1}$	microlitres per kilogram
$\mu l\ l^{-1}$	microlitre per litre
LAR	leaf area ratio
LI	low irradiance
m	metre
m^{-2}	square metre
min	minute
$MJ\ m^{-2}$	megajoules per square metre
$MJ\ m^{-2}\ d^{-1}$	megajoule per square meter per day
mm	millimetre
mM	millimolar
$\mu mol\ m^{-2}\ s^{-1}$	micromol per square metre per second
$\mu mol\ cm^{-2}\ d^{-1}$	micromol per square centimetre per day
MPa	megapascal
MS	moderate stress
mV	millivolt
n	number of samples
2,5-NBD	2,5-norbornadiene
$nl\ g^{-1}FW\ h^{-1}$	nanolitre per gram fresh weight per hour

nm	nanometre
no.	number
NS	non-stress
PAR	photosynthetically active radiation
RH	relative humidity
rpm	revolutions per minute
s	second
SAM	S-adenosylmethionine
SED	standard error of the differences between means
SLA	specific leaf area
SON/T	high pressure sodium lamp
SS	severe stress
STS	silver thiosulphate
t	time
T_b	base temperature
T_{eff}	effective temperature
T_m	maximum temperature
T_o	optimum temperature
var.	variety
vs.	versus
W	watt
W m^{-2}	watts per square metre
WS	water stress
WUR	water use ratio
wt.	weight
ψ_l	water potential
ψ_p	turgor potential
ψ_s	osmotic potential
$\approx$	approximately

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Sweet pepper (*Capsicum annuum* L.) from the family Solanaceae is a tender perennial grown as an annual fruiting crop (Lincoln, 1987). Pepper plants have woody but brittle stems and an upright habit. Initially, the plant produces a single stem, but this soon branches into two. At the point of branching, one or more flower buds are produced and these are known as the primary or crown buds. After the production of one or two more leaves, each branch subdivides once more, again developing flower buds at each point of division. These are referred to as secondary buds or second layer flowers. Any form of stress that restricts the branching system is also bound to reduce the number of flowers. The plant structure is illustrated in Plate 1.1.1 (Smith, 1979).

Sweet pepper originated in South America, but is now grown throughout the world. It is among the most important 'vegetable' crops, ranking fifth in the vegetable area harvested on a global scale in 1991, with the largest cultivated areas being in the developing countries (FAO, 1992). Besides providing income in such areas, pepper fruits are also nutritionally valuable since they are rich in vitamin A and B, niacin, riboflavin and thiamine (Tindall, 1983). In Malaysia, the cultivation of sweet pepper is fast increasing in importance due to its market potential. Local demand for sweet pepper outweighed its production in 1990 by 98% (Hawa *et al.*, 1991), and this demand is expected to increase year by year. To meet Malaysia's increasing demand for sweet pepper, substantial quantities of the fruit are imported, which adds to the bill. To overcome this problem, peppers are grown in the humid, tropical lowland areas of Malaysia, replacing the highland areas in which land is increasingly

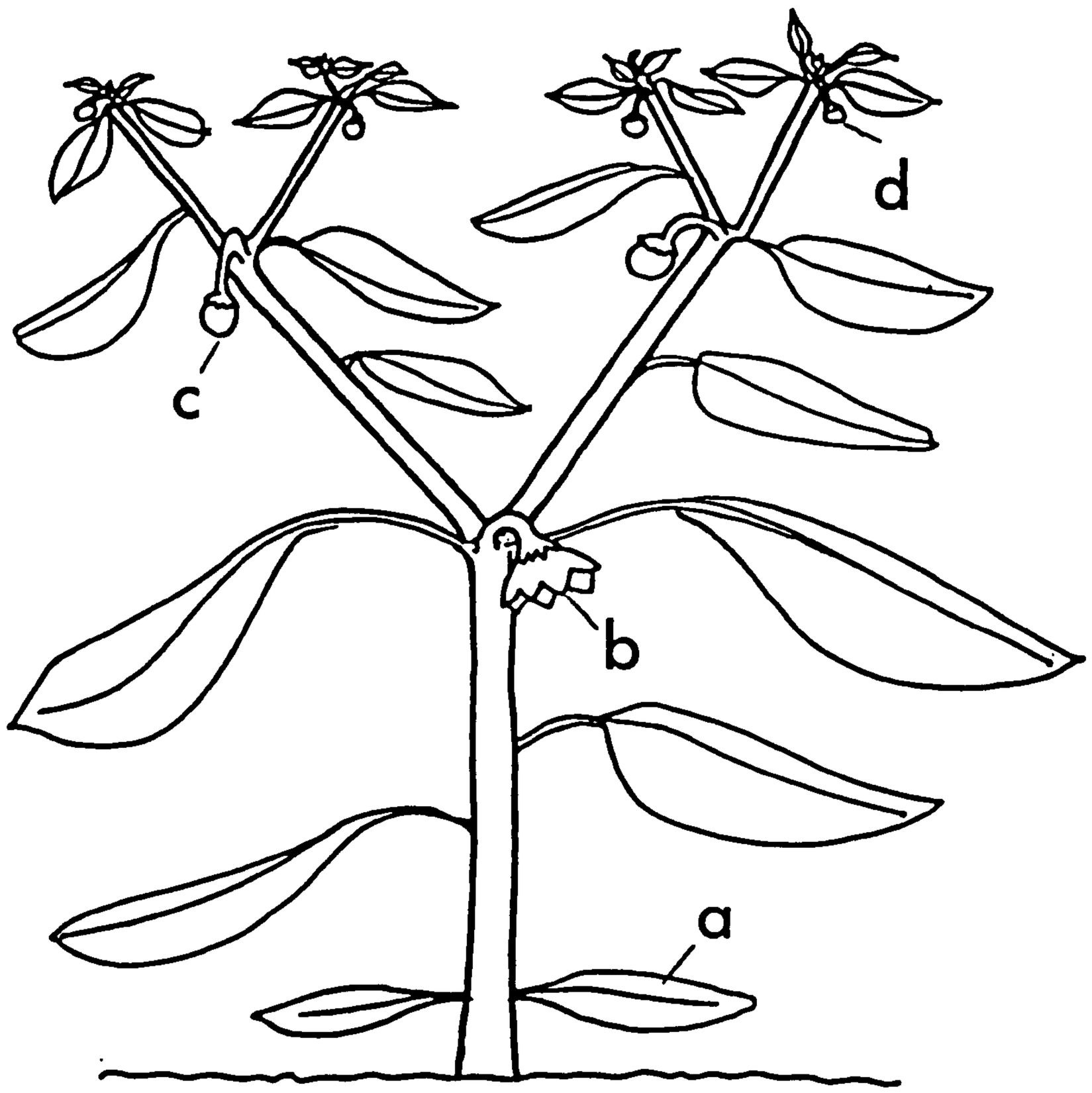


PLATE 1.1.1. *Young reproductive plant of sweet pepper showing (a) cotyledons, (b) first level or primary flower (crown), (c) second level or secondary flower and (d) third level or tertiary flower. Source: Smith, 1979.*

expensive.

Conventional production in the lowland areas is beset with many constraints. Environmental stresses such as high temperature, low light and lack of water are most often cited as causes for bud and flower abscission (Wien *et al.*, 1989a) and such conditions may occur under the rainshelters currently used for crop production in the lowlands. Since the rainshelters lack environmental control, the flower and bud abscission observed there may be attributable to increased temperature and reduced radiation within them, especially during the rainy season. Equally, shortage of water could be a major cause of abscission. The mean daily temperature in the lowlands is typically 26 - 27 °C, but under the rainshelters may increase by 3 - 7 °C. At the same time, the natural radiation penetrating the plastic covers of the shelters is reduced by almost 70% in the morning and late evening, and by 45% at noon (Hawa Jaafar, 1991, unpublished). As sustained growth and development of the reproductive sites until fruit is set is important in determining the success of early production, it is vital to establish how environmental conditions affect flower abscission and what the underlying mechanisms may be.

This thesis comprises a series of experimental studies conducted with the objective of determining the impact of environmental stresses, especially high temperature and water deficit, on reproductive development in sweet pepper, with special attention being given to the abscission of primary and secondary flowers. Investigations also focused specifically on the role of assimilate accumulation and partitioning and the endogenous growth regulator, ethylene, in mediating the observed stress effects on flower abscission. The hypothesis was that flower abscission would be induced by high temperature, low light and water stress and that these effects would be mediated by increases in endogenous ethylene and reduced carbohydrate supply to the developing flowers. Abscission is known to be promoted by enhanced ethylene production (Durieux *et al.*, 1983; Sexton *et al.*, 1985; Abeles *et al.*, 1992) and reduced assimilate partitioning to the flowers (Calvert, 1969; Halevy, 1984; Stirling *et al.*, 1989a).

1.2 Literature Review

The following sections review existing information on the effects of environmental conditions on reproductive growth and development in sweet pepper, with particular emphasis on flower abscission. Where information specifically relating to sweet pepper is limited, relevant research on other species is reviewed.

1.2.1 Temperature, irradiance and flower development

It is well established that temperature and irradiance both have a major influence on the growth and development of young reproductive plants (Kinet *et al.*, 1985; Morris and Newell, 1987; Aloni *et al.*, 1991b). Plant responses to temperature and irradiance vary between species and cultivars, and may be influenced by the levels of other environmental variables (Papadopoulos and Tiessen, 1983; Selander and Welander, 1984; Kinet *et al.*, 1985; McPherson *et al.*, 1985). For example, in some species temperature is critical in determining reproductive development up to anthesis or abscission of the buds (Fortanier and Zevenbergen, 1973; Dosser and Larson, 1981; Bernier *et al.*, 1981a), whilst in others temperature interacts strongly with irradiance to influence flower growth and development (Deli and Tiessen, 1969; Kinet *et al.*, 1985; Atherton and Harris, 1986).

High temperatures generally increase the rate of flower development, resulting in earlier anthesis (Kinet *et al.*, 1985). This effect has been recorded by several workers for grasses, herbaceous dicots and woody species (Calvert, 1964; Moe, 1972; Rawson and Bagga, 1979; Armitage *et al.*, 1981). Under near optimum growing conditions in a glasshouse, anthesis in the primary flowers of sweet peppers var. World Beater was accelerated at a mean daily temperature of 35 °C as compared to 12.5 °C (Cochran, 1936). Similarly, in a growth room study, flowering of tomato cv. Ohio MR-13 was delayed significantly at a mean air temperature of 16 °C as compared to 20.5 °C, whereas cv. Vendor was unaffected (Papadopoulos and Tiessen, 1983). The effect of high temperature on flowering in tomato was observed to be greater under

high irradiance conditions (Hurd and Cooper, 1967; 1970).

While high temperature accelerates flower development up to anthesis, it also increases flower and bud abscission in sweet pepper (Cochran, 1936, 1938; Song *et al.*, 1976; Rylski and Spigelman, 1982) and tomato (Calvert, 1969; Atherton and Harris, 1986; Wien *et al.*, 1989b). Similarly, Utsunomiya (1992) showed that a high mean temperature of 30.5 °C accelerated flower development up to anthesis in passion fruit, but also promoted extensive flower abscission after pollination. These results suggest that relatively high temperatures promote reproductive growth up to anthesis, but may also increase abscission after anthesis. The adverse effect of high temperatures after anthesis is also well illustrated by greenhouse-grown sweet pepper (Cochran, 1936). For example, at a mean air temperature of 18.5 °C, cv. World Beater exhibited the highest percentage fruit set, implying that abscission was reduced. However, when the mean temperature was increased to 24 °C, percentage abscission increased, with complete abscission of flowers and buds being observed at 35 °C (Cochran, 1936). Similar results obtained by Song *et al.* (1976) also showed that abscission increased as mean temperature was raised from 15.5 to 20.5 and finally to 35 °C.

Mean temperature seems to be more important than the variation between day and night temperatures in terms of its effects on flower development, anthesis and abscission (Kinet *et al.*, 1985; Atherton and Harris, 1986). The influence of temperature on developmental processes can be analysed using the thermal time concept (Garcia-Huidobro *et al.*, 1982; Stirling *et al.*, 1989b; Peltonen-Sainio, 1991; Slafer and Savin, 1991) and this approach can be used to predict the timing of developmental events during the life cycle of a crop. The calculation of thermal time is based on the linear rate/temperature response of developmental processes between base (T_b) and optimum (T_o) temperatures. Since cardinal temperatures for sweet pepper have not previously been reported, a small section of the investigation described here attempted to establish T_b, T_o and the maximum temperature (T_m) by means of a germination trial using a thermogradient plate under controlled environmental conditions (Chapter 4; Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*,

1988a). This technique has generally been found to produce satisfactory and reproducible results provided viable and non-dormant seeds of uniform size are used (Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*, 1988a). The number of degree days (°C d or thermal time) was calculated by accumulating the mean daily temperature above T_b. Subsequently the thermal time was used to relate the process of flower development (anthesis and abscission) to temperature.

Abscission at high temperatures may be attributable to competition for assimilates between flower buds and young leaves, as proposed by Dinar and Rudich (1985a) and Aloni *et al.* (1991b). The latter workers showed that, under high temperature conditions, the young leaves of sweet pepper plants appeared to be more effective in importing assimilates than adjacent flower buds. This view was further supported by results showing that ^{14}C-sucrose was partitioned in favour of young leaves as opposed to flowers (Aloni *et al.*, 1991b). In tomato, high temperature also reduced carbon transport from leaves by promoting the formation of callous plugs in the phloem of the petioles, and also inhibited starch hydrolysis within the leaves (Dinar *et al.*, 1983). Dinar and Rudich (1985a) later showed that the import of assimilates into flower buds and their conversion into starch were also inhibited. Increased abscission at high temperatures may also result from a failure of fruit set due to abnormal growth of the reproductive structures (Charles and Harris, 1972; Levy *et al.*, 1978; Polowick and Sawhney, 1985; Rylski, 1986).

Flower abscission at high temperatures is particularly severe under low irradiance conditions (Atherton and Othman, 1983; Papadopoulos and Tiessen, 1983; Picken, 1984; Rylski and Spigelman, 1986; Turner and Wien, 1994a). For example, up to 50% flower abscission was observed when tomato cv. Sonato was grown at a mean daily temperature of 20 °C and a mean irradiance of 4.8 MJ m^{-2} d^{-1}, but complete flower abortion occurred when irradiance was decreased to 2.5 MJ m^{-2} d^{-1} (Atherton and Othman, 1983). Kinet (1977a) also reported that the latter irradiance treatment induced substantial flower abscission. Low irradiance retards inflorescence development, accelerates and increases abscission in most species and may induce deformation of the flowers (Moe, 1972; Kinet *et al.*, 1985; Kinet, 1994). Abscission

of flowers and buds at low irradiance probably occurs because the reduced photosynthetically active radiation severely limits the growth of the whole plant (Atherton and Harris, 1986; Turner and Wien, 1994b) and increases competition for assimilates between the reproductive and vegetative structures (Halevy, 1987; Morris and Newell, 1987; Turner and Wien, 1994b). An alternative hypothesis is that the levels of the endogenous hormone, ethylene, may be altered (Tripp and Wien, 1989).

Growing glasshouse tomatoes at a relatively low mean temperature of 18 °C under low winter irradiance conditions reduced flower abscission and promoted fruit set (De Koning and Hurd, 1983), suggesting that lower temperatures may counteract the detrimental effects of low light, and that assimilate supply, redistribution, and utilisation may be essential factors in mediating temperature effects. This appears to be especially true at the time of appearance of visible buds (Kinet, 1977a; Bernier *et al.*, 1981b). Exposure to high temperatures at an earlier stage of the life cycle reduced the incidence of flower abscission, possibly by increasing the photosynthetic leaf area formed before the flowers were initiated (Calvert, 1969). Wien *et al.* (1989a) showed that, when sweet pepper plants were exposed to 80% shade during flowering, open flowers were the first reproductive organs to be shed, followed by flower buds. However, there is growing evidence that the increased flower abscission induced by the combination of high temperature and low irradiance is more likely to be caused by stimulation of endogenous ethylene production (Cameron and Reid, 1981; Tripp and Wien, 1989; Wien *et al.*, 1989b; Wien *et al.*, 1993; Abeles *et al.*, 1992).

It is apparent that there is a need to gain a better understanding of the interactive effects of temperature and irradiance on flower growth and development in young reproductive plants of sweet pepper. This was achieved by conducting the experiment described in Chapter 3, in which young sweet pepper plants were grown under different temperature conditions in combination with natural or reduced glasshouse irradiance. The time to anthesis and abscission and percentage abscission were examined to establish the impact of temperature-irradiance interactions on reproductive development in different varieties. The existence and extent of varietal

differences in response to temperature were examined further in a study of the cardinal temperatures for germination using the thermogradient plate.

1.2.2 Water stress

Water stress may take two extreme forms: stress due to an over-abundance of water which results in flooding, or stress resulting from a lack of water which causes water deficits. The investigations undertaken here were confined to stress induced by water deficits, which is sometimes defined as drought.

In simple terms, water deficits develop when water loss by transpiration exceeds absorption by the roots (Turner and Begg, 1981). At any stage of development, whether in the open field or under protected cultivation, plants may experience some degree of transient, midday water deficit during hot, sunny weather, even when growing in moist soil (Boyer *et al.*, 1980; Kramer, 1983). However, it is the development of long term deficits in plants that are progressively reducing the available soil water that is crucial for overall growth and productivity, as the consequent stress may cause severe disturbance of physiological processes and hence induce injury (Hsiao, 1973; Kramer, 1983).

Drought is one of the most important environmental factors limiting crop production as water deficits affect every aspect of plant growth. It is well established that water stress reduces growth and yield, and that the magnitude of the reduction is dependent upon the stage of development at which the stress occurs (Ney *et al.*, 1994). Different phases of growth may exhibit differing sensitivity to water stress (Salter and Goode, 1967; Kaufmann, 1972; Begg and Turner, 1976). Sweet pepper may be expected to demonstrate similar differential responses to water stress.

1.2.2.1 *Water stress and reproductive development*

The deleterious effects of water stress in plants are usually most pronounced in tissues and organs undergoing rapid growth and development (Salter and Goode, 1967; Slatyer, 1969; Hsiao, 1973; Fischer, 1973). The reproductive stages of growth are often, but not always, the most sensitive to water stress (Lewis *et al.*, 1974; Sionet and Kramer, 1977; Singh *et al.*, 1987), and, if water deficits occur at this time, yield is depressed more than if these occur at other growth stages (Kaufmann, 1972; Hale and Orcutt, 1987; Kirkham, 1990). Kaufmann (1972) divided the reproductive cycle into three stages when considering the effects of water stress on growth. The first is the flowering stage, beginning with the initiation of the flowers, anthesis and fertilisation, and culminating in fruit set. This phase largely determines the number of mature fruits produced, but not necessarily their individual size. The imposition of severe stress at any time during this stage may cause severe flower and bud abscission and hence yield loss. In the present study, stress was imposed at the appearance of the first flower buds. The other two phases of reproductive cycle defined by Kaufmann (1972) were the periods of fruit enlargement and fruit ripening; these were not specifically examined in the present study.

Plant water deficits have been reported by several workers to increase flower abscission and reduce fruit set. For example, water deficits during flowering and early pod development decreased yield in soybean (Momen *et al.*, 1979; Westgate and Peterson, 1993), primarily because of a decrease in pod number per plant resulting from increased flower and pod abortion (Shaw and Laing, 1966). In oats moderate or severe water deficits imposed after anthesis caused 57 - 80 and 89 - 90% of the fertile florets to be aborted respectively (Peltonen-Sainio, 1991). In maize, stress at anthesis may cause failure of pollination or poor development of the fertilised embryos (Herrero and Johnson, 1983; Westgate and Boyer, 1985).

In general, the abscission of flowers and juvenile fruit is increased and fruit or seed set are decreased by water stress. For example, water stressing cotton plants during early flowering caused severe leaf wilting and shedding of new flower buds, but had

no effect on existing flowers or boll retention (Grime *et al.*, 1970). Similar stress during the period of peak flowering caused extensive bud shedding and reduced boll retention, while late stress reduced current flowering and induced almost complete loss of bolls. In contrast, Saito and Ito (1967b) observed no effect on flower abscission when the water supply to tomato seedlings was restricted. Similarly, Dubetz and Mahalle (1969) observed no change in flower abscission in bush beans when soil water potential reached -0.8 MPa before, during or after flowering. In winter grown glasshouse tomato, a marked increase in flower bud abscission occurred before anthesis when plants were stressed after the largest bud on the inflorescence reached 4 mm (Atherton and Othman, 1983). However, when stress was applied at an earlier stage of development (largest bud 1 mm long), no significant effect on bud abscission was observed. In contrast, water stress was especially injurious to wheat when imposed during initiation of flower primordia and anthesis (Fischer, 1973; Sionet *et al.*, 1980). These findings suggest that there are specific stages of bud development which are more susceptible to water stress-induced abscission. Because flowering occurs over a defined period, Radin (1993) suggested that yield responds strongly to stress at anthesis. Investigations demonstrating the differential sensitivity of specific stages of bud development to water stress have been reported by other workers (Dubetz and Mahalle, 1969; Huang, 1978). The results obtained also suggest that some species are more sensitive than others and that the impact on reproductive development may depend on the timing of the stress.

The effects of water deficits on flowering are complex because the flowering process can be affected in many ways. The extent of the effects on abscission, for example, is affected by other environmental variables such as temperature and irradiance (Lawlor, 1979). For instance, Cochran (1936) demonstrated that the reduction in fruit set in water stressed sweet pepper resulting from increased flower abscission was greater when the daily mean temperature was increased to 24 °C. Similarly, Klapwijk and De Lint (1974) showed that water deficits imposed during flowering in tomato induced less flower abscission under low irradiance conditions. The same approach of restricting the watering of tomato plants during winter propagation has been practised by the commercial growers to produce 'hard ' or 'balanced' plants in which

flower abscission is greatly reduced (Cooper and Hurd, 1968). The purpose of imposing such intentional stress by controlling the water supply was to restrict vegetative growth without adversely affecting subsequent growth during the reproductive phase (De Koning and Hurd, 1983). Increased fruit set following a decrease in flower abscission has occasionally been observed when tomato plants were subjected to water stress during flowering (Rudich *et al.*, 1977) and some tolerance of water deficits has also been observed in sweet pepper (Hernandez-Armenta, 1985). According to Hernandez-Armenta (1985), the degree of moisture stress required to reduce fruit set significantly in sweet pepper appears to be great, since measurable increases in the abscission of reproductive structures occurred only when the plants were severely wilted. Similarly, Menzel *et al.* (1986) demonstrated a reduction in flower bud abscission prior to anthesis when passion fruit plants were stressed.

A limited number of investigations have shown that anthesis may be advanced in plants subjected to water stress during early vegetative growth (Cooper *et al.*, 1966; De Koning and Hurd, 1983; Wudiri and Henderson, 1985; Drinnan and Menzel, 1994; Jaafar *et al.*, 1994). For example, when greenhouse tomatoes were gradually water stressed after the appearance of the first flower buds, flowering was accelerated (De Koning and Hurd, 1983; Wudiri and Henderson, 1985). During water stress, accumulation of carbohydrates in the stems was increased and this was suggested to advance flowering by providing an enhanced carbohydrate supply to support the development of reproductive primordia (De Koning and Hurd, 1983). Observations of plants grown using the nutrient film technique further revealed that the enhancement of early inflorescence development by stress was accompanied by reductions in leaf expansion and stem extension (Cooper, 1976; Hurd and Graves, 1981). Similar results reported by De Koning and Hurd (1983) suggest that this effect of water deficits in promoting reproductive development at the expense of vegetative growth may be a common phenomenon.

Effects of water stress on flower number have been observed in several annual crops. For example, processing tomatoes receiving different quantities of water during vegetative growth exhibited a decrease in the number of flowers produced as the

severity of stress increased (Wudiri and Henderson, 1985). Similarly, Klapwijk and De Lint (1974) demonstrated that the number of flower buds initiated at the growing points of tomato plants stressed during winter propagation was reduced. In oats, drought was observed to decrease the number of fertile florets in the panicles of oats when watering was restricted when the first leaf began to unroll (Peltonen-Sainio, 1991).

However, water stress does not always reduce flower number. For example, Rudich *et al.* (1977) demonstrated that processing tomato plants receiving different rates of drip irrigation during vegetative growth showed no difference in the number of inflorescences produced. Similarly, the number of flower buds per node in apricots was not affected by water stress, although total flower number was reduced because of a decrease in the number of nodes bearing flowers (Jackson, 1969). De Koning and Hurd (1983) reported that the number of flowers reaching anthesis was increased when winter-sown tomato plants were subjected to restricted watering, while Kaufmann (1972) observed that fruit set was increased because of reduced flower abscission when pepper plants were stressed by reducing the osmotic potential of the nutrient solution from -0.29 to -0.62 MPa during the first week of flowering.

In addition to reducing flower number, water stress may also affect flower size, as in tomato (Saito and Ito, 1967b). Menzel *et al.* (1986) also observed a reduction in flower size in passion fruit following the imposition of moisture stress, although this did not lead to premature abscission, and suggested that these developing flower buds acquire some resistance to desiccation. According to Damptey and Aspinal (1976) and Yegappan *et al.* (1982), the smaller flower size in water stressed maize and sunflower plants may be attributable to the reduced rate of growth and final size of the inflorescence, an effect which might become more serious if the stress was imposed earlier during flowering and for a longer period, as observed in apple (Modlibowska, 1961). The observed effects of water stress on flower size may presage the consequent effects on fruit dry weight, as suggested for tomato by Gates (1955).

However, the reproductive stages are not always the most sensitive to stress

(Kirkham, 1990). A classic example was reported by Sionet *et al.* (1987) who exposed goosegrass (*Eleusine indica* (L.) Gaertn.) to water stress at the vegetative stage, the reproductive stage and during both stages. Plants subjected to water deficits during flowering showed the smallest decline in biomass during stress and a higher rate of growth after rewatering, as compared to plants stressed during vegetative growth or during both the vegetative and flowering stages.

The available evidence therefore suggests that some species are more resistant to the effects of water stress on flower abscission and fruit set than others. This apparent discrepancy may arise from the different approaches used to apply water stress, variation in the intensity of the stress imposed, its timing and duration, and interactions between treatments imposed and the other environmental factors. Since different species exhibit differential sensitivity to water stress, the responses observed during reproductive development may also affect time to anthesis and the severity of subsequent flower abscission. The experiments described in Chapter 5, were therefore carried out to determine the influence of the intensity and duration of water stress, and its interaction with irradiance, on flower development and abscission. The role of assimilate accumulation and partitioning in mediating the effects of water stress on flower development and abscission was also investigated.

1.2.2.2 *Water stress and vegetative growth*

Water stress imposed during vegetative growth may affect subsequent reproductive development to an extent that depends on the severity and duration of the stress imposed. In general, moderate water stress inhibits vegetative growth, particularly leaf expansion (Turner and Begg, 1981; Kramer, 1982; Kirkham, 1990), but reproductive development may be hardly affected (Kaufmann, 1972; Bradford and Hsiao, 1982).

It is well documented that expansion growth is extremely sensitive to water stress (Hsiao *et al.*, 1985; Hsiao and Jing, 1987) and that restriction of leaf expansion is

one of the first symptoms of stress (Kirkham, 1990). El-Sharkawy and Cock (1987) suggested that decreasing leaf area by slower growth and leaf rolling during dry weather is a mechanism which enables plants to reduce water loss by transpiration. Decreases in leaf area resulting from drought also have secondary effects because of consequent reductions in light interception and photosynthate production, which in turn contribute to the observed reduction in growth (Hale and Orcutt, 1987). Many recent studies have reported reductions in leaf growth resulting from water deficits (Sobrado and Turner, 1986; McIntyre, 1987; Guralnick and Ting, 1987). For example, water stress had a major effect on the leaf area of passion fruit, which was associated with reductions in the total number, dry weight and size of the leaves (Menzel *et al.*, 1986). Similar reductions in leaf area resulting from the production of fewer and smaller leaves or the shedding of older leaves during dry periods have been reported for groundnut and sorghum (Ong, 1984; Ong *et al.*, 1985; Huda *et al.*, 1987). The observed reductions in leaf expansion induced by water stress may imply either a reduction in cell expansion and/or a slower rate of cell division, as indicated by the longer plastochron, in tomato (De Koning and Hurd, 1983).

As for reproductive development, the nature and extent of the effects of water deficits on vegetative growth depend on the severity and duration of the stress (Chaves, 1991). Although the effects of mild stress on vegetative growth may be reversible upon rewatering, severe and/or prolonged water stress often permanently reduces plant growth (Bradford and Hsiao, 1982). For example, when tomato plants were mildly stressed, the total number of leaves per plant was increased, although the number of leaves below the first truss was unaffected (Cooper *et al.*, 1966). In contrast, the imposition of severe stress on sunflower plants directly affected the leaf primordia, leading to a permanent reduction in total leaf number (Marc and Palmer, 1976; Yegappan *et al.*, 1980). During severe and/or prolonged stress, there is also more opportunity for the initial responses to lead to secondary and tertiary effects. Acevedo *et al.* (1971) demonstrated that mild stress reduced vegetative growth in maize, but that growth resumed within seconds of rewatering, suggesting that the increase in tissue water status immediately promoted cell expansion. Similarly, Hoogenboom *et al.* (1987) observed that leaf size in soybean was reduced during

periods of moisture stress, but that the shoots of non-irrigated plants grew more rapidly after rain than those of irrigated plants, causing most of the growth reduction during the previous stress period to be restored. In contrast, withholding water for 7 d sharply decreased the rate of leaf expansion in bean (*Phaseolus vulgaris* L.) and no recovery was observed 3 d after rewatering (Markhart, 1985). The various vegetative organs may also respond differently to stress. For instance, the reductions in relative growth rates and dry weights following wilting were greater in the upper and younger leaves of tomato than in the lower and older leaves (Gates, 1955). However, the younger leaves were more tolerant of water deficits than older tissues in that they resumed active growth upon rewatering (Gates, 1964, 1968).

Water stress not only reduces leaf area but also often increases leaf thickness, thereby increasing the weight per unit area or specific leaf weight (Kramer, 1983). Hampton *et al.*, 1987) observed that thick leaves were associated with drought resistance in cotton genotypes which also possessed thick epidermal cell walls and cuticles. Retarded growth and development of other plant parts as a result of water deficits have also been documented. For example, stem diameter was decreased in tomato, indicating that cell expansion across the stem axis was affected by water stress (Cooper *et al.*, 1966; De Koning and Hurd, 1983). The number of branches was also reduced by water stress in sweet pepper (Wien *et al.*, 1989a), an effect which reduced the number of flowers produced because fewer flowering nodes were present.

In this thesis, investigations of the effects of water stress on vegetative growth, in particular leaf number and size, were carried out to determine the relative importance of the severity and duration of water stress. The consequences of drought-induced restrictions of vegetative growth for reproductive development were also examined.

1.2.3 Assimilate production and partitioning

Developing flowers are centres of growth which require continued supplies of assimilate. An acute shortage of carbohydrates resulting from unfavourable

environmental conditions during flower development promotes flower abscission in many species (Kinet *et al.*, 1985; Halevy, 1987; Kinet, 1994). The occurrence of stress-induced abscission under such conditions may result from the inability of young flower buds to compete successfully for available assimilates with the meristems and developing leaves at the shoot apex (Hussey, 1963; Calvert, 1969; Halevy, 1984), or with the roots (Cooper and Hurd, 1968). Several examples of flower abscission induced by environmental stresses such as low light and temperature extremes were described for tomato (Kinet *et al.*, 1978; Picken, 1984; Dinar and Rudich, 1985a, b), sweet peppers (Wien *et al.*, 1989a, b; Turner and Wien, 1994a; Jaafar *et al.*, 1994) and cotton (Guinn, 1974).

During water stress, gas exchange is frequently impaired by reduced stomatal conductances (Black *et al.*, 1985; Bennett *et al.*, 1987) and photosynthetic area is restricted by decreases in both leaf production and expansion (Ong *et al.*, 1985; Huda *et al.*, 1987). As a result, vegetative and reproductive growth are both affected by the consequent reduction in assimilate production, as indicated by the reduced total dry weight of passion fruit plants subjected to stress (Menzel *et al.*, 1986) and the flower abscission induced by the inability to compete successfully for inadequate assimilate supplies (Shillo and Halevy, 1976a). Similarly, reproductive growth in groundnut may be restricted by inadequate supplies of assimilates resulting from water stress (Stirling *et al.*, 1989a). These results suggest that when assimilate supplies are limited by stress, partitioning to reproductive organs, particularly young buds and flowers, may be reduced to the point where continued growth cannot be supported and premature abscission occurs.

Some stages of reproductive development appear to be more susceptible to stress-induced abscission than the others. For example, the most sensitive growth stage to water stress in tomato was observed to last for about 10 d from the time the buds became macroscopically visible, and restricted supplies of assimilates during this period caused complete abortion of the inflorescence, without affecting the growth of the young leaves (Kinet, 1977a). Gladiolus flower buds were also very sensitive to water stress and low temperature which caused abscission (Shillo and Halevy, 1976a,

b); mild water stress also decreased the partitioning of ^{14}C-assimilates to the inflorescence, but increased their transport to the competing sink, the corm.

Evidence to support the view that competition for assimilates may limit bud growth was obtained for tomato plants grown under low light conditions since removal of young leaves, particularly those which developed at the same time as the inflorescence, reduced bud abscision (Aung and Kelly, 1966; Saito and Ito, 1974; Kinet, 1977b). Aloni *et al.* (1991b) also showed that the partitioning of ^{14}C-sucrose to the buds, but not the young leaves of sweet pepper, was reduced by high temperature stress. The involvement of changes in assimilate partitioning between reproductive and vegetative structures during water stress in promoting the abscission of reproductive organs has not previously been investigated in sweet peppers.

1.2.4 Ethylene and water stress-induced flower abscission

As discussed above, certain environmental factors may promote extensive flower abscission (Menzel *et al.*, 1986; Kinet, 1994), presumably by reducing assimilate production or its partitioning to the flowers (Menzel, 1985; Halevy, 1987). Ethylene has also been implicated as the major cause of abscission in many species under stress conditions (Sexton *et al.*, 1985; Taiz and Zieger, 1991; Abeles *et al.*, 1992). Ethylene production in plants has often been found to increase during various types of stress, including low light (Durieux *et al.*, 1983; Wien *et al.*, 1989b), temperature extremes (Ohno, 1991; Wien *et al.*, 1993) and flooding (Kawase, 1976; Jackson, 1985). This increased ethylene has been called stress ethylene (Kirkham, 1990). Water stress also promotes ethylene production, as noted by McMichael *et al.* (1972), El-Beltagy and Hall (1974) and Stumpff and Johnson (1987). However, most studies of water stress-induced ethylene production have concentrated on the leaves, and have paid little attention to the flowers. A major objective of the study reported here was to investigate the role of ethylene in mediating the effects of water stress on flower abscission in sweet pepper.

1.2.4.1 *Stress-induced ethylene*

Apelbaum and Yang (1981) showed that ethylene production increased by more than 30-fold within 4 h of droughting plants and decreased rapidly thereafter. At the time of maximum ethylene production, a maximum 9% of the leaf fresh weight had been lost, but thereafter ethylene production declined as water loss continued. An increase in ethylene production from excised wheat leaves was also observed with increasing water stress until c. 10 - 13% of the tissue water had been lost (Wright, 1977; Kimmerer and Kozlowski, 1982). In contrast, Guinn (1976) found that a loss of 1.3% of the total moisture content from cotton bolls detached from partially droughted plants did not increase the rate of ethylene evolution, indicating perhaps that a greater loss of fresh weight was required to stimulate ethylene production. These data suggest that during water stress, ethylene production may increase as tissue fresh weight decreases until a certain maximum water loss is reached, beyond which further losses of fresh weight no longer increase ethylene evolution.

The involvement of stress-induced ethylene production in accelerating and increasing flower and leaf abscission is well documented (El-Beltagy and Hall, 1974; Jordan *et al.*, 1972; Guinn, 1976; Hoffman *et al.*, 1983), although the impact of stress on ethylene production differs between species and cultivars. Ethylene production by cotton petioles increased as leaf water potential decreased from -2.0 to -2.5 MPa (McMichael *et al.*, 1972), whilst abscission of the cotyledonary leaves occurred following rewatering of stressed cotton seedlings (Jordan *et al.*, 1972). Stumpff and Johnson (1987) reported that ethylene production rates increased slightly in response to an initial stress of -1.3 MPa, then declined until leaf water potential reached -1.6 MPa before increasing sharply at -2.5 MPa. A similar response was observed for intact *Vacia faba* plants, in which internal ethylene levels initially declined during moderate stress and then increased again under severe stress (El-Beltagy and Hall, 1974).

Reductions in osmotic potential have been reported to accompany the observed increases in ethylene production during water stress (Curtis, 1981; Miyamoto and

Kamisaka, 1987), and the application of ethylene apparently always reduces osmotic potential (Eisinger *et al.*, 1983; Kirkham, 1983, 1985; Miyamoto and Kamisaka, 1987). As plants may be able to maintain turgor at least partially by lowering their osmotic potential during periods of water stress, the observed increase in ethylene production during stress may be related to drought tolerance, as suggested by Zhang and Kirkham (1988). In contrast, Morgan *et al.* (1990), Narayana *et al.* (1991) and Eklund *et al.* (1992) found no increase in ethylene production in response to water stress. It has also been observed that endogenous ethylene concentrations were several times higher in petioles than in the leaf blade (McAfee and Morgan, 1971), whilst root tissue exhibited greater ethylene production than the needles of loblolly pine seedlings (Stumpff and Johnson, 1987). Increased ethylene production during water stress has also been observed in Freesia inflorescences (Spikeman, 1986) and carnation flowers (Borochov *et al.*, 1982). However, although the involvement of ethylene in leaf abscission during water stress is well documented, its role in the abscission of flowers is not clearly understood and no studies appear to have been conducted in this respect for sweet pepper.

Water stress has consistently been shown to increase endogenous ethylene levels and accelerate abscission. However, in order to implicate ethylene firmly in the acceleration of water stress-induced abscission, it is necessary to show that the endogenous ethylene level increases above the threshold concentration of 0.1-1 $\mu l\ l^{-1}$ that is normally effective when added exogenously (Sexton *et al.*, 1985). Burg (1968) suggested that rates of ethylene production in the range 3 - 5 $\mu l\ kg^{-1}$ FW h^{-1} would produce the necessary saturating concentration in the abscission zone. Tissues in which values within this range and where positive correlations between ethylene levels and abscission have been found include young cotton bolls (Guinn, 1976), lily buds (Van Meeteren and De Proft, 1982) and bean leaves (Jackson and Osborne, 1970; Jackson *et al.*, 1973). Several studies have shown that exogenous applications of ethylene may stimulate abscission (Morgan and Durham, 1980; Durieux *et al.*, 1983; Furutani *et al.*, 1989; Mason and Miller, 1991). This ethylene may be applied either as ethylene gas or as compounds which release ethylene within the plant tissues. One such compound which is commonly used to generate ethylene within the plant is 2-

chloroethylphosphonic acid (CEPA; Maynard and Swan, 1963; Yang, 1969).

1.2.4.2 *CEPA - an ethylene-releasing compound*

The ethylene releasing compound CEPA is believed to elicit physiological responses in two ways, i.e. through the direct action of the released ethylene and also through the stimulation of ethylene production within the plant tissues (Yang, 1969; Kays and Beaudry, 1987; Abeles *et al.*, 1992). In plants capable of autocatalytic production of ethylene, ethylene released from CEPA accelerates the endogenous synthesis of ethylene (Gupta and Anderson, 1989; Schierle *et al.*, 1989; Foster *et al.*, 1992), thereby promoting abscission (Furutani *et al.*, 1989). Autocatalytic ethylene production is due primarily to an increase in the activity of the ethylene-forming enzyme (EFE) which transforms ACC to ethylene (Abeles *et al.*, 1992) or increases ACC production (Riov and Yang, 1982a). In contrast, some plants are autoinhibitory and may exhibit reduced ethylene synthesis in response to ethylene application (Abdel-Gawad and Martin, 1973; Riov and Yang, 1982b). This may result either from reduced ACC levels caused by decreased ACC synthesis (enzyme in SAM conversion to ACC; Aharoni, 1985) or from an increase in the conjugation of ACC (Liu *et al.*, 1985; Philosoph-Hadas *et al.*, 1985).

The application of ethylene generating compounds effectively simulates the effects of water deficits by increasing internal ethylene production and accelerating and increasing abscission (Mason and Miller, 1991; De Munk *et al.*, 1992). Jackson *et al.* (1973) showed that the application of CEPA to bean petioles increased ethylene production just prior to abscission to levels similar to those suggested by Burg (1968) as being necessary to promote abscission, indicating that ethylene production may increase naturally prior to abscission in abscising organs. Application of a spray containing 2.1 mM ethephon (2-chloroethylphosphonic acid) to Easter lilies growing under a 92% irradiance reduction caused more extensive bud abscission than 4.2 mM ethephon (Mason and Miller, 1991). However, under natural glasshouse irradiance conditions, maximum abscission occurred following treatment with 4.2 mM ethephon.

Temperature also influences the rate of ethylene evolution from CEPA in such a way that flower and leaf abscission increase with temperature (Beaudry and Kays, 1988). Plants also tend to be much more sensitive to ethylene when under some form of stress (Bukovac *et al.*, 1971; Wilde and Edgerton, 1975; Kays and Beaudry, 1987), possibly due to the compounding effect of increased endogenous ethylene production resulting from the stress (Abeles *et al.*, 1992). For example, water stress is known to make cotton leaves more susceptible to abscission (Jordan *et al.*, 1972). These results suggest that, while the concentration of the ethylene generating compounds applied determines the potential severity of bud abscission, the prevailing macro- and micro- climatic conditions strongly influence the condition of the plants, and thus their response to applied ethylene.

To investigate whether increased ethylene production during water stress may be responsible for promoting flower abscission in sweet pepper, an experiment was carried out using the ethylene-releasing compound, CEPA. Its aim was to establish whether CEPA application increased ethylene production and induced flower abscission, thereby simulating the effects of water stress (Chapter 6).

1.2.4.3 *Inhibitors of ethylene-action*

The action of ethylene can be inhibited competitively by various chemicals, the most common of which are silver nitrate (Beyer, 1976) and silver thiosulphate (STS; Cameron and Reid, 1983; Reid, 1985; Veen, 1983; 1986). These chemicals are thought to act by blocking the binding sites for ethylene by combining with the ethylene receptor, thereby preventing the cells from responding to ethylene (Veen, 1986). Veen (1986, 1987) developed a model based on the concept that competition between ethylene and 2,5-NBD occurred at the primary receptor site, whereas that between silver and ethylene originated from competition between silver and copper atoms for an active site on the secondary enzymic sub-unit of the receptor. Beyer (1976) suggested that silver ions could replace copper within the receptor unit and might subsequently prevent binding between the primary receptor and ethylene by

modifying the receptor molecule, as has been suggested by Yang (1985).

Silver nitrate and STS are both persistent and specific in their action, although the usefulness of silver nitrate has been limited by its relative immobility within plant tissues. This immobility is believed to be caused by the participation of silver ions in cation-exchange processes at negatively charged sites on the xylem vessel walls (Veen and Van De Geijn, 1978; Veen *et al.*, 1980). Possibly because of heavy metal toxicity, phytotoxicity is generally induced following its application at effective concentrations, thereby increasing ethylene production relative to untreated control plants (Aharoni and Lieberman, 1979; Veen, 1983; Atta-Aly *et al.*, 1987). In contrast, silver complexed with thiosulphate is extremely mobile within plants because the silver chelates possess net negative charges (Veen and Van De Geijn, 1978) which enable the anti-ethylene effects of the silver to be preserved within the complex (Veen, 1979a, b). STS has been found to be transported up carnation stems at a rate of 2 m h^{-1} (Veen and Van De Geijn, 1978), while Reid *et al.* (1980) showed that treatments as short as 10 min with solutions containing as little as 1.0 mM Ag postponed senescence in carnation blooms from 5 to 10 d after cutting. As ethylene is involved in the regulation of senescence (Kao and Yang, 1983; Abeles *et al.*, 1992), the observed delay in senescence provides evidence for the effectiveness of STS in blocking the action of ethylene. STS is also less phytotoxic than silver nitrate (Veen and Van De Geijn, 1978), although it may become toxic at higher concentrations (Joyce *et al.*, 1990; Wang and Dunlap, 1990; Dostal *et al.*, 1991). For example, the uptake of more than 5 μmol of silver per carnation stem was found to be toxic, whereas 0.5 μmol Ag per stem provided maximum vase life (Reid *et al.*, 1980).

STS may also block the action of exogenous ethylene applications (Joyce *et al.*, 1990; Dostal *et al.*, 1991). In the former study, STS was applied to holly and mistletoe branches placed in distilled water and left overnight before being supplied with ethylene gas ($\approx$ 35 l h^{-1}) the following day. Leaf abscission was induced by the ethylene treatment within 3 d of application, whilst the same percentage abscission (60%) did not occur in the control until day 13. However, application of 0.2 - 2 μmol

Ag per branch prevented leaf abscission in mistletoe for up to 13 d after exposure to ethylene. However, at a higher concentration of 4 μmol Ag per branch, STS induced leaf abscission, presumably because of silver toxicity, or perhaps in response to the increased production of ethylene, as has been observed in vegetable tissues treated with Ag (Gavinlertvatana *et al.*, 1980).

In a simulated shipping study, Dostal *et al.* (1991) found that exposure to exogenous ethylene (1 - 10 μl l^{-1}) for $\geq$ 4 h caused 80 - 100% corolla abscission in cut blooms of New Guinea impatiens (*Impatiens x hawkeri* 'Sunfire'), whereas abscission was only 65% in the control. Plants pre-treated with 1 mM STS and subsequently exposed to simulated shipping conditions showed a reduced corolla abscission of 75 - 80%, whereas complete protection against corolla abscission was observed in plants treated with STS and exposed to exogenous ethylene. Application of 4 mM STS produced phytotoxic symptoms. Inhibitory effects of STS on ethylene or high temperature (26 °C)-induced abscission have also been observed in zygocactus plants (*Schlumbergera truncata*; Cameron and Reid, 1981), in which a foliar application of 2 mM STS significantly reduced flower and bud abscission. Application of 4 mM STS occasionally caused blistering of the leaves which eventually subsided to form dark depressions, whereas concentrations lower than 2 mM provided only partial protection against ethylene- and stress-induced abscission. The effectiveness of the 4 mM STS spray persisted until 28 d and was slightly greater for flowers than for buds.

These results demonstrate that STS is an effective inhibitor of ethylene-induced abscission, although its impact in reducing, preventing or increasing abscission depends on the concentration applied. Complete protection was provided by 2 mM STS, whereas higher concentrations were phytotoxic and lower concentrations provided only partial protection. The effectiveness of STS was apparent within 10 min of application, persisted for up to 28 d and did not appear to be affected by the method or duration of the STS treatment. For example, ethylene application one day after treating mistletoe with STS produced no leaf abscission (Joyce *et al.*, 1990). Similar total protection against abscission was also achieved in plants sprayed with STS to run off and kept for 7 - 28 d before exposing the flowers and buds to ethylene

or high temperature (Cameron and Reid, 1981; Dostal *et al.*, 1991).

Previous work using STS sprays has involved pre-treatment of cut flowers (Reid *et al.*, 1980; Veen and Kwakkenbos, 1983; Dostal *et al.*, 1991) or foliage (Wang and Dunlap, 1990; Joyce *et al.*, 1990) before exposing them to ethylene, sometimes in transport simulation studies. However, few studies have examined the protective influence of STS against stress-induced ethylene production in either pot or field grown plants, and the consequent abscission of plant organs, particularly flowers.

To date, Cameron and Reid (1981) have shown that spraying with 4 mM STS reduces flower and bud abscission in potted zygocactus plants exposed to stress-induced ethylene by keeping them in darkness at high temperature (26 °C) for 4 d. Cameron and Reid (1983) subsequently tested the protective influence of STS against flower abscission in other species of potted plant. Petal abscission in geranium seedlings (*Pelargonium hortorum* Bailey) was completely suppressed by foliar sprays containing 0.5 mM STS, while similar treatment of *Calceolaria herbeohybrida* Voss reduced flower drop from 83 to 22 % when plants were exposed to a 4 d drought in darkness at 25 °C. Bracteole drop in *Bougainvillea glabra* Chois caused by 3 d of water stress was also reduced by foliar treatment with 0.5 mM STS. STS has been found to be completely effective regardless of whether this applied to whole plants or only to individual developing inflorescences, and phytotoxic effects have not been observed at concentrations of 0.5 mM or less in any of the species examined (Cameron and Reid, 1983).

The available evidence suggests that high temperature and irradiance coupled with water stress may exert either beneficial or detrimental effects on growth and development in sweet pepper, depending on the severity and duration of the stress. Changes in assimilate production and partitioning during stress may not be the direct cause of flower abscission, especially during transient stress. There is also evidence to suggest that the flower abscission induced by environmental stresses may be mediated by increased endogenous ethylene production since it is clear that the application of silver ions, which are known to inhibit other ethylene-induced

responses completely or partially, suppresses the abscission of flowers and leaves. There is therefore considerable evidence that ethylene may be present at levels sufficient to induce or accelerate abscission during stress periods such as water stress. The primary aim of the present investigation was to establish the impact of environmental stresses, especially water deficits, on the growth and development of young sweet pepper plants, and to determine the mechanisms involved in mediating these stress effects, particularly on flower abscission.

CHAPTER 2

GENERAL MATERIALS AND METHODS

The experiments described in this thesis were carried out in controlled environment facilities at the University of Nottingham between January 1992 and March 1994. This chapter describes the materials and methods relevant to all experiments; specific modifications to standard procedures are described in the appropriate chapters.

2.1 Plant materials

Two F_1 hybrid varieties of sweet pepper (*Capsicum annuum* L.) were used in preliminary experiments: Bell Boy (Breeders' Seeds Ltd, Lancaster, UK) was selected because it had been widely used in previous experiments in temperate areas (pers. comm., Cullen, 1992) and Blue Star (Know-You Seed Co., Ltd, Taiwan) was chosen for its continuous high productivity and adaptation to tropical conditions (Hawa and Aziz, 1991).

2.1.1 Propagation

Germination tests were carried out prior to sowing to determine seed requirements. The seeds were thinly sown in flat trays containing Levington F2 Compost (Fisons Horticulture Ltd, Ipswich, UK) which were then placed on a propagating bed which provided a basal temperature of 24 °C in a glasshouse where the mean ambient temperature was 20 - 22 °C. To encourage germination, the trays were covered with black polythene which was removed when the cotyledons emerged. When the cotyledons had fully expanded, uniform seedlings were pricked out individually into

9 cm diameter pots containing Levington M2 potting compost and placed on benches in a glasshouse at a mean daily temperature of 20 - 22 °C, usually under natural lighting conditions (Plate 2.1.1).

When the third pair of true leaves was about 1 cm long (defined as the third true-leaf stage), the seedlings were transplanted into larger pots (6 or 12 l) containing Levington M2 compost. These pots were arranged in a glasshouse at a mean daily temperature of 26 ± 3 °C with ventilation at 29 °C. The plants received natural radiation, supplemented with 400 W high pressure sodium lamps (SON/T) between 0500 - 2300 h to provide an 18 h daylength and an additional irradiance of 2.5 MJ m^{-2} (PAR; 400-700 nm). Relative humidity (RH) was maintained above 65% by placing moist capillary mats on the glasshouse floor.

2.1.2 **Seedling management**

The seedlings were watered every morning throughout the growing period to maintain optimum growth using a standard nutrient solution containing Vitafeed NPK 214 (16:8:32; $N:P_2O_5:K_2O$) at a concentration of 0.5µl l^{-1}. However, neither the plants in the water stress treatments nor the unstressed controls received any nutrient solution during treatment. This practice was adopted to avoid the development of major nutritional differences between the water-stressed and well-watered plants during treatment. Spraying of pesticide was carried out whenever necessary, as recommended by the manufacturer. Red spider mites and aphids were controlled using Torque^R (fenbutatin oxide at 50% w/w by Zeneca) and Pirimor^R (pirimicarb at 50 % w/w by I.C.I.) respectively, sprayed fortnightly at a concentration of 5 g l^{-1} of water. Thrips were controlled using Hostaquicks^R (heptanophos by Hoechst) and Decis^R (deltamethrin by Hoechst) at a concentration of 7.0 - 7.5 ml l^{-1} of water.

PLATE 2.1.1. *Seedlings established in F2 compost (top) and transplanted to 0.6 l pots 4 d after germination (bottom).*


88
N/2
85
N/1


10 3'92

2.2 Reproductive development

2.2.1 Flower growth

Periodic measurements were carried out to determine treatment effects on reproductive growth, particularly in the primary (flowers borne at the first branching position) and secondary flowers (flowers borne at the second branching position; cf. Chapter 1). The measurements included the total numbers of flowers and flower buds and the times required to reach anthesis or abscission. From these observations, the percentages of flowers abscising or reaching anthesis were calculated.

2.3 Growth measurements

Periodic non-destructive measurements were carried out on the same plants to monitor the effects of temperature, irradiance and water stress on shoot growth. Destructive analyses were also carried out at the beginning, midway through and at the end of each experiment to provide information on shoot growth and development, particularly with regard to the accumulation and partitioning of dry matter to the different plant parts. Measurements of roots were not made because of the extreme difficulty of separating them from compost.

2.3.1 Non-destructive approaches

Various measurements of vegetative growth were taken, including plant height, leaf number and stem diameter. Plant height was recorded as the distance between the cotyledon and the shoot apex, while the length of the main stem was measured between the cotyledon and the point of branching. The number of leaves on the main stem (primary leaves) and the rest of the leaves on the shoots subtending the primary flowers which were longer than 1 cm (secondary leaves) were counted and totalled to

determine effects on leaf initiation. Stem diameter was measured 2 - 3 cm above the cotyledons using electronic callipers (Trimos Sylvac Metrology Ltd, London, UK) to establish whether stem thickness and dry matter content were related.

2.3.2 Destructive sampling

Vegetative and reproductive parameters that could not be measured non-destructively included leaf area and the dry weights of the various plant parts. At regular intervals during the experimental period, the shoots were severed at the soil surface, subdivided into flowers, fruits, leaves and stems and placed in paper bags. The samples were then oven-dried for 48 h at 84 °C, cooled and weighed.

Before oven-drying, the leaves were placed in polyethylene bags and kept in a refrigerator (5 °C in darkness) for no longer than 12 h before measuring leaf areas using a Li-Cor 3100 leaf area meter (Li-Cor, Lincoln, NE, USA). The primary leaves were detached and separated from the secondary leaves before being passed through the instrument, which had been previously calibrated using a standard calibration plate measuring 10 cm x 10 cm. This separation was carried out to determine treatment effects on the expansion of both primary and secondary leaves. A camera located above the area meter board recorded the leaf areas, which were displayed on a Phillips 25 cm Monitor. To ensure accurate measurements, the leaves were arranged within the field of view and overlapping of adjacent leaves was avoided. Specific leaf area (SLA) and leaf area ratio (LAR) were calculated from the measurements of total leaf area and leaf weight per plant. The length and width of the leaves of various sizes were also measured to establish a non-destructive empirical relationship with leaf area, as measured using the Li-Cor 3100 meter.

2.4 Environmental treatments

2.4.1 Temperature

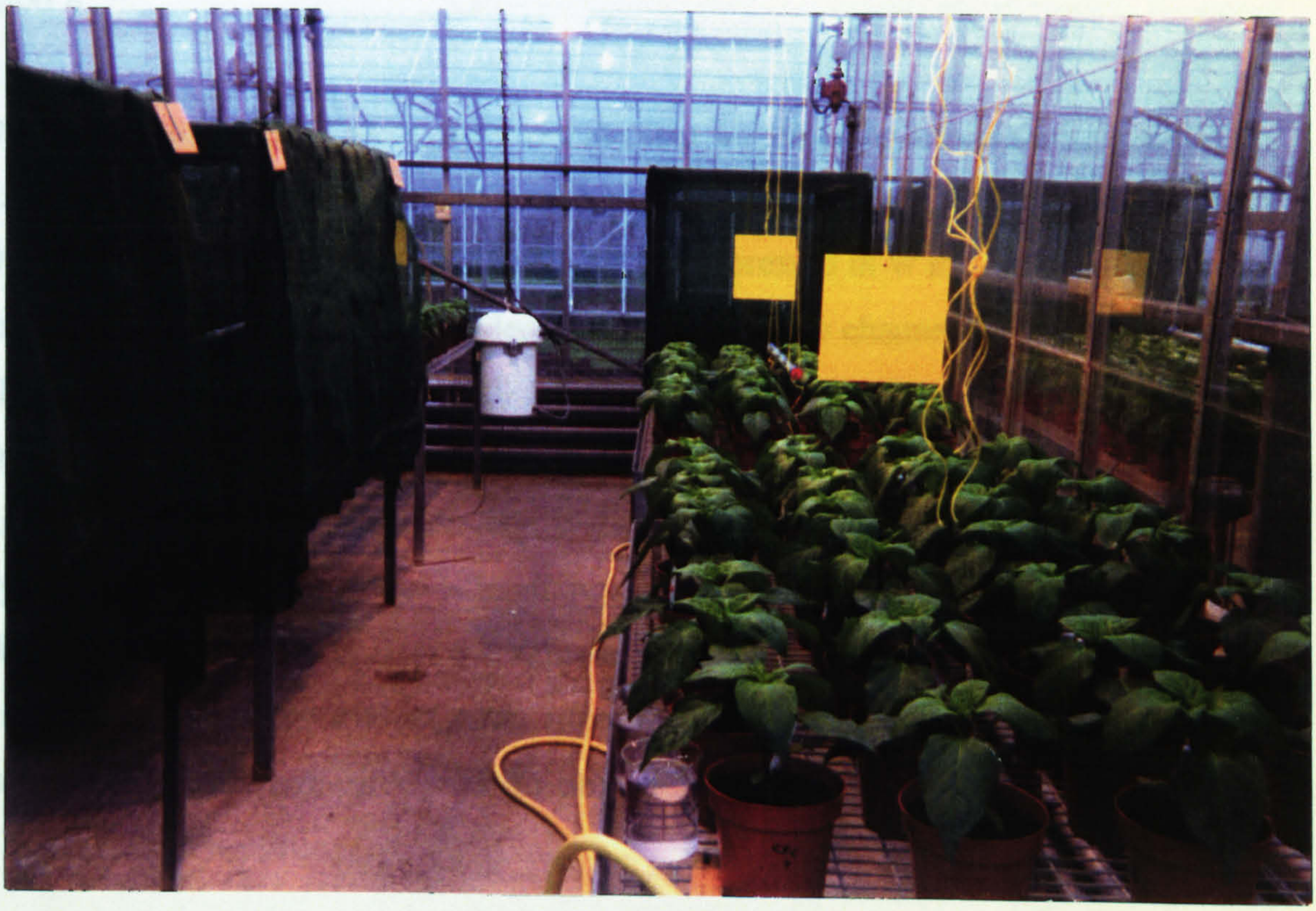
One week before commencing treatments, the temperature regime within the glasshouse was set to the required values and allowed to stabilise. Mean daily temperature for each treatment was set with a range of $\pm$ 3 °C, above which ventilation operated to reduce and below which the heating system operated to increase to temperature. Air temperature was measured at plant height using screened and aspirated "PT - 100" sensors and the values were recorded by a "Squirrel" Data Logger (Grant Instruments Cambridge Ltd, Cambridge, UK) at 30 min intervals. Due to a shortage of glasshouse compartments, each temperature treatment was allocated to a specific compartment, within which all the other treatments were randomly blocked and replicated. To avoid external errors between temperature treatments, the other controlled environmental and plant management factors were kept identical in all compartments. At the end of the experiment, accumulated total and mean daily temperatures during the experiment were calculated from daily records. The thermal time requirement for reproductive development was calculated using the cardinal temperatures obtained from the thermogradient study of germination (Section 2.4.4).

2.4.2 Irradiance

Two levels of irradiance were applied. The "high irradiance" (HI) treatment comprised the incident radiation received within the glasshouse, while the "low irradiance" treatment (LI) was achieved using green Rokolene netting providing 50% shade (Rokolene KDA, Rokocontainers, Nottingham, UK) suspended above and around the plants (Plate 2.4.1). Rokolene netting has previously been shown to have no effect on light quality (Meiri *et al.*, 1982). In both treatments the incident radiation comprised the natural glasshouse radiation, supplemented with 18 h of light from 400 W high pressure sodium lamps (SON/T) which provided an additional total radiation of approximately 5.4 MJ m^{-2} ($\approx$ 2.5 MJ m^{-2} PAR; 400 - 700 nm) between 0500 and

PLATE 2.4.1.(top) *Experimental layout showing the high (foreground) and low irradiance (background and left) treatments. The latter was provided by suspending green Rokolene netting above and around the plants.*

PLATE 2.4.2.(bottom) *Tube solarimeter suspended above the plants to measure incident radiation.*



2300 h.

Daily radiation receipts were measured at plant height using tube solarimeters (Plate 2.4.2; Green and Deuchar, 1985) calibrated against a Kipp Solarimeter (Delta-T Devices, Cambridge, UK). Measurements were expressed in W m^{-2} and later converted to MJ m^{-2}. The values were recorded using either single channel millivolt integrators (Type MV1, Delta-T Devices Ltd, Burwell, Cambridge, UK) or a Campbell CR10 Data Logger (Campbell Scientific Inc., Shepshed, UK) at 60 min intervals. In the former method, daily voltage readings were recorded and total radiation was calculated by dividing the cumulative voltage by the corresponding solarimeter constants, obtained by calibration against the Kipp solarimeter. Two solarimeters were allocated to each block. At the end of each experiment, accumulated total and mean daily radiation receipts were calculated for each treatment.

2.4.3 Thermogradient plate for germination studies

A thermogradient plate based on the design reported by Thompson (1970) and adapted by Garcia-Huidobro *et al.* (1982) was used to examine the thermal characteristics of seed germination. The thermogradient plate consisted of an aluminium alloy plate with dimensions of 70 x 50 cm, heated at one end by 12 5 W x 12 V resistance heaters fused to the plate. The other end of the plate was cooled by a refrigeration system. This comprised a refrigeration unit which cooled a tank filled with a 33% ethylene glycol solution. Fluid from this tank was circulated through pipe loops to the cold end of the plate at a rate controlled by a pump and a bypass valve. The pump and valve were controlled by a thermistor sensor and related to the hot end of the plate to achieve a uniform gradient over its entire length. Thus, different ranges and gradients of temperature could be obtained by changing the energy inputs or outputs to the heating and cooling systems. The entire thermogradient plate was housed in a polystyrene box to provide insulation. The system was accommodated in a growth room maintained at a mean temperature of 20 ± 0.5 °C.

