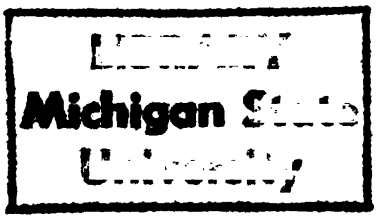

This is to certify that the

thesis entitled

Environmental and Physiological Factors Influencing the Response of Chrysanthemum morifolium cvs. "Bright Golden Anne" and "Circus" To Daminozide

presented by

John Enos Erwin

has been accepted towards fulfillment
of the requirements for

Master's degree in Horticulture

Ronald D. Heins

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Date April 1, 1986

Environmental And Physiological Factors Influencing The Response Of *Chrysanthemum morifolium* cvs. 'Bright Golden Anne' and 'Circus' To Daminozide

By

John Enos Erwin

Thesis

Submitted To
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Abstract

Environmental and Physiological Factors Influencing the Response of *Chrysanthemum morifolium* Ramat. cvs. 'Bright Golden Anne' and 'Circus' to Daminozide

Applications of daminozide to *Chrysanthemum morifolium* shoots early in development (less than 7 days after an internode reached 0.5 cm in length) stimulated internode cell division by as much as 120 %. However, cell elongation was retarded to such a degree that final internode length was reduced 30 to 40 %. Proximal internodes responded more strongly to daminozide than more distal internodes.

Retardation of final shoot length by a 5000 mg l^{-1} a.i. daminozide application to 'Bright Golden Anne' and 'Circus' shoots decreased 76 and 83 %, respectively, as day temperature increased from 10°C to 26°C. Increasing day temperature from 26°C to 30°C reversed this trend and increased daminozide retardation of shoot length. Photosynthetic photon flux did not influence plant response to daminozide.

Three methods were evaluated for modeling the effect of day temperature and daminozide concentration on shoot elongation. Method II predicted parameter estimates for the gompertz function through multilinear regression. When the parameter estimates were evaluated within the gompertz function shoot elongation over time was predicted. Method II showed the greatest potential for modeling stem elongation as influenced by day temperature and daminozide.

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Dedication

This thesis is dedicated to my grandparents without whos guidance and encouragement the completion of this work would not have been possible. It is only my hope that I may be as good a grandparent as my grandparents have been for me. I love them all very much.

Edward Enos Erwin

Sophie Blandina Erwin

Janis Albert Gedrovics

Zinaida Gedrovics

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As with everything I have undertaken, my family has supported and encouraged me throughout this project. I am deeply grateful to all of them.

I have been very fortunate throughout my life to have good friends which have always been there to help me when I need encouragement. I would especially like to thank Candice Shoemaker, Rob Berghage, Mike McCaffery, and Jim Eppink. In addition, I would also like to express my gratitude to Hoyt Emmons, whose enthusiasm and encouragement have always, and will always, be an inspiration to me.

I have also been fortunate to work with a wonderful major professor and friend, Royal Heins. Royal's patience and willingness to give support have been tremendous.

Lastly, I would like to thank the rest of my graduate committee, John Bukovac, William Carlson, and C. Alan Rotz, for their efforts and encouragement.

Literature Review

Cell Wall Structure and Expansion

Plant Cell Composition The primary plant cell wall of higher plants is composed of cellulose, a matrix of various compounds, and water (82,125). The cellulose molecule is composed of long chains of B- 1,4 linked glucose units (82,125). These chains of glucose are organized into highly ordered crystalline lattices which are held together by hydrogen bonds (82,125). When approximately one hundred cellulose molecules are organized into a lattice, they are collectively called a 'micelle'. Twenty or more micelles interwoven into a helical structure are called a microfibril. These microfibrils are imbedded in a matrix composed of pectins, protopectins (with a high hydroxyproline content), lignins, and hemicelluloses consisting of pentoses, arabinose, xylose, hexose, glucose, galactose and mannose (82,125). The hemicellulose sugars are organized into chains which form a highly hydrated hydrophilic gel. The degree of hydration of the gel is variable.

Cell Wall Expansion: For growth of a cell to occur the wall or microfibril network must yield to an applied stress (19,41,72,88,125). The degree of stress a microfibril network can tolerate before it yields is determined by the

coherence between, and the alignment of, the the microfibrils (19,70,88) . Coherence is dependent on the number of crosslinkages between the microfibrils. (19,70). Crosslinkages consist of both covalent and hydrogen bonds which contain sufficient potential energy to retain the integrity of the system. As the number of crosslinkages increases, cell wall rigidity increases and resistance to an applied force increases.

Alignment of the microfibrils also influences resistance to an applied force. Resistance to elongation is greatest on a plane parallel to the microfibril alignment (96) and least in a plane perpendicular to microfibril alignment. Extension of a cell wall would therefore be easiest in a plane with a low microfibril density, and perpendicular to the alignment of the microfibrils.

Since cell wall elongation is influenced by microfibril orientation in the cell wall, cell shape is determined to a great extent by microfibril orientation (96). Early in differentiation, all plant cells have a multinet, i.e. random, micrifibril orientation. When the microfibrils are deposited in a multinet manner isodiameteric growth results (19,96). However, as maturation progresses, differential placement of microfibrils may occur resulting in non-isodiameteric growth. (19,37,96). Cells with a transverse microfibril distribution elongate more longitudinally than laterally and appear as a cylinder (37,96). Cells with an

oblique orientation of microfibrils expand on that side of the cell with the least stress tolerance and elongate as a helix or spiral (37,88,96). Examples of spiraling growth are seen in Phycomyces sporangiophore cells and Trandscatia staminal hairs (37).

The shape of a cell may also be influenced by the stress from adjacent cells. The growth of a cell influenced by the stress from surrounding cells is said to undergo 'passive growth' (Fig. 1 (96)) (19). For instance, if an epidermal cell is isolated and allowed to expand in a suspension, it would develop a 'U' shape due to an oblique distribution of the microfibrils. Axial stress placed on the cell by adjacent cells in the whole plant results in a cylinderically shaped cell.

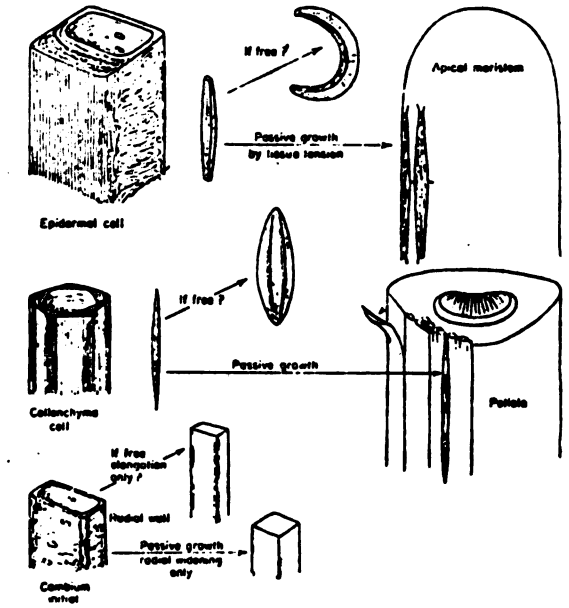


Fig. 20. Diagram of examples of passive growth.

Figure 1

Dynamics of Cell Wall Extension The cell wall with its microfibril network has a structure similar to a polymer (19,70). Therefore, a polymer system may be used to help understand the mechanical properties of the plant cell wall.

When a force is applied to a polymer, it undergoes a deformation (102,110,125). Upon release of the force the polymer may be permanently deformed or it may return to its original shape (19,70,102). The degree and mode of deformation is dependent on the extent of polymerization and crosslinkage (110). Linear polymers (i.e. vegetable oil) have few, if any, crosslinkages and the deformation is a linear function of time and is proportional to the magnitude of the stress (70). When the deformation of a material is both irreversible and a linear function of time (i.e. linear polymers), it is said to undergo viscous flow (19,70,110).

In contrast to linear polymers, deformation of rubbers which have a high degree of crosslinking is not a linear function of time and is often temporary, or elastic (19,70,102). Elastic extension involves the reorientation of configurations from a minimum potential energy to a configuration which has a greater potential energy (70). The energy used in changing a configuration is regained when the stress is removed. (19,110). Long polymers which are crosslinked such as cellulose microfibrils often undergo an

intermediate type of deformation called 'viscoelastic extension'. Viscoelastic extension contains both a viscous flow and an elastic component (19,70,102,125).

Viscoelastic Extension Viscoelastic substances, when placed under stress, undergo an initial phase of rapid elongation which is elastic (19,70). This elastic extension phase persists until a 'yield point' is reached (19,70). After the 'yield point' is reached the material will undergo a phase of extension called 'viscoelastic creep' (19,20,110). This phase is characterized by both a viscous flow component and an elastic component. The rate of elongation during this phase is constantly diminishing which ultimately approaches a horizontal asymptote (Fig. 2 (19)) (19,70).

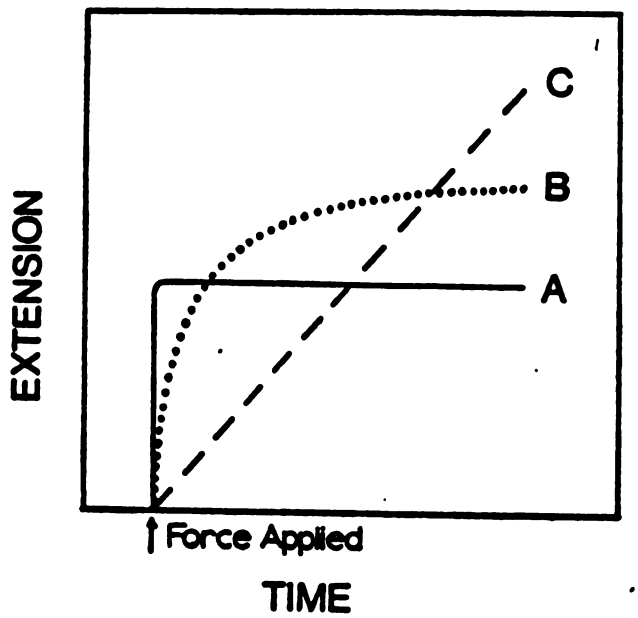


FIGURE 1. The relationship between extension and time for instantaneous elastic extension (A), viscoelastic extension (B), and viscous flow (C).

