EFFECTS OF LIGHT AND TEMPERATURE ON INFLORESCENCE DEVELOPMENT OF *HELICONIA STRICTA* 'DWARF JAMAICAN'

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ABSTRACT

Plants of *Heliconia stricta* 'Dwarf Jamaican' were grown under different light conditions: continuous long days (LD: 14 hr. daylength), continuous short days (SD: 9 hr. daylength) and those grown under LD until the plant reached a 3 or 4 expanded leaf stage then treated with 4 weeks of SD then returned to LD. Leaf length was measured on alternate days for each treatment. A Richards model was chosen to represent the leaf growth. There were no differences in leaf growth curves of different treatments within the same leaf position, but curves were different by leaf position. Common leaf growth curves for 3rd and 5th leaf were proposed.

After the 4 weeks of SD treatment, plants were grown in growth chambers under 4 different temperature conditions (18, 21, 24 and 28°C) with 14 hr days (LD). As night temperature increased from 18 to 28°C percent flowering decreased from 55% to 31% and percent flower bud abortion increased from 0% to 19.2%. Inflorescence abortion was observed 6 weeks after the start of SD when flower primordia were evident.

Plants grown under full sun, 40% sun, and 20% sun in ambient outdoor conditions after the start of SD, did not significantly differ in percent flowering or aborted apices.

Foliar ABA content of *H. stricta* was quantified by an indirect enzyme-linked immunosorbent assay (ELISA) specific for free (+)-abscisic acid (ABA). Effects of environmental factors on foliar ABA level were investigated. Foliar ABA level increased as temperature decreased. As light intensity was decreased from full sun to 20% sun foliar ABA increased. Foliar ABA does not seem to be involved in inflorescence abortion as abortion was less under conditions leading to high ABA levels. However, ABA was not analyzed in the pseudostem tissue where the reproductive development was occurring.

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CHAPTER 1

INTRODUCTION

Heliconia is a rather new cut-flower crop that has been introduced to tropic regions around the world during the past 10 years. However, there have been only a few horticultural studies of these plants. Research on *Heliconia stricta* 'Dwarf Jamaican' has been conducted at the University of Hawaii for almost 10 years partly because of its compactness and manageability. Moreover, it can be grown for pot plant as well as cut flower use. *H. stricta* 'Dwarf Jamaican' showed a seasonal flowering pattern with production higher in winter than in summer and was found to require a minimum of 4 weeks of short day (SD) for flower initiation (Criley and Kawabata, 1986). Only plants that had 3 or more leaves were susceptible to the initial stimulus. Plants with 4 initial leaves reached anthesis approximately 13 weeks after start of SD (Criley and Kawabata, 1986). Further experiments showed that decreasing night temperature during 4 weeks of SD from 25°C to 15°C increased the flowering percentage of pseudostems from 15.5% to 57.6% (Lekawatana, 1986). It was observed that pseudostems that did not flower were either in a vegetative phase or their inflorescences had been aborted.

Aborted pseudostems cause losses in flower production since each pseudostem is capable of producing only one inflorescence. This is not a problem in species that flower year-round such as *H. psittacorum* which has a high flowering percentage and multiplies very quickly. However, with species that flower seasonally and usually produce better quality inflorescences, such as *H. stricta* 'Dwarf Jamaican', *H. angusta* 'Holiday', and *H. wagneriana*, this problem of flower bud abortion is quite severe for cut flower production. If the percentage of flower bud abortion for these species can be reduced, there is a good

chance of retaining their existence as a cut flower crop because the market for cut flowers requires a stable supply (Criley and Lekawatana, 1994).

The research reported in this dissertation was undertaken to develop a better understanding of the environmental factors influencing flowering in *H. stricta* 'Dwarf Jamaican'; to continue studies on the physiological basis for flower initiation, development and abortion; and to determine if a relationship existed between abscisic acid (ABA) production in mature leaves and flower bud abortion.

The ultimate goal of this work is control of flower production to ensure a steady supply of cut heliconia flowers for the flower market of the world. *H. stricta* has served as the model plant for these studies, but it is hoped that the information gained in its study can be generaiized to other important cut flower heliconia species.

CHAPTER 2

LITERATURE REVIEW

HELICONIA

Heliconias have been popular conservatory plants, and interior plantscapers have begun to use them in containers and interior plantings. Recently, the cut flower market for Heliconias has expanded with much interest expressed by commercial growers in tropical area seeking crops for export. The intense interest in new potted flowering plants has also led to the development of heliconia as potted plants (Criley, 1991).

ECOLOGY

Most Heliconia species are found in the New World tropic from the Tropic of Cancer in Mexico and the Caribbean islands to the Tropic of Capricorn in South America. Only six species are found in the Pacific island tropics. Heliconia attain their most vigorous growth in the humid lowland tropics at elevations below 500 meters. Many species are found in middle elevation rain and cloud-forest habitats. Few species are found above 2,000 meters (Kress, 1984; Criley and Broschat, 1992).

TAXONOMY

Heliconia is a monotypic genus that is estimated to consist of 200-250 species (Berry and Kress, 1991). The taxa within the order Zingiberales have been debated for a long time, but the heliconias long were placed with the Musa complex (Criley and Broschat, 1992). Nakai (1941) suggested that the Heliconiaceae was distinct from the Musaceae, and recent studies and publications also accepted this classification (Tomlinson, 1962; Dahlgren and Clifford, 1982; Kress, 1984; Dahlgren *et al.*, 1985).

MORPHOLOGY

Heliconias are rhizomatous, perennial herbs with an erect, aerial, and stem-like tube called a pseudostem composed of overlapping leaf sheaths. The rhizome branches sympodially from buds at the base of the pseudostem. Leaves are alternately arranged and distichous (Berry and Kress, 1991; Criley and Broschat, 1992). A pseudostem is often composed of a specific and limited number of 5-9 leaves which may be influenced by cultural and environmental conditions (Criley and Broschat, 1992). Leaf blades are usually green; with some species they are tinted maroon or red underneath especially along the margin and midrib (Berry and Kress, 1991). The leaf apex is acute to acuminate with the base of the lamina unequal and usually obtuse to truncate (Criley and Broschat, 1992). The colorful inflorescence structure is the main attraction of Heliconia for ornamental and cut flower purposes. The inflorescence has either an erect or pendent orientation and is made up of peduncle, modified leaflike structures called inflorescence bracts (cincinnal bracts), the rachis, and a coil of flowers within each bract. The inflorescence bracts are usually red, yellow, or both, but are sometimes green or pink in some species. Each inflorescence bract contains a varying number of flowers, up to 50 depending on the species. The perianth is made up of three outer sepals and three inner petals united at the base and to each other in various ways. The flowers are bisexual, epigynous and strongly zygomorphic. There are five functional stamens and one staminode which is subulate or, to some degree, petaloid. The overy is inferior and 3-locular. Fruits of the New World species are blue in color while those of Pacific tropical species are red when mature.

RESEARCH

It was not until recently that Heliconia was grown commercially for cut flowers. Therefore, the basic knowledge of these plants is limited. However, there were some

studies with *H. psittacorum*, *H. stricta*, *H. chartacea* and *H. wagneriana* done in Hawaii and in Florida.

Increased nitrogen fertilizer rate to *H. psittacorum* yielded more inflorescences especially for plants grown in full sun compared to those under 60% shade (Broschat and Donselman, 1982, 1983).

H. psittacorum, *H.* X *nickeriensis*, *H. episcopalis*, *H. hirsuta*, *H.* X'Golden Torch', *H. chartacea* and some cultivars of *H. stricta* and *H. bihai* flower year-round and are considered to be day-neutral. *H. stricta* 'Dwarf Jamaican', *H. wagneriana*, and *H. aurantiaca* have been shown to initiate flowers under short days (Criley and Kawabata, 1986; Criley and Broschat, 1992) with 4 weeks of short days required at 15°C for flower initiation in *H. stricta* 'Dwarf Jamaican'. A minimum of 3 leaves must be present for this species to respond to photoperiodic stimuli (Criley and Kawabata, 1986). Research on *H. angusta* 'Holiday' showed that flower initiation was induced by long days (minimum of 13 hr. for 7 weeks) (Lekawatana, 1986; Sakai *et al.*, 1990; Kwon, 1992). A daylength requirement was proposed in the flower development of *H. chartacea* since large number of flowers were aborted from shoots that emerged from April to June (Criley and Lekawatana,1994).

Temperature is a limiting factor in the production of *H. psittacorum* in Florida. Growth and flower production declined as minimum temperature decreased from 21 to 10°C and ceased altogether at 10°C (Broschat and Donselman, 1983).

Postharvest life for some *H. psittacorum* cultivars is about 14-17 days, while flowers of other species often last less than one week (Criley and Broschat, 1992). *H. psittacorum* showed no improvement in vase life with different floral preservatives. However, the use of antitranspirants increased the vase life *of H. psittacorum* (Broschat, 1987).

Application of 2-(3,4-dichlorophenoxy)triamine (DCPTA) to *H. stricta* 'Dwarf Jamaican' increased number of inflorescences under full sun compared to 50% shade while

application of DCPTA to *H. caribaea* caused no increase in inflorescence production (Broschat and Svenson, 1994).

Growth retardants were used to control plant height in potted heliconias. Ancymidol was suggested for height control on *H. stricta* 'Dwarf Jamaican' (Lekawatana and Criley, 1989). Paclobutrazol, ancymidol, and uniconazole effectively decreased plant height of *H. psittacorum* making it suitable for potted plant use (Tjia and Jierwiriyapant, 1988; Broschat and Donselman,1988).

MODELS FOR GROWTH AND DEVELOPMENT

LEAF GROWTH

The simplest measure of size of an unfolding leaf often is its length. The exponential relationships of leaf length, volume, area, weight, etc. with time continue until after emergence from the enclosing sheaths and then decline, giving the S-shaped curves characteristic of post-primordial growth (Dale and Milthrope, 1983).

A number of mathematical models have been used to describe a change of area, length or weight (Y) with time (X) (Dale and Milthrope, 1983; Ratkowsky, 1983; Causton and Venus, 1981):

Logistic: $$Y = \frac{\alpha}{1+\exp(\beta - \gamma X)} \quad (2.1)$$

Gompertz: $$Y = \alpha . \exp[-\exp(\beta - \gamma X)] \quad (2.2)$$

Richards: $$Y = \frac{\alpha}{[1+\exp(\beta - \gamma X)]^{1/\delta}} \quad (2.3)$$

Morgan-Mercer-Flodin (MMF) $$Y = \frac{\beta\gamma + \alpha X^{\delta}}{\gamma + X^{\delta}} \quad (2.4)$$

Weibull: $$Y = \alpha - \beta . \exp(-\gamma X^{\delta}) \quad (2.5)$$

These growth rate curves start at some fixed point and increase monotonically to reach an inflection point; after this the growth rate decreases to approach asymptotically some final value (α). β, γ, and δ are parameters (Ratkowsky, 1983; Causton and Venus, 1981).

Logistic Model

The logistic model has been used extensively in the field of animal ecology for modeling the numbers of individuals within a population. In plant growth studies, the fact that the model is S-shaped has rendered it very popular. The model has been applied to many primary data such as single leaf growth, stem length, sugar content, flower number, etc. in many species such as cucumber cotton, asparagus, wheat, grape, etc. (Hunt, 1982).

The logistic model, 2.1, is the best known sigmoid model with asymptotes at $Y = 0$ and $Y = \alpha$. Of the other two model parameters, γ is a 'rate' parameter - a high value indicating a rapid rise of Y between the two asymptotes, and vice versa - and β/γ (β divided by γ) defines the value of X at the point of inflection (Causton and Venus, 1981).

Gompertz Model

The Gompertz model, 2.2, devised by Benjamin Gompertz in 1825, from work with animals and population studies, has three parameters arranged as a double exponent. The majority of applications of the Gompertz model in plant growth analysis has been connected with the modeling of the growth of individual organs, especially leaves (Hunt, 1982).

The parameters have the same general meaning as in the logistic model. The asymptotes are again at $Y = 0$ and $Y = \alpha$, but the value of Y at the point of inflection is α/e instead of $\alpha/2$ (Causton and Venus, 1981). Amer and William (1957) considered that the asymmetry of the Gompertz model was more appropriate to leaf growth data than the symmetry of the logistic model.

Richards Model

The Richards model, 2.3, (Richards, 1959) was first derived from one developed by Von Bertalanffy which was based on theoretical considerations of animal growth. This model is largely applied to single leaf growth (Causton and Venus, 1981). In contrast to both the logistic and Gompertz models that have fixed inflection points relative to the two asymptotes, the inflection point of a Richards model varies in location on the curve. This variability allows much flexibility in describing growth patterns. The Richards function often gives good representation of plant growth (Causton and Venus, 1981).

The Richards model has four parameters. The fourth parameter, δ, controls whether or not the model has an inflection, and if so where it occurs. With $\delta = -1$ no inflection is possible, while increasing the value of δ moves the point of inflection progressively higher up the curve (Hunt, 1982).

Weibull Model

The Weibull model, 2.5, has been put forward by Yang *et al.* (1978) as a flexible sigmoid empirical model for data in forestry, α being the asymptote, and γ and δ being scale and shape parameters, respectively.

Morgan-Mercer-Flodin Model

The Morgan-Mercer-Flodin model (MMF), 2.6, is derived from two well-known models in use in catalytic kinetic studies. When $\beta = 0$, MMF model reduces to the Hill model and when $\beta = 0$ and $\delta = 1$, it reduces to Michaelis-Menten rectangular hyperbola (Ratkowsky, 1983). The parameter β in this model allows the model to have a nonzero intercept on the Y-axis.

CHOICE OF GROWTH MODEL

If there are scientific reasons for preferring one model over the others, strong weight should be given to the researcher's reasons because the primary aim of data analysis is to explain or account for the behavior of the data, not simply to get the best fit. If the researcher cannot provide convincing reasons for choosing one model over others, then statistics can be used to evaluate various models. The smallest residual mean square and the most random-looking residuals should be chosen (Bates and Watts, 1988).

Stability of Parameter Estimates to Varying Assumptions About the Error Term

The first series of estimations were carried out assuming an additive error term, which means that models (2.1)-(2.5) were of the form

$$Y_{tM} = f(X_t,\theta) + e_{tA} \qquad (2.6)$$

where θ designates the vector of the parameters α, ß, and γ (and δ where appropriate) to be estimated, and e_{tA} is assumed to be iidN (independent identically distributed normal) with mean zero and unknown variance δ_A^2. The second series of estimations are carried out assuming a multiplicative error term, which means that models (2.1)-(2.5) are logarithmically transformed and are of the form

$$\log Y_{tM} = \log f(X_t,\theta) + e_{tM} \qquad (2.7)$$

where e_{tM} is assumed to be iidN with mean zero and unknown variance δ_M^2.

T-Test

Another useful criterion for examining the acceptability of a model is Student's t. The *t* value is the ratio of the parameter estimate to its standard error. The *t* values may be tested by reference to a Student's *t*-distribution with N - P degrees of freedom. A high *t* value tends to indicate that the estimate is well determined in the model; a low *t* value tends to indicate that the estimate is poorly determined (Ratkowsky, 1983).

Lack of Fit

When the data set includes replications, it is also possible to perform tests for lack of fit of the expected model. The data takes the form (Y_{qr},X_{qr}) where r represents the repetitions, $r = 1, ..., n_q$, at distinct locations $q = 1, ..., s$. Thus $\Sigma n_q = N$. These analyses are based on an analysis of variance in which the residual sum of squares (RSS) with (N-P) degrees of freedom (P = number of parameters) is decomposed into the replication sum of squares S_r

$$S_r = \sum_{q=1}^{s}\sum_{r=1}^{n_q}\left(Y_{qr} - \bar{Y}_{q.}\right)^2 \qquad (2.8)$$

with M degree of freedom ($Y_{qr} = \Sigma Y_{qr}/r_q$) and $M = \sum_{r=1}^{s} (r_q - 1)$ and the lack of fit sum of squares S_l = RSS - S_r with N-P-M degrees of freedom. The ratio of the lack of fit mean square to the replication mean square (2.9) is compared with appropriate value in the F table (Borowiak, 1989; Bates and Watts, 1988).

$$(S_l/N\text{-}P\text{-}M)/(S_r/M) \text{ with } F(N\text{-}P\text{-}M,M;\alpha) \qquad (2.9)$$

If no lack of fit is found (low F-value), then the lack of fit analysis of variance has served its purpose, and the estimate of σ^2 should be based on the residual mean square.

Considering the above criteria, Richards model is chosen as the most appropriate model for this studies.

STARTING VALUES FOR FITTING RICHARDS MODEL

The physical interpretability of many of the parameters means that crude initial estimates can often be obtained from a scatterplot of the growth data in the form of Y versus X. A visual estimate of the asymptote α, denoted α_0, may be obtained as the maximum value approached by the response at high values of X. To obtain an estimate δ_0 of δ, an estimate of point of inflection (X_F, Y_F) was used. Differentiating (2.3) twice with

respect to X, setting the resulting expression equal to 0, solving for X, and denoting it X_F, one obtains

$$X_F = \frac{(\beta - \log\delta)}{\gamma} \tag{2.10}$$

Substitution of (2.10) into (2.3) results in the following ordinate of the point of inflection:

$$Y_F = \frac{\alpha}{(\delta+1)^{1/\delta}} \tag{2.11}$$

An initial estimate of δ_0 may be obtained by solving (2.11) using estimates α_0 of the asymptote and of the point of inflection Y_F.

Initial estimates of ß and γ can be obtained by rewriting the model (2.3) as

$$\log\left[\left(\frac{\alpha}{Y}\right)^{\delta} - 1\right] = Z_0 = \beta - \gamma.X \tag{2.12}$$

Substituting α_0 and δ_0 into expression (2.12) give values of Z_0 corresponding to each pair values of $ß_0$ and γ_0, which together with α_0 and δ_0, may form a suitable set of initial parameter values for use with the Gauss-Newton algorithm (Causton and Venus, 1981; Ratkowsky, 1983; Seber and Wild, 1989).

BIOLOGICALLY RELEVANT PARAMETERS

Fitting Richards model yields estimates of the parameters α, ß, γ and δ; of which only α and δ can be considered to be biologically meaningful. Parameter α gives the asymptotic maximum size of the leaf. Parameter δ describes the shape of the curve. With δ = -1 no inflection was possible; increasing the value of δ moves the point of inflection progressively higher up the curve. The parameter ß has no biological significance; it is concerned with the positioning of the curve in relation to the time-axis. Finally, γ is a rate parameter related to the mean relative growth rate and the shape of the curve, but its

interpretation depends upon the value of δ (Causton and Venus, 1981; Hunt, 1982; Karlsson and Heins, 1994).

COMPARING PARAMETERS ESTIMATES

Curves for different sets of data can be compared or tested for invariance of some or all of the parameters (the null hypothesis is that the parameter(s) tested are not different among sets of data or treatments). Examination of the difference between the residual sums of squares (RSS) for the model making the least restrictive assumption about the parameters and that for other models with more restrictive assumptions about the parameters could be used to make a decision about parameter invariance. The following steps were adapted from Ratkowsky (1983) for comparing α, γ, and δ in different data sets (treatments).

A) Fit α, ß, γ, and δ to data sets in each data set (all data sets). Each of the data sets may be fitted individually. Their RSS are added together to produce a pooled RSSs. This provides the most general, or least restricted, model for carrying out subsequent tests.

B) Fit α, ß, γ, and δ to data sets in each of two sets of data to be compared (obtained from A.)

C) Fit a common α, ß, γ, and δ to each of the two sets of data to be compared.

D) Fit a common α to each of the two individual sets of data to be compared, but fit individual ß, γ, and δ.

E) Fit a common ß to each of the two individual sets of data to be compared, but fit individual α, γ, and δ.

F) Fit a common γ to each of the two individual sets of data to be compared, but fit individual α, ß, and δ.

G) Fit a common δ to each of the two individual sets of data to be compared, but fit individual α, β, and γ.

With the hypothesis of an invariant α, β, γ and δ (no difference of the 4 parameters across treatments), testing for invariance was done by taking differences between the RSSs obtained from step C and B finding the residual means square (RMS) and dividing by the RMS obtained from step A yielding an F-value whose significance is read from the F table using the degrees of freedom from step A as denominator.

Testing for individual invariants (α, β, γ or δ) and ignoring the others was performed by using the differences D-B, E-B, F-B, and G-B finding the RMS and dividing by the RMS obtained from step A resulting in the F-value.

ENVIRONMENTAL STRESS

WATER STRESS

Water stress affects many aspects of plant physiology, in particular the ABA content and the growth rate. Water deficit may influence growth via effects on several parameters such as the hydraulic conductivity of tissues, the osmotic properties of the cell, and the rheological properties of the cell wall (Ribaut and Pilet, 1991). In water stressed leaves, the level of ABA is often related to water potential, but turgor seems to be the essential parameter influencing ABA accumulation under a water stress condition.

In water stressed sunflower, the rise in ABA concentration in xylem under stress was a sequential response; the initial increase being derived from the roots, and the subsequent increase being at least partially derived from the stressed leaves. This second source of ABA is transported downwards in the phloem to the roots then transferred to the transpiration stream in the xylem (Creelman, 1989).

The primary site of action of ABA is on the outer surface of the plasmalemma of guard cells, it is the apoplastic ABA that is physiologically relevant (Creelman, 1989). There

are two possible ways to increase ABA concentrations in the apoplast in this region. These are: (a) an enhanced transport to the leaves of root-sourced ABA in transpiration stream, and (b) a rapid release of ABA from mesophyll compartments to the apoplast. The later response can be promoted by a small change in leaf water status (Hartung and Davies, 1991).

The transport of ABA in the apoplast of the leaf, from xylem to epidermis, is influenced among other things, by pH and the rate of ABA biosynthesis, metabolism and conjugation. Therefore, it does not necessarily follow that the ABA concentration to which guard cells respond is the same as that measured in the xylem sap (Neales and McLeod, 1991). By using enzyme-amplified immunoassay (ELISA), the ABA content of guard cells was found to be only 0.15% of the leaf ABA of *Vicia faba* L. (Harris *et al.*, 1988).

CHILLING STRESS

A chilling temperature can be defined as any temperature that is cool enough to produce injury but not cool enough to freeze the plant. For vast majority of plants, a chilling stress refers to any temperature below 10-15°C, and down to 0°C. Rice and sugar cane may suffer chilling injury at 15°C. At chilling temperatures, respiration rate may exceed the rate of photosynthesis, and this may lead to starvation eventually (Levitt, 1980 a).

A number of researchers have demonstrated increased ABA content following chilling exposure (Pan, 1990). Cooling roots of bean seedlings to 10°C resulted in an increase in the content of free ABA in the primary leaves and a reduction in their otherwise rapid growth (Smith and Dale, 1988). Exposure of chilling-sensitive cucumber seedlings to chilling temperatures caused a significant rise in the level of ABA. However, it was concluded that the increase of ABA was due to a temperature-induced water deficit and not to the low temperature *per se* (Capell and Dörffling, 1989).

HEAT STRESS

Temperature below the optimum temperature decreases growth rate of plants due to the depressing effect of temperature on the rate of chemical reaction. However, temperature above the optimum temperature also decrease growth rate which can not be explained by the direct effect of temperature on chemical reaction. The longer plants are exposed to the high temperatures, the longer it takes them to recommence growth. The temperature at which the rate of respiration equal the rate of photosynthesis is called the temperature compensation point. Respiration rate was higher than photosynthetic rate at high temperature. If plant temperature rises above the compensation point, the plant reserves will begin to be depleted and ultimately lead to starvation and death (Levitt, 1980a).

LIGHT STRESS

A level of illumination below the light compensation point can lead to a slow, indirect injury, due to starvation (decrease in carbohydrates). To avoid light deficit, plants can increase the total interception of light by increasing leaf area. Shade leaves are thin and have a low dry matter content, providing a maximum photosynthetic surface per unit dry matter. Resistance to light deficit is associated with a decrease in resistance to the temperature and water stress (Levitt, 1980b). However, plants grown under higher light intensity usually have smaller and thicker leaves than those under low light intensity (Whatley and Whatley, 1980).

ABSCISIC ACID

Most higher plant tissues are capable of synthesizing ABA which have been demonstrated in fruit tissues, seeds (embryo, cotyledon, endosperm), roots, stem and leaves. Within the cells of these tissues it appears likely that most of the ABA is synthesized in the plastids (Goodwin and Mercer, 1983).

ABA and its metabolites are very mobile. ABA can be transported over long distances in plants via phloem and xylem (Walton, 1980). However, in various species the most actively growing organs act as sinks for ABA. Young tissues have the highest levels of endogenous ABA. Older tissues such as cotyledons and primary leaves are weaker sinks but are strong exporters (Habick and Reid, 1988). Ross and McWha (1990) reported over 90% of ABA in the *Pisum sativum* plant was located in the young seed.

PHYSIOLOGY

Since its isolation in 1965, ABA has figured prominently in discussions on the regulation of plant development. Among other processes, there is evidence for an involvement of ABA in the induction and processes of dormancy (including abscission and senescence) and in many plant developmental responses to water deficit (Trewavas and Jones, 1991).

Flower Induction

Abscisic acid applications promote flowering in short day plants (Milborrow, 1984). ABA does not appear as a major determinant in the floral transition, except in some species. S-(+)-abscisic acid applied to short day *Phabitis nil* completely inhibited floral bud initiation (Kamuro *et al.*, 1990). High concentrations of ABA inhibited or delayed flowering in a number of species, but this effect was probably a result of an inhibitory effect on growth (Milborrow, 1984).

Increases in endogenous ABA were reported to promote flower initiation in short day plants and inhibit it in long day plants. However recent studies do not support earlier findings since it appears that there is no consistent relationship between photoperiod and ABA content in plant tissues (Bernier, 1988; Bernier *et al.*, 1981).

Flower Development

The ability of ABA to induce, promote or to accelerate flower abscission has been demonstrated in many species such as *Begonia*, *Gossypium*, *Linum*, *Rosa*, etc. (Addicott, 1983). Application of synthetic ABA to buds of tulip and differentiating flower buds of *Phaseolus vulgaris* resulted in bud blasting in tulip and abscission of many of the buds at later stages of development in *Phaseolus* (Bentley *et al.*, 1975; Kinet *et al.*, 1985).

Correlations of high levels of endogenous ABA with the abscission process were reported on cotton flowers and young fruits (Davis and Addicot, 1972; Guinn *et al.* 1990), bean flower buds (Bentley *et al.*, 1975) and Lupin flowers (Porter, 1977).

BIOCHEMISTRY

Naturally occurring abscisic acid (ABA; Figure 1) is exclusively the +(S)-enantiomer. The 2-*cis* double bond of ABA can be isomerized by light to give the biologically inactive 2-*trans* isomer (Neill and Horgan, 1987), which has been regarded as an artifact formed from ABA during extraction and isolation. However, *trans*-ABA is present in plant extracts obtained even under dim light (Hirai, 1986).

If plant extracts are hydrolyzed by alkali, the free ABA content of the extracts is increased. The source of this ABA is ABA-conjugates. At least two conjugates have been identified in plant tissues. The most prevalent compound is the glucose ester of ABA (ABAGE: (+)-abscisyl-ß-D-glucopyranoside); however, a second conjugate, 1'-*O*-glucoside (ABAGS: 1'-*O*-abscisic acid-ß-D-glucopyranoside), has also recently been characterized. There is no evidence that these conjugates act as a source of free ABA, since wilted plants accumulate ABA in the absence of a change in levels of ABA conjugates (Neill *et al.*, 1983; Roberts and Hooley, 1988).

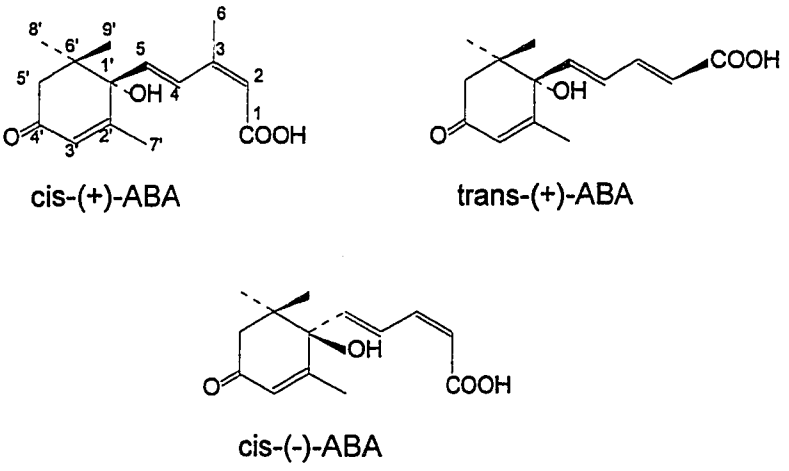

Figure 1. ABA structures

Extraction

Although ABA is chemically stable under a wide range of conditions (liquid N_2 to 70 °C, pH 2.0-11.0), extracts should receive the minimum exposure to light to prevent isomerization of ABA to its 2-trans isomer (Hirai, 1986; Parrry and Horgan, 1991b). ABA levels also rapidly change in response to drought. If fresh material is not extracted immediately, it is usually frozen in liquid N_2 and stored at -20°C (Neill and Horgan, 1987). Strong acid or basic conditions and heating should be avoided during extraction and isolation (Hirai, 1986).

Distilled water, 80% methanol, and 80% acetone have been used as solvents for extraction (Piaggesi *et al.*, 1991; Vernieri, 1989b; Daie and Wyse, 1982; Norman *et al.*, 1988; Neill and Horgan, 1987). The addition of antioxidants such as BHT (2,6-di-tert-butyl-4-methyl-phenol) at concentrations up to 100 mg/l has been recommended (Neill and Horgan, 1987).

Quantitation

Quantitative measurement of the endogenous levels of ABA is quite difficult because of its instability and low concentration in plants (ng/g fresh weight range). For the determination of ABA, several methods including bioassays and chromatographic procedures have been used. Detection limits range from that of UV spectroscopy at 1-3 μg, and optical rotary dispersion at 0.5 μg/ml, to high pressure liquid chromatography (HPLC) at 1-2 ng, gas chromatography (GC) with flame ionization detection (FID) at 10-100 ng, and GC/mass spectrometry and electron capture detection (ECD) at 10 pg - 50 ng (Weiler, 1979; Hirai, 1986). All of these analytical techniques require prior preparation of highly purified extracts which are achieved by one or more differential solvent extractions followed by at least one chromatographic step and often a derivative synthesis. The same degree of

purification is also required for all known ABA-bioassays. The sensitivity of the best bioassays was about 100-200 ng/ml (Weiler, 1979).

Recently, immunoassay for ABA has been confirmed as the most sensitive and selective detection method for ABA with detection limits as low as 2×10^{-16} mole (Harris and Outlaw, 1990). In theory, the assay should offer maximal specificity with minimal interference from extraneous compounds (Roberts and Hooley, 1988). Preparation of antigen and antiserum is a time-consuming process, but the advantage of the immunoassay method is that a number of crude samples without preliminary purification can be tested semiautomatically in a short time with high accuracy (Hirai, 1986).

Immunoassay

Historically, radioimmunoassays (RIA) comprised the first generation of immunoassays that were sensitive enough to cope with PGR at physiological levels. These assays made use of polyclonal antisera raised in rabbits. Tritium or iodine-125-labeled PGR or their derivatives were employed (Weiler *et al.*, 1986a). Immunoassay is based on the competition of a known amount of labeled antigen and an unknown amount of sample antigen for a limited number of high-affinity antibody binding sites. Monoclonal antibodies (MAbs) useful for immunoassay have to exhibit both high affinity and specificity. This combination has rarely been achieved for low molecular weight antigens such as ABA and other PGRs. Therefore, synthesis of a PGR-protein conjugate is necessary for an immune response, and this introduces changes in the structure of the PGR with which the animal immune system is confronted (Weiler, 1984).

By coupling the carrier to the PGR molecules at different sites, it is possible to generate antibodies exhibiting different selectivity (Roberts and Hooley, 1988). Bovine serum albumin (BSA), human serum albumin (HSA), and hemocyanin have been used for carrier proteins to be conjugated with a Hapten ABA. There are two ways of conjugation,

as shown in Figure 2. Antigen conjugated to C-4' of ABA through a hydrazone linkage is used for free ABA determination; antigen conjugated to C-1 of ABA through an amide bond is used for total ABA determination (Hirai, 1986). Antigen conjugated to C-1 of ABA through the carboxyl group did not discriminate between free ABA or C-1 conjugated ABA (Perata *et al.*, 1990).

Enzyme-linked immunosorbent assay (ELISA). The antibody is bound to a solid phase such as the well of a microtitre plate, and 'free' and enzyme-linked antigen molecules compete for the immobilized binding sites. At equilibrium, the 'free' phase is decanted and the quantity of 'bound' enzyme determined after the addition of the enzyme's substrate. Most commonly, the antigen is linked to alkaline phosphatase or horseradish peroxidase, since these enzymes exhibit high activity against substrates which produce products which are colored or fluorescent and are therefore readily quantifiable (Roberts and Hooley, 1988).

Indirect ELISA. This method employs the conjugation of the antigen to a protein which is immobilized to the walls of a support such as the well of a microtitre plate. 'Free' antigen and antibody are added to the reaction vessel, and the antibody molecules bind to either the immobilized or the 'free' antigen (Figure 3). The soluble antibody-antigen conjugate is decanted away. An enzyme-linked second antibody, which specifically recognizes the antiserum in which the primary antibody was raised, is introduced into the reaction vessel. The secondary antibody binds to the immobilized conjugate. After the liquid phase has been removed, the substrate of the enzyme linked to the secondary antibody is added and the amount of product quantified (Roberts and Hooley, 1988). Indirect ELISA was reported 5 to 10 times more sensitive than the direct procedure and was about 50 times more sensitive than GC-MS (Belefant and Fong, 1989).

Control of Assay Performance. A high degree of binding specificity does not guarantee a valid assay because of interference. Therefore, assay precision, reproducibility and accuracy need to be checked. The checks required reflect the sources of potential

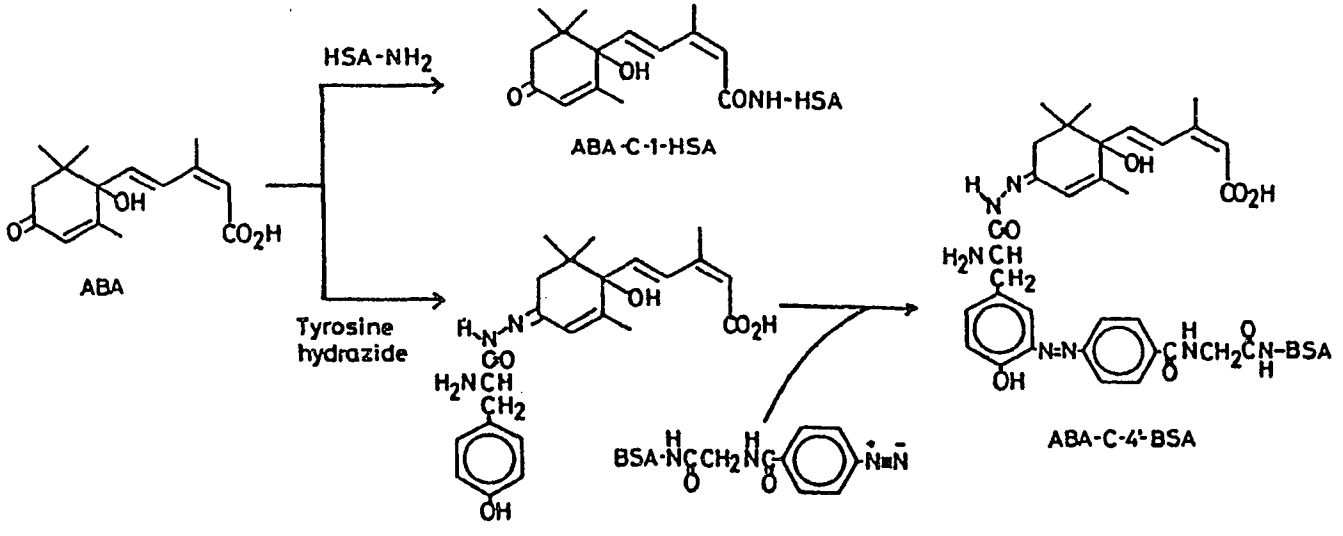

Figure 2. Synthesis of ABA-serum albumin conjugates, ABA-c-1-HSA and ABA-c-4'-BSA (Hirai, 1986). HSA = human serum albumin, BSA = bovine serum albumin

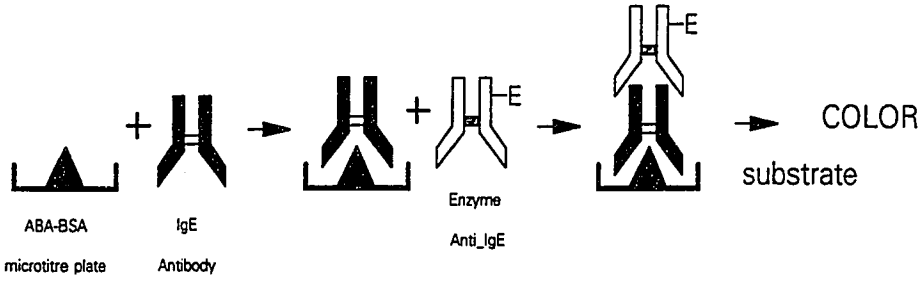

Figure 3. Indirect ELISA. Antibody binds to antigen (ABA-BSA) in the solid phase and is subsequently detected by the color which develops when an enzyme-labeled antibody binds to the complex. (IgE = antibody or immunoglobulin; Enzyme Anti-IgE = enzyme-labelled anti-immunoglobulin)

interference peculiar to immunoassays. The most relevant potential sources of interference in immunoassays are the following (Pengelly, 1986; Weiler, 1986; Weiler *et al.*, 1986b):

1. Compound antigenically (structurally) similar to the plant hormone under study.

2. The presence of excessive amounts of compounds which exhibit only weak cross-reactions.

3. The presence of antibody denaturing or desorbing agents, For example, high levels of phenolic compounds may partially denature antibodies; the presence of surfactants may likewise denature soluble antibodies or may desorb them from solid supports.

4. The presence of factors which prevent the binding of hormone to its binding site (e.g., by complexation).

5. The presence of contaminants which impair the quantitation step.

No single test for assay validity is absolutely safe. It is recommended to use the maximum number of the following controls when dealing with a new source of plant material (Weiler, 1986).

1. Losses of hormone during extract work-up will affect accuracy. Work-up losses are detected by use of radioactive hormone internal standards or by using hormone-spiked split extracts processed in parallel. This also compensates for any isomerization of *cis, trans*-ABA to *trans, trans*-ABA which might have occurred during extraction and assay (Weiler, 1986; Weiler, 1980).

2. Parallelism test of a plant extract dilution curve with the standard curve is a test for specificity. This can be done by performing a dilution series of the extracts and to show additivity, or parallelism to the standard curve. The plot will yield a line parallel to the standard curve if there is no interference (Daie and Wyse, 1982; Pengelly, 1986, Wang *et al.*, 1986).

3. Dilution analysis with internal standardization: increasing amounts of extracts are added to standards. Absence of interference is indicated if the data points (plot hormone

found vs hormone added) fall on a straight line parallel to each other and to the standard line. Information of quantitative recovery will also be obtained and values should be close to 100% recovery of the added hormone. Highly cross-reactive material may be overlooked this way (Mertens *et al.*, 1985; Vernieri *et al.*, 1989a; Weiler, 1986).

4. Successive approximation: This approach makes use of a series of different purification steps. This process is continued until an estimate is obtained that does not change on purification. An internal standard is used so sample losses encountered during purification can be assessed (Crozier *et al.*, 1986; Weiler, 1986).

Factors included in group 2 are best for checking a dilution analysis at various levels of added standard hormone. Deviation from uniformity (slope = 1) indicates interference. Cross reactants as defined under 1 will show up in this test if their dose response curves do not run parallel to the hormone standard curve. Cross reactants with tracer displacement curves parallel to the standard curve cannot by detected by this method. Cross reactants are best detected in immunohistograms of separated extracts (Weiler *et al.*, 1986b).

CHAPTER 3

LEAF GROWTH MODEL AND FLOWERING PROGRAM OF *HELICONIA STRICTA*

ABSTRACT

Heliconia stricta cv. Dwarf Jamaican plants were grown under: continuous long days (14 hr. daylength), continuous short days (9 hr. daylength), or grown under long days (LD) until the plants reached the 3 to 4 expanded leaf stage, then 4 weeks of short days (SD) and returned to long days. Plants grown under continuous SD and LD+SD until the 3-leaf stage had the highest flowering percentage (45 and 46%), while only 17% of plants grown under LD+SD until the 4-leaf stage flowered, and no flowers were produced in plants grown under continuous LD. Plants grown under LD until the 3 or 4-leaf stage flowered 13 weeks after the start of SD. The plants and inflorescences were more vigorous than those under continuous SD. Leaf length was measured on alternate days for each treatment and fitted to the Richards model. There were no differences in leaf growth curves of different treatments within the same leaf position (3^{rd}, 4^{th} and 5^{th}). By fitting relative leaf elongation and relative time to full leaf expansion to the Richards model, leaf growth curves of different leaf positions were shown to be significantly different. Common leaf growth curves for leaf positions 3-5 and a program for *H. stricta* 'Dwarf Jamaican' culture were proposed.

INTRODUCTION

Criley and Kawabata (1986) found that established *Heliconia stricta* cv. Dwarf Jamaican plants with 3 or more expanded leaves could be induced to flower in 13 weeks by growing them under a minimum of 4 weeks of short days. Continuous long days (LD) had a strong effect in prolonging the vegetative phase or inducing flower bud abortion in the first generation of shoots produced after potting, while continuous short days (SD) enhanced

flowering of pseudostems (Lekawatana, 1986). The effect of LD decreased with successive generations of daughter pseudostems as some plants did flower in continuous LD. The lengths of both inflorescence and pseudostem were longer in continuous LD than in SD.

The purpose of this experiment was to determine growth of plants raised under different daylength condition at different stages of development with the goal to develop a cultural program of *Heliconia stricta* 'Dwarf Jamaican' from potting to flowering.

MATERIALS AND METHODS

PLANT MATERIAL AND CULTURAL PRACTICES

Eighty-four rhizome pieces of *Heliconia stricta* cv. Dwarf Jamaican were propagated on June 20, 1988. Rhizome pieces including pseudostems were separated from the mother plants, and the roots removed. The pseudostem was cut to 5-cm lengths from the leaf sheath base, treated in a 55°C water bath for 5 minutes, dipped in fungicide solution (Dithane M45) and drained. The rhizomes were then held in plastic bags for 3 weeks at 20°C to stimulate root and shoot growth. They were planted in a 1:1 ratio (v/v) perlite and vermiculite medium and held under mist for 1 week. Rooted rhizome pieces were potted singly into a mixture of peat and perlite 1:1 ratio (v/v) in 15-cm pots on July 18, 1988 in a greenhouse at the Magoon greenhouse facility of the University of Hawaii. The potting medium was amended with dolomite, Micromax and treble superphosphate at the rates of 6.0, 1.0 and 0.6 kg per cubic meter, respectively. Plants were drip irrigated twice daily with nutrient solution, 200N-0P-200K (ppm).