During the week before each germination trial, a range of different constant temperatures was established along the plate by monitoring the temperatures until constant readings were achieved. Sets of three replicate plastic petri dishes (4.5 cm diameter) containing 30 seeds were placed at intervals along each of these constant temperature gradients. Surface temperatures on the plate were monitored using thermocouples housed in rectangular petri dishes at several points along the length of the plate. Temperature readings from these thermocouples were recorded daily at 1000 and 1800 h using a Comark Electronic Thermometer Type 1625 Cu/Con (Comark Electronics Ltd, Littlehampton, UK). On alternate days, temperatures at the centre of petri dishes placed along the same temperature gradient were also measured to check for any variation within the temperature gradient.

2.4.4 **Photoperiod**

An 18 h daylength was chosen to avoid having to draw opaque screens over the plants on a daily basis during summer in order to achieve a shorter photoperiod. In winter the 18 h daylength also partly offset the lower instantaneous fluxes and increased daily total irradiances to the level required for normal plant growth. However, to ensure that this long day regime was not detrimental for normal reproductive growth and development, a preliminary experiment was conducted to examine the effects of a range of daylengths (8, 10, 12, 16, 18 and 20 h) imposed at the third true leaf stage on flower initiation and development.

In this experiment, an automatic blackout system was used to control daylength by excluding all natural daylight when activated, by covering the entire glasshouse compartment with black canvas painted with a reflective aluminium coating on its outer side. The seedlings in all photoperiod treatments received natural radiation for 8 h daily (the shortest photoperiod treatment) between 0900 and 1700 h, after which the automatic blackout system was activated to exclude all natural radiation. In the longer photoperiod treatments, extended day lengths were achieved using 100 W incandescent lamps providing a PAR flux of about 6.8 μmol m^{-2} s^{-1} incident at plant

height, as measured using a cosine corrected pyranometer (Crump Scientific Products Ltd, Essex, UK). The photoperiod treatments were separated by black plastic sheets suspended between the benches. Mean daily temperature was 20 ± 3 °C, with ventilation at 24 °C.

The results obtained showed that the 18 h photoperiod had no detrimental effects on reproductive growth and development, for example, by inducing flower bud abscission. The time to flower initiation and anthesis in the longer photoperiod treatments was similar to the 12 h treatment, but was 2 -3 d slower than in the 8 and 10 h photoperiod treatments. The delaying effect of long daylength on flower initiation and anthesis in sweet pepper has been reported previously by Cochran (1938), and a similar response has also been observed in tomato (Hurd, 1973). Salisbury (1982) classified *Capsicum frutescens* L. as a quantitative short-day plant which flowers in any daylength, but better under short days. The influence of daylength on flowering has also been reported to be relatively unaffected by temperature, as reviewed by Schwabe (1971) and Vince-Prue (1975).

2.5 Imposition and management of water stress

2.5.1 Water stress treatments

The water stress treatments applied relied on measurements of pot weight to indicate the severity of the drought imposed and the levels of water to be supplied (Saito and Ito, 1967a; Klapwijk and De Lint, 1974; Wudiri and Henderson, 1985; Menzel *et al.*, 1986). Using this method, the soil moisture deficit imposed at different stages of growth may be accurately and reproducibly defined (Kramer, 1983). However, frequent weighing of pots and regular replacement of the water lost through evapotranspiration (ETP) on alternate days in the well watered treatment is necessary to avoid substantial variation in soil water content between rewaterings (Richards and Marsh, 1961; Plate 2.5.1).

PLATE 2.5.1. *Water stress experiment using 12 l pots partly enclosed in clear plastic bags to prevent loss of water drainage from the base of the pot.*

All pots initially received equal volumes of water to maintain them near to the predetermined pot capacity (0.26 litre water per litre of compost), and moisture lost by ETP was replaced on alternate days. A range of water stress treatments was imposed following the appearance of the first flower buds: replacement of water at 100% of ETP served as a "no stress" control (NS), whereas watering at different fractions of ETP provided a range of progressive stress treatments. ETP was determined by weighing the NS pots on alternate days using a 50 kg capacity mechanical balance (August Sauter Ebingen, Germany).

2.5.2 **Measurements of water stress**

The severity of stress was determined by measuring the components of plant water status, i.e., leaf water potential (ψ_l), osmotic potential (ψ_s) and turgor potential (ψ_p), and leaf gas exchange.

2.5.2.1 *Components of plant water relations*

Midday ψ_l was measured in the youngest fully expanded leaf in the upper canopy (fourth or fifth leaf from the apex) of four randomly selected plants using a portable pressure chamber (PMS Instruments, Corvallis, Oregon, USA; Scholander *et al.*, 1965). Leaves sampled from the different water stress treatments were taken from equivalent nodes on the main stem to avoid age effects on the values obtained (Ritchie and Hinckley, 1975). The leaf was excised at the base of the petiole using a sharp blade and immediately wrapped in moist tissue and placed in a plastic bag to reduce dehydration. It was then inserted into the pressure chamber with the cut petiole protruding through the rubber sealing gasket, which was lined with thin layer of silicon rubber to create a good seal. The pressure within the chamber was then increased using nitrogen gas from a portable cylinder until xylem sap reappeared at the cut surface of the petiole (Barrs, 1968). A portable microscope was used to aid observation of the end-point when the petiole first began to exude sap. The pressure

at the end-point was assumed to be equal to the average bulk water potential within the leaf. Each measurement took less than 2 min from excision of the leaf.

Immediately after measuring ψ_l, the leaves were rapidly frozen prior to measuring ψ_s using the cryoscopic method (Barrs, 1968; Slavik, 1974). ψ_p was obtained by difference between ψ_l and ψ_s, i.e.

$$\psi_p = \psi_l - \psi_s$$

After measuring ψ_l, each leaf sample was inserted into a clean 1.5 ml plastic Sarstedt epindorph vial presprayed with a freezing aerosol (Dichlorodifluoro-methane); the sample was then sprayed with freezing aerosol and placed in a deep-freeze for 24 h at -15 °C. This process destroyed turgor within the living tissue. A coolbox filled with ice was used to transport the samples from the glasshouse to the freezer. The samples were then thawed for 30 - 60 min at room temperature (Turner *et al.*, 1978; Turner, 1981) and the vials perforated with minute holes at their narrow ends before being placed in clean 7 ml sap collecting tubes. Sap was expressed by centrifuging at 2500 rev min^{-1} using a Mistrel 3000 Centrifuge MSE (Fisons Instrument, UK) for 15 min at 4 °C to minimise evaporative losses of from the tissue. 25 µl of the leaf extract was then transferred using a micropipette into a 0.5 ml microcentrifuge tube and leaf ψ_s determined using a Roebling Automatic Freezing-Point Osmometer (Hermann Roebling, Ketteweg, Berlin). The meter was regularly calibrated using fresh deionised water and standard salt solution (300 milliosmol kg^{-1} water). Care was taken to avoid all sources of measurement error (Squire *et al.*, 1981; Turner, 1981).

Osmotic potential provides a measure of the solute concentration within a cell or tissue (Turner, 1987). The output from the Roebling Osmometer is expressed in units of milliosmols and may be converted to MPa as follows (Jones and Gorham, 1983):

$$MPa = \frac{(mOsmol/1000 \times 0.0832 \times T\ °K)}{1000}$$

The freezing of tissue causes a breakdown of cell components, which in turn results in the mixing of fluid within the cell walls and xylem external to the cell (apoplast), with the cell contents (cell vacuole and cytoplasm; symplast). The osmotic potential of the apoplastic water is usually above -0.1 to -0.02 MPa, whereas the osmotic potential of the symplastic water is usually between -1 to -3 MPa (Turner, 1981). Thus the symplastic fluid is diluted by apoplastic water, which may comprise between 5 and 50% of the total volume of the water in leaves at full turgor (Slavik, 1974; Tyree, 1976; Grace and Russell, 1977; Wilson *et al.*, 1979). As the volume of apoplastic water is unlikely to vary greatly as the leaf dehydrates, the dilution of the symplastic fluid by apoplastic water is much greater in dehydrated tissue (Tyree, 1976), thereby introducing the possibility of significant underestimates in measurements of osmotic potential. To estimate the magnitude of this error, a correction factor for apoplastic dilution was calculated from the difference in the values for ψ_l and ψ_s in tissues which were visibly wilted ($\psi_p = 0$). Since the measured values of ψ_s and ψ_l carried out during water stress experiments agreed closely in fully wilted leaves, in which ψ_p is zero, errors in the estimation of ψ_s introduced by apoplastic dilution were assumed to be negligible.

2.5.2.2 *Leaf gas exchange*

Foliar CO_2 and H_2O fluxes were monitored using an LCA-3 portable infra-red gas analyser (IRGA; Analytical Development Corp. (ADC), Hoddesdon, Herts, UK) to measure relative humidity, leaf temperature, stomatal diffusive conductance, transpiration rate and net photosynthesis. The LCA-3 is an open or steady state differential system IRGA without temperature control, and was used with a Parkinson broad leaf cuvette (model PLC(B)) with a leaf area of 6.2 cm^2. The leaf chamber was designed to allow maximum air mixing, and included a Cu-Con thermocouple to read the temperature on the undersurface of the leaves and a quantum flux sensor to record the PAR flux. The air flow rate was set at 400 cm^3 min^{-1}. Values for leaf temperature, transpiration rate, relative humidity and intercellular CO_2 concentration were recorded shortly after enclosing the leaf within the chamber.

Periodic measurements of gas exchange were carried out between 1000 to 1400 h on fully expanded young leaves (fourth or fifth leaf) of similar physiological age. At least four leaves from five randomly selected plants per treatment per block were measured, for a period of 45 to 60 s before values were recorded. The data obtained were transferred into an IBM-compatible PC using an ADCDUMP programme and then imported into a Quattro-Pro programme for analysis. Water use ratio (WUR) was calculated by dividing the net photosynthesis rate by the corresponding transpiration rate. Possible errors introduced by variation in ambient CO_2 concentration within the glasshouse associated with the operator's respiration were minimised by drawing fresh air from about 3.5 m above ground level.

Relative humidity (RH) within the glasshouse was also determined using wet and dry bulb thermometry (Assman and Sling Psychrometers, Casella, London, UK). This instrument has a built-in clockwork fan which draws air over the wet and dry bulb thermometers to ensure adequate ventilation. The instrument was placed either under the canopy or at different locations within the glasshouse for 60 to 90 s before recording the readings from the wet and dry bulbs. The percentage RH of the air was then calculated from standard tables.

2.6 Preparation and application of growth regulatory chemicals

2-Chloroethylphosphonic acid (98% active ingredient; Lot No. 41H7707; Sigma Chemical Co., St. Louis, USA) was used as an ethylene-releasing substance and silver thiosulphate was applied to block the action of ethylene.

2.6.1 2-Chloroethylphosphonic acid - CEPA (Ethylene releasing substance)

A solution of 2-Chloroethylphosphonic acid (CEPA) was prepared on the day of the experiment by diluting the substance with distilled water and stored in a refrigerator (4 °C) between sprayings. Approximately 10 ml of the chemical was applied daily directly onto the primary flower buds when they reached the stage most sensitive to

water stress (diameter 4 mm), a treatment which has been found to induce early flower abscission in pepper (Section 5.3; Tripp and Wien, 1989). Since no liquid surfactant was added, to ensure good contact between the chemical and the flower buds, the buds were covered with cotton balls (each pre-weighed at 500 mg) and saturated with the chemical. The cotton balls remained on the flower buds throughout the treatment period except when carrying out flower counts.

2.6.2 Silver thiosulphate - STS (Ethylene-action inhibitor)

Silver thiosulphate solution (STS) was prepared as described by Reid *et al.* (1980). Mixing aqueous solutions of silver nitrate ($AgNO_3$) and sodium thiosulphate ($Na_2S_2O_3.5H_2O$) should result in essentially complete formation of the STS complex, implying stability (Ghosh, 1974).

Stock solutions of silver nitrate (0.1 M) and sodium thiosulphate (0.1 M) (Fisons Scientific Apparatus, Loughborough, UK) were stored in clear storage bottles at room temperature (Cameron *et al.*, 1985). STS was prepared on the day of the application by mixing known volumes of these solutions with deionised water to produce a concentration of 1.0 mM. Silver nitrate was then added slowly to sodium thiosulphate solution in the ratio 1:4 to avoid the formation of a black precipitate of silver sulphide (Ag_2S). The chemical was applied every three days by spraying 10 ml of the chemical onto the shoot system.

2.7 Ethylene measurements

Care was taken during all sampling and measuring procedures to avoid damage and consequent stress to the plant material as this might have caused the evolution of additional ethylene which would have adversely affected the reliability of the measurements of ethylene evolution induced by the treatments applied.

2.7.1 Sampling procedure

The rates of ethylene evolution were determined for detached flowers and leaves. Primary or secondary flowers and the fourth or fifth youngest leaves were used. The organs were excised at the pedicels or petioles using a sharp blade and quickly weighed before inserting their excised ends into 1 ml of 2% w:v plain agar contained in clean glass vials of known volume. The medium provided mechanical support and helped maintain moisture in the vials and thereby minimised additional stress on the excised tissue. The vials were sealed with subaseal rubber caps and the time of capping was recorded. 10 ml vials were used for flowers, and larger 30 ml vials for the leaf samples.

2.7.2 Gas chromatography and expression of results

Ethylene production from the excised samples was measured by gas chromatography (GC; PU 4500, PYE Unicam, Phillips, UK). The GC was fitted with an alumina F1 JJ column (JJ's Chromatography Ltd, Kings Lynn, Norfolk), maintained at an operating temperature of 110 °C, and equipped with a flame ionisation detector heated to 130 °C. Nitrogen and hydrogen were supplied as the carrier gases at approximately 500 (not critical) and 40 ml min^{-1} respectively. Prior entering the GC, they were passed through gas-purifying bottles to prevent foreign particles, water vapour and light hydrocarbons from contaminating the detector, thus helping to maintain a high detector temperature.

The chromatograph was linked to an ionisation amplifier and a variable speed chart recorder operating at 1 mV. Using the F1 column at the temperature stated, alumina was found to give good separation of ethylene from ethane and produced the largest detector response at low ethylene levels (Ward *et al.*, 1987). Since ethylene production was relatively small, the chart peak height was used to determine the concentration of the gas.

Ethylene evolved by the explants was allowed to accumulate in the sealed vial for 90 min before withdrawing a 1 ml sample of the gas from the vial using a 16 mm hypodermic needle fitted to a 1 ml plastic syringe and injecting it through a rubber septum into the GC column. When the relation between wounding and ethylene evolution was examined, measurements were made at 90 min intervals. After each reading, the samples were well aerated for 5 min then recapped prior to the next measurement. Before ethylene measurements from plant tissues were carried out, a calibration gas of 10 μl l^{-1} ethylene was injected from a cylinder into the GC, for use as a reference standard (McAfee and Morgan, 1971).

The rate of ethylene evolution was calculated from the ethylene concentration of the gas sample, the volume of the glass vial and the fresh weight of the sample, and was expressed in units of nl g^{-1} FW h^{-1}. Although not an SI unit, this term has been employed in this thesis due to its widespread usage and comprehension throughout the literature.

2.8 Experimental design and statistical analysis

Most experiments were randomised complete block experiments containing three blocks. In experiments involving more than two factors, a split-plot design was used. The treatments were randomised accordingly using a Table of Random Numbers. Data were analysed using the Genstat 5 analysis of variance (ANOVA Programme), which provided means and standard errors of the difference between means (SED) for all variables measured. Regression analysis was also carried out in some experiments. Significance was tested at the 0.1%, 1% and 5% levels.

CHAPTER 3

VARIETAL RESPONSES TO TEMPERATURE AND IRRADIANCE

INTRODUCTION

As in many other horticultural crops, reproductive growth and development in sweet pepper are very sensitive to environmental factors, and problems of poor fruit set resulting from flower abscission are common. It is well known that temperature and irradiance have a major influence on both early flower development and fruit set (Cochran, 1936; Rylski and Spigelman, 1982; Bakker, 1989). While high temperature has been observed to promote flower development up to anthesis in sweet pepper, it also increased the incidence of flower and bud abscission (Cochran, 1936; 1938). These losses were particularly severe when high temperature occurred in combination with low irradiance (Rylski and Spigelman, 1986), as was also observed in tomato (Atherton and Othman, 1983; Atherton and Harris, 1986; Halevy, 1987). However, the existence of inter-specific differences may influence crop responses to the interactive effects of temperature and irradiance on the earliness of flower development and susceptibility to abscission, thereby affecting percentage fruit set and final yield (Rudich *et al.*, 1977; Rylski, 1986; Bakker, 1989). The varietal differences that exist within species in resistance to abscision under adverse conditions may also form the basis for future breeding programmes since these may determine the extent to which flower growth and development are affected by the interactive effects of temperature and irradiance.

This chapter describes an experiment which examined how differing temperature-irradiance combinations influenced the growth and development of young reproductive plants of two varieties of sweet pepper, Bell Boy and Blue Star.

3.1 Influence of temperature and irradiance on reproductive growth and development

Previous work on the reproductive growth and development of sweet pepper has shown that, while the optimum temperature range for fruit set was 12 - 16 °C (Cochran, 1936), the marketable yield was low because the fruits were deformed (Rylski, 1986). Increasing the air temperature at which the plants were grown from 16 to 27 °C hastened anthesis but also promoted flower abscission, which reached 100% at 32 - 38 °C (Cochran, 1936). Rylski and Spigelman (1982) showed that high day-time temperatures of 28 to 32 °C in combination with a night temperature of 18 °C did not increase flower abscission, whilst a constant day and night temperature of 25 °C caused almost total flower drop. Many workers have suggested that low night temperatures are important in increasing fruit set (Cochran, 1936; Rylski and Spigelman, 1982; Rylski, 1986), but Bakker (1989) demonstrated that the 24 h mean temperature also has a significant influence on flower number and fruit set. Earlier work on tomato also demonstrated the importance of mean daily temperature in controlling flowering (Calvert, 1957). Since the interaction between temperature and irradiance during early plant growth may produce long-term effects on growth and development (Calvert, 1959), the effects described above may have been the result of different combinations of temperature and irradiance. In addition, the varieties used in different experiments may have differed in their sensitivity to the environmental conditions imposed.

The main aim of this preliminary glasshouse study was to examine the growth and development of two sweet pepper varieties subjected to different mean daily temperatures under two levels of irradiance, paying particular attention to post-anthesis flower abscission and fruit set. The variety showing the greater response to temperature and irradiance in terms of flower abscission, and the temperature treatment found to be most favourable for early growth but critical for flower abscission and fruit set would be selected for further studies of the impact of environmental conditions on reproductive growth and development.

3.1.1 Materials and methods

The experiment was conducted between 14 February and 22 May 1992. Seeds from two F_1 hybrid varieties, Bell Boy (BB) and Blue Star (BS), were sown in flat trays and uniform seedlings with fully expanded cotyledons were pricked out into 3.5 l pots six days after emergence. The methods of propagation and seedling management were as described in Sections 2.1.1 and 2.1.2. Plants were grown under natural glasshouse lighting conditions at a mean daily temperature of 20 ± 3 °C until they reached the third true leaf pair stage (≥ 1 cm), when the treatments were imposed.

Three different daily mean temperature treatments were set up (i.e. 26, 20 and 14 ± 3 °C), as explained in Section 2.4.1. Two irradiance levels were applied, high irradiance (HI) and low irradiance (LI), as described in Section 2.4.2. The experiment was designed as a 3 x 2 x 2 factorial in a Randomised Complete Block with three replicates, each containing 16 plants. Destructive and non-destructive growth analyses were carried out regularly to determine the effects of the treatments on reproductive and vegetative growth and development using the procedures described in Section 2.3. Daily observations were made of the number of flowers that reached anthesis or abscised. Flowers that did not drop within 15 d of reaching anthesis were classed as having set fruit (Cochran, 1924). Polynomial regression analysis was used to relate flower and vegetative development to cumulative incident radiation (total radiation) and cumulative temperature (total heat sum or thermal time in degree days; Atherton and Othman, 1983). Thermal time was calculated from the daily temperature records for the experimental period using the cardinal temperatures obtained from the germination trials described in Chapter 4 (T_b = 6.0 °C and T_o = 27.5 °C).

3.1.2 Results

Data for cumulative incident radiation, daily mean irradiance, thermal time, and mean, maximum and minimum temperatures between the emergence of the third true leaf pair stage (start of treatment) and the end of the experiment 50 d later are

summarised in Table 3.1.1. Cumulative incident radiation varied between 235.7 and 269.8 MJ m^{-2} in the high irradiance treatment, and between 114.0 and 122.6 MJ m^{-2} in the low irradiance treatment (45 - 48% of the high irradiance value). The mean daily irradiance varied between 4.7 and 5.4 MJ m^{-2} d^{-1} in the HI treatments and 2.3 - 2.4 MJ m^{-2} d^{-1} in the LI treatments. The target mean daily temperature for each temperature treatment was achieved quite closely in all treatments.

Reproductive development

Results for the effects of temperature and irradiance on flower growth and development in both varieties are summarised in Tables 3.1.2 and 3.1.3.

Time to flower bud emergence Blue Star was the first to show macroscopically visible flower buds when grown at 26 °C under high irradiance. These appeared about nine days after the treatment started i.e. after receiving about 33 MJ m^{-2} of total radiation or 180 °C d (mean daily irradiance, 4.9 MJ m^{-2} d^{-1}; mean daily temperature, 26.3 °C), almost five days earlier than in Bell Boy grown at high irradiance in the 20 °C treatment (9.4 vs. 14.2 d; $p<0.05$) and up to 11 d earlier than in the other treatments (Table 3.1.2). The chronological time to first flower bud appearance increased as temperature decreased from 26 to 20 and then to 14 °C, and from high to low irradiance (5.4 to 2.3 MJ m^{-2} d^{-1}), with var. Bell Boy consistently being slower than Blue Star. These results show that Blue Star responded well to the combination of high temperature (26 °C) and high irradiance in terms of promotion of earlier development of flower buds.

Time to anthesis In the 26 °C treatment Bell Boy and Blue Star reached first anthesis in the primary flowers at about 28 - 30 d under high irradiance conditions (c. 113 - 119 MJ m^{-2} or 566 °C d), 6 - 8 d earlier than when Bell Boy was grown under high irradiance at 20 °C ($p<0.001$; Table 3.1.2). As temperature decreased to 20 and 14 °C and irradiance was reduced (mean 2.4 MJ m^{-2} d^{-1}), the time taken for flowers to reach anthesis increased in both varieties (36 - 49 d; $p<0.001$). Under low irradiance conditions at 26 °C, Bell Boy took longer than Blue Star to reach first anthesis (58

TABLE 3.1.1. *Cumulative radiation, mean daily irradiance, thermal time and temperature conditions within the glasshouse between the third true leaf pair stage (start of treatment) and 50 d after treatment commenced.*

Treatment		Irradiance[c]		Temperature[d] (°C)			Thermal time[g]
TEMP[a] (°C)	IRR[b]	Cumulative (MJ m^{-2})	Daily mean (MJ m^{-2} d^{-1})	Daily mean	Max[e]	Min[f]	(°C d)
26	HI	243.9	4.9	26.3	29.8	23.4	1015.5
	LI	121.8	2.4				
20	HI	269.8	5.4	20.4	23.3	17.6	722.7
	LI	122.6	2.4				
14	HI	235.7	4.7	14.5	17.6	12.0	426.9
	LI	114.0	2.3				

a: Temperature treatment

b: Irradiance

c: Mean of six tube solarimeters

d: Calculated from values recorded at 30 min intervals

e: Mean maximum temperature

f: Mean minimum temperature

g: Calculated using T_b = 6.0 °C obtained in the germination trial

TABLE 3.1.2. *Effects of temperature, irradiance and variety on the time to first flower bud appearance and anthesis in primary and secondary flowers (n=24). SED denotes the Standard Error of the Difference between means.*

Treatment			Time to appearance of first flower 1[a] (d)	Time to first anthesis (d)	
TEMP (°C)	IRR	VAR		flower 1	flower 2[b]
26	HI	BB	$12.2_{(46.3)}$[c]	$29.7_{(119.5)}$	$30.3_{(126.6)}$
		BS	$9.4_{(33.1)}$	$28.3_{(113.4)}$	$29.7_{(119.5)}$
	LI	BB	$14.0_{(28.0)}$	$58.0_{(150.8)}$	$38.0_{(78.7)}$
		BS	$12.9_{(24.0)}$	$49.0_{(115.3)}$	$36.3_{(75.7)}$
20	HI	BB	$14.2_{(62.0)}$	$36.0_{(161.9)}$	$40.0_{(199.2)}$
		BS	$12.1_{(49.5)}$	$38.3_{(170.1)}$	$40.3_{(199.2)}$
	LI	BB	$14.5_{(28.7)}$	$48.0_{(171.5)}$	$50.3_{(120.7)}$
		BS	$14.0_{(28.7)}$	$48.3_{(171.5)}$	$51.0_{(122.6)}$
14	HI	BB	$19.3_{(70.1)}$	$57.0_{(283.7)}$	$54.3_{(259.2)}$
		BS	$15.8_{(60.2)}$	$56.0_{(277.1)}$	$55.7_{(277.1)}$
	LI	BB	$20.3_{(33.7)}$	$59.3_{(141.3)}$	$65.0_{(155.0)}$
		BS	$18.2_{(30.0)}$	$59.7_{(151.1)}$	$65.3_{(155.0)}$
SED(TEM)			0.11^{***}	0.76^{***}	0.80^{***}
SED(IRR)			0.11^{***}	0.62^{***}	0.72^{***}
SED(VAR)			0.11^{***}	0.62^{**}	0.51^{n}
SED(TEM*IRR)			0.17^{***}	1.08^{***}	1.09^{n}
SED(TEM*VAR)			0.17^{***}	1.08^{**}	1.03^{n}
SED(IRR*VAR)			0.16^{***}	0.88^{**}	0.88^{n}
SED(TEM*IRR*VAR)			0.26^{*}	1.53^{***}	1.43^{n}

a: Primary flowers
b: Secondary flowers
c: Values in parenthesis denote cumulative radiation (MJ m^{-2})
*, **, ***: significant at $p<0.05$, 0.01, 0.001
n: not significant

vs. 49 d). However, at 14 °C, both varieties required a similar period to reach anthesis regardless of irradiance level. These results show that, while an interaction between high temperature (26 °C) and high irradiance advanced flowering in the more responsive variety Blue Star, low temperature (14 °C) retarded flower development in both varieties to an extent which could not be compensated for by high irradiance. Observations of flower growth and development in the various temperature treatments showed that at 14 °C the flower buds were larger in diameter and the petals appeared to stick together and took longer to open (7 - 10 d; Plate 3.1.1). When the flowers finally opened, abnormalities were observed in the flower parts (Plate 3.1.2) and the fruits which eventually developed were deformed (Plate 3.1.3).

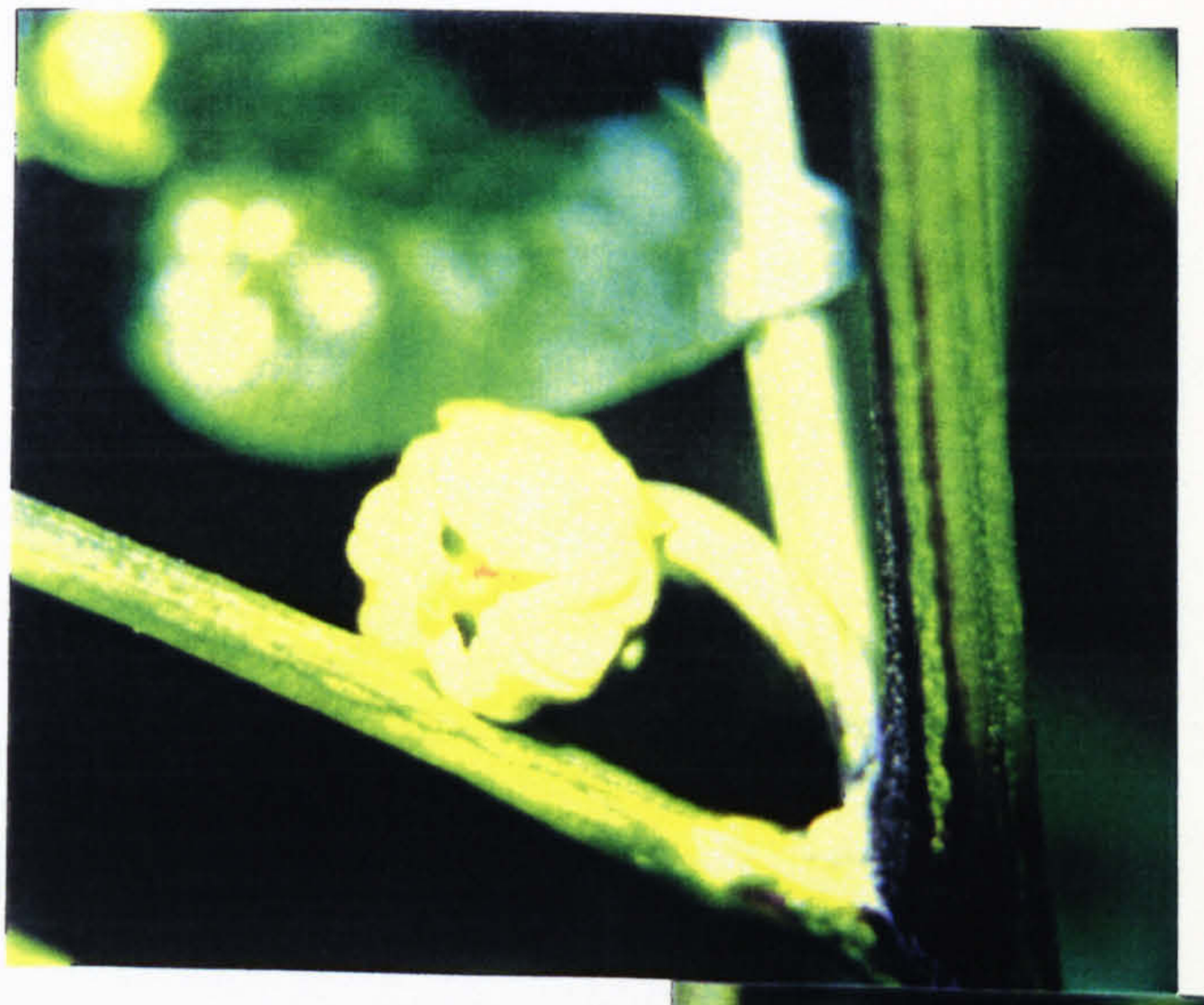
The time required for secondary flowers to reach first anthesis was also shorter under high temperature and high irradiance conditions ($p<0.001$; Table 3.1.2). At 26 °C, anthesis was about 10 - 15 d earlier than at 20 °C and up to 29 d earlier than at 14 °C. High irradiance accelerated anthesis by c. 9 d as compared to low irradiance.

Regression analysis showed a highly significant correlation between the mean total number of primary flowers reaching anthesis ($n=24$) and cumulative radiation or thermal time in some treatments ($p<0.001$). For example, a highly significant quadratic relationship existed between the mean total number of flowers reaching anthesis in Bell Boy and Blue Star and total radiation and thermal time in the 26 and 20 °C treatments at high irradiance (Figure 3.1.1a and b). Under low irradiance, a significant correlation was only achieved at 20 °C.

Flower number The mean total numbers of primary flowers produced ($n=12$) by Bell Boy and Blue Star were similar (11.0 - 14.7 flowers) at 26 °C and 20 °C when plants were grown under high irradiance (Table 3.1.3). However, under low irradiance and at 26 °C, only 1.0 - 3.0 flowers were produced in both varieties ($p<0.05$). Blue Star also produced very few flowers when grown at 14 °C under low irradiance (3.8 flowers). These results suggest that the 26 and 20 °C treatments were equally effective in promoting flower production at high irradiance, while low irradiance greatly reduced flower production in both the 26 and 14 °C treatments.

PLATE 3.1.1. *Stages of flower bud development to anthesis in the 14 °C temperature treatment (a - c). Maximum flower bud diameter was larger than in the 26 °C treatment (d) and the petals appeared to stick together.*

a



b

c

d

PLATE 3.1.2. *Abnormalities in flower structure in the 14 °C temperature treatment (a-c) as compared to the 20 and 26 °C treatments (d).*

a

b

c

d

PLATE 3.1.3. *Deformed developing fruitlets in the 14 °C temperature treatment, as compared with flowers from the other temperature treatments in Blue Star (top) and Bell Boy (bottom).*


MS H JAAFAR
6 9'92

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UK 1992
6 9'92

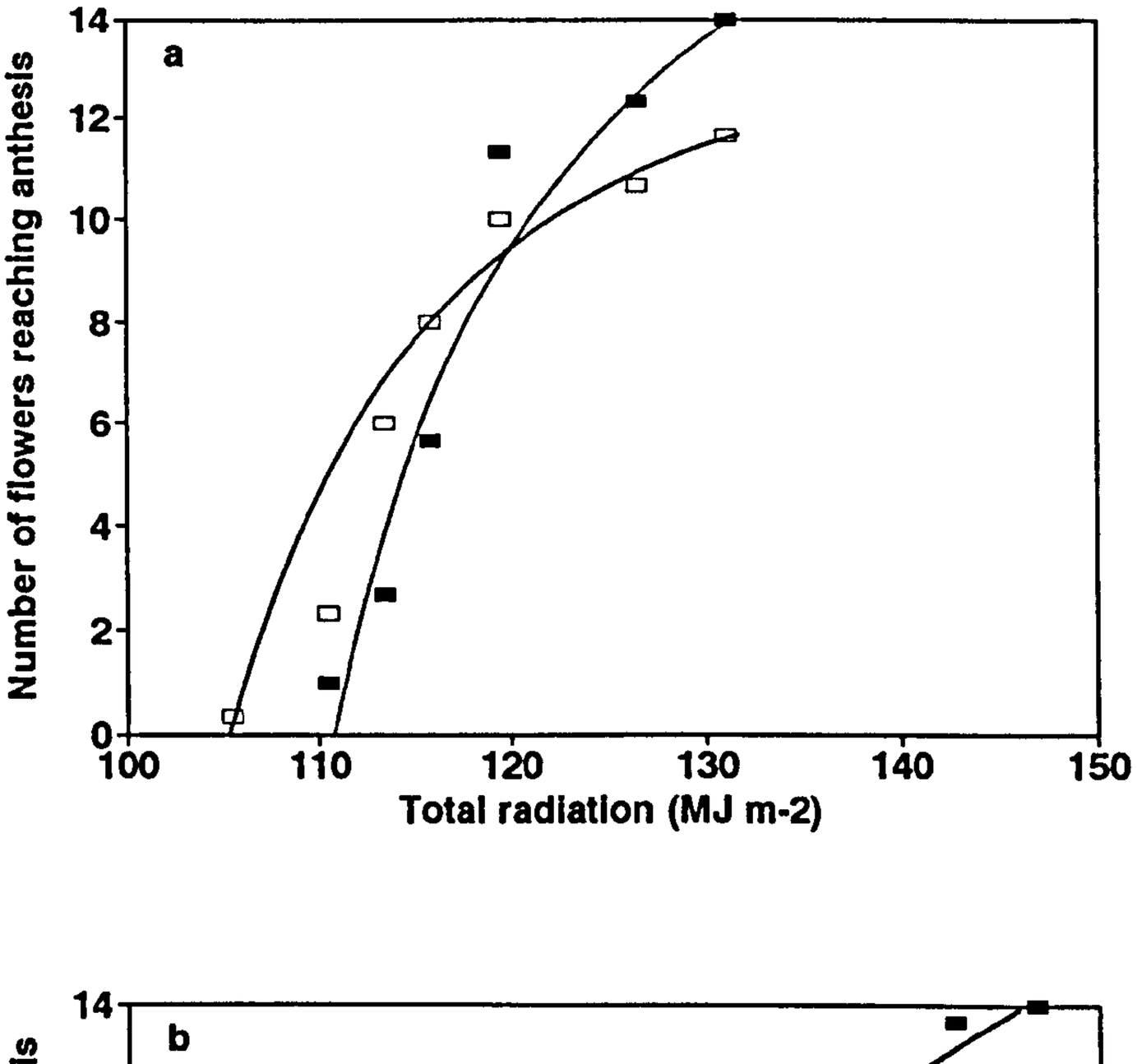
TABLE 3.1.3. *Effects of temperature, irradiance and variety on the growth of primary and secondary flowers (n=12). SED denotes the Standard Error of the Difference between means.*

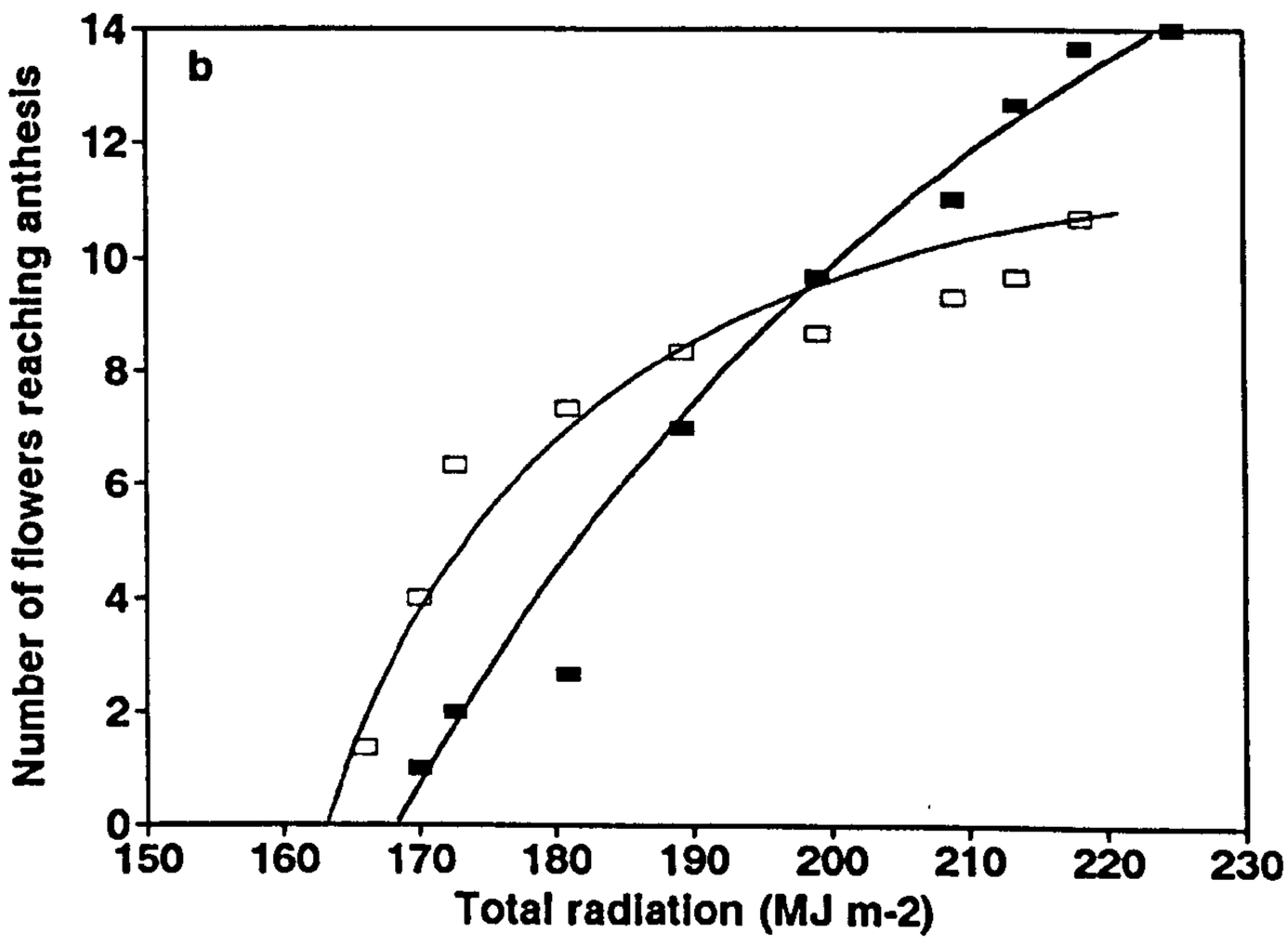
Treatment			Primary flower			Secondary flower	
TEMP (°C)	IRR	VAR	Total number	Number reaching anthesis	abscission (%)	Total number	Number reaching anthesis
26	HI	BB	14.0	13.7	14.2	26.3	26.3
		BS	11.7	11.7	17.2	24.0	24.0
	LI	BB	1.0	1.0	100.0	5.0	4.0
		BS	3.0	3.0	100.0	14.0	14.0
20	HI	BB	14.3	13.7	8.6	28.3	27.0
		BS	14.7	10.7	2.6	27.0	24.7
	LI	BB	14.7	11.7	34.3	22.0	22.0
		BS	11.0	9.7	52.3	22.3	22.3
14	HI	BB	14.3	9.7	38.0	25.3	24.7
		BS	9.0	8.7	7.4	25.3	24.7
	LI	BB	8.0	2.3	16.7	11.5	11.5
		BS	3.8	2.8	8.3	18.0	18.0
SED(TEM)			0.81^{***}	0.64^{***}	4.92^{***}	0.67^{***}	0.90^{***}
SED(IRR)			0.66^{***}	0.51^{***}	4.02^{***}	0.55^{***}	0.73^{***}
SED(VAR)			0.66^{***}	0.51^{n}	4.02^{***}	0.55^{**}	0.73^{**}
SED(TEM*IRR)			1.15^{***}	0.88^{***}	6.96^{***}	0.95^{***}	1.27^{***}
SED(TEM*VAR)			1.15^{*}	0.88^{n}	6.96^{***}	0.95^{*}	1.27^{*}
SED(IRR*VAR)			0.94^{n}	0.72^{*}	5.68^{***}	0.78^{***}	1.04^{***}
SED(TEM*IRR*VAR)			1.62^{*}	1.25^{n}	9.84^{***}	1.34^{**}	1.79^{*}

*, **, ***: significant at $p<0.05$, 0.01, 0.001
n: not significant

FIGURE 3.1.1a. *Relationship between the total number of flowers reaching anthesis and total radiation for Bell Boy (BB: ■) and Blue Star (BS: □) in those temperature and irradiance treatments where highly significant correlation coefficients were established ($p \leq 0.001$). n=24. HI: high irradiance; LI: low irradiance.*

Treatments	**Regression equations**
a: 26 °C + HI	BB: $Y = -540.94 + 8.47X - 0.03X^2$
	$R^2 = 0.96$
	BS: $Y = -310.74 + 4.94X - 0.02X^2$
	$R^2 = 0.96$
b: 20 °C + HI	BB: $Y = -102.41 + 0.87X + 0.00X^2$
	$R^2 = 0.98$
	BS: $Y = -150.45 + 1.52X - 0.00X^2$
	$R^2 = 0.91$
c: 20 °C + LI	BB: $Y = -368.77 + 6.18X - 0.02X^2$
	$R^2 = 1.00$
	BS: $Y = 685.66 - 12.61X - 0.06X^2$
	$R^2 = 1.00$


a
Number of flowers reaching anthesis
0
2
4
6
8
10
12
14
100
110
120
130
140
150
Total radiation (MJ m-2)


b
Number of flowers reaching anthesis
0
2
4
6
8
10
12
14
150
160
170
180
190
200
210
220
230
Total radiation (MJ m-2)

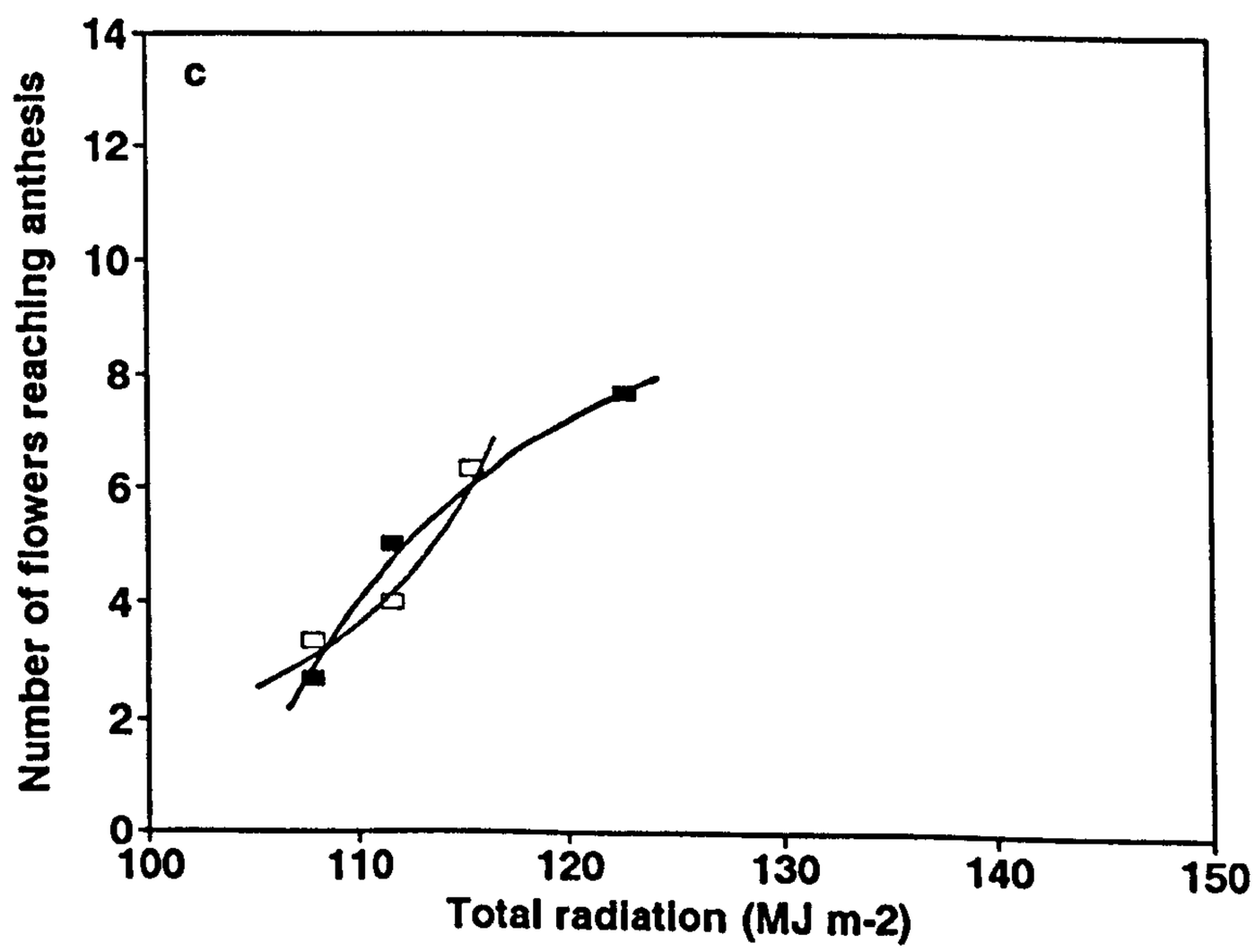
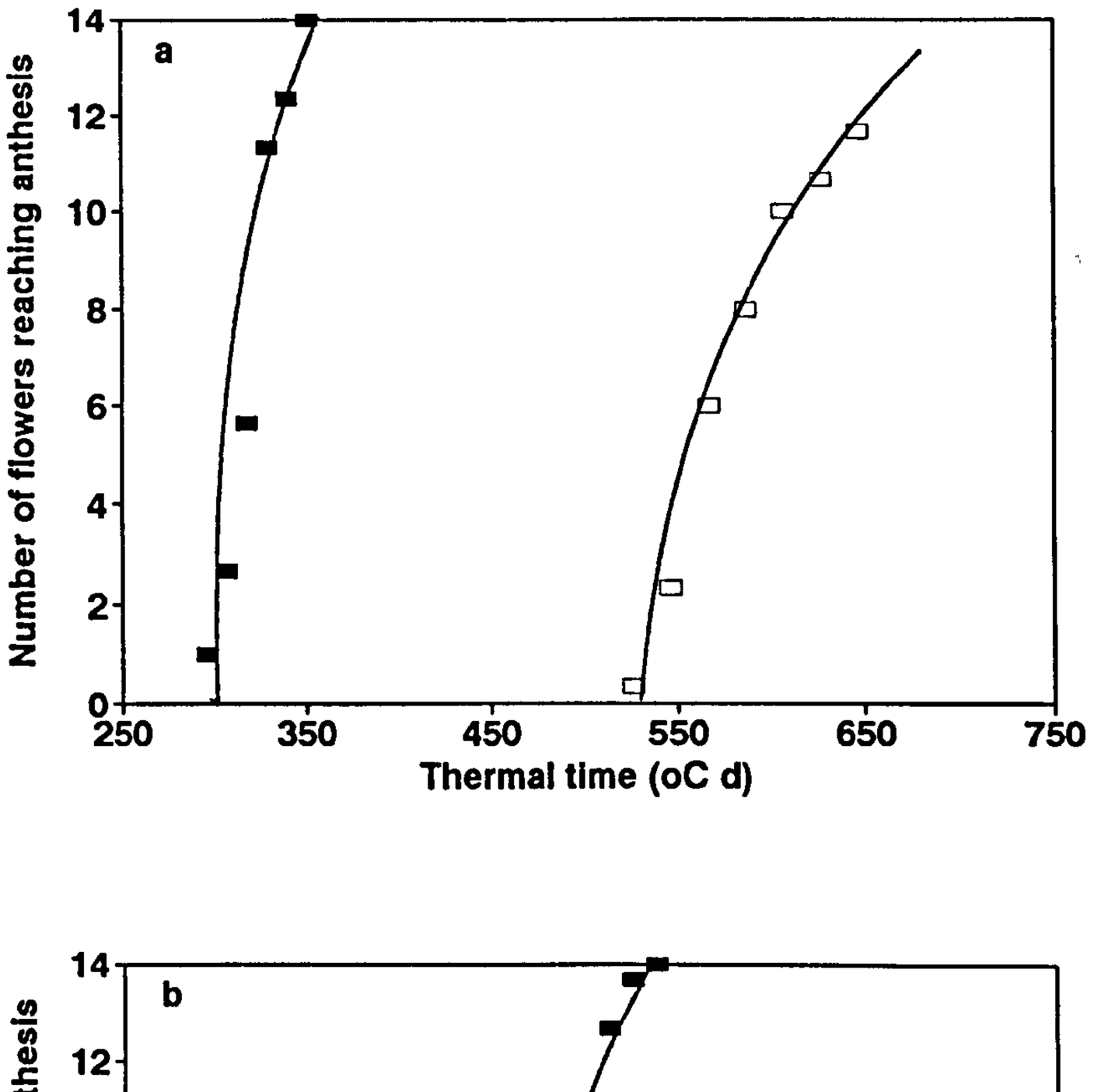
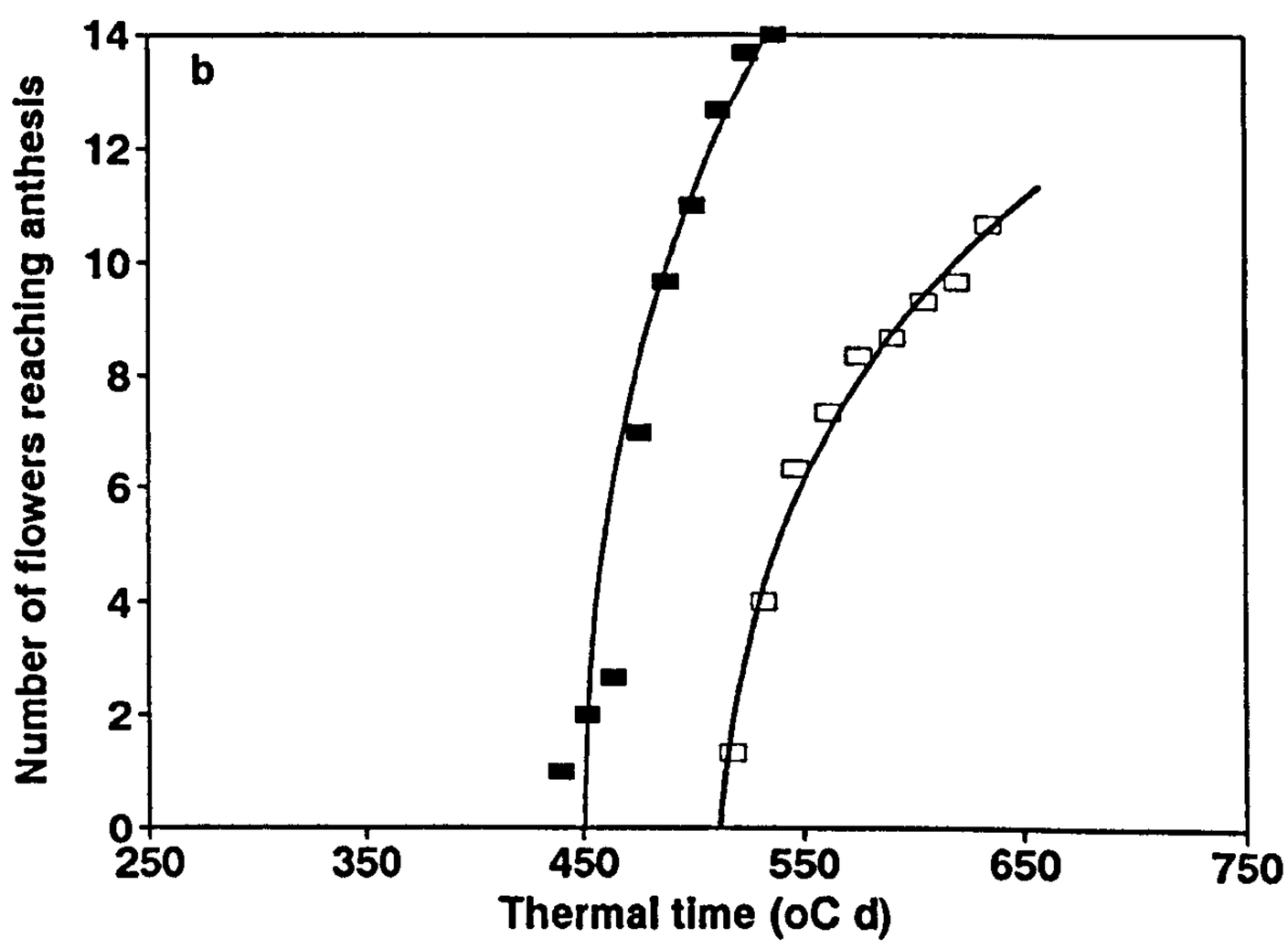
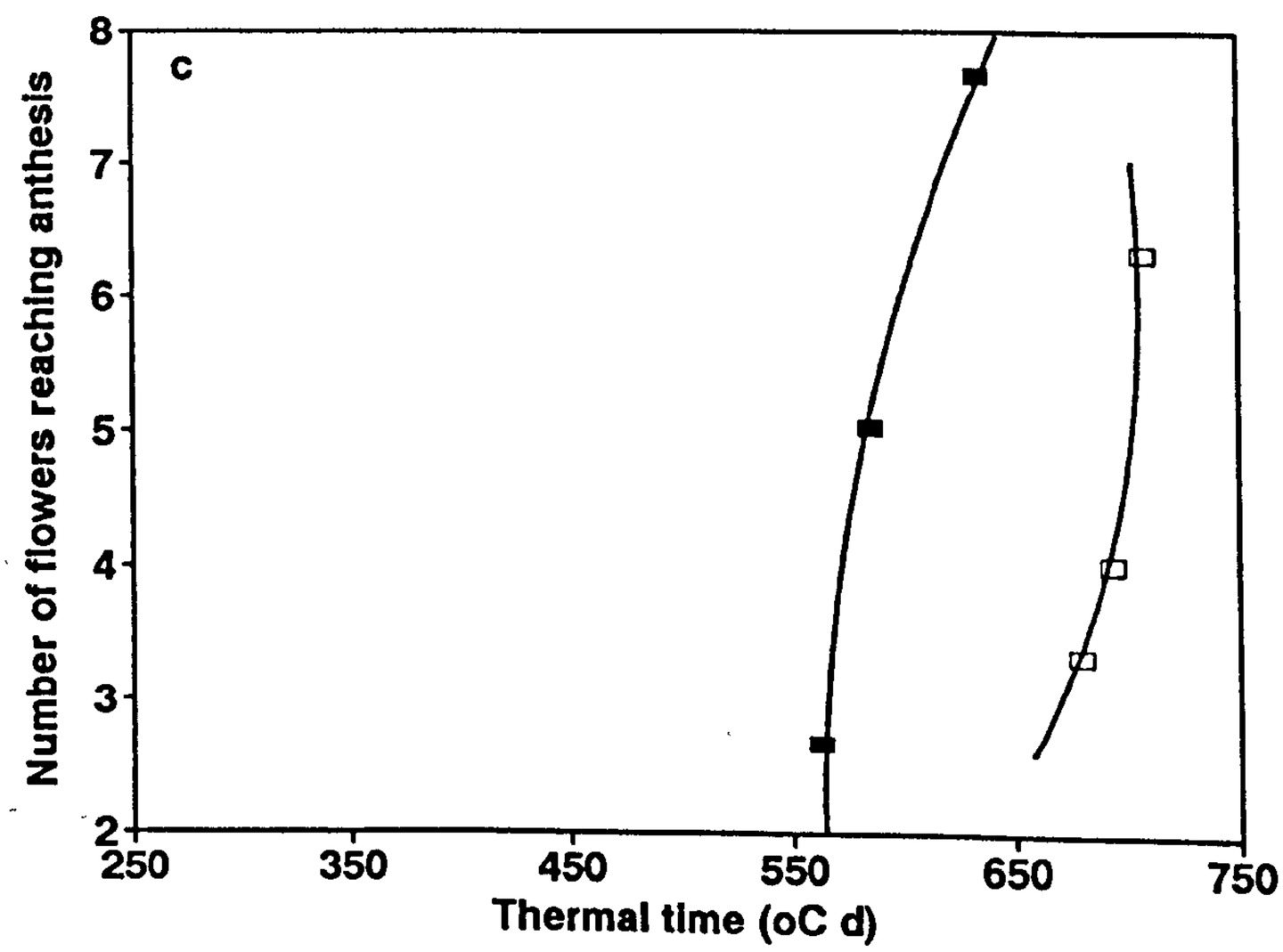

c
Number of flowers reaching anthesis
0
2
4
6
8
10
12
14
100
110
120
130
140
150
Total radiation (MJ m-2)

FIGURE 3.1.1b. *Relationship between the total number of flowers reaching anthesis and thermal time (°C d; total accumulated temperature) for Bell Boy (BB: ■) and Blue Star (BS: □) in those temperature and irradiance treatments where highly significant correlation coefficients were established ($p \leq 0.001$). n=24. HI: high irradiance; LI: low irradiance.*

Treatments	**Regression equations**
a: 26 °C + HI	BB: $Y = -188.24 + 0.96X - 0.00X^2$
	$R^2 = 0.96$
	BS: $Y = -252.79 + 0.79X - 0.00X^2$
	$R^2 = 0.99$
b: 20 °C + HI	BB: $Y = -242.89 + 0.88X - 0.00X^2$
	$R^2 = 0.97$
	BS: $Y = -260.22 + 0.86X - 0.00X^2$
	$R^2 = 0.97$
c: 20 °C + LI	BB: $Y = -256.50 + 0.81X - 0.00X^2$
	$R^2 = 1.00$
	BS: $Y = -1870.50 + 5.49X - 0.00X^2$
	$R^2 = 1.00$


a
Number of flowers reaching anthesis
0
2
4
6
8
10
12
14
250
350
450
550
650
750
Thermal time (oC d)


b
Number of flowers reaching anthesis
0
2
4
6
8
10
12
14
250
350
450
550
650
750
Thermal time (oC d)


c
Number of flowers reaching anthesis
2
3
4
5
6
7
8
250
350
450
550
650
750
Thermal time (oC d)

Flower number appeared to be more affected by low temperature and low irradiance in Blue Star. In contrast, Bell Boy consistently produced more flowers in all treatments (c. 14), although a decrease in flower number was also observed under low irradiance at 14 °C (Table 3.1.3).

The total number of primary flowers reaching anthesis was apparently affected both by interactions between temperature and irradiance and interactions between irradiance and variety. The total number of flowers reaching anthesis was greatly reduced under low irradiance at 26 and 14 °C ($p \leq 0.001$), primarily because of the low number of flowers produced initially. Under low irradiance, the total number of flowers reaching anthesis was reduced significantly in both varieties ($p \leq 0.05$).

As for the primary flowers, the total number of secondary flowers produced was also markedly reduced by low irradiance at 26 °C in both varieties ($p \leq 0.01$; Table 3.1.3). Blue Star also produced significantly more secondary flowers than Bell Boy under low irradiance at 14 °C, possibly because its thermal requirements for development were lower, or because it made more effective use of the available radiation to produce assimilates to support flower growth. The numbers of secondary flowers in both varieties were greater under high irradiance than under low irradiance conditions in all temperature treatments except 20 °C ($p \leq 0.01$). Similar results were obtained for the total numbers of secondary flowers reaching anthesis as for the production of secondary flowers.

Flower abscission Flower counts made 15 d after first anthesis showed that abscission of primary flowers occurred in all treatments. Complete flower abscission was observed in both varieties at 26 °C under low irradiance conditions ($p < 0.001$; Table 3.1.3; Plate 3.1.4), leading to total failure of fruit set. Better fruit set was obtained at 26 °C under high irradiance conditions where only 14 - 17% flower abscission was observed. Greater abscission percentages were recorded for plants growing under low irradiance at 20 °C (34 - 52%) or at 14 °C (17 - 38%). In the 14 °C treatment, abscission was lower ($p \leq 0.001$) in Blue Star than in any other treatment irrespective of irradiance level, with the exception of Bell Boy and Blue

PLATE 3.1.4. *Absence of primary and secondary flowers in the high temperature (26 °C), low irradiance treatment resulting from complete flower abscission.*

Star grown under high irradiance at 20 °C. Abscission of secondary flowers was not observed prior to 50 d after start of experiment, when the plants were harvested for final destructive analysis.

Vegetative growth and development

Effects on vegetative growth and development were measured in terms of leaf number (leaves ≥ 1 cm), leaf area, plant height and dry matter content at the time of first anthesis in the primary flowers of Bell Boy and Blue Star in the various temperature and irradiance treatments examined. The data obtained are summarised in Table 3.1.4.

Leaf number Primary leaf (leaves developing on the main stem) number was affected by an interaction between irradiance and variety. At first anthesis, plants of Bell Boy grown under low irradiance had significantly more primary leaves than Blue Star plants grown under either low or high irradiance conditions ($p \leq 0.05$; c. 12 vs. 10 leaves plant^{-1}; Table 3.1.4). The greater leaf numbers under low irradiance in Bell Boy may have been attributable to the delaying effect of low irradiance on flower initiation, which would have allowed more leaves to be initiated (Cockshull, 1979).