Figure 2

Extension beyond the yield point often results in scission of bonds (19,70,102). Scission occurs as a result of a bond configurations inability to retain the potential energy which it is receiving (70). Excessive scission of crosslinkages ultimately results in a tearing of the material (19,70). The stress required to tear a material is called the 'ultimate yield stress'. If the force is removed prior to tearing the material, the material undergoes an 'instantaneous elastic deformation' (19,70). The material will not, however, return to its original length if scission of bonds has occurred (70). The difference between the original length, and the length after the force has been applied is referred to as the 'plastic component' (19,70,125).

Whether a viscoelastic extension is reversible depends upon the history of the material (19,20,70). If a material has been extended previously, subsequent extension will be entirely elastic if the extension does not exceed the length of the previous Extension (19,20,70). If an Extension is beyond the length of the previous Extension both an elastic and a plastic component will occur (19,20,70). The ability of a polymer system to change from irreversible to reversible Extension is known as 'mechanical conditioning' (19).

The physical concepts of viscoelastic Extension studied in rheology have been found to apply to the behavior of

physical cell wall section extension. An intact plant cell can maintain a constant rate of Extension for up to twenty four hours (19). Viscoelastic Extension alone results in a continuously diminishing rate of elongation (19,70). Therefore, constant plant growth rate is composed of a series of small viscoelastic extensions with each subsequent Extension being greater than the previous one (19).

Biochemical Modification Cell elongation is composed of not only a viscoelastic Extension but also biochemical modification (19,46,90). This is best demonstrated by the sensitivity of elongation to excessively high temperatures, and metabolic inhibitors (20,46). Ray determined that the rate of elongation of Avena coleoptile segments increased 3.5 times in response to a temperature increase from 2 to 23 degrees centigrade. Metabolic inhibitors such as cyanide inhibit the increase in rate of elongation due to temperature (20,90,91). Hager determined that the elongation response seen when Helianthus annuus hypocotyl segments were placed in a buffer at pH 4 was completely inhibited by addition of 5 mM Cu^{+2} ions. He concluded that elongation must be a result of enzyme catalyzed reactions with a pH optima of approximately 4 (46). In contrast, Cleland has found that neither temperature or metabolic inhibitors had any significant effect on physical cell wall Extension of Avena (19).

Turgor Pressure The force which drives cell elongation is the internal hydrostatic pressure of the cell, or tugor pressure (19,70,80). This force is variable and dependent on the size, shape, and water potential of the cell (82).

The larger the size or diameter of a cell the greater the total stress which is placed on the cell wall (19,82). This relationship is given by the equation:

$$\frac{r\ P}{2\ \Delta\ r} = \text{Cell Wall Stress}$$

Where r = radius of the cell
P = internal pressure of the cell (bars)
Δr = wall thickness

The relationship is demonstrated by comparing a cell with a four centimeter diameter (Valonia) and one with a 4 micrometer diameter (Chlorella) (82). The total pressure exerted on the Valonia cell wall is one thousand times greater than the pressure exerted on the Chlorella cell wall (82).

The shape of the cell determines if the hydrostatic pressure will be distributed equally or preferentially to any side of the cell (70,82,96). For instance, the stress on the side walls and end walls of a cylinder shaped cell (Nitella) may be separated into a transverse and a longitudinal stress:

$$\text{Transverse Cell Wall Stress} = \frac{r\,P}{2\Delta r} \qquad \text{Longitudinal Cell Wall Stress} = \frac{r\,P}{\Delta r}$$

The force exerted on the side walls is twice that which is exerted on the end walls (82).

The water potential of a cell is inversely proportional to the force which is exerted on the cell wall. Therefore, those cells with a lower osmotic potential (more negative) will have a greater tendency to elongate than cells with higher osmotic potentials due the greater turgor pressure and the resulting increase in stress on the cell wall (79).

Cell elongation will only occur if the turgor pressure inside the cell is greater than some critical value (19,41,42). In an expanding cell this critical value is variable and dependent on the turgor pressure, i.e. the critical value changes as the turgor pressure changes (19,41,42). Typically the difference between the critical value and the turgor pressure is approximately .2 bars. If the turgor pressure in the cell is dropped via an external osmoticum, growth of a cell will initially cease (19,41,42). After a period of time, which is proportional to the drop in turgor pressure, the critical value drops and growth resumes at a rate similar to its previous rate (19,41,42). If the turgor pressure is increased to its original value, growth acceleration will occur for a short time (41,42). The additional length 'gained' during this burst of growth

will often be equal to that which was initially lost with the drop in turgor.

The dependence of cell elongation on a critical turgor suggests that wall loosening and/or wall Extension may be dependent on a critical turgor pressure (19). The absence of a critical turgor pressure for physical cell wall extension of Avena coleoptile sections suggests that it is not the wall extension process which is the limiting factor (19). Auxin induced wall loosening has, however, been found to have a critical turgor suggesting that the biochemical modification of the cell wall may be dependent on a critical turgor (19).

The dependence of cell elongation on a critical turgor pressure has led to the hypothesis that cell elongation involves a reversible crosslinkage cleavage which requires a minimum pressure for scission to occur and subsequent sliding of the microfibrils along each other and reformation of bonds (19). If that pressure is not present, the bonds will reform in their original positions. Similar behavior is seen in rubbers where some catalysts are only effective if the material is in an extended state (110).

Conclusion The plant cell wall has physical properties which make it structurally similar to polymers. Basic concepts of rheology may be used to describe the behavior of a cell wall sections when placed under stress. The plant cell elongates through a series of small viscoelastic

extensions. If a stress is greater than the yield stress the cell may undergo plastic deformation followed by a biochemical modification resulting in a permanent increase in size. For cell elongation to occur the turgor pressure of the cell must be greater than a critical value needed for elongation.

Horomonal Responses

Auxin Auxin increases cellular elongation by increasing the plasticity of the cell wall (9,34,79,86,107). The mechanism involved in induction of accelerated elongation by auxin is still controversial. The theory, which has gained widest acceptance is the 'acid-growth hypothesis'(91).

A number of recent studies have supported that both the low pH induced growth observed by Bonner (9,32,90), and auxin induced growth as having the same mechanism for inducing elongation, i.e. acidification of the cell wall and/or cytoplasm (33,46,53,90,92). This acidification may stimulate the activity of acid labile enzymes with low pH optima to cleave bonds between microfibrils (46). This reduction in coherence facilitates cell enlargement by allowing the microfibrils to slide along each other. The theory is supported by the following:

1. the discovery that auxin causes a drop in the pH of both the cytoplasm and the cell wall (21,32,34,53,91,111).
2. the pH drop occurs immediately before auxin stimulated elongation (20,45,84,89,109).
3. Specific wall bound enzymes, glycosidases, have been found to have low pH optima (45,77,108).
4. Metabolic inhibitors and reduced temperatures inhibit both auxin induced cell elongation and low pH induced elongation (19,21,32,46,90).

A secondary effect of auxin induced wall loosening is to decrease the cell wall pressure (34,62,79). This results in an increase in the turgor pressure which allows for continued Extension of the cell. This increase in turgor pressure occurs only when the turgor pressure is above the critical threshold value to enable bond cleavage to occur (18).

The acid growth theory is not supported by Pope who has observed that the auxin induced elongation and the acid growth stimulation of elongation have different pH optima which suggests that they may act through two separated mechanisms (86). In addition, Barkley and Leopold have shown that green pea stems respond strongly to an auxin treatment but not to an acidic buffer treatment (33).

Gibberellins Gibberellic acid has been shown to increase cell wall extensibility (55) in Avena internode (1,55), lettuce hypocotyl (55,106), and etiolated pea epicotyl segments (55). In addition, gibberellins may in some cases cause an increase in synthesis of IAA or decrease in destruction of IAA oxidase. Gibberellins promote cell wall synthesis. The promotion of cell wall synthesis has been found to occur simultaneously with the initiation of gibberellin induced elongation (55). Of the cell wall constituents, polysaccahides are preferentially synthesized in response to gibberellins. The mechanism by which gibberellins increase Extension has not been found to involve acidification as is seen with auxin (55). Instead gibberellins have been found to increase the concentration of calcium ions in the protoplast (55). Since the presence of calcium ions in the cell wall is believed to decrease the extensibility of the wall, an influx of such ions into the protoplast from the cell wall is thought to increase the extensibility of the cell wall (55). There is also evidence that GA may reduce the osmotic potential of the cell cytoplasm , and as a result, increase the turgor pressure and as a result, the stress applied to the cell wall (55).

Ethylene Ethylene affects cell division (67), cell expansion, auxin transport, auxin synthesis (27), GA response to tissue (27), and crosslinking of microfibrils (27) in plant cells (82). Cell division and D.N.A. synthesis (27) are inhibited in the apical hook of etiolated pea seedlings after an application of 50 mg l^{-1} ethylene (67) while in some aquatic plants ethylene stimulated cell division (82).

In terrestrial plants, ethylene has been shown to inhibit longitudinal expansion of cells, while promoting isodiameteric or radial expansion (4,27,61,67,82). The decrease in longitudinal expansion results from reorientation of microtubules from radial to longitudinal (27,67,82). Therefore, radial expansion was favored to longitudinal (67).

Ethylene also retarded both lateral auxin transport (67) and polar auxin transport (4,27,66,67) and enhanced indoleacetic acid oxidase activity (67). Ethylene also reduced both the amount of auxin present within the plant tissue and the mobility of that auxin. This was accomplished through ethylene enhanced IAA oxidase activity and retardation of polar auxin transport (4,67).