TREATMENT SETUP

After potting, plants were divided into 4 groups (21 pots each) for 4 treatments:

Tr. 1: Plants grown under continuous long days (LD).

Tr. 2: Plants grown under LD until the 3-leaf stage (Aug. 22, 1988). This stage is when the third leaf has expanded and the fourth leaf has started to emerge. Then, the plants were moved into short days (SD) for 4 weeks and returned to LD.

Tr. 3: Plants were grown under LD until the 4-leaf stage (Sept. 2, 1988). This stage is when the fourth leaf has expanded and the fifth leaf has started to emerge. Then the plants were moved into short days (SD) for 4 weeks and returned to LD.

Tr. 4: Plants grown under continuous SD

Labels for these treatments have been abbreviated to:

Tr. 1: conLD; Tr. 2: $LD_{3L}+SD$; Tr. 3: $LD_{4L}+SD$; Tr. 4: conSD;

SD was provided by placing plants under an automatic black cloth shading system from 5:00 p.m. to 8:00 (9-hr. photoperiod). Plants under LD were also under the shading system. However, they were given LD by supplementing daylength with incandescent illumination from 5:00 p.m. to 10:00 p.m. with 60-W lamps placed 1.3 m above the pots to give 14 hr. daylength (LD). One month after potting, plants that were not uniform were removed, leaving 15 pots in conLD, 13 pots in $LD_{3L}+SD$, 17 pots in $LD_{4L}+SD$, and 11 pots in conSD.

DATA COLLECTION

Lengths of each leaf from soil line to top of the plants were measured every other day from time of emergence until those leaves stopped growing. A total of 9,228 leaf length data points were recorded, averaging 20 data points per leaf. Time of inflorescence emergence and anthesis, peduncle and inflorescence lengths, and number of cincinnal bracts were recorded. Plants were discarded after anthesis. The experiment was

terminated on December 10, 1988. For pseudostems that did not show an inflorescence, a determination of status (vegetative or aborted) was then made by dissecting the stems.

Photosynthetically active radiation (PAR) in the 400 to 700 nm waveband was measured by a LI-COR quantum sensor model LI-190SZ. The light sensor and an air temperature sensor were connected to a Datapod model DP211 (Omnidata Int., Inc., Utah). Data were averaged over 5 minutes intervals and recorded every 60 minutes. The unit of PAR is micromoles per second per square meter (average daily maximum PAR was 449.3 μmole.s^{-1}.m^{-2} with a range of 40-680 μmol.s^{-1}.m^{-2}). The average minimum and maximum temperatures throughout the experiment were 22.8°C (range: 19-25°C) and 34.7°C (range: 27-41.5°C), respectively. A summary of the weather data is presented in Appendix B: Figures 1-2.

STATISTICAL ANALYSIS

Chi-Square

Chi-Square tests for independence were used in analyzing quantitative data such as number of pseudostems in each status (flowered, vegetative or aborted). The null hypothesis was that the differences among the ratios were not significant. The null hypothesis was rejected when the significance probability was 0.05 or less. If the null hypothesis was rejected, a chi-square test for a fixed ratio hypothesis was performed for the ratio of pseudostem numbers in each status. The test was done on different pairs of pseudostem numbers within each status. The null hypothesis was that the ratio of pseudostems in each status between two daylength treatments was not significantly different. This test enabled the comparison of numbers of pseudostem among different daylength treatments within a status.

Covariance Analysis and Comparison of Regression lines

In this experiment, daylengths were the primary treatments, but since leaves emerged sequentially during treatment over different periods of time, this was also considered a source of variation. The analysis of covariance was applied to this experiment by including leaf position on the pseudostem in the model as a covariate. When the covariate is measured after the treatments have been applied, it is important to determine if the behavior of the covariate is substantially influenced by the treatments applied. If the treatments significantly affect the covariate, the use of the covariance analysis takes on a different role. Instead of being used to reduce experimental error, it is now used to assist in the interpretation and characterization of the treatment effects upon the character of interest in much the same way that regression and correlation analyses are used (Gomez and Gomez, 1976). Testing for heterogeneity of slopes is an extension of covariance analysis (Freund *et al.*, 1986). In this regression model the continuous measured variable was number of leaves. A qualitative variable, daylength treatment, enabled the data to be stratified into groups, with different regression coefficients for linear and quadratic effects assigned to each treatment. This regression model tested whether the regression coefficients were constant over groups (daylength treatments). A model sequence approach was used for each response variable, the most general model including terms for common intercept, linear, and quadratic differences among daylength treatments (Allen and Cady, 1982). Testing progressed until reduced models were found that described the data adequately. The overall goodness of fit of reduced models is described in figures represented by the model r^2. Single degree of freedom contrast coefficients were used to compare intercepts and regression coefficients among each daylength treatment. If two or more treatments were not significantly different as to intercept, slope, or curvature, they were presented as a single regression equation.

Growth Model Fitting

Least-square estimates of model parameters were calculated by the Gauss-Newton method in nonlinear regression (NLIN) procedure of statistical analysis system (Freund and Little, 1986; SAS Institute Inc., 1987; Appendix C: Programs 1-10). Model selection was done using sample leaf length data from the 4th leaf of plants which flowered in LD_{3L}+SD. The selection was based on scientific reasons, stability of parameter estimates to varying assumptions about the error term, lack of fit test, and Student's t-test as described in chapter 2.

After a model was selected, leaf length data for each leaf position (2nd to 6th) of the plants in each treatment were fitted to it to study the growth curves. Estimated parameters of models among treatments within the same leaf position were compared using the method described in chapter 2 (Appendix C: Programs 11-14).

Growth curves among leaf positions were compared by transforming leaf expansion time and leaf length to relative scales from 0 to 1. This method facilitated the comparisons of different leaf lengths among leaf positions and the different time frames from emergence (T=0) to fully expanded (T=1). Leaf length at emergence time was assigned 0 and fully expanded, 1. Estimated parameters of leaf growth models among different leaf positions were then compared.

Richards Model Parameters

By fitting Richards equation (3.1) the change of leaf length (Y) with time (X) can be described. The model yields estimates of the parameters α, β, γ and δ.

Richards model:
$$Y = \frac{\alpha}{\left[1+\exp(\beta-\gamma X)\right]^{1/\delta}} \qquad (3.1)$$

Parameter α gives the asymptotic maximum size of the leaf. Parameter δ describes the shape of the curve. With δ = -1 no inflection was possible; increasing the value of δ moves

the point of inflection progressively higher up the curve. The constant β has no biological significance; it is concerned with the positioning of the curve in relation to the time-axis. Finally, γ is a rate constant related to the mean relative growth rate (RGR) (3.2) and the shape of the curve but its interpretation depends upon the value of δ (Causton and Venus, 1981; Hunt, 1982; Karlsson and Heins, 1994).

$$\text{Mean relative growth rate (RGR):} \quad = \quad \frac{\gamma}{\delta+1} \qquad (3.2)$$

Generally RGR is the rate of growth per unit weight of plant (Charles-Edwards *et al.*, 1986). In this experiment it will be referred to as rate of leaf growth per unit leaf length.

RESULTS

PSEUDOSTEM STATUS

The pseudostems grown under conLD did not flower. Those grown under LD_{3L}+SD and conSD had higher flowering percentage than those grown under LD_{4L}+SD (Table 1 and Figure 4). However, pseudostems grown in conLD had a higher percentage of vegetative pseudostems than those in conSD. There was very low percentage of vegetative pseudostems in plants grown under LD_{3L}+SD and LD_{4L}+SD. Percentage of flower bud abortion was highest in plants grown in LD_{4L}+SD while there was no flower bud abortion in plants grown under conSD (Table 1).

NUMBER OF LEAVES SUBTENDING THE INFLORESCENCE

Pseudostems grown under LD_{4L}+SD had significantly more subtending leaves (7 lvs.) than those grown under LD_{3L}+SD and conSD (6 lvs; Table 2, Appendix A:Table 1). However, those grown under conLD produced up to 8 leaves (Table 2).

Table 1. Flowering status of *H. stricta* pseudostems under different daylength treatments. The distribution of pseudostems in each status were significantly different among treatments with Chi-square = 39.242 (df = 6), and p = 0.0001.

	Number and (percentage) of pseudostem				
Treatment	Total	Vegetative	Flowering	Aborted	Flw. + Abrt.
conLD	15	12 (80.0)a[z]	0 (0) b	3 (20) b	3 (20) b
LD_{3L}+SD	13	0 (0) b	6 (46.1)a	7 (53.8)a	13(100) a
LD_{4L}+SD	17	1 (5.9)b	3 (17.6)b	13 (76.5)a	16 (94.1)a
conSD	11	6 (54.5)a	5 (45.4)a	0 (0) b	5 (45.4)b

[z]Separation of number of pseudostems in each status (column) by Chi-Square.

Table 2. Production and lengths of *H. stricta* inflorescences under different daylength treatments.

	Number			Length (cm)			
Treatments	Inflorescence	leaf	bract	last leaf	Peduncle	Inflorescence	Inf + Ped.
conLD	0	8 a[z]	-	41.9 a	-	-	-
LD_{3L}+SD	6	6 c	2.0 a	37.1 b	14.7 b	17.5	32.2
LD_{4L}+SD	3	7 b	1.7 a	41.9 a	17.2 a	14.9	32.1
conSD	5	6 c	1.0 b	34.4 b	14.7 b	14.4	29.0
Significance of F value		0.0	0.0001	0.0023	0.0224	NS	NS

[z]Mean separation in columns by Duncan's multiple range test at 5% level.

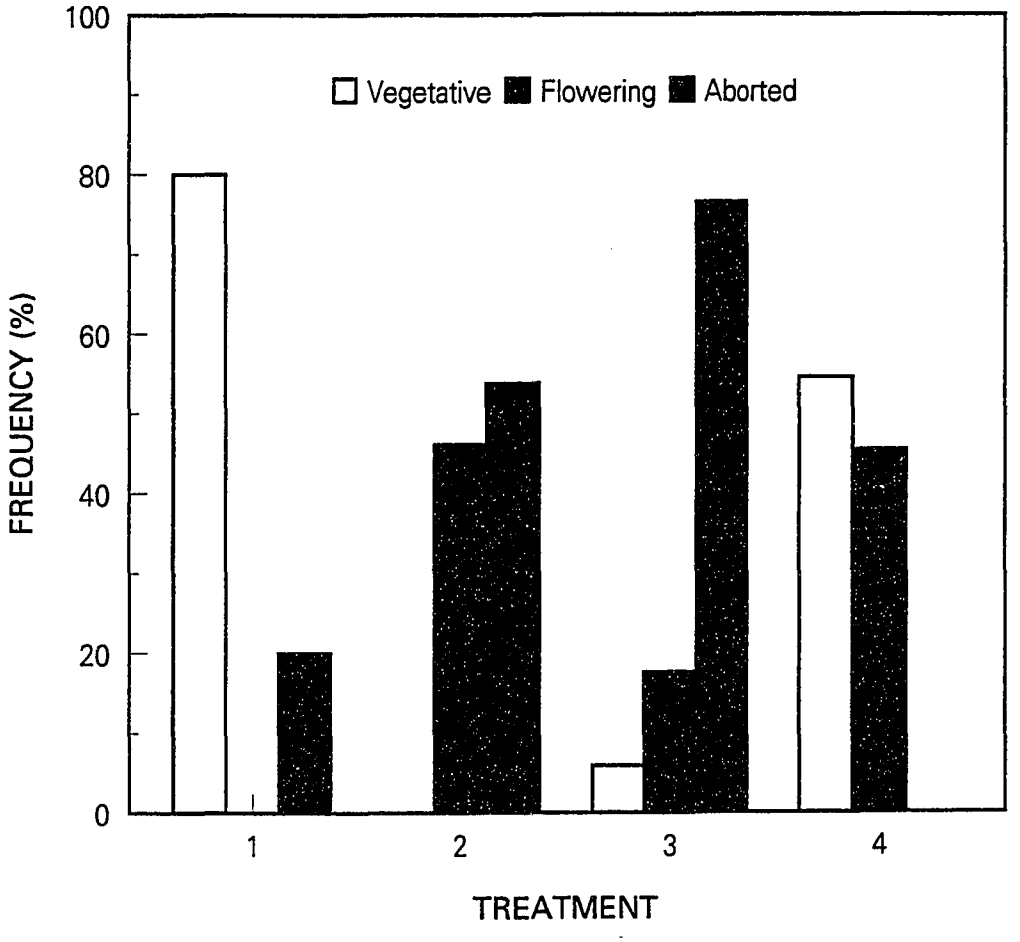

Figure 4. The percentage of all harvested *Heliconia stricta* showing vegetative, aborted or flowering status in different treatments (tr.1 = conLD, tr.2 = LD3L+SD, tr.3 = LD4L+SD, tr.4 = conSD).

FLOWERING

Inflorescence Characteristics

Cincinnal bract count for flowered plants grown under conSD was significantly less (1 br.) than for those grown under LD_{3L}+SD and LD_{4L}+SD (approx. 2 br.; Table 2). There was no significant difference among treatments on the overall length of the inflorescence (inflorescence and peduncle combined) (Table 2, Appendix A:Tables 3-4). However the length of the subtending leaves and the peduncle length of plants grown under LD_{4L}+SD was significantly longer than those grown under LD_{3L}+SD or those under conSD (Table 2, Appendix A:Tables 2-6).

Time to Flower

Plants grown under conSD required less time from potting to anthesis (15 wks) compared to those grown under LD_{3L}+SD and LD_{4L}+SD (18 and 19 wks.; Table 3).

PLANT GROWTH

Leaf length of plants grown under conSD was significantly shorter than those grown under conLD, LD_{3L}+SD and LD_{4L}+SD. Leaf position had significant linear components with leaf length at the 5% level and the length increased with successive leaf position (Figure 5, Appendix A:Table 13).

Time from potting to leaf emergence of plants grown under conSD was significantly less than those grown under conLD, LD_{3L}+SD and LD_{4L}+SD. Leaf position had a highly significant quadratic effect on the time from potting until any given leaf emergence at the 1% level (Figure 6, Appendix A:Table 14).

The time increment between successive leaves of plants grown under conSD was significantly less than those grown under conLD, LD_{3L}+SD and LD_{4L}+SD. Leaf position had significant quadratic components with days to produce each leaf at the 1% level

Table 3. Time from potting and from start of SD to inflorescence emergence and anthesis.

Treatments	Infl. No	Time					
		Potting to last. leaf emergence week and (day)	last leaf to infl. emergence week and (day)	Infl. emergence to anthesis week and (day)	Potting to anthesis week and (day)	SD to Infl. emergence week and (day)	SD to anthesis week and (day)
conLD	0	-	-	-	-		
LD_{3L}+SD	6	9.5 (68.3) b[z]	2.6 (19.0) ab	5.8 (41.6) a	18.0 (129.0) a	7.2 (52.3) b[y]	13.0 (94.0)
LD_{4L}+SD	3	12.0 (85.6) a	2.6 (20.6) a	4.6 (29.3) b	19.3 (135.7) a	8.7 (61.3) a	13.3 (90.7)
conSD	5	8.2 (60.6) b	2.2 (14.8) b	4.4 (29.6) b	14.8 (105.0) b	- -	
Significance of F value		0.0003	0.028	0.0006	0.0001	0.0125	NS

[z]Mean separation in columns by Duncan's multiple range test at 5% level.

[y]Mean separation in columns by t-test.

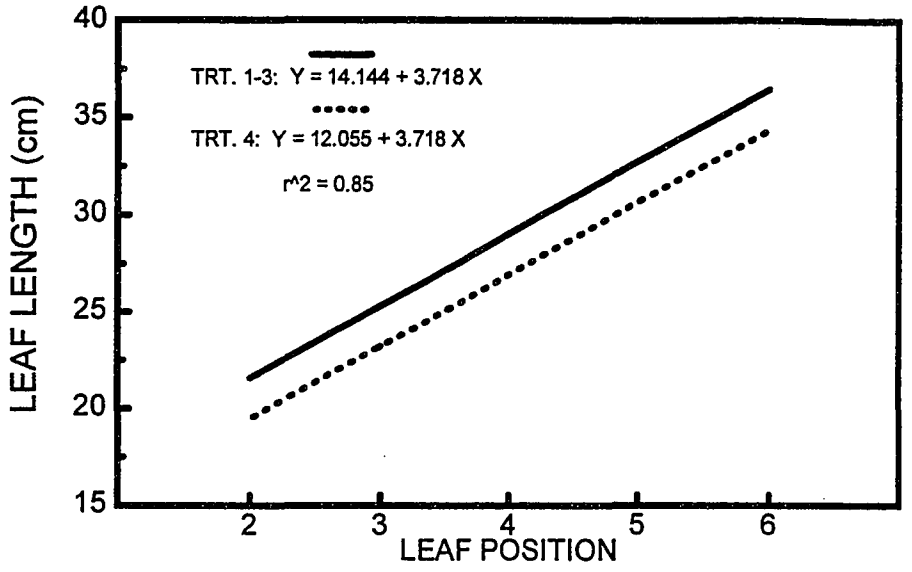

Figure 5. Influence of daylength treatment and leaf position on leaf length of *H. stricta* (tr.1 = conLD, tr.2 = LD3L+SD, tr.3 = LD4L+SD, tr.4=conSD).

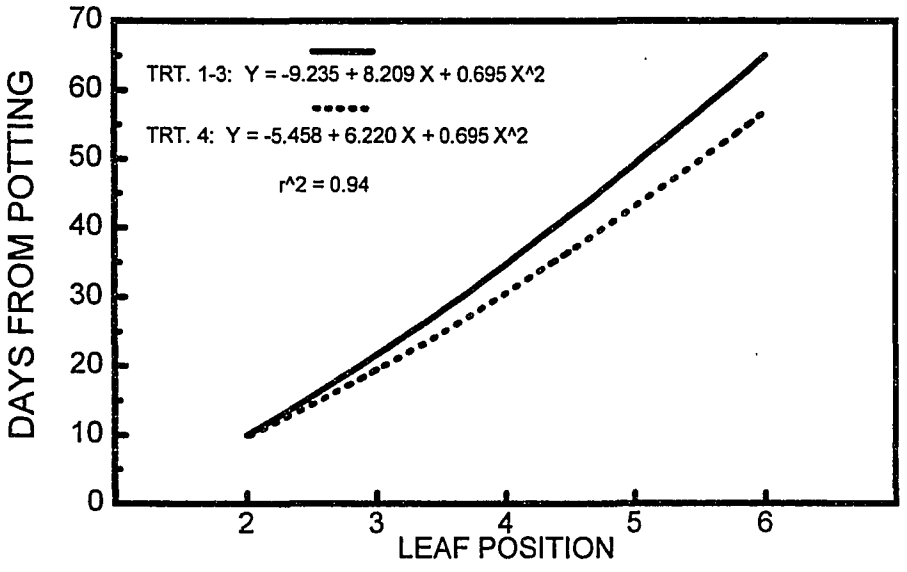

Figure 6. Influence of daylength treatment and leaf position on time from potting to leaf emergence of *H. stricta* (tr.1 = conLD, tr.2 = LD3L+SD, tr.3 = LD4L+SD, tr.4=conSD).

(Figure 7, Appendix A:Table 15). The time required to produce each leaf increased minimally from leaf 3 to leaf 4. However, substantially more time was needed to produce leaves 5 and 6.

Significantly longer time was needed for plants grown under LD_{4L}+SD (12 wks.) to produce the last subtending leaf (7^{th} lf) than for those grown under LD_{3L}+SD and conSD (6^{th} lf) 9.5 and 8.2 wks., respectively (Table 3, Appendix A:Tables 7-12).

Rate of leaf unfolding

Plants grown under conLD, LD_{3L}+SD and LD_{4L}+SD had a significantly higher rate of leaf unfolding (calculated from the length differences from leaf emergence to fully expanded divided by the period of time) than those under conSD. Leaf position accounts for significant differences in the rate of leaf unfolding as leaf number increases in a way that has a quadratic asymptote at the 1% level (Figure 8, Appendix A:Table 16).

GROWTH MODEL

Model Selection

Table 4 shows the least square (LS) estimates of the parameters of Gompertz, Logistic, Richards, Morgan-Mercer-Flodin (MMF), and Weibull models for a data sets of the 4^{th} lf of flowered plants grown under LD_{3L}+SD, for both additive and multiplicative error assumptions. For the 3-parameter models, the logistic model had a lower residual variance (σ^2) than the Gompertz model. However, the Richards model had the lowest residual variance. With regard to the stability of the LS estimates, all of the estimates were relatively stable (little variation) for all parameters except that for parameter γ of the MMF model.

T-values for the parameter estimates for each of the five models are presented in Table 5. The *t* value is the ratio of the parameter estimate to its standard error. A high

Table 4. Parameter estimates of growth models, additive and multiplicative errors.

	Three-parameter models				Four-parameter models					
	Gompertz (2.1)		Logistic (2.2)		Richards (2.3)		Morgan-Mercer-Flodin (2.4)		Weibull type (2.5)	
Parameter	Add.	Mult.	Add.	Mult.	Add.	Mult.	Add.	Mult.	Add.	Mult.
α	29.7614	29.9634	29.6206	29.7210	29.4126	29.4166	29.6564	29.8066	29.4179	29.4240
β	1.0190	0.9657	1.7177	1.6179	4.1424	3.6333	13.5798	13.1462	16.5460	16.7323
γ	0.1726	0.1531	0.2218	0.2049	0.4137	0.3805	421.9601	179.8439	0.0171	0.0207
δ	-	-	-	-	4.4762	3.9057	3.0801	2.6847	1.9028	1.8176
σ^2	1.0998	0.0019	0.8629	0.0014	0.6750	0.0010	0.7968	0.0012	0.6970	0.0009

Table 5. Student's t-values, as the ratios of the parameter estimates to their standard errors.

Parameter	Gompertz (2.1)	Logistic (2.2)	Richards (2.3)	MMF (2.4)	Weibull (2.5)
α	234.09	297.97	325.17	245.34	322.22
β	38.90	28.98	5.55	42.46	42.98
γ	29.06	32.64	8.08	2.41	3.77
δ			5.31	16.07	16.68

α - maximum leaf length, γ related to mean RGR, δ describes the shape of curve, β -highly correlated with γ and δ

Table 6. Lack of fit analysis for different models fitted to plants in trt. 1 and trt. 2.

	Gompertz (2.1)	Logistic (2.2)	Richards (2.3)	MMF (2.4)	Weibull (2.5)
RSS	152.87	119.95	93.16	105.97	92.71
F	3.58	1.73	0.22	1.08	0.28
P	< 0.01	< 0.05	NS	NS	NS

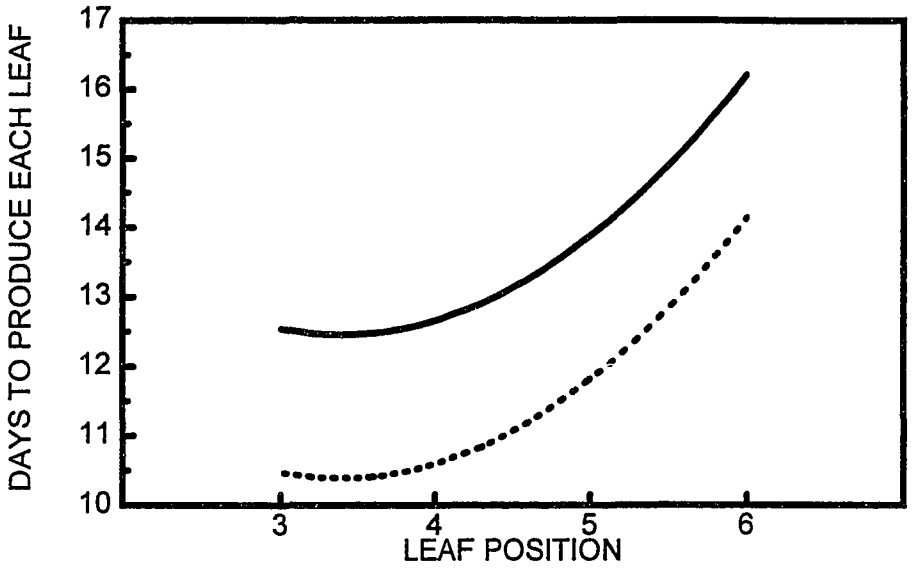

Figure 7. Influence of daylength treatment and leaf position on time frame between successive leaves, starting with the time for the appearence of leaf 3 after the emergence of leaf 2 (tr.1 = conLD, tr.2 = LD3L+SD, tr.3 = LD4L+SD, tr.4=conSD).

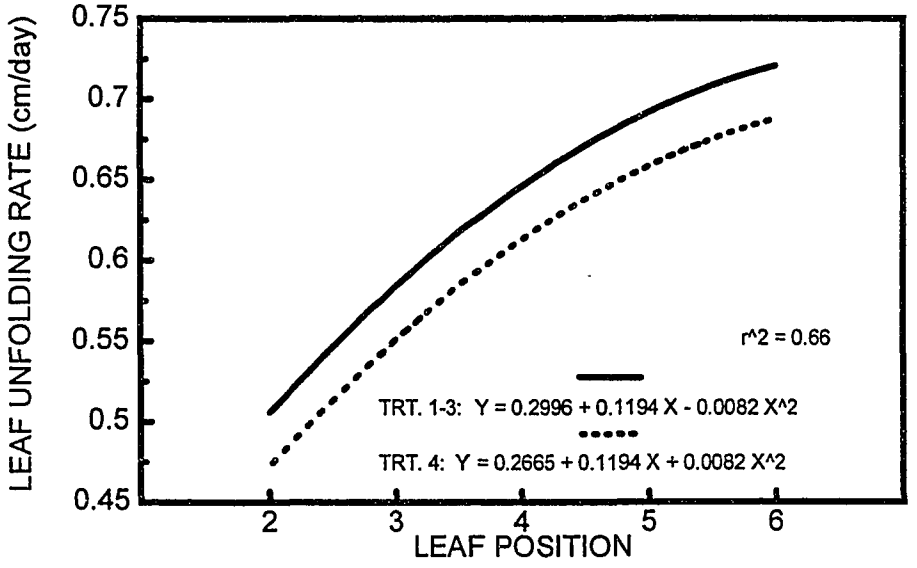

Figure 8. Influence of daylength treatment and leaf position on rate of leaf unfolding from leaf emergence to fully expanded in cm/day of *H. stricta* (tr.1 = conLD, tr.2 = LD3L+SD, tr.3 = LD4L+SD, tr.4=conSD).

t value tends to indicate that the estimate is well determined in the model, a low *t* value tends to indicate that the estimate is poorly determined (Ratkowsky, 1983). These values were relatively high for the estimates of the 3-parameter models. For the Richards model, the t-values associated with the estimates of α and γ were higher than those of MMF and Weibull models. However, the t-values associated with the estimates of β and δ were relatively lower than those of MMF and Weibull models.

No lack of fit for leaf length data was found in the 4-parameter models (Richards, MMF and Weibull). However, there was significant lack of fit in the 3-parameter models (Gompertz and logistic) (Table 6).

Largely because of its application to single leaf growth (Causton and Venus, 1981) and the results of the above selection criteria, the Richards model was selected for fitting the leaf length data.

Comparing Parameters within Each Leaf Position

Leaf length data of plants under different treatments and leaf position (Figure 9) were fitted to the Richards model (Appendix A:Tables 17-36). Parameters β, γ, and δ were all highly correlated (greater than 0.95). The correlation of α with other parameters was smaller and negative. Because of the high correlation among β, γ and δ, together with lack of biological meaning of the first two, only the α and δ would be discussed.

The maximum leaf length (α) of plants grown under conLD, LD_{3L}+SD, LD_{4L}+SD leaf 2 to leaf 4 was longer than those grown under conSD (Table 7). Leaf 5 and leaf 6 of plants grown under LD_{3L}+SD were shorter than those grown under conLD and LD_{4L}+SD but were longer than those under conSD. There was no significant difference for parameter β and δ among treatments within each leaf position (Appendix A:Tables 37-71). This is summarized in Figure 10 as there was no significant different in the shape of the growth curves among treatments within each leaf position, although maximum leaf length was different.

Table 7. Parameter estimates of Richards function on leaf length and time after leaf emergence of different daylength treatments from the 2nd leaf to the 6th leaf.

		Parameter			
Leaf position	Treatment	α	β	γ	δ
2nd	conLD	21.60 a[z]	3.647 a	0.363 a	3.604 a
	LD_{3L}+SD	22.33 a	5.286 a	0.451 ab	4.980 a
	LD_{4L}+SD	22.45 a	3.438 a	0.337 a	3.616 a
	conSD	19.89 b	0.612 a	0.259 b	1.026 a
3rd	conLD	25.12 a	4.740 a	0.414 a	4.708 a
	LD_{3L}+SD	24.83 a	5.242 a	0.457 a	5.181 a
	LD_{4L}+SD	25.56 a	2.931 a	0.309 a	3.035 a
	conSD	23.21 b	2.554 a	0.328 a	2.766 a
4th	conLD	28.64 a	2.238 a	0.297 a	2.369 a
	LD_{3L}+SD	28.92 a	2.928 a	0.308 a	2.998 a
	LD_{4L}+SD	29.76 a	3.176 a	0.336 a	3.271 a
	conSD	27.29 b	3.487 a	0.400 a	3.840 a
5th	conLD	33.86 a	1.917 a	0.234 a	1.887 a
	LD_{3L}+SD	32.67 b	3.261 a	0.279 a	3.232 a
	LD_{4L}+SD	34.20 a	2.950 a	0.269 a	2.856 a
	conSD	31.16 c	3.062 a	0.340 a	3.268 a
6th	conLD	37.45 a	1.745 a	0.206 b	1.932 a
	LD_{3L}+SD	35.81 b	3.610 a	0.309 a	4.075 a
	LD_{4L}+SD	37.16 a	3.046 a	0.261 ab	3.307 a
	conSD	34.14 c	2.150 a	0.216 ab	2.413 a

[z]Parameter estimates separation in columns of each leaf position by F-test at 5% level.
α = maximum leaf length, γ related to mean RGR, δ describes the shape of curve, β highly correlated with γ and δ

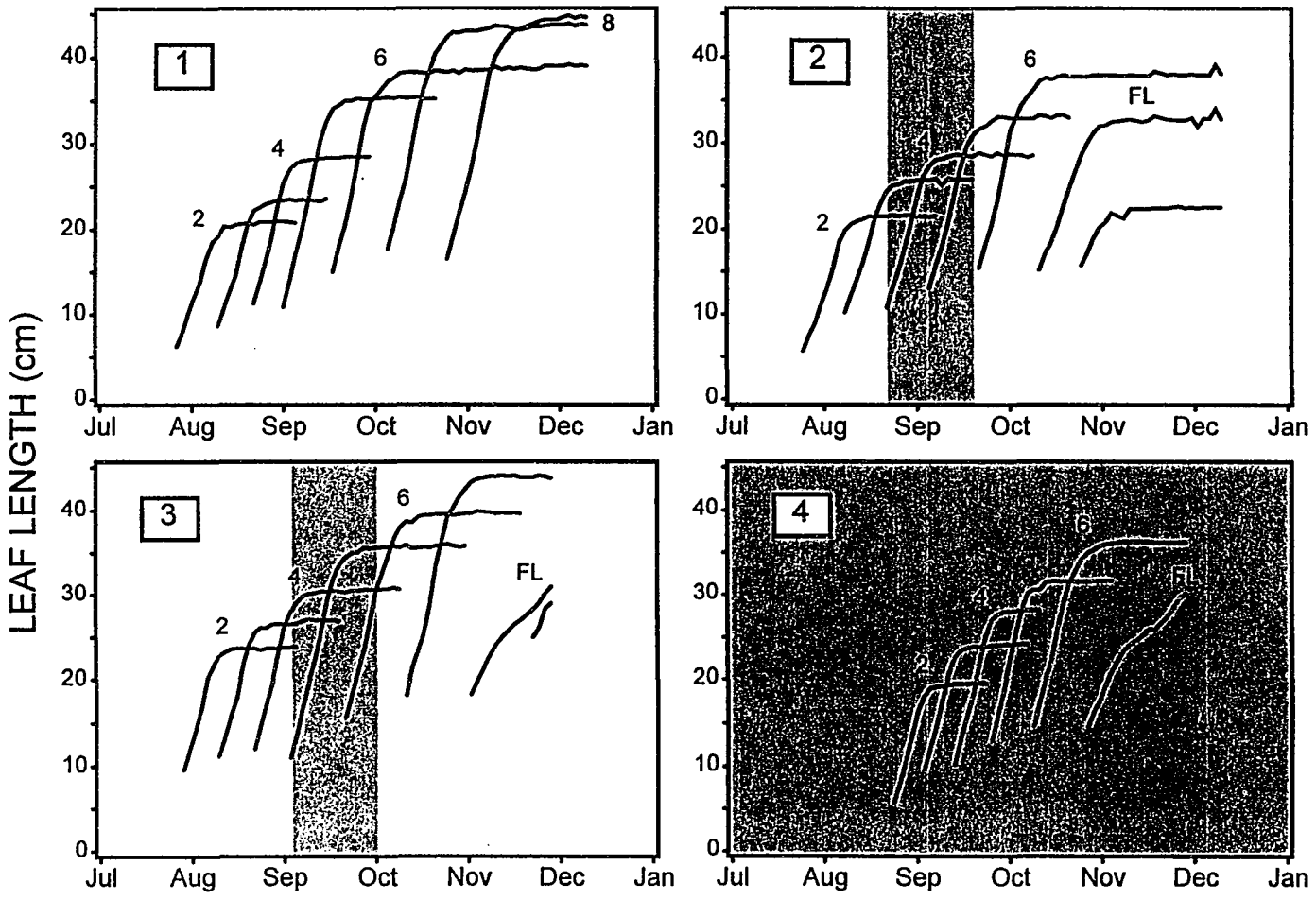

Figure 9. Raw data plot of length of individual leaves (numbered 2 to 6, 7 or 8) in sample plants *H. stricta* grown under different treatment. 1 = A vegetative plant under con-LD, 2 = A flowered plants under LD3L+SD, 3 = A flowered plants under LD4L+SD, and 4 = A flowered plants under conSD. Shaded area represents a period of SDs.

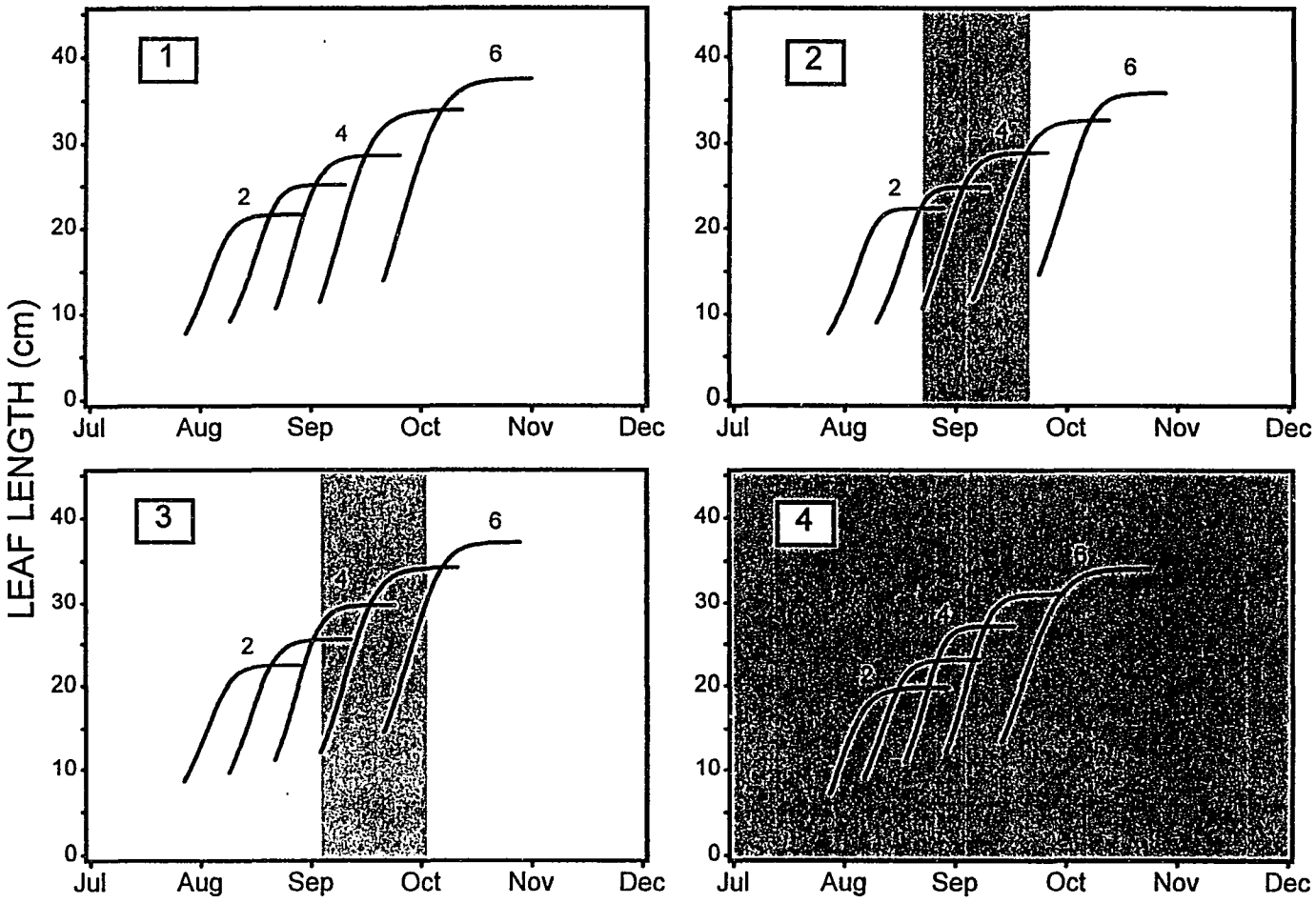

Figure 10. Richards curves fitted to the length of individual leaves (numbered 2 to 6) in *H. stricta* grown under different treatment. 1 = Plants under con-LD, 2 = Plants under LD3L+SD, 3 = Plants under LD4L+SD, and 4 = Plants under conSD. Shaded area represents a period of SDs.

Results of fitting leaf length to Richards model of vegetative pseudostems from conLD and flowered pseudostems from LD_{3L}+SD, LD_{4L}+SD and conSD are shown in Table 8 (Appendix A:Table 72-83). Flowering pseudostems grown under LD_{4L}+SD had significantly longer maximum leaf length (α) than the flowering pseudostems grown under LD_{3L}+SD, the vegetative pseudostems grown under conLD or the flowering pseudostems grown under conSD respectively within each leaf position (lf.4 to lf. 6). There was no significant difference for parameter δ among different treatments (Appendix A:Tables 84-104). However there was a trend in leaf 6 that parameter δ of plants in conSD was less than plants in other treatments resulting in a flatter curve as shown in Figure 11.

Comparing Parameters of Different Leaf Positions

Since there were no significant differences in estimated parameters for growth models among treatments for leaf position 3, 4 and 5, the possibility of fitting a common leaf growth curve for each leaf position was investigated. This was done by transforming leaf length and time to fully expanded to relative length and time. Results of fitting relative length and time are shown in Table 9 (Appendix A:Tables 105-107). Parameter estimates for leaf growth curves for each position (3-5) were significantly different (Appendix A:Tables 108-112). Mean RGR of the 5^{th} leaf, calculated from γ and δ, was greater (6.7) than those in 4^{th} and 3^{rd} leaf (5.1) resulting in a steeper slope for the 5^{th} leaf than the 4^{th} and 3^{rd} leaf (Figure 12).

Common Growth Curve for 3^{rd} to 5^{th} Leaf

Since leaf growth curves of positions 3-5 were significantly different, but were common among treatments (tr.1-tr.3) within each position, common growth curves were fitted leaf for positions 3-5 across tr. 1 to tr. 3 as follows (Figure 13, Table 10, Appendix A:Tables 112-114):

Table 8. Parameter estimates of Richards function on leaf length and time after leaf emergence of different daylength treatments of each pseudostem status (Flowered: LD_{3L}+SD, LD_{4L}+SD and conSD, Vegetative conLD) from the 4th leaf to the 6th leaf.

Leaf position	Treatment	Status	Parameter α	β	γ	δ
4th	conLD	Veg.	28.00 c[z]	3.052 a	0.369 a	3.273 a
	LD_{3L}+SD	Flw.	29.31 b	4.315 a	0.399 a	4.405 a
	LD_{4L}+SD	Flw.	30.21 a	3.900 a	0.398 a	4.175 a
	conSD	Flw.	28.59 c	4.863 a	0.493 a	5.130 a
5th	conLD	Veg.	33.45 b	2.214 a	0.252 b	2.153 a
	LD_{3L}+SD	Flw.	33.05 b	4.061 a	0.332 ab	4.188 a
	LD_{4L}+SD	Flw.	35.00 a	4.033 a	0.345 ab	3.717 a
	conSD	Flw.	32.41 c	5.190 a	0.489 a	5.785 a
6th	conLD	Veg..	37.18 b	1.344 ab	0.194 b	1.596 a
	LD_{3L}+SD	Flw.	37.23 b	3.648 a	0.313 a	4.229 a
	LD_{4L}+SD	Flw.	38.91 a	3.910 a	0.309 a	4.276 a
	conSD	Flw.	34.90 c	0.395 b	0.137 b	0.981 a

[z]Parameter estimates separation in columns of each leaf position by F-test at 5% level.
α = maximum leaf length, γ related to mean RGR, δ describes the shape of curve, β highly correlated with γ and δ

Table 9. Parameter estimates for Richards model on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) of different leaf position from the 3rd leaf to the 5th leaf.

Leaf position	Parameter				mean RGR
	α	β	γ	δ	
3rd	0.9926	2.0293a	9.2333a	0.7891a	5.1608
4th	0.9966	0.0716b	8.0966a	0.2341b	6.5607
5th	0.9989	-0.0542b	8.2884a	0.2308b	6.7341

[z]Parameter estimates separation in columns of each leaf position by F-test at 5% level.
α = maximum leaf length, γ related to mean RGR, δ describes the shape of curve, β highly correlated with γ and δ

Table 10. Parameters estimates of Richards function on leaf length and time after leaf emergence of different leaf position from the 3rd leaf to the 5th leaf.