In contrast, the total number of leaves, including leaves on the branches (secondary leaves), was significantly influenced by the interaction between temperature and irradiance ($p \leq 0.001$). The maximum number of leaves was recorded at 26 °C under high irradiance conditions (32.7 - 39.3 leaves plant^{-1}), whilst only 12.7 - 16.7 leaves plant^{-1} were produced at 14 °C or 18.0 leaves plant $^{-1}$ under low irradiance at 20 °C ($p < 0.001$; Table 3.1.4). Regression analysis showed a highly significant fit ($p < 0.01$) for all curves relating total leaf number to total accumulated radiation and thermal time in both varieties (Figure 3.1.2). Total leaf number exhibited significant correlations with both total radiation and thermal time in all temperature regimes examined under both levels of irradiance.

Leaf area The combination of high temperature (26 °C) and high irradiance

TABLE 3.1.4. *Effects of temperature, irradiance and variety on vegetative growth and total shoot dry weight per plant at first anthesis (n=12). SED denotes the Standard Error of the Difference between means.*

Treatment			Leaf number		Leaf area (cm^{-2})		Plant height	Shoot dry
TEMP (°C)	IRR	VAR	Leaf 1[a]	Total[b]	Area 1	Total	(cm)	weight (g)
26	HI	BB	11.3	39.3	1092	1740	29.5	17.9
		BS	10.3	32.7	1201	1656	29.7	17.9
	LI	BB	12.3	24.3	978	1367	37.6	13.8
		BS	9.7	25.3	854	1261	39.1	15.2
20	HI	BB	11.0	23.7	957	1205	22.5	14.7
		BS	10.7	27.7	900	1251	22.7	15.2
	LI	BB	12.3	18.0	988	1099	31.9	12.4
		BS	11.0	18.0	963	1019	33.2	11.6
14	HI	BB	11.3	14.3	460	466	12.1	11.8
		BS	10.7	12.7	417	421	13.0	12.1
	LI	BB	11.7	14.7	458	473	15.1	10.4
		BS	10.0	16.7	486	558	16.3	10.5
SED(TEM)			0.29^{n}	1.52^{***}	49.1^{***}	48.0^{***}	0.75^{***}	0.28^{***}
SED(IRR)			0.24^{n}	1.24^{***}	40.1^{n}	39.2^{***}	0.61^{***}	0.23^{***}
SED(VAR)			0.24^{***}	1.24^{n}	40.1^{n}	39.2^{n}	0.61^{n}	0.23^{n}
SED(TEM*IRR)			0.41^{n}	2.15^{***}	69.4^{*}	67.9^{***}	1.06^{***}	0.40^{**}
SED(TEM*VAR)			0.41^{n}	2.15^{n}	69.4^{n}	67.9^{n}	1.06^{n}	0.40^{n}
SED(IRR*VAR)			0.33^{*}	1.75^{n}	56.7^{n}	55.5^{n}	0.86^{n}	0.32^{n}
SED(TEM*IRR*VAR)			0.58^{n}	3.04^{n}	98.1^{n}	96.1^{n}	1.50^{n}	0.56^{n}

a: Primary leaves growing from the main stem
b: Total includes secondary leaves
*, **, ***: significant at $p<0.05$, 0.01, 0.001; n: not significant

FIGURE 3.1.2a: *Relationship between leaf number per plant and total radiation for Bell Boy (BB) and Blue Star (BS) at high (HI: BB: ■; BS: □) and low (LI: BB: ▲; BS: X) irradiance in the various temperature treatments. n=24.*

Treatments	**Regression equations**
a: 26 °C + HI	BB: $Y = 5.76 - 0.26X + 0.01X^2$
	$R^2 = 0.98$
	BS: $Y = 5.54 - 0.25X + 0.01X^2$
	$R^2 = 0.98$
26 °C + LI	BB: $Y = 4.43 - 0.18X + 0.01X^2$
	$R^2 = 0.98$
	BS: $Y = 4.92 - 0.38X + 0.02X^2$
	$R^2 = 0.97$
b: 20 °C + HI	BB: $Y = 3.11 + 0.06X + 0.00X^2$
	$R^2 = 0.99$
	BS: $Y = 3.12 + 0.05X + 0.00X^2$
	$R^2 = 1.00$
20 °C + LI	BB: $Y = 3.12 + 0.07X + 0.00X^2$
	$R^2 = 0.99$
	BS: $Y = 3.01 + 0.08X + 0.00X^2$
	$R^2 = 1.00$
c: 14 °C + HI	BB: $Y = 2.75 + 0.08X - 0.00X^2$
	$R^2 = 0.98$
	BS: $Y = 2.81 + 0.07X - 0.00X^2$
	$R^2 = 0.97$
14 °C + LI	BB: $Y = 2.89 + 0.13X - 0.00X^2$
	$R^2 = 0.99$
	BS: $Y = 2.99 + 0.09X - 0.00X^2$
	$R^2 = 0.97$

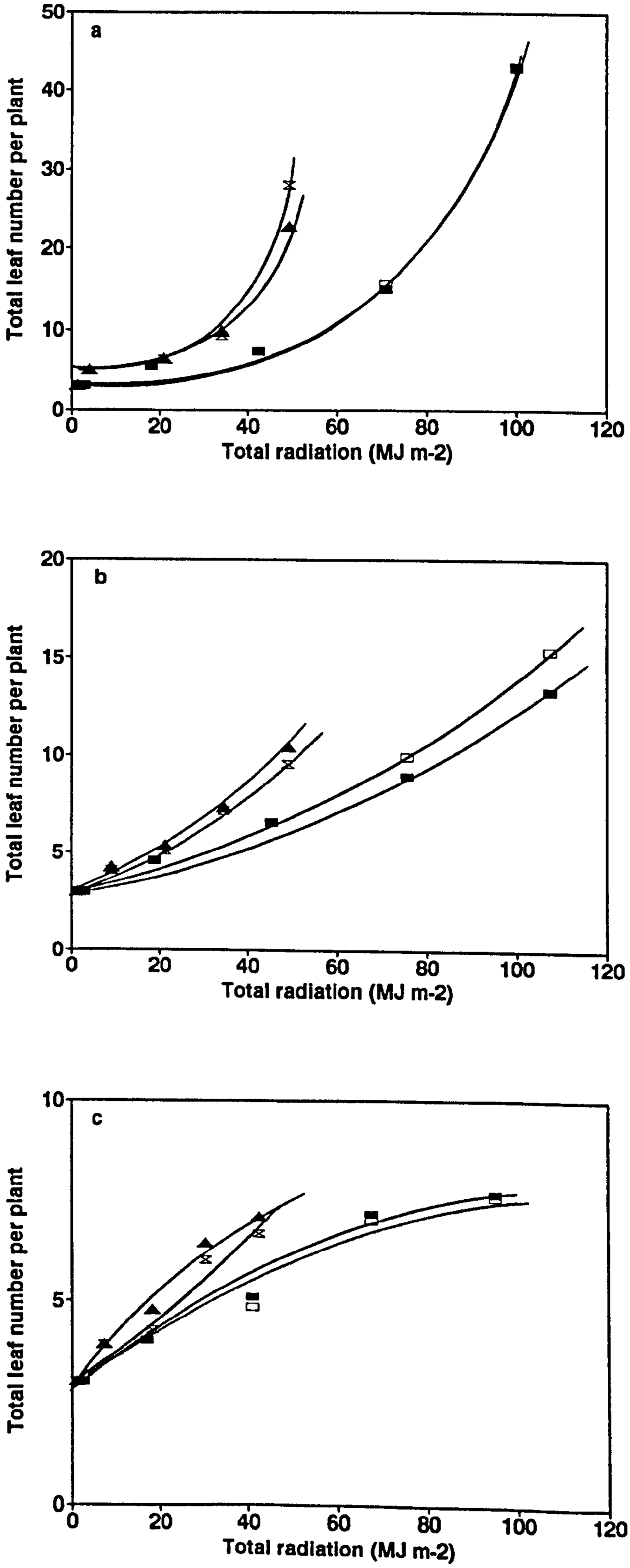
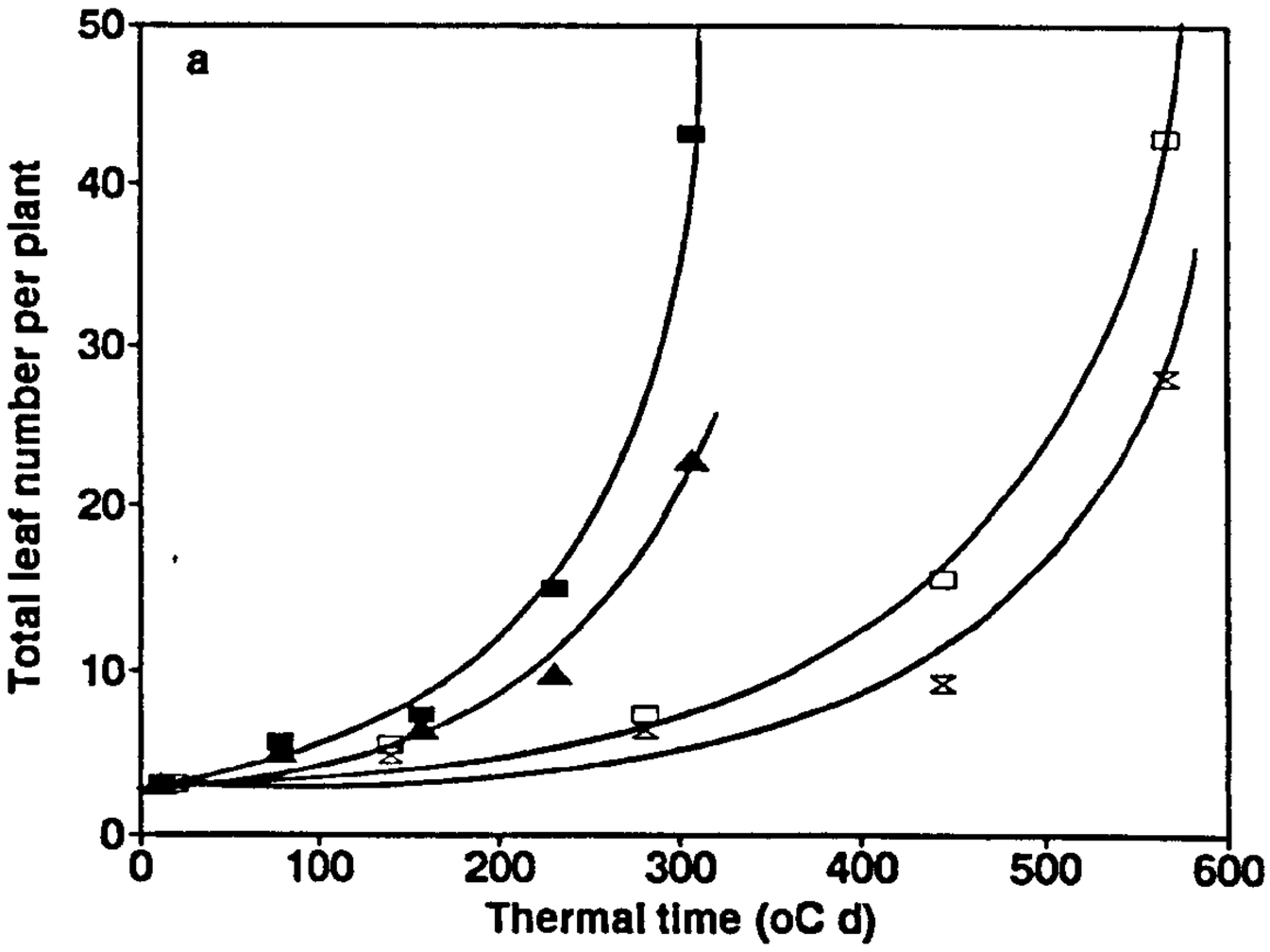
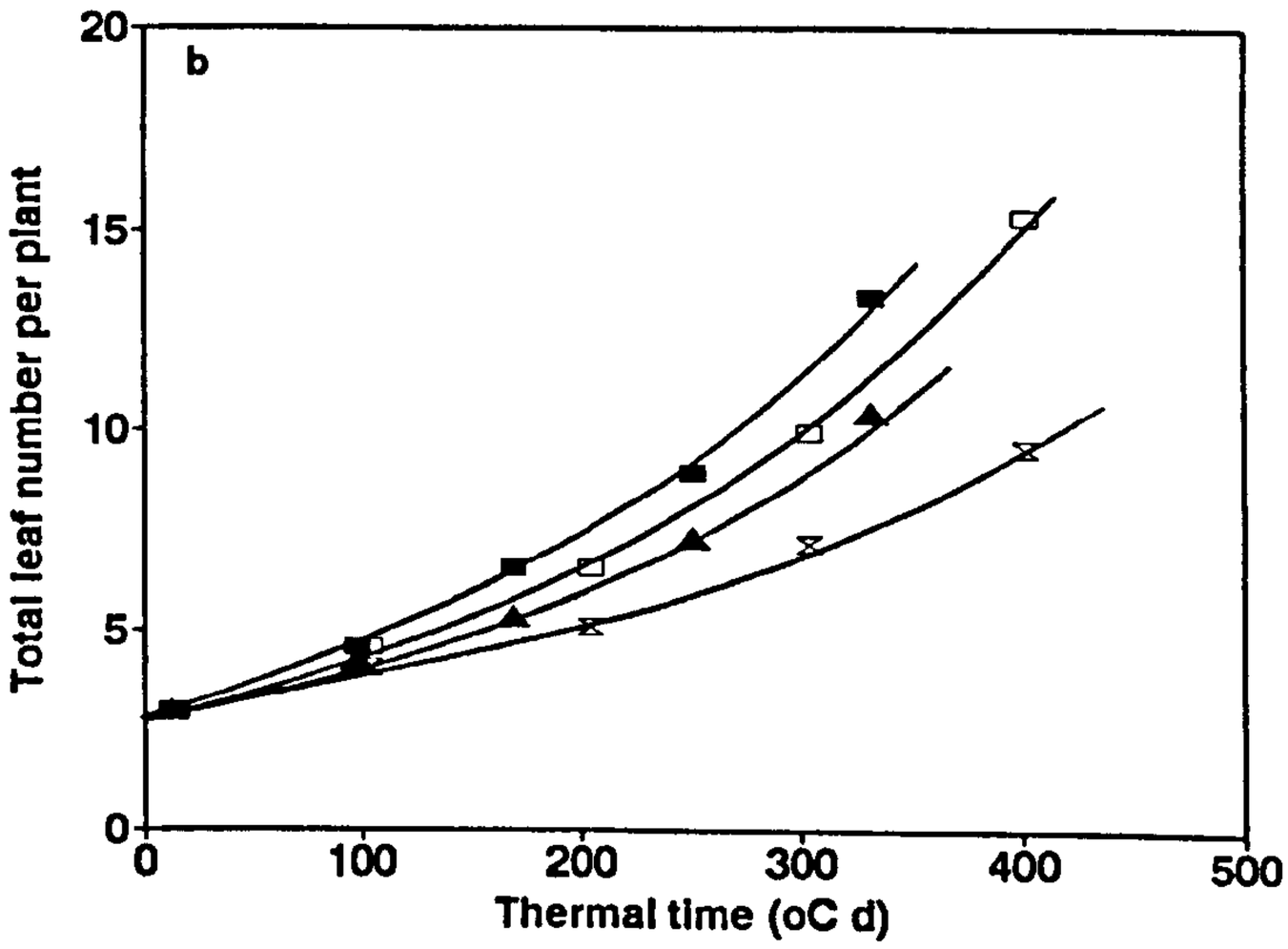
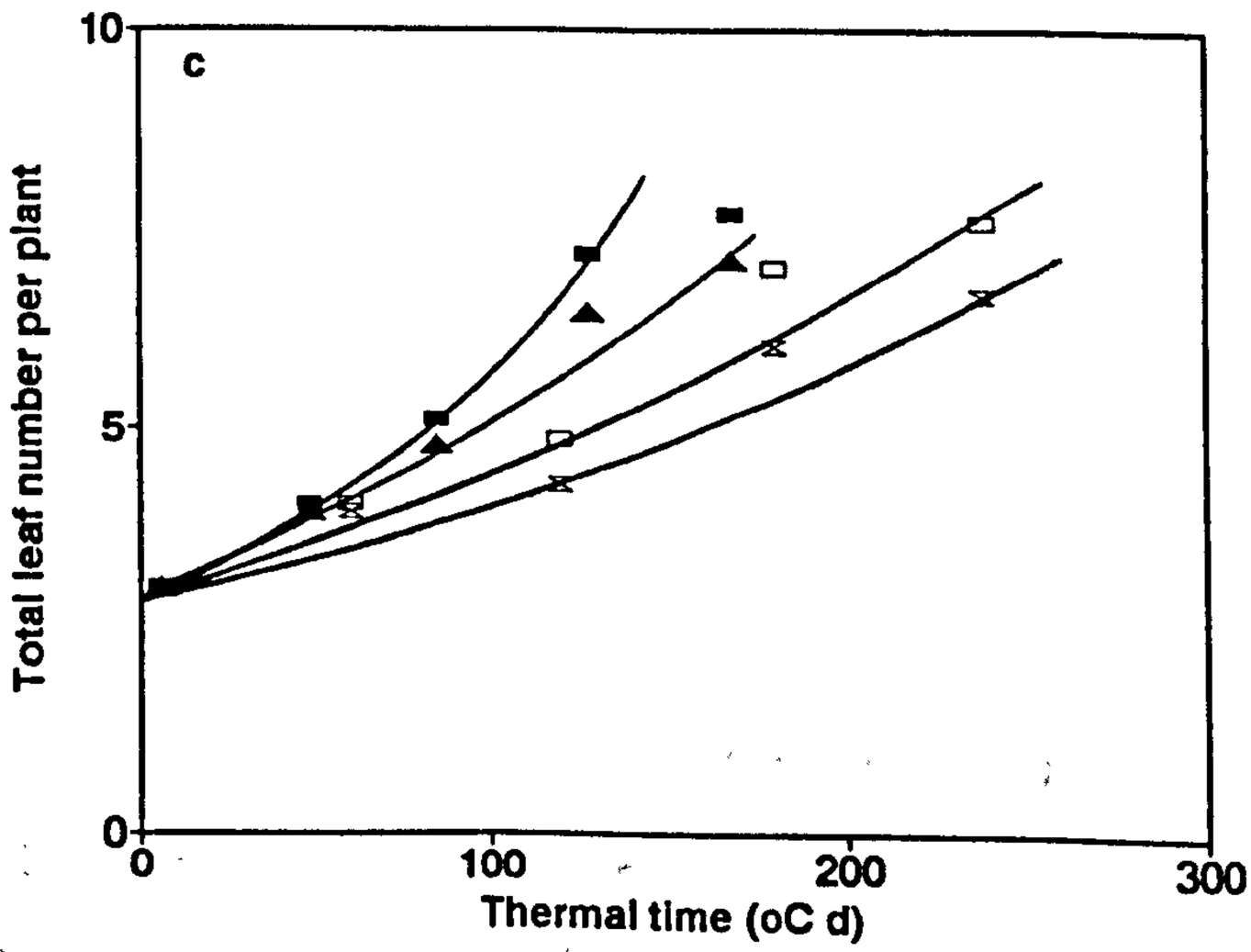

a
b
c
Total leaf number per plant
Total radiation (MJ m-2)
0
20
40
60
80
100
120
5
10
15
50

FIGURE 3.1.2b: *Relationship between leaf number per plant and thermal time for Bell Boy (BB) and Blue Star (BS) at high (HI: BB: ■; BS: □) and low (LI: BB: ▲; BS: X) irradiance in the various temperature treatments. n=24.*

Treatments	**Regression equations**
a: 26 °C + HI	BB: $Y = 6.35 - 0.12X + 0.00X^2$
	$R^2 = 0.96$
	BS: $Y = 6.40 - 0.06X + 0.00X^2$
	$R^2 = 0.95$
26 °C + LI	BB: $Y = 4.42 - 0.04X + 0.01X^2$
	$R^2 = 0.96$
	BS: $Y = 5.39 - 0.04X + 0.00X^2$
	$R^2 = 0.91$
b: 20 °C + HI	BB: $Y = 2.99 + 0.01X + 0.00X^2$
	$R^2 = 1.00$
	BS: $Y = 3.15 + 0.00X + 0.00X^2$
	$R^2 = 1.00$
20 °C + LI	BB: $Y = 3.04 + 0.01X + 0.00X^2$
	$R^2 = 1.00$
	BS: $Y = 3.01 + 0.01X + 0.00X^2$
	$R^2 = 1.00$
c: 14 °C + HI	BB: $Y = 2.68 + 0.03X - 0.00X^2$
	$R^2 = 0.97$
	BS: $Y = 2.77 + 0.02X - 0.00X^2$
	$R^2 = 0.97$
14 °C + LI	BB: $Y = 2.79 + 0.03X - 0.00X^2$
	$R^2 = 1.00$
	BS: $Y = 2.93 + 0.01X - 0.00X^2$
	$R^2 = 0.97$


a
Total leaf number per plant
0
10
20
30
40
50
0
100
200
300
400
500
600
Thermal time (oC d)


b
Total leaf number per plant
0
5
10
15
20
0
100
200
300
400
500
Thermal time (oC d)


c
Total leaf number per plant
0
5
10
0
100
200
300
Thermal time (oC d)

increased the area of primary leaves, while low temperature (14 °C) retarded leaf expansion to produce the smallest leaf areas regardless of irradiance ($p<0.05$; Table 3.1.4). The leaf area produced at 26 °C under low irradiance was similar to that produced at 20 °C under both high and low irradiance. As for total leaf number, total leaf area measured at first anthesis was also markedly increased ($p\leq0.001$) by high irradiance at 26 °C, and declined as temperature and irradiance decreased, except at 14 °C where the marked reductions in total leaf area (c. 3 - 4 fold) were unaffected by low irradiance (Table 3.1.4). This may imply that irradiance was not limiting for leaf area expansion at 14 °C. It also appears that an increase in the area of individual leaves partially compensated for the reduced leaf number produced under low irradiance conditions at 26 and 20 °C.

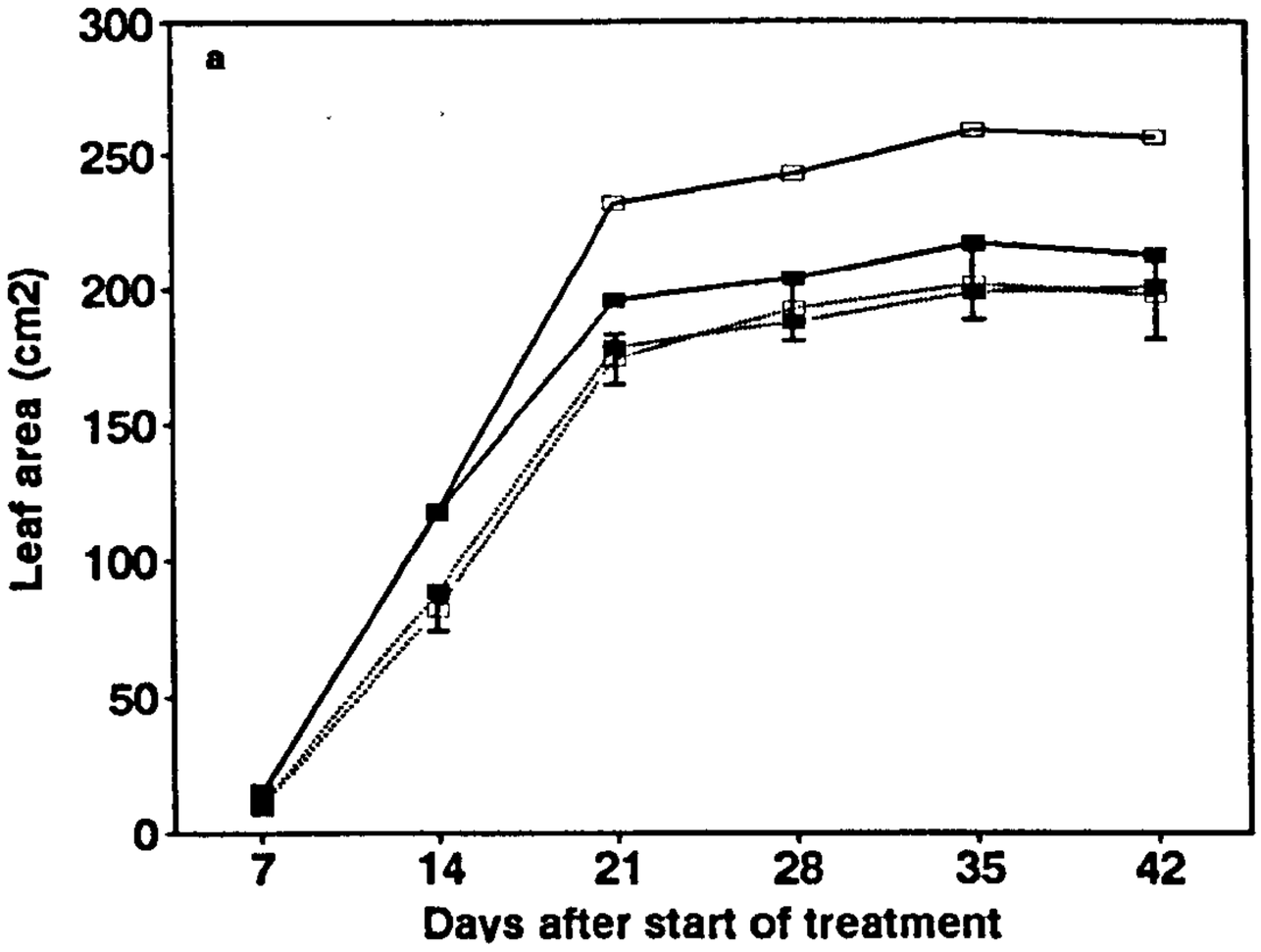
Leaf area was significantly greater at high temperature (26 °C) on day 7 than at low temperature (14 °C; $p\leq0.01$; Figure 3.1.3). By day 14, leaf area was significantly increased by the combination of high temperature and high irradiance ($p<0.01$), and by 21 d leaf area was greater in plants of Blue Star grown at 26 °C under high irradiance than in the other treatments examined ($p\leq0.01$).

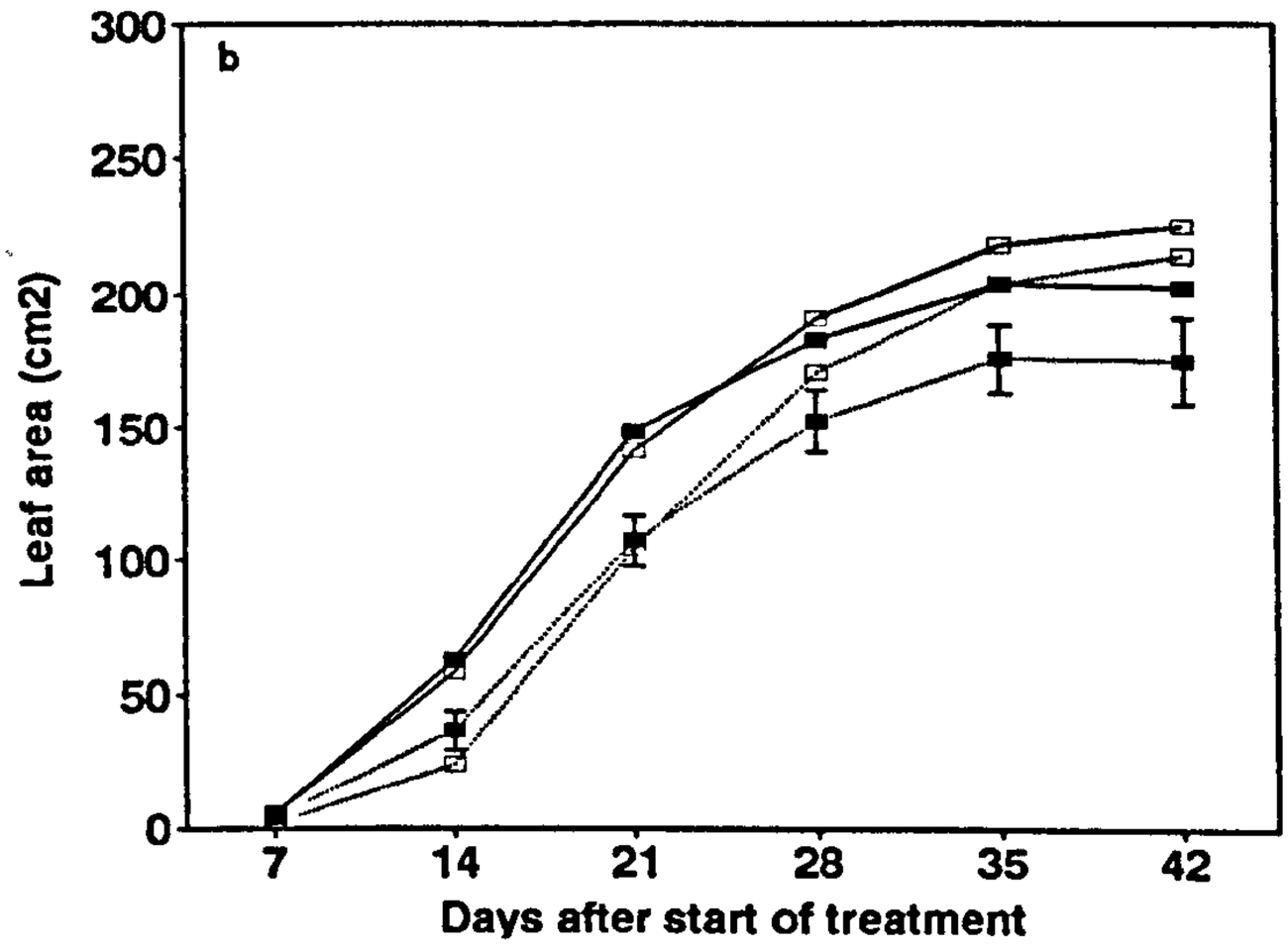
Plant height Plant height was greatly increased by low irradiance at 26 °C, and decreased as temperature declined to 20 and then to 14 °C ($p\leq0.001$; Table 3.1.4; Plate 3.1.5). At 14 °C, plant height was greatly reduced regardless of the irradiance, reaching only 12 - 16 cm as compared to 22 - 39 cm at the higher temperatures.

Shoot dry weight Dry weight at first anthesis was increased by the combination of high temperature and irradiance (Table 3.1.4). Total shoot dry weight was greatest (c. 18 g plant^{-1}; $p\leq0.01$) at 26 °C under high irradiance but decreased with decreasing temperature and at low irradiance. Dry weight was lowest at 14 °C under low irradiance.

Reproductive and vegetative growth depend not only on total dry matter production but also its distribution to individual plant parts. Dry matter distribution during early plant growth was not studied in the present experiment but was examined in the

FIGURE 3.1.3. *Effects of temperature and irradiance on the final area of the third pair of true leaves in Bell Boy (■) and Blue Star (□) under high (_) and low (...) irradiance at 26 (a), 20 (b) and 14 °C (c). n=24. Bars represent the Standard Error af the Difference between means.*


a
300
250
200
150
100
50
0
Leaf area (cm2)
7
14
21
28
35
42
Days after start of treatment


b
300
250
200
150
100
50
0
Leaf area (cm2)
7
14
21
28
35
42
Days after start of treatment

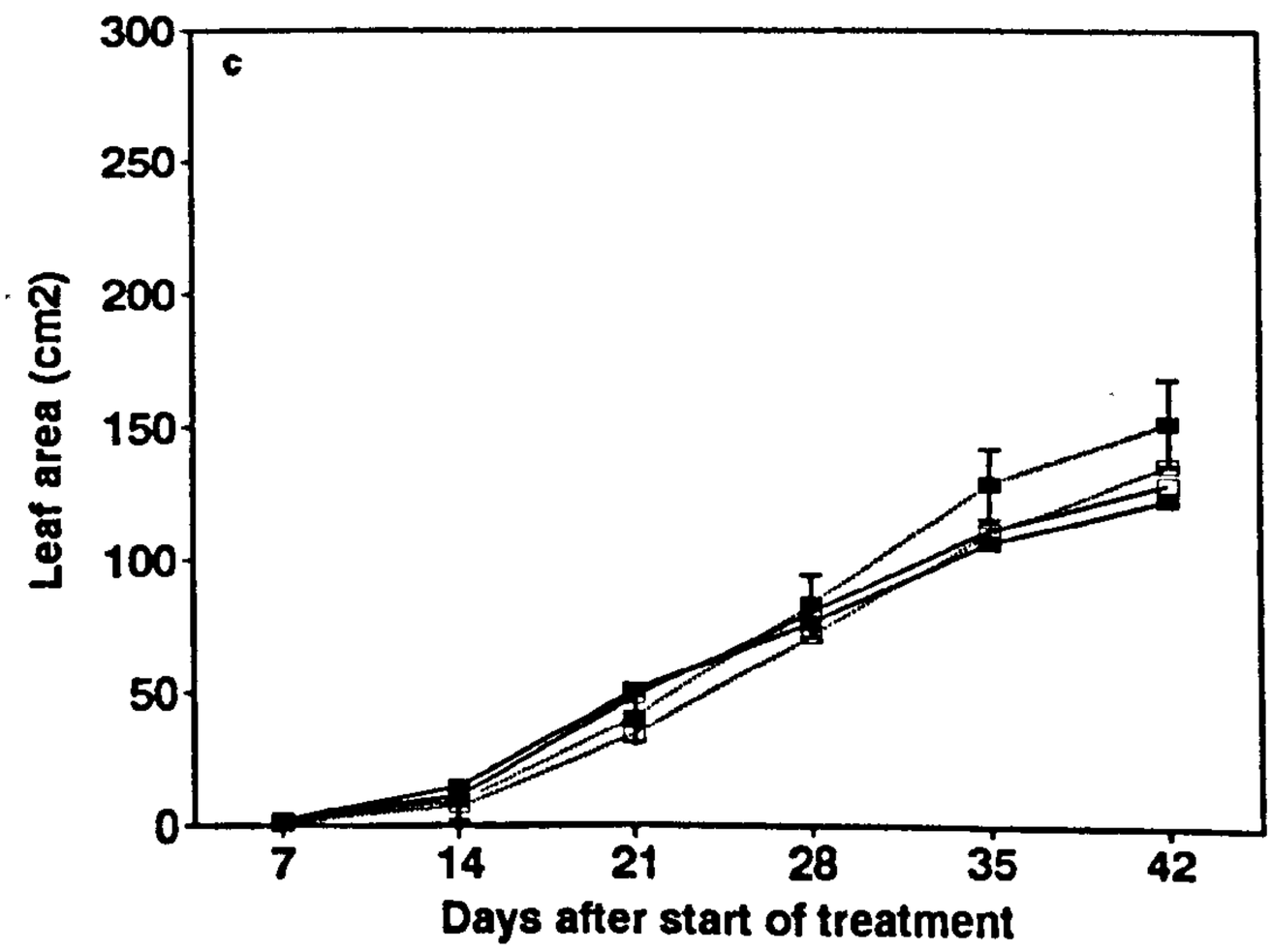

c
300
250
200
150
100
50
0
Leaf area (cm2)
7
14
21
28
35
42
Days after start of treatment

PLATE 3.1.5. *Promotion of shoot extension by low irradiance at high temperature (26 °C) at 21 DAT (a) and 49 DAT (b). At 28 DAT (c), plant height under high irradiance varied between temperature treatments: 14 ° (left), 20° (middle) and 26°C (right).*

b

C

experiments described in Chapter 5.

Fruit formation At the end of the experiment, the effects of temperature and irradiance on fruit formation were examined. Fruit harvested from plants grown at 14 °C was all abnormal regardless of irradiance conditions, as compared to fruit from the 26 or 20 °C treatments (Plate 3.1.6). The nature of the fruit abnormalities is shown in Plate 3.1.7. The effects of low night temperature on fruit formation have been discussed previously by Rylski (1973; 1986).

3.2 Discussion

The main aim of this preliminary study was to examine the influence of a range of temperature and irradiance treatments on the growth and development of two sweet pepper varieties. Varietal differences in response to temperature and irradiance were demonstrated between first flower bud emergence and anthesis. The combination of high temperature and high irradiance promoted earlier emergence of the primary flower buds in Blue Star than in Bell Boy. Conversely, lower temperatures delayed bud emergence more in Bell Boy, especially under low irradiance conditions.

These results are consistent with previous reports of the beneficial interactive effects of high temperature and irradiance in accelerating flower development in sweet pepper (Cochran, 1936; Deli and Tiessen, 1969; Rylski, 1972) and tomato (Calvert, 1959; Hussey, 1963). In a growth room study, Deli and Tiessen (1969) showed that a relatively high night temperature (18 °C) combined with an illuminance of 1600 ft-c promoted earlier flower initiation in sweet pepper cv. California Wonder, 62 d after imposing the treatment. Reduction of illuminance to 800 ft-c and night temperature to 12 °C delayed flowering. In a growth room study of tomato, Calvert (1959) showed that the initiation of the first flower was accelerated by a combination of high temperature (25 °C) and high illuminance (10000 lux) in cv. Ailsa Craig. As the illuminance was decreased to 2500 lux, flower initiation was delayed by up to 29 d. These results suggest that the effect of high temperature in hastening the initiation of

PLATE 3.1.6. *Mature normal fruits (a) from Bell Boy (left) and Blue Star (right) and (b) ripe fruits from Blue Star at 80 DAT, produced in the higher temperature treatments.*

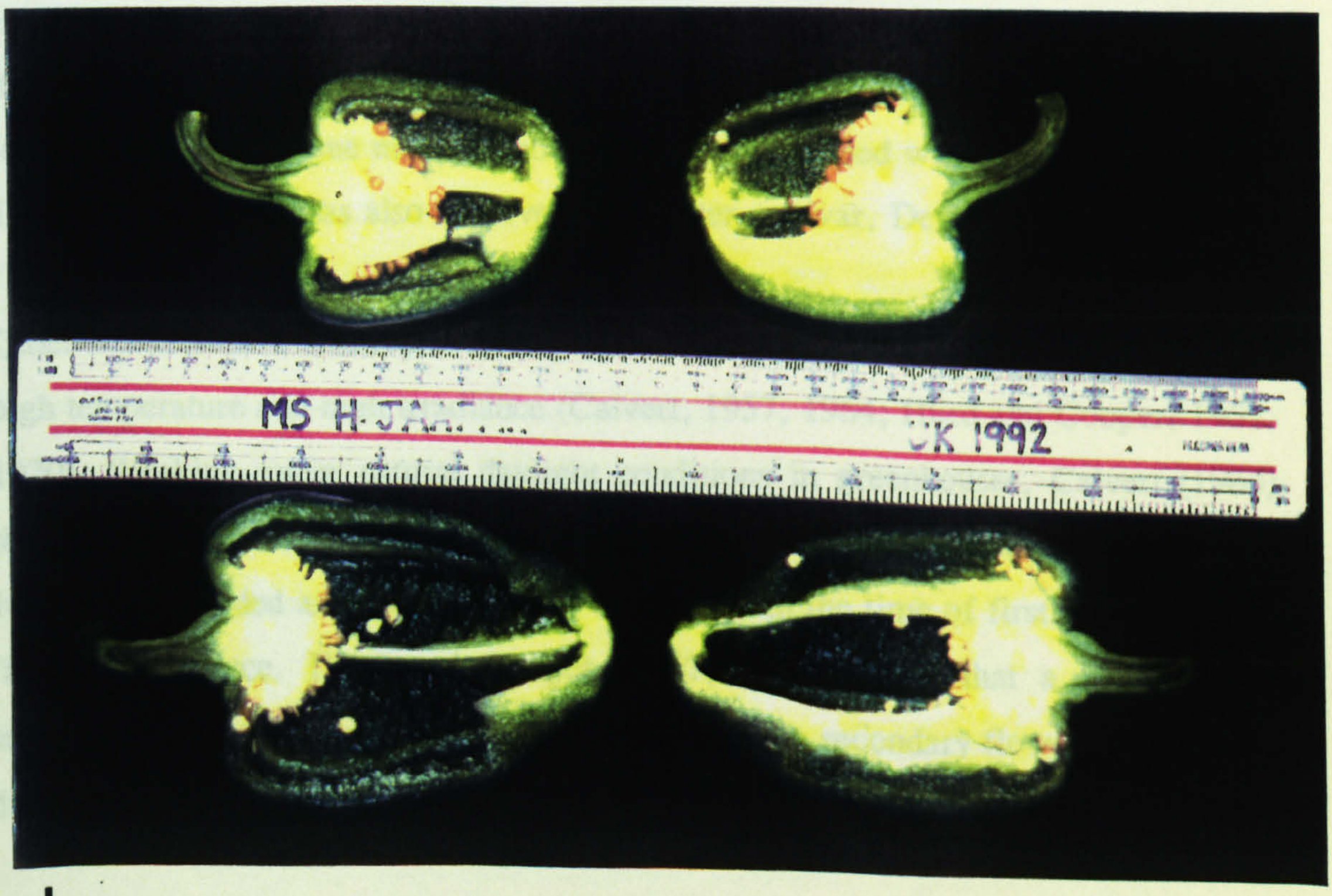
b

a

PLATE 3.1.7. *Abnormal fruits from the 14 °C temperature treatment. (a) Blue Star, (b) Bell Boy, (c) cross section from Blue Star at 14 °C and (d) from Bell Boy (top) and Blue Star (bottom) at 20 or 26 °C.*

b

c



d

the first flower is more rapid at high irradiance in at least some varieties.

The growth and development of the first flower once it had been initiated was also influenced by the aerial environment. Anthesis was hastened more in Blue Star than in Bell Boy by the combination of high temperature and high irradiance. However, at high temperature and low irradiance, anthesis was delayed by 28 - 30 d in Bell Boy, a similar delay to that induced by low temperature (14 °C) in both varieties regardless of irradiance. Anthesis in the secondary flowers was enhanced by both high temperature and high irradiance. Consistent with these results, the important influence of temperature in determining flower development following initiation has also been demonstrated in sweet pepper var. World Beater by Cochran (1936) who showed that, when other factors were maintained near optimum for growth, anthesis in primary flowers was accelerated at higher day/night temperatures (38/32 °C) as compared with lower temperatures (15/10 °C) by up to 80 d. Cochran attributed the earlier production of buds and blossom at high temperature partly to more rapid vegetative growth. Anthesis in the first and second flowers of cv. California Wonder was also accelerated (by 51 and 58 d respectively) when a high night temperature (25 °C), was combined with natural glasshouse irradiance; lower night temperatures of 10 - 15 °C delayed anthesis in both categories of flower (Rylski, 1972). The delaying effect of low temperature became more pronounced when combined with a low irradiance of 1.54 MJ m^{-2} d^{-1}, as was also observed in another cultivar, Delphin (Bakker, 1989).

Other workers have also reported that anthesis may be hastened by a combination of high temperature and high irradiance (Calvert, 1957, 1964; Hurd and Cooper, 1967, 1970). Working under natural daylight conditions in greenhouses, Calvert (1964) showed that flowers developed more rapidly at mean air temperatures of 20 than at 16 °C and recorded an advancement of up to 12 d in the time of first anthesis in the first inflorescence. Hurd and Cooper (1967, 1970) found that a higher mean temperature of 15 °C also advanced the opening of the secondary flowers by up to 18 d as compared to a lower temperature of 10 °C.

In addition to the more rapid flower development observed in the present study, the

total numbers of primary and secondary flowers produced were also increased in both varieties by the combination of high temperature (20 - 26 °C) and high irradiance, but were generally decreased at low irradiance in all the temperature treatments examined. Varietal differences were most obvious at low irradiance, under which conditions the fewest primary and secondary flowers were observed in Blue Star at low temperature, and in Bell Boy at high temperature. The number of primary flowers reaching anthesis was greater in both varieties at high as compared to lower temperatures under high irradiance, but fewer flowers were produced under low irradiance irrespective of temperatures. Atherton and Othman (1983) have previously established a close relationship between the number of flowers reaching anthesis in tomato and total accumulated radiation similar to that found in the present study. A close relationship was also observed in the present work between the number of flowers reaching anthesis and thermal time. Flower growth and development have also been shown to depend closely on thermal time by other workers (Ketring and Wheless, 1989; Bagnall and King, 1991). The numbers of secondary flowers reaching anthesis followed a similar trend to the primary flowers. These results are consistent with those of Cochran (1924, 1936), who found that more flowers were produced when plants of sweet pepper var. World Beater were grown in warm conditions (19 - 24 °C) than in intermediate (14 - 19 °C) or cool conditions (10 - 14 °C); in the latter treatment, the plants produced only one blossom on average.

Although the combination of high temperature and irradiance hastened flower development, it also promoted flower abscission in both Blue Star and Bell Boy, and consequently reduced fruit set. These results are consistent with those of Atherton and Othman (1983) who observed 50% flower abscission when tomato cv. Sonato was grown at temperatures 16 - 20 °C under a similar mean daily irradiance (4.8 MJ m^{-2} d^{-1}) to that used in the present study.

Adverse effects of high temperature on flower abscission have been reported for sweet pepper by many workers (Cochran, 1936; Wien *et al.*, 1989a, 1989b; Aloni *et al.*, 1991b). Cochran (1936) found that flower abscission in greenhouse-grown sweet pepper var. World Beater was increased when temperature was raised from 21/16 °C

(day/night) to 27/21 °C, but all other factors were maintained close to the optimum for growth. Aloni *et al.* (1991b) found that, under natural glasshouse lighting conditions, a high temperature regime of 25/35 °C (day/night) caused complete flower bud abscission in sweet pepper cv. Maor, whereas only c. 50% abscission occurred at 35/25 °C and no abscission took place in plants grown at 18/25 °C. These workers suggested that the increased bud abscission at high temperature resulted from competition for assimilates between flower buds and young leaves. Under such conditions, the young leaves appear to be more effective than adjacent flower buds in importing assimilates, a view supported by results showing that ^{14}C-sucrose was partitioned in favour of young leaves as opposed to flowers.

Other workers (Quagliotti, 1979; Rylski, 1986; Atherton and Harris, 1986) have attributed increased flower abscission at high temperature to the incomplete development of floral organs at anthesis. This is not likely to have been the cause of flower abscission in the present study since abscission occurred 10 -13 d after anthesis and the abscinded flowers showed no structural abnormalities. Instead, the observed abscission may have originated from competition for assimilates between the rapidly developing reproductive organs and the young leaves, in which the former may be more adversely affected than the latter which may provide stronger sinks for assimilates (Dinar *et al.*, 1983; Halevy, 1987; Rao *et al.*, 1992).

Complete flower abscission was observed in Blue Star and Bell Boy at high temperature and low irradiance, implying that both varieties were sensitive to this flower abscission-inducing environment. Complete truss abortion in tomato cv. Sonato was also demonstrated by Atherton and Othman (1983) when a shading treatment was applied at the bud visible stage under glasshouse conditions to reduce total radiation by 75% at an air temperature of 16 - 24 °C. The mean daily irradiance in Atherton and Othman's study was similar both to that used in the present work and that adopted by Kinet (1977a) in growth rooms to achieve a high percentage flower abortion.

According to Atherton and Harris (1986), complete flower bud abortion or flower

abscission after anthesis may indicate that the quantity of photosynthetically active radiation available to support the growth is severely limiting. This may in turn limit assimilate availability (Atherton and Othman, 1983), thereby increasing competition between the reproductive organs and vegetative sinks for assimilates (Hussey, 1963; Halevy, 1987; Morris and Newell, 1987). Turner and Wien (1994a) found that the partitioning of dry matter to young leaves in sweet pepper was unchanged by low light conditions as compared to the reproductive organs, indicating that the latter may be more affected by low light conditions and may therefore provide weaker sinks for assimilates. This was further supported by the work of Cooper and Hurd (1968), who showed that carbon dioxide enrichment under low irradiance-high temperature conditions in winter accelerated flower growth in the first inflorescence and reduced flower abortion. It has also been suggested that complete flower abscission may involve enhanced production of ethylene by the shaded flower buds, as demonstrated by Wien and Yipin (1989).

Apart from its effects on the reproductive organs, the interaction of temperature and irradiance also influenced vegetative growth and development. Such effects are important since vegetative growth influences subsequent reproductive growth and development, and hence yield (Rylski, 1972). The present study has shown that high temperature and high irradiance increased total leaf number, leaf area and total shoot dry weight, but decreased stem elongation. The effect of light on leaf number has previously been reported to be greater at high (25 °C) than at low (15 °C) temperature (Hussey, 1963). Nilwilk (1981) showed that supplementing natural glasshouse radiation with 0.49 MJ m^{-2} d^{-1} from HPLR lamps (400 W) increased leaf number in sweet pepper cv. Bruinsma Wonder by three. As temperature increased from 19 to 22 °C, leaf area was also increased by supplementary radiation (Nilwilk, 1981), corroborating the results of Newton (1963) and Milthorpe and Newton (1963) who also observed that total leaf area was increased by higher radiation.

Greater shoot elongation under low light conditions has previously been reported in sweet pepper, an agreement with the present observations of increased plant height under low irradiance conditions, especially at higher temperatures. During a summer

shading experiment, Rylski and Spigelman (1986) found that as irradiance decreased from 600 to 318 cal cm^{-2} d^{-1}, plant height increased from 29.9 to 40.2 cm in cv. Maor as a result of both increased internode elongation and node number. Othman (1984) examined young reproductive plants of tomato cv. Sonato in a glasshouse and demonstrated that total leaf number per plant was increased and plant height was decreased under high irradiance at day/night temperatures of 24/18 °C. Consistent with the present results, he also obtained a close direct correlation between total incident radiation over a 30 d period and leaf number per plant. Since the total number of leaves produced under low irradiance was significantly lower than at high irradiance, the priority of vegetative over reproductive growth under low irradiance conditions may originate from the promotion of stem extension rather than leaf production.

Total shoot dry matter reflects the carbohydrate status of the shoot system. An acute shortage of carbohydrate, indicated by the decrease in total shoot dry weight, often leads to complete arrestment of flower development and total flower abscission (Halevy, 1987; Morris and Newell, 1987). In the present study, a significant reduction in shoot dry weight was observed in plants grown at high temperature and low irradiance, which was accompanied by complete flower abscission. A lower percentage flower abscission was recorded under high irradiance/high temperature conditions and this was accompanied by a greater shoot dry weight. In a glasshouse study, Turner and Wien (1994b) found that a low light treatment (320 - 360 μmol m^{-2} s^{-1}) at day/night temperatures of 24/18 °C significantly reduced shoot dry weight in two sweet pepper cultivars, Shamrock and Ace. Turner and Wien also reported that the partitioning of assimilates in the low light treatment favoured the young leaves as opposed to the reproductive organs, a conclusion consistent with several other reports (Dinar and Rudich, 1985a, b; Morris and Newell, 1987; Halevy, 1987). Since dry matter partitioning was not examined in the experiment reported here, it could not be ascertained whether dry matter was preferentially partitioned in favour of the vegetative as opposed to the reproductive parts, and whether this may have been partly responsible for flower abscission. However, changes in shoot dry matter distribution are examined in Chapter 5.

The results presented here demonstrate the existence of varietal differences between Bell Boy and Blue Star in their responses to temperature and irradiance. Under high irradiance, var. Blue Star proved to be more responsive to high temperature than Bell Boy, resulting in significantly earlier bud emergence and anthesis, although the latter effect was not significant. This varietal difference in the time required for flower development may reflect differences in the cardinal temperatures of the varieties since a variety with lower base and higher optimum temperatures would exhibit more rapid development under high temperature conditions (Ong and Monteith, 1985). The differences in cardinal temperatures between Bell Boy and Blue Star are examined in Chapter 4.

3.3 Conclusions

1. Emergence of the first primary flower bud was accelerated more by the combination of high temperature (26 °C) and high irradiance (4.9 MJ m^{-2} d^{-1}) in Blue Star than in Bell Boy. Low irradiance (2.4 MJ m^{-2} d^{-1}) delayed bud emergence to a greater extent in Bell Boy than in Blue Star, especially at low temperature.

2. Anthesis was hastened by the combination of high temperature and high irradiance in Blue Star, although the time to anthesis was not significantly different from Bell Boy. Anthesis was delayed more by the combination of high temperature and low irradiance in Bell Boy than in Blue Star, the effect of which was comparable to the delaying effect of low temperature (14 °C) in both varieties regardless of irradiance. Anthesis in the secondary flowers was enhanced by both high temperature and high irradiance, with no varietal difference in response.

3. The total numbers of primary and secondary flowers were increased in both varieties under high temperature-high irradiance conditions, and there was a corresponding increase in the total numbers of secondary flowers reaching

anthesis. However, the number of primary flowers reaching anthesis was increased only under high temperature-high irradiance conditions in both varieties, or in Bell Boy under high irradiance irrespective of temperature. The number of primary flowers reaching anthesis was also closely related to total radiation and thermal time.

4. Although the combination of high temperature and high irradiance accelerated flower development up to anthesis, the abscission of primary flowers was increased in both varieties relative to the 20 °C, high irradiance treatment. Complete abscission occurred under high temperature-low irradiance conditions.

5. Vegetative growth and development were strongly affected by an interaction between temperature and irradiance. The combination of high temperature and high irradiance increased total leaf number and area, but decreased stem growth. Total shoot dry matter declined as temperature and irradiance decreased.

CHAPTER 4

CARDINAL TEMPERATURES AND THERMAL TIME FOR GROWTH AND DEVELOPMENT

INTRODUCTION

An understanding of varietal differences in response to temperature not only serves as a tool for cultivar selection, but also allows the calculation of thermal time scales to facilitate comparison of growth and developmental rates and permit prediction of development processes such as bolting and flowering in carrot (Atherton *et al.*, 1990) and celery (Ramin and Atherton, 1991), curd initiation in cauliflower (Hand and Atherton, 1987) and leaf initiation (Kristensen *et al.*, 1985; Elphinstone *et al.*, 1988). The use of thermal time to analyse the growth and development of sweet pepper has not previously been reported, probably due to the lack of comprehensive information on its temperature responses. Work on seed germination in seven pepper cultivars (Coons *et al.*, 1989) only established the existence of varietal differences in the optimum and maximum temperatures, but did not examine the base temperature, a vital aspect in any calculation of thermal time.

The main aim of this Chapter was to determine cardinal temperatures for the two varieties of sweet pepper used in this study. When the rate of germination (the reciprocal of time to 50% germination; $1/t = 0.5$) is a linear function of temperature, the cardinal temperatures (base, optimum and maximum temperatures $\approx T_b$, T_o and T_m, respectively) for germination may be established and subsequently used to calculate the thermal time required for germination (Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*, 1988a). The base temperature (T_b) for germination may differ from that for other developmental processes; for example, the T_b value for reproductive development is normally higher than that for vegetative development (Angus *et al.*, 1981a, b; Leong

and Ong, 1983; Slafer and Savin, 1991). T_b may also differ substantially between species or cultivars (Weilgolaski, 1974; Mohamed *et al.*, 1988b; Squire, 1990) and may even exhibit considerable variability for the same process (Leong and Ong, 1983). In contrast, the results of Ong (1983a, b) for a single variety of millet grown in temperature-controlled glasshouses indicate that T_b was conservative for many processes and growth stages. To ensure reliable assessments of thermal time, it is important to establish accurate values for T_b, and one way of achieving this is to determine T_b under controlled conditions (Squire, 1990).

4.1 Determination of base, maximum and minimum temperatures for germination

The method for examining the effect of temperature on germination rates using a thermogradient plate under controlled environment conditions has generally been found to be satisfactory and reproducible (Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*, 1988b), provided viable and non-dormant seeds are used. The analysis of development in relation to thermal times derived using this approach is also possible (Mohamed *et al.*, 1988a). The main objective of the present study was to determine the base, maximum and optimum temperatures for germination in the sweet pepper varieties, Bell Boy and Blue Star. The values obtained were used to relate the rate of germination to thermal time.

4.1.1 Materials and methods

Two germination trials were carried out using a thermogradient plate as described in Section 2.4. The first trial was carried out using eight different constant day and night temperatures at intervals of 5 °C within the range 5 - 45 °C . Two further constant temperatures (26 and 29 °C) were included to achieve better resolution around the optimum of 25 - 30 °C for germination in sweet pepper (Coons *et al.*, 1989). The results from the first experiment showed that the seeds failed to germinate either at the

upper end of the temperature range (35 - 45 °C) or at the lower end (5 - 10 °C), with very high germination percentages (97 - 99%) being observed between 15 and 30 °C. Based on these results, a second experiment was conducted using a range of eleven more closely spaced (2 °C intervals) constant day/night temperatures (14 - 34 °C) to avoid the temperature extremes used in the previous experiment. The results from the two experiments were combined to give a more detailed understanding of the influence of temperature on germination.

The experiments began on 27 January 1993 and 12 March 1993 and were continued for 30 d, although germination ceased after 20 d. Seeds of Bell Boy (UK) and Blue Star (Taiwan) of uniform size (≈ 2 mm) were selected and arranged in sterilised 4.5 cm diameter plastic petri dishes lined with two layers of Whatman No.1 filter paper and moistened with 2 ml of distilled water. Relatively small petri dishes were used to reduce the possible temperature variations which may occur in larger petri dishes (Jackson, 1982). Thirty seeds from each variety were distributed uniformly within each petri dish. Three replicates were used for each variety at every constant temperature examined. Each set of six petri dishes was then covered and placed at the appropriate point along the thermogradient plate; their temperatures were checked every day as described in Section 2.4. The petri dishes were opened daily at the same time to count and remove germinated seeds and add more water if necessary. Seeds were scored as having germinated when 1 mm of radicle had emerged from the seed coat (Coons *et al.*, 1989).

Fractional germination was calculated daily by dividing the cumulative number of germinated seeds by the total number of seeds used. The values obtained showed how the rate of seed germination was affected by the various constant temperatures applied. The rate of germination was calculated as the reciprocal of the time taken for half the population to germinate. This was then plotted as a linear regression against temperature; extrapolation of the regression lines to the temperature axis was used to establish the minimum and maximum temperatures, while the point where the two regression lines met was used to identify the optimum temperature for germination in each variety. Using these cardinal temperatures, the thermal time required for

germination and rate of germination were calculated as described in Section 4.1.2. The results were expressed as the mean of the three replicates for each variety and temperature treatment.

4.1.2 Results

No seed germination was observed at 5, 10 or 40 °C.

Cumulative germination (G)

Figure 4.1.1 shows the timecourses for cumulative germination in both varieties examined, Bell Boy and Blue Star. The onset of visible germination (i.e. radicle emergence) varied with temperature in both varieties and occurred between 1 and 8 d after the treatments commenced. In Blue Star, seeds germinated first at 27 °C (1 d), 3 d earlier than the first germination in Bell Boy, which occurred after 4 d at 22 °C. Germination was slowest in the 15 and 35 °C treatments, in which the seeds began to germinate between day 8 and 9 in Bell Boy. Maximum germination was similar in both varieties (≥ 0.90 - 0.95) at all temperatures except 15 °C (0.63) in Bell Boy and 35 °C (0.73) in Blue Star, although Bell Boy required a longer period (15 - 19 d) than Blue Star (7 - 14 d) to reach maximum germination.