Plant Cell Differentiation and Development

The Apical Meristem The apical meristem is the progenitor of all aerial plant parts (97,98). It is located at the distal tip of a stem and is composed of a group of meristematic cells organized into a hemisphereical or conical dome from a few microns to several millimeters in size. Two distinct layers are present in the meristem (97,98): the tunic, and the corpus (Fig. 3 (98)).

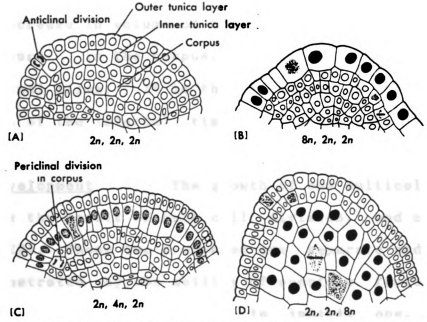


Figure 7-2. Diagrams of longitudinal sections of the promeristem region of shoot apices of *Datura*. A: Normal, untreated plant with all cells normal diploid. **B–D:** Different types of periclinal chimeras in plants after recovery from treatment with colchicine. Polyploidy occurs in the outer tunica layer only (**B**), in the inner tunica layer only (**C**), or in the corpus (**D**). Note orientation of anticlinal cell division in **A** and periclinal division in **C**. [Adapted from S. Satina, A. F. Blakeslee, and A. G. Avery, in *American Journal of Botany*, 27: 895, 1940.]

Figure 3

The tunic is composed of the outermost one to four cell layers of the meristem. Cell division within the tunic is through aniticlinal division, i.e. perpendicular to the nearest outer wall, and results in an increase in the surface area of the meristem (98). Periclinal division, parallel to the nearest outer wall, only occurs when leaves are being initiated. Differentiation within this tissue ultimately leads to the development of the epidermis.

The corpus is located below the tunic and is composed of randomly organized cells which undergo both anticlinal and periclinal division (82). Division in the corpus leads to an increase in volume of the meristem. As differentiation occurs in the corpus, the cells are organized into longitudinal files. It is this tissue which leads to the formation of the internal tissues of the plant.

Cell Development The growth of any multicellular organism is the result of both cell division, and cell elongation (29). These two processes are separate and distinct as demonstrated by the ability of specific factors, i.e. low fluence rates of light, to inhibit one, cellular division, but not the other (97). Typically both processes occur simultaneously within growing systems although Extension of the stem in the early phases of internode development is predominantly a result of cell division (97). The later phases of extension are primarily a result of cel-

lular elongation. The duration of both cell division and cell elongation is determined by genetic predetermination, and external factors which influence these processes through internal growth regulators (63,97). Two external environmental factors of particular importance are light and temperature.

The Influence Of Light On Stem Elongation

The Effect Of Light Intensity On Stem Elongation Plant shoot length is reduced by exposure to white light (64,79,109,116,118). Went determined that an exposure for 100 minutes to orange light (>750 nm) with a fluence of 3 ergs cm^{-1} sec^{-2} retarded elongation of the first and second internodes of etiolated Avena seedlings while stimulating elongation of the third and fourth internodes. Overall elongation was retarded as the final total stem length was 46% less than that of plants grown in the dark. The stimulation of the more distal internodes was attributed to a 'compensatory mechanism' in the plant which responded to a retardation of the more proximal internodes (116).

Kinetic growth studies by Thompson determined the response of an internode was not dependent on its position on the plant as Went had suggested, but rather, on the internode's developmental stage when it was exposed to

light (40,111). Exposure of an actively dividing cells to white light reduced the duration of cell division in the internode, but increased the rate at which division occurred. Exposure of older elongating internodes to light where elongation was primarily occurring through cell elongation was found to reduce the duration of the cell elongation phase, and as a result, the length of the individual cells (39,109). Therefore, the overall reduction of elongation observed by Went may have been a result of an acceleration in maturity of the individual cells (39,109), which resulted in fewer (Fig. 4 (11)) and shorter (Fig. 5 (11)) cells within an internode.

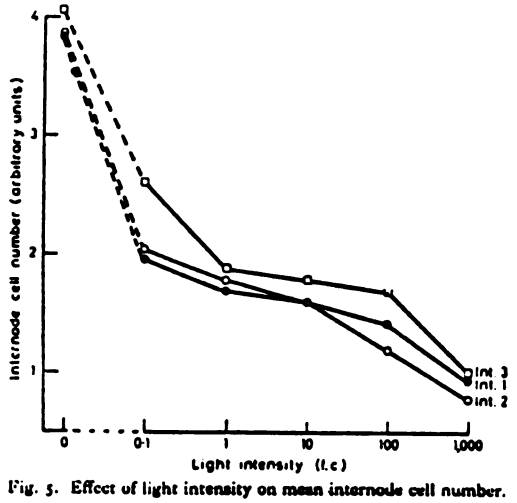


Fig. 5. Effect of light intensity on mean internode cell number.

Figure 4

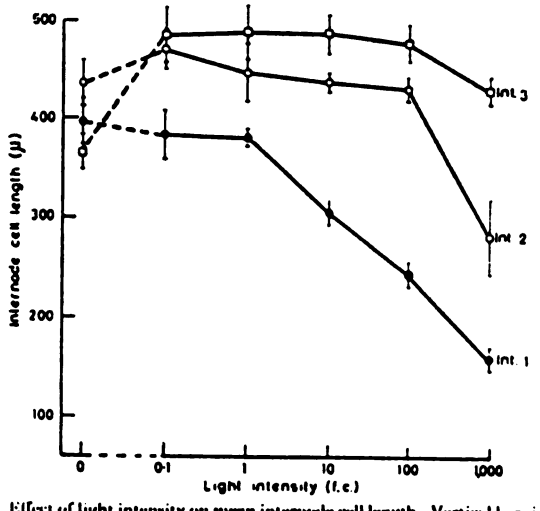


Fig. 6. Effect of light intensity on mean internode cell length. Vertical bars indicate the fiducial limits at the P = 0·05 level.

Figure 5

An actively growing plant is composed of several internodes, each of which may be in a different developmental stage at any given time (29,64). Therefore, exposure to light may influence both cell elongation and cell division in different degrees, with the overall response depending on the developmental stage of the individual internodes (Table 1 (109)) (109).

TABLE 5. *Dimensions and numbers of phloem cells (sieve elements). Values are means for 8–10 cells in each of 5 plants. Other specifications as for Table 3*

	Cell dimensions (μ)					
	Int. 1	Int. 2	Int. 3	Int. 4	Int. 5	Int. 6
White	82.0 × 11.2	91.2 × 10.4	87.0 × 10.3	64.3 × 9.6	44.0 × 7.9	35.7 × 6.4
Red	130.8 × 11.2	125.7 × 10.0	84.4 × 9.1	67.8 × 8.6	49.9 × 7.3	37.6 × 6.5
Dark	149.4 × 12.8	120.4 × 10.0	149.7 × 10.2	63.3 × 8.3	41.0 × 7.4	29.2 × 6.8
	Calculated number of cells per internode					
White	207.7	169.9	208.0	59.1	19.5	9.0
Red	243.1	288.0	630.3	126.8	26.1	7.2
Dark	613.8	745.8	605.2+	62.1	18.8	5.3

Table 1

The irradiance which the plant perceives throughout the day plays a major role in stem elongation (Fig. 6 (117)). Seventy to ninety percent of tomato stem elongation occurred during the night (117,118). The slowest rate of

Extension occurred at noon (117), and the maximum occurred just prior to dawn (117). The maximum rate of elongation that occurs in the dark is dependent on the light fluence prior to the dark period (65). An abrupt drop in the rate of elongation occurs as soon as the plant is exposed to daylight, which is consistent with the theories of blue and red light retardation (117,118).

In contrast to stem elongation, Van Volkenburgh determined that white light increases cell wall extensibility and rapid cell elongation in Phaseolus vulgaris leaves. He suggested that light stimulated H^+ excretion from leaf cells in the presence of light and elongation via the acid growth hypothesis (112,113).

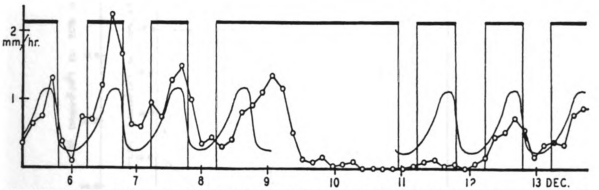


Fig. 1. Growth rate of tomato stems (ordinate in mm. per hour, mean of two plants), kept continuously at 26.5°C., either in natural daylight or in darkness. Periods of darkness indicated by heavy line along top of graph. Dates of consecutive days marked at noon on abscissa. Smooth line gives the mean of all measurements from December 5–8 and December 12–14; line connecting the circles gives the actual growth rate.

Figure 6

<u>The Effects Of Light Quality on Stem Elongation</u> Stem elongation can be profoundly influenced by both light fluence (38,108) and light quality (28,38). Until recently, most plant photomorphogenic responses were believed to be influenced only by phytochrome. It has become increasingly apparent that there are two photoreceptor pigments: a photoreceptor in the blue region and phytochrome.

Retardation of stem elongation can be achieved by exposure of plant tissues to blue light (400-500nm)(Fig. 7 (28)) (15,28,38,94,108).