Leaf position	Parameter				mean RGR
	α	β	γ	δ	
3rd	24.8746	5.9529	0.4803	5.6761	0.0719
4th	29.1951	2.8156	0.2957	2.6222	0.0816
5th	34.2175	2.7999	0.2501	2.5035	0.0714

α = maximum leaf length, γ related to mean RGR, δ describes the shape of curve, β highly correlated with γ and δ

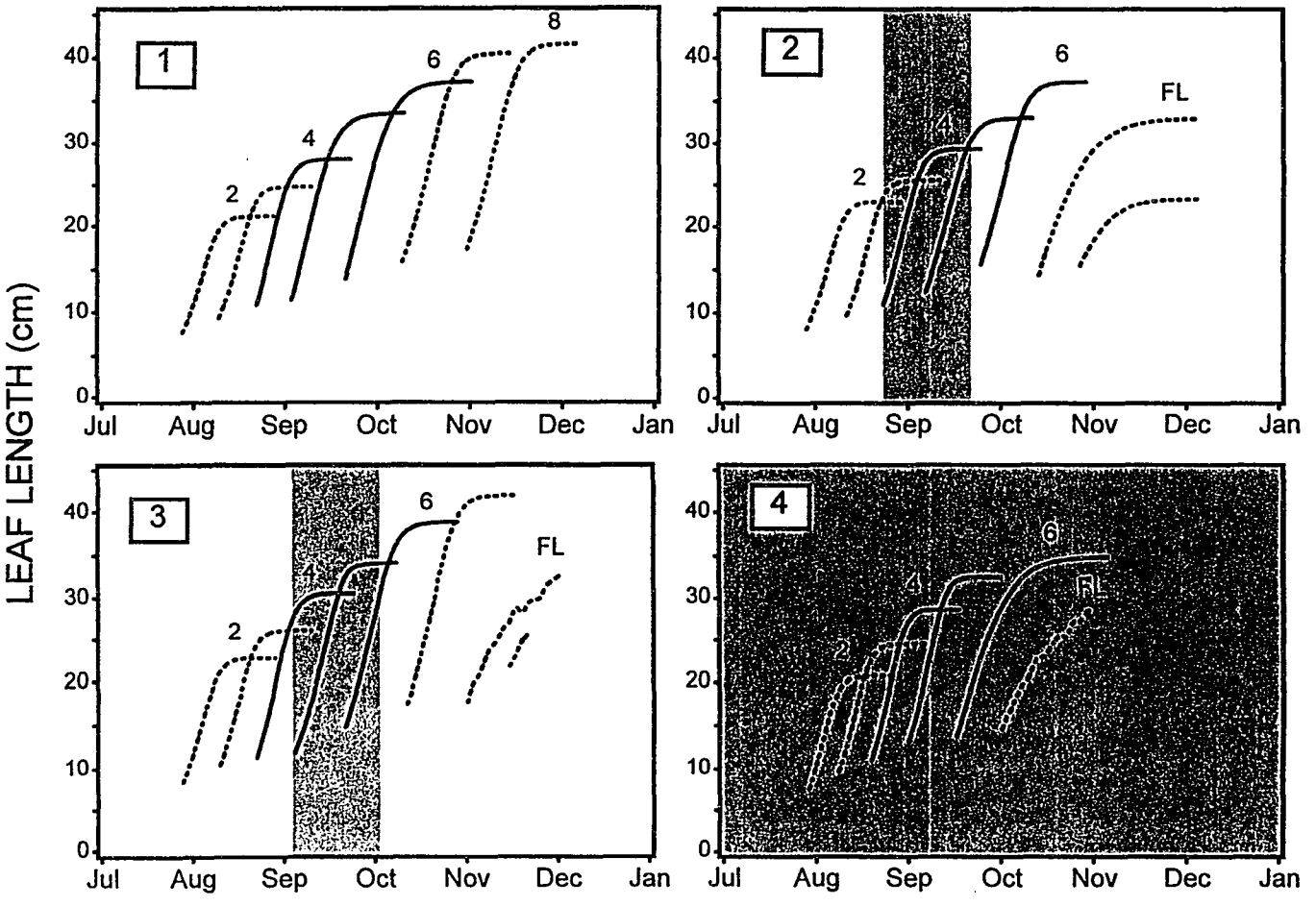

Figure 11. Richards curves fitted to the length of individual leaves (numbered 2 to 6) in *H. stricta* grown under different treatment. 1 = Vegetative plants under con-LD, 2 = Flowered plants under LD3+SD, 3 = Flowered plants under LD4L+SD, and 4 = Flowered plants under conSD. Shaded area represents a period of SDs. Dot lines represents length of leaf 2, 3, 7 and 8 or first and second cincinal bract. This lines were not fitted to Richards curve.

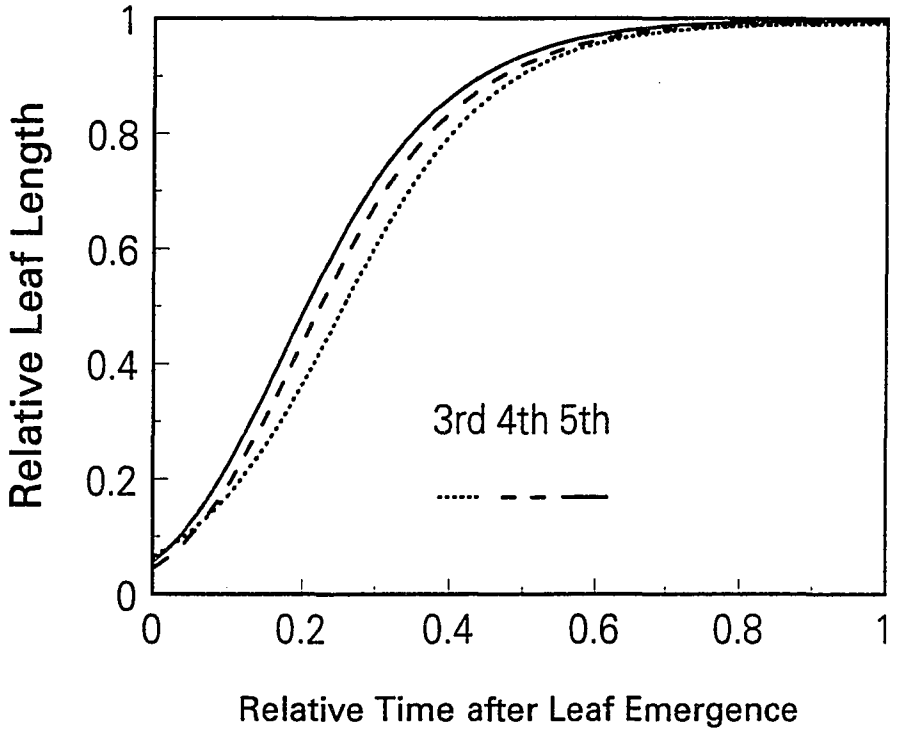

Figure 12. Richards curve fitted to relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) of different leaf position from the 3rd leaf to the 5th leaf.

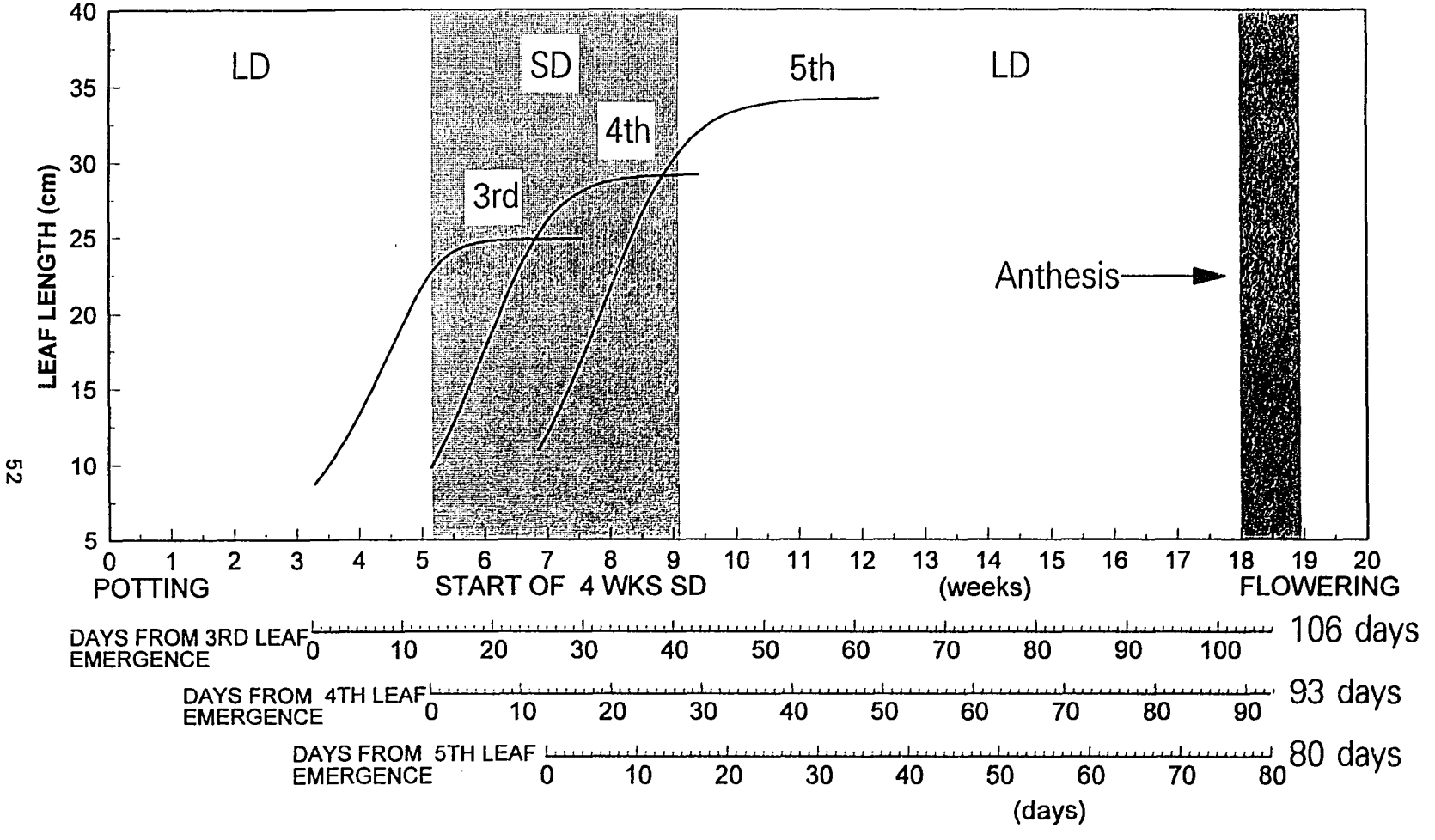

Figure 13. Program for H. srticta 'Dwarf Jamaican' from potting until anthesis under conditions similar to the experiment..

Leaf growth curve for 3rd leaf $$Y = \frac{24.87}{[1+\exp(5.95-0.48X)]^{1/5.67}} \quad (3.3)$$

Leaf growth curve for 4th leaf $$Y = \frac{29.19}{[1+\exp(2.81-0.29X)]^{1/2.62}} \quad (3.4)$$

Leaf growth curve for 5th leaf $$Y = \frac{34.22}{[1+\exp(2.79-0.25X)]^{1/2.5}} \quad (3.5)$$

Y = leaf length (cm.), X = time after leaf emergence (days)

DISCUSSION

FLOWER INDUCTION PERIOD

In a previous study, Lekawatana (1986) found that plants grown under LD had already produced a total of 6 leaves when 2 leaves had been expanded. The apical meristem either developed as an inflorescence if SD were applied as early the 3 leaf stage or produced a 7th leaf before reproductive transformation if SD were applied later in development as in the 4-leaf stage. However, the first cincinnal bract of plants treated with SD at the 3 leaf stage has a small leaf blade attached at the distal end. Plants with 4 expanded leaves were treated with 4 weeks of SD, 12 days later than those having 3 leaves, after completion of the transition period from the 3-leaf stage. The seventh leaf of these plants was in a leaf primodium stage and could be converted to a bract of an inflorescence if SD were applied early enough. However, if SDs were applied while the seventh leaf had started to develop, a leaf-like cincinnal bract was the result.

If SDs were applied while the seventh leaf was more developed as in LD_{4L} + SD, the primodium continued to develop as a full 7th leaf. As the 8th leaf primordia had not been formed at the initiation point of SD treatment of LD_{4L} + SD, the apical meristem then transformed into a reproductive phase to form complete inflorescences. This conclusion was derived from Lekawatana's (1986) studies on the apical meristem of heliconia growing

under LD at different stages of development and from the plot in Figure 7 of time required to produce each leaf. It would take more than 10 days from 7th leaf emergence to 8th leaf emergence assuming that the leaf primordia emergence correlated with the leaf emergence.

Plants grown under continuous SD produced 6 leaves before flowering and produced only one cincinnal bract per inflorescence. Plants grown under LD continued to produce up to 9 leaves and no flowers at the time of harvest. These results implied that plants were susceptible to floral stimulus before reaching the 3-leaf stage since plants grown under SD flowered earlier than those treated with SD at the 3-leaf stage. This also showed that a period of apical competence for floral initiation ranges from before the 3-leaf stage to sometime after the 4-leaf stage. This was in agreement with previous suggestion that pseudostems with 2 to 3 expanded leaves up to 5 leaves were capable of differentiating into inflorescences if exposed to SD (Lekawatana, 1986; Criley and Kawabata, 1986).

FLOWERING

This experiment confirmed that the first generation of *Heliconia stricta* pseudostems grown under LD condition until 3 or 4 fully expanded leaves were evident and then given a floral stimulus (4 weeks of SD) could be induced to flower in 13 weeks after the start of SD.

Flowering percentage of pseudostems grown under con SD (46.1%) was similar to those reported by Lekawatana (1986; 42.5%) under similar condition. Heliconia grown from a single rhizome piece have little food reserve to begin with. Young emerging new shoots of the second generation pseudostem might be a stronger sink than the developing inflorescence of the first generation pseudostem. Therefore, plants grown from a single rhizome piece had lower flowering percentage of pseudostems than those of well established plants.

Plants grown under conSD initiated flowers as soon as their apical meristems were competent for initiation, while those under $LD_{3L}+SD$ and $LD_{4L}+SD$ were still vegetative. However, inflorescence development of plants grown under SD was inferior than those grown under $LD_{3L}+SD$ and $LD_{4L}+SD$.

Plants treated with SD at 3 leaf stage had higher percentage of flowering than those treated at 4 leaf stage. Due to the small sample size, this results was inconclusive despite the significant differences.

The plants and inflorescences of plants grown under $LD_{3L}+SD$ and $LD_{4L}+SD$ produced more bracts (2) than those grown under continuous SD (1). However, the number of cincinnal bracts (1 or 2 in this experiment) was fewer than those of well established plants (2 or 3: Lekawatana, 1986; Criley and Kawabata, 1986). This might be explained as follows:

a) Plants under conSD had smaller and shorter leaves than those under $LD_{3L}+SD$ and $LD_{4L}+SD$. Therefore, plants under conSD had less leaf area and, presumably, less assimilates.

b) Under limited assimilates, the young flower bud constitutes a weaker sink compared with the vegetative apices, developing leaves and it competes poorly with them for the available assimilates (Halevy, 1984). After heliconia plants were given flower initial stimulus, 3 or 4 cincinnal bracts may be produced within the inflorescence, but not all cincinnal bracts will be fully developed due to limited assimilates.

c) Plants materials in this experiment were first generation of pseudostems planted from single rhizome pieces while those in previous experiments (Lekawatana, 1986; Criley and Kawabata, 1986) were from plants that were well established in pots. Therefore, well established plants might have more food reserves in the

rhizomes to support inflorescence development than those recently planted as a single rhizome piece.

PLANT GROWTH

Plants grown under conSD produced significantly shorter and smaller leaves compared to those grown under conLD, $LD_{3L}+SD$, and $LD_{4L}+SD$. This is in agreement with other results which showed that plants grown under LD are usually taller with longer internodes and larger leaves, which are often lighter green in color than those grown under SD (Whatley and Whatley, 1980; Vince-Prue and Tucker, 1983).

Number of leaves produced after SD for plants grown under $LD_{3L}+SD$, and $LD_{4L}+SD$ is constant at 3 leaves. This reflects the number of leaves that are already produced by the plants but have not fully expanded yet. The conSD pseudostems must have had 6 leaves/leaf primodia when flower initiation occured.

Plant grown under conLD remained vegetative and could produced up to 8 to 9 leaves. However, leaf emergence interval was longer as more leaf were produced (Figure 9).

RICHARDS MODEL

The Richards model for leaf length estimated the parameter α well. There were no differences for growth parameters across treatments within each leaf position of 3^{rd}, 4^{th} and 5^{th} except for the asymptote (α: maximum leaf length). However, these 3 leaf positions have different growth curves as the 5^{th} leaf has a steeper curve than do leaves 4 and 3 (Figure 12). It means that the 5^{th} leaf has the fastest development rate (cm/day) as shown by the leaf unfolding rate that had quadratic response to leaf position and mean relative growth rate.

HELICONIA STRICTA 'DWARF JAMAICAN' FLOWERING PROGRAM

From the above findings, parameters of the Richards models for 3rd, 4th, and 5th leaf were common among treatments with the exception for α (maximum leaf length); plants grown under continuous SD had the shortest leaf length. However parameters of Richards model for each leaf position were different. Therefore, general leaf growth models for H. stricta 'Dwarf Jamaican' fitted for each leaf position (3, 4 and 5) are presented in Table 10 (eq. 3.3, 3.4, 3.5). This general leaf growth model can serve as a reference for heliconia grown under similar conditions of temperature and light. If leaves 3, 4 and 5 of heliconia plants are not fully expanded, one can calculate leaf age after emergence (3.6) and estimate how many days remain before anthesis time by referring to Figure 13.

$$\text{Time after leaf emergence (X)} = \frac{\beta - \ln\left[\left(\frac{\alpha}{Y}\right)^{\delta} - 1\right]}{\gamma} \quad \text{days} \qquad (3.6)$$

Y = length from soil line to the top of leaf blade in cm.

or

$$\text{X for } 3^{rd} \text{ leaf} = \frac{5.95 - \ln\left[\left(\frac{24.87}{Y}\right)^{5.67} - 1\right]}{0.48} \quad \text{days} \qquad (3.8)$$

$$\text{X for } 4^{th} \text{ leaf} = \frac{2.81 - \ln\left[\left(\frac{29.19}{Y}\right)^{2.62} - 1\right]}{0.29} \quad \text{days} \qquad (3.9)$$

$$\text{X for } 5^{th} \text{ leaf} = \frac{2.79 - \ln\left[\left(\frac{34.21}{Y}\right)^{2.5} - 1\right]}{0.25} \quad \text{days} \qquad (3.10)$$

A time table for raising *H. stricta* 'Dwarf Jamaican' is proposed (Figure 13) from potting to anthesis under the condition of this experiment. Prior to that, 4 weeks are needed for propagation from a single stem rhizome piece. However, plants grown under continuous SD will have shorter leaves and flower 5 weeks earlier.

CHAPTER 4

EFFECT OF TEMPERATURE ON INFLORESCENCE DEVELOPMENT AND ABSCISIC ACID LEVELS IN *H. STRICTA*

ABSTRACT

Plants of *Heliconia stricta* Huber 'Dwarf Jamaican' were treated with four temperatures (18°C, 21°C, 24°C and 28°C) under an 14 hr. daylength after an initial floral induction stimulus of 4 weeks of SD at 21°C. Free (+)-abscisic acid (ABA) content of mature leaves was measured by indirect enzyme-linked immunosorbent assay (ELISA). Increased night temperature decreased percent flowering (from 55% to 31%) and increased the percent aborted pseudostems from 0% to 19.2%. However, temperature during this period had no influence on percent reproductive pseudostems (flowering + aborted). Lower temperatures during inflorescence development increased levels of ABA in *H. stricta* 'Dwarf Jamaican' leaves from 264.6 ±18.8 ng/g dry wt. at 28°C to 441.0 ± 42.3 ng/g dry wt. at 18°C. A lower level of ABA was found in leaves of aborted pseudostems (285.5 ± 55.7 ng/g dry wt.) compared to that found in leaves of inflorescence developing pseudostems (386.9 ± 37.3 ng/g dry wt.). The smallest developing inflorescence that was found to be aborted was 2 cm long and was found 6 weeks after start of SD.

INTRODUCTION

Flower bud abortion in Heliconia has been found in many species such as *H. angusta*, *H. chartacea* and *H. stricta* (Lekawatana, 1986; Criley and Lekawatana, 1994; Kwon, 1992). Lekawatana (1986) reported that pseudostems grown under different night temperatures (18-28°C) at the time of flower initiation (4 weeks of short days) showed no difference in percent flower bud abortion. If one could induce these plants to flower year

round, reducing the percent of flower bud abortion would be beneficial to the cut flower industry.

Roles of ABA in promoting or inhibiting flower abortion have not been fully understood. Trewavas and Jones (1991) stated that exogenous application of ABA increased flower abscission which was similar to the effect of water stress. Furthermore, ABA was found to inhibit flower initiation in several short day plants and long-day plants grown under inductive conditions (Bernier, 1988). Increased ABA levels paralleled the increase of ethylene which was responsible to the onset of irreversible wilting in carnation petals (Hanley and Bramlage, 1989).

Several environmental factors affect the endogenous level of abscisic acid (ABA) in plant tissues. Most prominent is the effect of drought stress, but other kinds of stress such as aeration stress and temperature extremes have been reported to change the level of ABA (Addicott, 1983). Exposure to low temperature causes a rise in the level of ABA of some plants (Capell and Dörffling, 1989).

The common methods of quantification of ABA are by high-pressure liquid chromatography with UV detection (HPLC-UV), gas chromatography with flame ionization detection (GC-FID) or gas chromatography with electron capture detection (GC-ECD), all of which require rigorous cleanup procedures to reduce contaminants. Immunoassay provides a technique to use a selective antibody (Ab) to identify and quantify the low physiological concentrations of ABA in unpurified plant extracts (Weiler, 1979).

Mertens *et al.* (1983) developed a specific monoclonal antibody (MAb) directed at free ABA. This MAb is now available commercially (Idetek, 1985). Norman et al. (1988) developed an indirect ELISA procedure which requires less commercial MAb than the direct procedure the company suggested. This provided a rapid, sensitive, and efficient technique for ABA quantification.

high temperature was also reported to promote flower abortion (Kinet et al., 1985). This experiment was carried out to investigate the effect of temperature during flower bud development on percent abortion and ABA content in *Heliconia stricta* 'Dwarf Jamaican'. An indirect ELISA procedure for free ABA was adapted for analyzing ABA content in Heliconia leaves and apices. This procedure was adapted from those used by USDA (Hawaii) researchers for analyzing ABA content in sugarcane leaves, which in turn, were based on Norman et al. (1988) and Walker-Simmons (1987). Assay sensitivity, precision and specificity were verified.

MATERIALS AND METHODS FOR INDIRECT ELISA PROCEDURE

PLANT MATERIAL

Heliconia stricta 'Dwarf Jamaican' plants were grown in 15-cm pots in a greenhouse at the Magoon greenhouse facility of the University of Hawaii. The average minimum and maximum temperatures during the growing period (December 1989-January 1990) were 20.4°C (range: 15.5-22.5°C) and 32.8°C (range: 22.5-36°C), respectively

Apex tissues (1 cm. in length) used in this experiment was selected from plants with 3-4 leaves. Twenty pieces of apex tissue were harvested and immediately frozen in liquid N_2, then powdered and lyophilized. Three apical tissues per sampling time were analyzed through ELISA, and the resulting ABA concentrations were calculated.

Leaves used in this experiment were selected from the top mature leaves of plants with 3-4 expanded leaves. Ten leaves were harvested. Leaf blades were stripped from their midribs. Each sample was placed in a plastic tube and immediately stored in liquid nitrogen (-70 °C). Leaves were then powdered by grinding in a precooled mortar and pestle with liquid nitrogen then lyophilized. The lyophilized samples were then stored in plastic

tubes at -20 °C. Three leaf tissues per sampling time were analyzed through ELISA, and the ABA concentrations were calculated.

ABA EXTRACTION

Leaf Samples

Heliconia leaf samples (0.5 g dry wt.) were ground in 10 ml of 80% acetone with a Tissumizer (Tekmar). The extract was suction-filtered through Whatman No. 1 filter paper. The residue was extracted twice more with 10 ml of 80% acetone. The volume of the combined supernatant was brought up to 25 ml with 80% acetone.

Then 0.5 ml of the supernatant was placed in 3 ml glass tube and dried in a Speed Vac Concentrator (Savant). The dry extract was resuspended to 0.01 g dry weight tissue/ml with TBST (Tris-buffered saline with Tween 20). Norman et. al (1998) reported that the use of 0.2-µm Lid/x filter improved ELISA values compared to no filter or other filter procedures (silica Sep Paks, centrifugation and other filters). Therefore, aliquots of the extract were passed through a 0.2-μm Lid/x nylon 66 syringe filter (Genex Corp.) twice. This extract solution was then ready to be tested by ELISA.

Apex Tissue

Since the apex tissue samples were much smaller than the leaf samples, only 0.1 g. dry wt. was used. Heliconia apex tissue samples (0.1 g dry wt.) were ground in 2 ml of 80% acetone with a glass tube and a pestle. The extract was centrifuged to separate the supernatant. The residue was extracted twice more with 2 ml of 80% acetone. The volume of the combined supernatant was brought up to 5 ml with 80% acetone. Subsequent procedures were identical to the method described for leaf samples.

ELISA MATERIALS

Buffer

TBST (Tris-buffered saline with Tween 20): 6 g of Tris [tris(hydroxymethyl)-aminomethane], 0.2 g of $MgCl_2$, 8.8 g of NaCl, 0.5 ml of Tween 20, and 0.1 g of sodium azide, pH 7.5.

Bicarb: 50 mM $NaHCO_3$ with sodium azide, 0.1 g/L, pH 9.6.

Standards

A stock solution 0.01 g (+)*cis-trans* ABA (Sigma Chemical Co.) was prepared in 100 ml of MeOH. This was further diluted with TBST to 10, 20, 40, 80, 160, 300 and 5000 pg/100 μL.

ABA-4'-TH-BSA conjugate

(±)-ABA-4'-tyrosyl-hydrazone and *p*-aminohippuric acid substituted BSA were prepared and coupled to form an ABA-4'-TH-BSA conjugate according to Weiler (1980; conjugate solution was obtained from K. Pitz, USDA). The solution contained about 1.4 mg of conjugate/ml and was stored in 0.5 ml aliquots at -20°C. A 0.5 ml aliquot was diluted to 20 μg/ml with bicarb buffer for coating microtitration plates.

Monoclonal Antibody

Two mg of MAb to free *cis-,trans*-(+)-ABA (Idetek, Inc.) were dissolved in 2.0 ml of TBST. This stock solution was diluted to 0.8 μg/ml with TBST just before use.

MAb stored at -20°C for several years showed no obvious deterioration. However, storage at 4°C for only a few days resulted in some reduction of activity, and freezing and thawing caused measurable loss in activity. Dilute Ab preparations lose significant amounts

of activity by adsorption onto plastic surfaces; thus, polypropylene tubes are preferred to polystyrene (Zola, 1987).

Antimouse Alkaline Phosphatase Conjugate

Just prior to use, 0.85 g of PEG 8000 was added to 21 ml of TBST buffer and then 27 µl of rabbit antimouse alkaline phosphatase (RaMAP) conjugate (Sigma Chemical Co.) was added. The enzyme activity doubles between 25 °C and 37 °C (Kemeny, 1991).

Substrate

Five tablets of p-nitrophenyl phosphate (p-NPP; 5 mg/tablet plus filler) were dissolved in 25 ml of bicarb buffer previously warmed to 37 °C. Hydrolysis of the p-NPP occurs at temperatures above 30 °C (Kemeny, 1991).

Microtitration Plates

Immulon 2 flat bottom, polypropylene 96-well microtitration plates (Dynatech Laboratories, Inc.) were utilized. The outer wells of the plate were not used.

ELISA PROCEDURE (FIGURE 14)

Coating of Wells with ABA-4'TH-BSA Conjugate

A 200 µl aliquot of the conjugate was added to each well of the microtitration plates. Plates were covered with parafilm, wrapped in foil, and incubated at 4°C overnight.

The binding of proteins to plastic depends on time and temperature. For convenience coating is usually done at 4°C overnight. The optimum pH for binding immunoglobulin is pH 9.6 (Kemeny and Chantler, 1988).

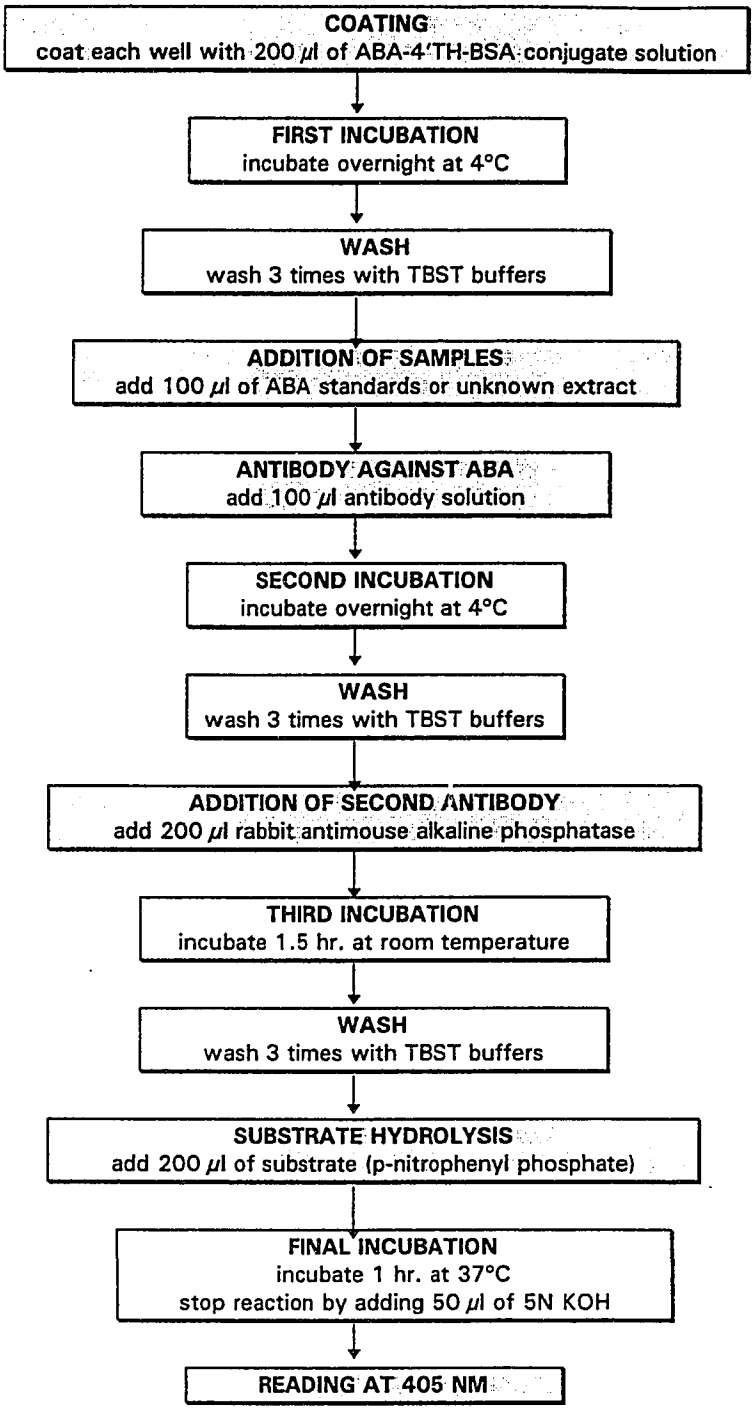

Figure 14. Flow chart of ELISA procedures.

Addition of ABA Samples

Plate wells coated with conjugate were washed three times with TBST buffer. The final washing solution was left in the plates for 10 min. and then discarded. Each well was filled with 100 μl of one of the eight ABA standards, TBST buffer or leaf tissue extract (representing 1 mg of leaf dry weight). All standards, apex and leaf tissue extracts were replicated three times (3 wells). Leaf tissue extract was diluted to 1 (undiluted) and 1/2 dilution. One column contained excess ABA (5,000 pg) for nonspecific binding (NSB). Absorbance reading from this column was used as a correction number. Another column contained TBST buffer only for a maximum absorbance reading (B_0). When absorbance readings from this column (B_0) reached 1 the final incubation was stopped.

Addition of MAb

One hundred μl of diluted MAb were added to each sample or standard. Plates were covered with parafilm, wrapped in foil, and incubated at 4°C overnight.

Since binding of ABA to MAb is pH dependent, and binding decreases at pH 6.0 or below but is not affected in the range of pH 6.0-9.0 (Daie and Wyse, 1982), a pH of 7.5 was used in the assays.

Addition of the Second Antibody

Wells were washed three times with TBST. Two hundred μl of Rabbit antimouse alkaline phosphatase conjugate containing PEG were added to each well. Plates were incubated for 1.5 h at room temperature in the dark.

Measurement of Alkaline Phosphatase

Wells were washed three times with TBST. Two hundred μl of substrate (p-nitrophenyl phosphate) solution were added to each well. Plates were incubated for around

1 h until the absorbance of control sample containing no ABA (B_0) measured at 405 nm was approximately 1.0. The incubation was stopped by adding 50 μl of 5 N KOH, after which the sample absorbance was measured at 405 nm with Microplate Reader Model 450 (Bio-Rad Lab.). Since the method used was indirect ELISA, the absorbance reading of the samples is inversely proportional to the amount of ABA in the original sample.

ELISA DATA PROCESSING

Absorbance readings (optical density: O.D.) for the ABA standards were converted to percent binding (B/ B_0%, 4.1) and then logit transformed (4.2).

$$B/B_0\% = (\text{Standard or Sample O.D.} - \text{NSB O.D.} \times 100) / (B_0 \text{ O.D.} - \text{NSB O.D.}) \quad (4.1)$$

$$\text{Logit.}B / B_0 = \text{Ln}\left(\frac{B / B_0\%}{100 - B / B_0\%}\right) \quad (4.2)$$

NSB = Non Specific Binding

B_0 = Maximum absorbance reading

B = Absorbance reading for ABA standard series

Standard curves were linearized by plotting logit-log transformed data against the Ln of ABA added (Vernieri et al., 1989a; Parata et al., 1990). All sample absorbance readings were converted to B/B_0 % , and logit transformed; ABA concentration was extrapolated from the linear regression line of the ABA standard curve (Hanley and Bramlage, 1989). There were 3 replicates of each sample.

DETERMINING CONJUGATE CONCENTRATION

Three concentrations of ABA-4'-TH-BSA conjugate (5, 10 and 20 µg/ml) were used to determine the optimal concentration range to be used in the routine ELISA. The concentrations of MAb and second Ab were held constant at 0.8 μg/ml MAb and 1.29 μl/ml second Ab. The goal was to obtain an optical reading of 1.0 of B_0 (blanks containing only TBST) within 1 hour of the final incubation. Two microtitration plates were used for each

concentration and followed the above ELISA procedure. Only the ABA standards and TBST blank were used with 6 wells of each per plate.

DETERMINING REPRODUCIBILITY OF THE ELISA OUTPUT

Eight microtitration plates were processed through the ELISA procedure using 20 μg /ml ABA-4'-TH-BSA conjugate (coating), 0.8 MAb μg/ml Mab, 6 levels of ABA standard. and 1.29 μl/ml second Ab. The absorbance readings among plates were analyzed to determine whether the readings were significantly different among plates.

SPECIFICITY TEST

A test for specificity in immunoassays is to test for parallelism of a plant extract dilution curve with the standard curve (Daie and Wyse, 1982). Any interfering substances in the leaf extract should change the slope of the curve (Pengelly, 1986). Therefore heliconia leaf extract, diluted to 5 levels of concentration (1, 1/2, 1/4, 1/8, and 1/16), was added to a microtitration plate as unknowns in triplicate, and ABA was determined through ELISA. The linear regression line of the logit B/B_0 on log of leaf extract dilution was analyzed. Parallelism was then evaluated by comparing the slope of the leaf extract dilution to the standard slope of the regression equations.

PERCENT RECOVERY

One hundred ng/g dry wt. of *cis-,trans-*(+)-ABA (Sigma Chemical Co.) were added as an internal standard to each of 5 dry leaf samples (0.5 g dry wt). These samples, together with those without added ABA, were analyzed through routine ELISA. The percent recovery was calculated (4.3) to confirm the specificity and accuracy of the assay (Daie and Wyse, 1982).

$$\% \text{ recovery} = \frac{SA - S0}{A} \times 100 \qquad (4.3)$$

A = Amount of ABA added to sample

SA = ABA conc. from sample with added ABA

S0 = ABA conc. from sample without ABA added

MATERIALS AND METHODS FOR THE EXPERIMENT

PLANT MATERIALS

Three hundred rhizome pieces of *Heliconia stricta* 'Dwarf Jamaican' were propagated on September 20, 1989. Rhizome pieces including pseudostems were separated and the roots removed. Pseudostems were cut to 5 cm in length from the leaf sheath base, treated in a 55°C water bath for 5 minutes, dipped in fungicide (Dithane M-45) and drained. The rhizomes were then held in plastic bags for 3 weeks at 20°C to stimulate root and shoot growth. They were then rooted in metal trays containing perlite and vermiculite 1:1 ratio (v/v) in a growth chamber at the Pope laboratory of the University of Hawaii. The environmental conditions were: photoperiod: 14 hours (long day: LD) using a combination of fluorescent and incandescent lamps, 214 $\mu mol.m^{-2}.s^{-1}$; temperature 25/20° C Day/Night (D/N). On November 2, 1989 rooted rhizome pieces were potted into 15-cm pots with 3 plants/pot for a total of 100 pots. The potting medium was a mixture of peat and perlite 1:1 ratio (v/v) amended with dolomite, Micromax and treble superphosphate at the rates of 6.0, 1.0 and 0.6 kg per cubic meter, respectively. Plants were continued in growth chambers under the same condition and were hand-watered daily with nutrient solution 200N-200P-200K (ppm) throughout the experiment. When approximately 50% of plants had reached the 3 to 4 expanded leaf stage (January 16, 1990), they were subjected to short day (8 hour daylength, SD) at 25/20 °C D/N temperature for 4 weeks to provide the flower initiation stimulus to these plants (Criley and Kawabata, 1986). Throughout the experiment plants were provided with adequate water, therefore the effect of water stress was minimized.

TREATMENT SETUP

After 4 weeks in SD (February 18, 1990) heliconia plants were placed in 4 growth chambers with different day and night temperatures (D/N) as follows:

Trt. 1. 20/15 °C

Trt. 2. 23/18 °C

Trt. 3. 26/21 °C

Trt. 4. 30/25 °C

$$\text{Average daily temperature} = \frac{(14 \times DT) + (10 \times NT)}{24} \qquad (4.4)$$

DT = day temperature, NT = night temperature

Only the average daily temperature (calculated by eq. 5.1), 18°C, 21°C, 24°C and 28°C, will be used throughout this chapter to represent treatments 1, 2, 3, and 4, respectively. The photoperiod was 14 hours (long day: LD) using combination of fluorescent and incandescent lamps, 214 $\mu mol.m^{-2}.s^{-1}$. Treatments were terminated on May 31, 1990 at 19 weeks after the start of SD.

DATA COLLECTION

At the beginning of SD the expanded leaf number of all pseudostems (250 pseudostems) was determined. During the SD induction period (January 16 - February 17, 1990) 6 plants (2 pots) were removed each week for sample collection. From February 18 to April 6, 1990, 6 plants (two pots) were removed from each chamber every 7 days for sample collection (except weeks 5 and 9 after the start of SD).

Leaf blades of the topmost mature leaves of each shoot were selected for ABA analysis (180 samples). Tissue around the apical meristematic region (~1.5 cm long) of each shoot was fixed with FAA for shoot status determination (255 samples). The number of expanded leaves when collected was recorded for each sample.

At the end of the experiment (May 31, 1990) the growing status of the remaining plants (total of 43 pseudostems with approximately 9 plants or 3 pots per treatment) was determined (vegetative, elongated, flowering or aborted).

SHOOT STATUS DETERMINATION

Two hundred and fifty-five samples of shoot apical meristems were collected to determine their developmental status through microscopy of thin longitudinal sections. Tissues to be examined were fixed in FAA solution (formalin-aceto-alcohol) and dehydrated in a graded series of ethyl alcohol-tertiary butyl alcohol (TBA) solutions (Johansen 1940). Infiltration with Parowax and embedding in Paraplast followed a standard paraffin embedding technique (Johansen 1940). Longitudinal sections were made on a rotary microtome at 20 micrometer thickness. Tissues were then stained with 0.05% toluidine blue O (Sakai, 1973). The status of each meristem was determined (vegetative, elongate, flower, or aborted) (Lekawatana, 1986).

STATISTICAL ANALYSIS

The statistical analysis was by SAS general linear model (PROC GLM) analysis of variance with mean separation by t-test or contrast (SAS Institute, 1987). Quantitative data such as meristem and shoot status were analyzed by Chi-Square test for independence with the null hypothesis that the differences among the ratios in each temperature treatment were not significant.

RESULTS FOR THE ELISA PROCEDURE

ASSAY SENSITIVITY AND PRECISION

Standard curves for ELISA were obtained by plotting absorbance at 405 nm versus the log of ABA concentration in the assay. The lower detection limit was 10 pg/100 μl and the assay concentrations ranged from 10-300 pg/100 μl. To improve the sensitivity of

standard curves, coating concentration, MAb dilution, second Ab dilution and the duration of the incubations could be adjusted. With MAb and second Ab at constant levels (0.8 μg/ml MAb and 1.29 μl/ml second Ab), an increase in coating concentration (ABA-4'-TH-BSA conjugate; 5, 10, and 20 μg/ml) decreased time for B_0 (blanks) absorbance reading to reach 1.0. Curves of similar shape were obtained either at 10 μg/ml with a 108 min. incubation, or 20 μg/ml with a 60 min. incubation (Figure 15). The latter were used as a standard concentration for ABA-4'-TH-BSA conjugate.

Figure 16 demonstrated the high day-to-day reproducibility of the assay using identical dilutions of antibody, conjugate, and ABA solutions (8 consecutive assays (Appendix A:Table 115). There was no significant difference among assay means and slopes at the 5% level. The coefficient of variation was -13.5% and n=8 (Appendix A:Table 116).

SPECIFICITY

Leaf Samples

When *Heliconia* leaf extract was used in a serial dilution (1, 1/2, 1/4, 1/8, and 1/16), the curve (Figure 17, Appendix A:Table 118) was parallel to the standard curve (Figure 20, Appendix A:Table 117) with both slopes = -2.57 (Figure 20). This confirmed the absence of interference.

When known amounts of ABA (100 ng/g dry wt.) were added to leaf extracts as internal standards, the recovery was 92.1 ± 2.3 %. This confirmed the specificity and accuracy of the assay (Daie and Wyse, 1982).