Rate of germination

The germination rates for Bell Boy and Blue Star derived from the cumulative fractional germination curves are shown in Figure 4.1.2. Blue Star exhibited a higher rate of germination over most of the temperature range, particularly between 25 and 29 °C (0.20 vs. 0.14 d^{-1}). When separate regressions were calculated for the sub- and supra-optimum temperature ranges, extremely close linear relationships ($p<0.001$) between germination rate and temperature were obtained for both varieties (Figure 4.1.2). The experimental data show that germination rate increased with temperature from 8.5 to 23.0 °C in Bell Boy, and from 6.0 to 27.5 °C in Blue Star, but thereafter

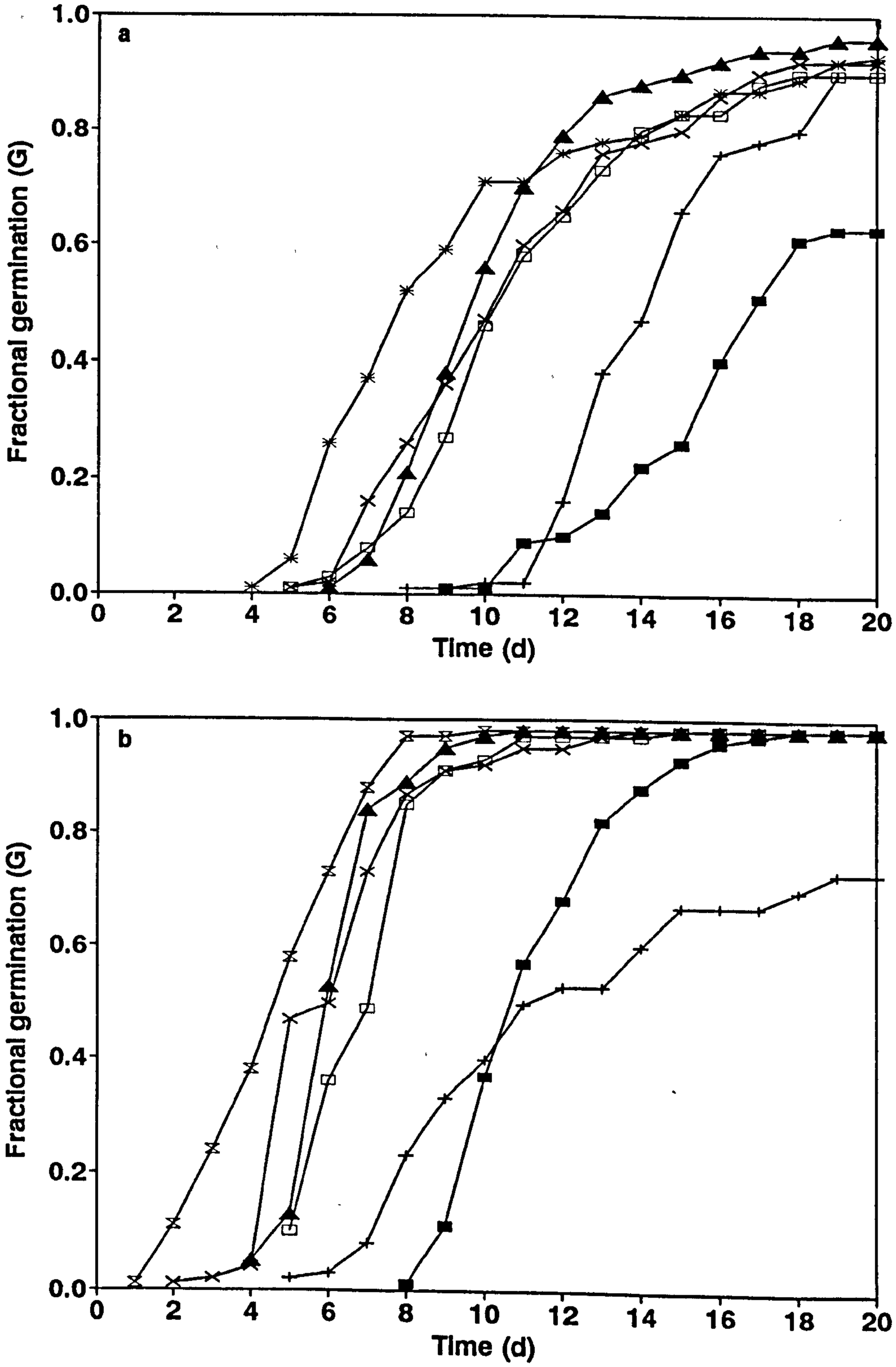


FIGURE 4.1.1. *Timecourse of cumulative germination for varieties Bell Boy (a) and Blue Star (b) at different constant temperatures:* ■, *15 °C;* □, *20 °C;* *, *22 °C;* ▲, *25 °C;* X, *27 °C;* x, *30 °C;* +, *35 °C. n=3.*

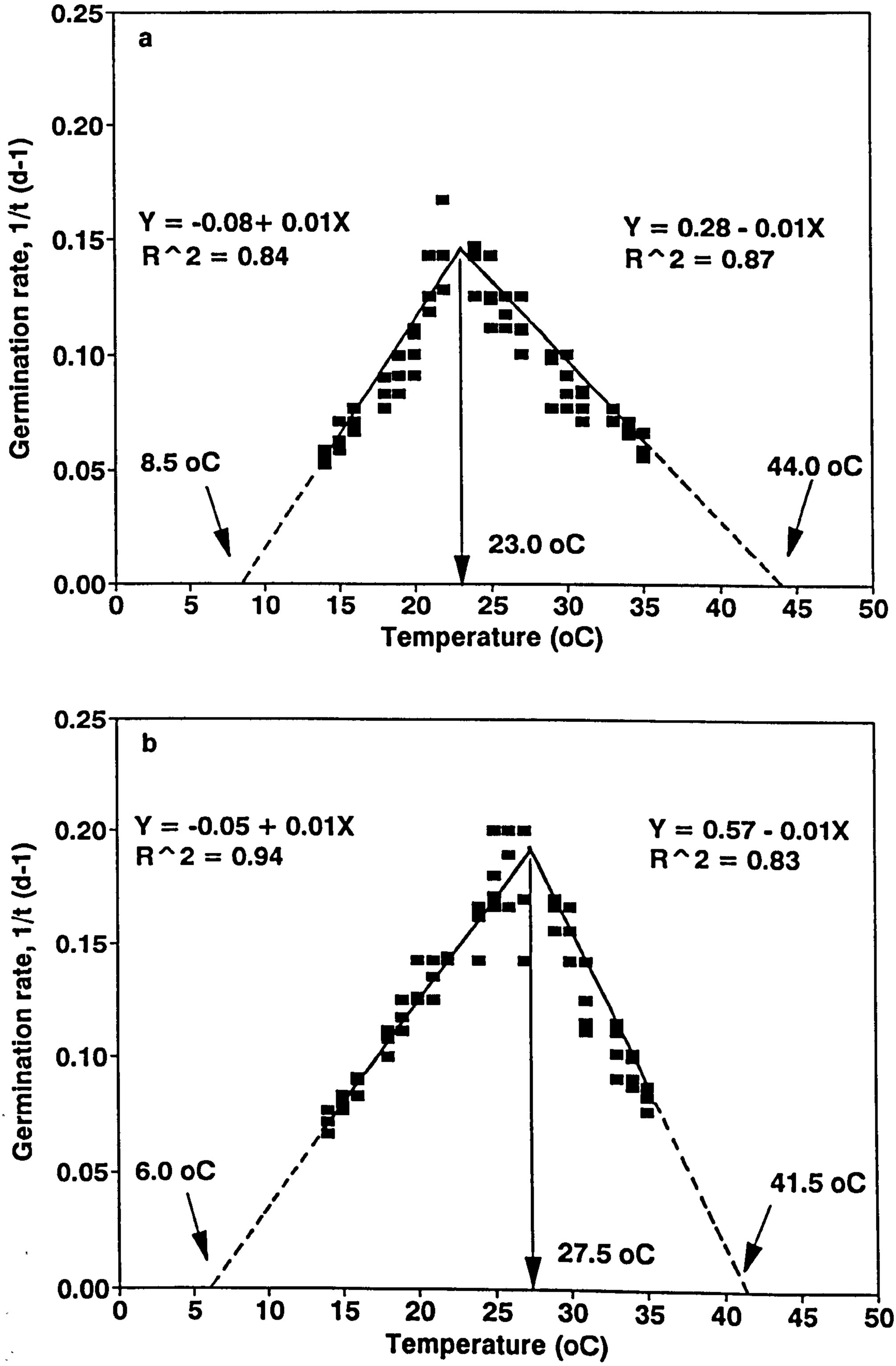


FIGURE 4.1.2. *Relationship between the rate of germination (1/t to G = 0.5 d^{-1}) and temperature for varieties Bell Boy (a) and Blue Star (b). The lines in (a) and (b) are linear regressions ($p<0.001$). $n=3$.*

declined sharply to maximum of 44.0 and 41.5 °C in Bell Boy and Blue Star respectively.

Cardinal temperatures

Extrapolation of the germination rate-temperature regression lines allowed the base (T_b), optimum (T_o) and maximum (T_m) temperatures to be estimated for both varieties. The cardinal temperatures estimated from Figure 4.1.2 for Bell Boy were T_b = 8.5 °C, T_o = 23.0 °C and T_m = 44.0 °C. For Blue Star, the equivalent values were T_b = 6.0 °C, T_o = 27.5 °C and T_m = 41.5 °C.

Thermal time for germination

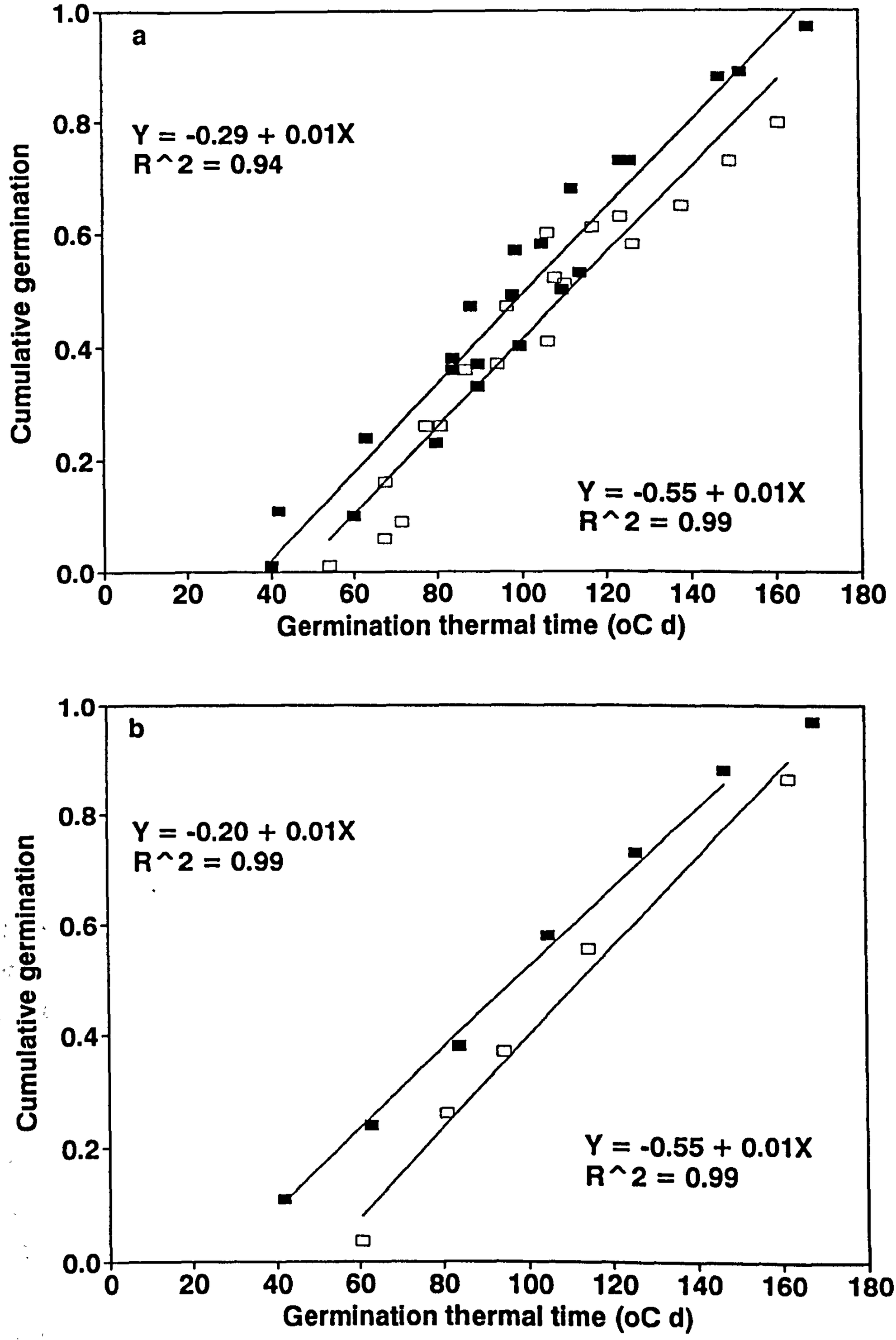
For temperatures below T_o, the thermal time required for germination, θ_g, defined as the product of time in days t, and the effective temperature ($T - T_b$) was calculated as follows:

$$\theta_g = (T - T_b)\, t$$

where T is the constant temperature used and T_b is the base temperature for germination in each variety (Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*, 1988b). When temperature was above the optimum, the temperature values were converted to their equivalent 'effective' temperature (T_{eff}) in the sub-optimum range using the method of Craigon *et al.* (1990). Cumulative germination within the temperature range up to T_o is shown as a function of thermal time in Figure 4.1.3a for both Bell Boy and Blue Star. The minimum θ_g required for germination varied between varieties, and was c. 50 °C d in Bell Boy and 40 °C d in Blue Star. The minimum θ_g for 90% germination was c. 150 °C d in Blue Star, whereas Bell Boy required c. 160 °C d to reach 80% germination. When linear regression analysis was carried out to examine the relation between cumulative germination and thermal time at near-optimum constant temperatures of 22 °C for Bell Boy and 27 °C for Blue Star (Figure 4.1.3b), highly significant relationships were obtained. The rate of germination was also linearly related to thermal time above the base temperature for each variety ($p<0.001$;

FIGURE 4.1.3a. *Relationship between cumulative germination at constant temperatures between 15 and 27 °C and thermal time (°C d) calculated above a base temperature of 8.5 °C in var. Bell Boy (□) and 6.0 °C in var. Blue Star (■). The lines are linear regressions ($p<0.001$). $n=3$.*

FIGURE 4.1.3b. *Relationship between cumulative germination and thermal time (°C d) at near-optimum constant temperatures of 22 °C for Bell Boy (□) and 27 °C for Blue Star (■). The lines are linear regressions ($p<0.001$). $n=3$.*


a
Y = -0.29 + 0.01X
R^2 = 0.94
Y = -0.55 + 0.01X
R^2 = 0.99
Cumulative germination
Germination thermal time (oC d)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
b
Y = -0.20 + 0.01X
R^2 = 0.99
Y = -0.55 + 0.01X
R^2 = 0.99
Cumulative germination
Germination thermal time (oC d)

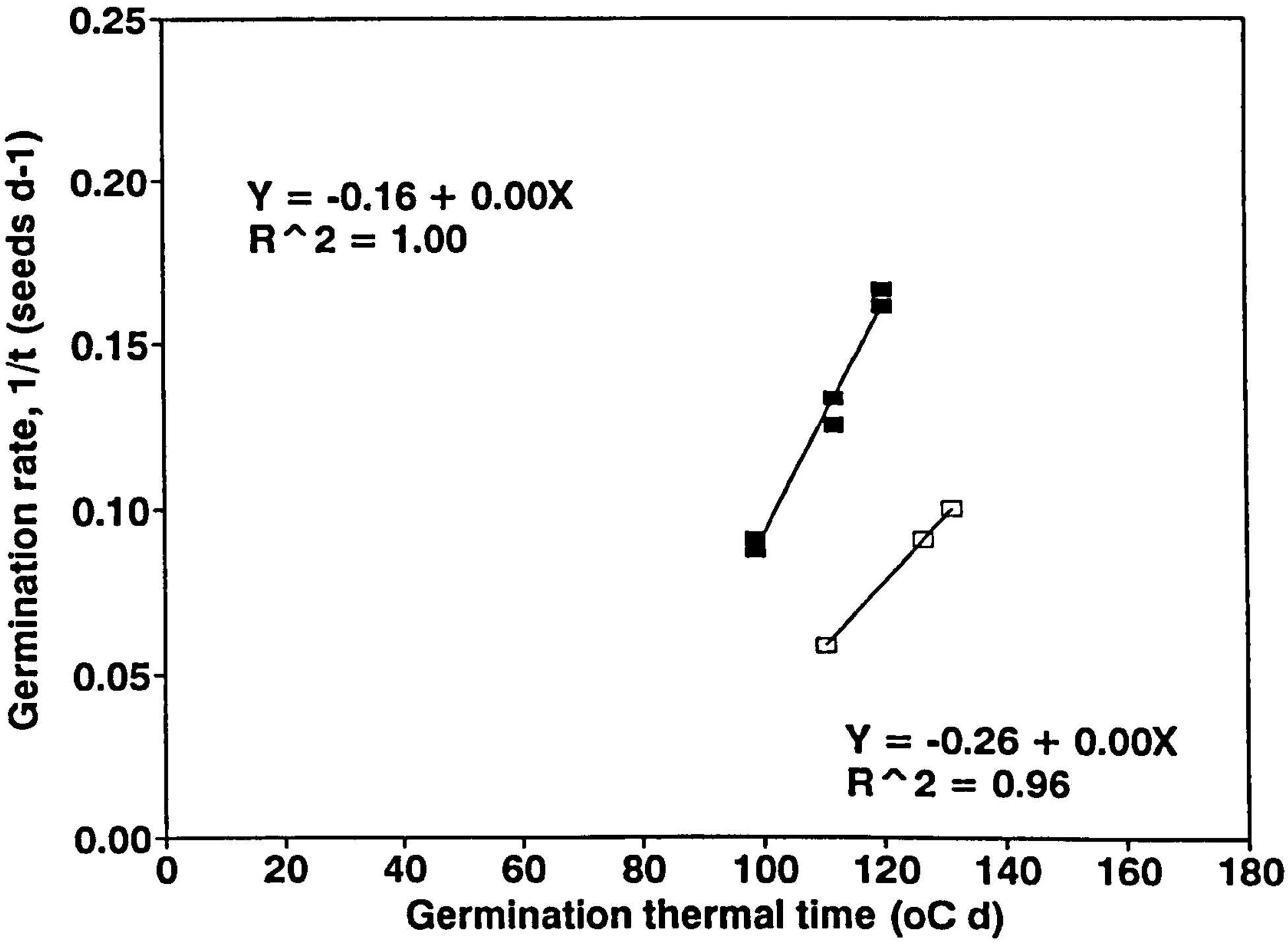


FIGURE 4.1.4. *Relationship between germination rate and germination thermal time (°C d) above the base temperatures for var. Bell Boy (□; T_b = 8.5 °C) and Blue Star (■; T_b = 6.0 °C). The lines are linear regressions ($p<0.001$). n=3.*

Figure 4.1.4). Germination began first in Blue Star after a thermal time of about 120 °C d, as opposed to 132 °C d in Bell Boy; germination rate was also more rapid in Blue Star.

4.2 Discussion

Germination of sweet pepper seed under controlled conditions on a thermogradient plate demonstrated similarities and differences in the responses of the two varieties examined to various constant temperature treatments between 15 and 35 °C. No germination was observed outside this range, possibly because dormancy was induced when seeds were exposed to lower or higher temperatures (Coons *et al.*, 1989). Since tetrazolium tests were not carried out, the possibility that reduced seed viability contributed to germination failure below 14 and above 35 °C cannot be excluded. Blue Star showed a higher fractional germination than Bell Boy at all temperatures except 35 °C, at which a longer period was required to reach maximum fractional germination (0.73 on day 20; Figure 4.1.1). In contrast, the lowest fractional germination in Bell Boy was recorded at 15 °C. The slower germination at temperatures between 20 and 30 °C in Bell Boy may reflect an inherent lack of vigour or an adaptive response to the environment in which this genotype evolved.

The linear relationships between the rate of germination and temperature established for both varieties (Figure 4.1.2) resemble those reported previously for other species (Garcia-Huidobro *et al.*, 1982; Mohamed *et al.*, 1988a). Germination rate increased linearly with temperature between T_b and T_o and thereafter decreased linearly until T_m was reached. The base and maximum temperatures for Blue Star were 2.5 °C lower than in Bell Boy, although its optimum temperature was over 4 °C higher at 27.5 °C. Both varieties, however, exhibited a similar range of 35.5 °C between their base and maximum temperatures. The observed varietal differences in cardinal temperatures may reflect their differing climatic origins. For example, varieties with low T_b and high T_m values may have evolved in regions which experience extremes of climatic variation, whilst varieties from more temperate environments often show a narrower

range between the base and maximum temperatures (Mohamed *et al.*, 1988b). Since Blue Star is widely cultivated in tropical regions and Bell Boy in temperate areas, it was anticipated that the former would be adapted to tropical climates and the latter to temperate zones. However, since both varieties exhibited similar T_b (6 - 8.5 °C) and T_m values (41.5 - 44 °C), it is possible that both may have developed in a mediterranean climate but are suitable for growing in either cooler or warmer areas. This suggestion is supported by the similar range between T_b and T_m, which indicates that both varieties evolved under climatic conditions where the diurnal temperature variation is large (Squire, 1990). Similar values of T_b and T_m have been demonstrated for some groundnut cultivars (Mohamed *et al.*, 1988b).

The germination tests suggest that Blue Star may be slightly more heat tolerant due to its lower T_b (6.0 °C) but higher T_o (27.5 °C; Ong and Monteith, 1985). These differences imply that the rate of development of Blue Star should be faster than that of Bell Boy at higher temperatures. This might explain the more rapid development observed in Blue Star during early reproductive growth in the 26 °C treatment described in Chapter 3. The observed variation in the rate of germination also has important implications for crop production in areas where rapid development and heat tolerance favour survival.

Provided that T_b had been accurately determined under controlled conditions, this value is often applicable to other developmental processes (Leong and Ong, 1983; Morrison *et al.*, 1989; Squire 1990), although some workers have suggested that T_b may be higher during the reproductive phase than during the vegetative phase (Weigolaski, 1974; Angus *et al.*, 1981b; Slafer and Savin, 1991). By accumulating the mean daily temperature above the base for the appropriate period, the thermal time required for specific growth and development stages can be predicted (Atherton *et al.*, 1990; Craigon *et al.*, 1990). Thermal time is an extremely useful concept because it allows the development of crops at different locations and in different seasons to be compared (Ong and Monteith, 1985; Squire, 1990). Often, the same variety of a crop is grown at a number of different sites and thus requires different amounts of chronological time, but similar amounts of thermal time to reach maturity. Thus,

although the chronological duration for any particular developmental process is shortest at the optimum temperature and lengthens at temperatures below or above this value, the thermal duration for the same process remains the same at each location.

Using the T_b value obtained from seed germination trials, the fractional emergence of groundnut and millet seeds was satisfactorily transformed into thermal time (Mohamed *et al.*, 1988b). Correlation of cumulative germination at all the temperatures below T_o and cumulative germination at temperatures close to the optimum with thermal time produced highly significant linear relationships in both varieties examined in the present study, especially when optimum temperatures were used (Figure 4.1.3). The minimum thermal time needed to initiate germination was 40 °C d in Blue Star and 55 °C d in Bell Boy, whilst a maximum germination fraction of 0.95 was obtained after 165 °C d in Blue Star. Under the optimal temperature environment of 27 °C, Blue Star required a lower thermal time than Bell Boy to initiate germination. Differences in the rate of germination between Blue Star and Bell Boy can also be examined using the germination thermal time. The thermal time required to achieve specific germination rates was consistently lower in the former, ranging from 100 to 120 °C d for 0.09 - 0.17 seeds d^{-1}, as compared to 0.06 - 0.10 seeds d^{-1} for the greater thermal time of 110-130 °C d.

Several previous attempts have been made to relate crop development to thermal time above the base temperature (Ketring and Wheles, 1989; Slafer and Savin, 1991). In a study of flowering and bolting in carrot grown under growth room, glasshouse and field environments, Craigon *et al.* (1990) demonstrated a linear relationship between the rate of progress to flower bud visibility and the number of vernalising degree days accumulated when non-juvenile plants were grown in constant temperature rooms. However, Slafer and Savin (1991) were unable to establish a unique base temperature for all developmental stages in wheat, and suggested that T_b varies in a manner which reflects adaptation to low temperatures during early developmental phases and to warmer temperatures during reproductive development (Angus *et al.*, 1981a, b). An alternative suggestion is that T_b may be highest during periods of growth when metabolic activity is greatest, such as the pre-anthesis and grain filling stages.

The present study of the cardinal temperatures for germination in two varieties of sweet pepper has made it possible, at least tentatively, to relate the rate of growth and development to thermal time. The present work was not intended to provide a detailed understanding of the relation between accumulated thermal time and development, but rather to determine whether varietal differences in cardinal temperatures may be responsible for the observed varietal differences in response to temperature. Since it has been shown that the rate of development may be related to thermal time, this may open an area where the prediction of growth and development in sweet pepper using the concept of accumulated thermal time above the base temperature can provide a useful tool for planning glasshouse or protected production of sweet pepper throughout the year. In the experiments described in Chapters 5 and 6, thermal time analysis was used to separate the effects of temperature and water stress and examined the influence of applications of chemicals on developmental processes in sweet pepper variety Blue Star, particularly time to anthesis and flower abscission. Although the T_b value established in this germination trial may not be identical to that during reproductive phase, its use was better than adopting the arbitrary assumption that $T_b = 0$ °C or some other value.

4.3 Conclusions

1. Germination in the two sweet pepper varieties examined occurred between 15 - 35 °C, but did not occur outside this temperature range.

2. The cardinal temperatures established from the linear relationships between the germination rate and temperature showed that T_b, T_o and T_m were respectively 8.5, 23.0 and 44.0 °C for Bell Boy and 6.0, 27.5 and 41.5 °C for Blue Star.

3. Cumulative fractional germination and germination rate were correlated with thermal time above the appropriate base temperature for each variety, although varietal differences in the developmental processes were apparent. Thus, the minimum thermal time required to initiate germination in Bell Boy and Blue

Star was 55 and 40 °C d respectively, whilst the thermal time required to achieve a germination rate of 0.09 - 0.17 seeds d^{-1} was 100 - 120 °C d in Blue Star, as compared to 0.06 - 0.10 seeds d^{-1} for a greater thermal time of 110 - 130 °C d in Bell Boy.

CHAPTER 5

INFLUENCE OF WATER STRESS ON GROWTH AND DEVELOPMENT

INTRODUCTION

In Chapter 3, it was shown that high temperature (26 °C) promoted the earlier initiation and development of primary flowers in Blue Star when combined with high irradiance, but that this treatment also increased flower abscission. Reducing glasshouse irradiance when the third true leaf was expanding retarded reproductive development more than vegetative growth and induced complete flower abscission. The failure of the flowers to set fruit may have been at least partly attributable to competition between the vegetative and reproductive components for the limited assimilate supplies available (Atherton and Harris, 1986; Halevy, 1987). Any improvement of reproductive development under low irradiance therefore requires a proportionate balance of growth to be maintained within the plant. A restriction on vegetative growth, possibly induced by withholding water, may reduce competition by producing small 'hard plants', thereby producing a more balanced pattern of growth (De Koning and Hurd, 1983). Many commercial growers regularly practise 'balancing' of growth in early-sown greenhouse tomatoes (Cooper and Hurd, 1968; Calvert, 1969; De Koning and Hurd, 1983), usually by subjecting them to periods of water stress.

The experiments described in this Chapter were designed to determine the influence of water stress on the early growth and development of the reproductive organs, paying particular attention to the abscission of the primary and secondary flowers. They also examined the possible role of changes in dry matter distribution within plants induced by water stress in modifying reproductive growth and development in sweet pepper. The hypothesis being tested was that water stress would enhance flower

growth and development by altering assimilate partitioning in favour of the flowers, thereby reducing flower abscission.

5.1 Effects of water stress and irradiance on growth and development after the first bud-visible stage

Water stress frequently has deleterious effects on the reproductive development of horticultural crops (Atherton and Othman, 1983; Wudiri and Henderson, 1985), the extent of which depends on the developmental stage when it is imposed (Salter and Goode, 1967; Kaufmann, 1972; Begg and Turner, 1978). For example, water stress imposed shortly after the flower buds become visible causes flower abscission (Wudiri and Henderson, 1985). In contrast, water stress may prolong the period of flower development under conditions of low irradiance, thus reducing abscission (Klapwijk and De Lint, 1974), an effect which was suggested to originate from an increase in the quantity of carbohydrate stored in the stems (De Koning and Hurd, 1983).

The following experiment was conducted to investigate the extent to which the imposition of water stress and low irradiance under glasshouse conditions reduces flower abscission through changes in assimilate distribution which favour early flower development.

5.1.1 Materials and methods

The experiment was conducted between 22 July and 20 October 1992. Two seeds of Blue Star were sown in each inverted pyramid cell of Speedling trays (2.5 x 2.5 x 7.2 cm deep) containing Levington F2 Compost and then managed as described in Section 2.1. Each cell was thinned to leave one seedling seven days after sowing. At the stage when the third true leaves became visible (17 days after germination), healthy seedlings of uniform size were pricked out into 9 cm diameter pots containing Levington M2 compost. These were then placed on benches measuring 146 x 78 x

75 cm (length x width x height) in a glasshouse where the environmental conditions had been pre-set approximately one week before use (Section 2.1). Twenty three days after germination, uniform seedlings were transplanted into 12 l black plastic pots containing M2 compost. The experiment was a split-plot 2 x 4 factorial replicated three times, and each treatment contained 42 plants.

Before imposing water stress, the pots were maintained as described in Section 2.5. Following the appearance of the first flower buds (26 d after germination), water stress treatments were imposed using the method described in Section 2.5. Four watering treatments were applied: replacement of water at 100% of ETP (evapotranspiration) served as a "no stress" control (NS), while watering at rates of 75, 50 and 25% of ETP provided progressive "medium stress" (MS), "high stress" (HS) and "severe stress" (SS) treatments. Two light treatments were also included, as described in Section 2.4. These were a "high irradiance" treatment (HI) which comprised the incident radiation received within the glasshouse plus supplementary light and a "low irradiance" treatment (LI) which provided c. 33% of HI (Plate 5.1.1).

Non-destructive measurements were taken throughout the experimental period to determine the timecourse of treatment effects. The numbers of flowers present were counted daily and records of the times required for primary and secondary flowers to reach anthesis or abscission were compiled and expressed in terms of both chronological and thermal time (T_b = 6.0 °C). Periodic destructive analyses were conducted to establish treatment effects on the timecourses of vegetative and reproductive growth and development, as described in Section 2.3. Environmental conditions within the glasshouse were recorded throughout the experimental period and accumulated total radiation (Section 2.4) and thermal time (Chapter 4) were calculated. Stomatal diffusive conductance, net photosynthesis, transpiration and the water use ratio of individual leaves were measured at weekly intervals using an LCA-3 portable IRGA (cf. Section 2.5) to examine the effects of progressive stress. Midday water potentials (ψ_l) were measured weekly in the youngest fully expanded leaf of four plants selected randomly from each treatment per block using a pressure

a

PLATE 5.1.1. *NS-LI (a) and NS-HI (b) at 7 DAT.*

b

chamber. These samples were then used to measure osmotic potential (ψ_s), and turgor potential (ψ_p) was calculated as the difference between the water and osmotic potentials. The procedures involved were described in Section 2.5.

5.1.2 Results

Environmental conditions

Irradiance During the 50 d period between the appearance of visible flower buds and final harvest, total radiation receipts for plants in the HI and LI irradiance treatments were 457 and 139 MJ m^{-2} respectively (Figure 5.1.1a). These values corresponded to mean daily irradiances of 9.1 and 2.8 MJ m^{-2} d^{-1}, with ranges of 6.4 -13.3 and 2.1 - 4.2 MJ m^{-2} d^{-1} in the HI and LI treatments respectively (Figure 5.1.1b). The high irradiance treatment allowed normal development of the flowers to anthesis, but low irradiance induced flower abortion. The radiation received by plants in the LI treatment over the entire experimental period was only 30-34% of that in the HI treatment.

Temperature Over the same period, total accumulated thermal time was about 983.5 °C d, which was equivalent to a mean daily value of 20.1 °C d (Figure 5.1.2a). Thermal time was calculated from the mean daily air temperatures and the cardinal temperatures for germination determined earlier (cf. Chapter 4). The daily maximum and minimum temperatures fluctuated between 28.8 - 35.2 and 20.2 - 24.6 °C respectively. However, the variation in the mean daily temperature was smaller, ranging between 25.6 - 27.0 °C (Figure 5.1.2b).

Stomatal diffusive conductance In general, stomatal conductance was decreased significantly ($p<0.001$) by water stress at both high and low irradiance. Stomatal conductances were significantly lower in the LI treatment ($p<0.001$), particularly at 14 and 21 d after the start of the treatment (DAT; Figure 5.1.3) and in the most severely stressed plants ($p<0.001$).

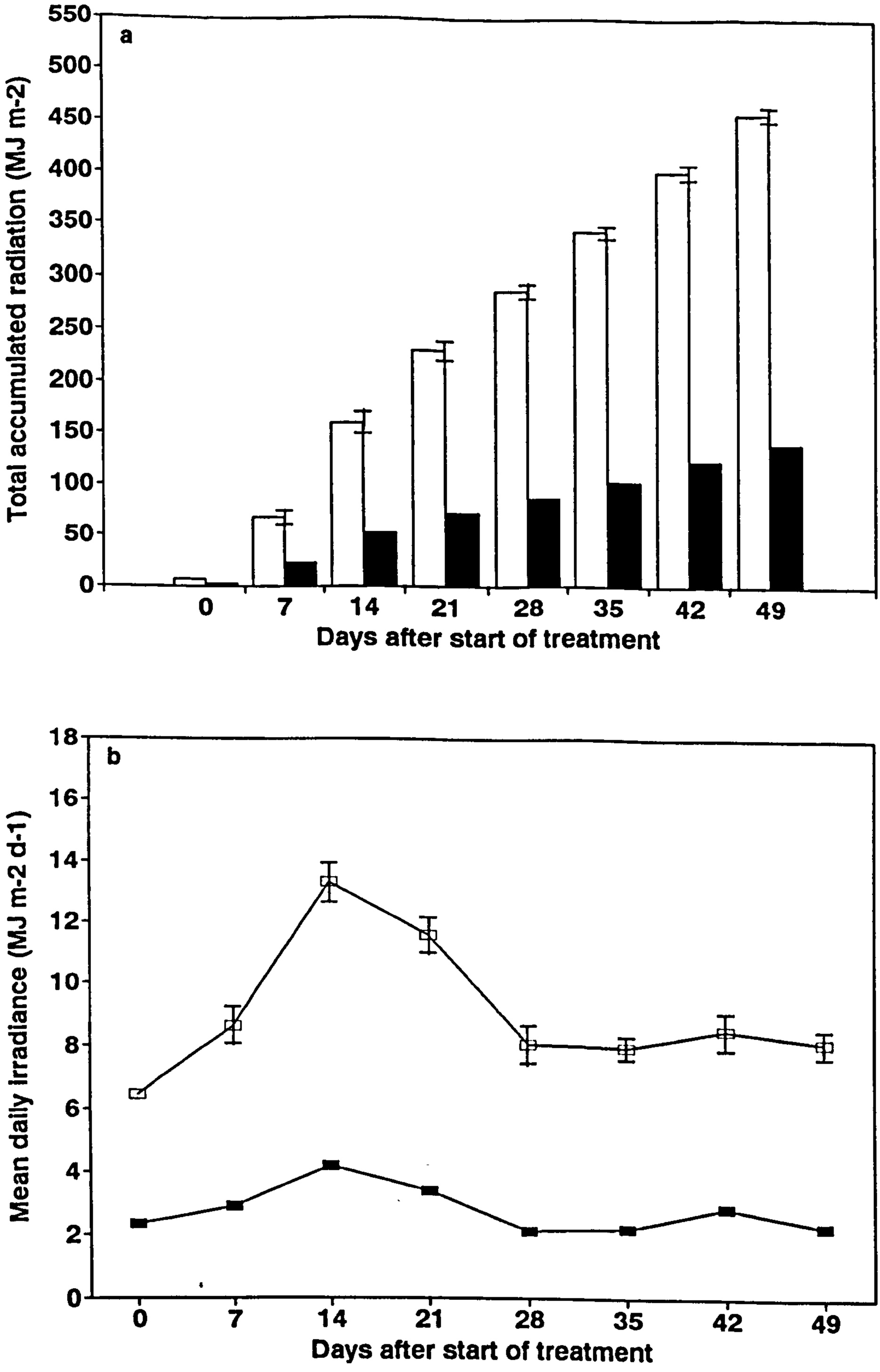


FIGURE 5.1.1. *Timecourses of (a) total accumulated radiation and (b) mean daily irradiance under glasshouse conditions.* □, *HI;* ■, *LI. n=6. Bars represent the Standard Error of the Difference between means.*

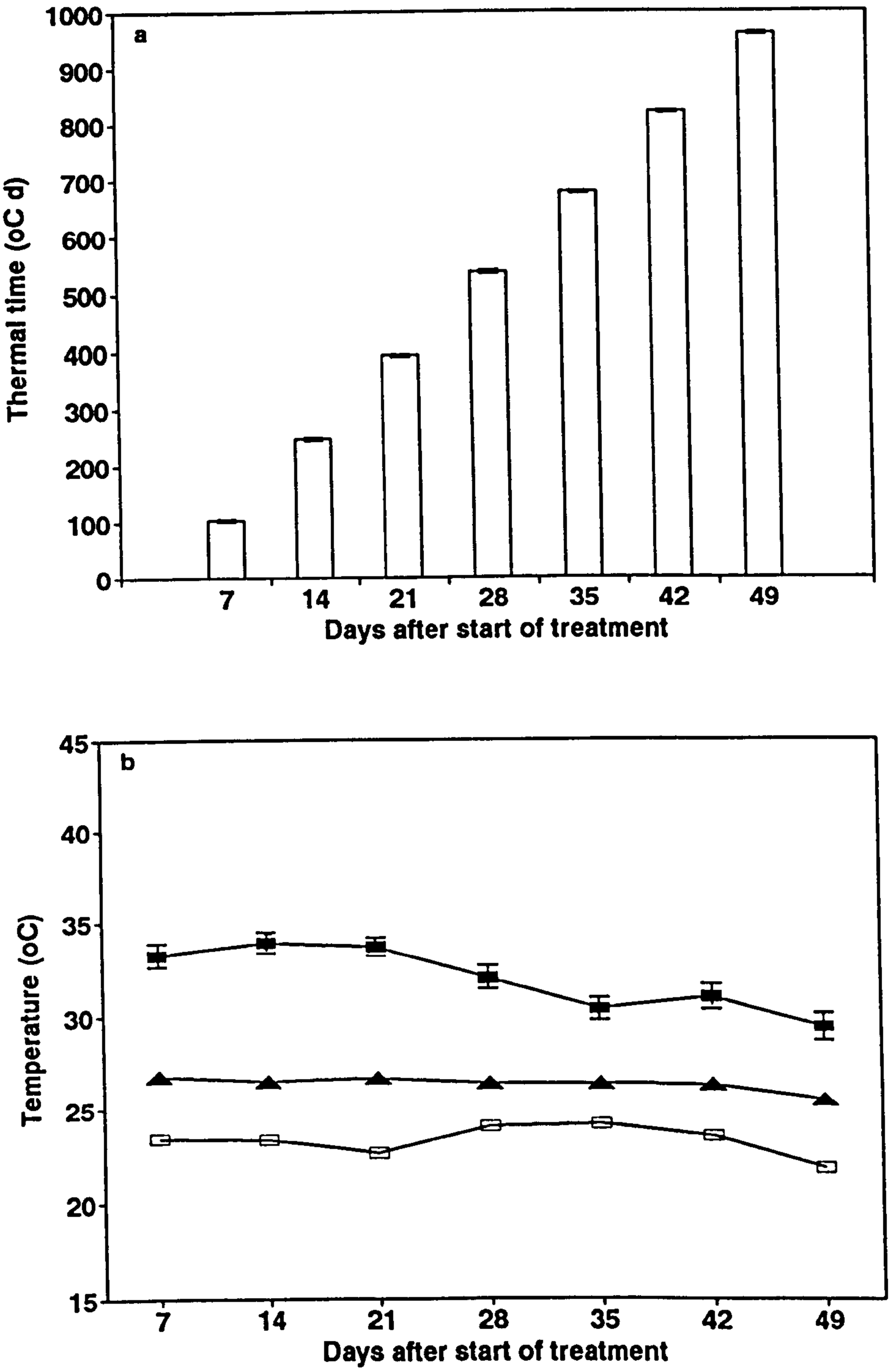


FIGURE 5.1.2. *Timecourses of (a) accumulated thermal time (°C d) and (b) weekly mean temperature.* ■, *maximum temperature;* □, *mean temperature;* ▲, *minimum temperature. n=3.*

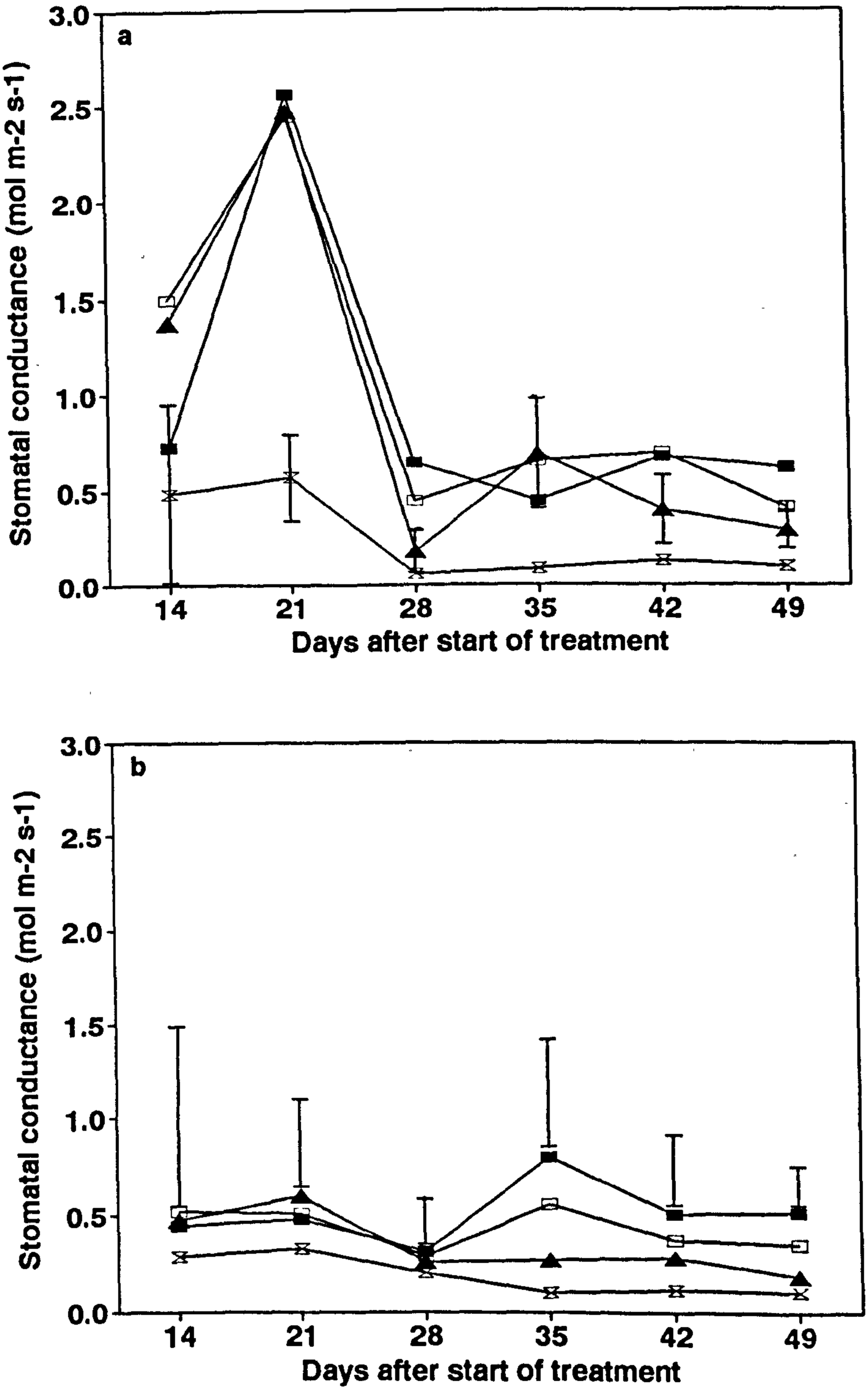


FIGURE 5.1.3. *Timecourses showing the effects of water stress on stomatal conductance under (a) high (HI) and (b) low irradiance (LI) conditions.* ■, *NS;* □, *MS;* ▲, *HS;* X, *SS. n=12. Bars represent the Standard Error of the Difference between means.*

Net photosynthesis and transpiration Assimilation by the leaves did not differ significantly between treatments, although there was a general trend for photosynthesis to decline towards the end of the experiment (Figure 5.1.4). At 14 and 21 DAT, during a period of relatively high solar radiation receipts, net photosynthesis was higher in the HS than in any of the other watering treatments ($p<0.05$). Transpiration also showed no significant difference between treatments and the timecourses of changes corresponded closely with those for net photosynthesis (data not presented).

Water use ratio (WUR) The instantaneous water use ratio of single leaves was calculated as the ratio of CO_2 uptake to H_2O loss determined from the IRGA measurements. There was no significant difference in WUR between watering treatments under either HI or LI conditions, although the values were consistently higher in the HS treatment under LI conditions (Figure 5.1.5). However, the pooled data for all watering treatments clearly show that WUR was much lower in the LI treatment, and decreased sharply towards the end of the experimental period ($p<0.001$). These results indicate that low irradiance did not improve the efficiency of water use during the assimilation of CO_2, but instead its adverse effect became more pronounced as the experiment progressed.

Flower and reproductive development

Anthesis was not reached in either primary or secondary flowers under LI conditions as the developing flower buds began to abscind from 13 DAT onwards. For this reason, analysis of the effects of water stress on the time required for the first primary and secondary flowers to reach anthesis was restricted to the HI treatment.

Observations during the first 30 DAT showed that the SS treatment induced early anthesis in the primary flowers at 15 DAT, which corresponded to a thermal time of 329 °C d ($p<0.05$; Table 5.1.1). However, this was immediately followed by almost complete flower abscission (97%; Table 5.1.2). Time to anthesis in the secondary flowers was not affected by the water stress treatments imposed. The percentage

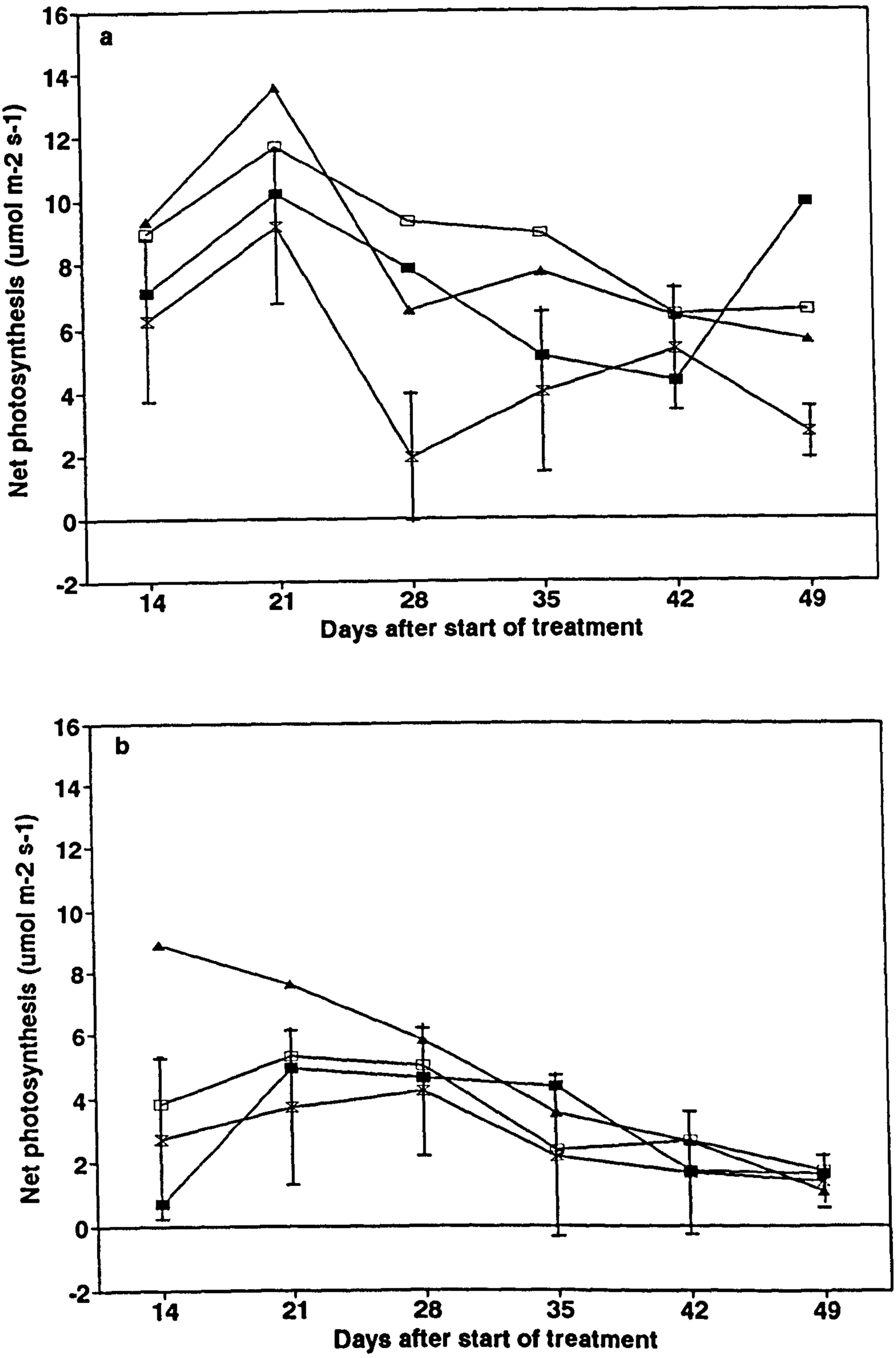
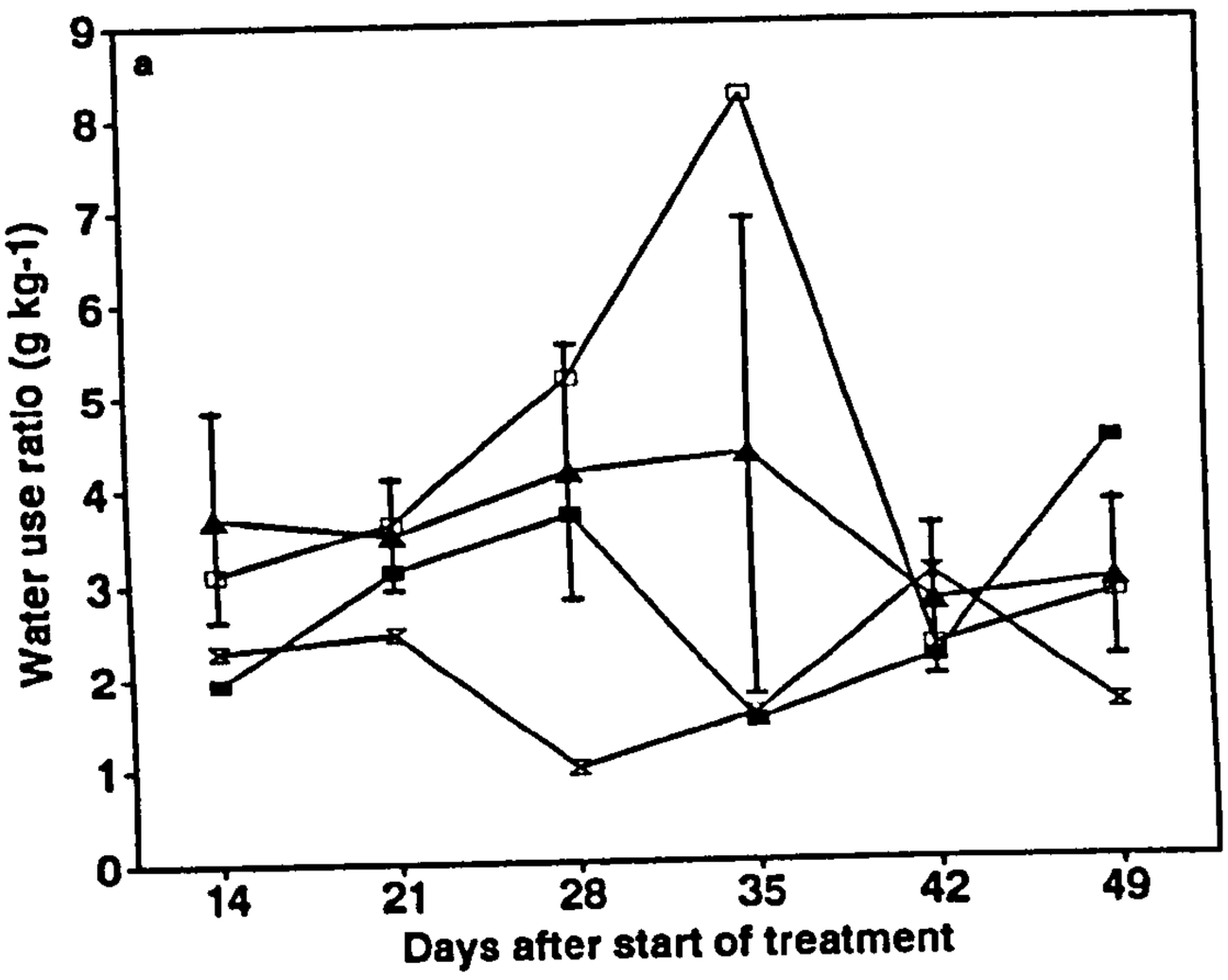
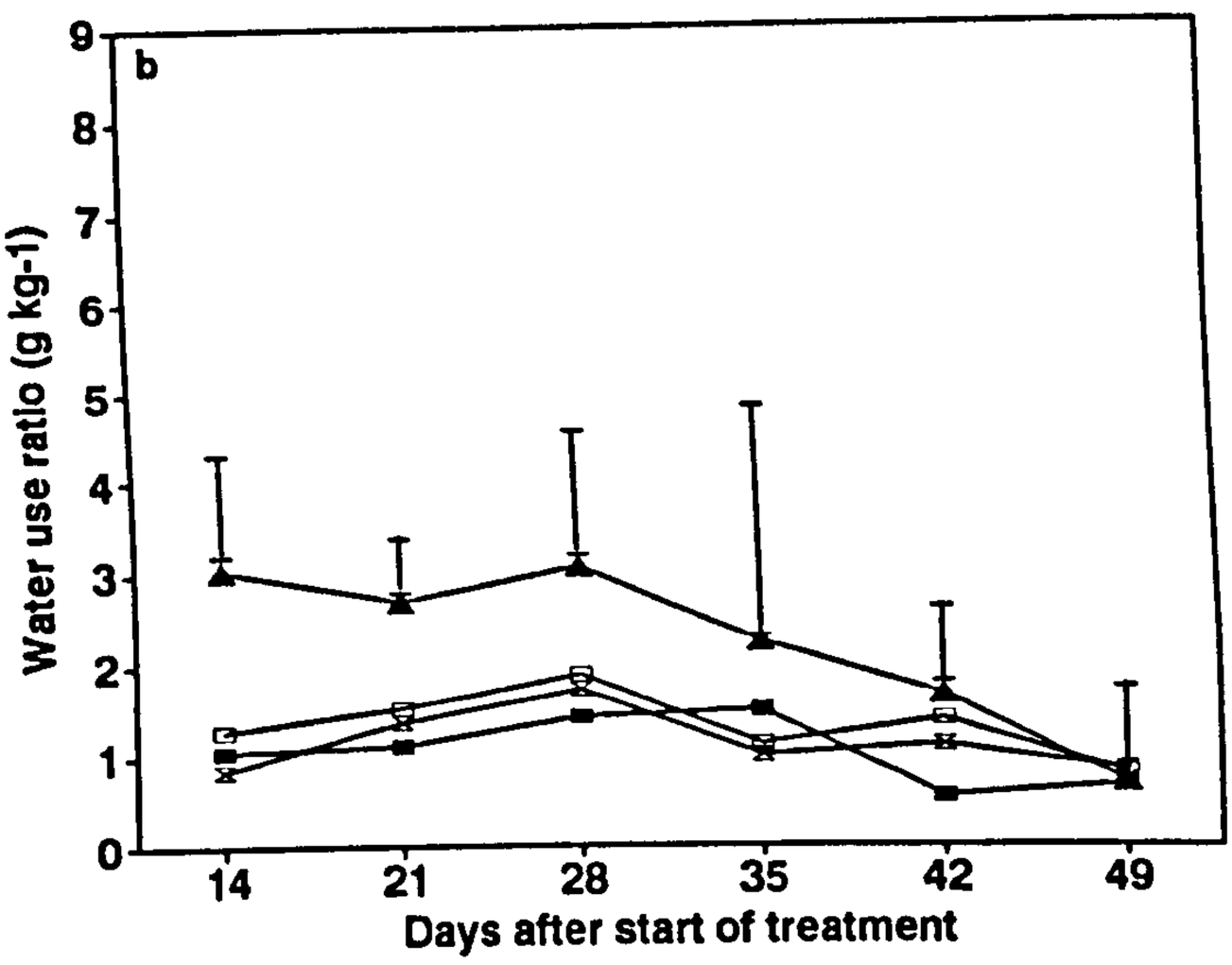


FIGURE 5.1.4. *Timecourses showing the effects of water stress on net photosynthesis under (a) high (HI) and (b) low irradiance (LI) conditions.* ■, *NS;* □, *MS;* ▲, *HS;* ⧖, *SS. n=12. Bars represent the Standard Error of the Difference between means.*

FIGURE 5.1.5. *Timecourses showing the effects of water stress on water use ratio under (a) high (HI) and (b) low irradiance (LI) conditions.* ■, *NS;* □, *MS;* ▲, *HS;* ⧖, *SS. c shows the pooled data for all water stress treatments;* ■, *HI;* □, *LI. n=12 (a and b) or n=24 (c). Bars represent the Standard Error of the Difference between means.*


a
Water use ratio (g kg-1)
0
1
2
3
4
5
6
7
8
9
14
21
28
35
42
49
Days after start of treatment


b
Water use ratio (g kg-1)
0
1
2
3
4
5
6
7
8
9
14
21
28
35
42
49
Days after start of treatment

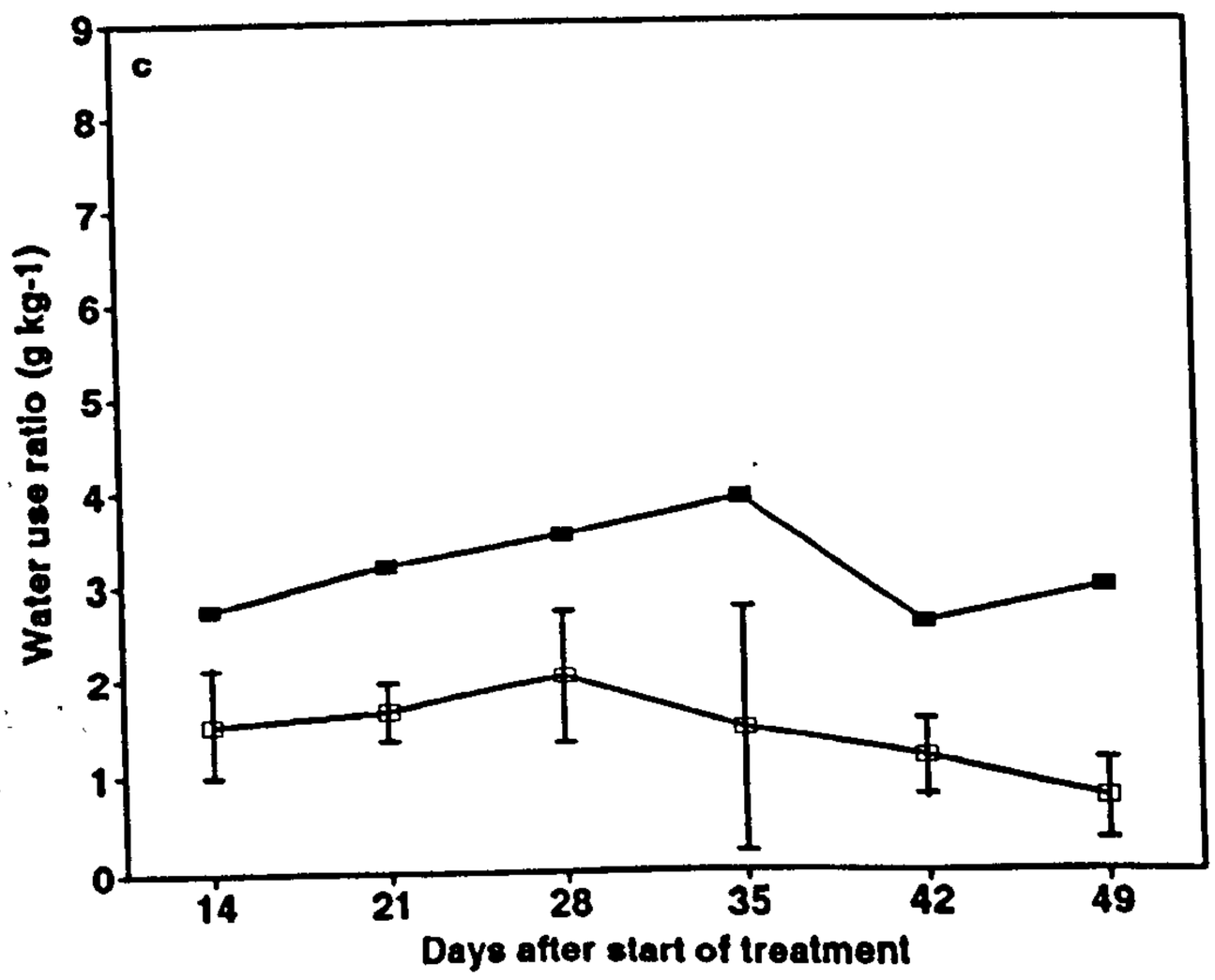

c
Water use ratio (g kg-1)
0
1
2
3
4
5
6
7
8
9
14
21
28
35
42
49
Days after start of treatment

TABLE 5.1.1. *Effects of irradiance and water stress on the time to first anthesis and first flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Irradiance (I)	Water stress (WS)	Days to first flower				Degree days to first flower			
		anthesis		abscission		anthesis		abscission	
		1[a]	2[b]	1	2	1	2	1	2
High	NS	18	19	20	20	391	418	438	480
	MS	19	20	22	21	411	431	466	459
	HS	18	20	19	20	384	425	418	439
	SS	15	20	16	20	329	432	357	432
Low	NS	-	-	14	13	-	-	301	294
	MS	-	-	14	13	-	-	301	287
	HS	-	-	14	14	-	-	315	301
	SS	-	-	14	13	-	-	308	281
SED(I)				1.09^{*}	0.66^{**}			22.44^{*}	4.39^{***}
SED(WS)		0.97^{*c}	0.45^{nsc}	1.18^{ns}	1.75^{ns}	20.15^{*c}	9.29^{nsc}	24.24^{ns}	25.67^{ns}
SED(I*WS)				1.81^{ns}	2.24^{ns}			37.21^{ns}	31.74^{ns}

a: primary flowers
b: secondary flowers
c: analyses restricted to water stress effect only
* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$
ns: not significant

TABLE 5.1.2. *Effects of irradiance and water stress on the total number of flower buds per plant and percentage anthesis and abscission of the primary and secondary flowers (n=18). SED denotes the Standard Error of the Difference between means.*

Irradiance (I)	Water stress (WS)	Flower number		% anthesis		% abscission	
		1[a]	2[b]	1	2	1	2
High	NS	2.2	2.8	47	82	31	34
	MS	1.6	2.7	41	72	84	32
	HS	1.5	2.8	38	70	89	40
	SS	2.1	2.6	35	77	97	50
Low	NS	2.3	2.2	0	0	100	100
	MS	2.3	2.3	0	0	100	100
	HS	2.4	2.2	0	0	100	100
	SS	2.3	2.2	0	0	100	100
SED(I)		0.01^{***}	0.09^{*}	3.67^{**}	5.01^{**}	2.74^{*}	3.98^{**}
SED(WS)		0.19^{ns}	0.18^{ns}	8.45^{ns}	6.13^{ns}	5.22^{***}	3.98^{ns}
SED(I*WS)		0.24^{ns}	0.24^{ns}	10.98^{ns}	9.03^{ns}	6.95^{***}	6.29^{ns}

a: primary flowers
b: secondary flowers
* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$
ns: not significant

anthesis values for primary and secondary flowers showed little effect of water stress under high irradiance, whereas under the LI conditions, all primary or secondary flower buds failed to reach anthesis (Table 5.1.2; $p<0.01$). Low irradiance also significantly increased the number of primary flowers ($p<0.001$), but also encouraged earlier bud abscission in both primary ($p<0.05$) and secondary ($p<0.01$) flowers (Table 5.1.1). In contrast, the number of secondary flowers was decreased by the LI condition ($p<0.05$). Complete flower abscission occurred in all watering treatments under LI conditions, and high percentage abscission values were observed for the primary flowers in the SS-HI, HS-HI and MS-HI treatments, with abscission being significantly lower in the NS-HI treatment (Table 5.1.2; $p<0.001$). In the secondary flowers, the watering treatment had little effect on percentage flower abscission, but the LI treatment again caused 100% abscission ($p<0.01$).

At final harvest (50 DAT), the number of other flowers (excluding primary and secondary flowers) and the dry weights of other flowers (bud diameter > 2 mm) and of fruits developed from primary and secondary flowers were measured (Table 5.1.3). The number of other flowers was significantly increased by the HS and MS treatments and by the HI conditions ($p<0.05$), and the corresponding dry weights were also higher ($p<0.01$ and 0.05 respectively; Table 5.1.3). The unstressed plants produced fewest 'other' flowers, possibly due to the suppression of later flowering by the fast developing fruits in this treatment (Ho and Hewitt, 1986). The dry weight of fruit formed from the primary and secondary flowers was significantly greater in the NS treatment under HI conditions, followed successively by the MS-HI and HS-HI treatments ($p<0.05$). However, no fruits were formed due to complete flower abscission under SS-HI and all LI conditions. The flowers produced in the severely stressed treatments were invariably smaller in size and had shorter petioles.