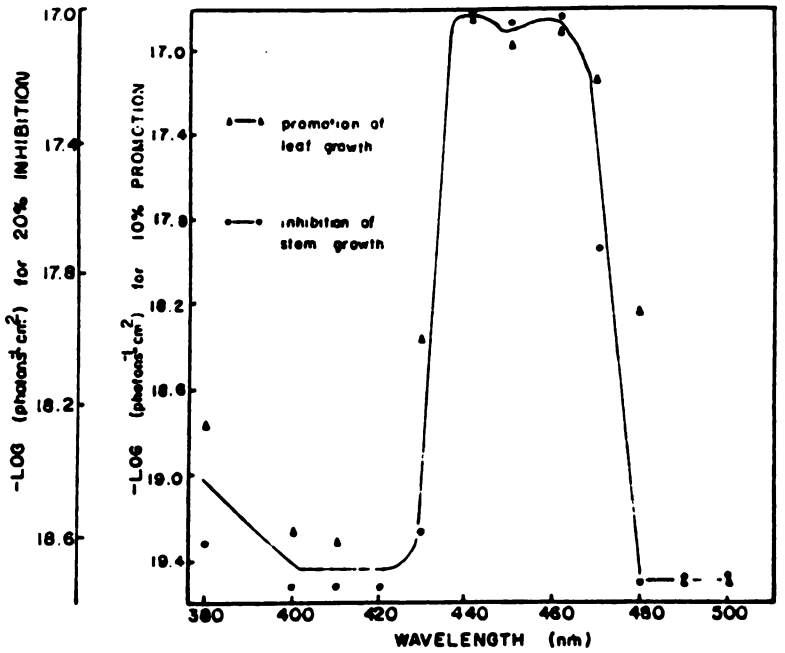


Figure 7

Retardation of cucumber hypocotyls began five minutes after an exposure to white light and persisted for thirty minutes (38). Growth resumed at a rate similar to that previous to the exposure (38) . The extent of the retardation may range from 50- 95% with a fluence of 11 umol s^{-1} m^{-2} blue light and is species dependent. Retardation increased with increasing fluence rate. The relationship between fluence rate and retardation is log linear in cucumber and lettuce (39,64,108) (Fig. 8).

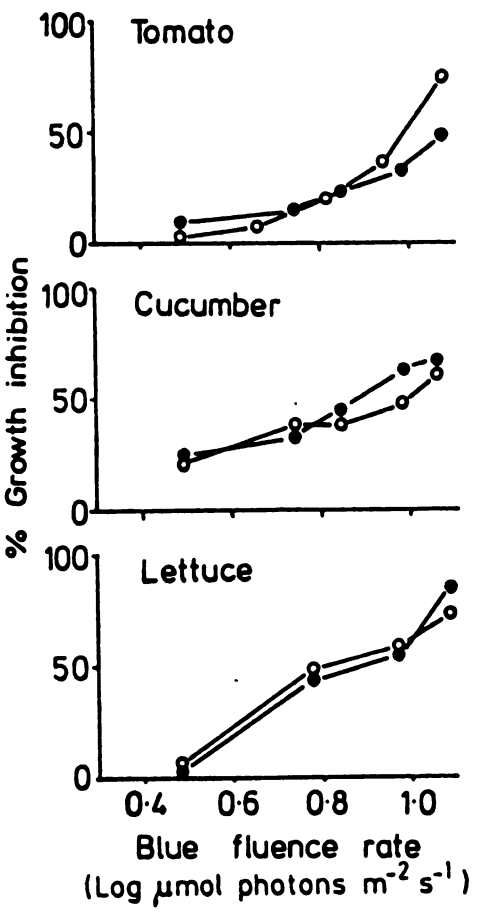


Fig. 2. Fluence-response curves for blue light inhibition of hypocotyl extension in de-etiolated cucumber, lettuce and tomato in the presence of SOX at 210 μmol photons $m^{-2}s^{-1}$ o—o or 150 μmol photons $m^{-2}s^{-1}$ ●—●. Results were calculated as

Figure 8

Exposure of plant tissues to red light (600-710nm) also leads to retardation of elongation (28,39)(Fig. 9 (28)). In most cases, the degree of retardation is equivalent to that seen with blue light (15,25,28,38,39,108). The period between termination of an exposure and initiation of retardation is, however, considerably longer than that seen with blue light (39). Exposure of <u>Cucumis sativus</u> seedlings to 8 W m^{-2} red light for 1 hour did not produce any significant response until 5-6 hours after the exposure (38). The degree of retardation increased from 5 to 10 hours after an exposure, there after the plant returned to a growth rate equal to that prior to a red light exposure.

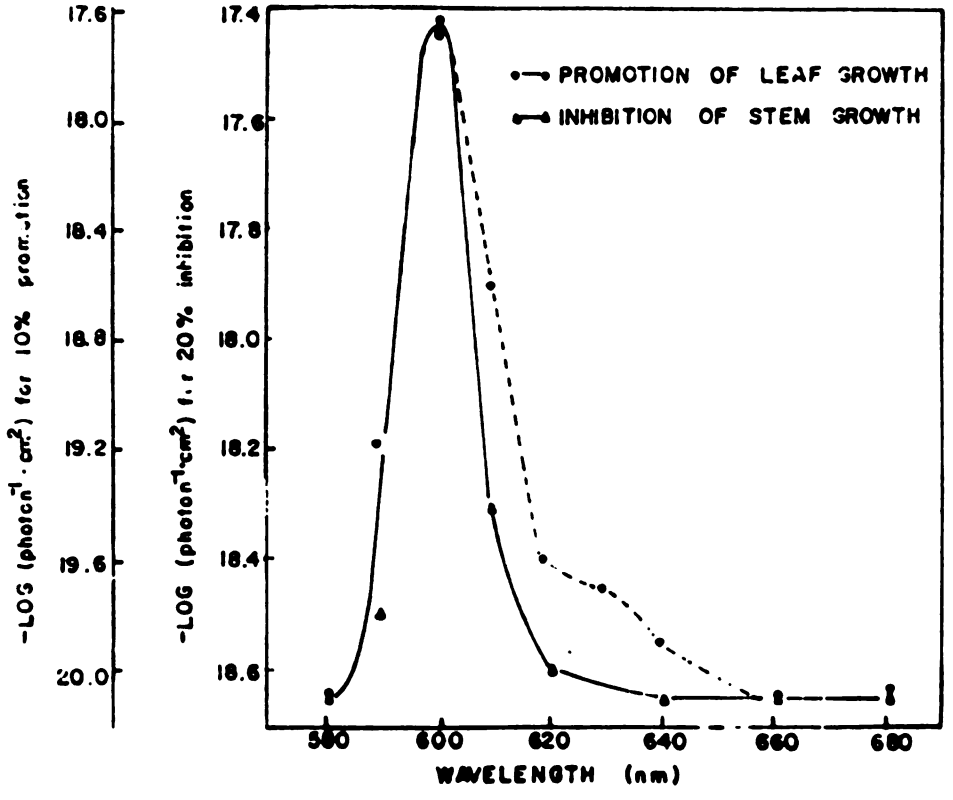


Figure 9

Exposure of seedlings to both blue and red light, i.e. white light, for 1 hour resulted in a dual kinetic response (Fig. 10). The seedlings underwent an immediate retardation of elongation which persisted for 20 minutes (64). After which growth returned to the rate previous to the exposure (39). Approximately two hours after the exposure a second period of retardation of elongation occurred (39).

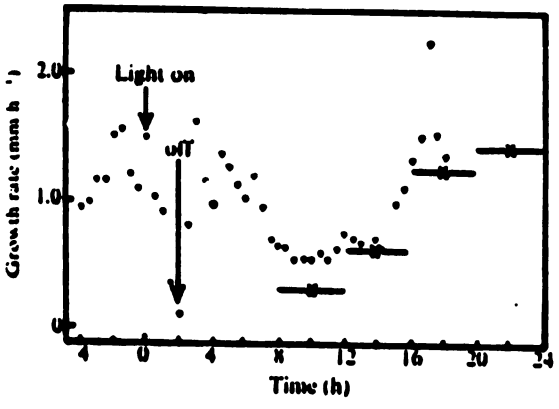


Fig. 3 Effect of a brief white light irradiation. The white light source consisted of 20-W 'daylight' tubes. (Fluence rate as in Table 2.) Crosses mark the mean growth rates calculated every 4 h (shown by the horizontal bar) of similarly treated seedlings, measured by ruler ($n = 12$). As in Fig. 1*b* these indicate that the seedlings merely recover their growth rate, showing no entrained rhythm.

Figure 10

The location of the blue and red photoreceptors are believed to be in two separate areas, the hypocotyl and the cotyledons, (39). The blue light retardation response acts directly on the hypocotyl (38,39,64). Exposure of the

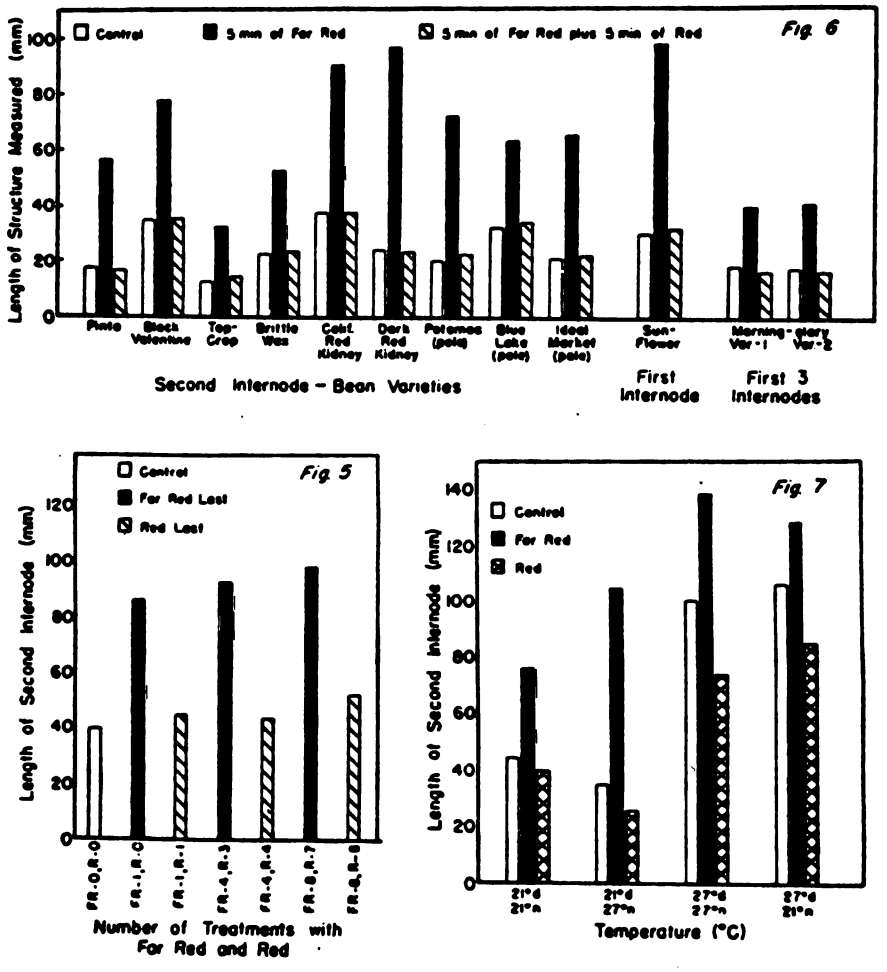
cotyledons to blue light does not enhance or prolong the response (38). In contrast, the phytochrome, or red light retardation response is reduced in magnitude but not duration if tissue other than the cotyledons are exposed to white light (38).