Shoot apex samples

When *Heliconia* apex extract was used in a serial dilution (1, 1/2, 1/4 and 1/8), the curve (Figure 18, Appendix A:Table 119) was not parallel to the standard curve (Figure 21,

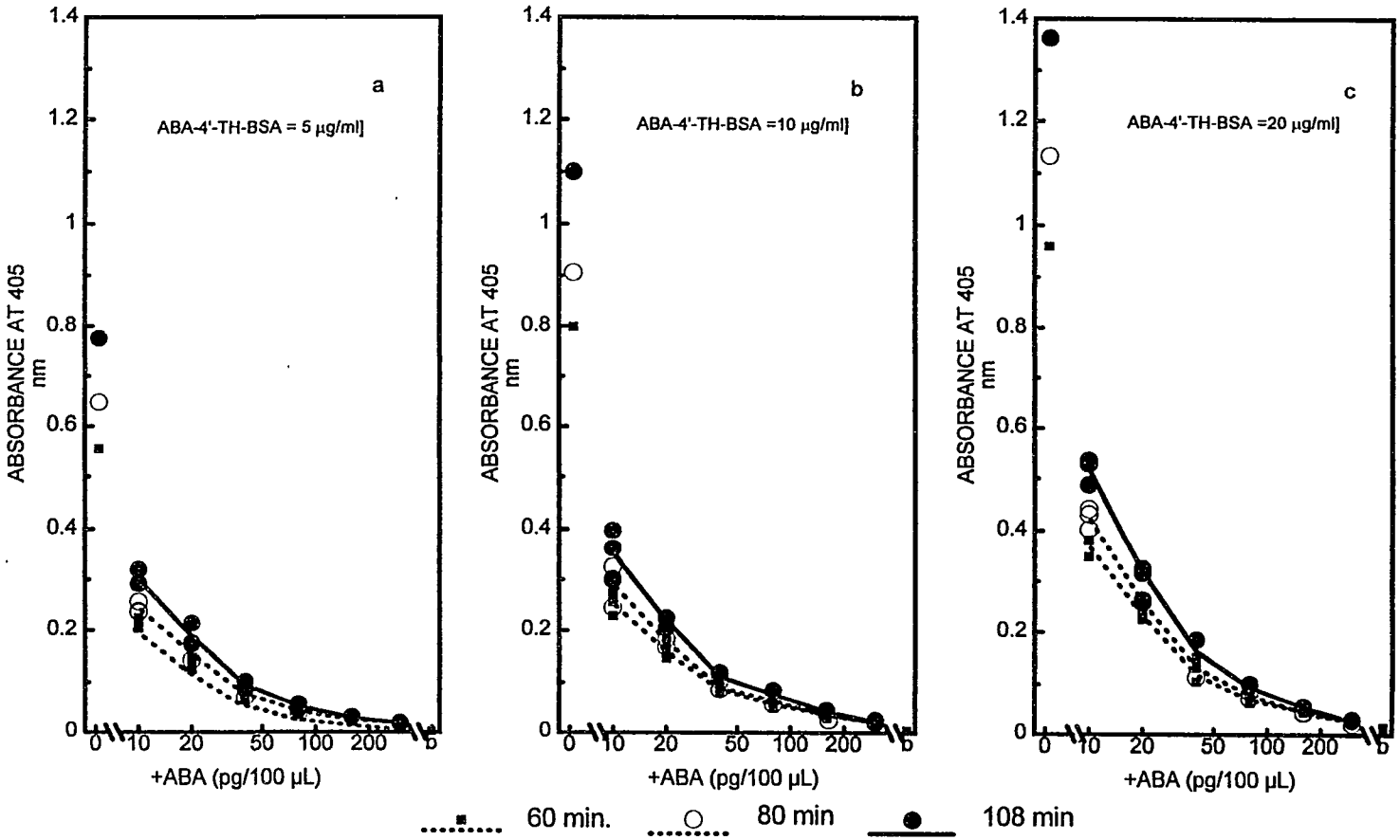

Figure 15. The effect of varying the coating concentration of the ELISA standard curve for free +ABA. Microtitration plates coated with ABA-4'-TH-BSA conjugate at: a) 5 μg/ml; b) 10 μg/ml; c) 20 μg/ml. After development the absorbance at 405 nm was read after 60 min, 80 min, and 108 min.

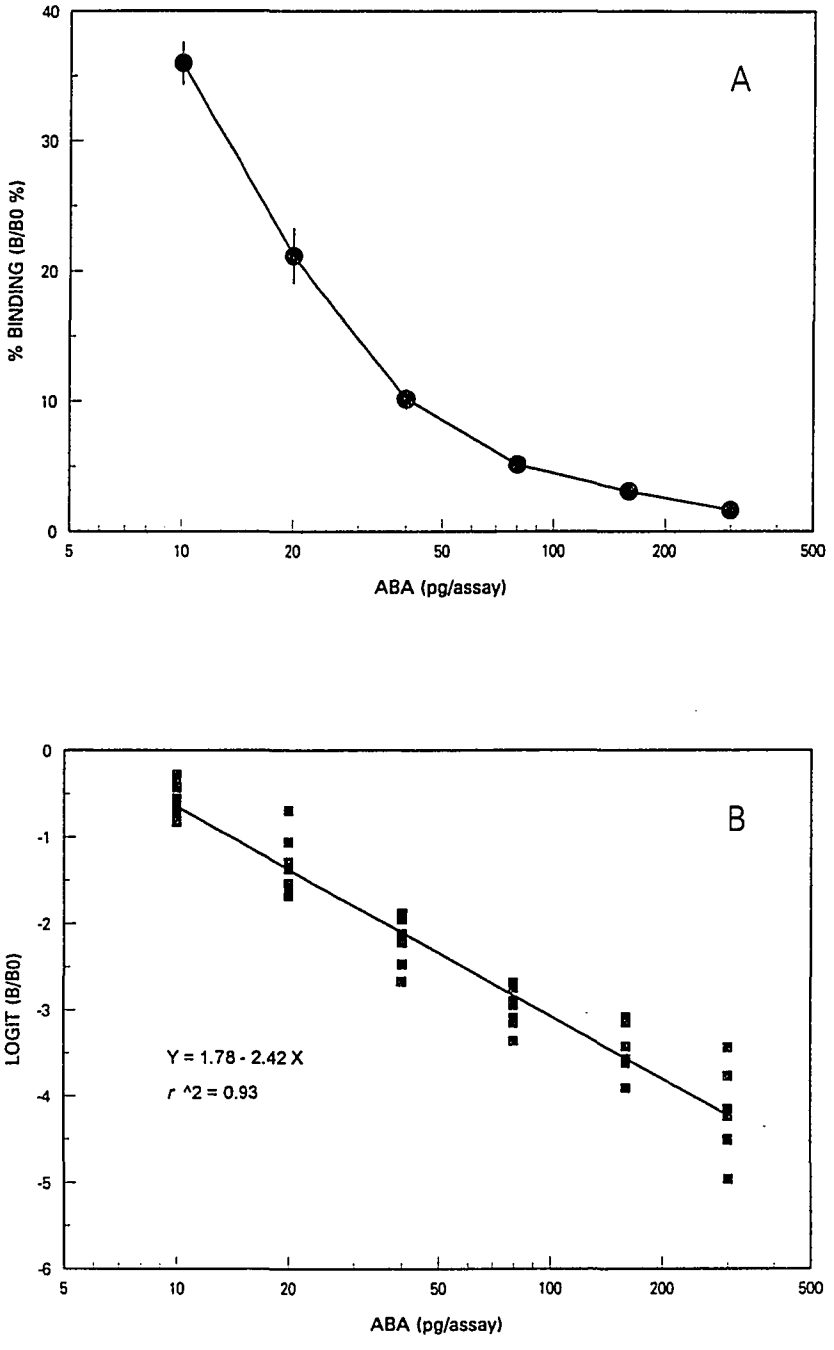

Figure 16. Standard curve for ELISA of free ABA displaying: a) average percent binding and ABA concentration and b) LOGIT and ABA concentration both were constructed from n = 8 consecutive assays to show day-to-day reproducibility. C.V. = -13.52

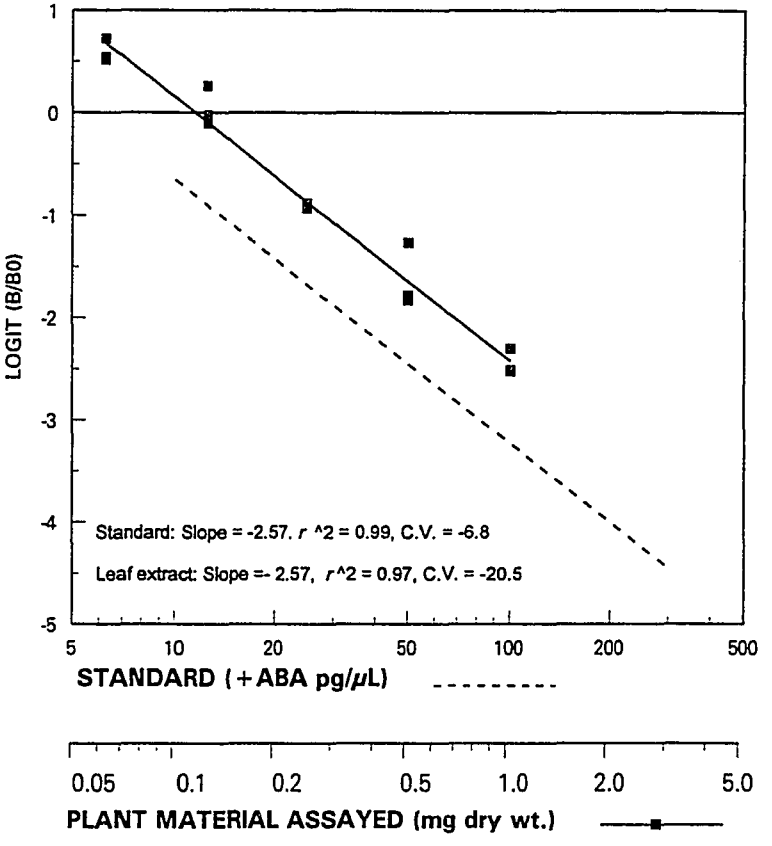

Figure 17. Parallelism of *Heliconia stricta* leaf extract dilution curves and ABA standard curves as determined by ELISA. X axes are log expression.

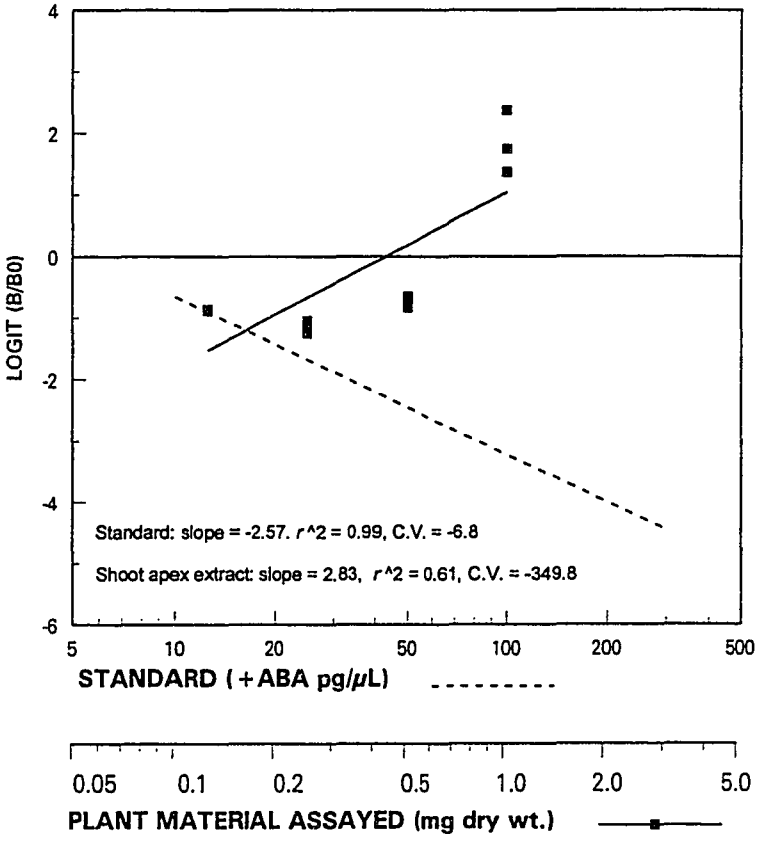

Figure 18. Parallelism of *Heliconia stricta* shoot apex extract dilution curves and ABA standard curves as determined by ELISA. X axes are log expression.

Appendix A:Table 119) with leaf extract slopes = 2.836 and standard curve = -2.574 (Figure 18). This indicated interference. More attempts were made but without improvement of the results. With this high interference, it was decided to drop the ELISA for shoot apex from the rest of the experiments.

QUANTIFICATION OF ABA IN HELICONIA LEAF TISSUE

ABA levels in mature *Heliconia* leaves from 10 plants grown in greenhouse condition, using an indirect ELISA ranged from 91.44 to 372.15 ng/g dry wt. with a mean of 219.7 ± 22.5 ng/g dry wt.

The assay reported was reliable and reproducible with standard and leaf extracts. This indirect ELISA method coupled with the discriminatory power of the MAb offered an efficient method for further investigation of the physiological functions of ABA in *Heliconia* leaves.

RESULTS FOR THE EXPERIMENT

ABA LEVELS BEFORE AND DURING SD

ABA content in heliconia leaves was not significantly different at the 5% level before (Jan. 15) and during SD (Jan. 22, Jan. 29, Feb. 5, and Feb. 12) (300.3 ± 13.0 and 326.6 ± 31.9 ng/g leaf dry wt., respectively). There were no significant differences at the 5% level among samples taken from leaves of pseudostems with different number of expanded leaves (3-6 leaves) from these two periods (Appendix A:Table 120).

EFFECTS OF TEMPERATURE TREATMENTS COMBINED OVER 4 TO 11 WEEKS AFTER SD

Temperature Effects on Foliar ABA Levels and Pseudostem Status

Foliar ABA levels taken at harvest were not significantly different at the 5% level for the different growing stages (vegetative, elongated, flowering, or aborted) within each temperature condition (Figure 19, Appendix A:Table 121) or across the temperature

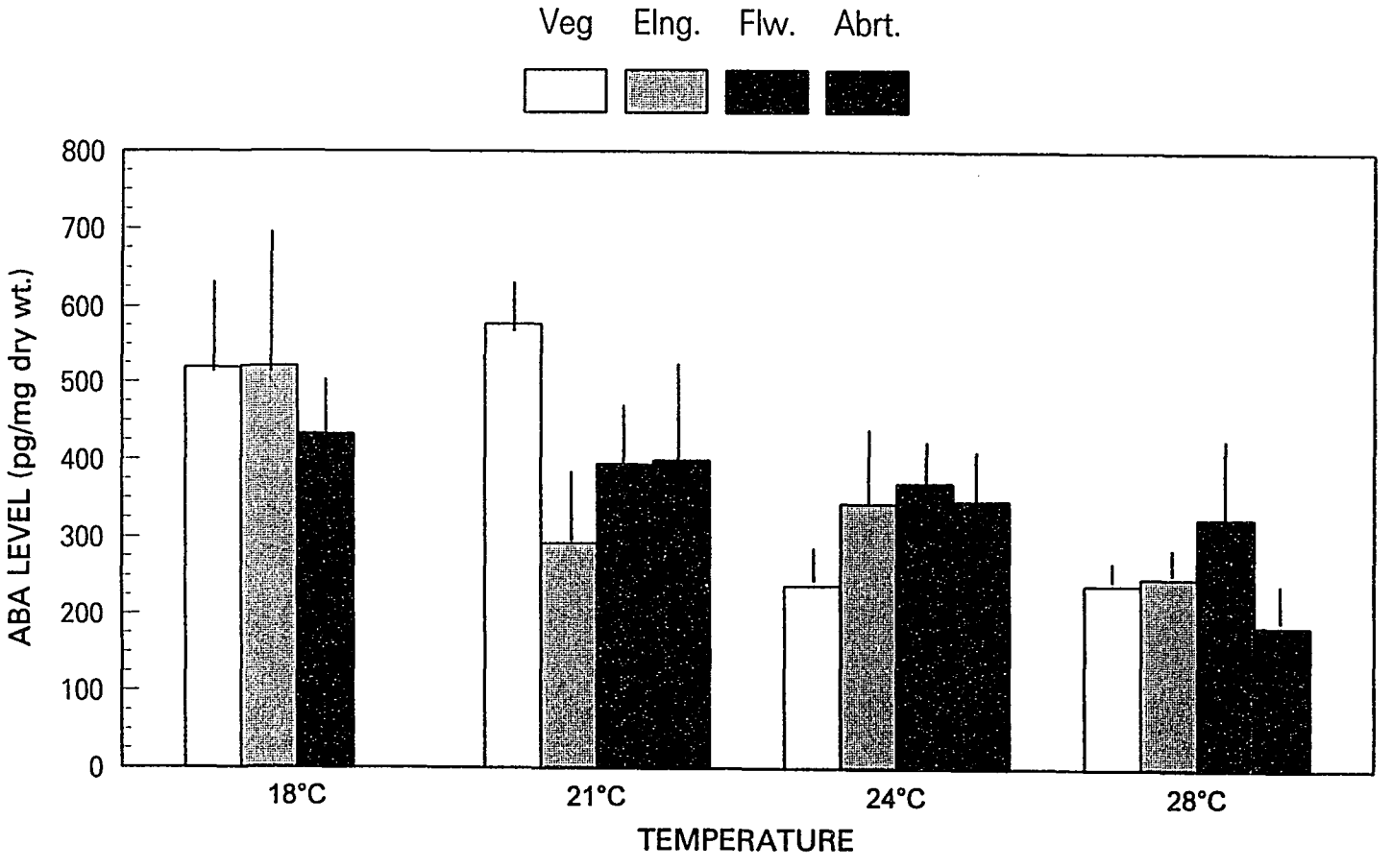

Figure 19. Leaf ABA levels of *Heliconia stricta* at different stages of growth (vegetative, elongated, flowering and aborted pseudostems) and different temperature conditions. Bars indicate mean ± SE.

condition (Appendix A:Table 122). Across all temperature conditions, foliage of flowering pseudostems had the highest ABA level at 386.9 ± 37.3 ng/g dry wt. while foliage of aborted pseudostems contained the lowest ABA level at 285.5 ± 55.7 ng/g dry wt (Figure 20). Foliage of vegetative and elongated pseudostems was intermediate at 349.5 ± 47.7 and 334.6 ± 52.2 ng/g dry wt. of ABA, respectively.

The foliar ABA content of heliconia grown under different temperature conditions (across all growth stages) had a significant linear effect at 5% level (Appendix A:Table 121). An increase in average daily temperature led to a decrease in foliar ABA content (18 °C: 441.0 ± 42.3, 21°C: 339.5 ± 29.8, 24°C: 331.5 ± 20.8, and 28°C: 264 ± 18.8 ng/g dry wt.; Figure 21; Appendix A:Table 123).

Temperature Effects on Pseudostem Status

For a period of 4 to 11 weeks after the start of SD, temperature treatments had a significant effect on the proportion of flowering, elongated, vegetative and aborted pseudostems (Figure 22, Appendix A:Table 124). At the lower temperatures, the percentage of flowering pseudostems increased from 31% at 28°C to 55% at 18°C. The percent aborted pseudostems increased from none at 18°C to 19.2% at 28°C. However, there was no significant difference at the 5% level in the proportion of reproductive shoot stages (flowering plus aborted apices) among different temperature treatments (Appendix A:Table 124) with an average of 50.2 % reproductive stage.

Foliar ABA Levels and Expanded Leaf Number at Harvest

Foliar ABA content from the topmost mature leaf, exhibited a quadratic relationship with the position of leaves on the pseudostems when samples were taken (averaged over all 4 temperature conditions and developmental stages; Figure 23, Appendix A:Table 125). ABA level decreased from 438.1 ± 45.6 ng/g dry wt. at the 4-leaf stage to 287.8 ± 19.3

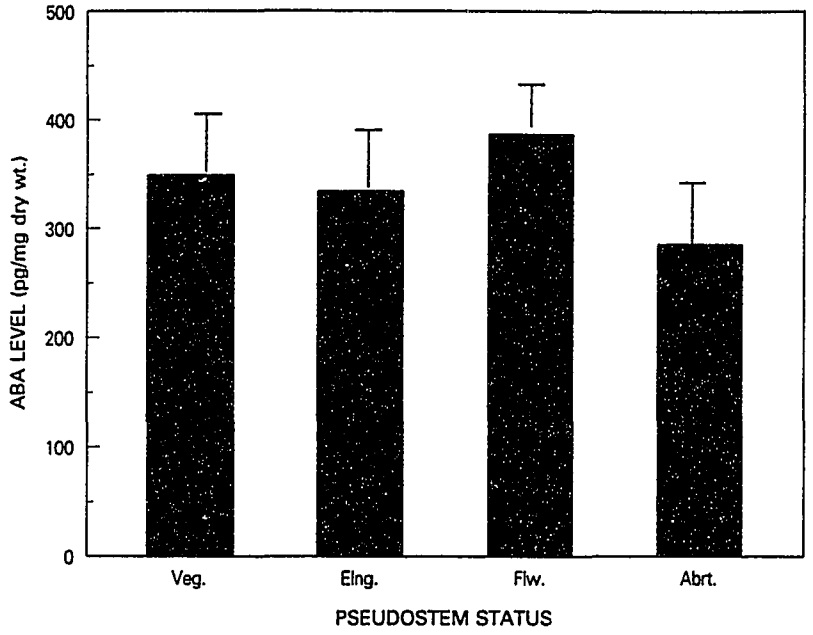

Figure 20. Concentration of ABA in leaf tissue from *Heliconia stricta* pseudostems pooled across all temperatures during 4 to 11 weeks after start of SD (Veg. = vegetative, Elng. = elongated, Flw. = flowering, Abrt. = aborted). Bars indicate mean ± SE.

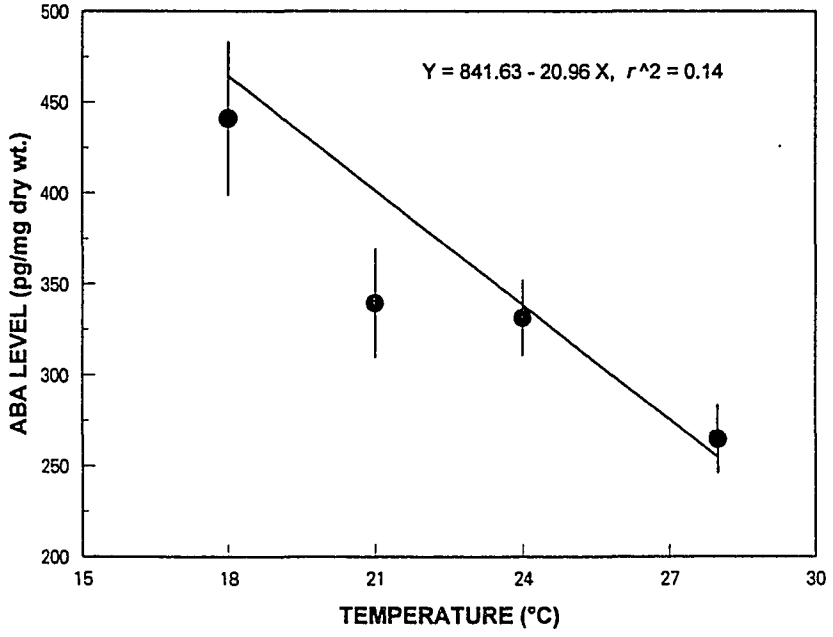

Figure 21. Effect of average daily temperatures on leaf ABA levels averaged over all growth stages for 4 to 11 weeks after start of SD. Bars indicate mean ± SE.

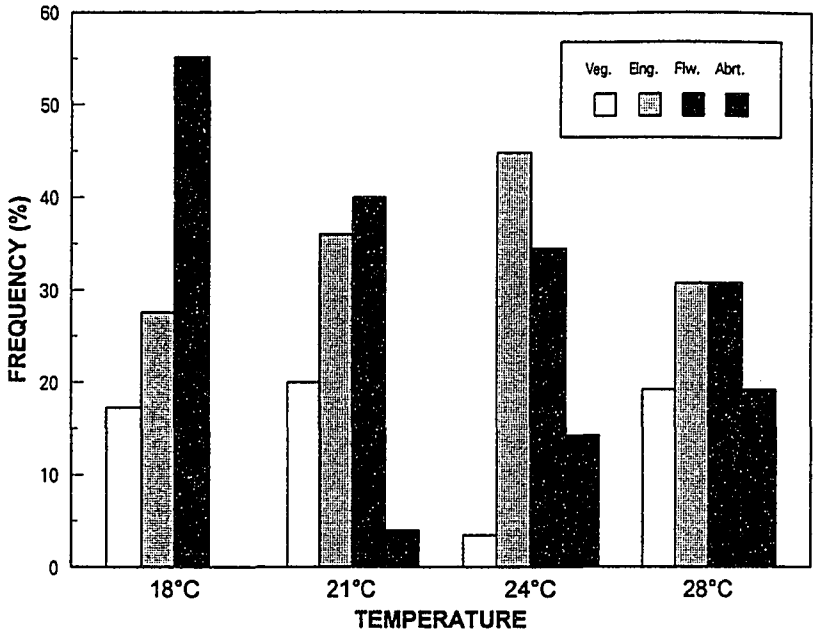

Figure 22. Effect of temperatures during a period 4 to 11 weeks after the start of SD on percentage of pseudostems: showing vegetative (Veg.), elongated (Elng.), flowering (Flw..) or aborted (Abrt.) pseudostem.

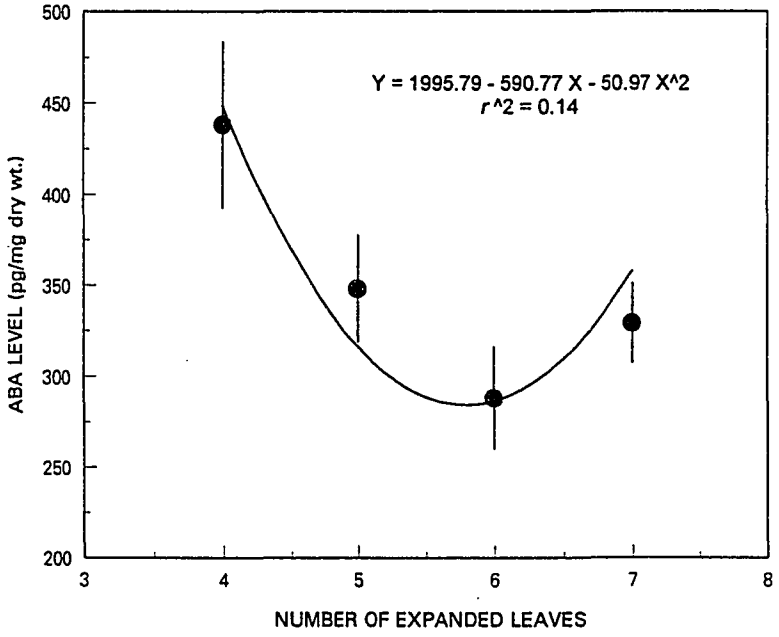

Figure 23. Leaf ABA levels of *Heliconia stricta* pseudostems with different number of expanded leaves. Bars indicate mean ± SE.

ng/g dry wt. at the 6-leaf stage then increased to 329 ± 22.1 ng/g dry wt. at the 7-leaf stage.

EFFECT OF TEMPERATURE TREATMENTS AT DIFFERENT TIMES OF DEVELOPMENT

Pseudostems with 2 to 5 leaves at the Start of SD

During the 11 weeks after the start of SD, pseudostems with 4 and 5 leaves at the start of SD showed signs of apical meristem elongation in the second week of SD while those with fewer than 4 expanded leaves did not elongate until 4 weeks after the start of SD (Figure 24). Flower primordia were found at 3 weeks after the start of SD in plants with 5 expanded leaves at start of SD, at 4 weeks after SD in plants with 3 and 4 expanded leaves and not until after 4 weeks after the start of SD for plants with 2 leaves. Evidence of flower bud abortion was found 6 weeks after the start of SD in shoots with 2, 3, and 4 leaves at start of SD but not until 10 weeks after the start of SD in shoots with 5 expanded leaves at start of SD (Figure 24).

Foliar ABA content of plants with different numbers of leaves at the start of SD fluctuated over time (averaged over all 4 temperature conditions and developmental stages). However, the patterns of peaks and valleys for pseudostems with 3-4 expanded leaves at start of SD were quite similar with a dip at 3 weeks after start of SD and a peak at 4 weeks.

Pseudostem with 3 To 6 Leaves at Time of Sampling

During the 11 weeks after the start of SD, pseudostems with 5 and 6 expanded leaves at sampling showed apical meristem elongation in the second week of SD while those with fewer than 5 expanded leaves did not elongate until 4 weeks after the start of SD (Figure 25). However, flower primodia were found in pseudostems with 6 expanded leaves at 3 weeks after the start of SD while those with 3-5 expanded leaves showed flower primordia at 4 weeks after the start of SD. The first sign of flower bud abortion was

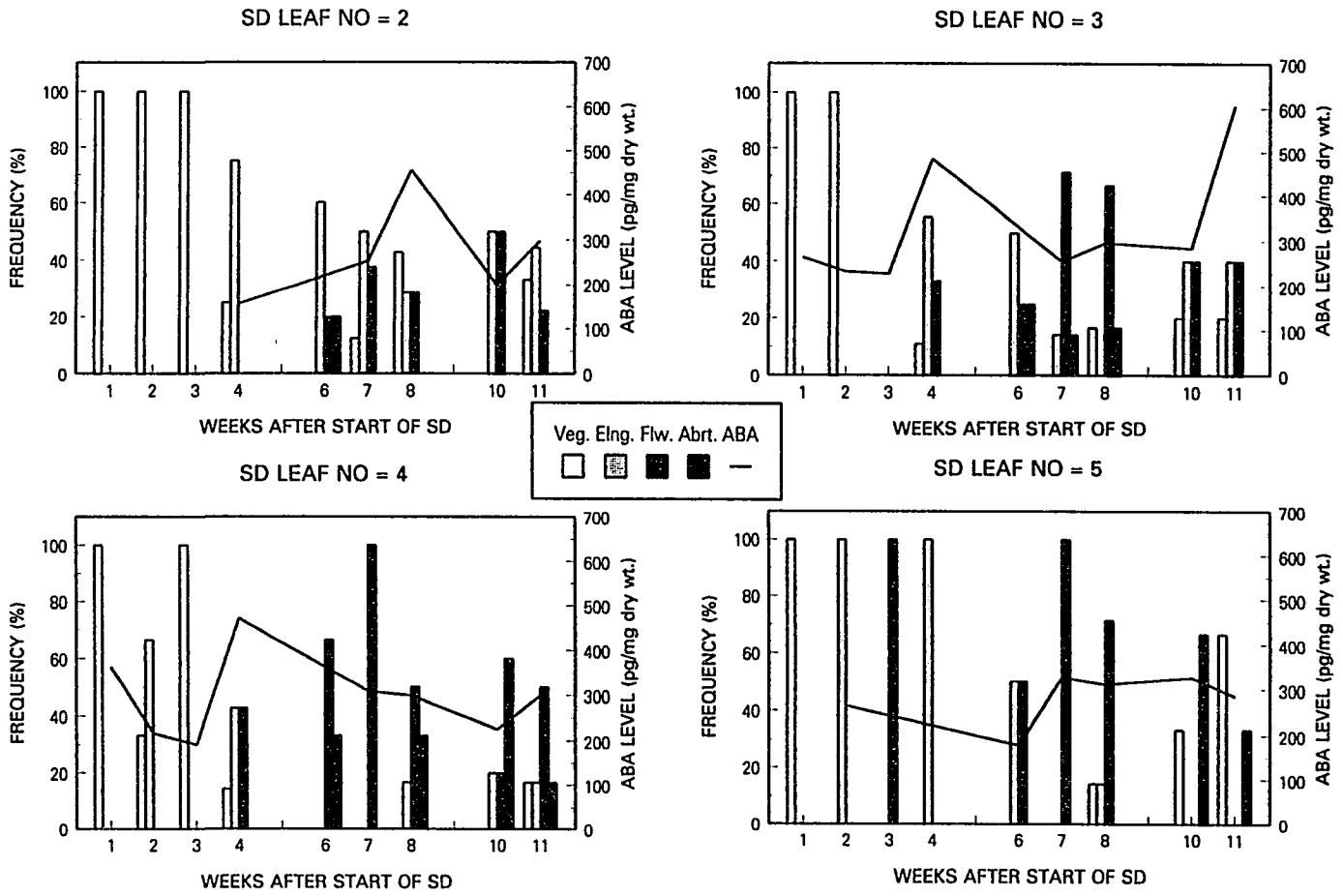

Figure 24. Leaf ABA levels (line) and percentage of pseudostems (bars) showing vegetative (Veg.), elongated (Elng.), flowering (Flw.) or aborted (Abrt.) at different time period in weeks after start of short day (8 hr.) with different numbers of expanded leaves at start of short day.

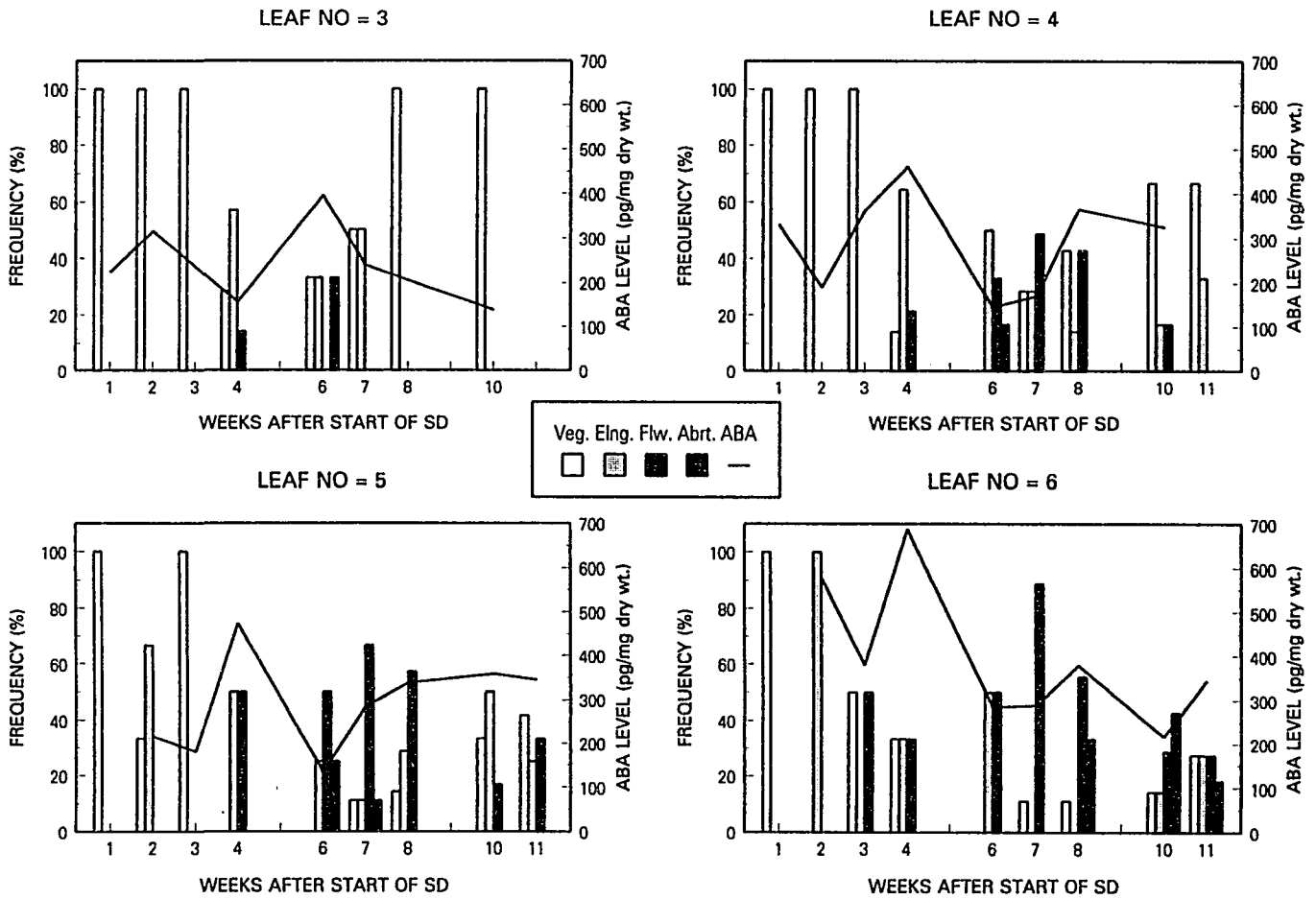

Figure 25. Leaf ABA levels (line) and percentage of pseudostems (bars) showing vegetative (Veg.), elongated (Elng.), flowering (Flw.) or aborted (Abrt.) at different time period in weeks after start of short day (8 hr.) with different numbers of expanded leaves at the time samples were taken.

found at 6 weeks after start of SD in shoots with 3, 4 and 5 expanded leaves but not until 8 weeks after the start of SD for shoot with 6 expanded leaves.

The foliar ABA content of plants with different expanded leaf numbers fluctuated over time (average over all 4 temperature conditions and developmental stages). However, the patterns of peaks and valleys for pseudostems with 4-6 expanded leaves at harvest were quite similar with a peak at 4 weeks and a dip at 6 weeks after the start of SD.

Pseudostem Status and Temperature Treatments

At 4 weeks after the start of SD, pseudostems in all treatments showed signs of flower primodia formation (Figure 26). Flower bud abortion occurred 6 weeks after the start of SD for pseudostems growing at 24°C and 28°C while those at 21°C showed signs of flower bud abortion at 7 weeks.

Foliar ABA levels of pseudostems grown under 18°C and 21°C fluctuated highly with a dip at 7 and 6 weeks after the start of SD respectively. Foliar ABA of pseudostems grown at 24°C and 28°C was more constant and peaked at 8 weeks similar to those grown under 18°C and 21°C (Figure 26).

TEMPERATURE AND FOLIAR ABA CONTENT MODEL

Considered across all leaf counts and weeks after the start of SD, the mean foliar ABA content of plants grown at 18°C and 21°C was significantly higher than for plants grown at 24°C and 28°C D/N at 5% level (Figure 27, Appendix A:Table 126). Statistical differences between treatments were found for the interactions with the straight-line effect of leaf number at the start of SD and the quadratic effects of time after SD. Foliar ABA content increased linearly with increasing leaf number at the start of SD (Appendix A:Table 126.). Foliar ABA content of plants grown under 18°C and 21°C exhibited a quadratic relationship with time after the start of SD with bottom of the curve around 7-8 weeks (Figure 28). Foliar ABA levels of plants grown under 24°C and 28°C exhibited a different

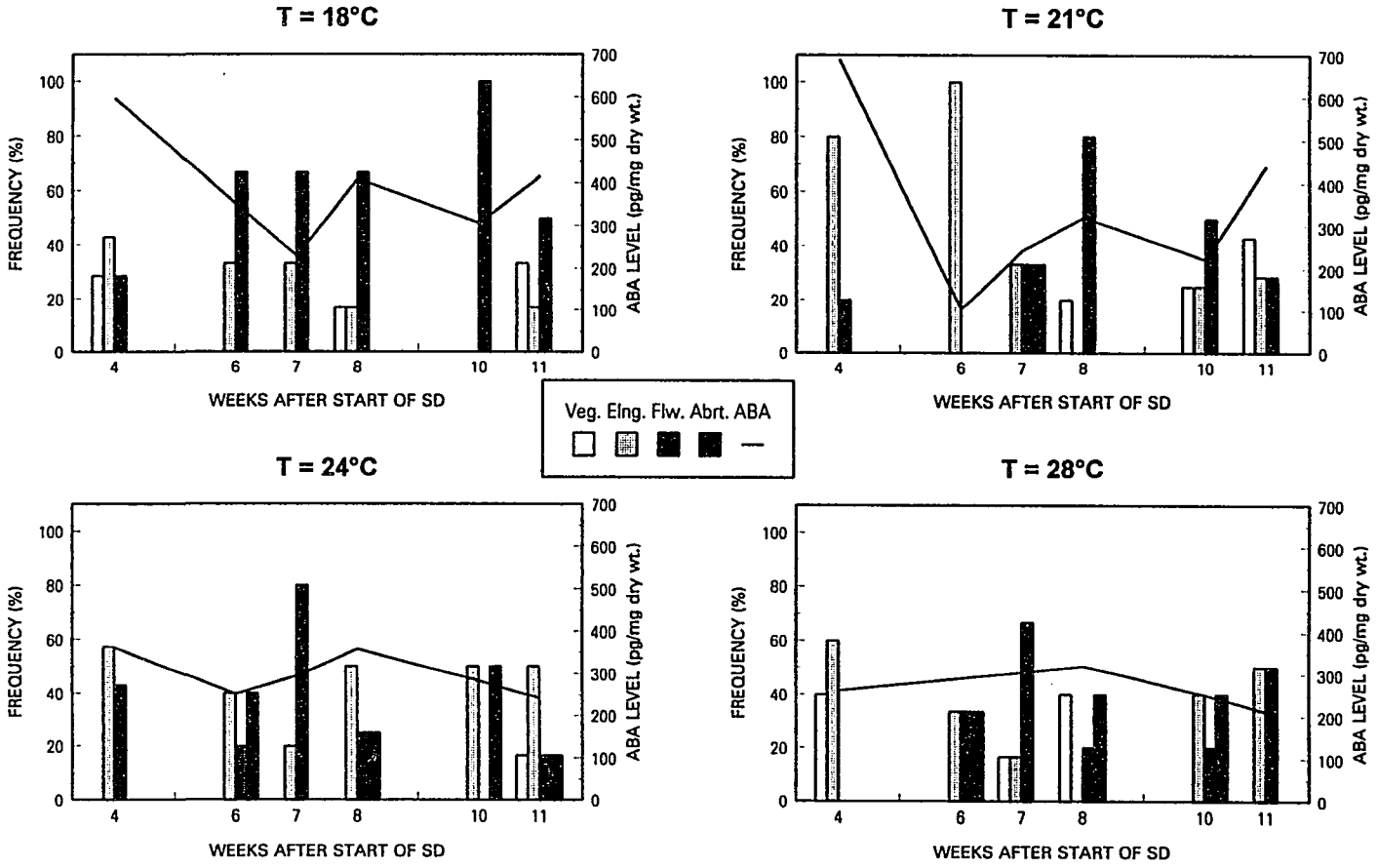

Figure 26. Leaf ABA levels (line) and percentage of pseudostems (bars) showing vegetative (Veg.), elongated (Elng.), flowering (Flw.) or aborted (Abrt.) at different time period in weeks after start of short day (8 hr.) in each temperature treatment.

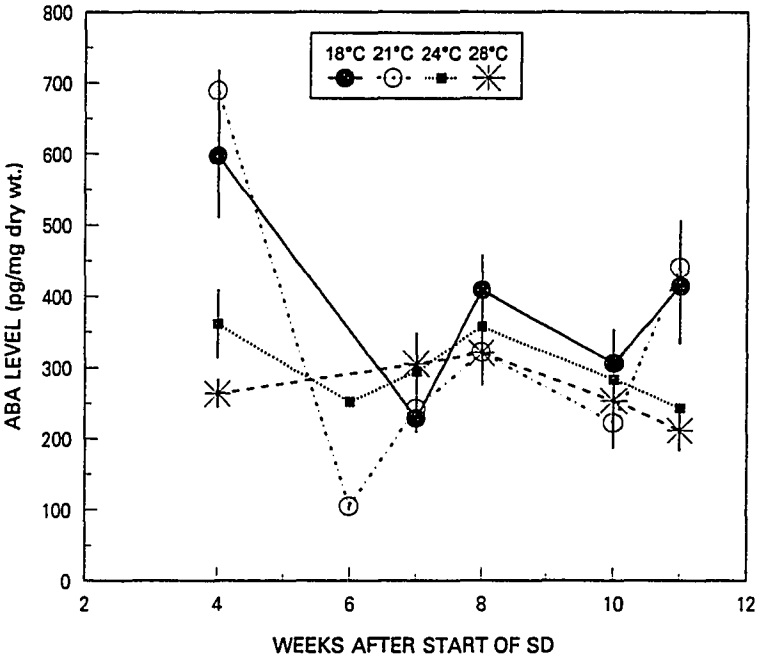

Figure 27. Concentration of ABA in leaf tissue from *Heliconia stricta* pseudostems at different average daily temperatures (18°C, 21°C, 24°C, and 28°C) during 4 to 11 weeks after start of SD. Bars indicate mean ± SE.