Vegetative growth and development

The effects of irradiance and water stress on vegetative growth and development at the end of the 50 d experimental period are summarised in Tables 5.1.4 and 5.1.5.

TABLE 5.1.3. *Effects of irradiance and water stress on the total numbers and dry weights of flowers and fruits per plant at final harvest (n=18). SED denotes the Standard Error of the Difference between means.*

Irradiance (I)	Water stress (WS)	Other flowers[a] (number)	Dry weight (g)	
			other flowers	fruits[b]
High	NS	39	0.6	20.4
	MS	54	0.9	8.7
	HS	60	0.7	0.9
	SS	47	0.4	0.0
Low	NS	30	0.3	0.0
	MS	47	0.4	0.0
	HS	45	0.4	0.0
	SS	22	0.2	0.0
SED(I)		2.7*	0.05*	1.71*
SED(WS)		6.3*	0.06**	2.42*
SED(I*WS)		8.2ns	0.09ns	3.29*

a: excluding primary and secondary flowers
b: from primary and secondary flowers
* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$
ns: not significant

Leaves Leaf growth and development were more severely reduced by water stress ($p<0.001$) than by low irradiance, but there was no significant interaction ($p<0.01$; Table 5.1.4). Total leaf number was reduced in the severe stress treatment relative to the NS control by 39 and 44% at high and low irradiance, but was increased in the MS treatment by 47 and 5% respectively. The secondary leaves (i.e. those growing from the point of branching subtending the primary flowers) were affected by severe stress in a similar manner to total leaf number ($p<0.001$). Leaf dry weight was 40 and 47% lower in the SS-HI and SS-LI treatments than in the NS control and 31% and 46% higher in the corresponding MS treatments. Leaf area was significantly reduced in the HS and SS treatments and exhibited a ranking of MS > NS > HS > SS. Low irradiance also reduced total leaf number and the number of secondary leaves per plant ($p<0.01$), although total leaf dry weight and leaf area were not significantly affected. At the end of the experiment, the basal leaves showed no symptoms of senescence or abscission due to prolonged progressive stress.

Leaf areas were significantly reduced by the SS and HS treatments and specific leaf area (SLA) was significantly lower in all water stress treatments ($p<0.01$) or at HI ($p<0.05$), indicating either that the leaves were thicker or their density was greater, perhaps because of increased wall thickness. The leafiness of the plants, as indicated by their Leaf Area Ratio (LAR), was decreased in all stress treatments under HI conditions, but was significantly greater in the NS and MS than in the HS and SS treatments under LI conditions ($p<0.01$).

Stems The effects of irradiance and water stress on plant growth are presented in Table 5.1.5. Total shoot and stem dry weights were significantly reduced in the SS treatment under both HI and LI conditions, with the smallest values being recorded in the SS-LI treatment ($p<0.001$). The SS treatment also greatly reduced total plant height, branch numbers and stem diameter ($p<0.001$) under both high and low irradiance conditions. The shorter plant height in the SS treatment resulted from a significant reduction in shoot extension from the point of branching to the shoot tip ($p<0.001$). The LI treatment consistently produced taller plants with fewer branches ($p<0.05$), a smaller stem diameter and reduced stem dry weights ($p<0.01$).

TABLE 5.1.4. *Effects of irradiance and water stress on leaf growth and development at final harvest (n=18). SED denotes the Standard Error of the Difference between means.*

Irradiance (I)	Water stress (WS)	Leaves per plant				SLA[b]	LAR[c]
		total (no.)	secondary[a] (no.)	dry wt. (g)	area (cm^2)	(cm^2 g^{-1} leaf)	(cm^2 g^{-1} plant)
High	NS	123	114	15.9	5921	376	97
	MS	181	172	20.9	6172	300	90
	HS	111	102	15.3	4189	277	92
	SS	75	66	9.5	2250	239	82
Low	NS	94	85	11.8	6098	516	220
	MS	99	90	17.2	6517	404	193
	HS	86	77	12.5	4530	374	157
	SS	53	45	6.3	2275	364	133
SED(I)		3.6^{**}	3.54^{**}	1.7^{ns}	191.8^{ns}	27.3^{*}	13.78^{*}
SED(WS)		13.8^{***}	13.81^{***}	1.7^{***}	243.2^{***}	32.0^{**}	9.72^{***}
SED(I*WS)		17.3^{ns}	17.28^{ns}	2.7^{ns}	354.3^{ns}	47.8^{ns}	18.21^{**}

a: Secondary leaves above the primary flowers

b: Specific Leaf Area (cm^2 g^{-1} leaf)

c: Leaf Area Ratio (cm^2 g^{-1} plant)

* significant at $p<0.05$

** significant at $p<0.01$

*** significant at $p<0.001$

ns: not significant

TABLE 5.1.5. *Effects of irradiance and water stress on total shoot dry weights at 25 and 50 DAT and on shoot characteristics at final harvest (n=18). SED denotes the Standard Error of the Difference between means.*

Irradiance (I)	Water stress (WS)	Total shoot[a] dry wt. at DAT (g)		Plant height (cm)	Total branch number	Stems		
		25	50			length[b] (cm)	dry wt. (g)	Diameter (mm)
High	NS	18.0	62.3	103	87	73	25.7	14
	MS	19.0	58.2	103	97	73	27.7	13
	HS	18.1	37.1	87	79	58	20.1	12
	SS	14.9	21.9	65	64	40	11.9	10
Low	NS	7.4	23.1	110	52	75	11.1	11
	MS	7.6	30.2	113	65	80	12.6	10
	HS	8.6	21.7	107	59	73	11.9	9
	SS	8.5	13.9	85	44	49	7.5	8
SED(I)		0.7^{***}	2.8^{*}	2.6^{*}	5.3^{*}	2.1^{ns}	1.0^{**}	0.1^{**}
SED(WS)		0.6^{ns}	1.9^{***}	3.0^{***}	4.3^{***}	3.3^{***}	0.9^{***}	0.3^{***}
SED(I*WS)		1.0^{**}	3.6^{***}	4.5^{ns}	7.5^{ns}	4.6^{ns}	1.5^{***}	0.4^{ns}

a: root dry weight not included

b: length from the point of branching to the tip of the shoot

* significant at $p<0.05$

** significant at $p<0.01$

*** significant at $p<0.001$

ns: not significant

Dry matter distribution

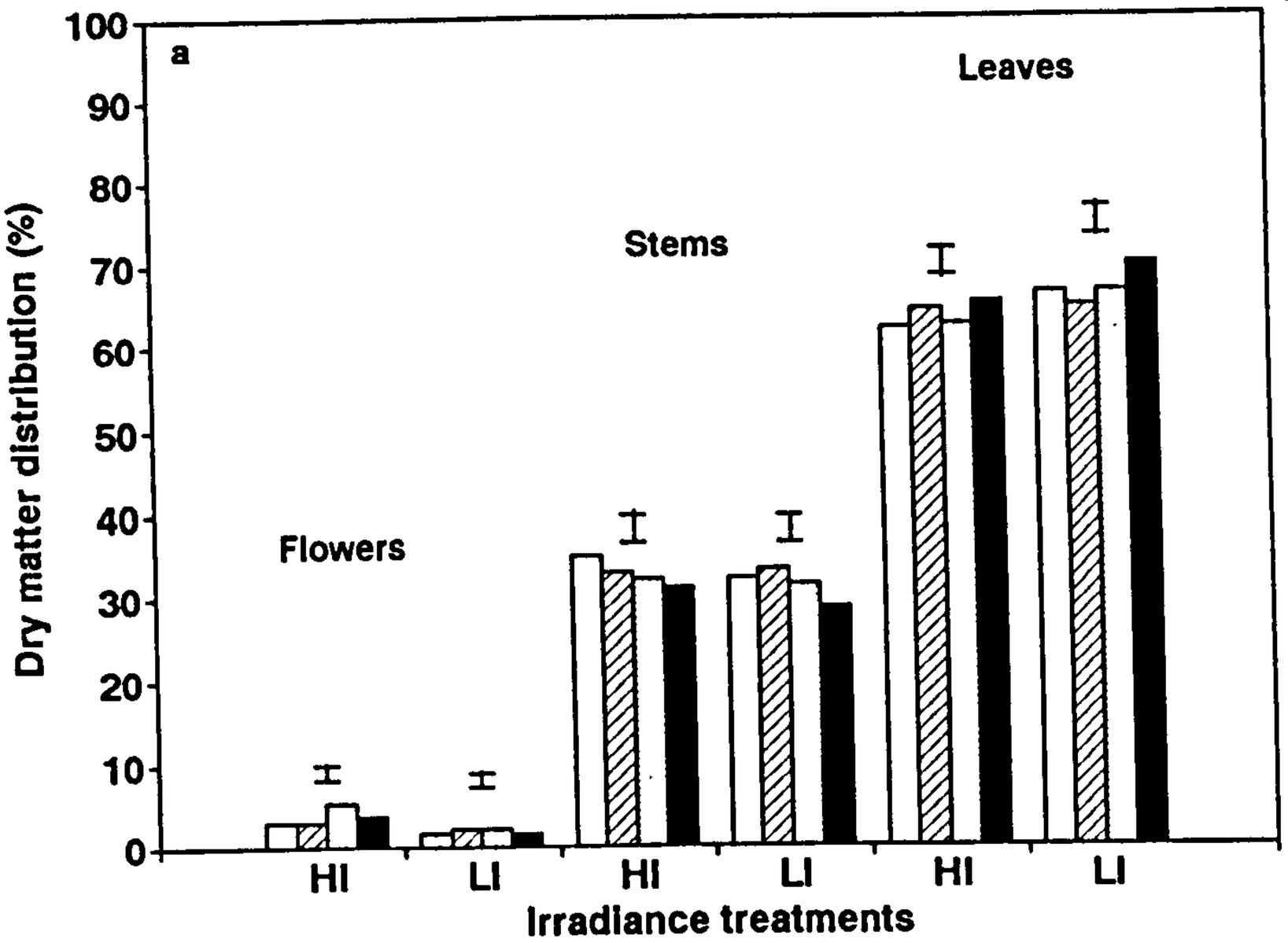
Total shoot dry weight was reduced by the interaction between LI and SS (Table 5.1.5). At 25 DAT, shoot dry matter was significantly lower in all watering treatments under LI conditions and in the SS-HI treatment than in the NS-HI control ($p<0.01$). This reduction in dry weight was even more pronounced at 50 DAT.

In general, the percentage of dry matter partitioned to the flowers+fruits and stems increased during the treatment period, while that partitioned to the leaves decreased (Figure 5.1.6). At 25 DAT, dry matter distribution within the plant was significantly affected by irradiance ($p<0.01$), but not by either water stress or any interaction between irradiance and water stress. Low irradiance promoted a greater accumulation of dry matter in the leaves at the expense of the flowers and stems. The percentage dry matter present in the leaves at 25 DAT was invariably greater than in the stems and flowers.

At 50 DAT, dry matter distribution was influenced by the interaction between irradiance and water stress, except in the stems, which were more sensitive to water stress. Dry matter partitioning to the leaves was greater under LI than HI conditions, particularly in the MS-LI and NS-LI treatments. Under HI conditions, the highest percentage of dry matter was partitioned to the severely stressed leaves ($p<0.01$), while the reverse occurred for the partitioning of dry matter to the flowers+fruits. High irradiance increased dry matter distribution to the flowers+fruits in the NS-HI treatment, followed by the MS-HI, HS-HI and SS-HI treatments ($p<0.01$). Plants in the severe and high water stress treatments also accumulated a greater proportion of their dry matter into the stems.

Plant water relations

Leaf water (ψ_l), osmotic (ψ_s) and turgor potentials (ψ_p) were all significantly decreased by water stress ($p<0.001$; Figure 5.1.7); osmotic potential was also reduced by high irradiance ($p<0.001$; Figure 5.1.8). The extent of the reductions in



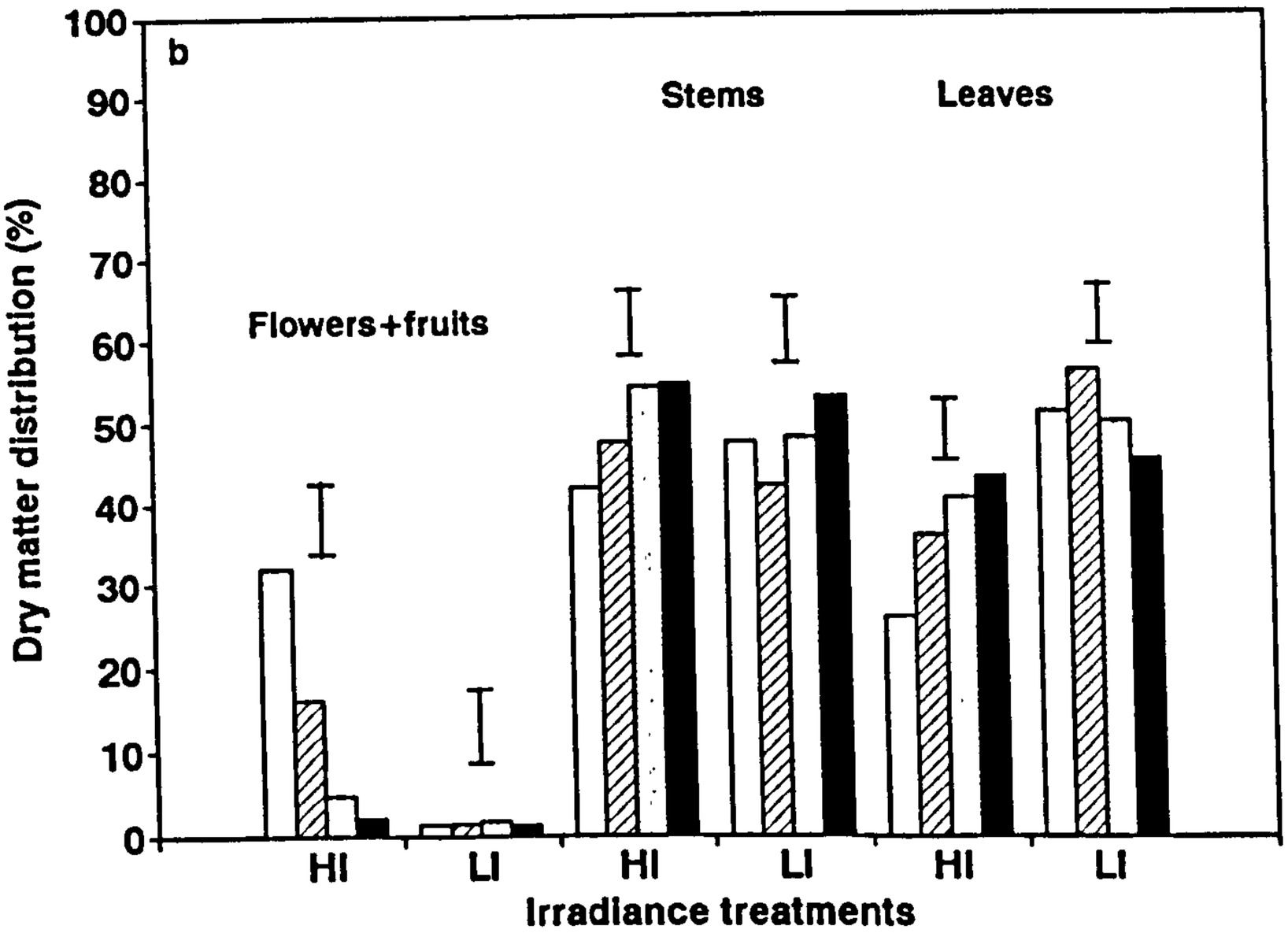
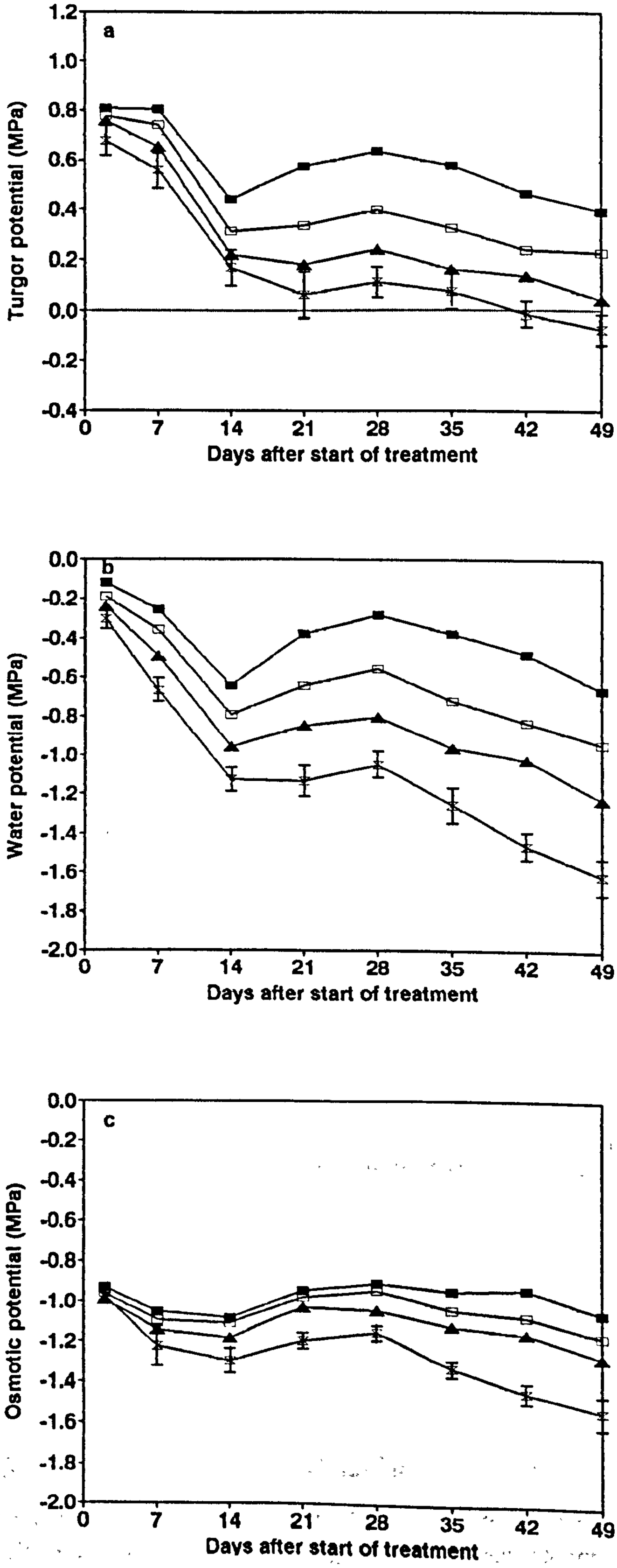


FIGURE 5.1.6. *Effects of water stress on dry matter distribution at (a) 25 DAT and (b) 50 DAT under high (HI) and low irradiance (LI) conditions.* □, *NS;* ▨, *MS;* ⊡, *HS;* ■, *SS. n=18. Bars represent the Standard Error of the Difference between means.*

FIGURE 5.1.7. *Effects of water stress on the timecourses for the components of leaf water potential.* ■, *NS;* □, *MS;* ▲, *HS;* ⧗, *SS. n=24. Bars represent the Standard Error of the Difference between means.*


a
Turgor potential (MPa)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
7
14
21
28
35
42
49
Days after start of treatment
b
Water potential (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
Days after start of treatment
c
Osmotic potential (MPa)
Days after start of treatment

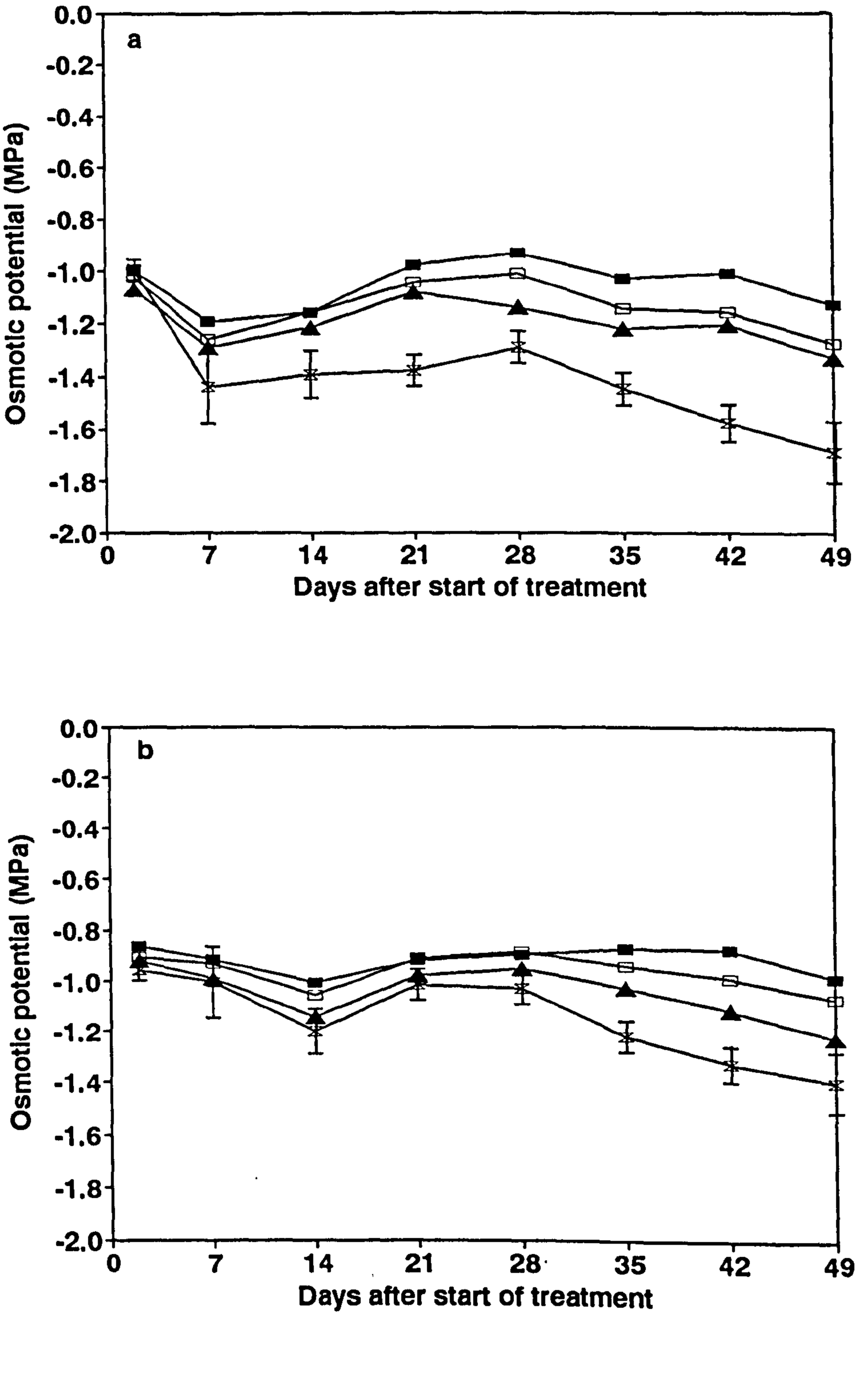


FIGURE 5.1.8. *Effects of (a) high (HI) and (b) low irradiance (LI) conditions on the timecourses for osmotic potential.* ■, *NS;* □, *MS;* ▲, *HS;* X, *SS. n=24. Bars represent the Standard Error of the Difference between means.*

the components of water potential consistently followed the treatment order SS > HS > MS > NS. As the stress period progressed, the values for each component gradually decreased and the treatment effects increased (Figure 5.1.7); for instance, ψ_l in SS decreased from -0.30 to -1.62 MPa during this period. The significantly lower values for ψs in the SS treatment relative to the NS control suggest that some osmotic adjustment occurred in response to water stress and that this may have minimised the reduction in cell turgor. However, noticeable reductions in turgor were apparent in all treatments by 14 DAT, when the ψ_l values ranged from -0.64 MPa in NS to -1.13 MPa in SS. Leaf turgor potentials determined immediately prior to anthesis and the abscission of primary flowers in SS-HI were 0.10 and -0.01 MPa respectively. The corresponding ψ_l values were -1.29 MPa during anthesis and -1.39 MPa during flower abscission, while the ψ_s values were -1.39 and -1.38 MPa, respectively.

5.2 Effects of the severity and duration of water stress imposed at macroscopic flower bud-visible stage on dry matter distribution during flower development

Experiment 5.1 examined the effects of three levels of stress applied progressively after the flower bud-visible stage (BVS). It was evident that, although the imposition of stress at BVS accelerated anthesis in the primary flowers, prolonged severe water stress under natural glasshouse irradiance conditions resulted in severe flower abscission. Although shoot biomass and vegetative growth were reduced in plants showing flower abscission, the results obtained provide little information on the pattern of dry matter distribution prior to anthesis and flower abscission. It is known that the restriction of vegetative growth by stresses imposed when both flowers and leaves are developing may increase the supply of assimilates to the flowers, thereby promoting their development and reducing the incidence of flower abscission (Halevy, 1975, 1987). However, the imposition of severe or prolonged stress over this period may also reduce assimilation sufficiently to induce the abscission of flowers (Russell and Morris, 1982; Halevy, 1987; Morris and Newell, 1987). In contrast, mild or short

duration stress induces limited and reversible effects which may be rapidly reversed upon watering by a transitory phase of more rapid growth than in the unstressed control plants (Bradford and Hsiao, 1982).

The aim of the following experiment was to examine the effects of the severity and duration of water stress on dry matter distribution between the vegetative and reproductive organs, and to establish the effects on flower abscission.

5.2.1 Materials and methods

Seeds of variety Blue Star were sown on 1 February 1993. Propagation and growth conditions were as described in Section 2.1. When the seedlings had produced their third pair of true leaves (1 cm long; 22 d after germination), they were transplanted from 9 cm diameter pots into 12 l black plastic pots containing Levington M2 compost. The water stress treatments were imposed 29 d after germination following the procedure described in Experiment 5.1.

At first appearance of macroscopic flower buds (8 - 10 true leaf stage), the pots were randomly allocated to four treatments, each containing 28 pots. The treatments included a control (NS), moderate stress (MS), high stress (HS) and severe stress (SS) treatments. Two weeks after imposing the treatments, half of the plants in each treatment were rewatered and kept near pot capacity until the end of the experiment. Thus, from this point onwards there were seven treatments: a well watered control (NS) plus three levels of stress severity (MS, HS, SS) and two stress durations (long duration stress (LD) imposed from BVS until the end of experiment (35 d) and short duration stress (SD) imposed for the first two weeks after BVS). The treatments were arranged in a Randomised Complete Block Design containing three replicates; each treatment comprised 14 pots.

Growth measurements and destructive sampling were carried out at regular intervals as described in Section 2.3 to determine the effects of the various treatments on dry

matter production and distribution and flower abscission. The experiment lasted for 35 d.

5.2.2 Results

During the experimental period, the total irradiance received within the glasshouse was 365 MJ m^{-2}, with a mean of 10.4 MJ m^{-2} d^{-1}, which was sufficient to support normal flower growth and development. Total accumulated thermal time was 587 °C d, with a daily mean of 16.8 °C d. Relative humidity was maintained at approximately 70%.

Flower growth and development

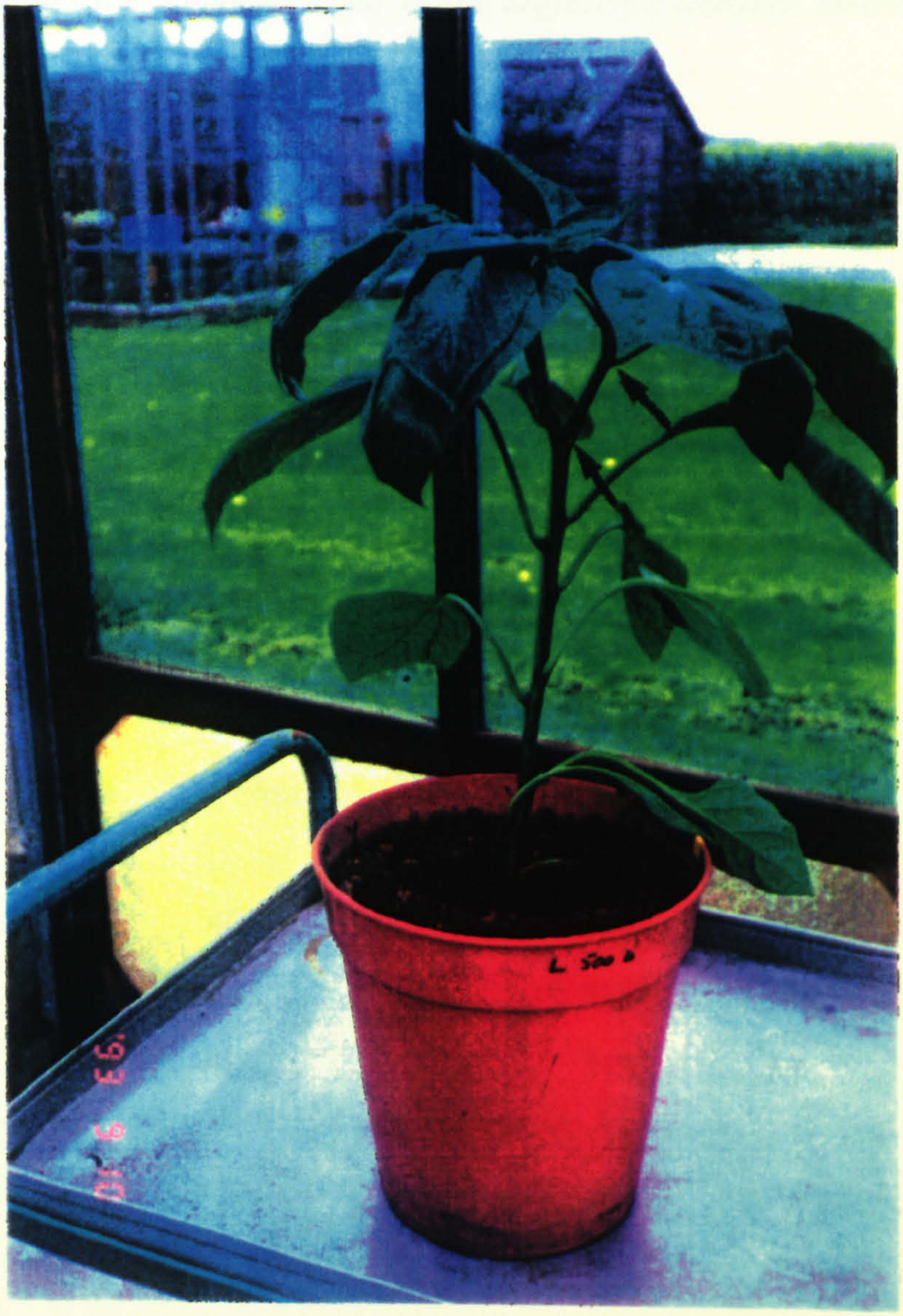
The primary and secondary flowers were both affected either by the severity of water stress or the interaction between the duration and severity of stress (Plate 5.2.1). The duration of stress alone had little effect on flower growth and development.

Primary flowers The thermal time required to reach anthesis was influenced by the interaction between the duration and severity of water stress. When the SS treatment was imposed for an extended period (35 d), first anthesis in the primary flowers was accelerated by 28 °C d as compared to the unstressed control (2 d; Table 5.2.1). No acceleration of anthesis was observed in the SD treatment. As in the previous experiment (Experiment 5.1), the accelerated anthesis was soon followed by increased flower abscission in the SS treatment, regardless of the stress duration ($p<0.05$; Table 5.2.1).

Secondary flowers Although the thermal time to first anthesis was not significantly affected by the treatments imposed, the secondary flowers showed earlier abscission in the long duration SS treatment than in the NS control, by 72 °C d ($p<0.05$). Percentage abscission was significantly higher in the HS (c. 43%) and SS (c. 30%) treatments ($p<0.001$) than in the NS control irrespective of stress duration. As for the primary flowers, the SS-SD treatment did not enhance anthesis or delay flower

a

PLATE 5.2.1. *NS-LD (a) and SS-LD (b) at 25 DAT. Arrows showing the absence of primary and secondary flowers in the SS-LD as compared to NS-LD.*



b

TABLE 5.2.1. *Effects of the duration and severity of stress on flower development (n=18). SED denotes the Standard Error of the Difference between means.*

Duration	Water stress (WS)	Thermal time (°C d) to first				Abscission (%)	
		anthesis		abscission			
		flower1[a]	flower2[b]	flower1	flower2	flower1	flower2
	NS	$351_{(22)}$[c]	$402_{(25)}$	$425_{(26)}$	$503_{(30)}$	40	14
Long	MS	$368_{(23)}$	$402_{(25)}$	$437_{(27)}$	$497_{(30)}$	39	27
(35 d)	HS	$357_{(22)}$	$397_{(24)}$	$448_{(27)}$	$521_{(31)}$	59	53
	SS	$323_{(20)}$	$380_{(23)}$	$396_{(24)}$	$431_{(26)}$	73	20
Short	MS	$346_{(21)}$	$402_{(25)}$	$424_{(26)}$	$515_{(31)}$	49	20
(14 d)	HS	$346_{(21)}$	$402_{(25)}$	$431_{(26)}$	$515_{(31)}$	44	37
	SS	$357_{(22)}$	$402_{(25)}$	$430_{(26)}$	$515_{(31)}$	54	39
SED(NS)		6.0^{ns}	7.9^{ns}	7.9^{ns}	16.9^{ns}	7.7^{ns}	6.7^{*}
SED(NS*D)		6.4^{ns}	8.5^{ns}	8.4^{ns}	18.0^{*}	8.3^{ns}	7.2^{ns}
SED(NS*WS)		6.8^{*}	9.0^{ns}	8.9^{**}	19.1^{*}	8.8^{*}	7.6^{***}
SED(NS*D*WS)		7.9^{***}	10.4^{ns}	10.3^{**}	22.1^{*}	10.1^{ns}	8.8^{ns}

a: primary flowers

b: secondary flowers

c: numbers in parentheses indicate DAT

* significant at $p<0.05$

** significant at $p<0.01$

*** significant at $p<0.001$

ns: not significant

abscission significantly.

Vegetative development

Measurements made immediately prior to first anthesis in the primary flowers showed a progressive decrease in total leaf area and branch number as the severity of stress increased, irrespective of its duration (Table 5.2.2). Long duration severe stress decreased leaf expansion by 9% ($p<0.01$) and the number of branching nodes by 80% ($p<0.05$) relative to the unstressed NS control. A similar effect was observed in the short duration, severe stress treatment. Total leaf number was not significantly affected by the treatments imposed, although there was a trend of decreasing values with increasing severity of stress. This restriction in vegetative development may have reduced competition for assimilates by the developing vegetative organs, thereby favouring the initial development of flowers in SS-LD. However, the equivalent short duration stress treatment (SS-SD) did not appear to hasten flower development, probably because of greater competition for assimilates by new leaves produced after the stress ended.

By first abscission, there were no longer any significant treatment effects on vegetative development, although total leaf number, leaf area and branch number were invariably lower in SS-LD than in the NS control.

Dry matter production and distribution

Figures 5.2.1 and 5.2.2 show the effects of the duration and severity of water stress on the timecourses of dry matter production and distribution. Their individual effects and the interaction between the duration and severity of stress had little influence on dry matter production and distribution. Dry matter accumulation (g) in the leaves and stems, but not the flowers, was decreased by severe stress ($p<0.05$; Figure 5.2.1) just prior to anthesis (18 DAT). Immediately after first abscission (25 DAT), no significant reductions in dry matter accumulation were observed for any of the shoot components in the SS treatment. Following more prolonged stress, the dry weights of the leaves

TABLE 5.2.2. *Effects of the duration and severity of water stress on vegetative growth and development before first anthesis and after first abscission (n=12). SED denotes the Standard Error of the Difference between means.*

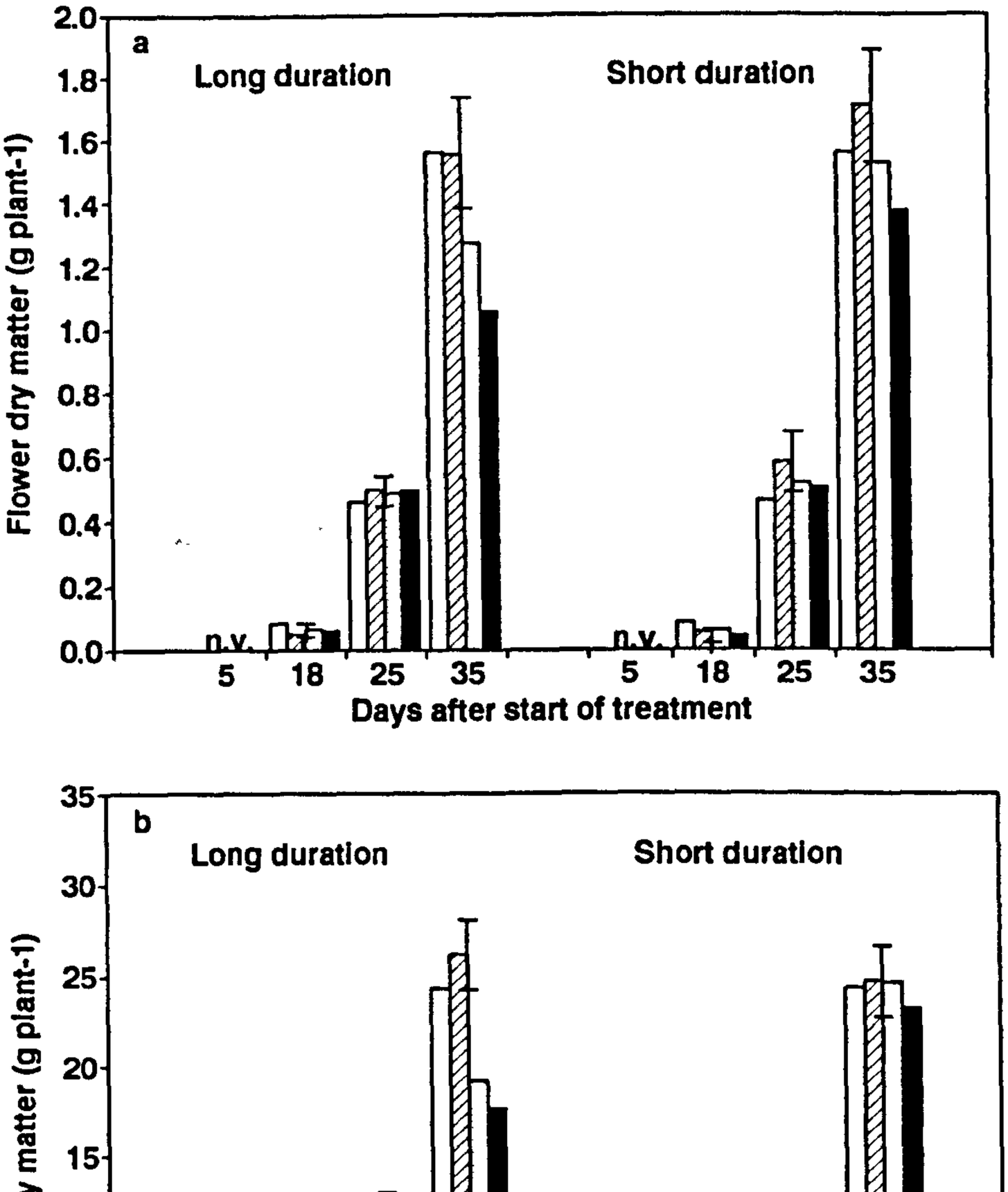
Duration	Water stress (WS)	Before anthesis (plant^{-1})			After abscission (plant^{-1})		
		leaf no.	leaf area (cm^2)	branch no.	leaf no.	leaf area (cm^2)	branch no.
	NS	26	1348	5	63	3280	41
Long	MS	26	1402	6	63	3191	39
(35 d)	HS	24	1248	2	57	3839	30
	SS	23	1220	1	54	2567	30
Short	MS	28	1436	6	56	3143	33
(14 d)	HS	25	1247	2	56	2933	31
	SS	21	1149	2	58	2934	27
SED(NS)		2.2^{ns}	61.6^{ns}	1.4^{ns}	5.7^{ns}	211.2^{ns}	4.6^{ns}
SED(NS*D)		2.3^{ns}	65.9^{ns}	1.6^{ns}	6.1^{ns}	225.8^{ns}	4.9^{ns}
SED(NS*WS)		2.5^{ns}	69.9**	1.6*	6.4^{ns}	239.5^{ns}	5.2^{ns}
SED(NS*D*WS)		2.9^{ns}	80.7^{ns}	1.9^{ns}	7.4^{ns}	276.5^{ns}	6.0^{ns}

* significant at $p<0.05$

** significant at $p<0.01$

ns: not significant

FIGURE 5.2.1. *Effects of the duration and severity of water stress on the timecourses of dry matter accumulation (g plant^{-1}) by (a) flowers, (b) leaves and (c) stems. □, NS; ▨, MS; ▩, HS; ■, SS. n=12. Bars represent the Standard Error of the Difference between means. n.v. denotes negligible values ≤0.05 g plant^{-1}.*


a
Long duration
Short duration
Flower dry matter (g plant-1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
n.y.
5
18
25
35
Days after start of treatment
b
Leaf dry matter (g plant-1)
35
30
25
20
15
10
5
0
c
Stem dry matter (g plant-1)

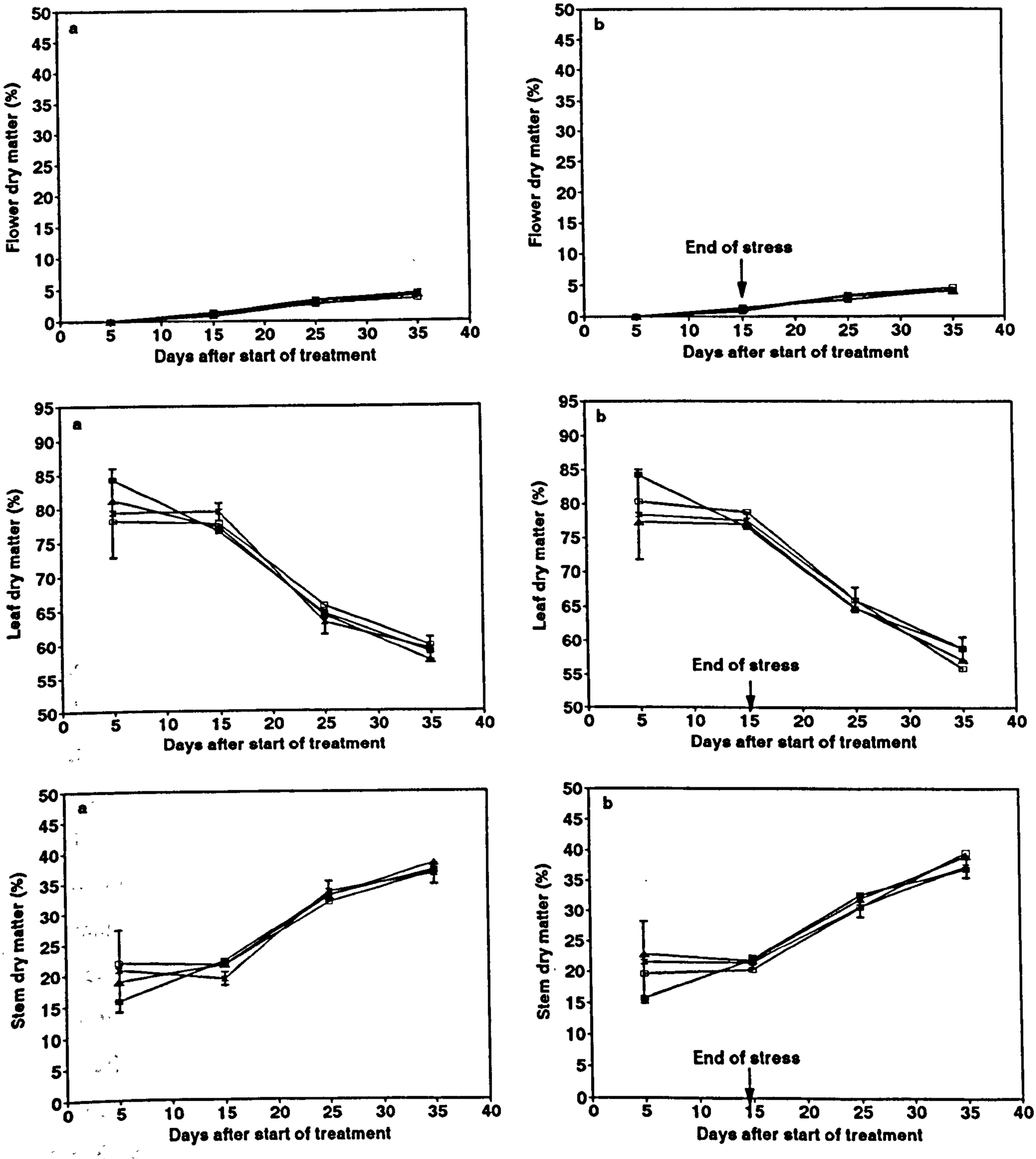


FIGURE 5.2.2. *Effects of the duration and severity of water stress on the timecourses of dry matter distribution (%) during (a) long duration and (b) short duration stress.* ■, *NS;* □, *MS;* ▲, *HS;* ⧖, *SS. n=12. Bars represent the Standard Error of the Difference between means.*

and stems at day 35 were greatly reduced in both the SS and HS treatments under LD conditions ($p<0.001$; Figure 5.2.1). The accumulation of assimilates by the flowers at 35 DAT was also significantly reduced by both prolonged (LD) and severe stress (SS).

Dry matter distribution (%) to the leaves, stems and flowers was not significantly affected by the treatments imposed either before anthesis (18 DAT) or shortly after abscission (25 DAT; Figure 5.2.2). This indicates that, although dry matter production before anthesis was reduced by the stress, its partitioning between plant organs remained unaffected. Although there were no significant treatment effects, dry matter distribution to the flowers and stems increased with time, whereas that to the leaves decreased. Similar trends were also observed for dry weight accumulation.

Water stress reduced shoot dry matter shortly before anthesis by 12 - 20% in the SS treatment ($p<0.05$; Table 5.2.3). This reduction resulted primarily from decreases in the dry weights of the leaves and stems (9 - 20 and 23% respectively), whereas the flowers were apparently unaffected by stress at this time. By first flower abscission, shoot dry weight was no longer significantly affected by the treatments imposed (Table 5.2.3). Similarly, the dry weights of the flowers, leaves and stems did not differ significantly between treatments, although the values for leaves and stems were again consistently lower in the more stressed treatments. With greater replication, these trends might well have proved significant. These results imply that, although the early restriction of vegetative development by stress may have increased the supply of available assimilates to the flowers, thereby promoting anthesis, the abscission of the primary flowers could not be linked directly with reductions in either total shoot dry weight or the dry matter content of the leaves.

Plant water relations

Figure 5.2.3 shows the timecourses for the components of leaf water potential (ψ_l, ψ_s and ψ_p) as influenced by the duration and severity of water stress. ψ_l in SS plants and ψ_s in the SS-LD treatment had both decreased significantly by 14 DAT relative to the NS controls, but then increased again by 21 DAT. ψ_p was similarly affected by the

TABLE 5.2.3. *Effects of the duration and severity of water stress on total shoot dry weight and dry matter distribution before anthesis (18 DAT) and after flower abscission (25 DAT; n=12). SED denotes the Standard Error of the Difference between means.*

Duration	Water stress (WS)	Tissue dry weight (g plant^{-1})							
		Before anthesis				After abscission			
		total shoot	flower	leaf	stem	total shoot	flower	leaf	stem
	NS	6.0	0.1	4.6	1.3	17.8	0.5	11.5	5.8
Long	MS	6.0	0.1	4.6	1.3	18.1	0.5	11.9	5.7
(35 d)	HS	5.4	0.1	4.2	1.2	15.6	0.5	10.1	5.1
	SS	5.3	0.1	4.2	1.0	14.8	0.5	9.4	4.9
Short	MS	6.2	0.1	4.9	1.3	17.5	0.6	11.5	5.4
(14 d)	HS	5.4	0.1	4.2	1.2	16.7	0.5	10.8	5.4
	SS	4.8	0.0	3.7	1.0	15.7	0.5	10.4	4.4
SED(NS)		0.37^{ns}	0.02^{ns}	0.29^{ns}	0.08^{ns}	1.07^{ns}	0.04^{ns}	0.79^{ns}	0.35^{ns}
SED(NS*D)		0.40^{ns}	0.02^{ns}	0.31^{ns}	0.08^{ns}	1.15^{ns}	0.04^{ns}	0.85^{ns}	0.38^{ns}
SED(NS*WS)		0.38^{*}	0.02^{ns}	0.33^{*}	0.09^{*}	1.22^{ns}	0.04^{ns}	0.90^{ns}	0.40^{ns}
SED(NS*D*WS)		0.47^{ns}	0.02^{ns}	0.38^{ns}	0.11^{ns}	1.40^{ns}	0.05^{ns}	1.04^{ns}	0.46^{ns}

* significant at $p<0.05$

ns: not significant

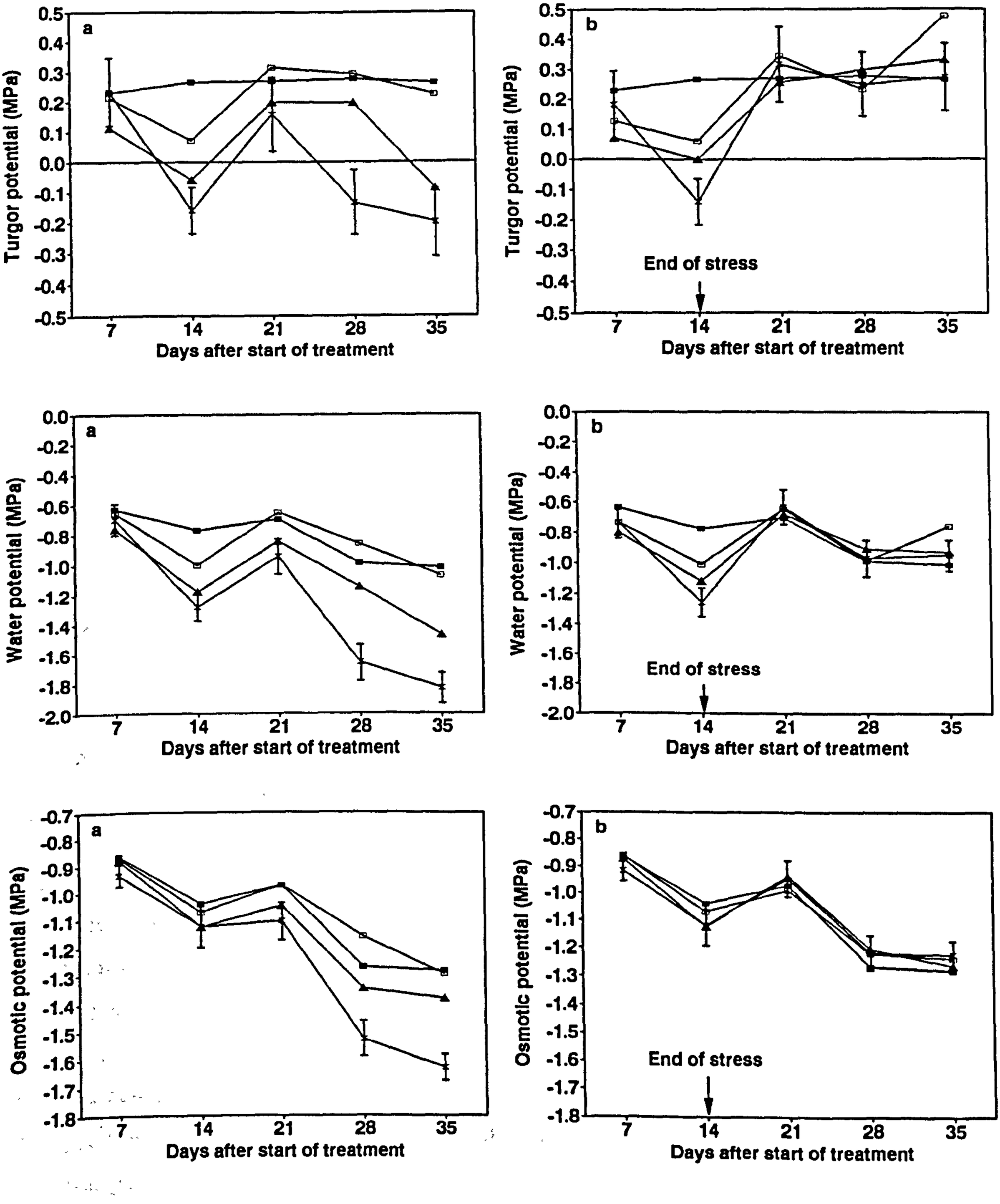


FIGURE 5.2.3. *Effects of the duration and severity of water stress on the timecourses of changes in the components of water relations during (a) long duration and (b) short duration stress.* ■, *NS;* □, *MS;* ▲, *HS;* X, *SS. n=12. Bars represent the Standard Error of the Difference between means.*

duration and severity of stress. Immediately after anthesis (21 DAT), ψ_s in SS-LD was significantly lower than in the NS control (-1.10 vs. -0.98 MPa; $p<0.05$). ψ_l in SS-SD increased from -1.27 to -0.64 MPa between 14 and 21 DAT, 6 d after releasing the stress, and smaller increases were also observed in the HS-SD and MS-SD treatments. Smaller increases in ψ_l also occurred under LD conditions during this period, even though stress had not been relieved in these treatments. The values for all plant water relations components decreased more severely in SS-LD than in the other watering treatments after first flower abscission (28 DAT). ψ_p decreased to -0.15 MPa ($p<0.01$) and ψ_s to -1.53 ($p<0.05$) in SS-LD, resulting a decline in ψ_l to -1.64 MPa ($p<0.001$). As stress continued to 35 DAT, ψ_l, ψ_s and ψ_p all decreased further in SS-LD, reflecting the increasing severity of the water stress conditions. After the release of stress under SD conditions (14 DAT), the values for all water relations components increased initially in the water stress treatments and then remained stable for the remainder of the experimental period.

5.3 Differential sensitivity to water stress according to the stage of flower bud development

The previous experiment described the influence of the duration and severity of progressive water stress imposed at the flower-bud visible stage (BVS) on subsequent flower development. Between 264 - 424 °C d after BVS, the total numbers of branches and leaves produced, branch and leaf dry weights, and total shoot dry weights were all significantly reduced by stress. These effects appeared to be associated with an acceleration of anthesis in the primary, but not in the secondary flowers. Since plants frequently show differential sensitivity to stress at different stages of growth (Salter and Goode, 1967), it was anticipated that subjecting pepper plants to stress at different stages of reproductive growth would have differential effects on flower development.

The experiment reported here was designed to explore the effects of water stress imposed at defined stages of flower bud growth, starting from the early flower bud-

visible stage, on subsequent vegetative and reproductive growth and development in sweet pepper.

5.3.1 Materials and methods

Seeds of the F_1 hybrid variety Blue Star were sown on 11, 14 and 17 May, 1993 so that plants at defined stages of flower bud growth could be exposed simultaneously to water stress. In this way, all plants would experience the same radiation and temperature conditions during the treatment period. The experiment ended on 29 July 1993, 40 d after the treatments commenced. Methods of propagation and environmental control were as described in Section 2.1.

When the cotyledons had fully expanded, the seedlings were pricked out individually into 9 cm diameter pots containing M2 Levington Compost and placed in a glasshouse under natural irradiance conditions with a mean daily temperature of 20 - 22 °C. When the third pair of true leaves were about 1 cm long (315 - 350 °C d after germination), the seedlings were transplanted into 23 cm diameter pots (6 l) containing M2 compost. The plants were then placed in a glasshouse with a mean daily temperature of 26 ± 3 °C under natural glasshouse lighting conditions, supplemented by high pressure sodium lamps (SON/T) between 0500 - 2300 h to provide an 18 h daylength and an additional total irradiance of 4.6 MJ m^{-2}.

Before imposing the water stress treatments, all plants were watered and maintained as described in Section 2.5. The water stress treatments, high stress (HS) and severe stress (SS), commenced when the flower buds had reached diameters of 0.5 - 1.0 mm (stage BS3), 2.0 - 2.5 mm (BS2) and 3.5 - 4.0 mm (BS1). An unstressed (NS) treatment with flower buds at stage BS3 was included as a control. The method of stress imposition using pot weighing was described in Section 2.5.

Periodic destructive growth analyses were carried out to determine effects on both reproductive and vegetative growth and development using the procedures described

in Section 2.2. The experiment was a 2 by 3 factorial arranged as a Randomised Complete Block Design, replicated three times. Each treatment contained 15 plants in each replicate.

5.3.2 Results

The total radiation received by plants during the 40 d period between imposing water stress and final harvest was 522 MJ m^{-2}, equivalent to a mean daily irradiance of 13.1 MJ m^{-2} d^{-1}. Over the same period, the total number of degree days accumulated (assuming T_b = 6.0, T_o = 27.5 and T_m = 41.5 °C) was 780 °C d, equivalent to a daily mean of 19.5 °C d (cf. Chapter 4).

Flower and reproductive development

Development of the primary and secondary flowers was not affected by any interaction between water stress and the stage of bud development. Instead, the thermal time required to reach first flower abscission in both types of flower was strongly affected ($p \leq 0.001$) by the individual effects of the bud stage when stress was imposed and the water stress treatment involved (Table 5.3.1). In the analysis of variance, examination of the thermal times required to reach abscission was confined to the water stress treatments, as no flower abscission was observed in the NS control.

Primary flowers The largest flower buds (BS1: 4.0 mm) reached anthesis first, and were also the first to show abscission in both the SS and HS treatments ($p < 0.001$; Table 5.3.1). The thermal time (°C d) between anthesis and first flower abscission was also significantly shorter in the primary flowers ($p < 0.01$) when stress was imposed at BS1 or BS2 than at BS3, supporting the view that the most advanced stages of flower bud development are more susceptible to abscission than newly developed flower buds. The more rapid abscission induced by the imposition of stress at stage BS1 was accompanied by an increase in percentage flower abscission (77 and

TABLE 5.3.1. *Effects of water stress applied at various stages of flower bud development on degree days (°C d) to first anthesis and first flower abscission and the thermal time intervals between anthesis and first flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress (WS)	Bud diameter (mm)	°C d to anthesis		°C d to abscission		Interval (°C d) between anthesis and abscission	
		Flower1[a]	Flower2[b]	Flower1	Flower2	Flower1	Flower2
NS		$372_{(18)}$[c]	$442_{(22)}$				
High stress (HS)	1.0	$373_{(18)}$	$442_{(22)}$	$487_{(24)}$	$577_{(29)}$	114	135
	2.5	$308_{(15)}$	$360_{(18)}$	$373_{(18)}$	$445_{(22)}$	64	85
	4.0	$256_{(12)}$	$308_{(15)}$	$322_{(16)}$	$410_{(20)}$	66	102
Severe stress (SS)	1.0	$354_{(17)}$	$423_{(21)}$	$461_{(23)}$	$486_{(25)}$	107	63
	2.5	$308_{(15)}$	$366_{(18)}$	$341_{(17)}$	$454_{(23)}$	32	88
	4.0	$256_{(12)}$	$322_{(16)}$	$301_{(15)}$	$334_{(16)}$	45	12
SED(NS)		4.51^{*}	5.71^{ns}				
SED(WS)		6.94^{ns}	7.90^{ns}	12.71^{ns}	21.30^{*}	13.82^{ns}	16.74^{**}
SED(BS)		8.50^{***}	9.67^{***}	15.57^{***}	26.10^{***}	16.92^{**}	20.50^{ns}
SED(WS*BS)		12.02^{ns}	13.68^{ns}	22.01^{ns}	33.81^{ns}	23.93^{ns}	28.99^{ns}

a: primary flowers

b: secondary flowers

c: numbers in parentheses indicate DAT

* significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$

ns: not significant

92% for the HS and SS treatments; $p<0.05$; Table 5.3.2); the corresponding values for stress imposed at BS2 and BS3 were 52 - 88% and 25 - 83% respectively. Imposition of severe stress at any stage of flower development caused greater flower abscission than the HS treatment (83 - 92% and 25 - 77% respectively; $p<0.001$; Table 5.3.2).

Secondary flowers As for the primary flowers, the largest buds (BS1) reached anthesis first in the SS treatment, although this was followed by early abscission ($p<0.001$; Table 5.3.1). The thermal time between anthesis and abscission was significantly shorter in SS (12 - 88 °C d) than in the HS (85 - 135 °C d) treatment ($p<0.01$). Percentage flower abscission was increased by the SS treatment to 75 - 100% ($p<0.001$; Table 5.3.2).

When plants with newly developed (BS3: 1.0 mm) or the most advanced flower buds (BS1: 4.0 mm) were exposed to water stress, the number of primary flowers per plant was increased ($p<0.01$) from 1.2 to 1.5 - 1.7 flowers plant^{-1} for the BS1 treatment, whilst the number of secondary flowers decreased from 3.3 to 2.3 - 2.5 flowers plant^{-1} ($p<0.05$; Table 5.3.2) for the BS3 treatment. It also appeared that the lower number of primary flowers in the BS1 as compared to the BS3 treatment was offset by a greater number of secondary flowers, and vice versa. However, similar flower numbers to the unstressed control were recorded when plants were stressed when the flowers buds were at BS2 (2.5 mm).

The dry weight of flowers+fruit at final harvest was significantly reduced ($p<0.001$) by the water stress treatments (Table 5.3.4) and there was no interaction between water stress and the stage of bud development when this was imposed. The dry weight of flowers+fruit in the HS and SS treatments was reduced by approximately 47 and 96% relative to the NS control. The greatly reduced flowers+fruit dry weights in the SS treatment suggest that the plants were unable to sustain flower development after anthesis under prolonged severe stress.