Conclusion There appears to be two photoreceptor pigments involved in retardation of stem elongation by light, the blue photoreceptor and phytochrome (11,38,39,64,108). This conclusion is supported by the following observations:

1. Retardation of stem elongation by both blue light (400-500nm) and red light (600-710nm) (39).
2. Response kinetics differ between red and blue light (39).
3. Differential sensitivity of specific tissues (cotyledon, hypocotyl) to either blue or red light (39,108). This suggests different locations of the photoreceptors.
4. The species specific sensitivity seen in some cases (lettuce, mustard) to only blue light or red light (39,81).

The fluence dependence of the blue light retardation and the lack of a fluence dependence of the retardation response from phytochrome suggests that the purpose of a blue photoreceptor may be to detect changes in fluence rate (94). The photomorphogenic responses seen with small shifts in the Pfr/Pr ratio of phytochrome suggests that the primary role of phytochrome may be in perceiving changes in light quality (80).

Stimulation of Elongation by Far Red Light Far red light (710-800nm) stimulates stem elongation in a number of plant species (15,25,39,83)(Fig. 11 (25)). A five minute exposure to far red light after an eight hour exposure to white light increased internode elongation 400% in Phaseolus.



FIGS. 5-7.—Mean lengths of indicated parts. Fig. 5, Pinto bean plants treated with various numbers of alternating 5-minute periods of far-red and red radiant energies; treatments given at beginning of dark period with far red first in each instance. Fig. 6, bean, sunflower, and morning-glory plants treated with indicated irradiations at beginning of 16-hour dark periods. Fig. 7, Pinto bean plants grown with different combinations of day and night temperatures and exposed to 5 minutes of far red or to 5 minutes of red radiation at beginning of 16-hour dark periods.

Figure 11

Elongation increased as a log linear function of a decrease in the photoequilibria to a Pfr/Pr ratio of 0.3 (39). The magnitude of the elongation response was reduced as the time between the termination of the white light exposure and initiation of the far red exposure prior to a far-red exposure increased. The elongation response was also reduced as the length of the white light exposure prior the a far-red exposure increased (115), and the elongation response was enhanced as the white light fluence rate increased. The stimulation of elongation was reversible if the 5 minute exposure to far-red light was followed by a five minute exposure to red light (600-710nm) (25). Morgan has determined that the importance of the end of day exposure increases as the photoperiod decreases (80). The rapidity of this induction and its reversibility strongly suggests the involvement of phytochrome as a photoreceptor (25).

Far-red stimulation of elongation is not restricted to an end of day exposure. Comparisons between plants grown under white light supplemented with far red light and plants treated with an end of day exposure showed that 80% of the resulting elongation response was a result of the far-red to red ratio during the day (80).

The magnitude of the elongation response to the red far red ratio is species specific (81). Species native to open grasslands such as Veronica persica and Senecio vul-

garis where phytochrome photoequilibria tend to be higher below the canopy tend to respond more strongly to a reduction in photoequilibria than species native to lower canopies of woodlands such as Oxalis acetosella and Geum urbanum where phytochrome photoequilibria tend to be lower (81).

Hormonal Basis For The Effects of Light on Stem Elongation

Application of red light to Avena coleoptile segments retarded elongation after 120 minutes, and reduced cell wall plasticity after 180 minutes (96). Exposure of Avena coleoptile cell walls has shown that a 4-hour exposure to red light reduced auxin induced proton excretion (96). Galston determined that Avena coleoptile segments contain less extractible auxins after an exposure to red light than before the exposure (40).

Lockhart suggested that light reduces the a decrease in the level of endogenous gibberellins within Phaseolus vulgaris cv. Mores's Progress #9 (69). Kende and Lang compared the gibberellin content of dwarf (only expressed in the presence of light) and normal Pharbitis (60) and concluded that light influenced the response of plant tissues to gibberellins and not the total gibberellin content of the plant (55,60).

The Effects Of Light On Growth Inhibitors Until recently, it was believed that responses to environmental stimuli were primarily dependent on the concentration of growth 'promoters' present within a system at any particular time i.e. auxins and gibberellins. It is now understood that there are substances within a plant which inhibit 'promoter' response (102,64,59,57,58,48). Therefore, plant growth results from a coordinated interaction between promoter and inhibitor concentrations (102,64,57,58,48).

The process of cell elongation promotes synthesis of peroxidase by the cell (58). Peroxidase has at least two functions, deactivation of indolacetic acid and the participation in polymerization of phenols into lignins (58). Lignins are the primary component of a cell wall which make its size permanent. Secondary effects of peroxidase promotion include reduction of internal auxin levels, reduced influence of intracellular auxin upon cellular elongation and stimulation of biosynthesis of phenolic compounds.

The discovery of natural phenolic activity within the cell promoted research into the differences in the concentrations of phenols that may exist in either genetically or environmentally dwarfed plants. In studies on Pisum sativum, the concentration of quercetin-3-glycosyl-p-coumarate (QGC), a phenolic precursor, was found to increase significantly under environments of high light intensity (57,40). Dramatic increases in phenolic precursors

were also noted in dark versus light grown plants (11).

Phenols can be divided into two groups, the polyphenols such as chlorogenic, caffeic, ferulic, and protocatechuic acids which suppress IAA oxidase acivity, and monophenols such as paracoumaric, parahydroxybenzoic, vanillic, stringic, phloretic, and quercietic acids which stimulate I.A.A. oxidase synthesis (78). When stimulatory and inhibitory phenols are present together in the plant the inhibitory function is found to predominate (78).

Light stimulates cell elongation in younger tissues but inhibits elongation in older tissues. Polyphenols are produced initially in the cell (57). However, as the cell ages, monophenols are synthesized (57). This would result in stimulation of I.A.A. oxidase synthesis and a resultant cessation of growth (40,57). The rapidity of monophenol synthesis is dependent upon genetic predetermination and/or environmental factors.

Environmental factors which have been found to induce phenol synthesis are nitrogen deficiency, and cool temperatures, and light (103). In each of these situations, monophenols are synthesized (57).

Phenols may also reduce the ability of a plant to translocate gibberellins and/or cytokinins and reduce the ability of the plant tissue to respond to gibberellins (57,58).

The Effects Of Temperature On Stem Elongation

The optimal temperature for development of different parts of a plant or the whole plant at any given time in its development varies (22,114). Cline observed that the shoot-root ratio declined as both day and night temperature decreased with Scrophularia (22). In Chrysanthemum a decline in the shoot-root ratio was found to be primarily dependent on a decrease in night temperature and was enhanced by decreasing day temperature (56). Went determined that Lycopersicum esculentum plants had a temperature optima for stem elongation of 26 C day and night temperature early in their development (<30 cm). As the plants matured (>30 cm), the optimal night temperature for stem elongation decreased to 18° C (117). A similar decline in optimal night temperature with maturity was observed with Capsicum, Phaseolus, Antirrhinum, and Chrysanthemum (117). This indicated that the processes which may be limiting for elongation late in plant development that occur during the day had a higher temperature optima than those processes which occur during the night (Fig. 12 (117)). Although the night temperature is more critical with stem elongation in Lycopersicum, day temperature has been found to be more critical than night temperature with stem elongation of Phaseolus, Chrysanthemum, and Lilium (31,56,117).

When a cool day temperature (15° C) is given in com-

bination with a warm night temperature (24° C) stem elongation has been found to be severely reduced (93). In addition, a chlorosis of the leaves may occur. Warm climate plants such as corn, sorghum, and hemp exhibited a more severe leaf chlorosis response than cool climate plants such as rye, nasturtium, and bluegrass (95).

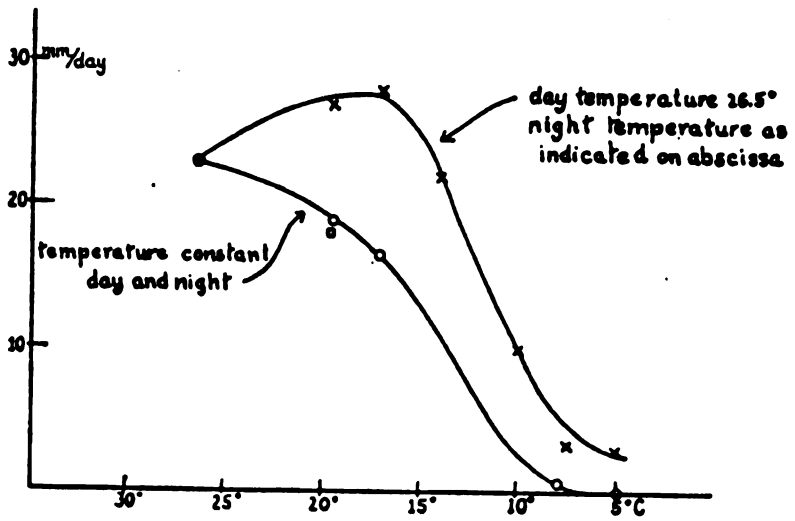


Figure 12

Miller proposed that the change in temperature optima for stem elongation as a plant matures was related to the change in the available photosynthates within the plant. As plants grow the proportion of plant tissues respiring to plant tissues photosynthesizing increased (116). As a result, respiration may become a more limiting factor in an older plant growth than on younger plants (116). Since the

Q_{10} of respiration ranges from 2 to 30 a small decline in temperature would result in a significant reduction in respiration (116).