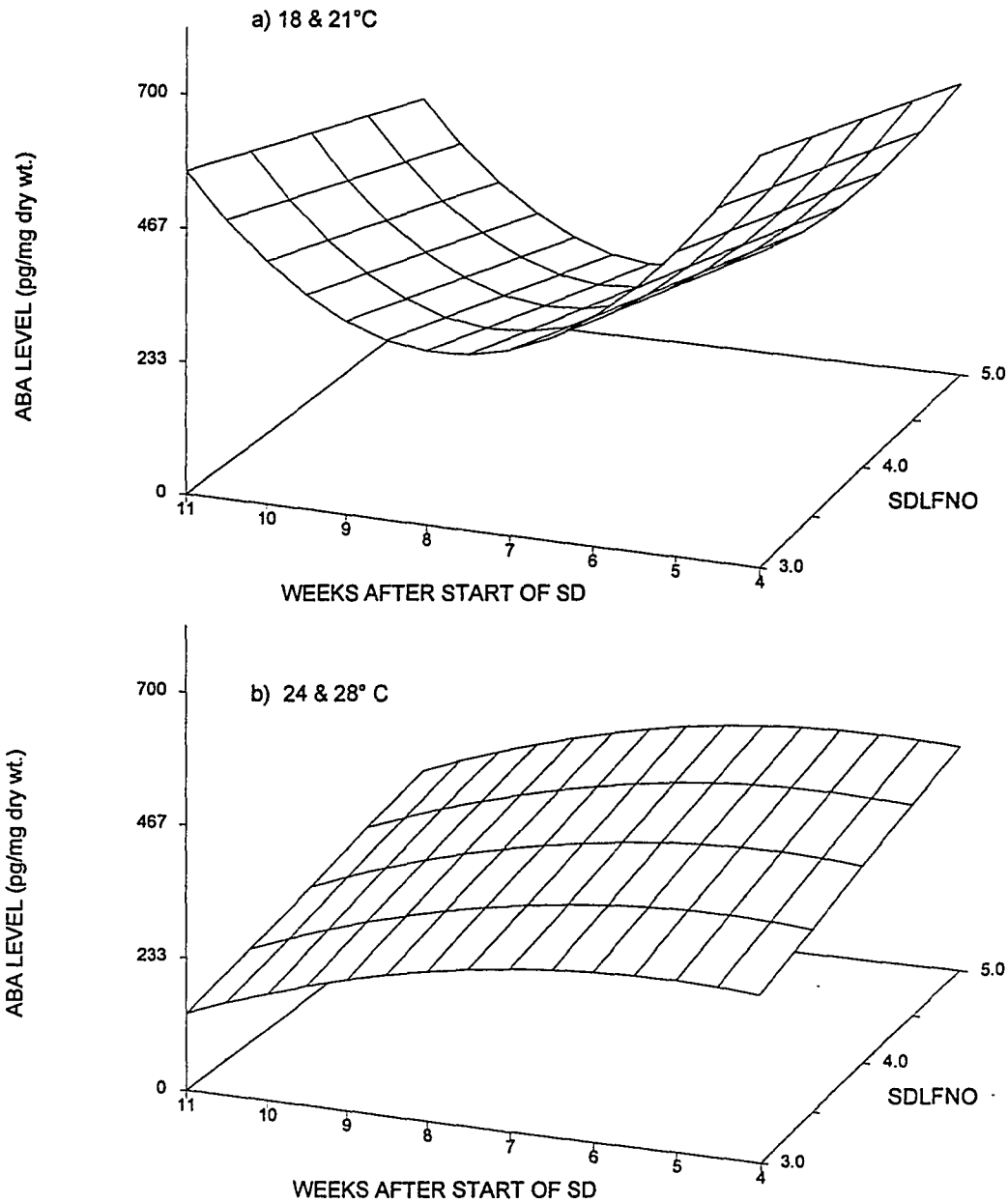

Figure 28. The comparison of leaf ABA level responses of *Heliconia stricta* under 18-21°C (a) and 24-28°C (b). Statistical differences between treatments were found for the interactions with the straight-line effects of leaf number at start of SD (SDLFNO) and the quadratic effects of time after start of SD (Appendix Table 123).

quadratic relationship with time after start of SD curve with the top of the curve around 6-7 weeks after the start of SD (Figure 28).

SHOOT STATUS AT THE END OF THE EXPERIMENT

At the termination of the experiment (20 weeks after start of SD), plants grown at 18 °C yielded the highest percent flowering (61%) while those grown at 21, 24 and 28 °C flowered at the rate of 50%, 33%, and 27% respectively. The higher the temperature the more flower buds were aborted, ranging from 7% at 18°C to 27% at in 28°C (Appendix A:Table 127).

Average time to flower from the start of SD for all pseudostems grown at 18°C and 21°C was 18 weeks which was one week later than those grown at 24°C and 28°C. This was in good agreement with Lekawatana (1986).

CHARACTERISTICS OF FLOWER BUD DEVELOPMENT

Figures 29-31 showed apical longitudinal sections of *H. stricta* 'Dwarf Jamaican' grown at 18, 21, 24 and 28°C under 14 hr daylength after an initial floral induction stimulus of 4 weeks of SD at advance stage of development. Before SD, the apical meristem remained vegetative (Figure 29A). Two to 3 weeks after the start of SD, pseudostem elongation was observed (Figure 29B,C). Four weeks after the start of SD, the first and the second cincinnal bracts were distinguishable (Figure 30A). At 6 weeks after the start of SD, flower primordia were conspicuous (Figure 30B). At 11 weeks after the start of SD, flower primordia in the first cincinnal bract were almost 1 cm in length (Figure 31C).

There were however, some pseudostems that did not develop into stages described above, but remained in vegetative (Figure 32A,C), early flower development stage (Figure 32B) or aborted (Figure 31A,B,C).

Figure 29. Apical longitudinal section of *H. stricta* 'Dwarf Jamaican' treated with an initial floral induction stimulus of 4 weeks of SD at different stages of development. Bar equal 500 µm.

A. Vegetative pseudostem with 4 visible, expanded leaves and 6 total leaves produced before the start of SD.

B. Pseudostem elongation commenced with 5 visible, expanded leaves and 6 total leaves produced 1 weeks after the start of SD. (4 leaves at the start of SD)

C. Pseudostem elongation commenced with 5 visible, expanded leaves and 6 total leaves produced 2 weeks after the start of SD. (4 leaves at the start of SD)

(L = leaf number, B = cincinnal bract, P= unidentified primodium, FP = flower bud primodium, PD = peduncle)

A
B

Figure 30. Apical longitudinal section of *H. stricta* 'Dwarf Jamaican' treated with four temperatures (18, 21, 24 and 28°C) under 14 hr daylength after an initial floral induction stimulus of 4 weeks of SD at different stages of development. Bar equal 500 µm.

A. Pseudostem with 5 visible, expanded leaves and 6 total leaves produced 3 weeks after the start of SD (4 leaves at the start of SD). The first and second cincinnal bracts were evident.

B. Pseudostem with 5 visible, expanded leaves and 6 total leaves produced 4 weeks after the start of SD (4 leaves at the start of SD). The first flower primordium was evident.

C. Pseudostem with 5 visible, expanded leaves and 6 total leaves produced 6 weeks after the start of SD (4 leaves at the start of SD; 25°C LD). The second flower primordium was evident.

(L = leaf number, B = cincinnal bract, P= unidentified primodium, FP = flower bud primodium, PD = peduncle)

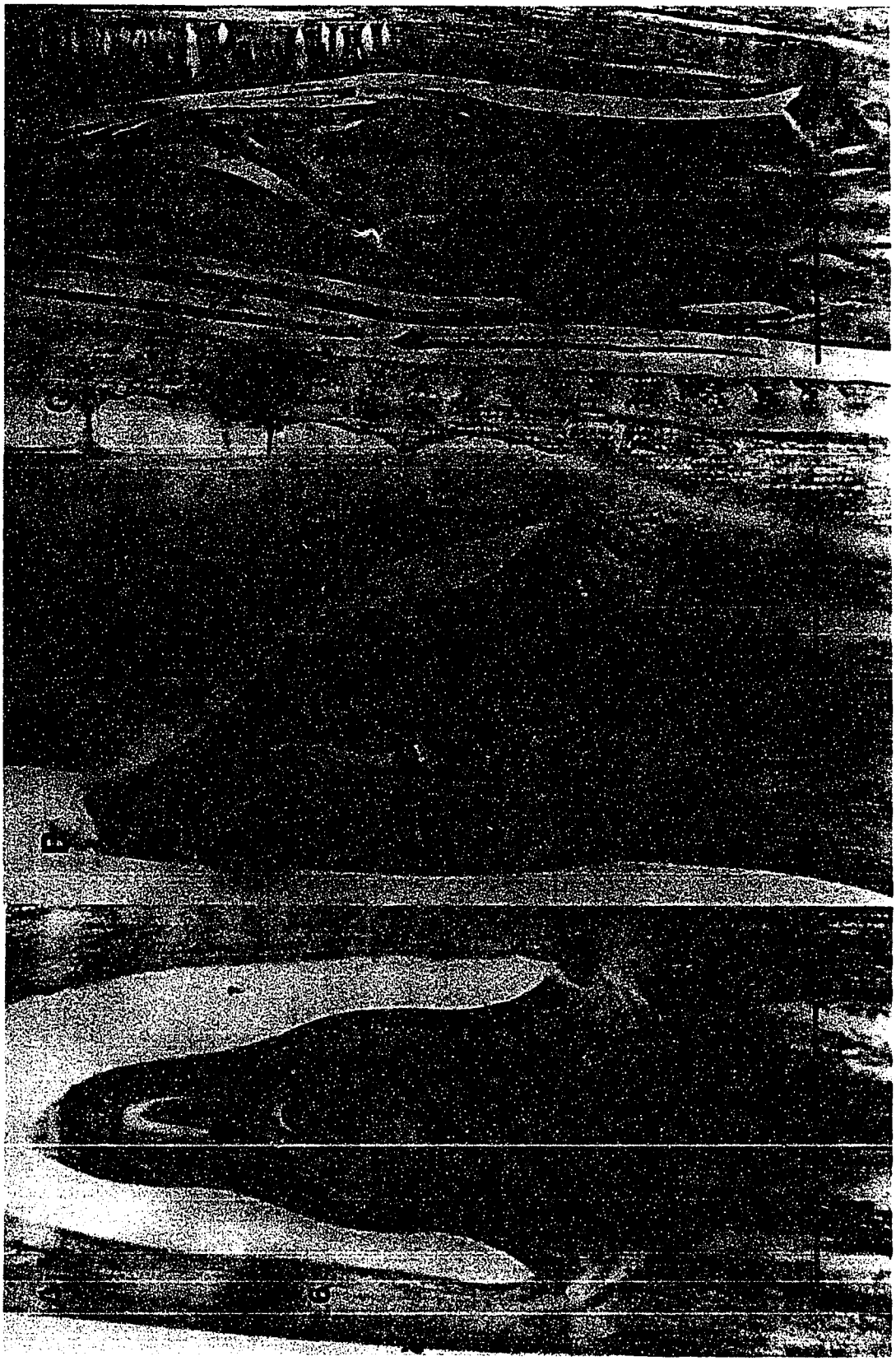

Figure 31. Apical longitudinal section of *H. stricta* 'Dwarf Jamaican' treated with four temperatures (18, 21, 24 and 28°C) under 14 hr daylength after an initial floral induction stimulus of 4 weeks of SD at different stages of development. Bar equal 500 μm.

A. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 7 weeks after the start of SD (4 leaves at the start of SD; 25°C LD). The third flower primordium was evident.

B. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 8 weeks after the start of SD (4 leaves at the start of SD; 25°C LD). The first flower primordium had differentiated flower parts.

C. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 11 weeks after the start of SD (4 leaves at the start of SD; 18°C LD). The inflorescence increased in size.

(L = leaf number, B = cincinnal bract, P= unidentified primodium, FP = flower bud primodium, PD = peduncle)

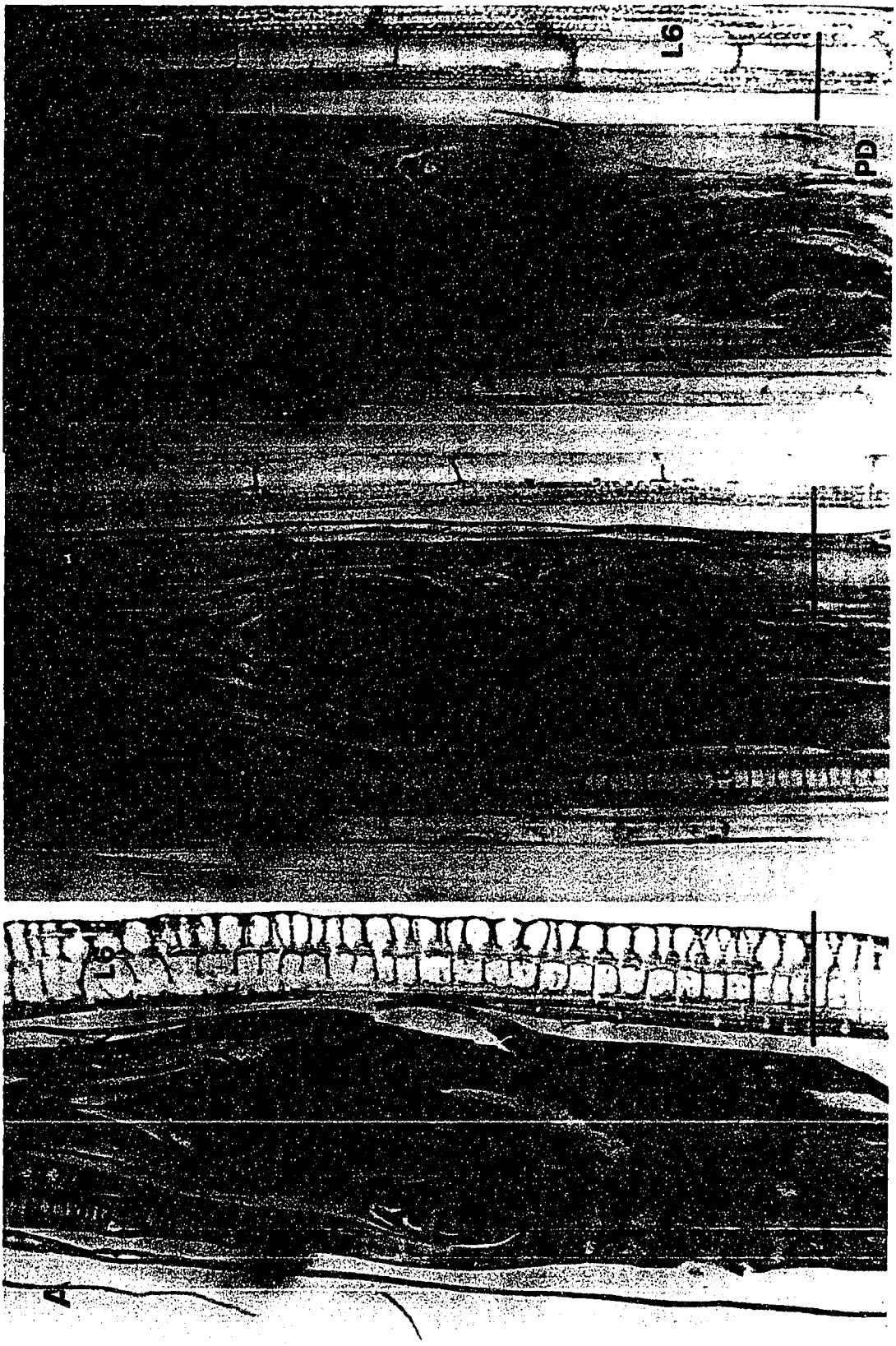
L6
PD
A

Figure 32. Apical longitudinal section of *H. stricta* 'Dwarf Jamaican' treated with four temperatures (18, 21, 24 and 28°C) under 14 hr daylength after an initial floral induction stimulus of 4 weeks of SD at different stages of development. Bar equal 500 µm.

A. Vegetative pseudostem with 6 visible, expanded leaves and 8 total leaves produced 11 weeks after the start of SD. (4 leaves at the start of SD; 18°C LD

B. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 8 weeks after the start of SD (4 leaves at the start of SD; 25°C LD). The inflorescence was in an early stage of development.

C. Vegetative pseudostem with 6 visible, expanded leaves and 6 total leaves produced 10 weeks after the start of SD (4 leaves at the start of SD; 18°C LD).

(L = leaf number, B = cincinnal bract, P= unidentified primodium, FP = flower bud primodium, PD = peduncle)

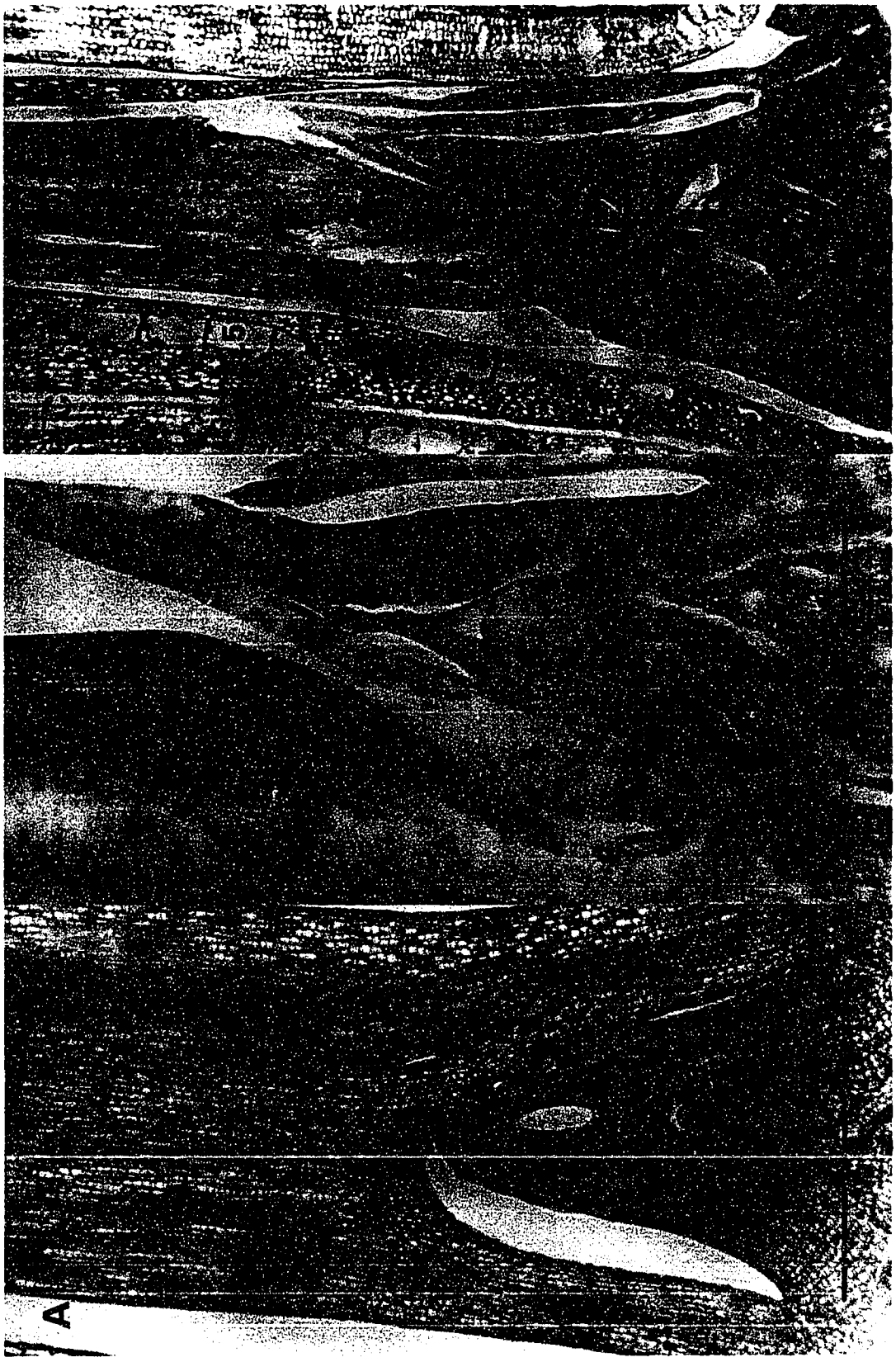
A

Figure 33. Apical longitudinal section of *H. stricta* 'Dwarf Jamaican' treated with four temperatures (18, 21, 24 and 28°C) under 14 hr daylength after an initial floral induction stimulus of 4 weeks of SD showing various stages of flower bud abortion. Necrotic cells were not stained (purplish blue) with toluidine blue were brown in color. Bar equal 500 μm.

A. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 10 weeks after the start of SD (3 leaves at the start of SD; 21°C LD). Failure to stain (brown color) in elongating peduncle reflected early stage of inflorescence abortion. Note the inflorescence was stained normally.

B. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 10 weeks after the start of SD (4 leaves at the start of SD; 21°C LD). In elongating inflorescence, some part of rachis failed to stain. Note the flower primordia were stained normally.

C. Pseudostem with 6 visible, expanded leaves and 6 total leaves produced 11 weeks after the start of SD (4 leaves at the start of SD; 25°C LD). In elongating inflorescence, some part of rachis failed to stain. Note the flower primordia were stained normally.

(L = leaf number, B = cincinnal bract, P= unidentified primodium, FP = flower bud primodium, PD = peduncle)

S1
S2
S3
L6

In aborted inflorescences, necrotic cells were not stained (purplish blue) with toluidine blue O but were brown in color. The location of necrotic tissue was from the tip of the inflorescence to the top part of peduncle (Figure 33). The smallest developing inflorescence that was found to be aborted was 2 cm long (from the tip of the inflorescence to the base of peduncle) and was found as early as 6 weeks after start of SD in plants growing at 28°C.

DISCUSSION

After the initial SD stimulus, pseudostems grown under high temperature developed a high percent of flower bud abortion. This is in agreement with studies in other plants such as snap bean, tulip, tomato , Iris, citrus etc (Konsens *et al.*, 1991; Kinet *et al.*, 1985). Kinet *et al.* (1985) generalized that the higher the temperature, the higher the percent abortion.

In a study by Lekawatana (1986), *Heliconia stricta* 'Dwarf Jamaican' were given an initial stimulus of 4 weeks of SD at 15, 20 and 25°C night temperature and later were grown until flowering in ambient temperatures with a mean night temperature of 20.2°C. There was no significant difference in percent of aborted shoots among temperature treatments. Plants grown at 20°C night temperature during SD, similar to this experiment, yielded similar percent flowering plus flower bud abortion pseudostems (52%). Furthermore, percent reproductive pseudostems (flowering + aborted) among temperature treatments of this experiment were not significantly different (mean of 50%). This demonstrates a consistent response of the species to temperature during SD. These two experiments indicate the following:

1) As night temperature decreased, from 25°C to 15°C, during the initial stimulus period (4 weeks of SD) the percent of reproductive pseudostem increased from 31% to

78% (Setapong, 1986). Night temperature during initiation does not have an influence on percent flower bud abortion.

2) As night temperature increased from 15°C to 25°C after the initial stimulus (4 weeks of SD) the percent that finally flowered decreased from 61% to 27% and the percent aborted pseudostems increased from 8% to 27%. However, night temperature during the development period did not influence the total percent reproductive pseudostem initiated.

Flower bud abortion was not found in plants grown at 18°C and was found 7 weeks after the start of SD for plants grown at 21°C. In plants grown at 24°C and 28°C, flower bud abortion found from 6 weeks after the start of SD.

When the average air temperature was increased, foliar ABA content decreased linearly. Similar findings were found in bean and cucumber seedlings (Smith and Dale, 1988; Capell and Dörffling, 1989). The rise in ABA content in leaf is most likely to be due to local synthesis in the leaf itself, although the stimulus behind this, which presumably must emanate from the roots, remains unknown (Smith and Dale, 1988).

The quadratic surfaces for foliar ABA content regressed on initial leaf number and weeks after the start of SD together with the ABA content regressed on number of expanded leaves indicated that, with progress of time and the increase in leaf number, the foliar ABA content of the top mature leaf tended to decrease at first and increase later on. The results are similar to Ross and McWha (1990) found that the ABA content of *Pisum sativum* leaflets toward the base of the plant was greater than at a higher position in the plant. However, the high levels of ABA could also be correlated with senescence. Ross and McWha (1990) reported that a high percentage of total ABA in pea was present in its reproductive tissues.

Due to the limitation of our knowledge on the influence of ABA on flower bud abortion and its mechanism, it can only be stated that high temperature during

inflorescences development decreased level of ABA in *H. stricta* 'Dwarf Jamaican' leaves and that a lower level of ABA was found in the foliage of aborted pseudostems compared to that found in developing inflorescences.

CONCLUSION

1. Temperatures of 18-21°C improved reproductive success both in terms of greater percent bud set and lower rate of abortion compared to plants grown at 24-28°C.

2. Flower bud abortion in *H. stricta* 'Dwarf Jamaican' was observed 6 weeks after the start of SD when the developing inflorescence was approximately 2 cm and flower primordia were conspicuous. The appearance of flowers coincides with the onset of abortion of the inflorescences and a lower ABA level in the foliage.

3. The hypothesized role for ABA as a stimulus to flower bud abortion appears to be unsupported as flower bud set was greater under conditions leading to high ABA levels in the foliage. However, ABA level in the foliage may have no relationship to ABA level at the meristem.

CHAPTER 5

EFFECT OF LIGHT INTENSITY ON INFLORESCENCE ABORTION AND ABSCISIC ACID LEVELS IN *H. STRICTA*

ABSTRACT

Plants of *Heliconia stricta* Huber 'Dwarf Jamaican' were grown under three light intensity treatments of full sun, 40% sun, and 20% sun in ambient outdoor conditions after an initial floral induction stimulus of 4 weeks of SD at 26/21°C. Free (+)-abscisic acid (ABA) content of mature leaves was measured by indirect enzyme-linked immunosorbent assay (ELISA). Changes in light intensity did not significantly affect percent flowering or percent aborted pseudostems. Decreased light intensity during inflorescence development increased the levels of ABA *in H. stricta* leaves from 88.8 ± 11.5 pg/mg dry wt. in full sun to 276.4 ± 39.1 and 219.5 ± 22.4 pg/mg dry wt. in 40% and 20% sun respectively. Different pseudostem statuses (vegetative, aborted or flowering) showed no significant difference relative to leaf ABA level.

INTRODUCTION

Flower bud abortion in Heliconia has been found in many species such as *H. angusta*, *H. chartacea* and *H. stricta* (Criley and Lekawatana, 1994; Lekawatana, 1986). Reports on some Heliconia species (*H. psittacorum* and *H. angusta*) stated that increased light intensity increased flower production (Broschat and Donselman, 1982, 1983; Kwon, 1992).

In most species increased photosynthesis in leaves is the major system that promotes flower development. The young developing flower bud is a major sink for assimilates under favorable growing conditions, when the metabolites essential for its growth are in ample supply (Halevy, 1987). Under stress conditions with an inadequate

supply of assimilates, the young flower bud constitutes a weaker sink compared with the vegetative apices, developing leaves, fruits or storage organs, and it competes poorly with them for the available assimilates (Halevy, 1984). This was found to be the case for conditions of light, temperature and water stress . In the developing bud, these environmental stresses promoted abortion, blasting or abscission of the flower buds, while other organs were only slightly affected (Kinet and Sachs, 1984; Halevy, 1987).

Several environmental factors increase the endogenous level of abscisic acid (ABA) in plant tissues. Most prominent is the effect of drought stress, but other kinds of stress such as aeration stress and temperature extremes have been reported to increase the level of ABA (Addicott, 1983). At the time the study initiated, no reference on the effect of light intensity on ABA levels in plants was found.

This experiment was carried out to investigate the effect of light intensity during flower bud development on percent flower bud abortion and ABA content in foliage of *Heliconia stricta* 'Dwarf Jamaican'

MATERIALS AND METHODS

PLANT MATERIAL AND CULTURAL PRACTICE

Two hundred rhizome pieces of *Heliconia stricta* cv. Dwarf Jamaican were propagated on March 25, 1991. Rhizome pieces including pseudostems were separated, and the roots removed. The pseudostem was cut to 5 cm in length from the leaf sheath base, treated in a 55°C water bath for 5 minutes, dipped in fungicide (Dithane M45) and drained. The rhizomes were then held in plastic bags for 3 weeks at 20°C to stimulate root and shoot growth. They were planted in perlite and vermiculite 1:1 ratio (v/v) and held under mist for 1 week. Three rooted rhizome pieces were potted per 15-cm pot on April 18, 1991, and placed in a greenhouse at the Magoon greenhouse facility of the University of Hawaii. The potting medium was a mixture of peat and perlite 1:1 ratio (v/v) amended

with dolomite, Micromax and treble superphosphate at the rates of 6.0, 1.0 and 0.6 kg per cubic meter, respectively. Plants were drip irrigated twice daily with nutrient solution, 200 N-0P-200K (ppm). On June 17, 1991, when forty-six percent of pseudostems were in the 3-leaf stage and 54% were in the 4-leaf stage, plants were given a flower initiation stimulus by subjecting them to short day conditions in growth chambers for 4 weeks from June 17, 1991 to July 15, 1991. The growth chamber conditions were: photoperiod 8 hour daylength using a combination of fluorescent and incandescent lamps with photosynthetically active radiation (PAR) measured 214 $\mu mol/s^{-1}/m^{-2}$ and temperature 25/20°C day/night (D/N). The average minimum and maximum temperatures in the greenhouse prior to growth chambers were 21.6°C (range: 19.0-23.5°C) and 31.8°C (range: 25.5-36.0°C), respectively. Average daily maximum PAR was 609.28 $\mu mole.s^{-1}.m^{-2}$ with a range of 240-1,000 $\mu mol.s^{-1}.m^{-2}$.

TREATMENT SETUP

After SD treatment (July 15, 1991) 50 pots of *H. stricta* plants were placed in 3 different shading treatments at the magoon area as follows:

Trt.1 - Full sun (average 1,476.2 $\mu mol.sec^{-1}.m^{-2}$ at 1:00 pm)

Trt.2 - 40% sun (average 591.9 $\mu mol.sec^{-1}.m^{-2}$ at 1:00 pm)

Trt.3 - 20% sun (average 262.6 $\mu mol.sec^{-1}.m^{-2}$ at 1:00 pm)

Two shading conditions were made by covering a bench with structures made by wooden frames sized 185 X 150 X 180 cm. These structures were covered with saran cloth with different percent shading (20% and 40% of sunlight). Fourteen pots each were placed under the two structures and another 15 pots were placed on a bench in full sun (100% sun). Plants were hand-watered daily with a nutrient solution 200N-200P-200K (ppm). Throughout the experiment plants were provided with adequate water, therefore the effect of water stress was minimized.

DATA COLLECTION

At the start of SD the expanded leaf number was recorded for all 192 pseudostems. On June 25 (1 pot); July 2 (1 pot) and July 15 (3 pots), pseudostems were collected. After 4 weeks SD, 2 pots of each shade treatment were collected on August 8; August 17; August 25; and September 2. These represent 8, 9, 10, and 11 weeks after the start of SD. Then pseudostems were dissected to determine their status (vegetative, flowering, aborted). Twenty-five leaf blades of the topmost mature leaves were collected from each treatment at each harvest for foliar ABA analysis.

At the end of the experiment (October 25, 1991) the status (vegetative, elongate, flowering or aborted) of 16 pots of *Heliconia stricta* were determined. Length of pseudostems were measured from base to the tip of the last leaves. Length of inflorescences were measured from base to the tip of inflorescences and number of cincinnal bracts were counted.

Photosynthetically active radiation (PAR: $\mu mol.s^{-1}.m^{-2}$) in the 400 to 700 nm waveband was measured by a Licor quantum sensor model LI-200S. Three light sensors and 3 air temperature sensors placed approximately 50 cm above bench in the middle of each treatment were connected to a Datalogger model LI-1000. Data were averaged over 5 minutes intervals and the way of the 12 intervals were recorded every 60 minutes. Average PAR readings, throughout the experiment period, at 1:00 pm for the different shading treatments from full sun, 40% sun and 20% sun were 1,476.2, 591.9 and 262.6 $\mu mol.sec^{-1}.m^{-2}$ which equaled 100%, 40.1% and 17.8% of the solar intergral respectively. Average air temperatures measured at 1:00 PM in the full sun, 40% sun and 20% sun treatments were 30.4 ± 0.2, 28.7 ± 0.2, and 27.8 ± 0.1°C respectively. Average temperatures measured at 5:00 am in the full sun, 40% sun and 20% sun treatments were 22.4 ± 0.2, 22.3 ± 0.1, and 22.4 ± 0.1°C. Summaries of the weather data are presented in Appendix: B Figures 3-6.

EXTRACTION AND DETERMINATION OF ABA LEVEL

Leaf blades were stripped from their midribs. Each sample was placed in a plastic tube and immediately stored in liquid nitrogen (-70 °C). Leaves were then powdered by grinding in a precooled mortar and pestle with liquid nitrogen then lyophilized (freeze dried). The lyophilized samples were then stored in plastic tubes at -20 °C. The samples were extracted and their ABA content determined using an indirect enzyme-linked immunosorbent assay (ELISA) as described in Chapter 4. With this method the percent recovery was more than 90%. The standard used was (+) *cis-trans*-ABA (Sigma Chemical Co.) and the Monoclonal Antibody (MAb) was MAb to free *cis-,trans*-(+)-ABA (Idetek, Inc.). Samples were tested in triplicate (3 wells for each sample).

STATISTICAL ANALYSIS

The statistical analysis was by SAS general linear model (PROC GLM) analysis of variance with mean separations by the t-test and contrast (SAS Institute, 1987). Quantitative data such as shoot status were analyzed by Chi-Square test for independence, based on the null hypothesis that the differences among the ratios were not significant.

RESULTS

ABA LEVELS DURING SD

ABA content of the combined 3rd and 4th leaf blades of heliconia during SD (4 weeks) was 296.9 ± 37.4 pg/mg leaf dry wt. (n=12).

EFFECT OF SHADINGS FOLLOWING SD

Shading Effects on ABA levels

Foliar ABA content of Heliconia grown under 20% and 40% sun (219.5 ± 22.4 and 276.4 ± 39.1 pg/mg dry wt.) were significantly higher than for those grown under

full sun (88.8 ± 11.5 pg/mg dry wt.) with mean separation by DMR-test (Figure 34, Appendix A:Table 128).

Shading Effect on Pseudostem Status

For a period of 4 to 11 weeks after start of SD, different light regimes had no significant effect on the proportion of flowering, vegetative or aborted pseudostems (Appendix A:Table 129). Percentage distribution of pseudostems was in a range of 76-78% flowering, with 4-6% aborted and 17-20% vegetative (Figure 35).

Foliar ABA Levels and Pseudostem Status

Foliar ABA levels taken from pseudostems of different reproductive status (vegetative, flowering, and aborted) were not significantly different at 5% level (Appendix A:Table 130). However, foliage of flowering pseudostems contained the lowest ABA level at 181.2 ± 34.8 pg/mg dry wt. while foliage of aborted and vegetative pseudostems had the highest ABA level at 306.9 ± 5.9 and 247.5 ± 58.9 pg/mg dry wt respectively (Figure 36).

Foliar ABA Levels and Expanded Leaf Number at Harvest

Leaf ABA content exhibited a quadratic relationship with number of expanded leaves when samples were taken (averaged over all 3 shade conditions and developmental stages; Figure 37, Appendix A:Table 131). ABA level decreased from 339.6 ± 30.8 pg/mg dry wt. at 4-leaf stage to 175.2 ± 6.3, 222.7 ±43.9, and 154.6 ± 16.5 pg/mg dry wt. at 5, 6, and 7 leaf stage respectively. Then ABA level increased to 306.9 ± 5.9 pg/mg dry wt. at 8 leaf stage.

Pseudostems Status at the Time samples were Taken

During SD (1-4 weeks) all pseudostems appeared to be in a vegetative stage (Figure 38). However, by 8 weeks after start of SD many pseudostems were

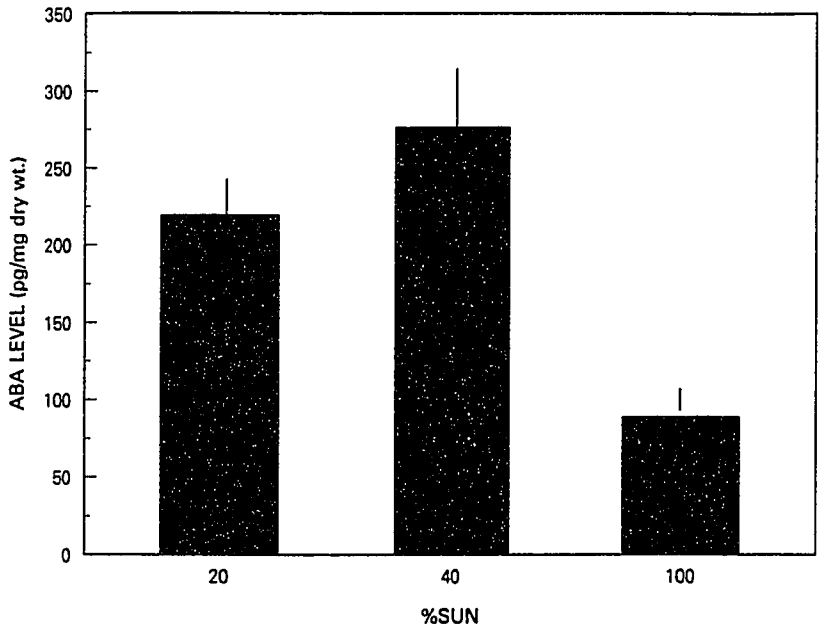

Figure 34. Effect of shading (20, 40 and 100% sun) on leaf ABA levels. Bars indicate mean ± SE.

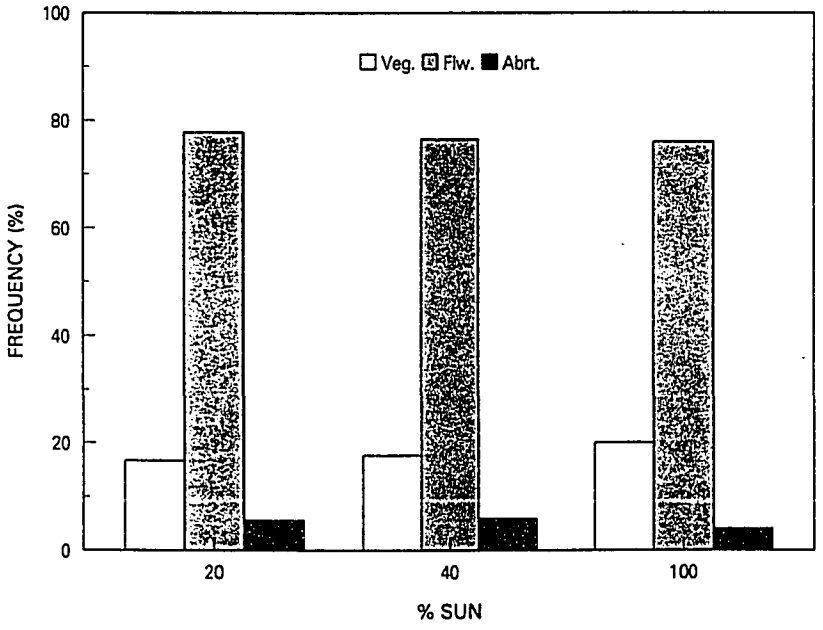

Figure 35. Effect of shading (20, 40 and 100% sun) on percentage of pseudostems showing vegetative (Veg.), flowering (Flw.) or aborted (Abrt.) apices 8-11 weeks (accumulative over 4 weeks period) after start of SD.

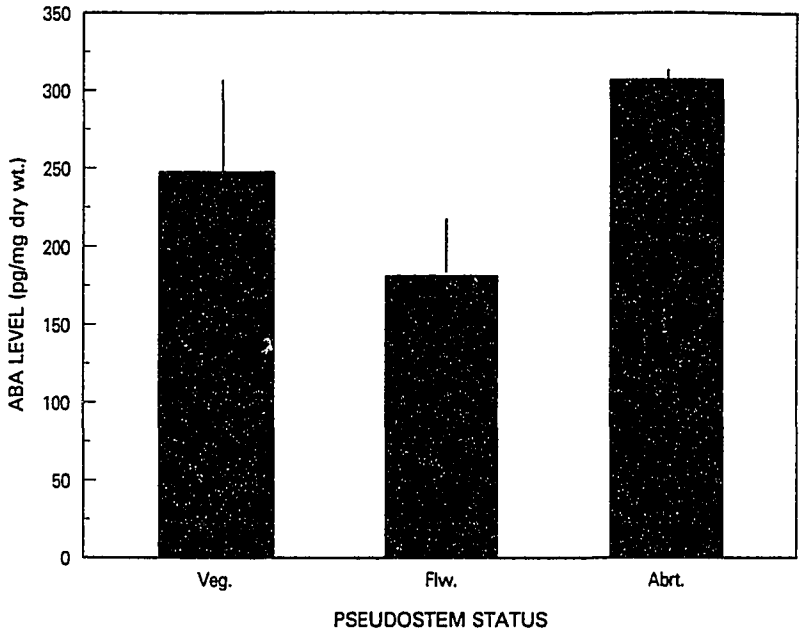

Figure 36. Concentration of ABA in leaf tissue from vegetative (Veg.), flowering (Flw.), or aborted (Abrt.) *Heliconia stricta* pseudostems apices based on average of stems sampled over 4 to 11 weeks after start of SD. Bars indicate mean ± SE.

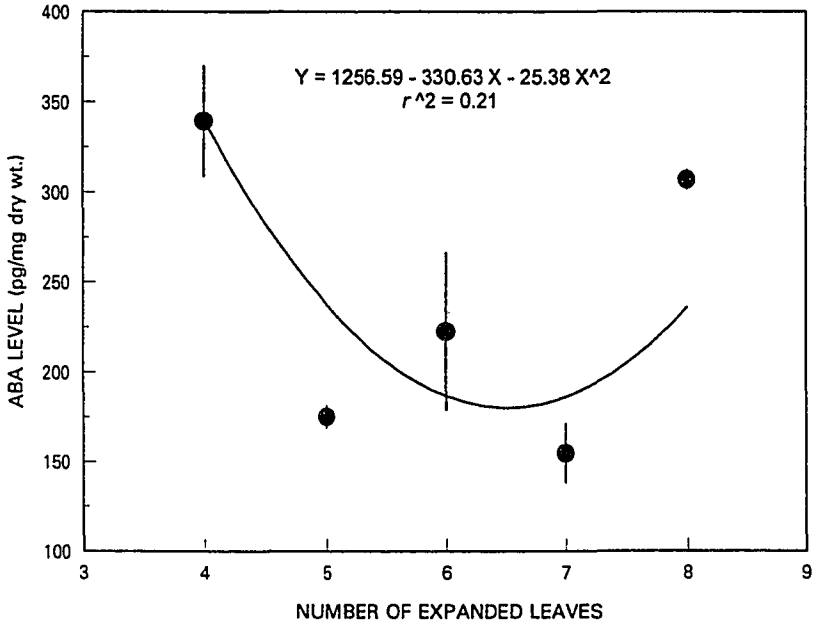

Figure 37. Leaf ABA levels of most recently matured leaf of *H. stricta* pseudostem with different number of expanded leaves based on average of stems sampled over 4 to 11 weeks after start of SD. Bars indicate mean ± SE.