TABLE 5.3.2. *Effects of water stress applied at various stages of flower bud development on total flower numbers per plant and percentage abscission of primary and secondary flowers (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress (WS)	Bud diameter (mm)	Total flower no. plant^{-1}		Abscission (%)	
		Flower1[a]	Flower2[b]	Flower1	Flower2
NS	1.0	1.2	3.3	6	4
High stress (HS)	1.0	1.7	2.5	25	23
	2.5	1.2	3.0	52	50
	4.0	1.5	2.7	77	34
Severe stress (SS)	1.0	2.0	2.3	83	100
	2.5	1.3	3.0	88	75
	4.0	1.7	2.4	92	90
SED(NS)		0.13^{*}	0.21^{**}	9.69^{***}	10.07^{***}
SED(NS*WS)		0.14^{ns}	0.23^{ns}	10.36^{***}	11.72^{***}
SED(NS*BS)		0.15^{**}	0.24^{*}	10.98^{*}	12.43^{ns}
SED(NS*WS*BS)		0.17^{ns}	0.28^{ns}	12.68^{ns}	14.36^{ns}

a: primary flowers
b: secondary flowers
* significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$
ns: not significant

TABLE 5.3.3. *Effects of water stress applied at various stages of flower bud development on vegetative growth and development measured after first flower abscission (17 d; n=9). SED denotes the Standard Error of the Difference between means.*

Water stress (WS)	Bud diameter (mm)	Total plant^{-1}		Total leaves plant^{-1}		Total branches plant^{-1}
		height (cm)	shoot dry wt. (g)	area (cm^2)	no.	
NS	1.0	40.8	19.8	3470	58.3	30
High stress (HS)	1.0	38.4	16.9	2749	54.7	25
	2.5	41.7	16.2	2580	41.3	20
	4.0	47.8	20.3	3183	59.3	35
Severe stress (SS)	1.0	36.0	13.4	2056	36.3	17
	2.5	40.5	15.3	2465	43.0	22
	4.0	44.2	17.5	2538	44.0	21
SED(NS)		1.08^{ns}	0.48^{***}	87.2^{***}	2.16^{***}	1.1^{***}
SED(NS*WS)		1.15^{*}	0.51^{***}	93.2^{***}	2.30^{***}	1.2^{***}
SED(NS*BS)		1.22^{***}	0.54^{***}	98.9^{***}	2.44^{***}	1.3^{***}
SED(NS*WS*BS)		1.41^{ns}	0.63^{*}	114.2^{**}	2.82^{***}	1.5^{***}

* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$
ns: not significant

TABLE 5.3.4. *Effects of water stress applied at various stages of flower bud development on vegetative growth and development and flower+fruit dry weight at final harvest (40 d; n=9). SED denotes the Standard Error of the Difference between means.*

Water stress (WS)	Bud diameter (mm)	Total plant^{-1}		Total leaves plant^{-1}		Total branches plant^{-1}	Flower +fruit dry wt. (g)
		height (cm)	shoot dry wt. (g)	area (cm^2)	no.		
NS	1.0	59.3	60.7	4674	88.6	77	19.7
High stress (HS)	1.0	56.1	37.7	3537	65.6	49	9.9
	2.5	65.0	41.0	3854	72.0	56	10.2
	4.0	62.3	42.6	3833	71.6	58	11.5
Severe stress (SS)	1.0	48.3	24.4	3118	57.1	36	0.5
	2.5	52.4	26.5	3141	61.6	37	0.6
	4.0	53.0	26.2	3150	57.7	38	0.9
SED(NS)		1.98^{ns}	1.68^{***}	220.3^{***}	4.47^{***}	3.31^{***}	1.60^{***}
SED(NS*WS)		2.11^{***}	1.80^{***}	235.5^{**}	4.78^{**}	3.53^{***}	1.71^{***}
SED(NS*BS)		2.24^{**}	1.90^{ns}	249.8^{ns}	5.07^{ns}	3.75^{ns}	1.82^{ns}
SED(NS*WS*BS)		2.59^{ns}	2.20^{ns}	288.4^{ns}	5.88^{ns}	4.33^{ns}	2.10^{ns}

* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$
ns: not significant

Vegetative growth and development

The measurements of vegetative growth and development made after flower abscission (17 DAT) showed that total leaf area, leaf and branch numbers and total shoot dry weight were all reduced in the SS treatment relative to the NS-BS3 control, irrespective of the stage of bud development when stress was imposed; these reductions were particularly severe in the SS-BS3 treatment ($p<0.05$ - 0.001; Table 5.3.3). However, in the HS-BS1 treatment, no significant reductions in vegetative growth were observed relative to the NS control; instead, higher total branch numbers and shoot dry weights were observed ($p<0.001$ and 0.05 respectively). It appears that, although the imposition of the SS treatment on plants with the smallest flower buds (BS3) restricted vegetative growth and development more severely than when severe stress imposed at more advanced bud stages, flower abscission was not accelerated to the same extent as in the SS-BS1 treatment. Conversely, although vegetative growth and development in the HS-BS1 treatment were not restricted by the water stress, flower development after anthesis was not promoted since abscission occurred earlier than in the NS control or SS-BS3 treatments. This may imply that the reproductive organs of plants with the largest developing buds (BS1) were more sensitive than the vegetative parts to the SS or HS treatments, which advanced anthesis but promoted early flower abscission, and that the restricted vegetative growth in the SS treatment may reflect the impact of the severe stress. The plants with the smallest buds (BS3) when the stress treatments were imposed and those subjected to the SS treatment were significantly shorter than the NS-BS3 control ($p<0.001$; Table 5.3.3).

At final harvest (40 DAT), no interactive effect between water stress and the stage of bud development when stress was first imposed were detected for vegetative growth and development. Plant height, total shoot dry weight, total leaf number and area and total branch number were all significantly reduced by water stress, especially under SS conditions ($p<0.01$ - 0.001; Table 5.3.4). Vegetative growth was reduced more by prolonged severe stress than by high stress relative to the unstressed control, but the stage of bud development when stress was imposed had no significant effect

on most of the vegetative characters examined. However, plants that were stressed at an earlier growth stage (BS3) were significantly shorter than the BS3 control plants ($p<0.01$).

Shoot dry matter and distribution

The effect of applying water stress at different stages of flower development on total shoot dry matter production is shown in Figure 5.3.1. The interactive effects of water stress and bud stage first became apparent at 17 DAT, when shoot dry matter in the SS-BS3 treatment was significantly lower than in the other treatments ($p<0.05$). Thereafter, the difference in biomass between treatments increased due to the individual effects of the water stress treatments and stages of bud development when stress was imposed. Shoot dry matter was most severely reduced in the SS and BS3 treatments ($p<0.001$).

Dry matter distribution (DMD; % of total shoot dry weight) to the leaves and stems was strongly affected by the individual effects of both water stress and the stage of bud growth when the stress was imposed ($p<0.001$). DMD to the flowers during early stress (17 DAT) was affected by the interactive effects of water stress and stage of bud growth ($p<0.01$) in such a way that dry matter distribution to the flowers was increased in SS-BS1 plants relative to the NS-BS3 control and the other treatments. DMD to the stems and flowers+fruits generally increased during the experimental period with the exception of DMD to the flowers+fruits under SS conditions, where a progressive decrease was observed regardless of bud stage. DMD to the leaves decreased throughout the experimental period (Figure 5.3.2). DMD to the leaves was significantly lower ($p<0.05$) when stress was imposed at BS1 (largest bud) than in the other treatments up to 17 DAT (1 - 2 d after first abscission). Conversely, DMD to the stems was 14% higher in BS1 than in BS2, and 25% higher than in BS3 or the NS-BS3 control ($p<0.001$). DMD to the flowers was lower in the SS-BS1 treatment than in any other treatment ($p<0.01$). As the stress progressed, DMD to the leaves increased in the SS treatment relative to the NS-BS3 control ($p<0.001$), whereas DMD to the stem was significantly greater than in the NS-BS3 control only at final

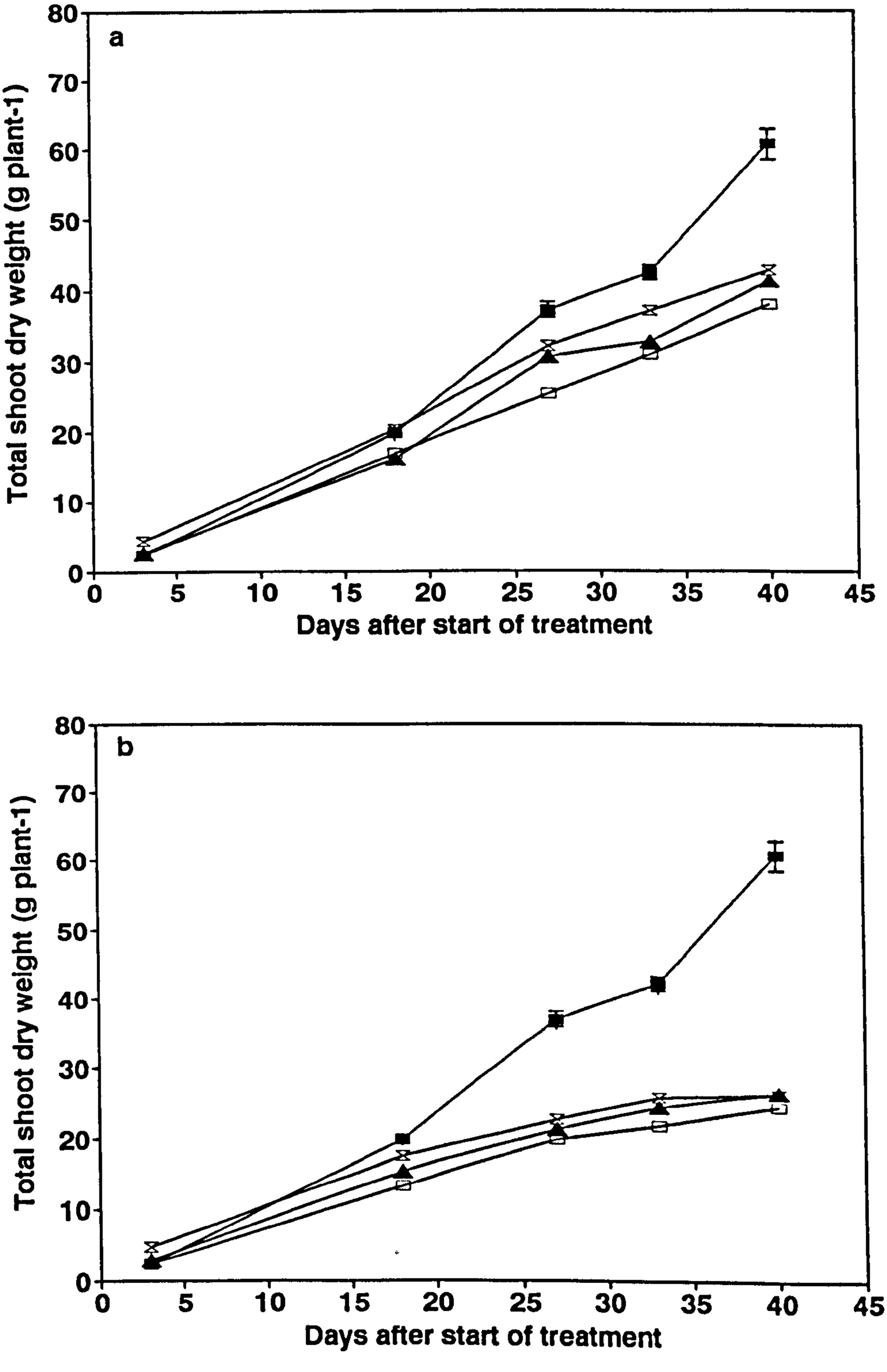


FIGURE 5.3.1. *Effects on total shoot dry weight of a) high and b) severe water stress treatments applied at different stages of bud growth:* ■, *NS;* □, *BS3;* ▲, *BS2;* ⧖, *BS1. n=9. Bars represent the Standard Error of the Difference between means.*

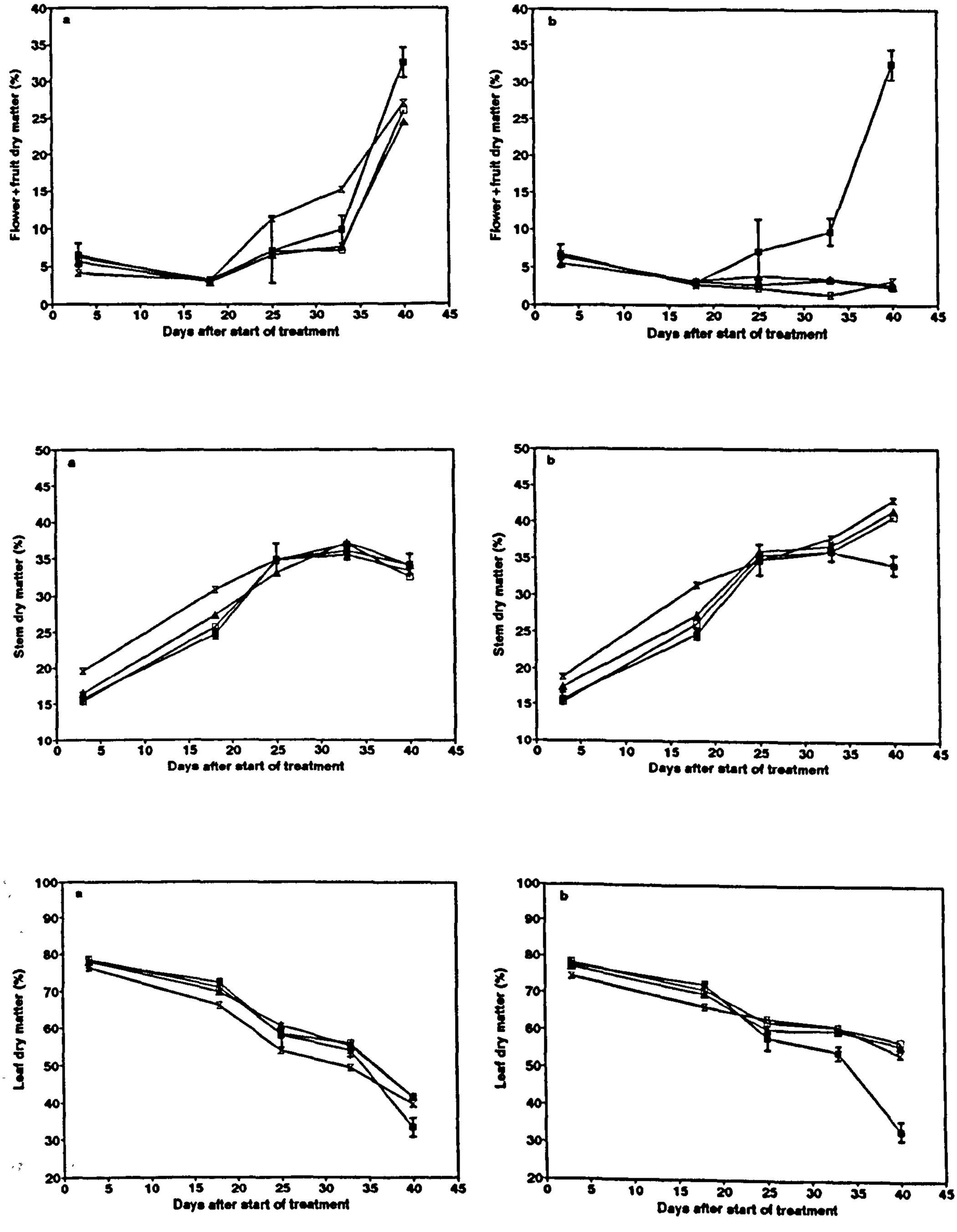


FIGURE 5.3.2. *Effects on dry matter distribution to the various shoot components of a) high and b) severe water stress treatments applied at different stages of bud growth:* ■, *NS;* □, *BS3;* ▲, *BS2;* ⧖, *BS1. n=9. Bars represent the Standard Error of the Difference between means.*

harvest ($p<0.001$). There was a simultaneous marked reduction ($p<0.001$) in DMD to the flowers+fruit relative to the NS control under SS conditions, regardless of the bud stage when stress was applied (Figure 5.3.2). Much smaller reductions in DMD to the flowers+fruits were observed under HS conditions.

Water relations

In general, plant water relations were more strongly influenced by the water stress treatment than by the stage of bud growth, with no interactive effects. Leaf water (ψ_l), osmotic (ψ_s) and turgor potentials (ψ_p) were all significantly lower in the SS treatment than in the HS and unstressed control treatments ($p<0.001$; Figure 5.3.3). By 11 DAT, ψ_p and ψ_s had decreased sharply in all of the water stress treatments relative to the NS-BS3 control ($p<0.001$), resulting in a decline in ψ_l. As the stress progressed, ψ_p and ψ_l increased in the HS but decreased in the SS treatments. The values for the SS treatment continued to decline to minimum values of -0.35 and -2.14 MPa for ψ_p and ψ_l at 22 DAT, during which period $\geq$ 50% flower abscission occurred. During the same period, ψ_s in SS-BS1 also reached its lowest value of -1.9 MPa ($p<0.01$). Towards the end of the experiment (34 DAT), ψ_p in the SS treatment appeared to increase slightly, although not significantly, whilst ψ_s continued to decline to the lowest recorded value of -2.0 MPa in SS-BS1. Correspondingly, the values of ψ_l in the SS treatment increased slightly to c. -2.0 MPa. The lower values of ψ_s in the SS treatment suggest that some osmotic adjustment occurred in response to water stress, which may have helped to maintain cell turgor.

Correlations

Linear regressions were calculated to establish the relationships between the percentage abscission of primary flowers and turgor potential (Figure 5.3.4), and flower abscission and DMD (Figure 5.3.5). Under conditions of severe stress, increasing flower abscission in BS2 was closely associated with decreasing turgor ($R^2=0.99$; $p<0.001$), whereas under conditions of high stress, increasing flower abscission in BS3 was apparently correlated with increasing turgor ($R^2=0.88$). No

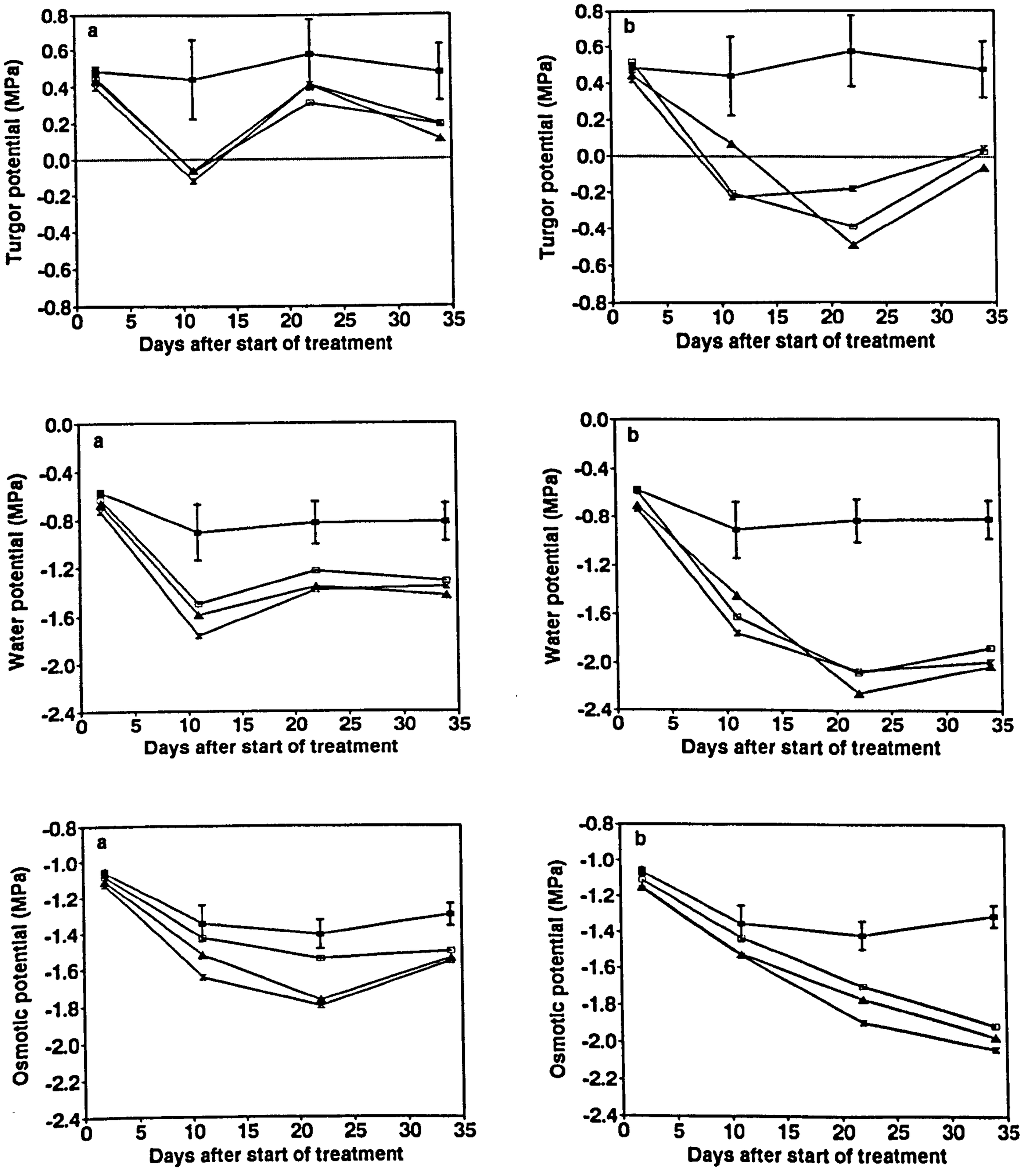


FIGURE 5.3.3. *Changes in turgor, water and osmotic potentials of leaves with time in response to a) high and b) severe water stress treatments applied at different stages of bud growth:* ■, *NS;* □, *BS3;* ▲, *BS2;* ⧖, *BS1. n=12. Bars represent the Standard Error of the Difference between means.*

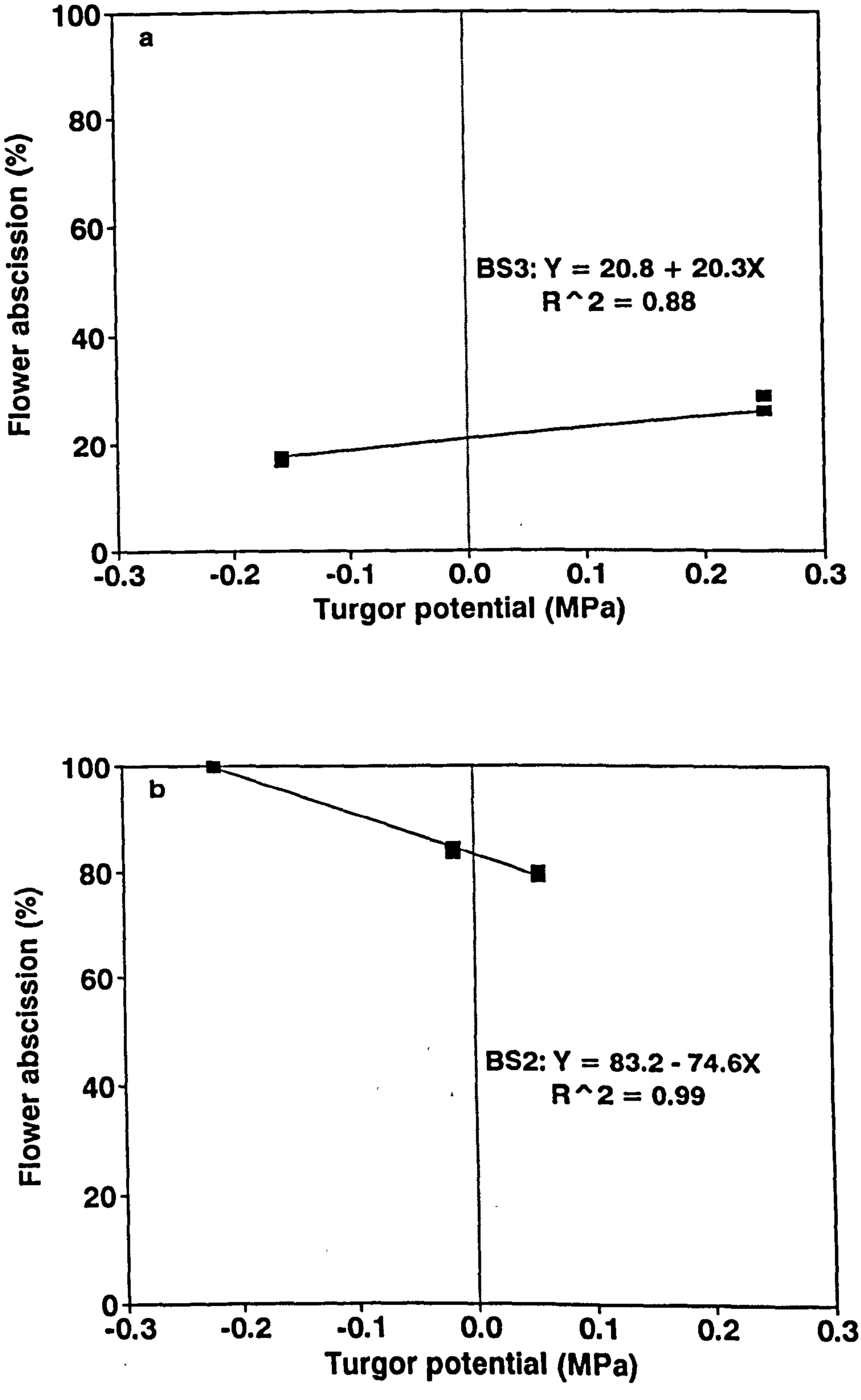


FIGURE 5.3.4. *Relationship between percentage abscission of primary flowers and turgor potential as influenced by a) high and b) severe water stress treatments applied at different stages of bud growth. n=12. Lines are the linear regressions.*

strong relationships were established for the other treatments.

DMD to the leaves was negatively correlated with turgor in both the NS control and SS treatments at BS1 (Figure 5.3.5; R^2=0.99; $p \leq 0.001$), but was positively correlated in the HS treatment at BS3 (R^2=0.96). DMD to the stems decreased as turgor declined when SS was imposed at BS1 or BS2 (R^2=0.99; $p<0.01$). Relatively close relationships also existed between DMD to flowers+fruit and turgor when of SS and HS conditions were imposed at BS3 (R^2=0.94) or BS1 (R^2=0.88).

5.4 Discussion

The overall aim of the experiments described in this Chapter was firstly to determine the extent to which water stress affects flower development and abscission and secondly to establish whether changes in dry matter distribution had any role in mediating these effects. The hypothesis was that the early imposition of water stress would promote flower growth and development and reduce abscission by increasing assimilate partitioning to the flower buds and subtending stem.

Restriction of vegetative growth by the imposition of water stress under low irradiance conditions (daily mean: 2.8 MJ m^{-2} d^{-1}) did not improve early reproductive growth and development. Complete abscission of both primary and secondary flowers occurred under low irradiance and there was no interactive effect of water stress, suggesting that low irradiance had a greater effect on flower development than water stress. Complete flower truss abortion has been reported previously for early-grown glasshouse tomatoes receiving similar daily mean quantities of radiation and there was again no beneficial effect of water stress on reproductive development in these studies (Klapwijk and De Lint, 1974; Othman, 1984; Halevy, 1987). The detrimental effects of low irradiance on flower growth were discussed in Chapter 3 (e.g. Atherton and Othman, 1983; Atherton and Harris, 1986; Halevy, 1987), and are thought to originate from limited supplies of assimilates to the flower buds (Saito and Ito, 1967a, 1972; Picken, 1984; Morris and Newell, 1987; Turner and Wien, 1994a). Under such conditions, flower initiation (Hussey, 1963) and development (Calvert, 1969) are more severely affected

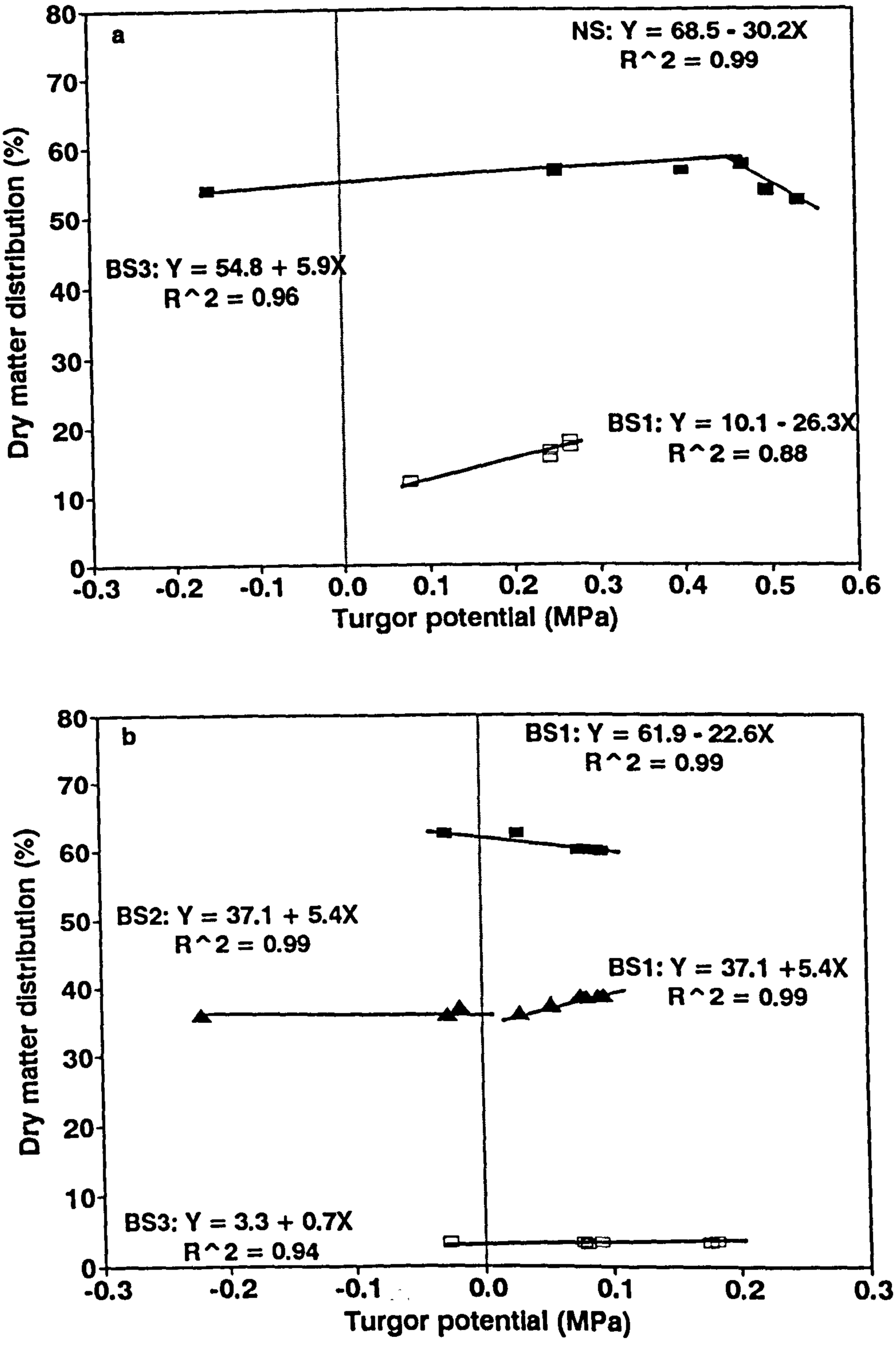


FIGURE 5.3.5. *Relationship between dry matter distribution and turgor potential as influenced by a) high and b) severe water stress treatments applied at different stages of bud growth:* ■, *leaf;* □, *flowers+fruit;* ▲, *stem. n=12. Lines are the linear regressions.*

than vegetative growth, bringing about severe flower abscission (Turner and Ewing, 1988; Wien *et al.*, 1989b; Turner and Wien, 1994a). In contrast, De Koning and Hurd (1983) showed that restriction of water supplies to winter-grown glasshouse tomatoes advanced flowering by four days when the greenhouse atmosphere was enriched with 1000 vpm CO_2 during the daylight period. This may reflect increased assimilation resulting from the additional CO_2 and also more favourable partitioning of that assimilate to the flowers.

Under relatively high irradiance conditions (daily mean 9.1 - 13.1 MJ m^{-2} d^{-1}), the development of primary, but not secondary flowers was accelerated when water stress was imposed at the time of appearance of the first flower buds. This hastening effect of severe stress was also influenced by the duration of stress. Severe stress gradually imposed over a prolonged period (i.e. uninterrupted stress from the stage when the first flower buds became visible to final harvest), or when applied at the most advanced stage of flower bud development (diameter: 4.0 mm) advanced anthesis in the primary flowers.

Faster flower development in response to water stress has been reported for several crops, including processing tomato (Wudiri and Henderson, 1985), coffee (Alvim, 1960; Drinnan and Menzel, 1994) and bamboo (Alvim, 1964). When tomatoes were gradually stressed after the appearance of the first flower buds, flowering was accelerated (Wudiri and Henderson, 1985), and Cooper *et al.* (1966) and De Koning and Hurd (1983) also observed earlier anthesis when tomato plants were sparingly watered. Nakata and Suehisa (1969) showed that, when water was not limiting, flowering of *Litchi chinensis* occurred on only 50% of the branches, but that water stress caused flowering on 75 - 95% of the branches, and also induced earlier flowering. In contrast, some delaying effect of water stress on flower development has also been reported for tomato (Gates, 1955; Klapwijk and De Lint, 1974; Atherton and Othman, 1983) and wheat (Angus and Moncur, 1977). These conflicting results may be attributable to the different methods of imposing water stress adopted in the present and previous studies, since sudden and/or severe stress was employed by all of these workers.

The earlier anthesis induced by water stress was immediately followed by flower abscission in the present study. This was most dramatic when stress was applied at the most advanced stage of flower bud development. Thus, plants stressed after the largest bud in the inflorescence had reached 4 mm showed a marked increase in abscission, whereas the number of buds aborting was not significantly affected when stress was applied at earlier stages of development, an observation supported by previous work (Atherton and Othman, 1983). Rudich *et al.* (1977) also observed that irrigation during early flowering reduced flower abortion. The newly developed flower buds seemed to be more resistant to water stress-induced abscission, possibly because of their more adaptable nature (Menzel *et al.*, 1986). A similar response was observed in passionfruit, in which the newly developed flower buds appeared to acquire some resistance when exposed to severe water stress, and exhibited no premature abscission, although flower size was reduced (Menzel *et al.*, 1986).

As prolonged stress enhanced only the initial development of flowers before hastening their abscission, shortening the water stress period might be expected to allow flower development to anthesis. However, the results obtained in the present study showed that shortening the stress duration did not hasten anthesis or delay abscission, although there was some evidence that the percentage abscission of primary flowers was reduced.

Flower development was not enhanced after rewatering, as had been expected in view of previous reports of a recovery in growth in other species following the alleviation of drought (Acosta Gallegos and Kohashi Shibata, 1989; Munier-Jolain *et al.*, 1993; Ney *et al.*, 1994). This may have been due to increased competition for assimilates between the flowers and vegetative organs, since more new leaves were formed after the stress ended. According to Acevedo *et al.* (1971), the release of stress effected a transitory phase of more rapid growth than the unstressed steady-state rate, with the result that there was no net reduction in leaf elongation over the entire period. Indeed, there are reports that growth may resume within seconds of rewatering plants due to promotion of cell expansion (Acevedo *et al.*, 1971; Bradford and Hsiao, 1982). Consistent with the present results, shoot growth in *Coffea arabica* L. also showed a

significant increase after rewatering plants, in which ψ_l had declined to -2.5 MPa, as compared to plants in which ψ_l was maintained above -0.5 MPa (Drinnan and Menzel, 1994).

Expansive growth has been noted to be the process most sensitive to water stress (Hsiao *et al.,* 1985; Hsiao and Jing, 1987) and restriction of leaf expansion growth is one of the earliest symptoms of water stress (Kriedemann, 1986; Kirkham, 1990). Consistent with this statement, total leaf areas and branch numbers were both decreased during the accelerated anthesis of primary flowers by severe stress observed in the present study. This reduction in vegetative growth was accompanied by a decrease in total shoot dry weight which resulted from reductions in dry matter accumulation by both the leaves and stems. The dry weight of the flowers was not affected. The observed reduction in vegetative growth may have been at least partly responsible for the accelerated anthesis under water stress, due to the consequent reduction in the availability of assimilate to support the growth of both vegetative and reproductive organs (De Koning and Hurd, 1983; Halevy, 1987).

The results obtained here, are not entirely consistent with this hypothesis since no significant reductions in vegetative development, shoot dry weight or its partitioning were observed at the time of first flower abscission in the severely stressed plants as compared to the unstressed plants, although a trend of declining dry matter distribution to the vegetative parts was apparent. However, a significant decrease in total shoot dry weight became apparent two days after abscission, which was again accompanied by a reduced partitioning of dry weight to the leaves and an increase to the stems. From these results, it appears that there may be a transitional period under severe stress conditions when flower abscission is not caused directly by a decrease in total assimilate production or competition for the available assimilates between vegetative and reproductive organs. These results therefore suggest that other mechanisms may operate to regulate flower abscission under progressive stress conditions. In support of this view, many workers have suggested that water stress may inhibit flower development by altering the balance of endogenous growth substances which may trigger abscission (Hsiao, 1973; Menzel, 1985; Kirkham, 1990).

Prolonged severe water stress reduced leaf number and area even more than low irradiance. Consequently, total shoot dry weights became increasingly reduced relative to the unstressed control, with an even greater proportion of dry matter being partitioned to the stems of severely and highly stressed plants than to the leaves and flowers and fruits. In contrast, a greater proportion of the total dry matter was accumulated in the leaves under low irradiance conditions at the expense of flowers and stems. As the duration of the stress increased under low irradiance conditions, more dry matter was partitioned to the stems of severely and highly stressed plants than to the leaves and flowers+fruits. Shoot extension was also restricted by water stress, resulting in shorter plants with fewer branches and thinner stems. Water stress and low irradiance both increased leaf thickness. Prolonged severe stress has also been reported by other workers to retard vegetative and reproductive growth and change the pattern of dry matter distribution (De Koning and Hurd, 1983; Menzel *et al.*, 1986; Turner, 1991).

The increasing severity of water stress in the present study is apparent from the progressive reductions in ψ_l, ψ_s and ψ_p, the intensity of which reflected the severity of the stress imposed. A temporary osmotic adjustment shortly after imposition of water stress was followed by noticeable reductions in leaf turgor in all treatments and wilting became apparent between 11 and 22 DAT, when ψ_l had fallen to between -1.2 and -1.8 MPa.

Osmotic adjustment has been suggested as a mechanism of drought tolerance (Turner and Begg, 1981; Morgan, 1984; Ludlow and Muchow, 1988), and has been reported previously in water stressed sweet pepper (Aloni *et al.*, 1991a; Wullschleger and Oosterhuis, 1991). Reductions in osmotic potential in response to water stress have been proposed to play a significant role in turgor maintenance (Zimmermann, 1978; Turner, 1986), thereby enabling a range of growth processes to be at least partially maintained (Morgan, 1984; Turner, 1986). Consistent with the present study, osmotic adjustment has also been suggested to be effective in reducing the impact of water deficits on growth only during short term stress (Toft *et al.*, 1987; Kirkham, 1990).

During anthesis, there was a tendency for almost all components of plant water potential to decrease during severe stress, and the values declined sharply during first abscission. However, the reductions in the water relations components were not consistent, e.g. ψ_p was similar (0.22 MPa) during both anthesis and abscission. Although correlation analyses showed there was no close relationship between flower abscission and ψ_p, reductions in leaf turgor and water potential were observed during flower abscission, consistent with reports for severely stressed millet and groundnut (Squire *et al.*, 1983; Black *et al.*, 1985); stomatal conductances also generally decline during severe stress (Black *et al.*, 1985; Menzel *et al.*, 1986). Although decreased stomatal conductance during periods of water stress may limit net photosynthesis (Schulze and Hall, 1982; Chaves, 1991), the decline in stomatal conductance observed in the present study did not appear to reduce net photosynthesis, as inferred from the non-significant effects of water stress on total shoot dry weight and its partitioning during abscission discussed above. Transpiration was also not affected by water stress. According to Kirkham (1990), it is possible that non-stomatal factors may be involved in maintaining higher photosynthetic rates under water stress conditions, as observed in wheat (Johnson *et al.*, 1987) and sunflower (Cox and Jolliff, 1987). Furthermore, although correlation analysis showed that DMD was closely related to ψ_p in some treatments, the relationship did not appear to be consistent.

These results suggest that the effects of water stress on anthesis and flower abscission cannot be directly related to changes in tissue water relations or dry matter distribution *per se*, but may be mediated by related water stress-induced changes which are more sensitive to the effects of stress. For example, increasing evidence suggests an important role for plant growth substances in the abscission of flowers (Hsiao, 1973; Miyamoto and Kamisaka, 1987; Kirkham, 1990). The role of ethylene in flower abscission in sweet pepper is examined in greater detail in Chapter 6.

5.5 Conclusions

1. Mean radiation levels of 9.1 - 13.1 MJ m^{-2} d^{-1} allowed normal development

of the flowers to anthesis, but complete abscission of the primary and secondary flower buds occurred under low irradiance conditions (2.8 MJ m^{-2} d^{-1}).

2. The development of primary flowers was accelerated when increasingly severe water stress was imposed at the appearance of the first flower bud. This effect was also dependent upon the duration of stress. Prolonged severe stress enhanced anthesis in the primary but not in secondary flowers.

3. The initial acceleration of reproductive development induced by severe water stress did not continue after anthesis, due to the early onset of extensive flower abscission. The most advanced stage of flower bud development at the time of imposing the stress was most susceptible to early abscission.

4. Shortening the duration of water stress did not hasten anthesis or reduce abscission, probably because of competition for assimilates by new leaves produced after the stress ended.

5. The accelerated development of primary flowers to anthesis caused by severe stress was accompanied by a decrease in vegetative growth (total leaf and branch numbers and leaf area). However, no reduction in the vegetative development was detected during first flower abscission.

6. Vegetative growth and development were more severely affected by the severe and prolonged water stress than by short stress or low irradiance. Shoot extension was restricted by severe stress and low irradiance, resulting in shorter plants with fewer branches and thinner stems. Water stress and low irradiance increased leaf thickness, whilst low irradiance increased leaf area, especially in unstressed or moderately stressed plants.

7. Shoot dry weight immediately prior to anthesis was reduced by severe stress, which decreased dry matter accumulation in both the leaves and stems.

However, at the onset of flower abscission, shoot dry weight and its partitioning were not affected by severe stress. This implies that, although the advancement of anthesis may have been associated with a decrease in dry matter accumulation in the leaves and stems, flower abscission was not directly related to any reduction in assimilates production or its distribution within the shoot.

8. Shoot dry weights were more severely affected by prolonged severe water stress than by low irradiance conditions. Under low irradiance, a greater proportion of the dry matter was partitioned to the leaves, whilst at high irradiance the reverse applied.

9. All components of water potential declined as the stress progressed. The extent of the reductions reflected the severity of stress treatment imposed. A temporary osmotic adjustment occurred soon after the imposition of water stress, during which the osmotic potential decreased sharply. Noticeable reductions in turgor were apparent in all treatments between 11 - 22 DAT. Low irradiance did not affect plant water relations with the exception of osmotic potential after prolonged severe stress.

CHAPTER 6

ROLE OF ETHYLENE IN MEDIATING THE EFFECTS OF WATER STRESS

INTRODUCTION

The young flower bud constitutes a weak sink in comparison with vegetative apices and developing leaves (Halevy, 1975, 1984). Under stress conditions which reduce assimilate supply, flower buds compete poorly for assimilates, often leading to bud abscission or the arrestment of flower development (Morris and Newell, 1987; Halevy, 1987). Environmental conditions or treatments that enhance the supply of assimilates to the flower buds or reduce vegetative growth generally reduce abscission and promote flower development (Halevy, 1987). Previous experiments (Chapter 5) showed that restriction of early vegetative growth through the gradual imposition of water stress shortly after the appearance of the first visible flower buds favoured their initial development, leading to earlier anthesis of the primary flowers. However, progressive water stress did not enhance the subsequent development of these and later flowers, which were lost by abscission. This may in part have been attributable to the reduced availability of assimilates within the plant (Menzel *et al.*, 1986; Stirling *et. al.*, 1989a, b; Wien *et al.*, 1989a). Stress-induced changes in the concentration and distribution of endogenous growth substances might also have been involved in promoting flower abscission (Halevy, 1985; Abeles *et al.*, 1992).

Ethylene appears to be a major promoter of flower abortion and abscission in many plant species under stress conditions (Halevy and Mayak, 1981; Sexton *et al.*, 1985). Its production in plants has often been found to increase during water stress (Jordan *et al.*, 1972; Kirkham, 1985) or stress induced by temperature extremes (Ohno, 1991) or low light (Durieux *et al.*, 1983). Although there is much information implicating

ethylene in stress-induced abscission, especially of vegetative organs, little attention has been directed to understanding its role and mechanisms of action in water stress-induced flower abscission.

The following experiments were designed to establish the role of ethylene in mediating the effects of water stress on flower development, particularly its involvement in initiating or accelerating flower abscission.

6.1 Ethylene production, dry matter accumulation and water relations in water stressed plants during flower development

Increased ethylene production is associated with a lowering of both leaf water (McMichael *et al.*, 1972) and osmotic potentials (Curtis, 1981; Stumpff and Johnson, 1987; Miyamoto and Kamisaka, 1987) and a marked reduction in leaf fresh weight (Apelbaum and Yang, 1981). The abscission of flowers and leaves is also known to increase with increasing ethylene production (McMichael *et al.*, 1973; Guinn, 1976; Curtis, 1981) to an extent related to the age and physiological state of the organs involved (Jackson *et al.*, 1973; Durieux *et al.*, 1983; Abeles *et al.*, 1992). Leaves and flowers generally become more susceptible to abscission as they age (Sexton *et al.*, 1985; Tripp, 1986). Responsiveness to ethylene may also be altered by water stress conditions, resulting in a marked acceleration of stress-induced abscission (Jordan *et al.*, 1972). If ethylene is involved in promoting flower abscission, endogenous ethylene levels may be expected to increase following the imposition of water stress and with increasing age.

The main purpose of the following experiment was to measure ethylene production during water stress and examine its role in accelerating flower abscission. Total shoot dry weight, the partitioning of dry matter and changes in plant water status were examined to detect any link with changes in ethylene production. Ethylene evolution at different stages of flower development was also determined.

6.1.1 Materials and methods

Seeds of *Capsicum* hybrid variety Blue Star were sown on 6 September 1993. Propagation and seedling maintenance were as described in Section 2.1. When the third true leaf pair was about 1 cm long (22 days after germination: DAG), the seedlings were transplanted into 6 l pots containing Levington M2 compost. Water stress treatments were imposed when the flower buds reached the stage of development most sensitive to stress, i.e. at a bud diameter of 4.0 mm (38 DAG). The method of imposing water stress treatment followed that explained in Section 2.5 and the treatment period lasted for 25 d (i.e. ending on 13 November 1993). The experiment comprised two treatments: severe stress (SS), which had previously been shown to exert a consistently detrimental effect by inducing premature flower abscission, and a non-stress (NS) control. The treatments were arranged in a Randomised Complete Block Design, blocked six times. Each treatment contained 96 plants.

Ethylene evolution was measured at specific stages of flower growth prior to abscission. The time to first anthesis and first abscission was predicted using thermal time calculations (cf. Chapter 5). Ethylene production rate or evolution from the leaves was measured to determine differences between treatments and establish how these were related to abscission. The method of ethylene measurement was as described in Section 2.7. Growth measurements and destructive analyses (Section 2.3), which included the determination of leaf water status (Section 2.5), coincided closely with the ethylene measurements to determine whether reproductive and vegetative growth were related to ethylene evolution.

Excising plant tissues for ethylene measurement causes a degree of wounding to the explants (Burg, 1968; Abeles *et al.*, 1992), which may compound the effects of stress on ethylene production. Therefore, tissues sampled just before flower abscission were used as test material to establish the timecourse of ethylene evolution by detached flowers and leaves and the time of peak production after sampling the tissues. These measurements were made to establish any differences in ethylene evolution between treatments and organs. Two initial measurements were made at 45 min intervals after

closing the incubation vials. Subsequent measurements were taken at 90 min intervals over a total period of 12 h. After each reading, the vials were aerated, recapped, and the presence of ethylene was verified by gas chromatography.

6.1.1 Results

Total accumulated radiation received between the appearance of first visible flower bud and final harvest was 321.8 MJ m^{-2}, with a daily mean of 7.85 MJ m^{-2} d^{-1}. Values for daily mean and accumulated thermal time were 19.0 and 780.8 °C d respectively. First anthesis and flower abscission in SS occurred at 436 and 537 °C d respectively. First anthesis in NS was observed at 497 °C d.

Ethylene production

The rates of ethylene evolution by primary flowers and leaves measured just before first anthesis and flower abscission in the NS and SS treatments are presented in Table 6.1.1. There was no significant increase in the rates of ethylene evolution from either the flowers or leaves of stressed plants immediately before anthesis. Ethylene evolution from the leaves was approximately 10-fold greater than in the flowers in both the NS and SS treatments, implying that the leaves may be a site for ethylene synthesis. Preceding the first abscission in SS, ethylene evolution from the primary flowers was approximately 8-fold greater than in the NS treatment ($p < 0.001$) and about 40-fold greater than that measured before anthesis (0.021 vs. 0.851 nl g^{-1} h^{-1}). Although ethylene production was lower in NS than in SS, there was still an increase of c. 6-fold as compared to the earlier measurements prior to anthesis, suggesting that ethylene production in flowers increases with age.

During the period before first abscission, higher rates of ethylene evolution were also observed in stressed as compared to unstressed leaves (0.299 vs. 0.140 nl g^{-1} FW h^{-1}; $p<0.05$) but these levels were approximately 3-fold lower than in the stressed flowers (Table 6.1.1). This may imply that developing flowers were more sensitive than the

TABLE 6.1.1. *Effects of watering treatments on the rates of ethylene evolution (nl g^{-1} FW h^{-1}) from the primary flowers and leaves sampled immediately before anthesis and flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress	Before first anthesis		Before first abscission	
	Flowers	Leaves	Flowers	Leaves
NS	0.016	0.142	0.106	0.140
SS	0.021	0.170	0.851	0.299
SED	0.005^{ns}	0.023^{ns}	0.110^{***}	0.015^{*}

* significant at $p<0.05$; *** significant at $p<0.001$; ns: not significant

TABLE 6.1.2. *Effects of watering treatments on shoot dry matter and its distribution immediately before first anthesis and flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress	Before first anthesis (g)				Before first abscission (g)			
	Total shoot	Flower	Leaf	Stem	Total shoot	Flower	Leaf	Stem
NS	6.4	0.3	4.5	1.6	9.0	0.3	6.2	2.5
SS	5.3	0.2	3.8	1.2	8.7	0.4	5.9	2.5
SED	0.33^{**}	0.10^{**}	0.23^{**}	0.10^{**}	0.27^{ns}	0.02^{*}	0.20^{ns}	0.08^{ns}

* significant at $p<0.05$; ** significant at $p<0.01$; ns: not significant

leaves to severe stress and that this was reflected by the higher rates of ethylene evolution. Ethylene evolution from the leaves increased as the duration of the stress period increased, from 0.170 before anthesis to 0.299 nl g^{-1} FW h^{-1} before abscission, although the increment was smaller than in the flowers. However, ethylene evolution from non-stressed leaves remained unchanged between anthesis and abscission at 0.140 nl g^{-1} FW h^{-1}, but was consistently higher than in the NS flowers both before anthesis and abscission. These results indicate that under unstressed growing conditions, the rate of ethylene evolution from the leaves is greater than that from the flowers, although this situation may be reversed in younger plants with less advanced 4 mm flower buds (Figure 6.1.1). The changing rate of ethylene evolution at different stages of flower development is clearly depicted in Figure 6.1.1.

Timecourse of ethylene production

Figure 6.1.2 shows the changing rates of ethylene evolution from excised flowers and leaves following abscission. The peak release of ethylene by flowers and leaves from stressed plants occurred 90 min after excision and evolution then gradually decreased during the remainder of the observation period in the flowers, but levelled off in the leaves around 270 - 360 min after excision. Flowers and leaves from the NS treatment took longer to reach a much lower peak ethylene evolution, some 180 min after excision. SS increased ethylene evolution from the flowers significantly throughout the timecourse, while a significant increase was only detected in the leaves during the peak period. The much higher rates of ethylene production from SS flowers until 720 min after excision may indicate that they possess higher levels of endogenous ethylene.

Dry matter distribution

Dry matter distribution between the components of the shoot is shown in Tables 6.1.2 and 6.1.3. As found in the previous experiment (Chapter 5.2), SS significantly reduced total shoot dry weight ($p<0.01$; c. 20%) immediately prior to anthesis and dry matter accumulation in the flowers, leaves and stems were all consistently reduced in the SS treatment (Table 6.1.2; $p<0.01$). However, dry matter distribution to these components

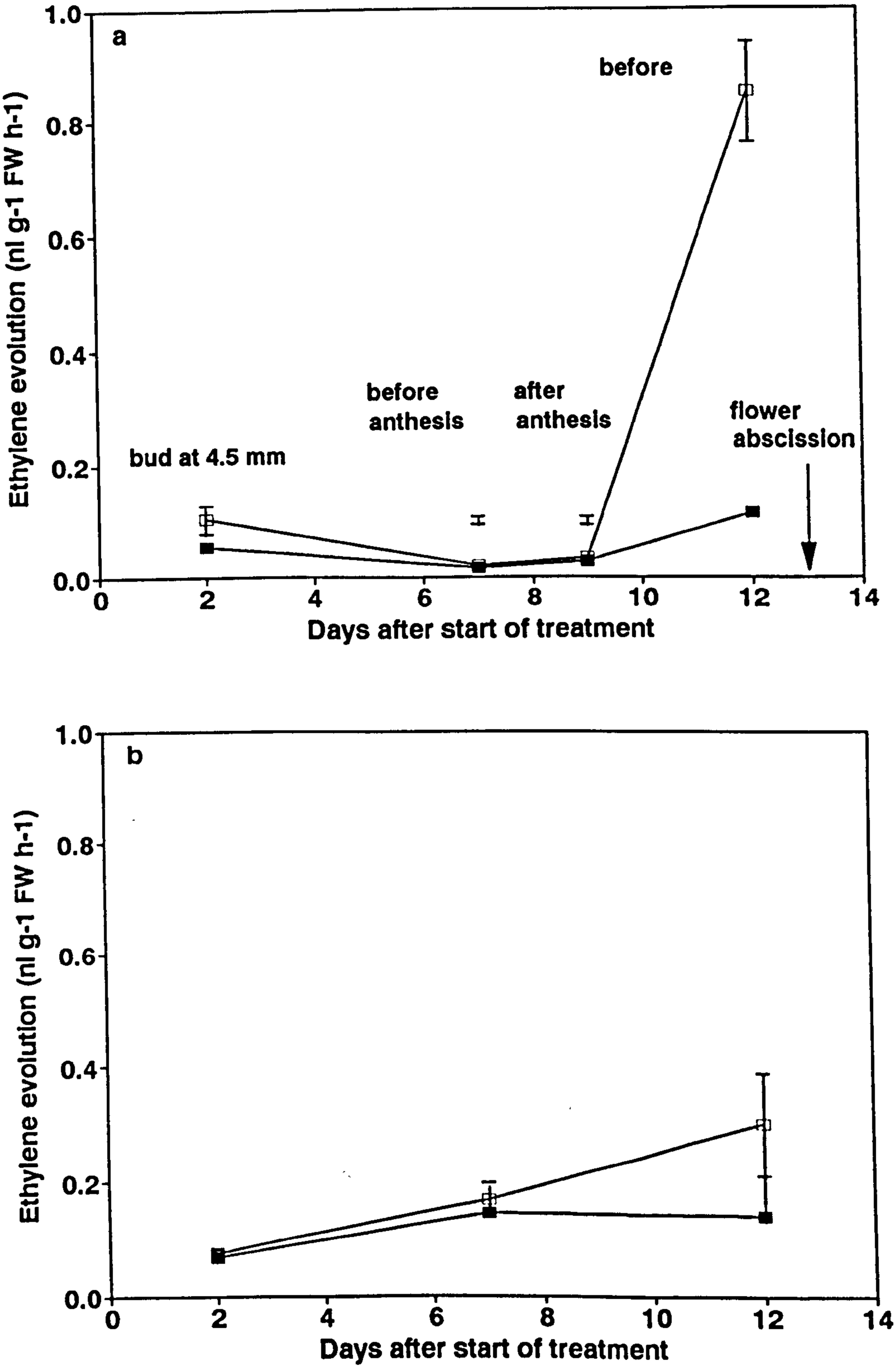


FIGURE 6.1.1. *Effects of water stress on the rates of ethylene evolution from flowers (a) and leaves (b).* ■, *NS;* □, *SS. n=18. Bars represent the Standard Error of the Difference between means.*

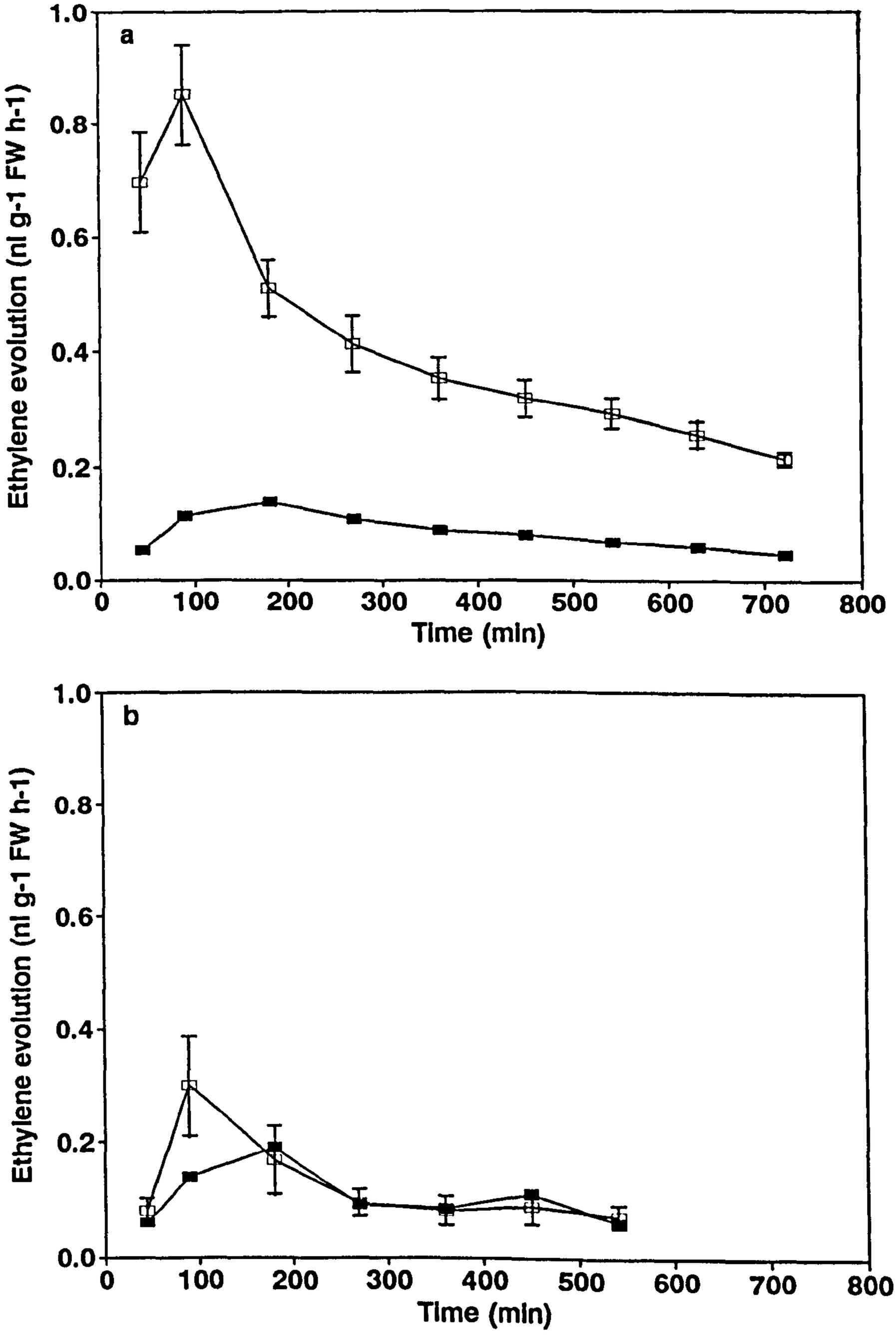


FIGURE 6.1.2. *Effects of water stress on the timecourse of ethylene evolution from excised flowers (a) and leaves (b) sampled before flower abscission.* ■, *NS;* □, *SS. n=18. Bars represent the Standard Error of the Difference between means.*

TABLE 6.1.3. *Effects of watering treatments on shoot dry matter and its distribution after first flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress	After first abscission (g)			
	Total shoot	Flower	Leaf	Stem
NS	13.0	0.4	8.1	4.5
SS	11.2	0.6	7.1	3.5
SED	0.35***	0.08***	0.22***	0.18***

*** significant at $p<0.001$

TABLE 6.1.4. *Effects of watering treatments on the components of plant water relations measured just before first anthesis and first flower abscission (n=18). SED denotes the Standard Error of the Difference between means.*

Water stress	Before first anthesis (MPa)			Before first abscission (MPa)		
	ψ_l	ψ_s	ψ_p	ψ_l	ψ_s	ψ_p
NS	-0.43	-1.02	0.58	-0.83	-1.09	0.27
SS	-0.78	-1.18	0.42	-1.29	-1.27	-0.02
SED	0.07***	0.09*	0.11ns	0.07***	0.04***	0.08***

* significant at $p<0.05$; *** significant at $p<0.001$; ns: not significant

expressed as a percentage of the total remained similar in both the NS and SS treatments (Figure 6.1.3). Prior to first abscission, no significant reduction was observed in total shoot, leaf or stem dry weights in the SS treatment (Table 6.1.2), although the dry weight of the flowers ($p<0.05$) was significantly increased, implying that more assimilates may have been directed to the developing flowers after anthesis. During the same period, the percentage of dry matter present in the leaves of NS plants was clearly reduced, while the fraction present in the stems was significantly increased (Figure 6.1.3; $p<0.01$). Immediately after first abscission, total shoot dry matter was significantly decreased in SS (Table 6.1.3; 11.2 vs. 13.0 g; $p<0.001$), as were leaf (7.1 vs. 8.1 g) and stem dry weights (3.7 vs. 4.5 g; $p<0.001$). However, flower dry weight was significantly increased. Neither total shoot dry matter production nor its distribution in SS plants (Figure 6.1.3) was correlated with the observed increase in the rate of ethylene production (Figure 6.1.3), suggesting that the abscission of primary flowers observed during severe water stress was not directly attributable either to the reduction in total shoot dry weight or to the altered pattern of dry matter distribution.

Plant water relations

Seven days after imposing the stress treatment (i.e. shortly before the first anthesis of primary flowers), ψ_l and ψ_s were both significantly decreased in SS, while ψ_p was unaffected (Table 6.1.4). However, by 12 d before first abscission, SS had caused significant reductions in all water relations components and leaves in the stressed treatment lost turgor entirely. The observed decrease in the values for all water relations components coincided with the marked increase in ethylene evolution which preceded abscission, although the observed changes were not linearly correlated.