Went and Bonner observed that Lycopersicum grown in daylight and placed in the dark elongated for approximately 16 to 24 hours after which the growth rate declined sharply. This decline in elongation was reversible by submerging individual leaves in a 10% sucrose solution (116,118). The resultant growth rate was proportional to the number of leaves submerged. An exposure to daylight was also found to stimulate elongation after an extended period of darkness (116). They concluded that the carbohydate content and/or translocation may be the limiting processes for stem elongation during the night on Lycopersicum greater than 30 cm tall (116).

The Influence Of Water On Stem Elongation

Water stressing a plant to such a point that cell turgor pressure decreases below some critical turgor pressure necessary for cell expansion reduces shoot length (2,8,104). The reduction in shoot length associated with water stress occurs primarily through a decrease in cell length and not cell number within a shoot (8,104). Reduction in cell length by a short period of water stress was

reversed within seconds after rewatering Maize plants (2). The speed of recovery following rewatering indicated that no metabolic processes were involved and that, initially, the retardation response was physical and not biochemical (2).

Prolonged water stress resulted in long term retardation of elongation (2,121,124). On a horomonal basis, water stress stimulated both ethylene and abscisic acid (ABA) biosynthesis (121,124). Ethylene reduces cell elongation through reorientation of microfibrils which results in isodiametric cell expansion (68). ABA induces stomatal closure which ultimately results in a reduction in available assimilates necessary for elongation (104). In addition to a reduction in assimilates, the transport of these assimilates was also restricted (104).

The opposite of water stress, or waterlogging, also inhibits shoot elongation (122). In contrast to water stress, waterlogging produces anaerobiosis in the roots which results in the transport of an ethylene precursor (ACC) from the roots to the shoots where ethylene is formed. As with water stress, isodiametric cell expansion is induced which ultimately results in a reduction in elongation (122).

Retardation Of Stem Elongation With Daminozide

The compound butanedioic acid mono (2,2-dimethyl hydrazide) (daminozide) is used as a commercial growth retardant on a number of ornamental crops (5,9,10,14,16,24,73,74,93,101,119,123). The plant response is typically characterized by a reduction in the length and increase in breath of the internodes and darker greener foliage (10). Daminozide is typically delivered as a foliar spray. Foliar absorption of daminozide is greatest on the newly expanding leaves (23). Studies by Dicks with Chrysanthemum morifolium showed that leaves on the lateral shoots absorbed 39% more daminozide than the older leaves on the mother shoot (23). Transport of daminozide from the leaves on the mother shoot was predominantly to those areas of the plant with the greatest mitotic activity, i.e. lateral shoots and roots (23,24). The movement of daminozide to the regions of more active growth suggests that daminozide is transported in the phloem (23). However, studies by Rothenberger showed that when daminozide was applied to the roots, it was transported to the apical meristem within one hour. This suggests that both phloem and xylem transport may occur (23). Undurraga observed ^{14}C labeled daminozide moved readily from the phloem to the xylem. The leakage appeared to initiate with an increase in cytoplasmic permeability (76). The daminozide induced

leakage may explain, in part, the ability of daminozide to depress utilization of respiratory energy necessary for the retention of solutes in the cell vacuole (76).

Retardation of stem elongation by daminozide in apple occurs primarily through a reduction of cell division (119). Wilde and Edgerton found a 66% reduction in mitotic activity 3 hours after an application of daminozide to the lateral shoots of apple (119). Subapical meristem activity was reduced 14% and after 24 hours, the mitotic activity in the subapical meristem was reduced an additional 54%. After 14 days, the number of cells in the apical meristem was 69% less than those plants which did not receive daminozide. Cell number in the subapical meristem was 28% of that of plants which did not receive daminozide. Therefore, daminozide is initially transported to the apical meristem after which it accumulates in the subapical meristems where it has been found to substantially reduce cell division (119). In contrast, an application of daminozide to an apple leaf increases the length of the palisade cells within the leaf.

The exact mode of action of daminozide is still under question (24,76). Dennis et al found that 100 ug/ml daminozide resulted in a 40% inhibition of the synthesis of kaurene from mavelonic acid (24). Ryugo and Sachs have determined that kaurene-19-ol synthesis from melavonic acid in peach ovules is also inhibited by daminozide (24). In

addition, an application of 10 mM daminozide to Pisum epicotyl tips has been found to block the conversion of trans- geranylgeranyl pyrophosphate to kauren-19-al. Ross and Brand determined that the total accumulation of gibberellins in vernalized hazel seeds was reduced by daminozide. The conclusion of the previous studies was that daminozide may reduce the total gibberellin content of the tissue through interfering with the biosynthetic pathway (24).

Menhennett has alternatively suggested that daminozide may modify the biological activity of the gibberellins in the plant (76,77). This theory is based on the ability of daminozide to reduce the activity and/or block the response of GA_9 (76). It was hypothesized that daminozide prevents the hydroxylation of GA_9 to GA_{20} (76), a more biologically active form.

Daminozide is not readily degraded in the plant (23,24). Dicks has determined that the quantity of daminozide in the plant remained constant for a minimum of 5 weeks in Chrysanthemum morifolium (24). Despite the relative stability of this compound in plant tissue, its duration of effectiveness is often limited (16,23,24,123). Zeevaart treated Pharbitis nil cotyledons with 5000 mg l^{-1} daminozide as a foliar application and following a period of retardation observed a resumption of growth rate similar to that previous to the application after approximately 25

days of retarded elongation (23,123). Only a small portion of the daminozide present in the plant was present in the upper shoot portion of the plant after 25 days (23,123). Zeevaart concluded that cell division in the upper shoot region diluted the concentration of the daminozide below a critical threshold concentration needed for retardation of elongation (123). Dicks called this loss of response to daminozide 'growing out' and quantified this response in Chrysanthemum with the equation:

$$(dmI^*/dt) = \frac{1}{1 + K(I - I^*)} \quad (dm/dt)$$

Where:
- (dmI^*/dt) = Retarded growth rate
- I = Concentration of inhibitor
- I^* = Threshold level of inhibitor
- K = Retardation constant
- (dm/dt) = Growth rate of controls

This equation was initially used for describing retardation of exponential growth in bacteria cultures (24). The studies with Chrysanthemum were not conducted under constant environmental conditions.

Other factors which have been shown to influence the response of a plant are physiological age (47), temperature and light levels (12,73,100,101). Schonherr and Buckovac determined that daminozide absorption increased as light intensity increased to 1.82 W m^2 and temperature increased to 35°C with Phaseolus vulgaris (100).

The Dynamics and Analysis of Plant Stem Elongation

Growth curves can describe the behavior of growth processes as they vary with time (30). The underlying form of a growth process over an extended period of time is normally curvilinear, and increasing with time (the first derivative varies and is positive) (17). The rate of increase in size for all growth processes ultimately approaches a horizontal asymptote as some factor necessary for growth becomes limiting or genetic predetermination causes a cessation of growth.

Typically the objective of fitting a growth curve to data is to obtain information from the growth curve parameters which may give insight into the growth process itself or to simply predict some factor as it increases with time (51). Insight into the true underlying growth process is often of primary importance to the biologist. Conversely, a statistician is concerned with the ability of an equation to describe a data set (51). A modeler of a living organism's growth must incorporate both views and judge a function's fulfillment of the 'biological expectations' of the modeler and the 'statistical exactitude' of a model in describing a data set. The degree to which either of these qualifiers is emphasized depends on the data set itself.

Linear Regression Linear regression is not based on underlying biological assumptions, i.e. proportionality between growth that has occurred and that which has yet to occur (26,30,36,89). Polynomials are also unable to predict an independent variable as it approaches an asymptotic value with an increase in the dependent variable to infinity (36,50,51). As a result, linear regression has traditionally been used to describe an independent variable within a limited range for the dependent variable.

Nonlinear Regression The difficulties associated with linear regression have led to an increase in the popularity of a group of functions which are based on biological assumptions of growth (26,30,89). In addition they are nonlinear in form and display asymptotic behavior (105). Some nonlinear models which have been found to be useful in describing stem elongation are:

Monomolecular (26,35,50,89)

General Form: $W = a (1 - be^{-cT})$

Where:

a = asymptotic value

a*(1-b) at (t=0) = initial size

c = rate constant

Characterized by:

1. No point of inflection
2. Curve is convex to the dependent variable axis
3. Relative growth rate declines linearly with time
4. Describes irreversible growth
5. Rate of growth is proportional to the growth yet to occur

Previously used in describing

1. The relationship between leaf area and temperature (43)
2. First order chemical reactions (35)
3. Cell expansion (35)
4. Regrowth studies on Festuca arundinacea (52)

Logistic (26,89,17,36)

General form: $W = a / (1 + be^{-cT})$

Where:

a = asymptote

W at t=0 is a(1-b) = initial size

c = rate constant

Characterized by:

1. Symmetric curve
2. Inflection at a / 2
3. Relative growth rate declines with time
4. Growth is proportional to the product
5. When t = 0 W = a/(1+b)
   t = a W = a

Previously used to describe:

1. Single leaf growth data on *Cucumis sativus* (44)
2. Animal population studies(52)
3. Disease progression (71)
4. Flower number analysis (87)
5. Seedling germination and growth (52)

Gompertz (17,26,35,85,89)

General form: $W = ae^{-be^{-cT}}$

Where:

a = asymptote

ae^{-b} at t=0 is initial size

c = rate constant

Characterized by:

1. Nonsymmeteric sigmoid function
2. Inflection at a / e
3. Relative growth rate is a declining linear function of the logarithm of the independent variable
4. Faster earlier growth with a slower approach to an asymptote