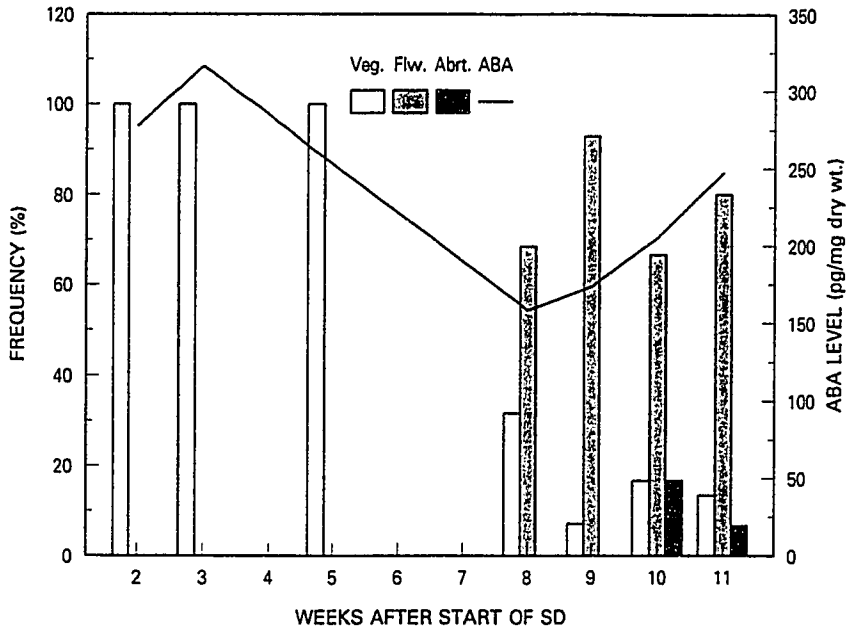

Figure 38. Percentage of pseudostems showing vegetative (Veg.), elongated (Elng.), flowering (Flw..) or aborted (Abrt.) apices and leaf ABA level (line) at the time samples were taken after the start of SD. No samples for weeks 6 and 7.

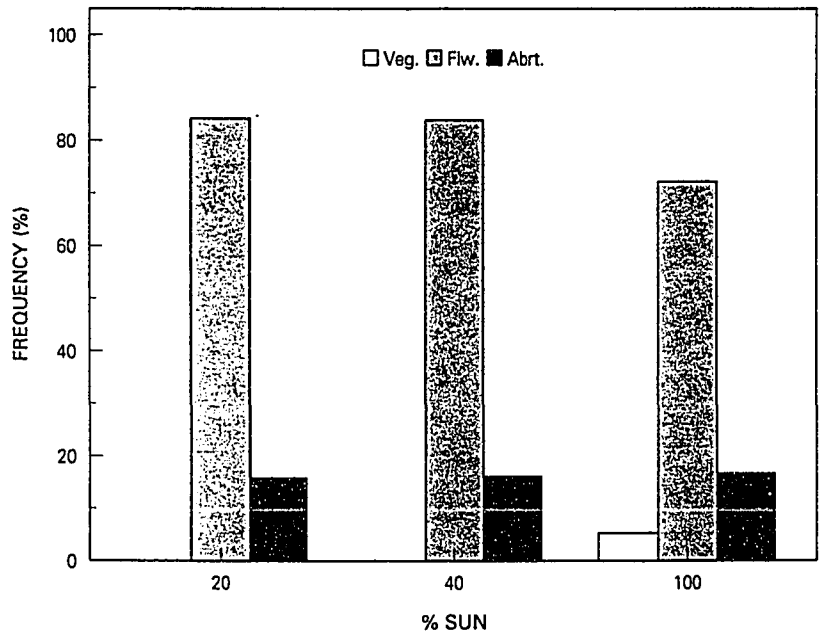

Figure 39. Effect of shading on percentage of pseudostems (bars) showing vegetative (Veg.), flowering (Flw..) or aborted (Abrt.) apices at time of experiment termination (18 weeks after the start of SD).

developing inflorescences. The developing inflorescences probably became visible during 6th and 7th weeks when no samples were taken. Inflorescences found at 8 week after SD were 0.3-2 cm long. Aborted inflorescences were visible at 10 weeks after start of SD.

SHOOT STATUS AT THE END OF THE EXPERIMENT

At the termination of the experiment (19 weeks after start of SD), different shade regimes had no significant effect on the proportion of flowering, vegetative or aborted pseudostems (Appendix A:Table 132). Percent flowering pseudostems of plants grown under 20% sun (84.2%) was slightly higher than those under 40% sun and full sun (77.4% and 78.9%, respectively, Figure 39). The percent of vegetative pseudostems was quite constant (5-7%). Plants under 20% sun had a lower percent abortion (10.5%) than those under 40% sun and full sun (16.1 and 15.8%, respectively).

FLOWERING PARAMETERS

There were no significant differences among shading treatments for time to anthesis (12.6 ±0.2 weeks after start of SD, Table 11 , Appendix A:Table 133), number of leaves subtending inflorescence (6.3 ± 0.1 leaves, Appendix A:Table 134), and number of cincinnal bracts within the inflorescence (2.2 ± 0.06 bracts, Appendix A:Table 135), at 5% level (Table 11). However, pseudostems of plants grown under 20% and 40% sun (54.9 ± 0.9 and 51.4 ± 0.7 cm) were significantly longer than those under full sun (44.5 ± 1.1 cm, Appendix A:Table 136). Inflorescence length for plants grown under 20% sun (33.5 ± 0.5 cm) were significantly greater than for those under 40% sun (31.9 ± 0.3 cm) and full sun (28.3 ± 0.6 cm, Appendix A:Table 137).

Leaf number at the start of SD had a significant positive linear relation with the final number of leaves before flowering (Figure 40, Appendix A:Table 138). Plants with

Table 11. Inflorescence and pseudostem length under different light intensity treatments.

Treatments	Time				
	Pseudostem length (cm.)	Time to anthesis (week)	No. of subtending leaves	Inflorescence length (cm)	No. of cincinnal bracts
20%sun	54.9a[z]	12.1a	6.4a	33.5a	2.2a
40%sun	51.4a	11.3a	6.1a	31.9b	2.3a
Full sun	44.5b	11.4a	6.7a	28.3c	2.1a

[z]Mean separation in columns by t-test at 5% level.

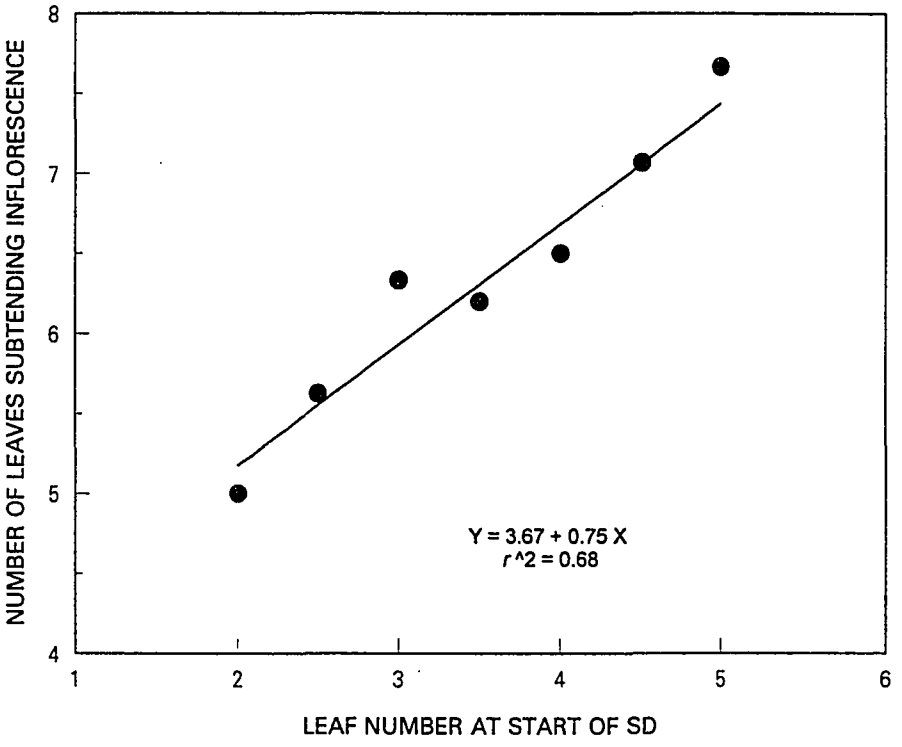

Figure 40. Effect of leaf number at the start of SD on number of leaves subtending inflorescence.

2 leaves at the start of SD added 3 leaves before flowering, while 5 leaf plants added an average of 2.6 leaves before flowering.

Leaf number at the start of SD had no significant linear relationship with number of weeks from start of SD to anthesis (Appendix A:Table 139).

DISCUSSION

This experiment produced no differences in proportion flowering and aborted pseudostems among different light intensity treatments. Many species of Heliconia were found to be light intensity limited (*H. psittacorum* and *H. angusta*) as an increase in light intensity increased flower production (Broschat and Donselman, 1982, 1983; Kwon, 1992). Broschat and Svenson (1994) reported that *H. stricta* 'Dwarf Jamaican' in full sun produced more flowers than those grown under 50% shade for a period of one year. However their finding was not conclusive since only 25% of the pseudostems in full sun flowered. This was probably due to the lack of a suitable short day induction period. The finding in this experiment suggests that *H. stricta* 'Dwarf Jamaican' can be grown under diverse light conditions without altering final percent flowering after receiving an initial 4 weeks of SD stimulus to induce flower initiation. The time from start of SD to anthesis was 12.6 weeks which was similar to the 13 weeks reported by Criley and Kawabata (1986) and in chapter 4 of this dissertation. The time to anthesis was not different among different light intensity treatments. However, increased light intensity significantly decreased plant height and inflorescence length. Lekawatana (1986) reported a flowering peak at 19 weeks after the start of SD. This may be due to plant materials having only 1-3 leaves at the time of SD, which postponed the susceptibility period.

ABA levels measured in leaves of aborted, vegetative or flowering pseudostems were not significantly different, similar to results of the temperature study in Chapter 4.

Foliage of plants grown under 20% and 40% sun contained higher leaf ABA than those grown under full sun. In temperature treatment 4 of Chapter 4, the environment (30/25°C D/N, PAR = 214 $\mu mol.s^{-1}.m^{-2}$) was similar to that of the 20% sun treatment of this chapter. Leaf ABA levels of the two similar treatments in different experiments were 264 ± 18.8 and 219.5 ± 22.4 pg/mg dry wt. for the high temperature of chapter 4 and low light of this experiment.

Foliar ABA content regressed on number of expanded leaves showed a quardratic relationship with the increase in leaf number. The foliar ABA content of the top mature leaf decreased with an increase in leaf number of the pseudostems. This result is similar to that in Chapter 4 and to Ross and McWha (1990) who fond that the ABA content of *Pisum sativum* leaflets toward the base of the plant was greater than at higher position in the plant. Foliar ABA content in this study was from the top most mature leaf of different stages of growth while those from Ross and McWha (1990) were from leaves locating on position of a plant. Therefore, interpretation has to be done carefully.

Fewer leaves were produced before flowering with plants that had more leaves at the start of SD. Plants with 2 to 3 leaves at the start of SD produced additional 3 leaves afterward while those with more than 3 leaves produced additional 2 leaves. A similar number of leaves subtended the inflorescences (6-7 leaves) no matter what the initial leaf count was. Bernier (1994) stated that most photoperiodic species, when shifted from noninductive to inductive conditions, went on initiating extra leaves before producing reproductive structures. However, *H. stricta* was reported to have already produced a total of 6 leaves at the time the second leaf expanded (Lekawatana, 1986).

Heliconia plants grown in full sun were shorter than those under shade. Cosgrove (1986) suspected that hormone metabolism was involved in the photoinhibition of pea stems by light. It was suggested that light might modify growth

in three potential ways: a reduction in GA synthesis, an increase in GA destruction, or a reduction in the plant's responsiveness to GA (Lockhart, 1959).

The anticipated differences in foliar ABA levels with stress of reduced light intensity did not parallel flower bud abortion under these conditions. Thus, it is not possible to conclude that a role for foliar-produced ABA exists in the abortion of the flower bud. However, since ABA was not analyzed in the pseudostem tissues where reproductive development was occurring, the question is far from settled.

The timing of the flower bud abortion appears to begin 10 weeks after the start of SD. The determination of shoot status was done by manual dissection. Therefore, the early stage of flower bud abortion might not be detected when compared with those in chapter 4 of this dissertation.

CHAPTER 6

CONCLUSION

With the attempt to control flower production of heliconia to ensure a steady supply of cut heliconia in the world market, we are just beginning to understand this plant through H. stricta 'Dwarf Jamaican' and other species. From these experiments and others we may conclude that:

PLANT GROWTH

LEAF LENGTH

Leaf growth parameters of *H. stricta* 'Dwarf Jamaican' were determined. Richard's growth curve were chosen to represent the leaf growth. The time required to produce each leaf increased minimally from leaf 3 to leaf 4. However, substantially more time was needed to produce leaves 5 and 6.

Environmental Effects

Light intensity affected *H. stricta* 'Dwarf Jamaican' growth. Plants grown under full sun were shorter than those under shade with smaller leaves and shorter petiole. It was suggested that light might modify growth in three potential ways: a reduction in GA synthesis, an increase in GA destruction, or a reduction in the plant's responsiveness to GA (Lockhart, 1959).

The number of leaves produced after SD for plants grown under LD_{3L}+SD, and LD_{4L}+SD was constant at 3 leaves. This reflected the number of leaves that already produced by the plants but not fully expanded yet.

The time increment between successive leaves of plants grown under conSD was significantly shorter than for those grown under conLD, LD_{3L}+SD and LD_{4L}+SD. Leaf

position had significant quadratic components with days to produce each leaf at the 1% level.

FLOWER INITIATION

The condition of flower initiation has been reported prior to these experiments. A minimum of 4 weeks of SD was required for flower initiation (Criley and Kawabata, 1986). During SD induction, decreased night temperature increased percent reproductive pseudostems (Lekawatana, 1986).

Flower initiation did not occur in plants grown under conLD and the plants remained vegetative and produced up to 8 to 9 leaves.

FLOWER DEVELOPMENT

H. stricta 'Dwarf Jamaican' responds well to a floral initial stimulus (4 weeks of SD) when plants have 3 or more leaves.

TEMPERATURE

As night temperature increased from 18°C to 28°C after the initial stimulus (4 weeks of SD) the percent of pseudostems that finally flowered decreased from 55% to 31%.

Average time to flower from the start of SD for all pseudostems grown at 18°C and 21°C was 18 weeks, which was one week later than those grown at 24°C and 28°C (under reduced energy of growth chamber condition).

LIGHT

After receiving an initial 4 weeks of SD stimulus to induce flower initiation, *H. stricta* 'Dwarf Jamaican' can be grown under diverse natural light conditions without altering final percent flowering. The percent flowering pseudostems for plants grown under

20% sun (84.2%) was slightly higher than for those under 40% sun and full sun (77.4% and 78.9%, respectively).

There was no different of time from start of SD to anthesis among shading treatments (12.6 weeks).

INFLORESCENCE ABORTION

The smallest developing inflorescence that was found to be aborted was 2 cm long and was at the stage when the second flower primordium was evident.

TEMPERATURE

The higher the temperature the more flower buds were aborted, ranging from 0% at 18°C to 19.2% at in 28°C in growth chamber condition.

Flower bud abortion was not found in plants grown at 18°C and was found 7 weeks after the start of SD for plants grown at 21°C. In plants grown at 24°C and 28°C, flower bud abortion found from 6 weeks after the start of SD.

LIGHT

There was no significant difference in inflorescence abortion for various shading treatments (natural light). Plants under 20% sun had a lower percent abortion (10.5%) than those under 40% sun and full sun (16.1 and 15.8%)

Flower bud abortion was detected by the 10^{th} week after the start of SD.

FOLIAR ABA LEVELS

Foliar ABA content of *H. stricta* 'Dwarf Jamaican' was successfully quantified by indirect ELISA. However, apex tissue ABA content was not reliably determined by this method due to interference such as impurity.

Foliar ABA level increased as temperature decreased. Foliar ABA level decreased as light intensity increased. ABA does not seem to induce flower bud abortion as flower bud

set was greater under conditions leading to high ABA levels in the foliage. However, since ABA was not analyzed in the pseudostem tissues where the reproductive development was occurring, this question is not settled.

PROGRAM FOR THE PRODUCTION OF FLOWERING H. STRICTA 'DWARF JAMAICAN'

Propagation: Clean rhizome pieces leaving 5 cm of pseudostem attached., dip or dust with fungicide., put in plastic bag at 20-25°C for 3 weeks to stimulate root and shoot growth.

Plant in a 1:1 ratio (v/v) perlite and vermiculite medium and held under mist for 1 week to increase root length.

Potting: Two rhizome pieces/15 cm pot. Place the rhizome pieces so that started eye just covered by the medium.

Medium: Well drained mixture of sphagnum peat and perlite. Amend with basic fertilizers: lime, superphosphate, minor elements according to normal practices. pH = 6.0 - 6.5.

Photoperiod: After pseudostems have developed 3 to 4 leaves, provide short day (SD: 8-9 hour of daylength) for 4 weeks.

Temperature: Before SD optimum temperature at 21°C

During SD optimum temperature at 15°C (night). High temperature increases percentage of aborted pseudostems.

After SD optimum temperature at 21°C

Light: Shading (20% sun to full sun) has no effect on flowering. Shortest plants are achieved in full sun light.

Watering: Daily

Timing: 4 weeks from propagation to potting

3 weeks from potting to develop 3 leaves (start of SD)

(5 weeks from potting to develop 4 leaves)

4 weeks of SD

13 weeks from start of SD to anthesis.

Note: - Prior to SD lower the temperature will slow down vegetative growth.

- During the flower development period (after the SD), lowering the temperature to 18°C will increase percentage flowering of pseudostems. However, longer time will be required for time to anthesis compared to those grown at 25°C.

APPENDIX A

TABLES

Table 1. ANOVA Effect of daylength treatments on number of leaves subtending inflorescence of H. stricta. CV = 0

Source	df	SS	MS	F	p
Daylength	2	2.3571	1.1786	99999.99	0.0000
Error	11	0.0000	0.0000		

Table 2. ANOVA Effect of daylength treatments on length of the last leaf subtending inflorescence of H. stricta. CV = 5.87

Source	df	SS	MS	F	p
Daylength	2	105.3966	52.6983	11.07	0.0023
Error	11	52.3720	4.7611		

Table 3. ANOVA Effect of daylength treatments on number cincinnal bracts of H. stricta. CV = 15.67

Source	df	SS	MS	F	p
Daylength	2	2.7619	1.3809	22.79	0.0001
Error	11	0.6667	0.0606		

Table 4. ANOVA Effect of daylength treatments on length of peduncle of H. stricta. CV = 7.62

Source	df	SS	MS	F	p
Daylength	2	14.7681	7.3734	5.47	0.0224
Error	11	14.8253	1.3477		

Table 5. ANOVA Effect of daylength treatments on length of inflorescence of H. stricta. CV = 20.19

Source	df	SS	MS	F	p
Daylength	2	29.4639	14.7319	1.45	0.2770
Error	11	112.0653	10.1877		

Table 6. ANOVA Effect of daylength treatments on length of inflorescence and peduncle combined of H. stricta. CV = 11.23

Source	df	SS	MS	F	p
Daylength	2	31.8463	15.9231	1.31	0.3090
Error	11	133.7880	12.1625		

Table 7. ANOVA Effect of daylength treatments on number of days to from potting to last leaf emergence of H. stricta. CV = 8.10

Source	df	SS	MS	F	p
Daylength	2	1187.6571	593.8285	18.81	0.0003
Error	11	347.2000	31.5636		

Table 8. ANOVA Effect of daylength treatments on number of days from time of last leaf emergence to inflorescence emergence of H. stricta. CV = 15.60

Source	df	SS	MS	F	p
Daylength	2	78.2476	39.1238	5.04	0.028
Error	11	85.4666	7.7696		

Table 9. ANOVA Effect of daylength treatments on number of days to from time of inflorescence emergence to anthesis of H. stricta. CV = 11.56

Source	df	SS	MS	F	p
Daylength	2	507.6571	253.8285	15.76	0.0006
Error	11	177.2000	16.1090		

Table 10. ANOVA Effect of daylength treatments on number of days to anthesis from potting of H. stricta. CV = 4.65

Source	df	SS	MS	F	p
Daylength	2	2299.0476	1149.5238	35.65	0.0001
Error	11	354.6666	32.2424		

Table 11. ANOVA Effect of daylength treatments on number of days to inflorescence emergence from started of SD treatments of H. stricta. CV = 6.89

Source	df	SS	MS	F	p
Daylength	1	162	162.0000	11.12	0.0125
Error	7	102	14.5714		

Table 12. ANOVA Effect of daylength treatments on number of days to anthesis from started of SD treatments of H. stricta. CV = 4.71

Source	df	SS	MS	F	p
Daylength	1	22.2222	22.2222	0.71	0.4269
Error	7	218.6666	31.2380		

Table 13. ANOCOVA Effect of daylength treatments and leaf position on leaf length of H. stricta. CV = 7.8

Source	df	SS	MS	F	p
Daylength (DL)	3	492.3735	164.1245	29.47	0.0001
LF	1	10424.5696	10424.5696	1871.63	0.0001
DL*LF	3	117.3620	39.1206	7.02	0.0001
LF2	1	19.0039	19.0039	3.41	0.0657
DL*LF2	3	52.7077	17.5692	3.15	0.0252
Error	296	1648.6545	5.5697		

Table 14. ANOCOVA Effect of daylength treatments and leaf position on days from potting to leaf emergence of H. stricta. CV = 13.34

Source	df	SS	MS	F	p
Daylength (DL)	3	670.3076	223.4358	10.21	0.0001
LF (Regr.)	1	97522.0437	97522.0437	4455.99	0.0001
DL*LF	3	383.7618	127.9206	5.84	0.0007
LF2 (Regr.)	1	371.8882	371.8882	16.99	0.0001
DL*LF2	3	2.9061	0.9687	0.04	0.9876
Error	263	5755.9141	21.8856		

Table 15. ANOCOVA Effect of daylength treatments and leaf position on days to produce each leaf from time of previous leaf emergence of H. stricta. CV = 17.50

Source	df	SS	MS	F	p
Daylength (DL)	3	164.7542	54.9180	8.54	0.0001
LF	1	1641.6439	1641.6439	255.26	0.0001
DL*LF	3	9.6376	3.2125	0.50	0.6829
LF2	1	227.3749	227.3749	35.36	0.0001
DL*LF2	3	27.1467	9.0489	1.41	0.2412
Error	256	1646.3826	6.4311		

Table 16. ANOCOVA Effect of daylength treatments and leaf position on leaf unfolding rate (cm/day) of H. stricta. CV = 13.34

Source	df	SS	MS	F	p
Daylength (DL)	3	0.05213	0.0173	5.55	0.0011
LF	1	1.5171	1.5171	484.16	0.0001
DL*LF	3	0.0237	0.0079	2.52	0.0583
LF2	1	0.0493	0.0493	15.75	0.0001
DL*LF2	3	0.0089	0.0029	0.95	0.4177
Error	256	0.08021	0.0031		

Table 17. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 2nd leaf of *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	112352.6739	28088.1684
Residual	300	1305.7160	4.3523
Total	304	113658.3900	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	21.6057	0.1762	1			
β	3.6473	1.4365	-0.3841	1		
γ	0.3630	0.0895	-0.4500	0.9846	1	
δ	3.6042	1.4975	-0.3860	0.9945	0.9691	1

Table 18. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 2nd leaf of *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	109913.3645	27478.3411
Residual	274	732.0854	2.6718
Total	278	110645.4500	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	2.3375	0.1363	1			
β	5.2869	1.5878	-0.3303	1		
γ	0.4517	0.1015	-0.3774	0.9910	1	
δ	4.9804	1.5722	-0.3346	0.9943	0.9778	1

Table 19. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 2nd leaf of *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	145400.7141	36350.1785
Residual	356	2600.1558	7.3038
Total	360	148000.8700	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	22.4544	0.2095		1		
β	3.4386	1.6649	-0.3874	1		
γ	0.3378	0.0987	-0.4552	0.9837	1	
δ	3.6168	1.8277	-0.3896	0.9948	0.9686	1

Table 20. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 2nd leaf of *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	52966.5255	1324.6313
Residual	170	468.6644	2.7568
Total	174	53435.1900	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	19.8910	0.2333	1			
β	0.6125	1.4813	-0.5254	1		
γ	0.2595	0.1596	-0.6432	0.9604	1	
δ	1.0269	0.9869	-0.5235	0.9986	0.9507	1

Table 21. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 3rd leaf of *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	148011.3659	37002.8414
Residual	294	905.5341	3.0800
Total	298	148916.9001	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	25.1283	0.1453	1			
β	4.7402	1.2990	-0.3477	1		
γ	0.4147	0.0834	-0.4006	0.9894	1	
δ	4.7087	1.3837	-0.3524	0.9946	0.9760	1

Table 22. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 3rd leaf of *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	139739.0939	34934.7734
Residual	277	742.6060	2.6809
Total	281	140481.7000	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	24.8301	0.1333	1			
β	5.2422	1.4402	-0.3215	1		
γ	0.4577	0.0962	-0.3684	0.9908	1	
δ	5.1814	1.5290	-0.3263	0.9945	0.9779	1

Table 23. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 3rd leaf of *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	190285.7668	47571.4417
Residual	360	2483.6031	6.8989
Total	364	192769.3700	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	25.5650	0.2055	1			
β	2.9313	1.2846	-0.3986	1		
γ	0.3093	0.0719	-0.4739	0.9804	1	
δ	3.0351	1.3491	-0.3993	0.9951	0.9646	1

Table 24. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 3rd leaf of *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	74161.4291	18540.3572
Residual	175	844.4708	4.8255
Total	179	75005.9000	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	23.2115	0.2768	1			
β	2.5544	1.5606	-0.4645	1		
γ	0.3281	0.0962	-0.5510	0.9782	1	
δ	2.7660	1.6459	-0.4658	0.9959	0.9643	1

Table 25. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	233300.0379	58325.0094
Residual	339	1677.5720	4.9485
Total	343	234977.6100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	28.6421	0.1682	1			
β	2.2387	0.9759	-0.3680	1		
γ	0.2972	0.0532	-0.4475	0.9748	1	
δ	2.3699	0.9631	-0.3677	0.99575	0.9587	1

Table 26. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	222047.3724	55511.8431
Residual	317	1033.0875	3.2589
Total	321	223080.4600	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	28.9269	0.1387	1			
β	2.9284	0.8752	-0.3478	1		
γ	0.3082	0.0483	-0.4171	0.9803	1	
δ	2.9989	0.9088	-0.3485	0.9949	0.9638	1

Table 27. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	294609.5966	73652.3991
Residual	398	970.9333	2.4395
Total	402	295580.5300	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	29.7674	0.1069	1			
β	3.1764	0.6820	-0.3451	1		
γ	0.3369	0.0406	-0.4116	0.9819	1	
δ	3.2713	0.7235	-0.3465	0.9947	0.9656	1

Table 28. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	100801.7829	25200.4457
Residual	166	701.9370	4.2285
Total	170	101503.7200	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	27.2928	0.2296	1			
β	3.4877	1.5436	-0.3559	1		
γ	0.4008	0.1088	-0.4234	0.98415	1	
δ	3.8407	1.7657	-0.3609	0.9952	0.9703	1

Table 29. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	342441.7918	85610.4479
Residual	363	1606.1481	4.4246
Total	367	344047.9400	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	33.8665	0.1566	1			
β	1.9173	0.6977	-0.3629	1		
γ	0.2344	0.2917	-0.4496	0.9713	1	
δ	1.8876	0.6065	-0.3605	0.9959	0.9540	1

Table 30. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	258829.9386	64707.4846
Residual	302	801.7013	2.6546
Total	306	259631.6400	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	32.6746	0.1359	1			
β	3.2614	0.6889	-0.3593	1		
γ	0.2793	0.0339	-0.4301	0.9823	1	
δ	3.2322	0.7105	-0.3607	0.9945	0.9658	1

Table 31. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	488877.8346	122219.4586
Residual	499	2636.9154	5.2844
Total	503	491514.7500	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	34.2042	0.1376	1			
β	2.9508	0.7464	-0.3275	1		
γ	0.2694	0.0356	-0.3946	0.9800	1	
δ	2.8561	0.7377	-0.3273	0.9945	0.9624	1

Table 32. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	2101233.9444	50308.4861
Residual	242	1032.2455	4.2654
Total	246	202266.1900	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	31.1602	0.1759	1			
β	3.0621	1.1208	-0.3297	1		
γ	0.3403	0.0679	-0.3978	0.9800	1	
δ	3.2689	1.2192	-0.3313	0.9948	0.9638	1

Table 33. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	485850.7930	121462.6982
Residual	404	1728.3869	4.2781
Total	408	487579.1800	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	37.4570	0.1367	1			
β	1.7456	0.6589	-0.3294	1		
γ	0.2060	0.0240	-0.4139	0.9686	1	
δ	1.9321	0.6118	-0.3281	0.9965	0.9526	1

Table 34. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	360423.1029	90105.7757
Residual	326	1581.0470	4.8498
Total	330	362004.1500	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	35.8116	0.1585	1			
β	3.6104	1.0505	-0.3073	1		
γ	.3091	0.0554	-0.3671	0.9839	1	
δ	.0751	1.2435	-0.3102	0.9947	0.9687	1

Table 35. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	668137.2106	167034.3026
Residual	559	2018.7993	3.6114
Total	563	670156.0100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	37.1652	0.1026	1			
β	3.0467	0.6160	-0.2994	1		
γ	0.2613	0.2858	-0.3617	0.9806	1	
δ	3.3074	0.6827	-0.3008	0.9949	0.9642	1

Table 36. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	206067.7969	51516.9492
Residual	224	815.0230	3.6385
Total	228	206882.8200	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	34.1470	0.2140	1			
β	2.1501	0.8172	-0.4742	1		
γ	0.2168	0.0333	-0.5668	0.9748	1	
δ	2.4138	0.8425	-0.4745	0.9966	0.9616	1

Table 37. RSS from fitting the 2nd leaf data of Heliconia on each treatment with common α, β, γ, and δ.

Treatment		M	df	RSS	RMS
ConLD		4	300	1305.7161	
3L-SD		4	274	732.0855	
4L-SD		4	356	2600.1558	
ConSD		4	170	468.6644	
(A)	Total	16	1100	5106.6218	4.6424

Table 38. RSS from fitting the 3rd leaf data of Heliconia on each treatment with common α, β, γ, and δ.

Treatment		M	df	RSS	RMS
ConLD		4	294	905.5341	
3L-SD		4	277	742.6061	
4L-SD		4	360	2483.6032	
ConSD		4	175	844.6032	
(A)	Total	16	1106	4976.2143	4.4993

Table 39. RSS from fitting the 4th leaf data of Heliconia on each treatment with common α, β, γ, and δ.

Treatment		M	df	RSS	RMS
ConLD		4	339	1677.5720	
3L-SD		4	317	1033.0875	
4L-SD		4	398	970.9333	
ConSD		4	166	701.9370	
(A)	Total	16	1220	4383.5298	3.5930

Table 40. RSS from fitting the 5th leaf data of Heliconia on each treatment with common α, β, γ, and δ.

Treatment		M	df	RSS	RMS
ConLD		4	363	1606.1482	
3L-SD		4	302	801.7613	
4L-SD		4	499	2636.9154	
ConSD		4	242	1032.2456	
(A)	Total	16	1406	6077.0105	4.3222

Table 41. RSS from fitting the 6th leaf data of Heliconia on each treatment with common α, β, γ, and δ.

Treatment		M	df	RSS	RMS
ConLD		4	404	1728.3869	
3L-SD		4	326	1581.0471	
4L-SD		4	559	2018.7994	
ConSD		4	224	815.0231	
(A)	Total	16	1513	6143.2565	4.0606

Table 42. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for conLD and 3L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	575	2051.33	
Common β	7	575	2039.66	
Common γ	7	575	2039.10	
Common δ	7	575	2039.11	
Common α,β,γ,δ	4	578	2067.34	
Individual α,β,γ,δ	8	574	2037.80	3.5502

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	29.54	7.38	1.589	NS
test of invariat α	1	13.52	13.52	2.913	NS
test of invariat β	1	1.86	1.86	0.400	NS
test of invariat γ	1	1.30	1.30	0.280	NS
test of invariat δ	1	1.31	1.31	0.282	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 43. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for conLD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	657	3955.08	
Common β	7	657	3905.91	
Common γ	7	657	3906.03	
Common δ	7	657	3905.88	
Common α,β,γ,δ	4	660	4005.81	
Individual α,β,γ,δ	8	656	3905.87	5.9541

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	99.94	24.98	5.380	**
test of invariat α	1	49.21	49.21	10.600	**
test of invariat β	1	0.045	0.045	0.009	NS
test of invariat γ	1	0.16	0.16	0.034	NS
test of invariat δ	1	0.01	0.01	0.002	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 44. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for conLD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	471	1864.40	
Common β	7	471	1781.32	
Common γ	7	471	1801.96	
Common δ	7	471	1780.35	
Common α,β,γ,δ	4	474	1971.52	
Individual α,β,γ,δ	8	470	1774.38	3.7753

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	197.14	49.28	10.615	**
test of invariat α	1	90.02	90.02	19.390	**
test of invariat β	1	6.95	6.95	1.497	NS
test of invariat γ	1	27.58	27.58	5.940	*
test of invariat δ	1	5.97	5.97	1.286	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 45. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for 3L-SD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	631	3333.26	
Common β	7	631	3334.96	
Common γ	7	631	3334.81	
Common δ	7	631	3334.57	
Common α,β,γ,δ	4	634	3378.36	
Individual α,β,γ,δ	8	630	3332.24	5.289

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	46.14	11.53	2.483	*
test of invariat α	1	1.02	1.02	0.219	NS
test of invariat β	1	2.72	2.72	0.586	NS
test of invariat γ	1	2.57	2.57	0.554	NS
test of invariat δ	1	1.33	1.33	0.286	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 46. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for 3L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	445	1438.36	
Common β	7	445	1214.96	
Common γ	7	445	1207.58	
Common δ	7	445	1211.96	
Common α,β,γ,δ	4	448	1597.74	
Individual α,β,γ,δ	8	444	1200.75	2.70

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	396.9	99.24	21.376	**
test of invariat α	1	237.61	237.61	51.182	**
test of invariat β	1	14.06	14.06	3.028	NS
test of invariat γ	1	6.83	6.83	1.471	NS
test of invariat δ	1	11.21	11.21	2.414	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 47. Comparison of fits for Heliconia 2nd leaf data to test invariance of α, β, γ and δ for 4L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	527	3437.44	
Common β	7	527	3075.32	
Common γ	7	527	3153.42	
Common δ	7	527	3075.11	
Common α,β,γ,δ	4	530	3506.84	
Individual α,β,γ,δ	8	526	3068.82	5.834

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	438.02	109.50	23.587	**
test of invariat α	1	368.62	368.62	79.402	**
test of invariat β	1	6.50	6.50	1.400	NS
test of invariat γ	1	84.60	84.60	18.223	**
test of invariat δ	1	6.29	6.29	1.355	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 37.

Table 48. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for conLD and 3L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	572	1654.47	
Common β	7	572	1648.31	
Common γ	7	572	1648.43	
Common δ	7	572	1655.14	
Common α,β,γ,δ	4	575	1655.54	
Individual α,β,γ,δ	8	571	1648.14	2.89

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	7.40	1.85	0.411	NS
test of invariat α	1	6.33	6.33	1.407	NS
test of invariat β	1	0.17	0.17	0.038	NS
test of invariat γ	1	0.29	0.29	0.064	NS
test of invariat δ	1	7.40	7.40	1.645	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 38.

Table 49. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for conLD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	655	3402.83	
Common β	7	655	3393.02	
Common γ	7	655	3392.60	
Common δ	7	655	3408.68	
Common α,β,γ,δ	4	658	3435.11	
Individual α,β,γ,δ	8	654	3389.14	5.18

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	45.96	11.49	2.553	*
test of invariat α	1	13.69	13.69	3.042	NS
test of invariat β	1	3.88	3.88	0.862	NS
test of invariat γ	1	3.46	3.46	0.769	NS
test of invariat δ	1	19.54	19.54	4.342	*

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 38

Table 50. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for conLD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	470	1854.34	
Common β	7	470	1753.64	
Common γ	7	470	1751.39	
Common δ	7	470	1752.52	
Common α,β,γ,δ	4	473	1987.47	
Individual α,β,γ,δ	8	469	1750.00	3.73

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	237.47	59.36	13.193	**
test of invariat α	1	104.34	104.34	23.190	**
test of invariat β	1	3.64	3.64	0.809	NS
test of invariat γ	1	1.39	1.39	0.309	NS
test of invariat δ	1	2.52	2.52	0.560	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 38

Table 51. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for 3L-SD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	638	3245.52	
Common β	7	638	3231.54	
Common γ	7	638	3231.73	
Common δ	7	638	3239.09	
Common α,β,γ,δ	4	641	3273.77	
Individual α,β,γ,δ	8	637	3226.21	5.06

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	47.56	11.89	2.64	*
test of invariat α	1	18.98	18.98	4.217	*
test of invariat β	1	5.33	5.33	1.185	NS
test of invariat γ	1	5.52	5.52	1.227	NS
test of invariat δ	1	12.88	12.88	2.862	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 38.

Table 52. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for 3L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	453	1676.80	
Common β	7	453	1591.85	
Common γ	7	453	1589.62	
Common δ	7	453	1590.45	
Common α,β,γ,δ	4	456	1770.78	
Individual α,β,γ,δ	8	452	1587.08	3.51

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	183.70	45.92	10.206	**
test of invariat α	1	89.72	89.72	19.940	**
test of invariat β	1	4.77	4.77	1.060	NS
test of invariat γ	1	2.52	2.52	0.560	NS
test of invariat δ	1	3.37	3.37	0.749	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 38.

Table 53. Comparison of fits for Heliconia 3th leaf data to test invariance of α, β, γ and δ for 4L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	536	3489.03	
Common β	7	536	3328.22	
Common γ	7	536	3328.17	
Common δ	7	536	3328.15	
Common α,β,γ,δ	4	539	3679.16	
Individual α,β,γ,δ	8	535	3328.07	

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	351.09	87.77	19.507	**
test of invariat α	1	160.96	160.96	35.774	**
test of invariat β	1	0.15	0.15	0.033	NS
test of invariat γ	1	0.10	0.10	0.022	NS
test of invariat δ	1	0.08	0.08	0.018	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 38.

Table 54. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for conLD and 3L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	657	2716.97	
Common β	7	657	2711.50	
Common γ	7	657	2710.72	
Common δ	7	657	2711.35	
Common α,β,γ,δ	4	652	2739.03	
Individual α,β,γ,δ	8	656	2710.66	4.13

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	28.37	7.09	1.973	NS
test of invariat α	1	6.31	6.31	1.756	NS
test of invariat β	1	0.84	0.84	0.233	NS
test of invariat γ	1	0.06	0.06	0.016	NS
test of invariat δ	1	0.69	0.69	0.192	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 39.

Table 55. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for conLD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	738	2660.71	
Common β	7	738	2650.24	
Common γ	7	738	2649.41	
Common δ	7	738	2650.04	
Common α,β,γ,δ	4	741	2680.54	
Individual α,β,γ,δ	8	737	2648.50	3.59

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	32.04	8.01	2.229	NS
test of invariat α	1	12.20	12.20	3.395	NS
test of invariat β	1	1.74	1.74	0.484	NS
test of invariat γ	1	0.91	0.91	0.253	NS
test of invariat δ	1	1.54	1.54	0.428	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 39.

Table 56. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for conLD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	506	2464.89	
Common β	7	506	2381.49	
Common γ	7	506	2382.89	
Common δ	7	506	2381.91	
Common α,β,γ,δ	4	509	2484.69	
Individual α,β,γ,δ	8	505	2379.51	4.71

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	105.18	26.295	7.318	**
test of invariat α	1	85.38	85.38	23.763	**
test of invariat β	1	1.98	1.98	0.551	NS
test of invariat γ	1	3.38	3.38	0.940	NS
test of invariat δ	1	2.4	2.4	0.668	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 39.

Table 57. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 3L-SD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	716	2014.74	
Common β	7	716	2004.14	
Common γ	7	716	2004.47	
Common δ	7	716	2004.14	
Common α,β,γ,δ	4	719	2030.82	
Individual α,β,γ,δ	8	715	2004.02	2.80

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	26.80	6.70	1.865	NS
test of invariat α	1	10.72	10.72	2.983	NS
test of invariat β	1	0.11	0.11	0.030	NS
test of invariat γ	1	0.45	0.45	0.125	NS
test of invariat δ	1	0.12	0.12	0.033	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 39.

Table 58. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 3L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	484	1852.60	
Common β	7	484	1735.39	
Common γ	7	484	1737.58	
Common δ	7	484	1735.73	
Common α,β,γ,δ	4	487	1894.33	
Individual α,β,γ,δ	8	483	1735.02	3.5922

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	159.31	39.83	11.085	**
test of invariat α	1	117.58	117.58	32.725	**
test of invariat β	1	0.37	0.37	0.103	NS
test of invariat γ	1	2.56	2.56	0.712	NS
test of invariat δ	1	0.71	0.71	0.197	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 39.

Table 59. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 4L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	565	2101.34	
Common β	7	565	1672.99	
Common γ	7	565	1674.09	
Common δ	7	565	1673.20	
Common α,β,γ,δ	4	568	2087.00	
Individual α,β,γ,δ	8	564	1672.87	2.96

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	414.13	100.53	27.979	**
test of invariat α	1	428.47	428.47	119.25	**
test of invariat β	1	0.12	0.12	0.033	NS
test of invariat γ	1	1.22	1.22	0.339	NS
test of invariat δ	1	0.33	0.33	0.091	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 39.