6.2 Effects of the ethylene releasing compound 2-chloroethylphosphonic acid on flower development

The previous experiment (Section 6.1) showed that flower abscission induced by

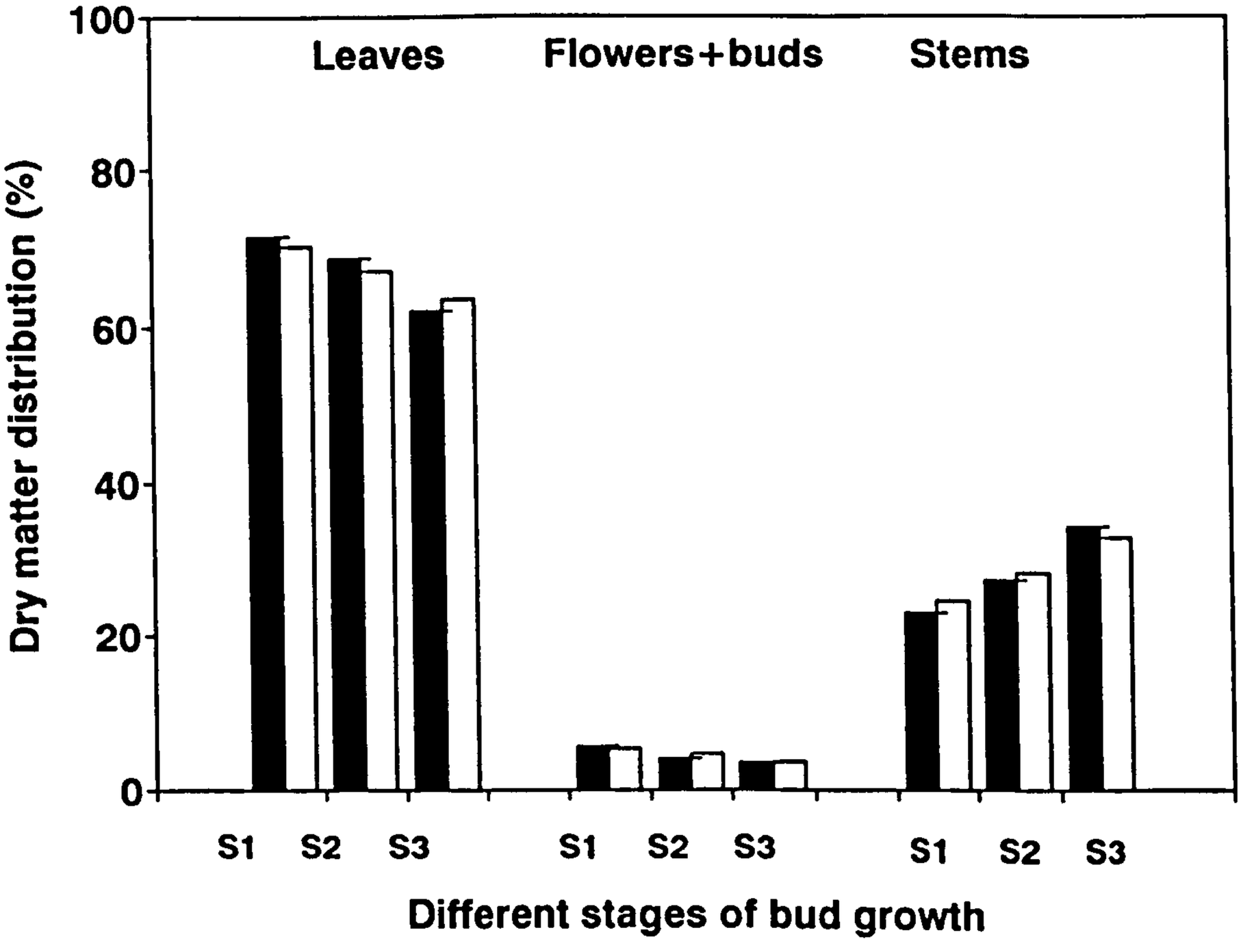


FIGURE 6.1.3. *Effects of water stress on percentage dry matter distribution within the shoot at three stages of growth. S1: before first anthesis; S2: before first abscission; S3: after first abscission.* ■, *NS;* □, *SS. n=18. Bars represent the Standard Error of the Difference between means.*

water stress is preceded by a dramatic increase in ethylene production. It was now necessary to determine whether ethylene triggered the observed abscission. The effects of exogenous ethylene on flower abscission have been reported by several authors (Kays and Beaudry, 1987; Furutani *et al.*, 1989; Mason and Miller, 1991). Tripp and

Wien (1989) showed that applications of the ethylene-releasing compound 2-chlororethylphosphonic acid (CEPA) increased bud abscission in sweet pepper. The present experiment was therefore designed to examine the extent to which applications of exogenous ethylene may substitute for water stress in promoting flower abscission in sweet pepper.

As ethylene is dispersed very rapidly in air, it was inappropriate to apply ethylene in a gaseous form in glasshouse experiments. This problem was overcome by using the ethylene-releasing compound, 2-chloroethylphosphonic acid (CEPA), which is readily absorbed, translocated and decomposed to produce ethylene after being sprayed onto plant tissues, thereby allowing the hormone to exert its effects (Kays and Beaudry, 1987; Abeles *et al.*, 1992).

6.2.1 Materials and methods

Seeds were sown on 16 October 1993 and the seedlings were pricked out into 9 cm pots 16 d later. The method of propagation, general maintenance of seedlings and glasshouse conditions were as described in Sections 2.1 and 2.4. The plants in all treatments were kept well watered to maintain optimum growth. The treatments were applied on 25 December 1993 when the secondary buds had reached a diameter of 4.0 mm. Since almost all of the primary flower buds remained dormant or withered immediately after they became visible, probably due to the low light conditions, the secondary flowers were used for treatments. The experiment continued until 2 January 1994.

Prior to this experiment, a preliminary trial was conducted to test a range of CEPA (98% a.i.) concentrations (100 - 1500 mg l^{-1} CEPA); these were either sprayed over the entire shoot or applied directly to flower buds with diameters of 1.5 - 4.0 mm. This preliminary trial was intended to determine the minimum concentration which induced abscission and the stages of flower development which were most sensitive to CEPA application. Since no surfactant was added, the flower buds were enclosed

in cotton balls (pre-weighed at 500 mg) to ensure good contact and the CEPA was applied to the cotton balls. These tests showed that the application of 1 mg flower^{-1} of CEPA using a 100 mg l^{-1} CEPA solution was sufficient to promote the formation of an abscission line on the pedicels of 4.0 mm diameter buds within two to three days of application. This was rapidly followed by increased flower abscission. Mature pepper leaves, however, were not capable of forming abscission layers in response to foliar applications of CEPA, although limited epinastic curvature was observed; in contrast, young developing leaves formed abscission lines at the base of their petioles and this was followed by leaf abscission (Plate 6.2.1). The rate of ethylene evolution from flower buds at this time was within the range observed under severe stress just before abscission. Based on these results, two concentrations of CEPA, 50 mg l^{-1} (0.5 mg CEPA flower^{-1}) and 100 mg l^{-1} (1 mg CEPA flower^{-1}), were applied daily to individual flower buds for 3 d; the control was sprayed with distilled water. The method of chemical preparation and application was as described in Section 2.6. The experiment was an Randomised Complete Block Design replicated three times, each containing of 75 plants.

Periodic flower counts and measurements of ethylene evolution from excised flowers and leaves were carried out throughout the treatment period to determine the sensitivity of flowers to exogenous ethylene and the rate of ethylene evolution from the leaves of plants whose flower buds had been treated with CEPA. Regression analysis of the values for percentage flower abscission and ethylene evolution rate was used to test for any possible relationship. Dry matter accumulation, its partitioning within the shoot and leaf water status were also examined to determine the effects of exogenous ethylene on these variables.

6.2.2 Results

During the period following first macroscopic bud appearance, accumulated irradiance measured using solarimeters was 167.9 MJ m^{-2}, with mean of 4.0 MJ m^{-2} d^{-1}. The accumulated thermal time was 831.4 °C d, with a daily mean of 19.8 °C d.

PLATE 6.2.1. *Preferential abscission of young developing leaves as compared to mature leaves following application of CEPA (top) and retention of young leaves in the control sprayed with distilled water (bottom). Arrows show the position of the abscinded leaves.*

Ethylene evolution rates

Figure 6.2.1 shows the timecourses of ethylene evolution from flowers and leaves after spraying with 50 and 100 mg l^{-1} CEPA. There was no significant increase in the rates of ethylene evolution from the flowers and leaves one hour after CEPA application (shown as Day 0), which may imply that CEPA absorption or its decomposition within the buds was slow (Yamaguchi *et al.*, 1971; Weaver *et al.*, 1972; Abeles *et al.*, 1992). No increase in ethylene evolution from the leaves, which were not sprayed with CEPA was detected, suggesting that there was no immediate response of these tissues to ethylene application to the buds, probably because there was no movement of CEPA from the flower buds to the leaves (Giulivo *et al.*, 1981).

Flowers From day 1 onwards, both CEPA treatments increased ethylene evolution from the flowers markedly compared to the control ($p<0.001$). Within 1 d of spraying with 100 mg l^{-1} CEPA, ethylene evolution increased sharply to a maximum of 0.94 nl g^{-1} FW h^{-1}, before decreasing to values below those produced in response to 50 mg l^{-1} CEPA. The maximum ethylene evolution induced by 50 mg l^{-1} CEPA (0.89 nl g^{-1} FW h^{-1}) occurred one day later than at the higher CEPA level, but thereafter the decline in ethylene evolution was similar in both treatments. These results imply that ethylene production is more rapid at the higher CEPA concentration.

Leaves Ethylene evolution following application of 100 mg l^{-1} CEPA was significantly higher than at 50 mg l^{-1} and both CEPA treatments increased gradually until day 3, before increasing sharply. By day 4, ethylene evolution had increased by approximately 14- and 9-fold in the 100 and 50 mg l^{-1} CEPA treatments respectively, relative to than in the control. The sharp increase in ethylene evolution from the leaves following the decline in evolution from the flowers could be due to two possibilities. Firstly, ethylene synthesis in the leaves might have been induced by the uptake of ethylene from the glasshouse atmosphere after being evolved from the flower pedicels following the formation of the abscission zone and during abscission (Burg and Burg, 1964, 1965; Solomos, 1989). Secondly, sweet pepper may be capable of autocatalytic production of ethylene, whereby ethylene released from CEPA accelerates the

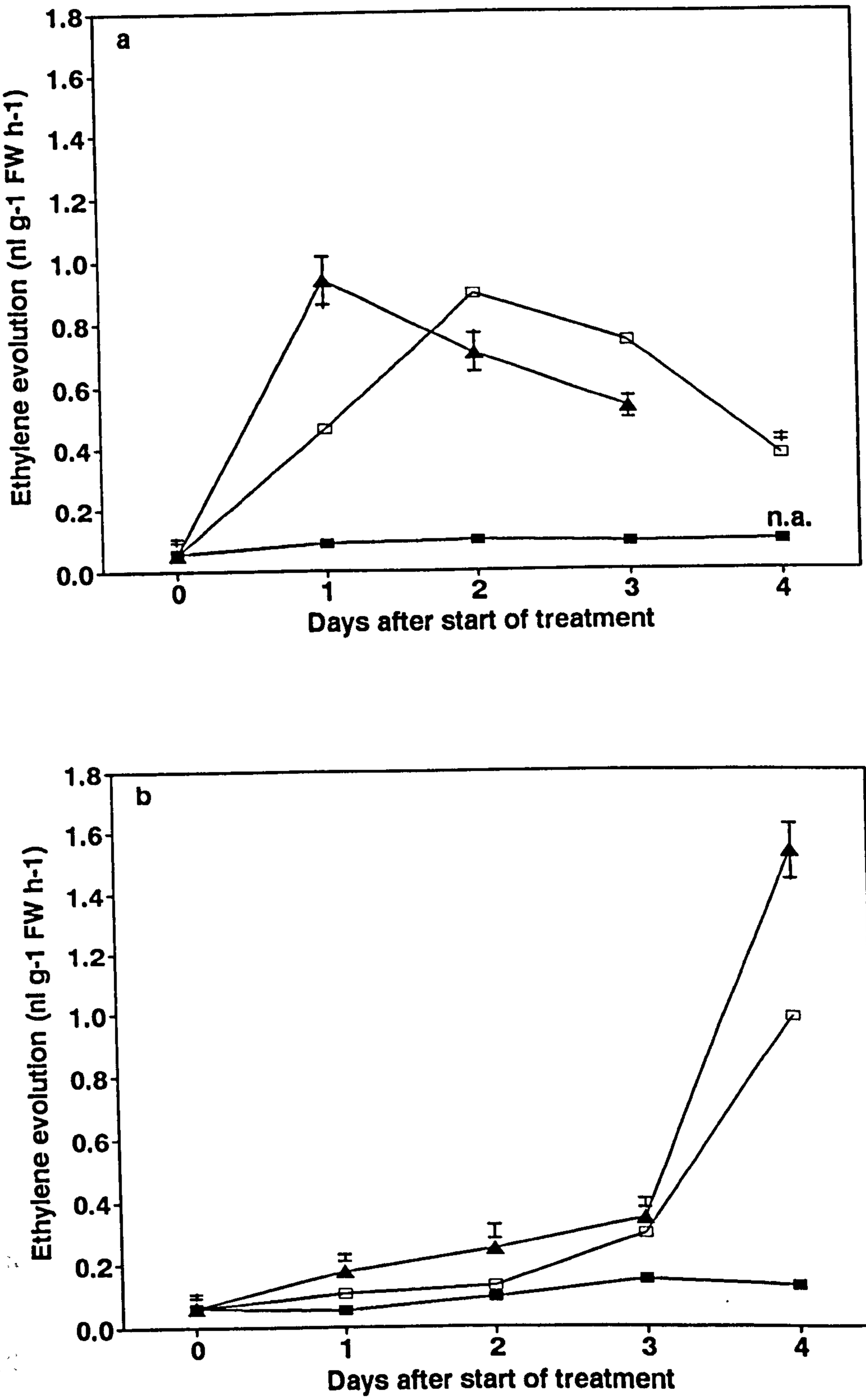


FIGURE 6.2.1. *Effects of 2-chloroethylphosphonic acid application on the rate of ethylene evolution from flowers (a) and leaves (b).* ■, *Control;* □, *50 mg l^{-1} (0.5 mg CEPA flower^{-1});* ▲, *100 mg l^{-1} (1.0 mg CEPA flower^{-1}. n=15. Bars represent the Standard Error of the Difference between means. n.a., data not available for the high concentration application at day 4 due to 100% abscission.*

endogenous synthesis of ethylene (Gupta and Anderson, 1989; Schierle *et al.*, 1989).

Flower abscission

Sweet pepper plants are clearly capable of producing an abscission zone in the pedicels of the flowers following CEPA application (Plate 6.2.2) and thereafter flower abscission began to occur. Earlier (day 1) and increased abscission was induced by 100 than 50 mg l^{-1} CEPA ($p<0.001$; Figure 6.2.2) following the formation of a visible abscission line. The line became distinct on day 2, when gentle pressure caused the flower to abscind easily. Almost complete flower abscission occurred during this period, which was preceded by a sharp increase in ethylene evolution from the flowers to 0.94 nl g^{-1} FW h^{-1} on day 1. By day 3, the pedicels had shrivelled and the flowers abscinded voluntarily.

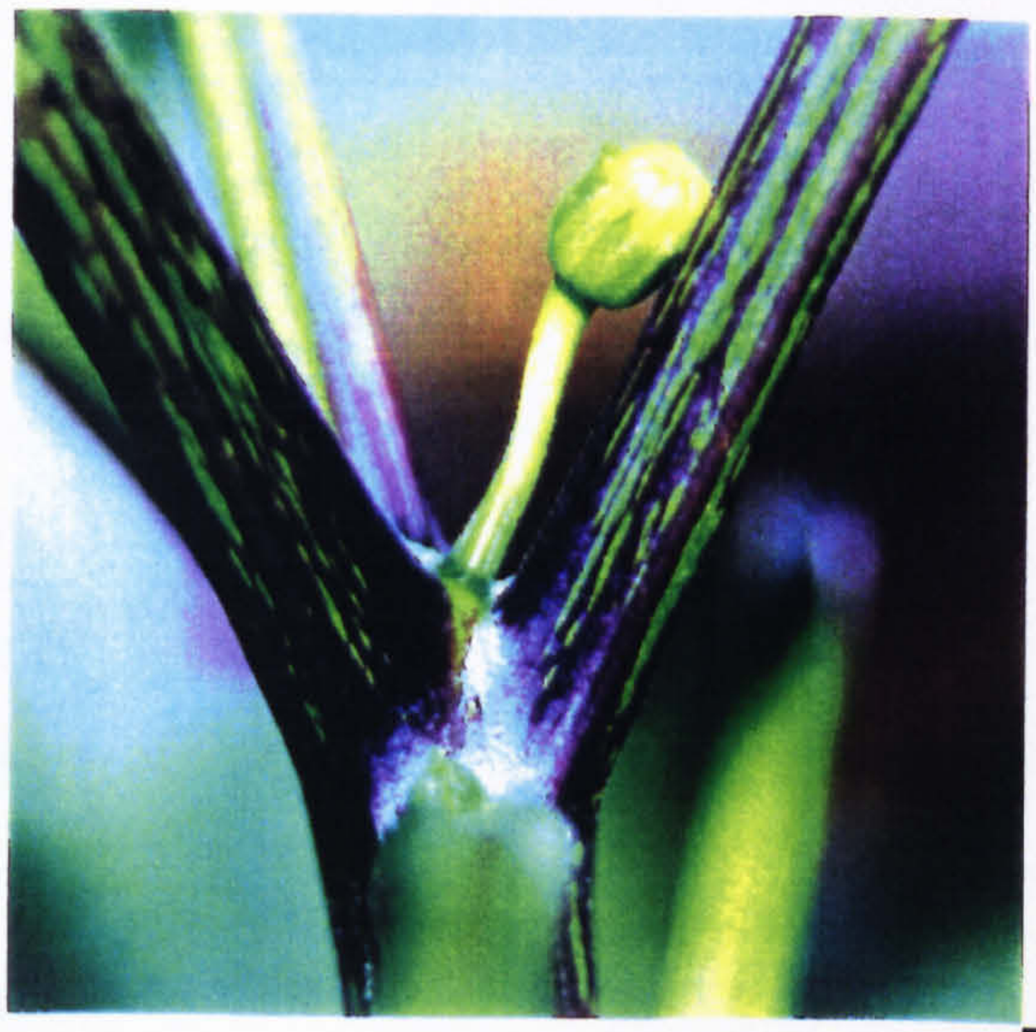
At the lower CEPA concentration, the formation of the abscission zone and the increase in percentage abscission occurred more gradually. The abscission line was first observed on day 2 and became distinct on day 3, and this was followed by a marked increase in abscission from 40 to 98%. As at the higher CEPA concentration, the increase in percentage abscission was preceded by an increase in the rate of ethylene evolution two days earlier.

Correlation

Figure 6.2.3 shows the highly significant ($p<0.001$) quadratic relationships between percentage flower abscission and ethylene evolution from flowers treated with 50 or 100 mg l^{-1} CEPA. Percentage flower abscission was lowest (c. 6%) when ethylene evolution was approximately 0.06 nl g^{-1} FW h^{-1} and maximum abscission (100%) occurred when ethylene evolution reached approximately 0.97 nl g^{-1} FW h^{-1} in the 50 mg l^{-1} and 0.55 nl g^{-1} FW h^{-1} in the 100 mg l^{-1} CEPA treatments. As ethylene evolution continued to increase in the 100 mg l^{-1} CEPA treatment, no simultaneous increase in percentage abscission was observed.

PLATE 6.2.2. *Sequence of flower bud abscission following the formation of an abscission zone at the base of pedicel following treatment with 100 mg l^{-1} CEPA. (a) 0 DAT; (b) 1 DAT (note distinct formation of the abscission layer); (c) 2 DAT; (d) 3 DAT.*

a



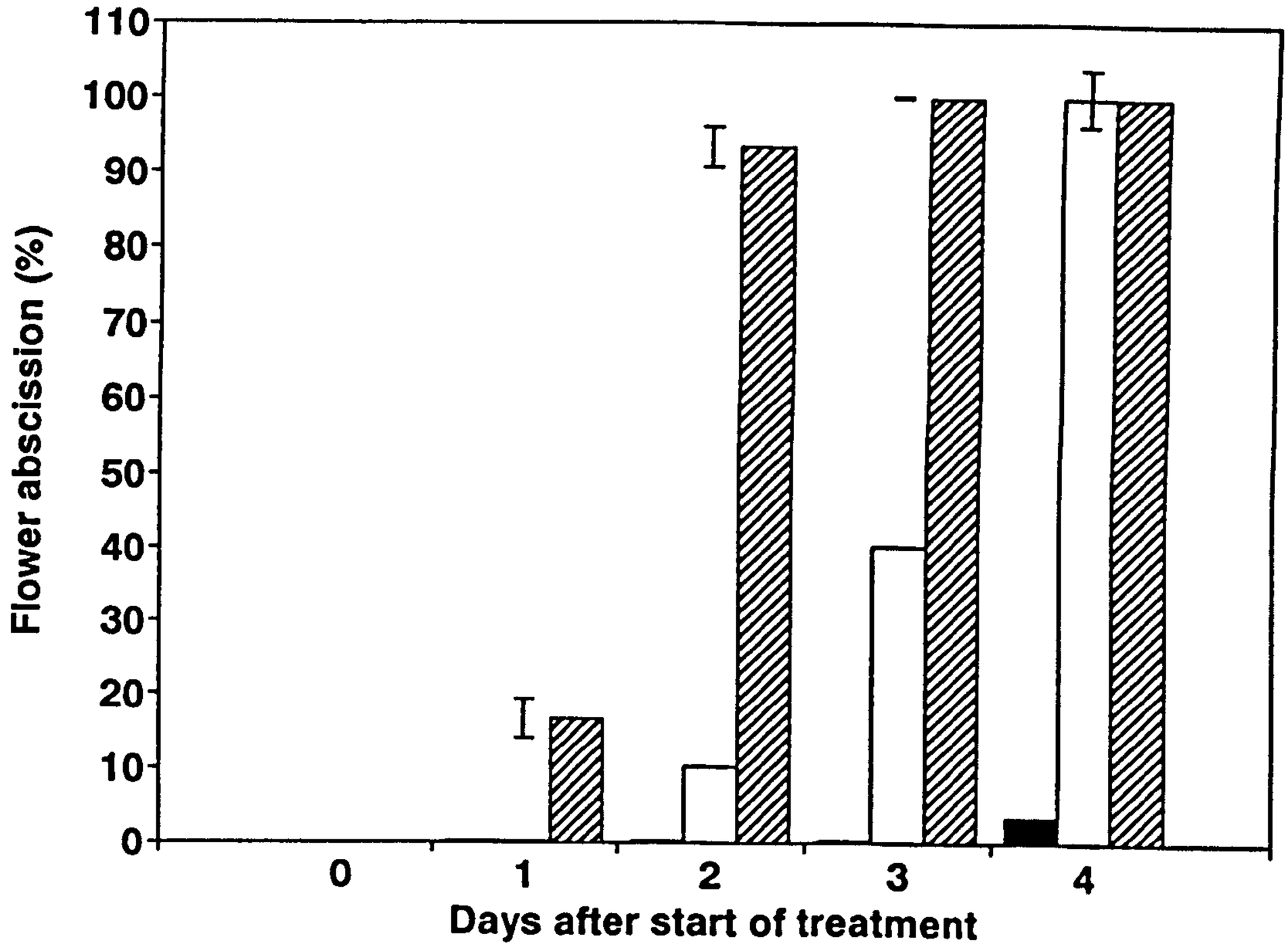
b

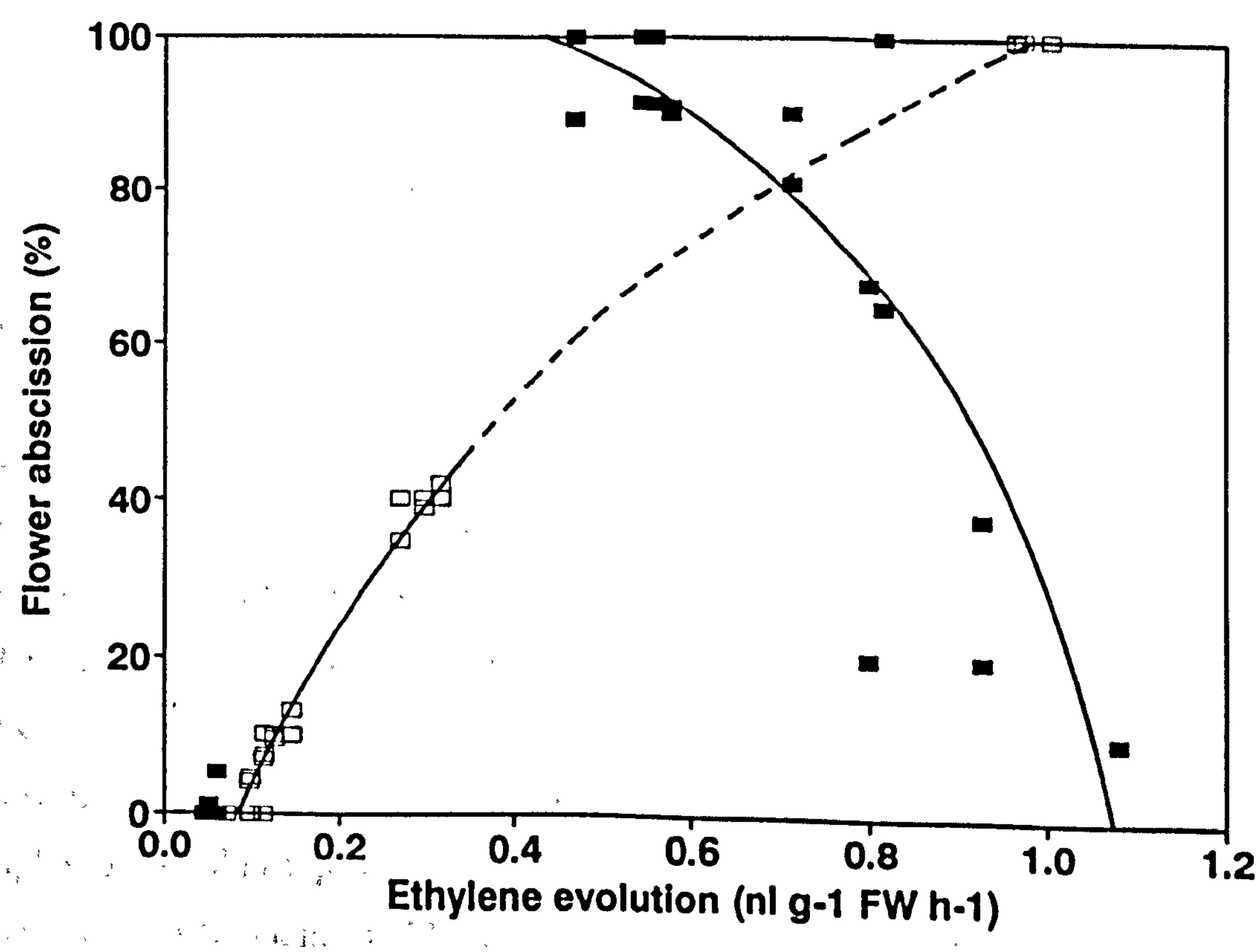
c

d

FIGURE 6.2.2. *Effects of 2-chloroethylphosphonic acid application on percentage flower abscission.* ■, *Control;* □, *50 mg* l^{-1} *(0.5 mg CEPA flower*$^{-1}$*);* ▨, *100 mg* l^{-1} *(1.0 mg CEPA flower*$^{-1}$*. n=15. Bars represent the Standard Error of the Difference between means.*

FIGURE 6.2.3. *Relationship between percentage flower abscission and the rate of ethylene evolution from flowers treated with 50 (□) and 100 (■) mg* l^{-1} *(0.5 and 1.0 mg CEPA flower*$^{-1}$ *respectively) 2-chloroethylphosphonic acid. n=15. Regression equations: 50 mg* l^{-1}*:* $Y = -16.16 + 213.90X - 97.26X^2$ *and 100 mg* l^{-1}*:* $Y = -18.64 + 402.61X - 368.62X^2$*;* $R^2 = 0.998$ *and 0.804* ($p<0.01$) *respectively.*


110
100
90
80
70
60
50
40
30
20
10
0
Flower abscission (%)
0
1
2
3
4
Days after start of treatment


100
80
60
40
20
0
Flower abscission (%)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Ethylene evolution (nl g-1 FW h-1)

Plant water status

Leaf ψ_l, ψ_s and ψ_p on day 4 decreased as the rate of CEPA application increased ($p<0.01$; Figure 6.2.4). A broadly similar pattern was observed 7 d after application ($p<0.01$), although ψ_s was not significantly reduced by the treatments imposed and was less negative than ψ_l in 100 mg l^{-1} CEPA treatment, causing a negative turgor of -0.13 MPa to be recorded. In the 50 mg l^{-1} CEPA treatment, which appeared to impose stress upon the plants more gradually, turgor was still retained at day 7, possibly because of osmotic adjustment. These results suggest that the application of CEPA to plants may simulate the effect of water stress on leaf water relations, where the nature of the response observed depends on the severity and duration of the stress applied. The more gradual stress imposed by the lower CEPA application also appeared to enhance the capacity of the leaves for osmotic adjustment.

6.3 Inhibition of ethylene action by silver thiosulphate

The involvement of endogenous and exogenous ethylene in flower abscission following the imposition of stress or the application of ethylene-generating sprays is well established (Halevy and Mayak, 1981; Sexton *et al.*, 1985; Abeles *et al.*, 1992). However, the action of ethylene can be competitively inhibited by various chemicals, including silver nitrate (Beyer, 1976) and silver thiosulphate (STS; Veen, 1983, 1986). These chemicals are thought to act by blocking binding sites for ethylene by combining with the ethylene receptor, thereby preventing the cells from responding to ethylene (Veen, 1986; Abeles *et al.*, 1992). Both chemicals are persistent and specific in their action, although the usefulness of silver nitrate has been limited by its relative immobility within plant tissues and the phytotoxicity which is generally induced following its application at effective concentrations. In contrast, silver complexed with thiosulphate is extremely mobile within the plant, is less phytotoxic (Veen and Van De Geijn, 1978) and remains active within plant tissues for extended periods (Reid *et al.*, 1980). At higher concentrations, however, it becomes phytotoxic (Wang and Dunlap, 1990).

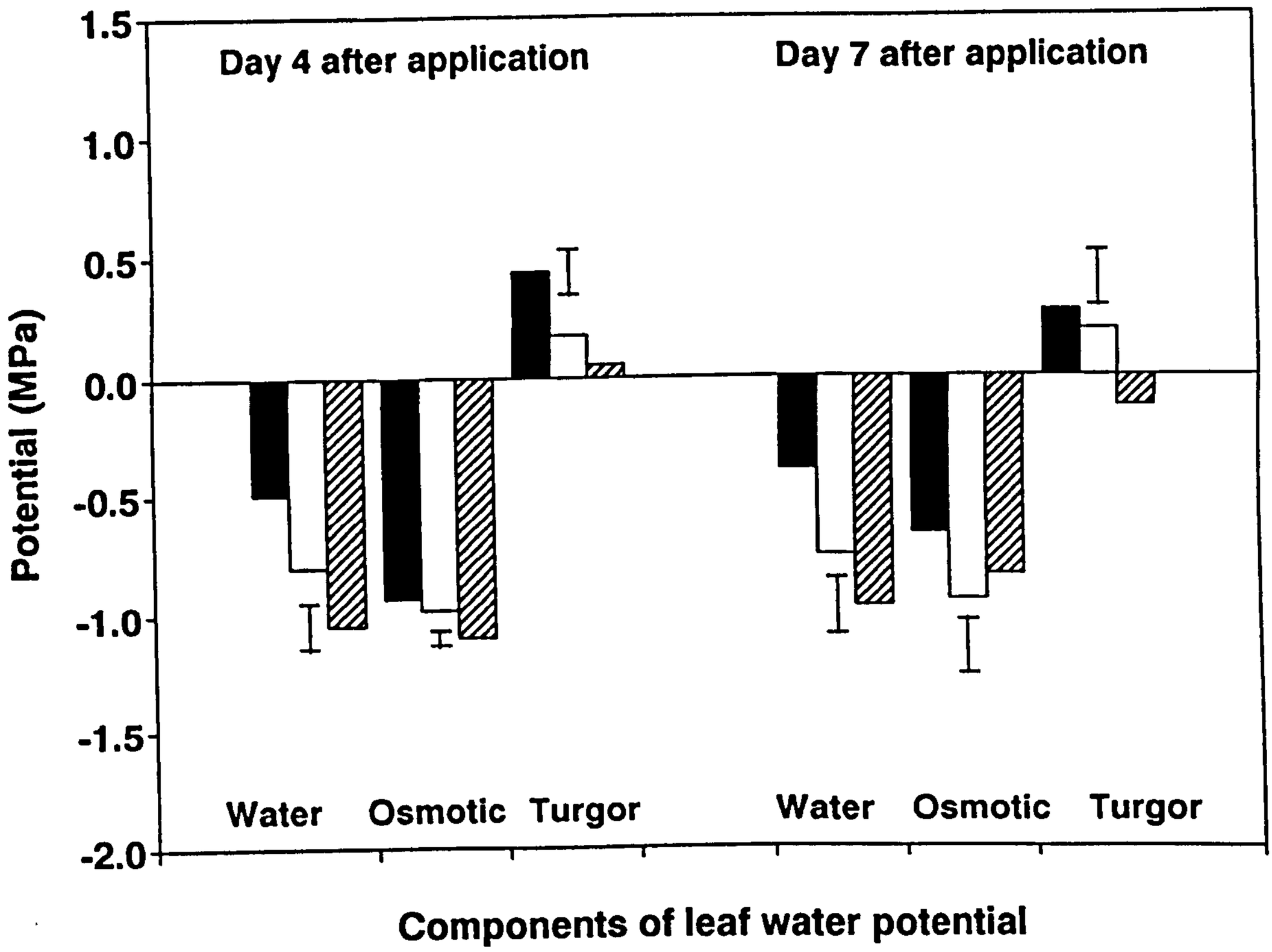


FIGURE 6.2.4. *Effects of 2-chloroethylphosphonic acid application on plant water status.* ■, *Control;* □, *50 mg* l^{-1} *(0.5 mg CEPA flower);* ▨, *100 mg* l^{-1} *(1.0 mg CEPA flower*$^{-1}$*. n=15. SED represents the Standard Error of the Difference between mans.*

In this section, two experiments were conducted using silver thiosulphate (STS) as an inhibitor of the promotory effect of ethylene on flower abscission. In the first experiment, STS was applied to plants subjected to water stress, while the second, flower buds pretreated with STS were supplied with the ethylene-generating substance,

2-chloroethlyphosphonic acid (CEPA). The main aim of these experiments was to test further the role of increased endogenous ethylene production in mediating the induction of flower abscission by water stress, as suggested by the preceding experiments.

6.3.1 Inhibition of water stress-induced ethylene production by silver thiosulphate

In Experiment 6.1, it was found that severe water stress increased flower abscission and dramatically increased the rate of ethylene production prior to abscission. Since ethylene is widely believed to be involved in mediating abscission, blocking its action with STS may be expected to prevent or reduce this process. The experiment described below was carried out to establish whether STS provided protection against water stress-induced flower abscission.

6.3.1.1 *Materials and methods*

Seeds were sown on 10 October 1993 and seedlings pricked out into 9 cm pots on 26 October 1993. The method of propagation and general maintenance of the seedlings and glasshouse conditions were as described in Section 2.1. As in Experiment 6.2, secondary flowers were used and treatments began when these reached a diameter of 3.5 - 4.5 mm (61 d after germination). Three treatments were imposed: no water stress (NS), severe stress (SS), and severe stress plus a spray treatment with 1 mM STS (SS+1). The plants were arranged in a Randomised Complete Block Design, blocked three times and with each treatment containing 36 plants. The experiment continued for 15 d after the treatments were imposed.

Water stress was imposed as described in Section 2.5, and STS was prepared as described in Section 2.6. Since STS was effective regardless of whether it was applied to whole plants or only to individual developing inflorescences (Cameron and Reid,

1983), c. 10 ml of 1 mM STS was applied as a foliar spray every three days. The control and severe stress treatments were sprayed with distilled water. Flower counts and measurements of ethylene evolution were carried out to determine the influence STS on ethylene production and flower abscission.

6.3.1.2 *Results*

Flower abscission

Limited flower abscission was observed 6 d after imposing the treatments in plants that were subjected to severe water stress, although this was not significantly greater than in the other treatments (Table 6.3.1). However, by 10 d after the treatment began, abscission had increased markedly to 61% in the severely stressed plants. In contrast, flowers from severely stressed plants treated with 1 mM STS spray exhibited complete flower retention, suggesting that this treatment was effective in preventing water stress-induced flower abscission (Plate 6.3.1).

Rate of ethylene evolution from flowers

Ethylene evolution from the flowers of severely stressed plants was significantly greater than in the other treatments (Table 6.3.1), at a time when 11 % of the flowers had abscinded. However, when severely stressed plants were treated with STS, there was no equivalent increase in ethylene evolution. By day 10, ethylene evolution had increased even further in the severely stressed plants, but this effect was again effectively blocked by STS ($p<0.001$). Indeed, ethylene evolution from STS-treated flowers was lower than in the control treatment. A small increase in ethylene production between days 6 and 10 was also observed in the NS control treatment, possibly reflecting an inherent increase associated with natural aging of the flowers.

TABLE 6.3.1. *Inhibitory effects of silver thiosulphate on flower abscission and on ethylene evolution from detached flowers (n=18). SED denotes the Standard Error of the Difference between means.*

Treatment	Flower abscission (%)		Ethylene evolution rate (nl g^{-1} FW h^{-1})	
	Days after start of treatment			
	6	10	6	10
NS	0	6	0.06	0.29
SS	11	61	0.37	0.89
SS+1	0	0	0.07	0.10
SED	4.54^{ns}	6.42^{***}	0.092^{*}	0.034^{***}

* significant at $p<0.05$
*** significant at $p<0.001$
ns: not significant

6.3.2 Inhibition of CEPA-induced ethylene production in flowers by silver thiosulphate

In Experiment 6.2 it was found that the application of exogenous ethylene in the form of 2-chloroethylphosphonic acid (CEPA) mimicked the effects of water stress by inducing increased flower abscission. In a recent study, Mason and Miller (1991) found that pre-treatment with STS reduced the abscission of flowers treated with CEPA. The object of the present experiment was to test whether STS could provide partial or complete protection against CEPA-induced flower abscission in sweet pepper.

PLATE 6.3.1. *Treatment with 1 mM STS prevents flower abscission in severely stressed plants. (a) SS plants + STS; (b) SS plants; (c) NS control.*

a

b

c

6.3.2.1 *Materials and methods*

Blue Star seeds were sown on 16 October 1993 and pricked out on 1 November 1993. The method of propagation and general maintenance of the seedlings and glasshouse conditions were as described in Section 2.1. The experiment was a factorial 2 x 2 and a control arranged in a Randomised Complete Block Design, blocked three times. The five treatments applied comprised two levels of CEPA (50 and 100 mg l^{-1} applied in 10 ml aliquots), with and without STS (0 and 1 mM in 10 ml aliquots) and a control sprayed with distilled water. Each treatment contained 12 plants.

The treatments were imposed on 25 December 1993 when the secondary flower buds had reached a diameter of 3.5 - 4.0 mm, and lasted for 10 d. The buds were sprayed with 10 ml of STS and one day later were covered with fresh cotton wool balls and sprayed with 10 ml of CEPA or distilled water. The buds were covered in cotton wool balls to ensure good contact with the chemical. Both chemicals were reapplied in the same way 3 d later. The STS and CEPA solutions were prepared as described in Section 2.6. Periodic flower counts were made to determine the effectiveness of STS in blocking the flower abscission induced by exogenous ethylene.

6.3.2.2 *Results*

Flower abscission

Table 6.3.2 clearly demonstrates the inhibitory effect of STS application on ethylene-induced flower abscission. Application of silver thiosulphate at a concentration of 1 mM completely inhibited flower bud abscission at both levels of CEPA ($p<0.001$) at all three sampling dates. During the same period, plants sprayed with CEPA and not protected with STS exhibited a marked increase in percentage abscission which increased with time and CEPA concentration (Plate 6.3.2). These results provide strong evidence that ethylene may mediate stress-induced abscission of flowers and buds.

TABLE 6.3.2. *Inhibitory effect of silver thiosulphate on flower abscission induced by 2-chloroethylphosphonic acid (n=18). SED denotes the Standard Error of the Difference between means.*

CEPA ($mg\ l^{-1}$)	STS (mM)	Flower abscission (%)		
		Days after STS application		
		3	5	7
Control		0	0	0
50	0	11	50	94
	1	0	0	0
100	0	83	100	100
	1	0	0	0
SED(Control)		5.56^{ns}	0.32^{***}	2.77^{***}
SED(Control*CEPA)		6.09^{***}	0.36^{***}	3.03^{***}
SED(Control*STS)		6.09^{***}	0.36^{***}	3.03^{***}
SED(CONTROL*CEPA*STS)		7.03^{***}	0.41^{***}	3.50^{***}

* significant at $p<0.05$

** significant at $p<0.01$

*** significant at $p<0.001$

ns: not significant

PLATE 6.3.2. *Treatment with 1 mM STS prevents flower abscission in plants sprayed with 100 mg l^{-1} CEPA. (a) CEPA + STS (28 DAT); (b) CEPA + STS (40 DAT); (c) CEPA (40 DAT).*

a

b

C

6.4 Discussion

The previous Chapter examined the growth responses of glasshouse-grown sweet pepper plants to progressive water stress imposed at various stages of flower bud development. Clear relationships were established between growth responses and the severity and duration of stress. The experiments described in this chapter investigated the possible role of ethylene in mediating the effects of severe stress on reproductive growth and development.

Progressive imposition of severe and prolonged water stress on reproductive plants when the first flower bud became macroscopically visible induced early and increased flower abscission. Although reductions in the availability of photoassimilates due to water stress have been suggested by other workers as a cause of abscission (Peltonen-Sainio, 1991), no consistent reduction in total shoot dry matter was observed in the work reported here. The observed decline in turgor shortly before flower abscission could not be related directly to flower abscission or shoot dry matter production, suggesting that other changes occurred much sooner and at lower stress levels than those associated with turgor loss (Hsiao, 1973).

A role for ethylene in stress-induced abscission in other species is well documented (Halevy and Mayak, 1981; Durieux *et al.*, 1983; Sexton *et al.*, 1985; Ohno, 1991; Abeles *et al.*, 1992). While ethylene production has often been found to increase following the imposition of water stress or drought in many species, ultimately resulting in leaf abscission (Jordan *et al.*, 1972; Michael *et al.*, 1972; El-Beltagy and Hall, 1974; Apelbaum and Yang, 1981; Kimmerer and Kozlowski, 1982; Kirkham, 1985), very few studies have examined the role of drought-induced ethylene production in flower abscission in horticultural crops. The work carried out here showed that water stress significantly increased ethylene evolution immediately prior to flower abscission to a level 40-fold greater than that measured before anthesis, and by up to 8-fold relative to unstressed plants. Ethylene evolution from flowers was also 3-fold greater than from leaves in water stressed plants, whereas the reverse applied in unstressed control plants. A similar involvement of increased ethylene production

immediately prior to the abscission of water stressed young cotton bolls has also been reported (Guinn, 1976). These observations suggest that the enhancement of ethylene production in flowers by water stress may have increased their susceptibility to abscission.

While water stress induced flower abscission, no simultaneous leaf abscission occurred in the older leaves. The lower rate of ethylene evolution by the leaves immediately prior to flower abscission suggests that the developing flowers of sweet pepper may have been more sensitive to water stress. The differential sensitivity to stress-induced ethylene production between plant organs and species (Sexton *et al.*, 1985) can often be attributed to the presence or absence of a differentiated class of target cells which form the separation layer - the line of abscission (Osborne, 1982; Abeles *et al.*, 1992). This line was observed in the present study to develop at the base of the pedicels just before the abscission. Similar abscission lines were not observed at the base of the petioles of mature leaves of sweet pepper, however, suggesting that the target cells were absent, or that endogenous ethylene levels did not reach the critical level for induction of leaf abscission. The observed increase in ethylene evolution with time from non-stressed flowers and leaves may indicate that ethylene production increased with the physiological age of individual flowers and the plants themselves (Sexton *et al.*, 1985; Abeles *et al.*, 1992). In non-stressed plants, the leaves also appeared to produce more ethylene than the flowers.

The findings so far have excluded direct roles for reductions in total shoot assimilate production or the components of water potential in promoting flower abscission in water stressed pepper plants. Instead, water stress-induced ethylene production was thought to be a more likely contributory factor to the flower abscission observed in stressed plants. To confirm this, a series of experiments was carried out on the assumption that, if the promotion of ethylene production by water stress was the causal factor for flower abscission in stressed plants, then administering ethylene-releasing substances to non-stressed plants should increase ethylene production and mimic the effects of water stress in inducing flower abscission. Total shoot dry matter and its partitioning to the leaves and stems did not decline markedly during the period of

increased ethylene evolution from flowers and therefore could not be correlated with the altered pattern of ethylene evolution. This further supports the view that reductions in assimilate production or dry matter partitioning to the leaves and stems were not directly involved in inducing flower abscission. Decreases in leaf water and osmotic potentials following increases in ethylene production have been reported previously (McMichael, 1972; Curtis, 1981; Stumpff and Johnson 1987; Miyamoto and Kamisaka, 1987) and the results obtained in the present study are consistent with these reports. However, these changes could not be related with ethylene evolution from flowers or leaves, implying that, although decreases in the components of water potential may indicate the severity of stress in plants, they are not necessarily closely coordinated with hormonal changes, which may be induced at much lower stress levels than those indicated by turgor potential.

The application of the ethylene-releasing compound (CEPA) to the flower buds of sweet pepper mimicked the effects of water stress. The resultant increase in ethylene evolution from the flowers was reflected by increased flower abscission prior to anthesis, but not by increased leaf abscission. This observation suggests that the flower buds were more sensitive to ethylene-induced abscission than the leaves, especially when sprayed with a relatively high concentration of CEPA. Highly significant quadratic correlations relating percentage flower abscission to ethylene evolution rate following the application of CEPA were established. Similar effects of exogenous ethylene on flower abscission have been described previously (Durieux *et al.*, 1982; Kays and Beaudry, 1987; Furutani *et al.*, 1989; Mason and Miller, 1991; Abeles *et al.*, 1992).

The concentration of the ethylene-releasing compound applied and the duration of exposure may both affect the response observed (Kay and Beaudry, 1987). In the present work, it was observed that applications of the higher CEPA concentration caused the abscission layer to form more rapidly at the base of the pedicels than at the lower concentration, and that this was followed by earlier and greater bud abscission. Mason and Miller (1991) also showed that applications of a high concentration of CEPA (4.2 Mm) caused greater bud abscission in glasshouse-grown Easter lilies than

a lower CEPA concentration (2.1 Mm). Increases in ambient temperature increase both the rate of ethylene evolution from CEPA (Bukovac *et al.*, 1971; Wilde and Edgerton, 1975), and the absorption of CEPA by plant tissues (Olien and Bukovac, 1982), which combine to determine the magnitude of the plant response. Thus, the much greater effect of CEPA in accelerating bud abscission prior to anthesis, especially at the higher concentration, as compared to the ethylene-induced flower abscission that occurred immediately after anthesis in water stressed plants suggests that the release of ethylene from CEPA may have been accelerated by the prevailing high glasshouse temperature (26 °C). In addition, sweet pepper plants may be capable of autocatalytic production of ethylene, whereby ethylene released from CEPA would accelerate the endogenous synthesis of ethylene (Gupta and Anderson, 1989; Schierle *et al.*, 1989; Foster *et al.*, 1992), thereby accelerating the abscission process (Furutani *et al.*, 1989). The present results also demonstrated the rapid formation of a distinct abscission layer in flower pedicels following the application of CEPA, especially at the higher concentration, indicating that the flower buds of sweet pepper plants are capable of forming an abscission line prior to stress-induced bud abscission (Abeles *et al.*, 1992). Similar results were reported by Wong and Osborne (1978) and Osborne (1982).

In green pepper and tomato, a major exit for endogenous ethylene to the external environment has been reported to be diffusion via the pedicels (Burg and Burg, 1964). Thus, the separation layer at the base of the pedicels may be an important exit point for ethylene from the abscinding flowers and, since leaves have a greater porosity in terms of the total number of stomata per unit area than fruit or flower buds, greater gas exchange between the tissue and the external environment is possible (Abeles *et al.*, 1992; Ben-Yehoshua *et al.*, 1985). This may provide an explanation for the abrupt increase in ethylene evolution from the leaves on the fourth day after CEPA application when all the flowers and buds had abscinded. Another possible explanation for the observed increase in ethylene evolution from leaves which had not been treated with CEPA is the capability of plants to undergo autocatalytic production of ethylene, whereby applications of exogenous ethylene have been shown to increase the levels of endogenous ethylene in plant tissues (Burg and Burg, 1964, 1965; Solomos, 1989). Although high ethylene evolution rates were detected for leaves treated with the higher

concentration of CEPA, there was no formation of a separation layer in the petioles of mature leaves. However, newly developed young leaves abscinded following the formation of an abscission zone, while a very mild epinastic curvature was seen in the older leaves. A similar preferential abscission of the younger leaves has been reported in pepper (Woltering, 1987) and cotton (Morgan, 1973; Beaudry and Kays, 1988)..

Water, osmotic and turgor potentials were all decreased following treatment with CEPA. In agreement with this observation, Kirkham (1985) found that the osmotic potentials of two genotypes of pearl millet grown under well watered conditions decreased following treatment with ethephon. Ishizawa and Esashi (1984) also found that ethylene promoted solute accumulation in the coleoptiles of rice (*Oryza sativa* L.), while Eisinger *et al.* (1983) reported that treatment with ethylene increased cell-sap osmolality in pea.

The results obtained strongly suggest that ethylene may mediate the impact of water stress on flower abscission, a supposition supported by the experiments involving the use of silver thiosulphate (STS) to block ethylene-induced abscission. These confirmed that ethylene has a major role in promoting flower or bud abscission in water stressed sweet pepper and that foliar application of STS one day after imposing water stress proved highly effective in blocking abscission. Although ethylene evolution increased significantly in severely stressed plants, no similar promotion of ethylene evolution was observed in plants treated with STS. Similarly, plants pre-treated with STS showed no abscission response to CEPA and this protective effect persisted for at least 7 - 10 d. The protective influence of STS against flower abscission has been reported previously (Cameron and Reid, 1983; Dostal *et al.*, 1991), although most previous studies have concentrated on stresses other than water stress. For instance, Cameron and Reid (1981) showed that foliar application of STS produced 80 - 90% retention of flowers and buds in zygocactus plants stressed by exposure to ethylene or 26 °C and darkness for four weeks after application; these treatments would otherwise have induced complete abscission of buds, flowers and leaflets. Cameron and Reid (1983) also demonstrated a marked effect of STS in preventing the abscission of floral organs and flowers in potted flowering plants. For example, petal abscission from geranium

flowers (*Pelargonium hortorum* Bailey) exposed to continuous light at 25 °C was completely suppressed by a 0.5 mM foliar spray of STS, while a similar treatment reduced flower drop from 83 to 22% in *Calceolaria herbeohybrida* Voss plants exposed to four days of drought in darkness at 25 °C. The protective influence of STS against stress-induced flower abscission was also observed when Easter lily was sprayed with ethephon following treatment with STS and then exposed to a 92% reduction in irradiance for 14 d (Mason and Miller, 1991). The effectiveness of the STS spray persisted for about two weeks after treatment. These results suggest that the STS applications have great potential as a means of reducing stress-induced abscission in potted flowering plants, including sweet pepper. However, further studies should be carried out to examine the effectiveness of STS as a preventative measure against flower abscission in soil-grown sweet pepper plants raised in protected shelters, or field-grown sweet pepper plants in the open. Such studies should also examine the subsequent fruiting performance of STS treated plants.

6.5 Conclusions

1. Water stress significantly increased ethylene evolution from sweet pepper flowers immediately prior to abscission to a rate 40-fold greater than that measured before anthesis. Ethylene evolution from the flowers was also increased by up to 8-fold as compared with unstressed control plants.

2. Ethylene evolution from the flowers was lower than from the leaves in unstressed plants, but in stressed plants, ethylene evolution from the flowers was 3-fold greater than from the leaves, implying that developing flowers were more sensitive to water stress. In unstressed plants, ethylene evolution from the flowers increased with age.

3. Shoot dry matter and its partitioning to the leaves and stems did not decline markedly during the period of increased ethylene evolution from the flowers, and therefore could not be correlated with the changing pattern of ethylene

evolution. This further supports the view that reductions in assimilate production or partitioning to the leaves and stems were not directly involved in inducing flower abscission.

4. The application of the ethylene-releasing compound 2-chloroethylphosphonic acid (CEPA) to flower buds increased ethylene evolution from the buds and mimicked the effects of water stress by increasing abscission. This observation strongly suggests that ethylene mediates the impact of water stress on flower abscission. Exogenous ethylene applications also promoted bud abscission prior to anthesis.

5. The severity of flower abscission following CEPA application was dependent upon the concentration applied. At 100 mg l^{-1} CEPA, almost 100% abscission occurred on day 2 after application, following a peak in ethylene evolution on day 1. Treatment with 50 mg l^{-1} CEPA induced complete abscission on day 4, following a peak in ethylene evolution on days 2 - 3 after application.

6. A highly significant quadratic correlation was established between percentage flower abscission and ethylene evolution following the application of CEPA.

7. Sweet pepper flowers were capable of forming abscission lines at the base of the pedicels following exposure to elevated endogenous ethylene concentrations caused either by water stress or exogenous ethylene application. Mature pepper leaves were incapable of forming abscission layers, although younger leaves developed abscission zones when sprayed with CEPA.

8. Foliar application of silver thiosulphate (STS) was highly effective in blocking the increase in ethylene evolution and preventing flower abscission in water stressed plants. Pre-treatment with STS also blocked the promotory influence of ethylene released from CEPA on flower abscission. The protective effects persisted for 7 - 10 d.

9. Although the components of water potential were all decreased during periods of increased ethylene production, these changes could not be consistently related to increased ethylene evolution from the flowers and leaves. These results imply that the relatively large decreases in water relations components observed during progressive water stress may not be useful in predicting the changes in ethylene production which are effective in promoting abscission; these may be induced by much smaller losses in water status or turgor.

CHAPTER 7

GENERAL DISCUSSION

The principal objective of this Chapter is to bring together the main conclusions from the various investigations carried out in this project. The overall aim was to determine the impact of environmental stresses, especially high temperature and water deficits, on reproductive development in sweet pepper, with special attention being given to abscission of the primary and secondary flowers since this has major implications for the economic value of the crop. The role of assimilate accumulation and partitioning and the endogenous growth regulator, ethylene, in mediating stress effects on flower abscission were also investigated. Throughout this work, the principal hypotheses were that flower abscission would be promoted by high temperature, low light and water stress, and that abscission is mediated by enhanced ethylene production and reduced partitioning of photosynthetic assimilates to the flowers.

The experiment which examined how differing temperature/irradiance combinations influenced the growth and development of young reproductive plants of two varieties of sweet pepper (Chapter 3) showed that a high mean daily temperature (26 °C) accelerated the development of the first primary flowers between the third true leaf stage and anthesis when combined with high irradiance (4.9 MJ m^{-2} d^{-1}), but subsequently increased abscission of the primary flowers as compared to the lower temperature treatments examined (20 and 14 °C). Development of the secondary flowers was accelerated by high temperature and high irradiance. At low temperature (14 °C), flower abscission was reduced, but the period between flower development and fruit set was longer than at higher temperatures and the fruits formed were abnormal. However, when high temperature was combined with low irradiance (2.4 MJ m^{-2} d^{-1}), complete abscission of the primary flowers occurred. The flower abscission observed under conditions of high temperature and high irradiance might

have resulted from competition for assimilates between the flower buds and young leaves, as was suggested by Aloni *et al.* (1991b). In this case, young leaves would appear to be more effective sinks than adjacent flower buds in importing assimilates (Dinar *et al.*, 1983; Aloni *et al.*, 1991b). Under conditions of low irradiance, the complete abscission observed before or after anthesis was attributable to the greatly reduced quantity of photosynthetically active radiation available to support growth (Picken, 1984; Atherton and Harris, 1986), which would have limited assimilate availability, thereby increasing competition between the reproductive and vegetative sinks for assimilates (Dinar *et al.*, 1983; Morris and Newell, 1987). However, it has also been suggested that flower abscission under high temperature or low irradiance conditions may be attributable to enhanced ethylene production in the flowers (Wien and Yipin, 1989; Wien *et al.*, 1993).

The results presented in Chapter 3 also indicate the existence of varietal variation in the responses of reproductive development in sweet pepper to differences in temperature. For example, flower emergence in var. Blue Star was faster than in Bell Boy at high temperature (26 °C), although the varietal differences in the time to anthesis were not significant. To relate flower development in sweet pepper to temperature, the thermal time concept was adopted. Thermal time is an extremely useful concept because it allows the development of crops at different locations and in different seasons to be compared (Ong and Monteith, 1985; Squire, 1990).

Since the cardinal temperatures for growth and development in sweet pepper (base, optimum and maximum temperatures; T_b, T_o and T_m respectively), appear not to have been reported previously, the cardinal temperatures for germination were calculated from the linear relationships between germination and temperature established using a thermogradient plate (Chapter 4). The values obtained for T_b, T_o and T_m were 6.0, 27.5 and 41.5 °C in Blue Star and 8.5, 23.0 and 44.0 °C in Bell Boy. These cardinal temperatures were subsequently used in Chapters 5 and 6 to calculate the predicted thermal times for anthesis and flower abscission. Although the cardinal temperatures obtained from germination trials may not be identical to those for flower development due to possible differences between the vegetative and reproductive stages of

development (Angus *et al.*, 1981a, b; Slafer and Savin, 1991), the use of a T_b value for germination in a given species in thermal time calculations is better than simply assuming that T_b is 0 °C or some other arbitrary value (Squire, 1990). Since the rate of development in sweet pepper was shown in the present work to be related to thermal time, the prediction of growth and development in this crop using the concept of accumulated thermal time above the base temperature may provide a useful tool for planning glasshouse or protected production throughout the year. Further studies are required however to check between possible differences in cardinal temperatures for the various stages of growth and development before confident predictions can be made.

Under low irradiance conditions, flower abscission may be reduced when plants are water stressed without inducing deleterious effects on the subsequent growth of the whole plant, thereby encouraging the reproductive phase (Cooper and Hurd, 1968; De Koning and Hurd, 1983). The possibility that flower abscission might be reduced by water stress under high temperature/low irradiance conditions was partly tested in Chapter 5, and the possible role of changes in dry matter distribution induced by water stress was examined. Restriction of vegetative growth by water stress imposed at the appearance of the first flower bud under low irradiance conditions did not improve early reproductive growth and development (Chapter 5.1); instead complete abscission of the primary and secondary flowers was observed under low irradiance conditions. These results suggest that low irradiance had a greater effect on flower development than water stress, consistent with the observations of Atherton and Othman (1983) and Halevy (1987).

Under high irradiance conditions, severe water stress enhanced flower development up to anthesis, but subsequently promoted early and increased abscission. In general, the development of primary, but not secondary, flowers to anthesis was accelerated by increasing the severity or duration of water stress (Chapter 5.2). In contrast, the abscission of both primary and secondary flowers was accelerated by increasing the intensity and duration of water stress. The high percentage abscission of primary flowers was largely offset by the reduced abscission of the secondary flowers.

Shortening the duration of water stress did not advance anthesis, or reduce abscission either in this or previous studies (Munier-Jolain *et al.*, 1993; Ney *et al.*, 1994). This may have been due to increased competition for assimilates between the flowers and vegetative organs since additional leaves were produced after the stress ended, resulting in a transitory phase of more rapid growth in the previously mildly stressed plants than the unstressed steady-state rate. Hence, there was no overall reduction in leaf elongation due to the promotion of cell expansion after rewatering mildly stressed plants (Acevedo *et al.*, 1971; Bradford and Hsiao, 1982). The most advanced stage of flower development at the time of imposing the stress also proved to be the most susceptible to early abscission (Chapter 5.3).

Examination of the growth analysis data immediately prior to anthesis showed that severe stress decreased dry matter accumulation in both the leaves and stems (Chapter 6.1). However, at the onset of flower abscission, shoot dry weight and its partitioning were not significantly affected by severe stress. This implies that, although the advancement of anthesis may have been associated with a decrease in dry matter accumulation in the leaves and stems, flower abscission was not directly related to any reduction in assimilate production or its distribution within the shoot.

The involvement of ethylene in the stress-induced abscission of leaves and other plant organs is well documented (Halevy and Mayak, 1981; Durieux *et al.*, 1983; Sexton *et al.*, 1985; Ohno, 1991; Abeles *et al.*, 1992), and increases in ethylene production have been found following the imposition of water stress in many plant species. Such increases in ethylene evolution are known to promote leaf abscission (Jordan *et al.*, 1972; Apelbaum and Yang, 1981; Kimmerer and Kozlowski, 1982; Kirkham, 1985), but a few studies have examined the role of ethylene in water stress-induced flower abscission. The present investigation (Chapter 6.1) has shown that, just prior to flower abscission in the severely stressed treatment, ethylene evolution from the flowers increased by up to 8-fold as compared with unstressed plants, and by 40-fold as compared to severely stressed plants measured before anthesis. Ethylene evolution from water stressed flowers was also 3-fold greater than from the leaves, whereas in unstressed control plants ethylene evolution from the flowers was lower than from the

leaves. These results imply that flowers may be more sensitive to water stress in terms of promotion of ethylene production prior to abscission. While water stress induced flower abscission, there was no simultaneous abscission of the older leaves, suggesting a role for ethylene in mediating flower abscission in sweet pepper.

Total shoot dry matter production and the partitioning of dry matter to the leaves and stems did not decline markedly during the period of increased ethylene evolution from flowers. Shoot dry matter production and partitioning also could not be correlated closely with the changing pattern of ethylene evolution. This further supports the view that changes in assimilate production or partitioning to the leaves and stems were not directly involved in inducing flower abscission. Decreases in leaf water and osmotic potentials following increases in ethylene production have been reported previously (Stumpff and Johnson, 1987; Miyamoto and Kamisaka, 1987), and the results from the present study are consistent with these reports. However, the changes in leaf water potential induced by severe stress were not correlated with those in ethylene evolution from flowers and leaves, implying that, although decreases in the components of water potential may be indicative of the severity of stress in plants, as observed in Chapter 5, they are not necessarily responsible for the observed hormonal changes, which may take place much sooner or at lower stress levels than those observed in the present study (Hsiao, 1973).