Previously used to describe:

1. Pelargonium leaf expansion(3)
2. Animal population studies(71)
3. Cotton hypocotyl elongation (71)
4. Germination studies (50)

Richard's (17,26,35,89,92)

General form: $W = a\left(1 + be^{(b-cT)}\right)^{-1/d}$

Where:

a = asymptote

b = initial size

c = rate constant

d = inflection point determinant

Characterized by:

1. sigmoid curve with variable inflection
2. Inflection point moves up the curve as the value of d increases from -1
   - d = 0 monomolecular
   - d = 1 gompertz
   - d = 2 logistic
3. Does not fit data with insufficient curvature approaching the upper asymptote

Previously used to describe:

1. Individual leaf growth (35,92)

Weibull's(12,71,72,84,89)

General form: $W = a\left(1 - e^{-(T/b)^{c}}\right)$

Where:

a = independent variable intercept

b = scale parameter inversely related to the rate of increase

c = shape parameter

Characterized by:

1. Sigmoid curve with a variable point of inflection
2. Shape parameter is independent of a and b parameters
3. When c< 3.6 curve skewed right
   c= 3.6 Symmetric inflection
   c> 3.6 curve skewed left
4. The curve may pass through the origin

Previously used to describe:

1. Disease progression in Phaseolus (13)
2. 'Time-to-failure' quality control investigations (70)
3. Cotton hypocotyl rot (84)
4. Differential pathogenicity of vectors (72)

The computational difficulties associated with the iterative techniques necessary for estimation of nonlinear parameters have limited their use. Recent advances in computer technology have eliminated many of these limitations. While the use of nonlinear functions has become more widespread, appropriate techniques for their evaluation has fallen behind the use of the functions themselves (52).

When evaluating a nonlinear function on its ability to describe a data set, the common assumptions employed with linear regression, i.e. normality and unbiased, independently distributed parameters, are not valid unless the sample population is very large (26,89). As a result, regression statistics based on t and F distributions may not be valid for small sample sizes typically seen in experiments (26,89). Bates and Watts and the Box methods are two methods which have been developed to evaluate nonlinear functions (89). From the statistics generated by these procedures the validity of conclusions based on t and F statistics can be determined (89). In addition, the confidence which may be placed in the parameter estimates can be calculated.

Stochastic distributions and Time Series Analysis Growth models for stem elongation typically make predictions for a specific quantity at some point in time, i.e. length, dry

weight (99). The validity of this quantity is, however, under question unless an entire population is sampled. Therefore, when developing models based on population samples, it is often of benefit to introduce a probability distribution to help account for variability (99). These probability distributions, often referred to as stochastic distributions, predict an exact value and an associated variance (99). The importance of such models increases as the variability of the data increases.

Another method of analysis which may be used to summarize a growth process with longitudinal studies, or studies in which a series of measurements are taken on the same subjects, is discrete time series analysis (49,52). Time series analysis, is of particular benefit in determining if there are any subtle relationships in the kinetics of growth of an individual plant, i.e. cyclic events, correlations between points, random components (49,52). Often these relationships may go unnoticed when data from individual plants is combined for standard regression analysis (49).

A model resulting from a time series analysis may combine a number of other models, e.g. Gompertz, logistic etc.. The parameters in these equations are not fixed as in standard regression analysis (49). Instead, they vary slowly and at random, and may be based on a previous predictions value (48).

Conclusion In conclusion, nonlinear regression functions are useful when prediction of some dependent variable, when growth is asymptotic within the time range studied. In addition, nonlinear parameter estimates based on large sample populations have biological significance in determining growth potential and the rate of growth which occurs as time varies.

Time series analysis and stochastic distributions may have potential in determining the mathematical nature of a growth process which may not be identifiable using standard regression analysis. This technique may produce more valid models for estimating population characteristics (49).

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Section I

Factors Influencing The Response

Of Chrysanthemum morifolium Ramat. cvs.

'Bright Golden Anne' and 'Circus' To Daminozide

Factors influencing the response of *Chrysanthemum morifolium* Ramat. 'Bright Golden Anne' and 'Circus' to daminozide.

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Abstract

Factors Influencing The Response Of *Chrysanthemum morifolium* Ramat. To Daminozide

Applications of daminozide to *Chrysanthemum morifolium* shoots early in internode developement stimulated internode cell division by as much as 120 percent however cell elongation was inhibited to such a degree that final internode length was reduced 30 to 40 percent.

Response of an internode to daminozide depended on the internode location on the shoot. The second stem segment from the shoot base was the most responsive segment to an daminozide application. daminozide effectiveness decreased as much as 70 percent as stem segments became more distal.

Multiple applications of daminozide to the same shoot were not additive in their retardation response. In general, inhibiition of internode elongation by daminozide was increased if the shoot had received a previous application of daminozide.

Introduction

The chemical daminozide reduces stem elongation in a number of plants (1) through retardation of cell division and elongation (8,11). The degree of reduction in final shoot length is dependent in part on the physiological age of the plant at the time of treatment (1,4,9). The maximum response of chrysanthemum to an daminozide application is reported to occur when an application is made 14 days after the initiation of short days (9). The relationship between the physiological age of a shoot and response to an daminozide has not been quantified in chrysanthemum under controlled environmental conditions. In addition, the response of an internode to multiple daminozide applications and the influence of internode position on the response to daminozide are not known. This paper quantifies the relationships between plant age, and the position of actively elongating internodes on a lateral shoot to the number and timing of daminozide applications in chrysanthemum.

Materials and Methods

General Procedures: Rooted cuttings of Chrysanthemum morifolium 'Bright Golden Anne' and 'Circus' were planted in a commercial potting mix (sphagnum peat, perlite, vermiculite) in 10.2 cm plastic pots. The cultivars 'Bright Golden Anne' and 'Circus' were chosen because of their commercial significance and to compare responses to daminozide of cultivars with different flower forms: decorative and daisy. All plants were placed in controlled environment chambers and grown with a photosynthetic photon flux (PPF) of 325 umol $s^{-1}m^{-2}$ supplied from cool white fluorescent lamps for 16 hr d^{-1}. Day and night temperatures were maintained at 20°C and 16°C, respectively. Plants were fertilized at each irrigation with 200 mg l^{-1} of N and K. After 7 days, the apical growing point was removed from each plant to promote branching and the photoperiod was reduced to 8 hr d^{-1} to induce flowering. Twelve days after the initiation of short days, lateral shoots other than the apical 3 of each plant were removed.

In experiments where daminozide was applied to a single leaf, the daminozide solution was applied as five 25 ul droplets to the leaf immediately below the stem segment which was being studied (Figure 1). A stem segment was defined as two adjacent internodes. Two internodes were

chosen as an experimental unit to reduce variability. Variability in length among internodes was great, especially in the more proximal internodes of the lateral shoot. By adding the lengths of two adjacent internodes, some of this variability was removed while still maintaining a system which would display more sensitivity than an entire shoot system (6). The second lateral shoot was used for all single leaf experiments. The droplets were applied using a digital Finnipippette (Cole-Palmer).

In whole plant experiments, the entire plant was sprayed to wet the foliage. Data were collected on total shoot length every five days with a 'MaxCal' Max-15 digital micrometer.

In all experiments treatments were applied and data were collected on both 'Bright Golden Anne' (BGA), and 'Circus' to study cultivar specificity.

Individual Experiment Procedures:

Experiment 1: Application To A Whole Plant During Development: Plants were treated with a foliar application of a 2500 mg l^{-1} a.i. daminozide solution 10, 20, 30, 40, or 50 days after the initiation of short days. The average dosage for each treatment date was 7.25, 16.25, 25.13, 27.30, or 27.30 mg $plant^{-1}$, respectively. The total length

of the second lateral shoot was measured every five days until elongation ceased. Cessation of elongation was cultivar and treatment dependent and occurred between 48 and 62 days after the initiation of short days.

The plants were randomized within the chambers at initiation of short days and every measurement day thereafter. The data were analyzed as a completely randomized design with six treatments and five plants per treatment.

Experiment 2: Application To A Single Internode During Development: The leaf below the second stem segment from the stem base was treated with 125 ul of a 2500 mg l^{-1} daminozide solution on 16, 19, 22, 25, 28, 31, 34, or 37 days from the initiation of short days. Data were collected on the length of the second stem segment every 3 days from day 15 to day 40 after the initiation of short days. At flower, the treated stem segments were removed and fixed in a 70% FAA solution. Cell length and number determinations were made on the xylem cells.

Plants were randomized within the chambers at the initiation of short days and every measurement day thereafter. The data were analyzed in a completely randomized design with nine treatments and five plants per treatment.

Experiment 3: Additive Responses Of Multiple Applications: One or two stem segments on a shoot were treated with 75 ul of a 3890 mg l^{-1} solution of daminozide in the combinations shown in Table 1. The leaf at the base of a stem segment was treated with the daminozide solution when the segment reached 0.5 cm in length. Data were collected on all stem segments every 4 days for 60 days from the initiation of short days.

The plants were randomized at the initiation of short days and every measurement day thereafter. Data were analyzed as a completely randomized design with 16 treatments and five plants per treatment.

Results

Experiment 1: Application To A Whole Plant During Development: Retardation of lateral shoot elongation decreased as the time of daminozide application occurred later in plant development (Table 2). With 'Circus', greatest total shoot retardation (43 %) occurred with an application on day 10 after which retardation associated with a daminozide application decreased rapidly until day 50 (Fig. 2). With BGA retardation from applications made on days 10, 20 and 30 were similar. Retardation of elongation

decreased rapidly from day 30 to day 60.

Retardation per microgram daminozide decreased faster than retardation of total elongation. The lack of response to an daminozide application after day 50 with Circus and day 60 with BGA was expected as elongation had ceased on plants which had received no daminozide application after these days.

In contrast to total retardation, post application elongation retardation, defined as the percent retardation of elongation that occurred following an daminozide application, increased for both Circus and BGA as the time of application occurred later in plant development (Table 2).