Table 60. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD and 3L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	666	2510.85	
Common β	7	666	2413.47	
Common γ	7	666	2410.67	
Common δ	7	666	2414.05	
Common α,β,γ,δ	4	669	2635.95	
Individual α,β,γ,δ	8	665	2407.85	3.62

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	228.10	57.03	13.194	**
test of invariat α	1	103.00	103.00	23.830	**
test of invariat β	1	5.62	5.62	1.300	NS
test of invariat γ	1	2.82	2.82	0.652	NS
test of invariat δ	1	6.2	6.2	1.434	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 61. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	863	4254.17	
Common β	7	863	4246.97	
Common γ	7	863	4245.14	
Common δ	7	863	4247.07	
Common α,β,γ,δ	4	866	4270.08	
Individual α,β,γ,δ	8	862	4243.06	4.92

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	27.02	6.76	1.564	NS
test of invariat α	1	11.11	11.11	2.570	NS
test of invariat β	1	3.92	3.92	0.906	NS
test of invariat γ	1	2.08	2.08	0.481	NS
test of invariat δ	1	4.01	4.01	0.927	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 62. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	606	3176.80	
Common β	7	606	2641.50	
Common γ	7	606	2648.48	
Common δ	7	606	2643.08	
Common α,β,γ,δ	4	609	3258.22	
Individual α,β,γ,δ	8	605	2638.39	4.3609

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	619.83	154.95	35.849	**
test of invariat α	1	538.41	538.41	124.568	**
test of invariat β	1	3.11	3.11	0.719	NS
test of invariat γ	1	10.09	10.09	2.334	NS
test of invariat δ	1	4.69	4.69	1.085	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 63. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 3L-SD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	802	3629.64	
Common β	7	802	3438.91	
Common γ	7	802	3438.73	
Common δ	7	802	3439.04	
Common α,β,γ,δ	4	805	3794.19	
Individual α,β,γ,δ	8	801	3438.62	4.29

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	355.57	88.89	20.566	**
test of invariat α	1	191.02	191.02	44.195	**
test of invariat β	1	0.29	0.29	0.067	NS
test of invariat γ	1	0.11	0.11	0.025	NS
test of invariat δ	1	0.42	0.42	0.097	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 64. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 3L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	545	1987.18	
Common β	7	545	1834.02	
Common γ	7	545	1836.41	
Common δ	7	545	1833.95	
Common α,β,γ,δ	4	548	2097.45	
Individual α,β,γ,δ	8	544	1833.94	3.37

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	263.51	65.87	15.239	**
test of invariat α	1	153.24	153.24	35.454	**
test of invariat β	1	0.08	0.08	0.018	NS
test of invariat γ	1	2.47	2.47	0.571	NS
test of invariat δ	1	0.01	0.01	0.002	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 65. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 4L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	742	4502.99	
Common β	7	742	3669.18	
Common γ	7	742	3673.13	
Common δ	7	742	3669.51	
Common α,β,γ,δ	4	745	4599.15	
Individual α,β,γ,δ	8	741	3669.16	4.95

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	929.99	232.49	53.789	**
test of invariat α	1	833.74	833.74	192.897	**
test of invariat β	1	0.03	0.03	0.006	NS
test of invariat γ	1	3.97	3.97	0.918	NS
test of invariat δ	1	0.35	0.35	0.080	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 40.

Table 66. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD and 3L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	731	3574.99	
Common β	7	731	3320.77	
Common γ	7	731	3325.69	
Common δ	7	731	3322.49	
Common α,β,γ,δ	4	734	3597.56	
Individual α,β,γ,δ	8	730	3309.43	4.53

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	288.13	72.03	17.740	**
test of invariat α	1	265.56	265.56	65.404	**
test of invariat β	1	11.34	11.34	2.793	NS
test of invariat γ	1	16.26	16.26	4.004	*
test of invariat δ	1	13.06	13.06	3.216	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 41.

Table 67. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	964	3758.42	
Common β	7	964	3754.84	
Common γ	7	964	3754.77	
Common δ	7	964	3755.35	
Common α,β,γ,δ	4	967	3762.40	
Individual α,β,γ,δ	8	963	3747.18	3.89

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	15.22	3.80	0.935	NS
test of invariat α	1	11.24	11.24	2.768	NS
test of invariat β	1	7.66	7.66	1.886	NS
test of invariat γ	1	7.59	7.59	1.869	NS
test of invariat δ	1	8.17	8.17	2.012	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 41.

Table 68. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	629	3257.75	
Common β	7	629	2543.95	
Common γ	7	629	2543.65	
Common δ	7	629	2544.21	
Common $\alpha,\beta,\gamma,\delta$	4	632	3702.45	
Individual $\alpha,\beta,\gamma,\delta$	8	628	2543.41	4.05

	df	change in RSS	MS	Fy	pz
test of invariat $\alpha,\beta,\gamma,\delta$	4	1159.04	289.76	71.364	**
test of invariat α	1	714.34	714.34	175.933	**
test of invariat β	1	0.54	0.54	0.133	NS
test of invariat γ	1	0.24	0.24	0.059	NS
test of invariat δ	1	0.80	0.80	0.197	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 41.

Table 69. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 3L-SD and 4L-SD.

Description of fit or test	M	df	RSS	RMS
Common α	7	886	3810.61	
Common β	7	886	3600.81	
Common γ	7	886	3602.63	
Common δ	7	886	3601.22	
Common $\alpha,\beta,\gamma,\delta$	4	889	3840.46	
Individual $\alpha,\beta,\gamma,\delta$	8	885	3599.85	4.07

	df	change in RSS	MS	Fy	pz
test of invariat $\alpha,\beta,\gamma,\delta$	4	240.61	60.15	14.814	**
test of invariat α	1	210.76	210.76	51.907	**
test of invariat β	1	0.96	0.96	0.236	NS
test of invariat γ	1	2.78	2.78	0.685	NS
test of invariat δ	1	1.37	1.37	0.337	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 41.

Table 70. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 3L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	551	2509.34	
Common β	7	551	2401.27	
Common γ	7	551	2405.07	
Common δ	7	551	2401.49	
Common α,β,γ,δ	4	554	2924.68	
Individual α,β,γ,δ	8	550	2396.07	4.36

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	528.61	132.15	32.546	**
test of invariat α	1	113.27	113.27	27.896	**
test of invariat β	1	5.20	5.20	1.280	NS
test of invariat γ	1	9.00	9.00	2.216	NS
test of invariat δ	1	5.42	5.42	1.334	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 41.

Table 71. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 4L-SD and conSD.

Description of fit or test	M	df	RSS	RMS
Common α	7	784	3491.59	
Common β	7	784	2836.41	
Common γ	7	784	2837.01	
Common δ	7	784	2836.05	
Common α,β,γ,δ	4	787	4061.42	
Individual α,β,γ,δ	8	783	2833.82	3.62

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	1227.6	306.9	75.585	**
test of invariat α	1	657.77	657.77	162.00	**
test of invariat β	1	2.59	2.59	0.637	NS
test of invariat γ	1	3.19	3.19	0.786	NS
test of invariat δ	1	2.23	2.23	0.549	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 41.

Table 72. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of non flowered *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	175948.0437	43987.0109
Residual	263	1122.1862	4.2668
Total	267	177070.2300	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	28.0046	0.1691	1			
β	3.0521	1.2056	-0.3309	1		
γ	0.3691	0.0797	-0.3965	0.9810	1	
δ	3.2739	1.3146	-0.3329	0.9949	0.9652	1

Table 73. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of flowered *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	103927.7656	25981.9414
Residual	142	195.5043	1.3767
Total	146	104123.2700	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	29.3179	0.1294	1			
β	4.3158	1.0689	-0.3155	1		
γ	0.3992	0.0685	-0.3679	0.9877	1	
δ	4.4059	1.1657	-0.3189	0.9943	0.9729	1

Table 74. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of flowered *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	56465.2043	14116.3010
Residual	69	27.1856	0.39399
Total	73	56492.3900	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	30.5370	0.0986	1			
β	3.7126	0.6888	-0.3261	1		
γ	0.3681	0.0430	-0.3848	0.9849	1	
δ	3.7623	0.7367	-0.3283	0.9943	0.9689	1

Table 75. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 4th leaf of flowered *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	40859.0522	10214.7630
Residual	60	27.0977	0.4516
Total	64	40886.1500	

			Correlation Matrix			
Parameter	estimate	Standard	α	β	γ	δ
α	28.5999	0.1228	1			
β	4.8635	0.9997	-0.3522	1		
γ	0.4930	0.0755	-0.4052	0.9904	1	
δ	5.1303	1.1239	-0.3593	0.9952	0.9791	1

Table 76. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of non flowered *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	248331.3266	62082.8316
Residual	270	1268.8733	4.6995
Total	274	249600.2000	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	33.4593	0.1876	1			
β	2.2148	0.8499	-0.3633	1		
γ	0.2522	0.0386	-0.4463	0.9741	1	
δ	2.1538	0.7756	-0.36146	0.9954	0.9566	1

Table 77. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of flowered *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	110224.9248	27556.2312
Residual	125	167.2751	1.3382
Total	129	110392.2000	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	33.0549	0.1506	1			
β	4.0611	0.8677	-0.3640	1		
γ	0.3328	0.0480	-0.4265	0.9866	1	
δ	4.1883	0.9533	-0.3681	0.9946	0.9722	1

Table 78. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of flowered *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	72761.8491	18190.4622
Residual	74	294.9608	3.9859
Total	78	73056.8100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	34.1093	0.3036	1			
β	6.1882	2.8743	-0.2974	1		
γ	0.4328	0.1638	-0.3365	0.9932	1	
δ	5.8219	2.9090	-0.3025	0.9475	0.9815	1

Table 79. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 5th leaf of flowered *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	108233.1632	27058.2908
Residual	115	303.7467	2.6412
Total	119	108536.9100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	32.4166	0.1855	1			
β	5.1909	2.0227	-0.2722	1		
γ	0.4895	0.1454	-0.3131	0.9908	1	
δ	5.7856	2.4047	-0.2771	0.9946	0.9781	1

Table 80. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of non flowered *Heliconia stricta* in conLD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	370433.0114	92608.2527
Residual	311	1498.9988	4.8199
Total	315	371932.0100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	37.1862	0.1658	1			
β	1.3446	0.8158	-0.3330	1		
γ	0.1949	0.0270	-0.4243	0.9638	1	
δ	1.5961	0.6997	-0.3315	0.9972	0.9492	1

Table 81. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of flowered *Heliconia stricta* in 3L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	201468.9703	50367.2425
Residual	165	594.4596	3.6027
Total	169	202063.4300	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	37.2388	0.1887	1			
β	3.6481	1.2584	-0.3024	1		
γ	0.3136	0.0671	-0.3625	0.9834	1	
δ	4.2297	1.5310	-0.3054	0.9948	0.9682	1

Table 82. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of flowered *Heliconia stricta* in 4L-SD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	104106.9137	26026.7284
Residual	80	105.2262	1.3153
Total	84	104212.1400	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	38.8178	0.1699	1			
β	3.9319	0.9603	-0.3290	1		
γ	0.2947	0.0473	-0.3864	0.9857	1	
δ	4.0918	1.0615	-0.3317	0.9944	0.9701	1

Table 83. Nonlinear regression for least-squares estimates of parameters of Richards function for length of 6th leaf of flowered *Heliconia stricta* in conSD as a dependent variable and time after leaf emergence as an independent variable.

Source	DF	Sum of Squares	Mean Square
Regression	4	91495.2114	22873.8028
Residual	97	278.6285	2.8724
Total	101	91773.8400	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	34.9091	0.4028	1			
β	0.3952	1.3605	-0.6307	1		
γ	0.1375	0.0279	-0.7477	0.9647	1	
δ	0.9819	0.9144	-0.6298	0.9992	0.9586	1

Table 84. RSS from fitting the 4th leaf data of Heliconia on each treatment and pseudostem status with common α, β, γ and δ.

Treatment	Status	M	df	RSS	RMS
ConLD	Veg.	4	263	1122.1863	
3L-SD	Fl.	4	142	195.5043	
4L-SD	Fl.	4	69	27.1856	
ConSD	Fl.	4	60	27.0977	
(A) Total		16	534	1371.9739	2.5692

Table 85. RSS from fitting the 5th leaf data of Heliconia on each treatment and pseudostem status with common α, β, γ and δ.

Treatment	Status	M	df	RSS	RMS
ConLD	Veg.	4	270	1268.873	
3L-SD	Fl.	4	125	167.275	
4L-SD	Fl.	4	74	294.960	
ConSD	Fl.	4	115	303.747	
(A) Total		16	584	2034.8562	3.4843

Table 86. RSS from fitting the 6th leaf data of Heliconia on each treatment and pseudostem status with common α, β, γ and δ.

Treatment	Status	M	df	RSS	RMS
ConLD	Veg.	4	311	1498.9989	
3L-SD	Fl.	4	165	594.4596	
4L-SD	Fl.	4	80	105.2262	
ConSD	Fl.	4	97	278.6285	
(A) Total		16	653	2477.3132	3.7937

Table 87. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 3L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	406	1406.7652	
Common β	7	406	1318.9962	
Common γ	7	406	1317.8588	
Common δ	7	406	1318.5706	
Common α,β,γ,δ	4	409	1434.5086	
Individual α,β,γ,δ	8	405	1317.6906	3.2535

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	116.8180	29.2045	11.3671	**
test of invariat α	1	89.0746	89.0746	34.6702	**
test of invariat β	1	1.3056	1.3056	0.5082	NS
test of invariat γ	1	0.1682	0.1682	0.0654	NS
test of invariat δ	1	0.8800	0.8800	0.3425	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of .Table 84

Table 88. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	333	1359.7421	
Common β	7	333	1149.6355	
Common γ	7	333	1149.3720	
Common δ	7	333	1149.4904	
Common α,β,γ,δ	4	336	1399.4104	
Individual α,β,γ,δ	8	332	1149.3719	3.4619

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	250.0384	62.5096	24.3304	**
test of invariat α	1	210.3702	210.3702	81.8816	**
test of invariat β	1	0.2636	0.2636	0.1026	NS
test of invariat γ	1	0.0001	0.0001	0.0000	NS
test of invariat δ	1	0.1245	0.1245	0.0484	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
The denominator for calculating F is obtained from line (A) of Table 84.

Table 89. Comparison of fits for Heliconia 4th leaf data to test invariance of $\alpha,\beta,\gamma,\delta$ for conLD (veg.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	324	1158.1751	
Common β	7	324	1150.6757	
Common γ	7	324	1150.5320	
Common δ	7	324	1150.4699	
Common $\alpha,\beta,\gamma,\delta$	4	327	1166.0118	
Individual $\alpha,\beta,\gamma,\delta$	8	323	1149.2840	3.5581

	df	change in RSS	MS	Fy	pz
test of invariat $\alpha,\beta,\gamma,\delta$	4	16.7278	4.1819	1.6277	NS
test of invariat α	1	8.8911	8.8911	3.4606	NS
test of invariat β	1	1.3917	1.3917	0.5417	NS
test of invariat γ	1	1.2480	1.2480	0.4857	NS
test of invariat δ	1	1.1859	1.1859	0.4616	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 84.

Table 90. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	212	263.3556	
Common β	7	212	222.8474	
Common γ	7	212	222.7941	
Common δ	7	212	222.8441	
Common $\alpha,\beta,\gamma,\delta$	4	215	285.4730	
Individual $\alpha,\beta,\gamma,\delta$	8	211	222.6899	1.0554

	df	change in RSS	MS	Fy	pz
test of invariat $\alpha,\beta,\gamma,\delta$	4	62.7831	15.6958	6.1092	**
test of invariat α	1	40.6657	40.6657	15.8281	**
test of invariat β	1	0.1575	0.1575	0.0613	NS
test of invariat γ	1	0.1042	0.1042	0.0406	NS
test of invariat δ	1	0.1542	0.1542	0.0600	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 84.

Table 91. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	203	232.9671	
Common β	7	203	222.6921	
Common γ	7	203	223.1578	
Common δ	7	203	222.7312	
Common α,β,γ,δ	4	206	241.9411	
Individual α,β,γ,δ	8	202	222.6021	1.1020

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	119.3390	29.8347	11.6124	**
test of invariat α	1	10.3650	10.3650	4.0343	*
test of invariat β	1	0.0900	0.0900	0.350	NS
test of invariat γ	1	0.5557	0.5557	0.2163	NS
test of invariat δ	1	0.1291	0.1291	0.0502	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 84.

Table 92. Comparison of fits for Heliconia 4th leaf data to test invariance of α, β, γ and δ for 4L-SD (fl.) and conSD (fl.).

Description of fit or test			M	df	RSS	RMS
Common α			7	130	107.6001	
Common β	7	130	54.6381			
Common γ			7	130	55.1550	
Common δ			7	130	54.7001	
Common α,β,γ,δ			4	133	123.2908	
Individual α,β,γ,δ			8	129	54.2834	0.4208

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	69.0074	17.2518	6.7148	**
test of invariat α	1	53.3167	53.3167	20.7522	**
test of invariat β	1	0.3547	0.3547	0.1380	NS
test of invariat γ	1	0.8716	0.8716	0.3392	NS
test of invariat δ	1	0.4167	0.4167	0.1622	SN

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 84.

Table 93. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 3L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	396	1442.4675	
Common β	7	396	1441.2927	
Common γ	7	396	1439.7295	
Common δ	7	396	1442.1992	
Common α,β,γ,δ	4	399	1452.6991	
Individual α,β,γ,δ	8	395	1436.1485	3.636

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	16.5506	4.1376	1.1875	NS
test of invariat α	1	6.3265	6.3265	1.18157	NS
test of invariat β	1	5.1442	5.1442	1.4764	NS
test of invariat γ	1	3.5810	3.5810	1.0277	NS
test of invariat δ	1	6.0437	6.0437	1.7345	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 85.

Table 94. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	345	1577.3317	
Common β	7	345	1576.4195	
Common γ	7	345	1572.3465	
Common δ	7	345	1574.8569	
Common α,β,γ,δ	4	348	1618.4625	
Individual α,β,γ,δ	8	344	1563.8342	4.5460

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	54.6283	13.6570	3.9196	**
test of invariat α	1	13.4975	13.4975	3.8738	*
test of invariat β	1	12.5853	12.5853	3.6120	NS
test of invariat γ	1	8.5123	8.5123	2.4430	NS
test of invariat δ	1	11.0227	11.0227	3.1635	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 85.

Table 95. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	386	1623.1695	
Common β	7	386	1580.2460	
Common γ	7	386	1586.8344	
Common δ	7	386	1582.3859	
Common α,β,γ,δ	4	389	1695.0953	
Individual α,β,γ,δ	8	385	1572.6201	4.0847

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	122.4752	30.6188	8.7876	**
test of invariat α	1	50.5494	50.5494	14.5077	**
test of invariat β	1	7.6259	7.6259	2.1886	NS
test of invariat γ	1	14.2143	14.2143	4.0795	*
test of invariat δ	1	9.7658	9.7658	2.8028	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 85

Table 96. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	199	489.2784	
Common β	7	199	464.2808	
Common γ	7	199	463.6188	
Common δ	7	199	463.3224	
Common α,β,γ,δ	4	202	513.9936	
Individual α,β,γ,δ	8	198	462.2359	2.3345

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	51.7577	12.9394	3.7136	**
test of invariat α	1	27.0425	27.0425	7.7612	**
test of invariat β	1	2.0449	2.0449	0.5869	NS
test of invariat γ	1	1.3829	1.3829	0.3969	NS
test of invariat δ	1	1.0865	1.0865	0.3118	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 85.

Table 97. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	241	484.5218	
Common β	7	241	471.6357	
Common γ	7	241	474.0895	
Common δ	7	241	471.9634	
Common α,β,γ,δ	4	244	560.2988	
Individual α,β,γ,δ	8	240	471.0219	1.9626

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	89.2769	22.3192	6.4056	**
test of invariat α	1	13.4999	13.4999	3.8745	*
test of invariat β	1	0.6138	0.6138	0.1762	NS
test of invariat γ	1	3.0676	3.0676	0.8804	NS
test of invariat δ	1	0.9415	0.9415	0.2702	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 85.

Table 98. Comparison of fits for Heliconia 5th leaf data to test invariance of α, β, γ and δ for 4L-SD (fl.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	190	678.1701	
Common β	7	190	598.9655	
Common γ	7	190	598.9126	
Common δ	7	190	598.7079	
Common α,β,γ,δ	4	193	797.6539	
Individual α,β,γ,δ	8	189	598.7076	3.1677

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	198.9463	49.7366	14.2745	**
test of invariat α	1	79.4625	79.4625	22.8058	**
test of invariat β	1	0.2579	0.2579	0.0740	NS
test of invariat γ	1	0.2050	0.2050	0.0588	NS
test of invariat δ	1	0.0003	0.0003	0.0000	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 85.

Table 99. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 3L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	477	2093.6301	
Common β	7	477	2104.2471	
Common γ	7	477	2107.6382	
Common δ	7	477	2105.8241	
Common α,β,γ,δ	4	480	2105.0575	
Individual α,β,γ,δ	8	476	2093.4584	4.3980

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	71.5991	17.8997	4.7182	**
test of invariat α	1	0.1717	0.1717	0.0452	NS
test of invariat β	1	10.7887	10.7887	2.8438	NS
test of invariat γ	1	14.1798	14.1798	3.7377	NS
test of invariat δ	1	12.3657	12.3657	3.2595	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 86.

Table 100. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	392	1701.8034	
Common β	7	392	1613.2738	
Common γ	7	392	1611.2547	
Common δ	7	392	1612.4604	
Common α,β,γ,δ	4	395	1761.4216	
Individual α,β,γ,δ	8	391	1604.2251	4.1029

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	157.1965	39.2991	10.3590	**
test of invariat α	1	97.5783	97.5783	25.7211	**
test of invariat β	1	9.0487	9.0487	2.3852	NS
test of invariat γ	1	7.0296	7.0296	1.8529	NS
test of invariat δ	1	8.2353	8.2353	2.1707	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 86.

Table 101. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for conLD (veg.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	409	2169.9944	
Common β	7	409	1778.9187	
Common γ	7	409	1783.5728	
Common δ	7	409	1778.5075	
Common α,β,γ,δ	4	412	2322.8729	
Individual α,β,γ,δ	8	408	1777.6274	4.3569

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	545.2455	136.3114	35.9309	**
test of invariat α	1	392.3670	392.3670	103.4259	**
test of invariat β	1	1.2913	1.2913	0.3404	NS
test of invariat γ	1	5.9454	5.9454	1.5672	NS
test of invariat δ	1	0.8801	0.8801	0.2320	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 86.

Table 102. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and 4L-SD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	246	778.7539	
Common β	7	246	699.7499	
Common γ	7	246	699.7898	
Common δ	7	246	699.6971	
Common α,β,γ,δ	4	249	804.4889	
Individual α,β,γ,δ	8	245	699.6858	2.8559

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	104.8031	26.2007	6.9064	**
test of invariat α	1	79.0681	79.0681	20.8419	**
test of invariat β	1	0.0641	0.0641	0.0169	NS
test of invariat γ	1	0.1040	0.1040	0.0274	NS
test of invariat δ	1	0.0113	0.0113	0.0029	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 86.

Table 103. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 3L-SD (fl.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	259	1205.5752	
Common β	7	259	885.8508	
Common γ	7	259	897.4158	
Common δ	7	259	885.3252	
Common α,β,γ,δ	4	262	1627.3746	
Individual α,β,γ,δ	8	258	873.0881	3.3840

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	754.2865	188.5716	49.7065	**
test of invariat α	1	332.4871	332.4871	87.6419	**
test of invariat β	1	12.7627	12.7627	3.3642	NS
test of invariat γ	1	24.3277	24.3277	6.4126	*
test of invariat δ	1	12.2371	12.2371	3.2256	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 86.

Table 104. Comparison of fits for Heliconia 6th leaf data to test invariance of α, β, γ and δ for 4L-SD (fl.) and conSD (fl.).

Description of fit or test	M	df	RSS	RMS
Common α	7	178	934.7006	
Common β	7	178	395.4407	
Common γ	7	178	400.5886	
Common δ	7	178	393.2637	
Common α,β,γ,δ	4	181	1152.3918	
Individual α,β,γ,δ	8	177	383.8547	4.3420

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	768.5371	192.1343	50.6456	**
test of invariat α	1	550.8459	550.8459	145.2002	**
test of invariat β	1	11.5860	11.5860	3.0540	NS
test of invariat γ	1	16.7339	16.7339	4.4109	*
test of invariat δ	1	9.4090	9.4090	2.4802	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 86.

Table 105. Nonlinear regression for least-squares estimates of parameters of Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) of 3 rd leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	589.0705	147.2676
Residual	917	3.8509	0.0042
Total	921	592.9214	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	0.9927	0.0040	1			
β	2.0293	0.3397	-0.5143	1		
γ	9.2333	0.4675	-0.6152	0.9666	1	
δ	0.7891	0.1324	-0.4872	0.9925	0.9342	1

Table 106. Nonlinear regression for least-squares estimates of parameters of Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) of 4 th leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	644.7933	161.1983
Residual	963	4.7378	0.0049
Total	967	649.5312	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	0.9966	0.0042	1			
β	0.0716	0.5999	-0.5004	1		
γ	8.0966	0.3979	-0.6413	0.9356	1	
δ	0.2341	0.1103	-0.4853	0.9984	0.9176	1

Table 107. Nonlinear regression for least-squares estimates of parameters of Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) of 5 th leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	612.4569	153.1142
Residual	905	7.5202	0.0083
Total	909	619.9772	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	0.9989	0.0057	1			
β	-0.0542	0.8291	-0.4918	1		
γ	8.2884	0.5487	-0.6346	0.9352	1	
δ	0.2308	0.1526	-0.4786	0.9987	0.9188	1

Table 108. RSS from fitting the 3rd, 4th and 5th leaf of Heliconia with common α, β, γ, and δ of Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1).

Treatment	M	df	RSS	RMS
3rd leaf	4	917	3.8509	
4th leaf	4	963	4.7379	
5th leaf	4	905	7.5202	
(A) Total	16	2785	16.1090	0.0058

Table 109. Comparing of fits for Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) to test invariance of β, γ, and δ for 3 rd and 4th leaf.

Description of fit or test	M	df	RSS	RMS
Common β	7	1881	8.6328	
Common γ	7	1881	8.6082	
Common δ	7	1881	8.6303	
Common α,β,γ,δ	4	1884	9.1484	
Individual α,β,γ,δ	8	1880	8.5888	0.0045

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	0.5596	0.1399	24.12	**
test of invariat β	1	0.0440	0.0440	7.58	*
test of invariat γ	1	0.0194	0.0194	3.34	NS
test of invariat δ	1	0.0415	0.0415	7.15	*

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) of Table 108.

Table 110. Comparing of fits for Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) to test invariance of β, γ, and δ for 3 rd and 5th leaf.

Description of fit or test	M	df	RSS	RMS
Common β	7	1823	11.4185	
Common γ	7	1823	11.5124	
Common δ	7	1823	11.4101	
Common α,β,γ,δ	4	1826	12.9990	
Individual α,β,γ,δ	8	1822	11.3711	0.0062

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	1.6280	0.4070	70.17	**
test of invariat β	1	0.0474	0.0474	8.17	*
test of invariat γ	1	0.1413	0.1413	24.36	**
test of invariat δ	1	0.0390	0.0390	6.72	*

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line Table 108.

Table 111. Comparing of fits for Richards function on relative leaf length (length at emergence = 0 and length at fully expanded = 1) and relative time (date of leaf emergence = 0 and date of leaf fully expanded = 1) to test invariance of β, γ, and δ for 4 th and 5th leaf.

Description of fit or test	M	df	RSS	RMS
Common β	7	1869	12.258114	
Common γ	7	1869	12.258507	
Common δ	7	1869	12.258027	
Common α,β,γ,δ	4	1872	12.573069	
Individual α,β,γ,δ	8	1868	12.258025	

	df	change in RSS	MS	Fy	pz
test of invariat α,β,γ,δ	4	0.315044	0.078761	13.5795	**
test of invariat β	1	0.000089	0.000089	0.0153	NS
test of invariat γ	1	0.000475	0.000475	0.0818	NS
test of invariat δ	1	0.0000002	0.0000002	0.0003	NS

zNonsignificant (NS) or significant at 5% (*) or 1% (**)
yThe denominator for calculating F is obtained from line (A) Table 108.

Table 112. Nonlinear regression for least-squares estimates of parameters of Richards function on leaf length and time after leaf emergence of 3 rd leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	287942.6371	71985.6593
Residual	628	2664.8929	4.2435
Total	632	290607.5300	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	24.8746	0.1359	1			
β	5.9529	1.2461	-0.4314	1		
γ	0.4803	0.0812	-0.4821	0.9927	1	
δ	5.6761	1.2977	-0.4348	0.9949	0.9812	1

Table 113. Nonlinear regression for least-squares estimates of parameters of Richards function on leaf length and time after leaf emergence of 4th leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	428850.2186	107212.5546
Residual	673	2029.4914	3.0155
Total	677	430879.7100	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	29.1951	0.1225	1			
β	2.8156	0.5089	-0.5091	1		
γ	0.2957	0.0269	-0.5943	0.9803	1	
δ	2.6222	0.4859	-0.5044	0.9955	0.9646	1

Table 114. Nonlinear regression for least-squares estimates of parameters of Richards function on leaf length and time after leaf emergence of 5th leaf position.

Source	DF	Sum of Squares	Mean Square
Regression	4	584504.7855	146126.1964
Residual	675	2884.0444	4.2727
Total	679	587388.8300	

			Correlation Matrix			
Parameter	Estimate	Standard Error	α	β	γ	δ
α	34.2175	0.1442	1			
β	2.7999	0.4946	-0.4928	1		
γ	0.2502	0.0221	-0.5795	0.9797	1	
δ	2.5035	0.4562	-0.4877	0.9952	0.9631	1

Table 115. ANOVA for regressing LOGIT on LOGCON of ABA standards compare among 8 plates. (PLATE).

Dependent Variable:	LOGIT	r2 = 0.9701	C.V. = -9.50	
Source	DF	Sum of Squares	F Value	Pr > F
PLATE	7	0.1228	0.32	0.9445
LOGCON	1	228.4621	4149.30	0.0001
LOGCON*PLATE	7	0.5907	1.53	0.1613
Error	134	7.3763		

Table 116. ANOVA for regressing LOGIT on LOGCON of ABA standards from 8 plates to obtain a standard curve.

Dependent Variable:	LOGIT	r2 = 0.9332	C.V. = -13.52	
Source	DF	Sum of Squares	F Value	Pr > F
LOGCON	1	220.0531	1973.94	0.0001
Error	148	16.4988		

Regression equations
LOGIT = 1.7802 - 2.4227 LOGCON

Table 117. ANOVA for regressing LOGIT on LOGCON of ABA standards to obtain standard curve for test of parallelism.

Dependent Variable:	LOGIT	r2 = 0.9882	C.V. = -6.7821	
Source	DF	Sum of Squares	F Value	Pr > F
LOGCON	1	24.4140	1002.05	0.0001
Error	12	0.2923		

Regression equations
LOGIT = 1.9225 - 2.5739 LOGCON

Table 118. ANOVA for regressing LOGIT on LOGWT with different dilution of leaf wt. to obtain curve for test of parallelism.

Dependent Variable:	LOGIT	r2 = 0.9775	C.V. = -20.52	
Source	DF	Sum of Squares	F Value	Pr > F
LOGWT	1	18.0171	566.10	0.0001
Error	13	0.4137		

Regression equations
LOGIT = -2.4192 - 2.5744 LOGCON

Table 119. ANOVA for regressing LOGIT on LOGWT with different dilution of shoot apex tissue to obtain curve for test of parallelism.

Dependent Variable:	LOGIT	r2 = 0.607	C.V. = -349.87	
Source	DF	Sum of Squares	F Value	Pr > F
LOGWT	1	10.9361	15.5	0.0028
Error	11	7.0555		

Regression equations

LOGIT = 1.0407 + 2.8364 LOGCON

Table 120. ANOVA for regressing leaf ABA level (ABA in ng/g lf. dry wt.) on number of leaves when sample were taken (LFNO) before, and during SD (SD)

Dependent Variable:	ABA	r2 = 0.0472	C.V. = 80.65	
Source	DF	Sum of Squares	F Value	Pr > F
SD	1	9009.31	0.17	0.6779
LFNO	1	11852.02	0.23	0.6339
SD*LFNO	1	109627.87	2.12	0.1513
Error	51	2633815.04		

Table 121. ANOVA and regression coefficients for regressing leave ABA level (ABA in ng/g lf. dry wt.) on temperature treatment (TEMP) compare with different shoot status (STA).

Dependent Variable:	ABA	r2 = 0.17	C.V. = 57.02	
Source	DF	Sum of Squares	F Value	Pr > F
STA	3	64534.50	0.51	0.6768
TEMP	1	403415.05	9.57	0.0030
STA*TEMP	3	70906.66	0.56	0.6431
TEMP*TEMP	1	5540.19	0.13	0.7183
Error	62	2614778.05		

Table 122. ANOVA for regressing leaf ABA level (ABA) on different temperature conditions (TEMP).

Dependent Variable:	ABA	r2 = 0.14	C.V. = 55.01	
Source	DF	Sum of Squares	F Value	Pr > F
TEMP	1	451647.57	11.51	0.0011
Error	11	2707526.89		

Regression equations

ABA = 841.63 - 20.96 TEMP

Table 123. Chi-square tests for comparing the effect of temperature treatment on ratio of vegetative, elongated, flowered and aborted samples collected during week 4-11 after the start of SD, using null hypothesis that there is no difference exist among the status. Within each column, number with the same letter are not significantly different ($P<0.05$, Chi-square test).

Treatment	Vegetative	Elongated	Flowered	Aborted	(Fl. + Ab.)
18	5 (17.2)a	8 (27.0)a	16 (55.2)ab	0 (0.0)c	16 (55.2)a
21	5 (20.0)a	9 (36.0)a	10 (40.0)bc	1 (4.0)bc	11 (44.0)a
24	1 (3.4)b	13 (44.8)a	10 (34.5)bc	5 (17.2)ab	15 (51.7)a
28	5 (19.2)a	8 (30.8)a	8 (30.8)c	5 (19.2)a	13 (50.0)a

Statistic	DF	Value	Prob	N
Chi-square	9	18.15	0.033	109

Table 124. ANOVA for leaf ABA level (ABA in ng/g leaf dry wt.) of different shoot status (STA)

Dependent Variable:	ABA	r2 = 0.0213	C.V. = 60.15	
Source	DF	Sum of Squares	F Value	Pr > F
STA	3	74355.39	0.54	0.6534
Error	78	3489127.09		

Table 125. ANOVA for regressing leaf ABA level (ABA in ng/g leaf dry wt.) on number of leave when sample were taken (LFNO).

Dependent Variable:	ABA	r2 = 0.1036	C.V. = 59.23	
Source	DF	Sum of Squares	F Value	Pr > F
LFNO	1	244675.09	6.08	0.0145
LFNO*LFNO	1	732888.65	18.21	0.0001
Error	210	8452284.71		

Estimated regression equations

ABA = 1995.79-590.77(LFNO)-50.97(LFNO*LFNO)

Table 126. ANOVA and regression coefficients for regressing foliar ABA level (ABA in ng/g lf. dry wt.) on number of leave at the start of SD (SDLFNO) and days after SD (TIM) compare with different temperature treatment (TEMP).

Dependent Variable:	ABA	r2 = 0.4519	C.V. = 47.82	
Source	DF	Sum of Squares	F Value	Pr > F
TEMP	3	824111.28	10.47	0.0001
SDLFNO	1	210724.47	8.03	0.0051
TIM	1	701191.25	26.73	0.0001
SDLFNO*TEMP	3	1094452.03	13.91	0.0001
TIM*TEMP	3	163614.81	2.08	0.1043
TIM*TIM	1	389146.52	14.83	0.0002
TIM*TIM*TEMP	3	878718.19	11.17	0.0001
Error	212	5167871.90		

Contrasts

Contrast	DF	Contrast SS	F Value	Pr > F
TEMP18&21 vs. 24&28	1	1775702.89	67.69	0.0001

Estimated regression equations

TEMP = 18&21: ABA = 2194.44 - 80.03(SDLFNO)-60.15(TIM) + 0.5469(TIMxTIM)
TEMP = 24&28: ABA = -38.42 + 64.52(SDLFNO) + 7.4891(TIM) - 0.1005(TIMxTIM)

Table 127. Chi-square tests for comparing the effect of temperature treatment on ratio of vegetative, flowered and aborted at the termination of experiment (20 weeks after the start of SD). Using null hypothesis that no difference exist among the status. Within each column, numbers with the same letter are not significantly different (P>0.05, Chi-square test).

Treatment	Vegetative	Flowered	Aborted	Flowered + Aborted
18	4 (30.7)a	8 (61.5)a	1 (7.7)a	9 (69.2)a
21	4 (40.0)a	5 (50.0)ab	1 (10.0)a	6 (60.0)a
24	4 (44.4)a	3 (33.3)ab	2 (22.2)a	5 (55.5)a
28	5 (45.4)a	3 (27.3)bc	3 (27.3)a	6 (54.6)a

Statistic	DF	Value	Prob	N
Chi-square	6	4.163	0.655	43

Table 128. ANOVA Effect of shading on leaf ABA level (ABA in ng/g lf. dry wt).

Dependent Variable: ABA C.V. = 55.8

Source	DF	Sum of Squares	F Value	Pr > F
STA	2	251543.2487	9.58	0.0004
Error	40	525409.8331		

Table 129. Chi-square tests for comparing the effect of shade treatment on ratio of vegetative, elongated, flowered and aborted from week 8-11 after started of SD. Using null hypothesis that no difference exist among the status. Within each column, numbers with the same letter are not significantly different (P>0.05, Chi-square test).

Treatment	Vegetative	Flowered	Aborted	Flowered + Aborted
20%sun	3 (16.7)a	14 (77.8)a	1 (5.6)a	15 (83.3)a
40%sun	3 (17.6)a	13 (76.5)a	1 (5.9)a	14 (82.3)a
100%sun	5 (20.0)a	19 (76.0)a	1 (4.0)a	20 80.0)a

Statistic	DF	Value	Prob	N
Chi-square	4	0.162	0.997	60

Table 130. ANOVA for leaf ABA level (ABA in ng/g lf. dry wt.) of different shoot status (STA)

Dependent Variable: ABA r2 = 0.0649 C.V. = 78.52

Source	DF	Sum of Squares	F Value	Pr > F
STA	2	59864.56	1.11	0.3416
Error	32	862175.37		

Table 131. ANOVA for regressing leave ABA level (ABA in ng/g lf. dry wt.) on number of leave when sample were taken (LFNO).

Dependent Variable:	ABA	r2 = 0.2072	C.V. = 55.85	
Source	DF	Sum of Squares	F Value	Pr > F
LFNO	1	148949.11	9.39	0.0034
LFNO*LFNO	1	66546.46	4.20	0.0456
Error	52	824597.54		

Estimated regression equations

ABA = 1256.5899-330.6298(LFNO)+25.3855(LFNO*LFNO)

Table 132. Chi-square tests for comparing the effect of shade treatment on ratio of vegetative, flowered and aborted at the termination of experiment (18 weeks after started of SD). Using null hypothesis that no difference exist among the status. Within each column, numbers with the same letter are not significantly different (P>0.05, Chi-square test).

Treatment	Vegetative	Flowered	Aborted	Flowered + Aborted
20%	1 (5.3)a	16 (84.2)a	2 (10.5)a	18 (94.7)a
40%	2 (6.5)a	24 (77.4)a	5 (16.1)a	29 (93.5)a
100%	1 (5.3)a	15 (78.9)a	3 (15.8)a	18 (94.7)a

Statistic	DF	Value	Prob	N
Chi-square	4	4.163	0.397	69

Table 133. ANOVA Effect of shades (Trt.) on number of weeks from the start of SD to anthesis (WKFL) of H. stricta

Dependent Variable:	WKFL	C.V. = 12.30		
Source	DF	Sum of Squares	F Value	Pr > F
Trt.	2	4.7654	1.17	0.3207
Error	39	79.3535		

Table 134. ANOVA Effect of shade (Trt.) on number of subtending leaves (SUBLF) of H. stricta

Dependent Variable:	SUBLF	C.V. = 15.59		
Source	DF	Sum of Squares	F Value	Pr > F
Trt.	2	3.3800	1.74	0.1847
Error	52	50.3654		

Table 135. ANOVA Effect of shade (Trt.) on number of cincinnal bracts (BRNO) of H. stricta

Dependent Variable:	BRNO		C.V. = 20.94	
Source	DF	Sum of Squares	F Value	Pr > F
Trt.	2	0.1591	0.37	0.6903
Error	46	9.8000		

Table 136. ANOVA Effect of shade (Trt.) on pseudostem height (HT) of H. stricta

Dependent Variable:	HT		C.V. = 7.29	
Source	DF	Sum of Squares	F Value	Pr > F
Trt.	2	797.4421	28.98	0.0001
Error	52	715.3517		

Table 137. ANOVA Effect of shade (Trt.) on inflorescence length (FLLGTH) of H. stricta

Dependent Variable:	HT		C.V. = 5.38	
Source	DF	Sum of Squares	F Value	Pr > F
Trt.	2	178.6619	30.80	0.0001
Error	47	136.3380		

Table 138. ANOVA for regressing number of subtending leaf at time of anthesis (SLFNO) on number of leaf at start of SD (LFNO).

Dependent Variable:	SLFNO	r2 = 0.68	C.V. = 7.46	
Source	DF	Sum of Squares	F Value	Pr > F
LFNO	1	21.1504	90.50	0.0001
Error	43	10.0495		

Estimated regression equations

SLFNO = 3.67+0.7535(LFNO)

Table 139. ANOVA for regressing time from SD to anthesis (WKSDFL) on number of leaf at start of SD (LFNO).