The promotion of flower abscission by water stress observed in the present work was preceded by a dramatic increase in ethylene production. To test whether ethylene is capable of triggering flower abscission, an experiment was carried out to determine the effects of exogenous ethylene on flower abscission (Chapter 6.2). Several authors have shown that the application of the ethylene-releasing compound 2-chloroethyl phosphonic acid (CEPA) increased bud and flower abscission (Tripp and Wien, 1989; Furutani *et al.*, 1989; Mason and Miller, 1991) following an increase in ethylene production.

The present study has shown that application of CEPA to the flower buds of sweet pepper mimicked the effects of water stress by increasing bud abscission and that the

extent of abscission induced by CEPA application was dependent upon the concentration applied. The higher concentration of CEPA (100 mg l^{-1}) induced almost complete and more rapid flower abscission (by day 2 after CEPA application) than the lower CEPA concentration (50 mg l^{-1}). At the lower CEPA concentration, abscission occurred more gradually and complete abscission was observed two days later than at the higher concentration. The effectiveness of ethylene-releasing compounds strongly suggests that ethylene is involved in mediating the promotory influence of water stress on flower abscission. A highly significant quadratic relationship between percentage flower abscission and ethylene evolution following the application of CEPA was also established. The flowers of sweet pepper were found to be capable of forming an abscission zone at the base of their pedicels following exposure to elevated endogenous ethylene concentrations resulting either from water stress or treatment with ethylene-releasing compounds. Mature pepper leaves, however, were incapable of forming abscission layers in response to these treatments, although limited epinastic curvature was observed. In contrast, young developing leaves formed abscission lines at the base of their petioles and this was followed by abscission. Similar preferential abscission of the younger leaves has also been reported by other workers (Woltering, 1987; Beaudry and Kays, 1988).

The results obtained firmly suggest that ethylene is involved in mediating the impact of water stress on flower abscission. Furthermore, the experiments involving the use of silver thiosulphate (STS) to block the action of ethylene in inducing abscission showed that a foliar application of 1 mM STS one day after imposing water stress (Chapter 6.3.1) or STS pre-treatment of plants sprayed with CEPA (Chapter 6.3.2) blocked the abscission response normally induced by both treatments. These results confirmed the role of ethylene in promoting flower and bud abscission in sweet pepper under water stress conditions. The protective influence of STS against flower abscission has been reported previously (Cameron and Reid, 1983; Dostal *et al.*, 1991), although most earlier studies concentrated on stresses other than water stress. The protective effect of STS against flower abscission observed in the present study persisted until the experiment was terminated, at least 10 d after application, but in other work, the protective influence of STS has been reported to persist for up to 28

d after first application (Cameron and Reid, 1981). These observations further suggest that STS treatment has great potential as a means of reducing stress-induced abscission in flowering pot plants, including sweet pepper. The results presented in Chapter 6 indicate that STS treatment blocks the action of stress-induced ethylene in promoting abscission. To validate this conclusion, further research is necessary to examine the effectiveness of STS as a protective measure against flower abscission in soil-grown sweet pepper plants raised in protected rainshelters or under field conditions. The use of other inhibitors of ethylene action should also be tested and the commercial feasibility of these compounds determined. In such studies, it would also be important to examine the subsequent fruiting performance of plants treated with protective agents.

The study has demonstrated the feasibility of using accumulated thermal time above the T_b obtained from germination studies to predict the time to anthesis and flower abscission. However, since T_b may differ between the vegetative and reproductive stages of development (Ebata, 1990; Slafer and Savin, 1991), further studies of cardinal temperatures at different stages of growth are required to obtain a better understanding of the thermal times required for specific developmental stages, particularly flower initiation and development. The suitability of these more precisely defined thermal times for field and protected rainshelter applications should be investigated to establish whether reliable predictions of the dates for various reproductive developmental stages can be obtained. Further studies of the influence of irradiance, water stress, endogenous ethylene levels and ethylene blocking compounds should also be carried out to determine the effects of these factors on the thermal time requirement for flower development, in particular flower abscission.

The work presented here dealt only with flowering and flower abscission in the first two inflorescences. Further studies might usefully examine later flowers that are developing whilst the plant is also carrying a fruit load.

BIBLIOGRAPHY

ABDEL-GAWAD, H.A. and MARTIN, G.C. (1973). The fate of 1,2-^{14}C(2-chloroethyl)phosphonic acid (ethephon) in peach. *HortScience,* 8, 125-127.

ABELES, F.B., MORGAN, P.W. and SALTVEIT, M.E. (1992). *Ethylene in Plant Biology.* Academic Press, New York.

ACEVEDO, E., HSIAO, T.C. and HENDERSON, D.W. (1971). Immediate and subsequent growth responses of maize leaves to changes in water status. *Plant Physiology,* 48, 631-636.

ACOSTA GALLEGOS, J.A. and KOHASHI SHIBATA, J. (1989). Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris* L.) cultivars. *Field Crops Research,* 20, 81-93.

AHARONI, N. (1985). Effect of silver ions and ethylene on auxin metabolism and auxin-induced ethylene production in tobacco leaf discs. *Physiologia Plantarum,* 63, 438-444.

AHARONI, N. and LIEBERMAN, M. (1979). Ethylene as a regulator of senescence in tobacco leaf discs. *Plant Physiology,* 64, 801-804.

ALONI, B., DAIE, J. and KARNI, L. (1991a). Water relations, photosynthesis and assimilate partitioning in leaves of pepper (*Capsicum annuum)* transplants: Effect of water stress after transplanting. *Journal of Horticultural Science,* 66, 75-80.

ALONI, B., PASHKAR, T. and KARNI, L. (1991b). Partitioning of [^{14}C]sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. *Annals of Botany,* 67, 371-377.

ALVIM, P.T. DE (1960). Moisture stress as a requirement for flowering of coffee. *Science,* 132, 354.

ALVIM, P. DE T. (1964). Tree growth periodicity in tropical climates. In: *The Formation of Wood in Forest Trees.* Ed. M.H. Zimmermann, pp. 479-496. Academic Press, New York.

ANGUS, J.F., CUNNINGHAM, R.B., MONCUR, M.W. and MACKENZIE, D.H. (1981a). Phasic development in field crops. I. Thermal response in the seedling phase. *Field Crops Research,* 3, 365-378.

ANGUS, J.F. and MONCUR, M.W. (1977). Water stress and phenology in wheat. *Australian Journal of Agricultural Research,* 28, 177-181.

ANGUS, J.F., MACKENZIE, D.H., MORTON, R. and SCHAFER, C.A. (1981b). Phasic development in field crops. II. Thermal and photoperiodic responses of spring wheat. *Field Crops Research,* 4, 269-283.

APELBAUM, A. and YANG, S.F. (1981). Biosynthesis of stress ethylene induced by water deficit. *Plant Physiology,* 68, 594-596.

ARMITAGE, A.M., CARLSON, W.H. and FLORE, J.A. (1981). The effect of temperature and quantum flux-density on the morphology, physiology, and flowering of hybrid geraniums. *Journal of the American Society for Horticultural Science,* 106, 643.

ATHERTON, J.G., CRAIGON, J. and BASHER, E.A. (1990). Flowering and bolting in carrot. I. Juvenility: cardinal temperatures and thermal times for vernalization. *Journal of Horticultural Science,* 65, 423-429.

ATHERTON, J.G. and HARRIS, G.P. (1986). Flowering. In: *The Tomato Crop: A Scientific Basic for Improvement.* Eds J.G. Atherton and J. Rudich, pp. 167-200. Butterworths, Robert Hartnoll Ltd, Cornwall, UK.

ATHERTON, J.G. and OTHMAN, S. (1983). Effects of irradiance and water stress on flower abortion in the glasshouse tomato. *Acta Horticulturae,* 134, 133-138.

ATTA-ALY, M.A., SALTVEIT, M.E. JR and HOBSON, G.E. (1987). Effect of silver ions on ethylene biosynthesis by tomato fruit tissue. *Plant Physiology,* 83, 44-48.

AUNG, L.H. and KELLY, W.C. (1966). Influence of defoliation on vegetative, floral and fruit development in tomatoes (*Lycopersicon esculentum* Mill.). *Proceedings of the American Society for Horticultural Science,* 89, 563-570.

BAGNALL, D.J. and KING, R.W. (1991). Response of peanut (*Arachis hypogaea*) to temperature, photoperiod and irradiance. 1. Effect on flowering. *Field Crops Research,* 26, 263-277.

BAKKER, J.C. (1989). The effects of temperature on flowering, fruit set and fruit development of glasshouse sweet peppers (*Capsicum annuum* L.). *Journal of Horticultural Science,* 64, 313-320.

BARRS, H.D. (1968). Determination of water deficits in plant tissues. In: *Water Deficits and Plant Growth.* Ed. T.T. Kozlowski, pp. 235-368. Academic Press, New York.

BEAUDRY, R.M. and KAYS, S.J. (1988). Effect of ethylene source on abscission of pepper plant organs. *HortScience,* 23, 742-744.

BEGG, J.E., and TURNER, N.C. (1976). Crop water deficits. *Advances in Agronomy* 28, 161-217.

BENNETT, A.B., SMITH, G.M. and NICHOLS, B.G. (1987). Regulation of climacteric respiration in ripening avocado fruit. *Plant Physiology,* 83, 973-976.

BEN-YEHOSHUA, S., BURG, S.P. and YOUNG, R. (1985). Resistance of citrus fruit to mass transport of water vapour and other gases. *Plant Physiology,* 79, 1048-1053.

BERNIER, G., KINET, J.M. and SACHS, R.M. (1981a). *The Physiology of Flowering - Volume I.* CRC Press, Boca Raton, Florida, USA.

BERNIER, G., KINET, J.M. and SACHS, R.M. (1981b). *The Physiology of Flowering - Volume II.* CRC Press, Boca Raton, Florida, USA.

BEYER, E.M. JR (1976). A potent inhibitor of ethylene action in plants. *Plant Physiology,* 58, 268-271.

BLACK, C.R., TANG, D.-Y., ONG, C.K., SOLON, A. and SIMMONDS, L.P. (1985). Effects of soil moisture stress on the water relations and water use of groundnut stands. *New Phytologist,* 100, 313-328.

BOROCHOV, A., MAYAK, S. and BROUN, R. (1982). The involvement of water stress and ethylene in senescence of cut carnation flowers. *Journal of Experimental Botany,* 33, 1202-1209.

BOYER, J.S., JOHNSON, R.R. and SAUPE, S.G. (1980). Afternoon water deficits and grain yields in old and new soybean cultivars. *Agronomy Journal,* 72, 981-986.

BRADFORD, K.J. and HSIAO, T.C. (1982). Physiological responses to moderate stress. In: *Physiological Plant Ecology II. Water Relations and Carbon Assimilation.* Encyclopedia of Plant Physiology. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler, pp. 246-324. Springer-Verlag, Berlin.

BUKOVAC, M.J., ZUCCONI, F., WITTENBACH, V.A., FLORE, J.A. and INOVE, H. (1971). Effects of (2-chloroethyl)phosphonic acid on development and abscission of maturing sweet cherry (*Prunus avium* L.). *Journal of the American Society for Horticultural Science,* 96, 777-781.

BURG, S.P. (1968). Ethylene, plant senescence and abscission. *Plant Physiology,* 43, 1503-1511.

BURG, S.P. and BURG, E.A. (1964). Biosynthesis of ethylene. *Nature,* 203, 869-870.

BURG, S.P. and BURG, E.A. (1965). Ethylene action and the ripening of fruits. *Science,* 148, 1190-1196.

CALVERT, A. (1957). Effect of the early environment on development of flowering in the tomato. I. Temperature. *Journal of Horticultural Science,* 32, 9-17.

CALVERT, A. (1959). Effect of the early environment on the development of flowering in tomato. II. Light and temperature interactions. *Journal of Horticultural Science,* 34, 154-162.

CALVERT, A. (1964). Growth and flowering of the tomato in relation to natural light conditions. *Journal of Horticultural Science,* 39, 182-193.

CALVERT, A. (1969). Studies on the post-initiation development of flower buds of tomato (*Lycopersicon esculentum*). *Journal of Horticultural Science,* 44, 117-26.

CAMERON, A.C., HEINS, R.D. and FONDA, H.N. (1985). Influence of storage and mixing factors on the biological activity of silver thiosulphate. *Scientia Horticulturae,* 26, 167-174.

CAMERON, A.C. and REID, M.S. (1981). The use of silver thiosulphate anionic complex as a foliar spray to prevent flower abscission of zygocactus. *Hortscience,* 16, 761-762.

CAMERON, A.C. and REID, M.S. (1983). Use of silver thiosulphate to prevent flower abscission from potted plants. *Scientia Horticulturae,* 19, 373-378.

CHARLES, W.B. and HARRIS, R.E. (1972). Tomato fruit set at high and low temperatures. *Canadian Journal of Plant Science,* 52, 497-506.

CHAVES, M.M. (1991). Effects of water deficits on carbon assimilation. *Journal of Experimental Botany,* 42, 1-16.

COCHRAN, H.L. (1924). Factors affecting flowering and fruit setting in the pepper. *Proceedings of the American Society for Horticultural Science* 29, 434-437.

COCHRAN, H.L. (1936). Some factors influencing growth and fruit-setting in the pepper (*Capsicum frustescens* L.). *Memograph, Cornell University Agriculture Experimental Station*, 190, 3-39.

COCHRAN, H.L. (1938). A morphological study of flower and seed development in pepper. *Journal of Agricultural Research,* 56, 395-419.

COCKSHULL, K.E. (1979). Effects of irradiance and temperature on flowering of *Chrysanthemum morifolium* Ramat. in continuous light. *Annals of Botany,* 44, 451-460.

COONS, J.M., KUEHL, R.O., OEBKER, N.F. and SIMONS, N.R. (1989). Seed germination of seven pepper cultivars at constant or alternating high temperatures. *Journal of Horticultural Science,* 64, 705-710.

COOPER, A. (1976). *Nutrient Film Technique of Growing Crops.* Grower Books, London.

COOPER, A.J. and HURD, R.G. (1968). The influence of cultural factors on arrested development of the first inflorescence of glasshouse tomatoes. *Journal of Horticultural Science,* 43, 243-248.

COOPER, A.J., HURD, R.G. and GISBOURNE, J.H. (1966). *Report of Glasshouse Crop Research Institute* (1965). pp. 145-152.

COX, W.J. and JOLLIFF, G.D. (1987). Crop-water relations of sunflower and soybean under irrigated and dryland conditions. *Crop Science,* 27, 553-557.

CRAIGON, J., ATHERTON, J.G. and BASHER, E.A. (1990). Flowering and bolting in carrot. II. Prediction in growth room, glasshouse and field environments. *Journal of Horticultural Science,* 65, 547-554.

CURTIS, R.W. (1981). Light requirement for silver nitrate inhibition of ethrel-induced leaf abscission from cuttings of *Vigna radiata* cultivar Jumbo. *Plant Physiology,* 68, 1249-1252.

DAMPTEY, H.B. and ASPINALL, D. (1976). Water deficit and inflorescence development in *Zea mays. Annals of Botany,* 40, 23-35.

DE KONING, A. and HURD, R.G. (1983). A comparison of winter-sown tomato plants grown with restricted and unlimited water supply. *Journal of Horticultural Science,* 58, 575-581.

DE MUNK, W.J., DUINEVELD, T. and VAN DER HULST, G. (1992). The production of Apeldoorn tulips after exposure to ethylene during storage of the planting stock. *Acta Horticulturae,* 325, 61-70.

DELI, J. and TIESSEN, H. (1969). Interaction of temperature and light intensity on flowering of *Capsicum frustescens* var. grossum cv. California Wonder. *Journal of the American Society for Horticultural Science*, 94, 349-357.

DINAR, M. and RUDICH, J. (1985a). Effect of heat stress on assimilate partitioning in tomato. *Annals of Botany* 56, 239-248.

DINAR, M. and RUDICH, J. (1985b). Effect of heat stress on assimilate metabolism in tomato flower buds. *Annals of Botany*, 56,249-257.

DINAR, M., RUDICH, J. and ZAMSKI, E. (1983). Effect of heat stress on carbon transport from tomato plants. *Annals of Botany*, 51, 97-103.

DOSSER, A.L. and LARSON, R.A. (1981). Influence of various growth chambers environments on growth, flowering, and senescence of *Tulipa generiara* L. cv Paul-Ritcher. *Journal of the American Society for Horticultural Science*, 106, 247-250.

DOSTAL, D.L., AGNEW, N.H., GLADON, R.J. and WEIGLE, J.L. (1991). Ethylene, simulated shipping, STS, and AOA affect corolla abscission of New Guinea Impatiens. *HortScience*, 26, 47-49

DRINNAN, J.E. and MENZEL, C.M. (1994). Synchronisation of anthesis and enhancement of vegetative growth in coffee (*Coffea arabica* L.) following water stress during floral initiation. *Journal of Horticultural Science*, 69, 841-849.

DUBETZ, S. and MAHALLE, P.S. (1969). Effect of soil water stress on bush beans *Phaseolus vulgaris* L. at three stages of growth. *Journal of the American Society for Horticultural Science*, 94, 479.

DURIEUX, A.J.B., KAMERBEEK, G.A. and VAN MEETEREN, U. (1983). The existence of a critical period for the abscission and a non-critical period for lasting of flower-buds of *Lilium* 'Enchantment'; Influence of light and ethylene. *Scientia Horticulturae*, 18, 287-297.

EBATA, M. (1990). Effective heat unit summation and base temperature on the development of rice plants. 1. A method determining base temperature and its application to the vegetative development of rice plants. *Japanese Journal of Crop Science*, 59, 225-232.

EISINGER, W., CRONER, L.J. and TAIZ, L. (1983). Ethylene-induced lateral expansion in etiolated pea stems. Kinetics, cell wall synthesis, and osmotic potential. *Plant Physiology*, 73, 407-412.

EKLUND, L., CIENCIALA, E. and HALLGREN, J.-E. (1992). No relation between drought stress and ethylene production in Norway spruce. *Physiologia Plantarum*, 86, 297-300.

EL-BELTAGY, A.S. and HALL, M.A. (1974). Effect of water stress upon endogenous ethylene levels in *Vicia faba*. *New Phytologist*, 73, 47-60.

ELPHINSTONE, E.D., REES, A.R. and ATHERTON, J.G. (1988). Temperature and development in *Iris* x *hollandica* during pre-planting storage. I. Leaf initiation. *Journal of Horticultural Science*, 63, 287-294.

EL-SHARKAWY, M.A. and COCK, J.H. (1987). Response of cassava to water stress. *Plant and Soil*, 100, 345-360.

FISCHER, R.A. (1973). The effect of water stress at various stages of development on yield processes in wheat. In: *Plant Response to Climatic Factors*. Ed. R.O. Slatyer, pp. 233-241. UNESCO, Paris.

FOOD AND AGRICULTURAL ORGANISATION. (1992). *Year Book of Production*. FAO. 1993.

FORTANIER, E.J. and ZEVENBERGEN, A. (1973). Analysis of the effects of temperature and light after planting on bud blasting in *Iris hollandica*. *Netherlands Journal of Agricultural Science*, 21, 145-162.

FOSTER, K.R., REID, D.M. and PHARIS, R.P. (1992). Ethylene biosynthesis and ethephon metabolism and transport in barley. *Crop Science*, 32, 1345-1352.

FURUTANI, S.C., JOHNSTON, E. and NAGAO, M. (1989). Anthesis and abscission of blue jade vine flowers treated with ethephon and AOA. *HortScience*, 24, 1042.

GARCIA-HUIDOBRO, J., MONTEITH, J.L. and SQUIRE, G.R. (1982). Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). I. Constant temperature. *Journal of Experimental Botany*, 33, 288-296.

GATES, C.T. (1955). The response of the young tomato plant to a brief period of water shortage. I. The whole plant and its principal parts. *Australian Journal of Biological Sciences*, 8, 196-215.

GATES, C.T. (1964). The effect of water stress on plant growth. *Journal of Australian Institute of Agricultural Science*, 30, 3-22.

GATES, C.T. (1968). Water deficits and growth of herbaceous plants. In: *Water Deficits and Plant Growth.* Ed. T.T. Kozlowski, pp. 135-190. Academic Press, New York.

GAVINLERTVATANA, P., REID, P.E. and WILKINS, H.F. (1980). Control of ethylene synthesis and action by silver nitrate and rhizobitoxine in petunia leaf sections cultured in vitro. *Journal of the American Society for Horticultural Science*, 105, 304-307.

GHOSH, J.C. (1974). Instability constant of $Ag(S_2O_3)_2^{3-}$ at 25°, 35° and 45°. *Journal of Indian Chemistry Society*, ll, 361-363.

GIULIVO, C., RAMINA, A., MAIA, A. and COSTA, A. (1981). Metabolism and translocation of 1,2-^{14}C-(2-chloroethyl)phosphonic acid in *Prunus persica* (L.) Batsch. *Scientia Horticulturae*, 15, 33-43.

GRACE, J. and RUSSELL, G. (1977). The effect of wind on grasses. lll. Influence of continuous drought or wind on anatomy and water relations in *Festuca arundinacea* Schreb. *Journal of Experimental Botany*, 28, 268-278.

GREEN, C.F. and DEUCHAR, C.N. (1985). On improved tube solarimeter construction. *Journal of Experimental Botany*, 36, 690-693.

GRIME, D.W., MILLER, R.J. and DICKENS, L. (1970). Water stress during flowering of cotton. *California Agriculture*, 24, 4.

GUINN, G. (1974). Abscission of cotton floral buds and bolls as influenced by factors affecting photosynthesis and respiration. *Crop Science*, 14, 291-293.

GUINN, G. (1976). Nutritional stress and ethylene evolution by young cotton bolls. *Crop Science*, 16, 89-91.

GUPTA, K. and ANDERSON, J.D. (1989). Influence of temperature on potentiation of cellulysin-induced ethylene biosynthesis by ethylene. *Plant Cell Physiology*, 30, 345-349.

GURALNICK, L.J. and TING, I.P. (1987). Physiological changes in *Portulacaria afra* (L.) Jacq. during a summer drought and rewatering. *Plant Physiology*, 85, 481-486.

HALE, M.G. and ORCUTT, D.M. (1987). *The Physiology of Plants Under Stress.* John Wiley and Sons, New York.

HALEVY, A.H. (1975). Light energy flux and distribution of assimilates as factors controlling the flowering of flower crops. In: *Proceedings of the XIXth International Horticultural Congress.* pp. 125-134. Warszawa.

HALEVY, A.H. and MAYAK, S. (1981). Senescence and postharvest physiology of cut flowers. *Horticultural Reviews*, 3, 59-143.

HALEVY, A.H. (1984). Light and autonomous induction. In: *Light and the Flowering Process.* Eds. D. Vince-Prue, B. Thomas and K.E. Cockshull, pp. 65-73. Academic Press, London.

HALEVY, A.H. (1987). Assimilate allocation and flower development. In: *Manipulation of Flowering.* Ed. J.G. Atherton, pp. 363-378. Butterworth, Robert Hartnoll Ltd, Cornwall, UK.

HAMPTON, R.E., OOSTERHUIS, D.M., STEWART, J.M. and KIM, K.S. (1987). Anatomical differences in cotton related to drought tolerance. *Arkansas Farm Research*, 36, 4.

HAND, D.J. and ATHERTON, J.G. (1987). Curd initiation in the cauliflower. I. Juvenility. *Journal of Experimental Botany*, 38, 2050-2058.

HAWA, J. and AZIZ, B. (1991). Vegetable production under rainshelters on BRIS. *Proceedings of the Soil Science Conference of Malaysia 1991*, Malaysian Soil Science Society, Genting Highlands, Malaysia.

HAWA, J., EMBI, Y. and REZUWAN, K. (1991). The potential and prospects of vegetable cultivation under simple construction in Malaysia. *Proceedings of the Cultivation Under Simple (Plastic/Glasshouse) Constructions in the Tropics and Sub-tropics*, Food and Fertiliser Technology Centre, Taichung, Taiwan.

HERNANDEZ-ARMENTA, R. (1985). *Fruit set of bell pepper varieties under heat and moisture stress, and the use of row cover as a fruit set screening technique*. M. Sc. Thesis, Cornell University, Ithaca, New York.

HERRERO, M.P. and JOHNSON, R.R. (1983). Drought stress and its effects on maize reproductive systems. *Crop Science*, 21, 105-110.

HOFFMAN, N.E., LIU, Y. and YANG, S.F. (1983). Changes in 1-(malonylamino)cyclopropane-1-carboxylic acid content in wilted wheat leaves in relation to their ethylene rates and 1-aminocyclopropane-1-carboxylic acid content. *Planta*, 157, 518-523.

HO, L.C. and HEWITT, J.D. (1986). Fruit development. In: *The Tomato Crop: A Scientific Basic for Improvement*. Eds. J.G. Atherton and J. Rudich, pp. 201-239. Butterworths, Robert Hartnoll Ltd, Cornwall, UK.

HOOGENBOOM, G., PETERSON, C.M. and HUCK, M.G. (1987). Shoot growth rate of soybean as affected by drought stress. *Agronomy Journal*, 79, 589-607.

HSIAO, T.C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology*, 24, 519-570.

HSIAO, T.C. and JING, J. (1987). Leaf and root expansive growth in response to water deficits. In: *Physiology of Cell Expansion During Plant Growth*. Eds. D.J. Cosgrove and D.P. Knievel, pp. 180-192. American Society of Plant Physiology, Rockville, MD.

HSIAO, T.C., SILK, W.K. and JING, J. (1985). Leaf growth and water deficits: Biophysical effects. In: *Control of Leaf Growth*. Ed. N.R. Baker, pp. 239-266. Cambridge University Press, Cambridge.

HUANG, W.L. (1978). Influence of soil-moisture deficit at different stages of growth on the development, yield, nutrients and water absorption of tomato plants. *Journal of Agricultural Research of China*, 27, 37-46.

HUDA, A.K.S., SIVAKUMAR, M.V.K., SRI RAMA, Y.V., SEKARAN, J.G. and VIRMANI, S.M. (1987). Observed and simulated responses of two sorghum cultivars to different water regimes. *Field Crops Research*, 16, 323-335.

HURD, R.G. (1973). Long-day effects on growth and flower initiation of tomato plants in low light. *Annals of Applied Biology*, 73, 221-228.

HURD, R.G. and COOPER, A.J. (1967). Increasing flower number in single-truss tomatoes. *Journal of Horticultural Science*, 42, 181-188.

HURD, R.G. and COOPER, A.J. (1970). The effect of early low temperature treatment on the yield of single inflorescence tomatoes. *Journal of Horticultural Science*, 45, 19-27.

HURD, R.G. and GRAVES, C.J. (1981). Controlling the water supply. *Grower*, 96, 17-22.

HUSSEY, G. (1963). Growth and development in the young tomato. ll. The effect of defoliation on the development of the shoot apex. *Journal of Experimental Botany*, 14, 393-407.

ISHIZAWA, K. and ESASHI, Y. (1984). Osmoregulation in rice coleoptile elongation as promoted by cooperation between IAA and ethylene. *Plant Cell Physiology*, 25, 495-505.

JAAFAR, H., BLACK, C.R. and ATHERTON, J.G. (1994). Water relations, dry matter distribution and reproductive development of sweet pepper (*Capsicum annuum*). In: *Aspects of Applied Biology 38, Efficiency of Water Use in Crop Systems*. Eds. M.C. Heath, T.M. Hess, T.J. Hocking, D.K.L. Mackerron and W. Stephens, pp. 299-306. The Association of Applied Biologists, Wellesbourne, UK.

JACKSON, D.I (1969). Effects of water, light and nutrition on flower-bud initiation in apricots. *Australian Journal of Biological Sciences*, 22, 69-75.

JACKSON, J.M., HARTLEY, C. and OSBORNE, D.J. (1973). Timing abscission in *Phaseolus vulgaris* by controlling ethylene production and sensitivity to ethylene. *New Phytologist*, 72, 1251-1260.

JACKSON, M.B. (1985). Ethylene and the responses of plants to excess water in their environment - A Review. In: *Ethylene and Plant Development*. Eds J.A. Roberts and G.A. Tucker, pp. 241-266. Butterworths, Robert Hartnoll Ltd, Cornwall, UK.

JACKSON, M.B. and OSBORNE, D.J. (1970). Ethylene, the natural regulator of leaf abscission. *Nature*, 225, 1019-1022.

JACKSON, P.G. (1982). *The Thermogradient and Field Establishment of Celeriac* (Apium graveolens *L. Rapaceum (Mill.) DC.* M. Phil. Thesis, University of Nottingham, UK.

JOHNSON, R.C., MORNHINWEG, D.W., FERRIS, D.M. and HEITHOLT, J.J. (1987). Leaf photosynthesis and conductance of selected *Triticum* species at different water potentials. *Plant Physiology*, 83, 1014-1017.

JONES, R.G.W. and GORHAM, J. (1983). Osmoregulation. In: *Physiological Plant Ecology lll - Response to the Chemical and Biological Environment.* Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler, pp. 35-58. Springer-Verlag, Berlin.

JORDAN, W.R., MORGAN, P.W. and DAVENPORT, T.L. (1972). Water stress enhances ethylene-mediated leaf abscission in cotton. *Plant Physiology*, 50, 756-758.

JOYCE, D.C., REID, M.S. and EVANS, R.Y. (1990). Silver thiosulphate prevents ethylene induced abscission in holly and mistletoe. *HortScience*, 25, 90-92.

KAO, C.H. and YANG, S.F. (1983). Role of ethylene in the senescence of detached rice leaves. *Plant Physiology*, 73, 881-885.

KAUFMANN, M.R. (1972). Water deficits and reproductive growth. In: *Water Deficits and Plant Growth.* Ed. T.T. Kozlowski, pp. 91-124, Academic Press, New York.

KAWASE, M. (1976). Ethylene accumulation in flooded plants. *Physiologia Plantarum*, 36, 236-241.

KAYS, S.J. and BEAUDRY, R.M. (1987). Techniques for inducing ethylene effects. *Acta Horticulturae*, 201, 77-116.

KETRING, D.L. and WHELESS, T.G. (1989). Thermal time requirements for phenological development of peanut. *Agronomy Journal*, 81, 910-917.

KIMMERER, T.W. and KOZLOWSKI, T.T. (1982). Ethylene, ethane, acetaldehyde and ethanol production by plants under stress. *Plant Physiology,* 69, 840-847.

KINET, J.M. (1977a). Effect of light conditions on the development of the inflorescence in tomato. *Scientia Horticulturea,* 6, 15-26.

KINET, J.M. (1977b). Effect of defoliation and growth substances on the development of inflorescence in the tomato. *Scientia Horticulturae,* 6, 27-35.

KINET, J.M. (1994). Environmental, chemical, and genetic control of flowering. *Horticultural Reviews,* 15, 279-334.

KINET, J.M., HURDEBISE, D., PARMENTIER, A. and STAINIER, R. (1978). Promotion of inflorescence development by growth substance treatments to tomato plants grown in insufficient light conditions. *Journal of the American Society for Horticultural Science,* 103, 724-729.

KINET, J.M., SACHS, R.M. and BERNIER, G. (1985). *The Physiology of Flowering.* CRC Press, Boca Raton, Florida, USA.

KIRKHAM, M.B. (1983). Effect of ethephon on the water status of a drought-resistant and a drought-sensitive cultivar of winter wheat. *Zeitschrift fur Pflanzenphysiology,* 112, 103-112.

KIRKHAM, M.B. (1985). Effect of ethephon on growth and water status of *Striga*-susceptible genotypes of pearl millet. *Field Crops Research,* 11, 219-231.

KIRKHAM. M.B. (1990). Plant responses to water deficits. In: *Irrigation of Agricultural Crops - Agronomy Monograph.* Eds. B.A. Stewart and D.R. Nielsen, pp. 323-342.

KLAPWIJK, D. and LINT, P.J.A.L. DE. (1974). Fresh weight and flowering of tomato plants as influenced by container type and watering conditions. *Acta Horticulturae,* 39, 237-247.

KRAMER, P.J. (1982). Water and plant productivity or yield. In: *Handbook of Agricultural Productivity.* R. M. Rechcigl, Jr., pp. 41-47. CRC Press, Boca Raton, Florida, USA.

KRAMER, P.J. (1983). *Water Relations of Plants.* Academic Press Inc., New York.

KRIEDEMANN, P.E. (1986). Stomatal and photosynthetic limitations to leaf growth. *Australian Journal of Plant Physiology,* 13, 15-31.

KRISTENSEN, S., FRIIS, E., HENRIKSEN, K. and MIKKELSEN, S.A. (1985). Applications of temperature sums in the timing of production of crisp lettuce. *Acta Horticulturae,* 198, 217-224.

LAWLOR, D.W. (1979). Effects of water and heat stress on carbon metabolism of plants with C3 and C4 photosynthesis. In: *Stress Physiology in Crop Plants.* Eds. H. Mussell and R.C. Staples, pp. 303-326. Wiley, New York.

LEONG, S.K. and ONG, C.K. (1983). The influence of temperature and soil water deficit on the development and morphology of groundnut (*Arachis hypogaea* L.). *Journal of Experimental Botany,* 34, 1551-1561.

LEVY, A., RABINOWITCH, H.D. and KEDAR, N. (1978). Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. *Euphytica,* 27, 211-218.

LEWIS, R.B., HILER, E.A. and JORDAN, W.R. (1974). Susceptibility of grain sorghum to water deficit at three growth stages. *Agronomy Journal,* 66, 589-590.

LINCOLN, C.P. (1987). *Vegetable Characteristics, Production and Marketing.* John Wiley and Sons, New York, USA.

LIU, Y., SU, L.-Y. and YANG, S.F. (1985). Ethylene promotes the capability to malonylate 1-aminocyclopropane-1-carboxylic acid and D-amino acids in climacteric tomato fruits. *Plant Physiology,* 77, 891-895.

LUDLOW, M.M. and MUCHOW, R.C. (1988). Critical evaluation of the possibilities for modifying crops for high production per unit of precipitation. In: *Drought Research Priorities for the Dryland Tropics.* Eds F.R. Bidinger and C. Johansen, pp. 179-211. ICRISAT, Patancheru, India.

MARC, J. and PALMER, J.H. (1976). Relationship between water potential and leaf and inflorescence initiation in *Helianthus annuus. Plant Physiology,* 36, 101-104.

MARKHART, A.H. (1985). Comparative water relations of *Phaseolus vulgaris* L. and *Phaseolus acutifolius* Gray. *Plant Physiology*, 77, 113-117.

MASON, M.R. and MILLER, W.B. (1991). Flower bud blast in easter lily is induced by ethephon and inhibited by silver thiosulphate *HortScience*, 26,1165-1167.

MAYNARD, J.A. and SWAN, J.A. (1963). Organophosphorus compounds. 1. 2-chloroethylphosphonic acid as phosphorylating agents. *Australian Journal of Chemistry*, 16, 596-608.

MCAFEE, J.A. and MORGAN, P.W. (1971). Rates of production and internal levels of ethylene in vegetative cotton plants. *Plant Cell Physiology*, 12, 839-847.

MCINTYRE, G.I. (1987). The role of water in the regulation of plant development. *Canadian Journal of Botany*, 65, 1287-1298.

MCMICHAEL, B.L., JORDAN, W.R. and POWELL, R.D. (1972). An effect of water stress on ethylene production by intact cotton petioles. *Plant Physiology*, 49, 658-660.

MCMICHAEL, B.L., JORDAN, W.R. and POWELL, R.D. (1973). Abscission processes in cotton: induction by plant water deficit. *Agronomy Journal*, 65, 202-204.

MCPHERSON, H.G., WARRINGTON, I.J. and TURNBULL, H.L. (1985). The effects of temperature and daylength on the rate of development of pigeonpea. *Annals of Botany*, 56, 597-611.

MEIRI, A., HOFFMAN, G.J., SHANNON, M.C. and POSS, J.A. (1982). Salt tolerance of two musk melon cultivars under two irradiance levels. *Journal of the American Society for Horticultural Science*, 107, 1168-1172.

MENZEL, C.M. (1985). Tuberisation in potato at high temperatures: interaction between temperature and irradiance. *Annals of Botany*, 55, 35-39.

MENZEL, C.M., SIMPSON, D.R. and DOWLING, A.J. (1986). Water relations in passionfruit: Effect of moisture stress on growth, flowering and nutrient uptake. *Scientia Horticulturae,* 29, 239-249.

MILTHORPE, F.L. and NEWTON, P. (1963). Studies on the expansion of the leaf surface. lll. The influence of radiation on cell division and leaf expansion. *Journal of Experimental Botany,* 14, 483-495.

MIYAMOTO, K. and KAMISAKA, S. (1987). Effects of water stress and ethylene on osmoregulation in the subhook region of pea epicotyls. *Biochemistry Physiology Pflanzen,* 182, 41-48.

MODLIBOWSKA, I. (1961). Effect of soil moisture on frost resistance of apple blossom, including some observations on 'ghost' and 'parachute' blossoms. *Journal of Horticultural Science,* 36, 186-196.

MOE, R. (1972). Effect of daylength, light intensity, and temperature on growth and flowering in roses. *Journal of the American Society for Horticultural Science,* 97, 796-800.

MOHAMED, H.A., CLARK, J.A. and ONG, C.K. (1988a). Genotypic differences in the temperature responses of tropical crops. I. Germination characteristics of groundnut (*Arachis hypogaea* L.) and pearl millet (*Pennisetum typhoides* S. & H.). *Journal of Experimental Botany,* 39, 1121-1128.

MOHAMED, H.A., CLARK, J.A. and ONG, C.K. (1988b). Genotypic differences in the temperature responses of tropical crops. II. Seedling emergence and leaf growth of groundnut (*Arachis hypogaea* L.) and pearl millet (*Pennisetum typhoides* S. & H.). *Journal of Experimental Botany,* 39, 1129-1135.

MOMEN, N.N., CARLSON, R.E., SHAW, R.H. and ARJMOND, O. (1979). Moisture stress effects on the yield components of two soybean cultivars. *Agronomy Journal,* 71, 86-90.

MORGAN, J.M. (1984). Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology,* 35, 299-319.

MORGAN, P.W. (1973). Regulation of ethylene as an agricultural practice in cotton. *Proceedings of Beltwide Cotton Production and Research Conference.* pp. 95-110.

MORGAN, P.W. and DURHAM, J.I. (1980). Ethylene production and leaflet abscission in *Melia azedarach* L. *Plant Physiology,* 66, 88-92.

MORGAN, P.W., HE, C.J. and POWELL, R.D. (1990). Does water deficit stress promote ethylene synthesis by intact plants?. *Plant Physiology,* 94, 1616-1624.

MORRIS, D.A. and NEWELL, A.J. (1987). The regulation of assimilate partition and inflorescence development in the tomato. In: *Manipulation of Flowering.* Ed. J.G. Atherton, pp. 379-391. Butterworths, Robert Hartnoll Ltd, Cornwall, UK.

MORRISON, M.J., MCVETTY, P.E.B. and SHAYKEWICH, C.F. (1989). The determination and verification of a baseline temperature for the growth of Westar summer rape. *Canadian Journal of Plant Science,* 69, 455-464.

MUNIER-JOLAIN, N.G., NEY, B. and DUTHION, C. (1993). Sequential development of flowers and seeds on the main stem of an indeterminate soybean. *Crop Science*, 33, 768-771.

NAKATA, S. and SUEHISA, R. (1969). Growth and development of *Litchi chinensis* as affected by soil-moisture stress. *American Journal of Botany,* 56, 1121.

NARAYANA, I., LALONDE, S. and SAINI, H.S. (1991). Water-stressed-induced ethylene production in wheat, fact or artifact?. *Plant Physiology,* 96, 406-410.

NEWTON, P. (1963). Studies on the expansion of the leaf surface. II. The influence of light intensity and daylength. *Journal of Experimental Botany,* 14, 458-482.

NEY, B., DUTHION, C. and TURC, O. (1994). Phenological response of pea to water stress during reproductive development. *Crop Science,* 34, 141-146.

NILWIK, H.J.M. (1981). Growth analysis of sweet pepper (*Capsicum annuum* L.) I. The influence of irradiance and temperature under glasshouse conditions in winter. *Annals of Botany,* 48, 129-136.

OHNO, H. (1991). Participation of ethylene in flower bud blasting induced by high temperature in *Cymbidium (Orchidaceae). Journal of the Japanese Society for Horticultural Science,* 60, 415-420.

OLIEN, W.C. and BUKOVAC, M.J. (1982). Ethephon-induced gummosis in sour cherry (*Prunus cerasus* L.). I. Effect of xylem function and shoot water status. *Plant Physiology,* 70, 547-555.

ONG, C.K. (1983a). Response to temperature in a stand of pearl millet (*Pennisetum typhoides* S. & H.). I. Vegetative development. *Journal of Experimental Botany,* 34, 322-336.

ONG, C.K. (1983b). Response to temperature in a stand of pearl millet (*Pennisetum typhoides* S. & H.). II. Reproductive development. *Journal of Experimental Botany,* 34, 337-348.

ONG, C.K. (1984). The influence of temperature and water deficits on the partitioning of dry matter in groundnut (*Arachis hypogaea* L.). *Journal of Experimental Botany,* 35, 746-755.

ONG, C.K., BLACK, C.R., SIMMONDS, L.P. and SAFFELL, R.A. (1985). Influence of saturation deficit on leaf production and expansion in stands of groundnut (*Arachis hypogaea* L.) grown without irrigation. *Annals of Botany,* 56, 523-536.

ONG, C.K. and MONTEITH, J.L. (1985). Response of pearl millet to light and temperature. *Field Crops Research,* 11, 141-160.

OSBORNE, D.J. (1982). *Plant Growth Substances.* Academic Press, London, New York.

OTHMAN, S. (1984). *Water Stress and Development in Young Reproductive Tomato Plants.* Ph.D. Thesis, University of Nottingham, UK.

PAPADOPOULOS, A.P. and TIESSEN, H. (1983). Root and air temperature effects on the flowering and yield of tomato. *Journal of the American Society for Horticultural Science,* 108, 805-809.

PELTONEN-SAINIO, P. (1991). Effect of moderate and severe drought stress on the pre-anthesis development and yield formation of oats. *Journal of Agricultural Science of Finland,* 63, 379-389.

PHILOSOPH-HADAS, S., MEIR, S. and AHARONI, N. (1985). Autoinhibition of ethylene production in tobacco leaf discs: Enhancement of 1-aminocyclopropane-1-carboxylic acid conjugation. *Physiologia Plantarum,* 63, 431-437.

PICKEN, A.J.F. (1984). A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *Journal of Horticultural Science,* 69, 1-13.

POLOWICK, P.L. and SAWHNEY, V.K. (1985). Temperature effects on male fertility and flower and fruit development in *Capsicum annuum* L. *Scientia Horticulturae,* 25, 117-127.

QUAGLIOTTI, L. (1979). Floral biology of *Capsicum* and *Solanum melongena.* In: *The Biology and Taxonomy of the Solanaceae.* Eds. H.G. Hawks, R.N. Lester and A.D. Skelding, pp. 399-418. Academic Press Inc., London.

RADIN, W.R. (1993). Water relations in controlled environments and the field. In: *Interacting Stresses on Plants in a Changing Climate.* Eds. M.B. Jackson and C.R. Black, pp 467-483. NATO, Springer-Verlag, Berlin, Heidelberg.

RAMIN, A.A. and ATHERTON, J.G. (1991). Manipulation of bolting and flowering in celery (*Apium graveolens* L. var. *dulce*). ll. Junenility. *Journal of Horticultural Science,* 66, 709-717.

RAO, N.K.S., BHATT, R.M. and ANAND, N. (1992). Effects of two temperature regimes on photosynthesis and growth in two cultivars of tomato (*Lycopersicon esculentum* Mill.). *Photosynthetica,* 26, 625-631.

RAWSON, H.M. and BAGGA, A.K. (1979). Influence of temperature between floral initiation and flag leaf emergence on grain number in wheat. *Australian Journal of Plant Physiology,* 6, 391-400.

REID, M.S. (1985). Ethylene and abscission. *Hortscience,* 20, 45-50.

REID, M.S., PAUL, J.L., FARHOOMAND, M.B., KOFRANEK, A.K. and STABY, G.L. (1980). Pulse treatment with the silver thiosulphate complex extends the vase-life of cut carnations. *Journal of the American Society for Horticultural Science,* 105, 25-27.

RICHARDS, S.J. and MARSH, A.W. (1961). Irrigation based on soil suction measurements. *Soil Science Society of America Proceedings,* 25, 65-69.

RIOV, J. and YANG, S.F. (1982a). Autoinhibition of ethylene production in citrus peel discs: Suppression of 1-aminocyclopropane-1-carboxylic acid synthesis. *Plant Physiology,* 69, 687-690.

RIOV, J. and YANG, S.F. (1982b). Effects of exogenous ethylene on ethylene production in citrus leaf tissue. *Plant Physiology,* 70, 136-141.

RITCHIE, G.A. and HINCKLEY, T.M. (1975). The pressure chamber as an instrument for ecological research. *Advances in Ecological Research,* 9, 165-254.

RUDICH, J., KALMAR, D., GEIZNEBERG, C. and HAVEL, S. (1977). Low water tensions in defined growth stages of processing tomato plants and their effects on yield and quality. *Journal of Horticultural Science,* 52, 391-399.

RUSSELL, C.R. and MORRIS, D.A. (1982). Invertase activity, soluble carbohydrates and inflorescence development in the tomato (*Lycopersicon esculentum* Mill.). *Annals of Botany,* 49, 89-98.

RYLSKI, I. (1972). Effect of the early environment on flowering in pepper (*Capsicum annuum* L). *Journal of the American Society for Horticultural Science,* 97, 648-651.

RYLSKI, I. (1973). Effect of night temperature on shape and size of sweet pepper (*Capsicum annuum* L.). *Journal of the American Society for Horticultural Science* 98, 149-152.

RYLSKI, I. (1986). Pepper (*Capsicum*). In: *Handbook of Fruit Set and Development.* Ed. S.P. Monselise, CRS Press Inc., Boca Raton, Florida, USA.

RYLSKI, I. and SPIGELMAN, M. (1982). Effects of different diurnal temperature combinations on fruit set of sweet pepper. *Scientia Horticulturae,* 17, 101-106.

RYLSKI, I. and SPIGELMAN, M. (1986). Effect of shading on plant development, yield and fruit quality of sweet pepper grown under conditions of high temperature and radiation. *Scientia Horticulturae,* 29, 31-35.

SAITO, T. and ITO, H. (1967a). Studies on the growth and fruiting in the tomato. IX. Effects of the early environmental conditions and the cultural treatments on the morphological and physiological development of flowers and the flower drop. (I). Effects of night temperature, light intensity and fertility of bed soil. *Journal of the Japanese Society for Horticultural Science,* 36, 195-205.

SAITO, T. and ITO, H. (1967b). Studies on the growth and fruiting in tomato. X. Effects of early environmental conditions and cultural treatments on the morphological and physiological development of flowers and flower drop. (2). Effects of watering, defoliation and application of gibberellin. *Journal of Japanese Society of Horticultural Science,* 36, 281-289.

SAITO, T. and ITO, H. (1972). Studies on the growth and fruiting in the tomato. Xlll. Effects of light intensity and fertility of bed soil on the development of flower especially that of the ovary and its locule. *Journal of Japanese Society of Horticultural Science,* 42, 310-316.

SAITO, T. and ITO, H. (1974). Studies on the growth and fruiting in tomato. XV. Role of mature leaves and immature leaves on the development of flower, especially that of the ovary. *Journal of Japanese Society of Horticultural Science,* 42, 310-316.

SALISBURY, F.B. (1982). Photoperiodism. *Horticultural Reviews,* 4, 66-105.

SALTER, P.J. and GOODE, J.E. (1967). Crop responses to water at different stages of growth. *Commonwealth Bureau of Horticultural Plant Crops,* Farnham Royal, Research Review 2.

SCHIERLE, J., ROHWER, F. and BOPP, M. (1989). Distribution of ethylene synthesis along the etiolated pea shoot and its regulation by ethylene. *Journal of Plant Physiology,* 134, 331-337.

SCHOLANDER, P.F., HAMMEL, H.T., BRADSTREET, E.D. and HEMMINGSEN, E.A. (1965). Sap pressure in vascular plants. *Science,* 148, 339-146.

SCHULZE, E.-D. and HALL, A.E. (1982). Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: *Physiological Plant Ecology II. Water Relations and Carbon Assimilation.* Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler, pp. 181-230. Springer-Verlag, Berlin.

SCHWABE, W.W. (1971). Physiology of vegetative reproduction and flowering. In: *Plant Physiology - A Treatise.* Ed. F.C. Steward, pp. 233-411.

SELANDER, C.S. and WELANDER, N.T. (1984). Effect of temperature on flowering in *Primula vulgaris. Scientia Horticulturae,* 23, 195-200.

SEXTON, R., LEWIS, L.N., TREWAVAS, A.J. and KELLY, P. (1985). Ethylene and abscission. In: *Ethylene and Development.* Eds. J.A. Roberts and G.A. Tucker, pp. 173-196. Butterworths, Robert Hartnoll Ltd, Cornwall, UK.

SHAW, R.H. and LAING, D.R. (1966). Moisture stress and plant response. In: *Plant Environment and Efficient Water Use.* Eds. W.H. Pierre, D. Kirkham, J. Pesek and R.H. Shaw, pp. 77-94. Madison, American Society of Agronomy.

SHILLO, R. and HALEVY, A.H. (1976a). The effect of various environmental factors on flowering of gladiolus. I. Light intensity. *Scientia Horticulturae,* 4, 131-137.

SHILLO, R. and HALEVY, A.H. (1976b). The effect of various environmental factors on flowering of gladiolus. III. Temperature and moisture. *Scientia Horticulturae,* 4, 147-155.

SINGH, D.P., SINGH, P., SHARMA, H.C. and TURNER, N.C. (1987). influence of water deficits on the water relations, canopy gas exchange, and yield of chickpea (*Cicer arietinum*). *Field Crops Research,* 16, 231-241.

SIONET, N. and KRAMER, P.J. (1977). Water potentials and stomatal resistance of sunflower and soy bean subjected to water stress during various growth stages. *Plant Physiology,* 58, 537-540.

SIONET, N., PATTERSON, D.T., COFFIN, R.D. and MORTENSON, D.A. (1987). Water relations and growth of the weed, goosegrass (*Elusine indica*), under drought stress. *Field Crops Research,* 17, 163-173.

SIONET, N., TEARE, I.D. and KRAMER, P.J. (1980). Effects of repeated application of water stress on water status and growth of wheat. *Physiologia Plantarum,* 50, 11-15.

SLAFER, G.A. and SAVIN, R. (1991). Developmental base temperature in different phenological phases of wheat (*Triticum aestivum). Journal of Experimental Botany,* 42, 1077-1082.

SLATYER, R.O. (1969). Physiological significance of internal water relations to crop yield. In: *Physiological Aspects of Crop Yield.* Eds J.D. Eastin, F.A. Haskins, C.Y. Sullivan and C.H.M. van Bavel, pp. 53-83. American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin, USA.

SLAVIK, B. (1974). *Methods of Studying Plant Water Relations.* Springer-Verlag, Berlin and New York.

SMITH, D. (1979). *Peppers and Aubergines.* Grower Guide 3, Grower Books, London.

SOBRADO, M.A. and TURNER, N.C. (1986). Photosynthesis, dry matter accumulation and distribution in the wild sunflower *Helianthus petiolaris* and the cultivated sunflower *Helianthus annuus* as influenced by water deficits. *Oecologia,* 69, 181-187.

SOLOMOS, T. (1989). A simple method for determining the diffusivity of ethylene in 'McIntosh' apples. *Scientia Horticulturae,* 39, 311-318.

SONG, K.W., PARK, S.K. and KIM, C.K. (1976). Studies on the flower abscission of hot pepper. *Research Report Office Rural Development,* 18, 9-32.

SPIKMAN, G. (1986). The effect of water stress on ethylene production and ethylene sensitivity of Freesia inflorescence. *Acta Horticulturae,* 181, 135-140.

SQUIRE G.R. (1990). *The Physiology of Tropical Crop Production.* C.A.B. International, Wallingford, UK.

SQUIRE, G.R., BLACK, C.R. and GREGORY, P.J. (1981). Physical measurements in crop physiology II. Water relations. *Experimental Agriculture,* 17, 225-242.

SQUIRE, G.R., BLACK, C.R. and ONG, C.K. (1983). Response to saturation deficit of leaf extension in a stand of pearl millet (*Pennisetum typhoides* S. & H.) II. Dependence on leaf water status and irradiance. *Journal of Experimental Botany,* 34, 856-865.

STIRLING, C.M., BLACK, C.R. and ONG, C.K. (1989a). The response of groundnut (*Arachis hypogaea* L.) to timing of irrigation. II. ^{14}C-partitioning and plant water status. *Journal of Experimental Botany,* 40, 1163-1173.

STIRLING, C.M., ONG, C.K. and BLACK, C.R. (1989b). The response of groundnut (*Arachis hypogaea* L.) to timing of irrigation. I. Development and growth. *Journal of Experimental Botany,* 40, 1145-1153.

STUMPFF, N.J. and JOHNSON, J.D. (1987). Ethylene production by loblolly pine seedlings associated with water stress. *Physiologia Plantarum,* 69, 167-172.

TAIZ, L. and ZEIGER, E. (1991). Ethylene and abscisic acid. In: *Plant Physiology,* pp. 473-489. Benjamin/Cummings Publishing Co. Ltd.

TYREE, M.T. (1976). Negative turgor pressure in plants: Fact or fallacy. *Canadian Journal of Botany,* 54, 2738-2746.

THOMPSON, P.A. (1970). Characterisation of the germination responses to temperature of species and ecotypes. *Nature,* 225, 827-831.

TINDALL, H.D. (1983). Solanaceae. In: *Vegetables in the Tropics.* pp. 347-387. MacMillan Press Ltd, Hong Kong.

TOFT, N.L., MCNAUGHTON, S.J. and GEORGIADIS, N.J. (1987). Effects of water stress and simulated grazing on leaf elongation and water relations of an east African grass, *Eustachys pappaloides. Australian Journal of Plant Physiology,* 14, 211-226.

TRIPP, K.E. (1986). *Varietal Differences in Stress-Induced Flower-Bud Abscission in Bell Pepper: Role of Ethylene*. M. Sc. Thesis, Cornell University, Ithaca, New York, USA.

TRIPP, K.E. and WIEN, H.C. (1989). Screening with ethephon for abscission resistance of flower buds in bell pepper. *HortScience,* 24, 655-657.

TURNER, L.B. (1991). The effects of water stress on the vegetative growth of white clover (*Trifolium repens* L.): Comparison of long-term water deficit and a short-term developing water stress. *Journal of Experimental Botany*, 42, 311-316.

TURNER, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil,* 58, 339-366.

TURNER, N.C. (1987). The use of the pressure chamber in studies of plant water status. In: *Proceedings of the International Conference on Measurement of Soil and Plant Water Status*. pp. 13-25. Logan, Utah, USA.

TURNER, N.C. and BEGG, J.E. (1981). Plant-water relations and adaptations to stress. *Plant and Soil,* 58, 97-131.

TURNER, N.C., BEGG, J.E. and TONNETT, M.L. (1978). Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. *Australian Journal of Plant Physiology,* 5, 597-608.

TURNER, A.D. and EWING, E.E. (1988). Effects of photoperiod, night temperature and irradiance on flower production in the potato. *Potato Research,* 31, 257-268.

TURNER, A.D. and WIEN, H.C. (1994a). Dry matter assimilation and partitioning in pepper cultivars differing in susceptibility to stress-induced bud and flower abscission. *Annals of Botany,* 73, 617-622.

TURNER, A.D. and WIEN, H.C. (1994b). Photosynthesis, dark respiration and bud sugar concentrations in pepper cultivars differing in susceptibility to stress-induced bud abscission. *Annals of Botany,* 73, 623-628.

UTSUNOMIYA, N. (1992). Effect of temperature on shoot growth, flowering and fruit growth of purple passionfruit (*Passiflora edulis* Sims var. *edulis*). *Scientia Horticulturae,* 52, 63-68.

VAN MEETEREN, U. and DE PROFT, M. (1982). Inhibition of flower bud abscission and ethylene evolution by light and silver thiosulphate in *Lilium*. *Physiologia Plantarum,* 56, 236-240.

VEEN, H. (1979a). Effects of silver on ethylene synthesis and action in cut carnations. *Planta,* 145, 467-470.

VEEN, H. (1979b). Effects of silver salts on ethylene production and respiration of cut carnations. *Acta Horticulturae,* 91, 99-103.

VEEN, H. (1983). Silver thiosulphate: an experimental tool in plant science. *Scientia Horticulturae,* 20, 211-224.

VEEN, H. (1986). A theoretical model for auto-ethylene effects of silver thiosulphate and 2,5-norbornadiene. *Acta Horticulturae,* 181, 129-134.

VEEN, H. (1987). Use of inhibitors of ethylene action. *Acta Horticulturae,* 201, 213-222.

VEEN, H., HENSTRA, S. and DE BRUYN, W.C. (1980). Ultrastructural localisation of silver deposits in receptacle cells of carnation flowers. *Planta,* 148, 245-250.

VEEN, H. and KWAKKENBOS, A.A.M. (1983). The effect of silver thiosulphate pre-treatment on 1-aminocyclopropane-1-carboxylic acid content and action in cut carnations. *Scientia Horticulturae,* 18, 277-286.

VEEN, H. and VAN DE GEIJN, S.C. (1978). Mobility and ionic form of silver as related to longevity of cut carnations. *Planta,* 140, 93-96.

VINCE-PRUE, D. (1975). *Photoperiodism in Plants.* McGraw-Hill, London, UK.

WANG, Y.T. and DUNLAP, J.R. (1990). Leaf abscission in *Radermachera sinica* in response to ethylene and silver thiosuphate. *Hortscience,* 25, 233.

WARD, T.M., WRIGHT, M., ROBERTS, J.A., SELF, R. and OSBORNE, D.J. (1987). Analytical procedures for assay and identification of ethylene. In: *Isolation of Plant Growth Substances.* Ed. J.R. Hillmann, pp. 135-151. Cambridge University Press, UK.

WEAVER, R.J., ABDEL-GAWAD, H.A. and MARTIN, G.C. (1972). Translocation and persistence of 1,2-^{14}C-(2-chloroethyl)phosphonic acid (ethephon) in Thompson seedless grapes. *Physiologia Plantarum,* 26, 13-16.

WEILGOLASKI, F.E. (1974). Phenology in agriculture. In: *Phenology and Seasonality Modelling.* Ed. H. Leith, pp. 369-381. Springer-Verlag, New York.

WESTGATE, M.E. and BOYER, J.S. (1985). Osmotic adjustment and the inhibition of leaf, root, stem, and silk growth at low water potential in maize. *Planta,* 164, 540-549.

WESTGATE, M.E. and PETERSON, C.M. (1993). Flower and pod development in water-deficient soybeans (*Glycine max* L. Merr.). *Journal of Experimental Botany,* 44, 109-117.

WIEN, H.C., ALONI, B., RIOV, J., GOREN, R., HUBERMAN, M. and HO, C.J. (1993). Physiology of heat stress-induced abscission in pepper. In: *Adaptation of Food Crops to Temperature and Water Stress.* Ed. C.G. Kuo, pp. 188-198. Asian Vegetable Research and Development Center, Taiwan, R.O.C.

WIEN, H.C., TRIPP, K.E., HERNANDEZ-ARMENTA, R. and TURNER, A.D. (1989a). Abscission of reproductive structures in pepper: causes, mechanisms and control. In: *Tomato and Pepper Production in the Tropics.* Ed. S.K. Green, pp. 150-165. Asian Vegetable Research and Development Center, Taipei, Taiwan, R.O.C.

WIEN, H.C., TURNER, A.D. and YANG, S.F. (1989b). Hormonal basis for low light intensity-induced flower bud abscission of pepper. *Journal of the American Society for Horticultural Science* 114, 981-985.

WIEN, H.C. and YIPIN, Z. (1989). Preventing low light stress-induced flower abscission of pepper (*Capsicum annuum* L.). *Plant Physiology,* suppl., 1094.

WILDE, M.H. and EDGERTON, L.J. (1975). Histology of ethephon injury on 'Montmorency' cherry branches. *HortScience,* 10, 79-81.

WILSON, J.R., FISCHER, M.J., SCHULZE, E.-D., DALBY, G.R. and LUDLOW, M.M. (1979). Comparison between pressure-volume and dewpoint-hygrometry techniques for determining the water relations characteristics of grass and legume leaves. *Oecologia,* 41, 77-88.

WOLTERING, E.J. (1987). Effects of ethylene on ornamental pot plants: A classification. *Scientia Horticulturae,* 31, 283-294.

WONG, C.H. and OSBORNE, D.J. (1978). The ethylene induced enlargement of target cells in flower buds of *Ecballium elaterium* and their identification by content of endoduplicated nuclear DNA. *Planta,* 139, 103-111.

WRIGHT, S.T.C. (1977). The relationship between leaf water potential and the levels of abscisic acid and ethylene in excised wheat leaves. *Planta,* 134, 183-189.

WUDIRI, B. and HENDERSON, D.W. (1985). Effects of water stress on flowering and fruit set in processing-tomatoes. *Scientia Horticulturae,* 27, 189-198.

WULLSCHLEGER, S.D. and OOSTERHUIS, D.M. (1991). Osmotic adjustment and the growth response of seven vegetable crops following water-deficit stress. *HortScience,* 26, 1210-1212.

YAMAGUCHI, M., CHU, C.W. and YANG, S.F. (1971). The fate of ^{14}C(2-chloroethyl) phosphonic acid in summer squash, cucumber and tomato. *Journal of the American Society for Horticultural Science,* 96, 606-609.

YANG, S.F. (1969). Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiology,* 44, 1203-1204.

YANG, S.F. (1985). Biosynthesis and action of ethylene. *HortScience,* 20, 41-45.

YEGAPPAN, T.M., PATON, D.M., GATES, C.T. and MULLER, W.J. (1980). Water stress in sunflower (*Helianthus annuus* L.). 1. Effect on plant development. *Annals of Botany,* 46, 61-70.

YEGAPPAN, T.M., PATON, D.M., GATES, C.T. and MULLER, W.J. (1982). Water stress in sunflower (*Helianthus annuus* L.). lll. Responses of Cypsela size. *Annals of Botany,* 49, 69-75.

ZHANG, J. and KIRKHAM, M.B. (1988). Ethylene production by maize and sorghum. *Plant Physiology,* 86(supplement), 156.

ZIMMERMANN, U. (1978). Physics of turgor and osmoregulation. *Annual Review of Plant Physiology,* 29, 122-148.