Dependent Variable:	SLFNO	r2 = 0.03	C.V. = 12.31	
Source	DF	Sum of Squares	F Value	Pr > F
LFNO	1	2.6313	1.29	0.2625
Error	40	81.4877		

APPENDIX B
FIGURES

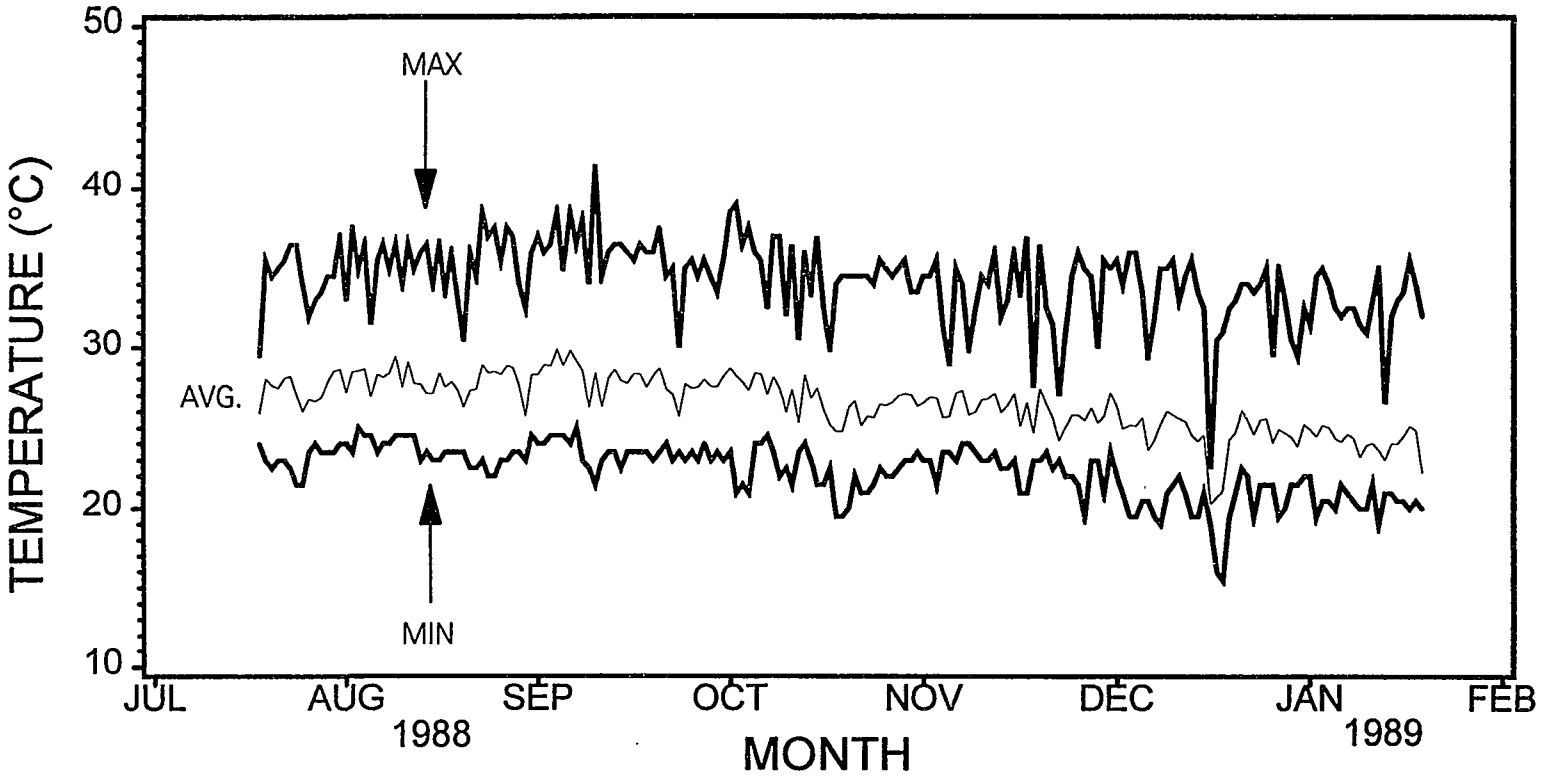

Figure 1. Daily maximum, minimum and average temperatures in °C at the inside of Magoon greenhouse facility of the University of Hawaii during 1988-1989.

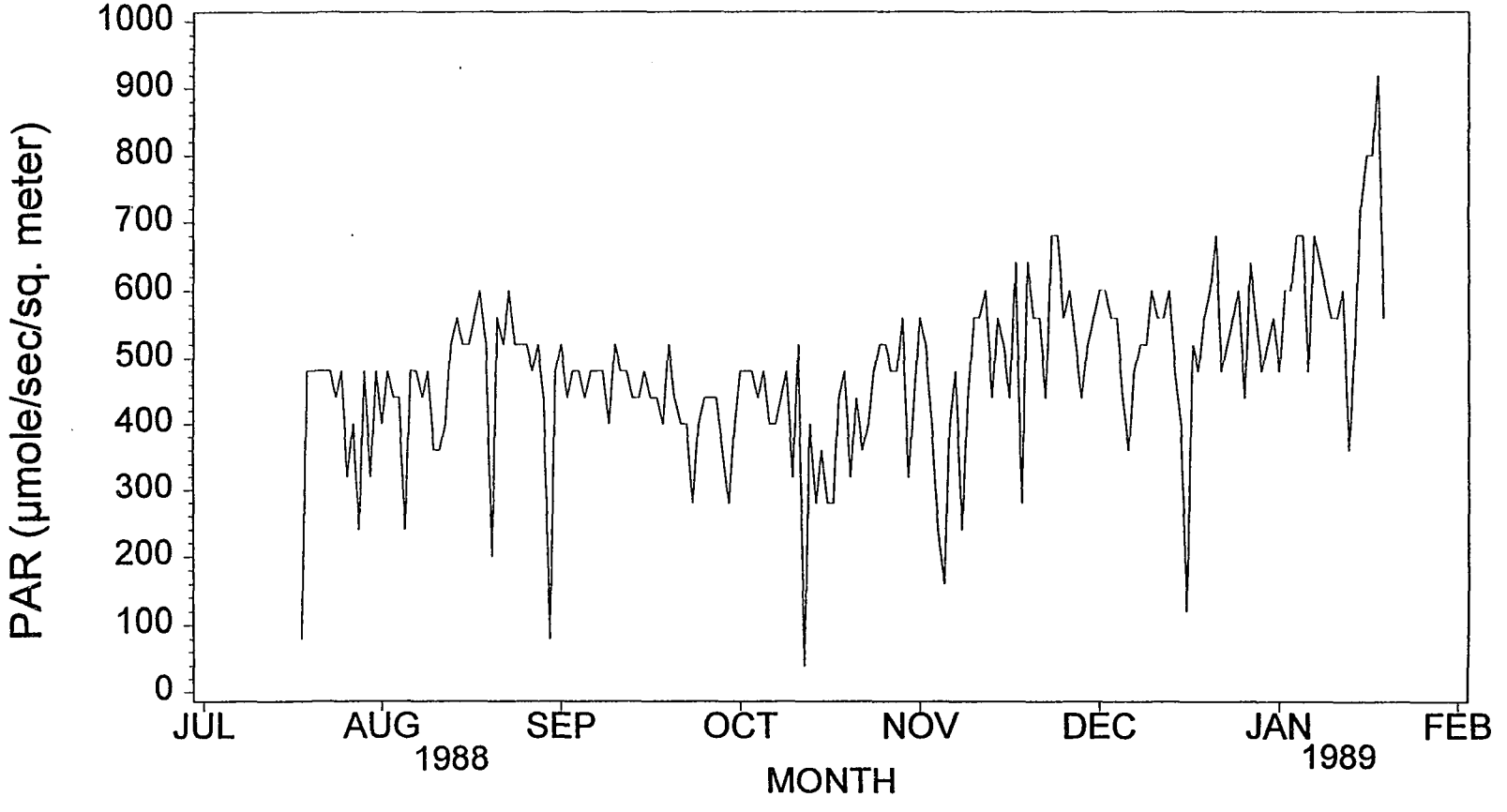

Figure 2. Daily maximum photosynthetically active radiation (PAR) in µmol/sec./sq.m. at the inside of Magoon greenhouse facility of the University of Hawaii during 1988-1989.

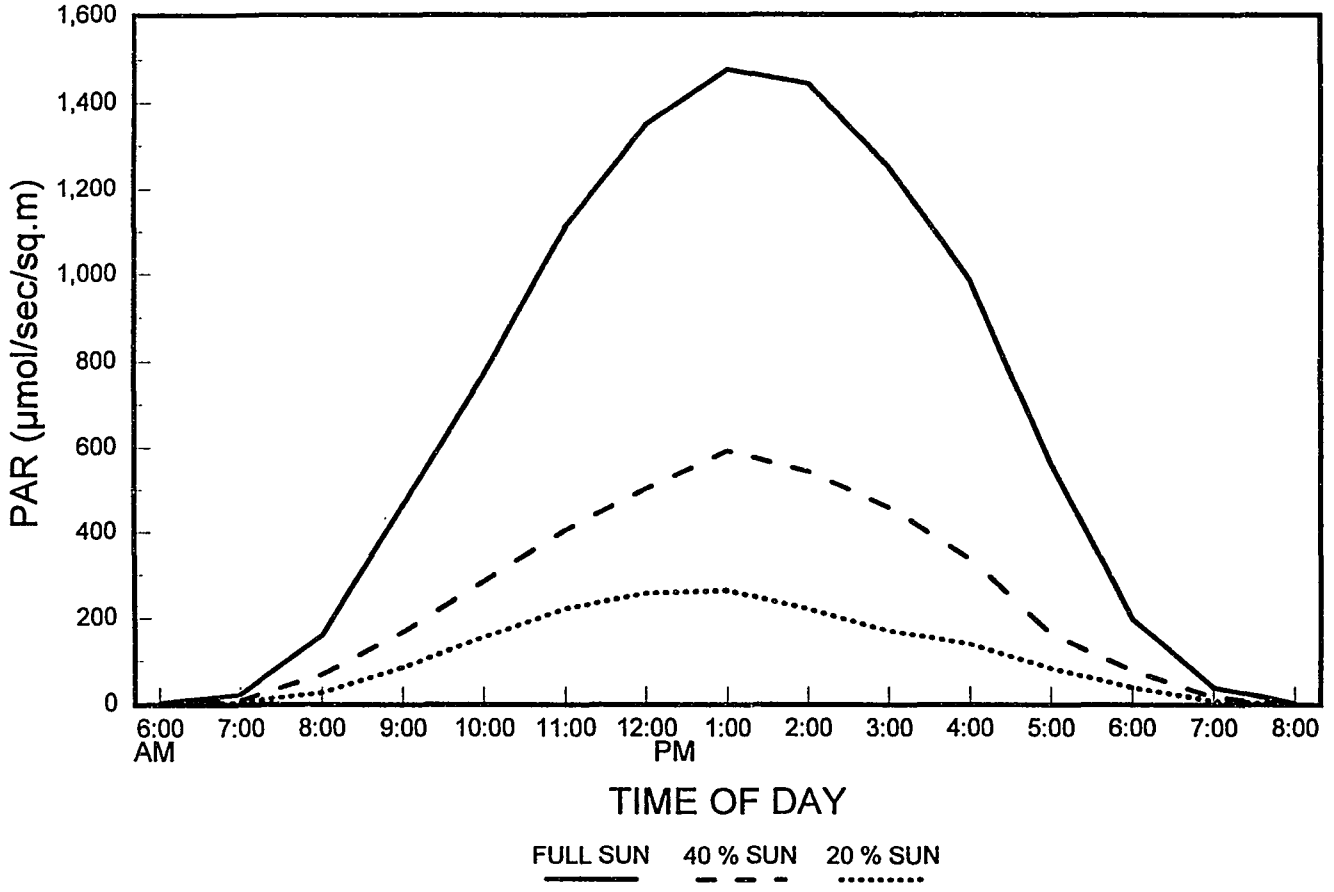

Figure 3. Hourly average photosynthetically active radiation (PAR) in µmol/sec./sq.m. in fullsun, 40% sun and 20% sun at the Magoon greenhouse facility of the University of Hawaii 1991.

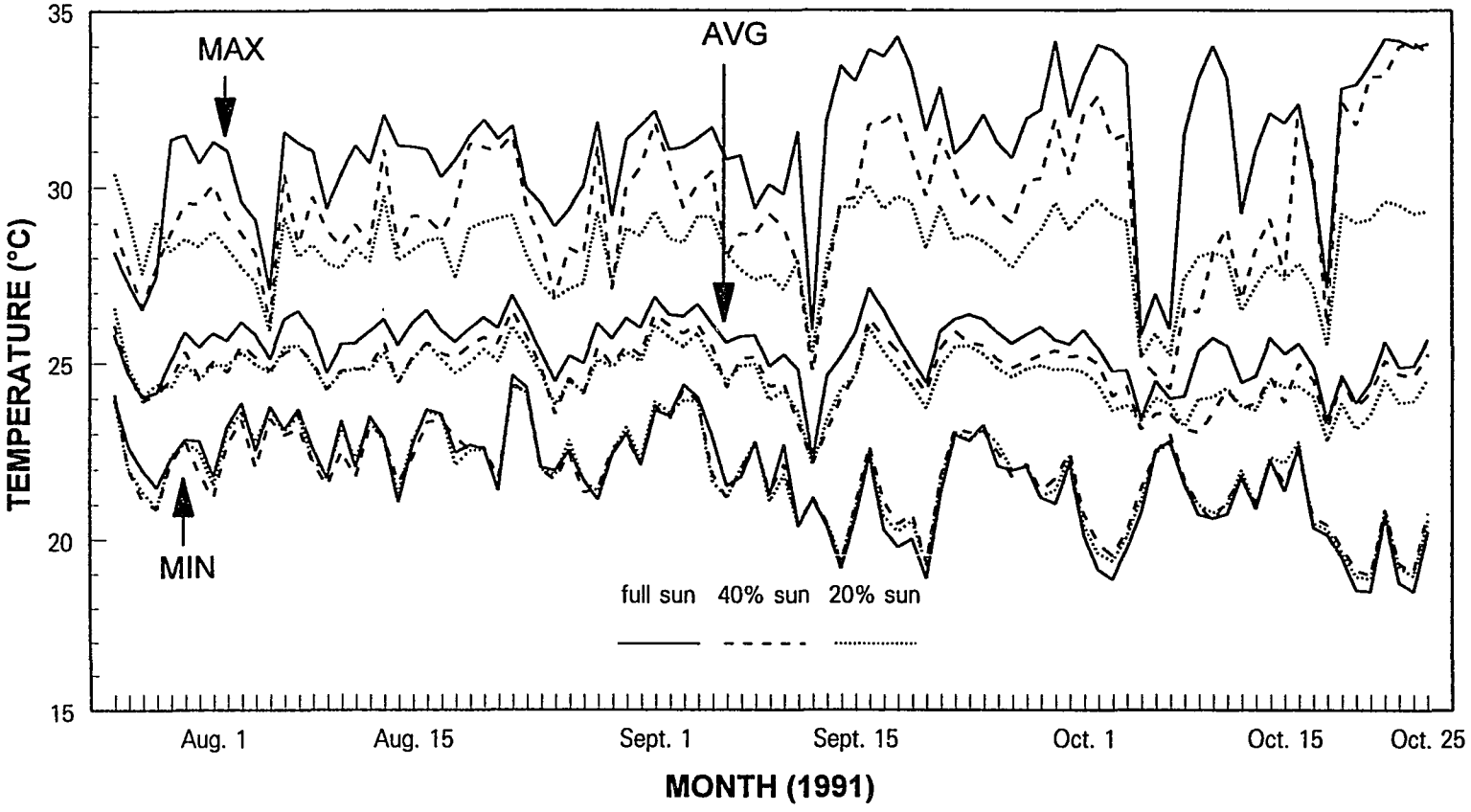

Figure 4. Daily maximum, minimum and average temperature in °C in ful lsun, 40% sun and 20% sun at the Magoon greenhouse facility of the University of Hawaii 1991.

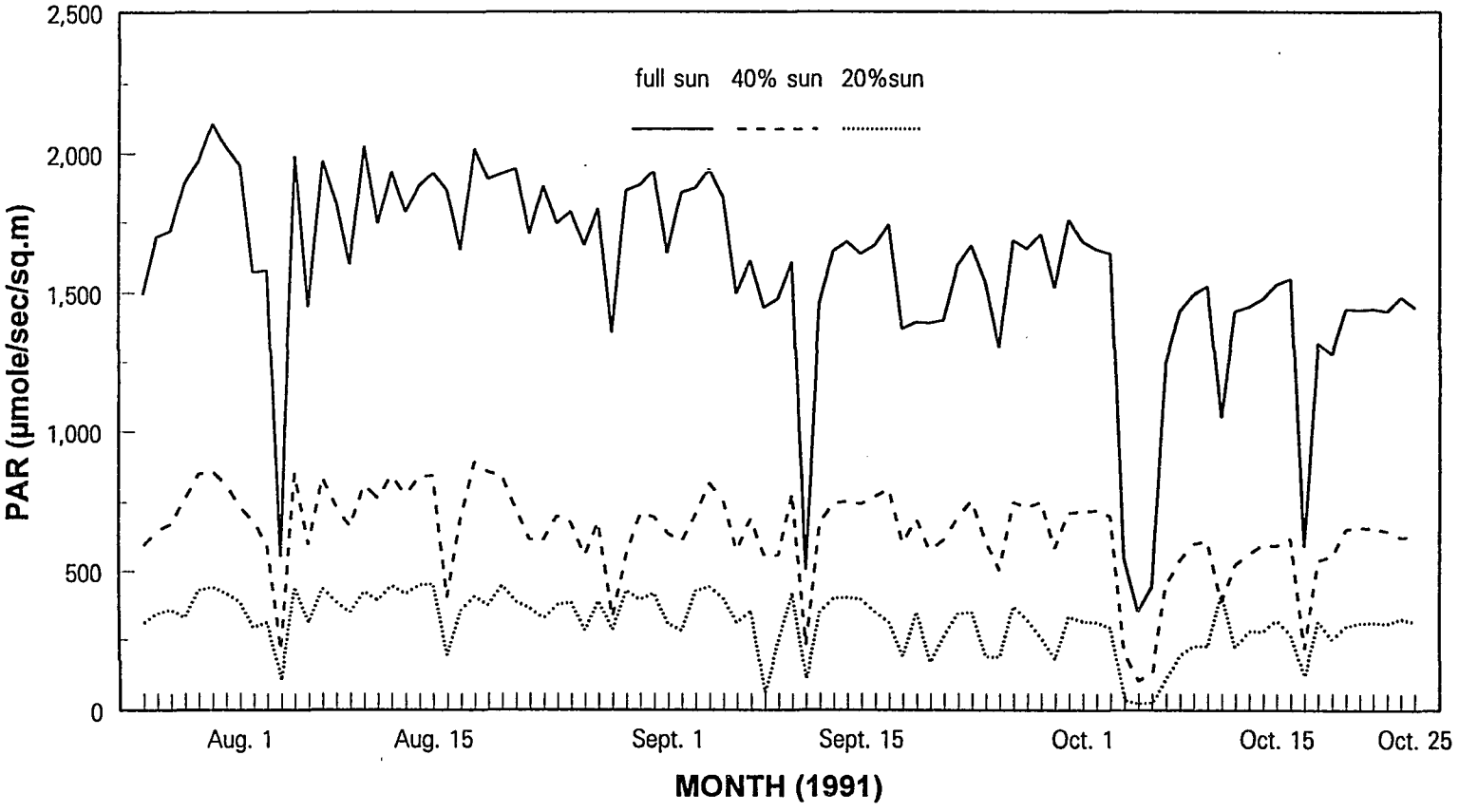

Figure 5. Daily maximum photosynthetically active radiation (PAR) in µmol/sec./sq.m. in full sun, 40% sun and 20% sun at the Magoon greenhouse facility of the University of Hawaii 1991.

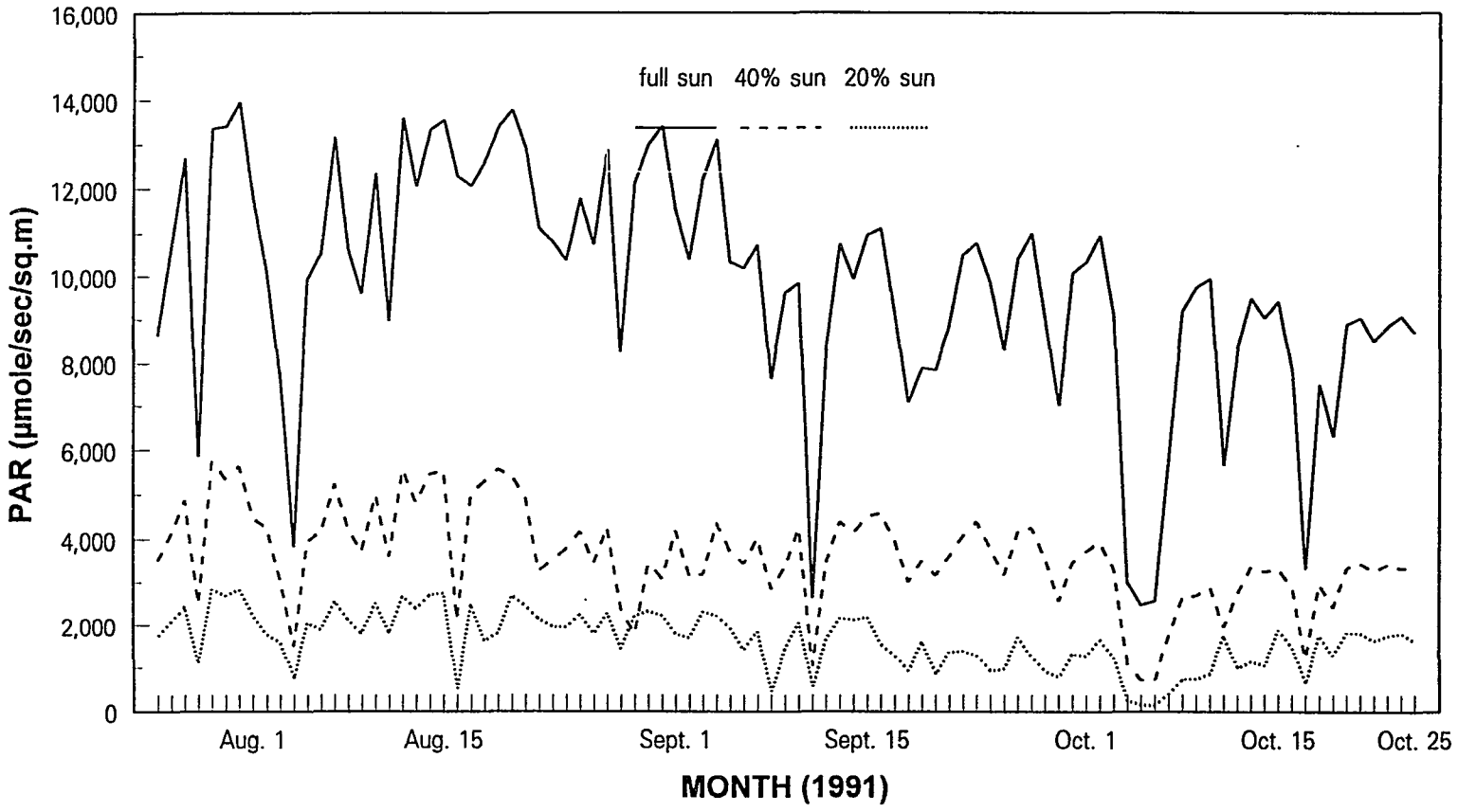

Figure 6. Daily average total photosynthetically active radiation (PAR) in µmol/sec./sq.m. in ful lsun, 40% sun and 20% sun at the Magoon greenhouse facility of the University of Hawaii 1991.

APPENDIX C

PROGRAMS

Program 1. A SAS program 'GOMPERTZ.SAS' for estimating parameters of the Gompertz model from leaf length (LENGTH) and time after leaf emergence (T). (A = α, B = β, AND K = γ)

```
*-------------------------------------------------------------*;

PROGRAM 'GOMPERTZ.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, AND K WERE PLACED IN PARAMETERS
      STATEMENT (PARMS)

*-------------------------------------------------------------*;


PROC NLIN DATA=SAVE.LEAFLG  METHOD = GAUSS;
PARMS  A = 29.0
       B = 9.3
       K = 0.3;
  U = -(K*T);
  Q = EXP(U);
  Z = EXP(-B*Q);
  MODEL LENGTH = A*Z;
  DER.A=Z;
  DER.B=-A*Z*Q;
  DER.K=A*B*Z*Q*T;
  TITLE 'GOMPERTZ MODEL';
RUN;
```

Program 2. A SAS program 'LG_GOMP.SAS' for estimating parameters of the Gompertz model from log of leaf length (LLGTH) and time after leaf emergence (T). (A = α, B = β, AND K = γ)

```
*------------------------------------------------------------*;

PROGRAM 'LG_GOMP.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, AND K WERE PLACED IN PARAMETERS
      STATEMENT (PARMS)

*------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LEAFLG  METHOD = GAUSS;
PARMS  A= 29.7
     B= 1.0
     K= 0.1;
  Q = EXP(-K*T);
  LLGTH = LOG(LENGTH);
  MODEL LLGTH = LOG(A) - (B*Q);
  DER.A=1/A;
  DER.B= -Q;
  DER.K= B*T*Q;
  TITLE 'GOMPERTZ MODEL  LOG';
RUN;
```

Program 3. A SAS program 'LOGISTIC.SAS' for estimating parameters of the logistic model from leaf length (LENGTH) and time after leaf emergence (T). (A = α, B = β, AND K = γ)

```
*------------------------------------------------------------*;

PROGRAM 'LOGISTIC.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, AND K WERE PLACED IN PARAMETERS
      STATEMENT (PARMS)

*------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LEAFLG METHOD = GAUSS;
PARMS  A=29.3
       B=30
       K=0.2;
  Q = -(K*T);
  U = EXP(Q);
  Z = 1+(B*U);
  ZSQ = Z**(-2);
  MODEL LENGTH = A/Z;
  DER.A=1/Z;
  DER.B=-A*U*ZSQ;
  DER.K= A*B*U*T*ZSQ;
  TITLE 'LOGISTIC MODEL';
RUN;
```

Program 4. A SAS program 'LG_LOGIS.SAS' for estimating parameters of the logistic model from log of leaf length (LLGTH) and time after leaf emergence (T). (A = α, B = β, AND K = γ)

```
*-------------------------------------------------------------*;

PROGRAM 'LG_LOGIS.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, AND K WERE PLACED IN PARAMETERS
      STATEMENT (PARMS)

*-------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LEAFLG  METHOD = GAUSS;
PARMS  A= 29.6
     B= 1.7
     K= 0.2;
 Q = EXP(-K*T);
 Z = 1 + (B*Q);
 M = LOG(Z);
 LLGTH = LOG(LENGTH);
 MODEL LLGTH = LOG(A) - M;
 DER.A=1/A;
 DER.B= -Q/Z;
 DER.K=(T*B*Q)/Z;
 TITLE 'LOGISTIC MODEL  LOG';
RUN;
```

Program 5. A SAS program 'RICHARDS.SAS' for estimating parameters of the Richards model from leaf length (LENGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*------------------------------------------------------------*;

PROGRAM 'RICHARDS.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      ========          ========      ==========================
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
      PARAMETERS STATEMENT (PARMS)

*------------------------------------------------------------*;


PROC NLIN DATA=SAVE.LEAFLG  METHOD = GAUSS;
PARMS  A = 29.4
       B = 4.2
       V = 0.4
       K = 4.4
  Q = B-(K*T);
  U = EXP(Q);
  Z = 1+U;
  M = LOG(Z);
  R = -1/V;
  MODEL LENGTH = A *(Z**R);
  DER.A= Z**R;
  DER.B= U*A*R*(Z**(R-1));
  DER.K= -T*U*A*R*(Z**(R-1));
  DER.V= A*(Z**R)*M/(V*V);
  TITLE 'RICHARDS MODEL';
RUN;
```

A sample output listing of "RICHARDS.SAS" program fitting the 4th leaf length of flowered *Heliconia* in trt. 3.

*---------- LF=4 ----------

Non-Linear Least Squares Iterative Phase Dependent Variable LENGTH Method: Gauss-Newton

Iter	A	B	K	V	Sum of Squares
0	32.000000	3.802726	0.286241	3.890597	154.450897
1	30.592414	1.432816	0.235301	1.448773	58.134962
2	30.553803	2.275998	0.274124	2.142648	51.420694
3	30.531991	3.294998	0.334997	3.202758	32.906728
4	30.539230	3.604540	0.360332	3.633066	27.276455
5	30.537866	3.696208	0.367071	3.744593	27.185963
6	30.537171	3.710681	0.368041	3.760359	27.185609
7	30.537065	3.712428	0.368154	3.762188	27.185606
8	30.537053	3.712626	0.368167	3.762395	27.185606

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable LENGTH

Source	DF	Sum of Squares	Mean Square
Regression	4	56465.204394	14116.301099
Residual	69	27.185606	0.393994
Uncorrected Total	73	56492.390000	
(Corrected Total)	72	2467.748767	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
A	30.53705287	0.09866806917	30.340215079	30.733890667
B	3.71262567	0.68889922480	2.338306648	5.086944685
K	0.36816729	0.04302862514	0.282327370	0.454007220
V	3.76239467	0.73676191013	2.292592026	5.232197323

Asymptotic Correlation Matrix

Corr	A	B	K	V
A	1	-0.326168579	-0.384890652	-0.328324902
B	-0.326168579	1	0.9849173579	0.9943596912
K	-0.384890652	0.9849173579	1	0.9689301965
V	-0.328324902	0.9943596912	0.9689301965	1

Program 6. A SAS program 'LG_RICH.SAS' for estimating parameters of the Richards model from log of leaf length (LLGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*-------------------------------------------------------------*;

PROGRAM 'LF_RICH.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
      PARAMETERS STATEMENT (PARMS)

*-------------------------------------------------------------*;


PROC NLIN DATA=SAVE.LEAFLG  METHOD = GAUSS;
PARMS  A=29.41265627
     B=4.14247176
     K=-0.4137904
     V = 4.47624117;
 Q = B+(K*T);
 U = EXP(Q);
 Z = 1+U;
 M = LOG(Z);
 LLGTH = LOG(LENGTH);
 MODEL LLGTH = LOG(A) - ((1/V)*M);
 DER.A=1/A;
 DER.B=-U/(V*Z);
 DER.K=(-T*U)/(V*Z);
 DER.V=LOG(Z)/V**2;
 TITLE 'RICHARDS MODEL  LOG';
RUN;
```

Program 7. A SAS program 'MMF.SAS' for estimating parameters of the Morgan-Mercer-Flodin model from leaf length (LENGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*-------------------------------------------------------------*;

PROGRAM 'MMF.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
      PARAMETERS STATEMENT (PARMS)

*-------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LEAFLG METHOD = GAUSS;
PARMS A = 29
    B = 10
    K = 400
    V = 7;
Q = T**V;
LT = LOG (T);
MODEL LENGTH = ((B*K)+(A*Q))/(K+Q);
DER.A = Q/(K+Q);
DER.B = K/(K+Q);
DER.K = Q*(B-A)/((K+Q)**2);
DER.V = K*LT*Q*(A-B)/((K+Q)**2);
OUTPUT OUT = SAVE.MMFT2F P=P R=R;
TITLE 'MMF MODEL';
RUN;
```

Program 8. A SAS program 'LG_MMF.SAS' for estimating parameters of the Morgan-Mercer-Flodin model from log of leaf length (LLGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*-------------------------------------------------------------*;

PROGRAM 'LG_MMF.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
      PARAMETERS STATEMENT (PARMS)

*-------------------------------------------------------------*;


PROC NLIN DATA=SAVE.LEAFLG METHOD = GAUSS;
PARMS A = 29.6
    B = 13.5
    K = 421.9
    V = 3.0;
Q = T**V;
LT = LOG (T);
LLGTH = LOG(LENGTH);
MODEL LLGTH = LOG((B*K)+(A*Q)) - LOG(K+Q);
DER.A = Q/((B*K)+(A*Q));
DER.B = K/((B*K)+(A*Q));
DER.K = (B/((B*K)+(A*Q)))-(1/(K+Q));
DER.V = ((A*Q*LT)/((B*K)+(A*Q)))-((Q*LT)/(K+Q));
OUTPUT OUT = SAVE.MMFT2FLG P=P R=R;
TITLE 'MMF MODEL  LOG';
RUN;
```

Program 9. A SAS program 'WEIBULL.SAS' for estimating parameters of the Weibull model from leaf length (LENGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*------------------------------------------------------------*;

PROGRAM 'WEIBULL.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE

      PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
      PARAMETERS STATEMENT (PARMS)

*------------------------------------------------------------*;

PROC NLIN DATA=SEVE.DATA METHOD=GAUSS;
PARMS  A = 29.0
       B = 21.5
       K = 0.4
       V = 3.6;
  Q = T**V;
  U = EXP(-K*Q);
  LT =LOG(T);
  MODEL LENGTH = A-(B*U);
  DER.A=1;
  DER.B = -U;
  DER.K = B*Q*U;
  DER.V = K*B*U*Q*LT;
  TITLE 'WIEBULL MODEL';
RUN;
```

Program 10. A SAS program 'LG_WEIB.SAS' for estimating parameters of the Weibull model from log of leaf length (LLGTH) and time after leaf emergence (T). (A = α, B = β, K = γ and V = δ)

```
*-------------------------------------------------------------*;

PROGRAM 'LG_WEIB.SAS'

INPUT FILE:        LEAFLG
DIRECTORY USED:  SAVE

     VARIABLE          TYPE          DESCRIPTION
     = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
     LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
     T                 NUMERIC       TIME AFTER LEAF EMERGENCE

     PARAMETER: STARTING VALUES OF A, B, K AND V WERE PLACED IN
     PARAMETERS STATEMENT (PARMS)

*-------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LEAFLG METHOD=GAUSS;
PARMS  A = 29.4
     B = 16.5
     K = 0.01
     V = 1.9;
 Q = T**V;
 U = EXP(-K*Q);
 LT =LOG(T);
 LLGTH = LOG(LENGTH);
 MODEL LLGTH = LOG(A-(B*U));
 DER.A=1/(A-(B*U));
 DER.B = -U/(A-(B*U));
 DER.K = B*Q*U/(A-(B*U));
 DER.V = B*K*U*Q*LT/(A-(B*U));
 TITLE 'WIEBULL MODEL  LOG';
RUN;
```

Program 11. A SAS program 'RIC_COMA.SAS' for fitting a common α to each of two groups of data for a Richards model.

```
PROGRAM 'RIC_COMA.SAS'

INPUT FILE:        LF4T1T3
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      =======           =======       ============================
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE
      TRTA              DISCRETE      TREATMENTS - 1: LD
                                                   2: LD+3IfSD+LD
                                                   3: LD+4IfSD+LD
                                                   4: SD

      PARAMETERS: - (A, B, K, AND V) COMMON PARAMETERS FITTED TO  2
                    GROUP OF DATA SET.
                  - (A1-A4, B1-B4, K1-K4, V1-V4) PARAMETERS FITTED TO
                    INDIVIDUAL GROUP OF DATA SET (TREATMENT 1 TO 4).
*-------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LF4T1T3;
PARMS  A = 28.4
       B1 = 2.24        B3 = 3.17
       K1 = 0.29        K3 = 0.34
       V1 = 2.37        V3 = 3.27;
IF TRTA = 1 THEN DO;  T1 = 1; T2 = 0; END;
IF TRTA = 3 THEN DO;  T1 = 0; T2 = 1; END;
B = (B1*T1)+(B3*T2);
K = (K1*T1)+(K3*T2);
V = (V1*T1)+(V3*T2);
Q = B-(K*T);
 U = EXP(Q);
 Z = 1+U;
 M = LOG(Z);
 R = -1/V;
 MODEL LENGTH = A *(Z**R);
 DER.A=Z**R;
 DER.B1= T1*U*A*R*(Z**(R-1));
 DER.B3= T2*U*A*R*(Z**(R-1));
 DER.K1= -T*T1*U*A*R*(Z**(R-1));
 DER.K3= -T*T2*U*A*R*(Z**(R-1));
 DER.V1= T1*A*(Z**R)*M/(V*V);
 DER.V3= T2*A*(Z**R)*M/(V*V);
RUN;
```

A sample output listing of "RICHARDS.SAS" program fitting the 4th leaf length of vegetative plants in Trt. 1 and flowered plants in trt. 3 with common α.

Non-Linear Least Squares Iterative Phase Dependent Variable LENGTH

Method: Gauss-Newton

Iter	A	B1	K1	V1	B3	K3	V3	Sum of Squares
0	28.400000	2.240000	0.290000	2.370000	3.170000	0.340000	3.270000	1461.956866
1	28.611601	1.181909	0.244851	1.388974	8.131626	0.684694	8.474461	1417.039040
2	28.737379	0.600621	0.223329	1.028947	9.010005	0.825176	9.984067	1359.929793
3	28.753842	0.429587	0.217405	0.923940	8.817745	0.809217	9.723954	1359.744388
4	28.755182	0.415043	0.216910	0.915094	8.821538	0.809490	9.727734	1359.742834
5	28.756371	0.402378	0.216481	0.907406	8.817394	0.809090	9.722676	1359.742210
6	28.756611	0.399823	0.216394	0.905858	8.816515	0.809005	9.721608	1359.742164
7	28.756744	0.398387	0.216346	0.904987	8.816361	0.808987	9.721389	1359.742149
8	28.756819	0.397579	0.216318	0.904497	8.816146	0.808965	9.721121	1359.742144

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable LENGTH

Source	DF	Sum of Squares	Mean Square
Regression	7	232202.87786	33171.83969
Residual	333	1359.74214	4.08331
Uncorrected Total	340	233562.62000	
(Corrected Total)	339	10678.77576	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
A	28.75681934	0.1606071942	28.440882302	29.072756387
B1	0.39757867	1.1190239897	-1.803699623	2.598856954
K1	0.21631827	0.0330507854	0.151302702	0.281333830
V1	0.90449732	0.6927188944	-0.458178612	2.267173260
B3	8.81614601	7.3966183577	-5.734047321	23.366339351
K3	0.80896539	0.6091500526	-0.389318825	2.007249608
V3	9.72112148	8.5259630217	-7.050652366	26.492895331

Asymptotic Correlation Matrix

Corr	A	B1	K1	V1	B3	K3	V3
A	1	-0.379693428	-0.487179056	-0.377768174	-0.126042481	-0.139283923	-0.130104202
B1	-0.379693428	1	0.9547782369	0.9986737095	0.0478575016	0.0528851901	0.0493997107
K1	-0.487179056	0.9547782369	1	0.9440062585	0.0614052568	0.06785621	0.0633840426
V1	-0.377768174	0.9986737095	0.9440062585	1	0.0476148377	0.0526170331	0.0491492269
B3	-0.126042481	0.0478575016	0.0614052568	0.0476148377	1	0.9968923405	0.996766484
K3	-0.139283923	0.0528851901	0.06785621	0.0526170331	0.9968923405	1	0.9902238238
V3	-0.130104202	0.0493997107	0.0633840426	0.0491492269	0.996766484	0.9902238238	1

Program 12. A SAS program 'RIC_COMB.SAS' for fitting a common β to each of two groups of data for a Richards model.

```
PROGRAM 'RIC_COMB.SAS'

INPUT FILE:        LF4T1T3
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      ========          ========      ==========================
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE
      TRTA              DISCRETE      TREATMENTS - 1: LD
                                                   2: LD+3IfSD+LD
                                                   3: LD+4IfSD+LD
                                                   4: SD

      PARAMETERS: - (A, B, K, AND V) COMMON PARAMETERS FITTED TO  2
                    GROUP OF DATA SET.
                  - (A1-A4, B1-B4, K1-K4, V1-V4) PARAMETERS FITTED TO
                    INDIVIDUAL GROUP OF DATA SET (TREATMENT 1 TO 4).

*-------------------------------------------------------------*;
PROC NLIN DATA=SAVE.LF4T1_3;
PARMS  B=2
     A1 = 28.64        A3 = 29.76
     V1 = 2.36         V3 = 3.27
     K1 = 0.29         K3 = 0.33;

IF TRTA = 1 THEN DO; T1 = 1; T2 = 0; END;
IF TRTA = 3 THEN DO; T1 = 0; T2 = 1; END;
A = (A1*T1)+(A3*T2);
K = (K1*T1)+(K3*T2);
V = (V1*T1)+(V3*T2);
Q = B-(K*T);
 U = EXP(Q);
 Z = 1+U;
 M = LOG(Z);
 R = -1/V;
 MODEL LENGTH = A *(Z**R);
 DER.A1=T1*Z**R;
 DER.A3=T2*Z**R;
 DER.B= U*A*R*(Z**(R-1));
 DER.K1= -T*T1*U*A*R*(Z**(R-1));
 DER.K3= -T*T2*U*A*R*(Z**(R-1));
 DER.V1= T1*A*(Z**R)*M/(V*V);
 DER.V3= T2*A*(Z**R)*M/(V*V);
RUN;
```

Program 13. A SAS program 'RIC_COMK.SAS' for fitting a common γ to each of two groups of data for a Richards model.

```
PROGRAM 'RIC_COMK.SAS'

INPUT FILE:        LF4T1T3
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE
      TRTA              DISCRETE      TREATMENTS - 1: LD
                                                   2: LD+3IfSD+LD
                                                   3: LD+4IfSD+LD
                                                   4: SD

      PARAMETERS: - (A, B, K, AND V) COMMON PARAMETERS FITTED TO  2
                    GROUP OF DATA SET.
                  - (A1-A4, B1-B4, K1-K4, V1-V4) PARAMETERS FITTED TO
                    INDIVIDUAL GROUP OF DATA SET (TREATMENT 1 TO 4).
*-------------------------------------------------------------*;

PROC NLIN DATA=SAVE.LF4T1_3;
PARMS  K = 0.473
       A1 = 28.64        A3 = 29.76
       B1 = 2.24         B3 = 3.17
       V1 = 2.37         V3 = 3.27;

IF TRTA = 1 THEN DO;  T1 = 1; T2 = 0; END;
IF TRTA = 3 THEN DO;  T1 = 0; T2 = 1; END;
B = (B1*T1)+(B3*T2);
A = (A1*T1)+(A3*T2);
V = (V1*T1)+(V3*T2);
Q = B-(K*T);
 U = EXP(Q);
 Z = 1+U;
 M = LOG(Z);
 R = -1/V;
 MODEL LENGTH = A *(Z**R);
 DER.A1= T1*Z**R;
 DER.A3= T2*Z**R;
 DER.B1= T1*U*A*R*(Z**(R-1));
 DER.B3= T2*U*A*R*(Z**(R-1));
 DER.K= -T*U*A*R*(Z**(R-1));
 DER.V1= T1*A*(Z**R)*M/(V*V);
 DER.V3= T2*A*(Z**R)*M/(V*V);
RUN;
```

Program 14. A SAS program 'RIC_COMV.SAS' for fitting a common δ to each of two groups of data for a Richards model.

```
PROGRAM 'RIC_COMV.SAS'

INPUT FILE:        LF4T1T3
DIRECTORY USED:  SAVE

      VARIABLE          TYPE          DESCRIPTION
      = = = = =         = = = = =     = = = = = = = = = = = = = = = = = = =
      LENGTH            NUMERIC       LENGTH OF LEAF MEASURED IN CM.
      T                 NUMERIC       TIME AFTER LEAF EMERGENCE
      TRTA              DISCRETE      TREATMENTS - 1: LD
                                                   2: LD+3IfSD+LD
                                                   3: LD+4IfSD+LD
                                                   4: SD

      PARAMETERS: - (A, B, K, AND V) COMMON PARAMETERS FITTED TO  2
                    GROUP OF DATA SET.
                  - (A1-A4, B1-B4, K1-K4, V1-V4) PARAMETERS FITTED TO
                    INDIVIDUAL GROUP OF DATA SET (TREATMENT 1 TO 4).

*-------------------------------------------------------------*;
PROC NLIN DATA=SAVE.LF4T1_3;
PARMS   V = 5.02
        A1 = 28.64      A3 = 29.76
        B1 = 2.24       B3 = 3.17
        K1 = 0.29       K3 = 0.33;

IF TRTA = 1 THEN DO;  T1 = 1; T2 = 0; END;
IF TRTA = 3 THEN DO;  T1 = 0; T2 = 1; END;
A = (A1*T1)+(A3*T2);
B = (B1*T1)+(B3*T2);
K = (K1*T1)+(K3*T2);
Q = B-(K*T);
 U = EXP(Q);
 Z = 1+U;
 M = LOG(Z);
 R = -1/V;
 MODEL LENGTH = A *(Z**R);
 DER.A1 = T1*Z**R;
 DER.A3 = T2*Z**R;
 DER.B1 = T1*U*A*R*(Z**(R-1));
 DER.B3 = T2*U*A*R*(Z**(R-1));
 DER.K1 = -T*T1*U*A*R*(Z**(R-1));
 DER.K3 = -T*T2*U*A*R*(Z**(R-1));
 DER.V = A*(Z**R)*M/(V*V);
RUN;
```

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