Experiment 2: Application To A Single Internode During Development: Retardation of elongation decreased as the time of daminozide application occurred later in the development of the second stem segment (Table 3). The retardation response was asymptotic for daminozide applications after day 13 with no difference in the final lengths of the second stem segments compared with plants which received no daminozide (Fig. 3). Although both cultivars responded to daminozide in a similar manner (Fig. 3), elongation of BGA stem segments was inhibited approximately 12-13% more than stem segments of Circus.

Final cell length decreased as applications of daminozide occurred earlier in development (Table 3). The retardation of cell elongation was accompanied by an increase in cell number. No stimulation of total stem length was observed with an application of daminozide to BGA on day 19, whereas, stem extension was stimulated 18% with Circus. The cell number and length data indicate that the stimulation resulting from a late application of daminozide resulted from an increase in cell elongation, not number. The magnitude of cell elongation retardation or cell number promotion in response to daminozide was at least 20% greater with BGA than Circus.

Experiment 3: Additive Responses To Multiple Applications: Response of a stem segment to daminozide applied to the leaf immediately below that segment decreased as a stem segment became more distal, then increased with the fifth stem segment (Table 4 and Fig. 4). The stem segments most sensitive to daminozide were the second and fifth. Applications of daminozide made to the second stem segment were three times more effective in inhibiting elongation than applications to the fourth stem segment (Table 4 and 5).

The retardation resulting from multiple applications was not additive. Eighty percent of the plants from treat-

ments receiving multiple treatments had greater overall retardation than the sum of the retardation resulting from applications made to separate stem segments on separate plants.

Discussion

Retardation of shoot elongation by daminozide varied with the physiological age of the shoot's component internodes, the position of the internodes on the shoot, and if previous applications of daminozide had been made to the shoot. Applications of daminozide early in internode development stimulated cell division, but inhibited cell elongation to such a degree that the ultimate length of the internodes was reduced. Applications late in development did not increase cell number but stimulated cell elongation in Circus but not BGA. To achieve any retardation of internode expansion these results indicate that it was necessary to apply daminozide no later than 10 days after an internode elongated to 0.5 cm in length.

Distal internodes displayed a smaller response to daminozide than more proximal internodes with the exception of stem segment 5 (Fig. 4). Some factor either enhanced the response of more proximal internodes to daminozide or some factors reduced the effectiveness of applications to distal

internodes. Hanks and Rees (3) reported the removal of a tulip flower early in development reduced total stem length by 59 percent. Retardation of elongation resulted from pedicel and more distal internode retardation (3). Application of indoleacetic acid (IAA) to the cut surface reversed the retardation while application of gibberellins did not (7). Studies by Op den Kelder showed that removal of the gynoecium alone could produce the same retardation of stem elongation associated with flower removal (7).

Stem elongation of tulip was also found to be inhibited by removal of leaves prior to anthesis (2). Retardation of stem elongation due to leaf removal resulted from retardation of the more proximal internodes. Application of ancymidol, a known gibberellin biosynthesis retardant (10), retarded internode expansion in the first internode but not the fourth (10). Based on these results Hanks suggested that two mechanisms control stem extension in tulip: 1) an auxin mediated system which is dependent on gynoecium auxin production and 2) a gibberellin mediated system dependent on stem and leaf gibberellin production (3).

Jeffcoat showed chrysanthemum flowers produce GA_3 and GA_9 and an auxin similar to IAA (5). Gibberellins similar to those produced by the flower were found in the leaves, while a different gibberellin complex (GA_4, GA_5, GA_6, or GA_7) was found in the stem. The activity of the latter gib-

berellins decreased with shoot age.

These findings suggest the presence of a differentially distributed growth promoter, i.e. auxin and/or gibberellins, present in higher concentrations in the more distal internodes of chrysanthemum. The increase in the response to daminozide of the fifth stem segment indicates that the concentration of the elongation promoter may decline late in development of the shoot. Jeffcoat observed that the growth promotive activity of the flower decreased significantly from stage 3 of flower development to stage 5 (from the time when the flower bud is spherical with 12 bracts around the rim to when the bud is flattened with 2 or 3 rows of floret primordia)(5). This period corresponds to the time period just prior to day 60 with BGA and 50 with Circus when the fifth stem segment is developing. These studies plus the data presented in this paper suggests that the flower, young leaves and the stem of the chrysanthemum produce growth promotive substances which are present in higher concentrations in the distal internodes. These substances reduce daminozide induced retardation. As the shoot develops and as the flower approaches stage 5, growth promoter production of the flower probably declines, and as no new leaves are produced, gibberellin activity probably declines. This decease in gibberellin activity may be responsible for the increase in retardation response observed in the fifth stem

segment as compared to the fourth stem segment.

The results of experiment 3 indicate that multiple applications of daminozide are not additive in their retardation of internode extension. The sum of the retardation of plants which received single applications to a stem segment was less than the total retardation resulting from plants which received multiple applications to the same segments.

Conclusions

1. Applications of daminozide to internodes in excess of 10 days old did not inhibit internode elongation and in some cases stimulated elongation primarily through stimulation of cell elongation.
2. Daminozide stimulated cell division but inhibited cell elongation to such a degree that the final length of an internode was less than if it had received no daminozide application.
3. Applications of daminozide to more distal internodes resulted in less retardation of elongation than that seen with a similar application to more proximal internodes. It was hypothesized that differential levels of a growth promoter present in higher concentrations in more distal internodes interfered with daminozide retardation of cell

elongation.

4. Multiple applications of daminozide were not additive in their response.

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Table 1. Treatment combinations designed to study the effect of daminozide applications to internodes at different positions on the plant and responses to multiple applications.

Segment [a]		Stem Segment Number					
First Application	Second Application	0	1	2	3	4	5
None	None	-	-	-	-	-	-
1	None	-	+	-	-	-	-
1	2	-	+	+	-	-	-
1	3	-	+	-	+	-	-
1	4	-	+	-	-	+	-
1	5	-	+	-	-	-	+
2	None	-	-	+	-	-	-
2	3	-	-	+	+	-	-
2	4	-	-	+	-	+	-
2	5	-	-	+	-	-	+
3	None	-	-	-	+	-	-
3	4	-	-	-	+	+	-
3	5	-	-	-	+	-	+
4	None	-	-	-	-	+	-
4	5	-	-	-	-	+	+
5	None	-	-	-	-	-	+

[a] Applications were made when stem segments were 0.5 cm in length.

Table 2. The effect of a single daminozide application at different stages in the development of Chrysanthemum morifolium 'Bright Golden Anne' and 'Circus' on final length of the second lateral shoot. Daminozide was applied as a foliar application to the whole plant until runoff.

Day Of Application [z]	Retardation(mm) Total	Retardation(mm) Per Microgram Daminozide applied	Total Retardation(%)[y]	Retardation of Post Application Elongation
		'Bright Golden Anne'		
10	10.0	1.37	25	28
20	11.0	0.67	28	34
30	11.4	0.45	29	44
40	8.8	0.32	22	53
50	4.3	0.16	11	66
60 [x]	-	-	-	-
Linear [w]	***	***	***	***
Quadratic	***	***	***	***
Cubic	N.S.	N.S.	N.S.	N.S.
		'Circus'		
10	7.8	1.08	43	51
20	4.5	0.28	25	42
30	4.4	0.18	24	52
40	3.1	0.11	17	62
50	0.7	0.03	4	42
60 [x]	-	-	-	-
Linear	***	***	***	***
Quadratic	N.S.	N.S.	N.S.	N.S.
Cubic	**	**	**	**

[z] Days after pinch and start of short days.
[y] Based on total second lateral shoot length.

[x] No application was given to these plants.
[w] Significant at P=.05(*), P=.01(**), P=.001(***), not significant(N.S.)

Table 3. The effect of daminozide application time on cell size and number in Chrysanthemum morifolium. Two hundred ninety two ug daminozide was applied as five 25ul droplets of a 2333 mg/l solution to the leaf at the base of the second stem segment. Tissue samples were taken from the treated internodes at the cessation of elongation.

Day Of Application	Percent Retardation of Final Length	Cell Length		Cell Number Internode^{-1}	
		Final[x]	% Change[y]	Final[w]	% Change[y]
		'Bright Golden Anne'			
-5	41	164	- 65	246	+ 84
1	24	179	- 62	285	+ 113
4	18	189	- 60	293	+ 119
7	13	198	- 58	293	+ 119
10	10	234	- 50	252	+ 88
13	-2	262	- 44	255	+ 90
16	-6	309	- 34	211	+ 57
19	9	328	- 30	184	+ 37
Control	-	469	-	134	-
Linear [z]	***	***		***	
Quadratic	***	***		***	
Cubic	***	***		***	
		'Circus'			
-5	30	200	- 9	150	+ 78
1	12	159	- 27	101	+ 20
4	3	180	- 18	99	+ 18
7	8	210	- 4	79	- 6
10	4	229	+ 5	77	- 8
13	-2	216	- 1	87	+ 4
16	-6	235	+ 7	83	+ 1
19	-18	197	- 10	100	+ 19
22	-	219	-	84	-
Linear [z]	***	***		**	
Quadratic	***	***		***	
Cubic	***	***		***	

[z] Significant at P=.05(*), P=.01(**), P=.001(***),not significant(N.S.)

[y] Percent change relative to untreated control.

[x] Mean cell length (microns)

[w] Cell number is calculated from the mean internode length divided by the mean cell length

Table 4. Percent retardation of Chrysanthemum morifolium cv. 'Bright Golden Anne' stem segments resulting from single and multiple applications of 292 ug of daminozide delivered as three 75ul droplets applied to the leaf immediately below the stem segment when it was 0.5 cm in length.

Segment [z]		Percent Retardation				
		Stem Segment Number				
First Application	Second Application	1	2	3	4	5
1	None	2	14	3	6	1
1	2	-	26	7	15	18
1	3	2	19	15	23	18
1	4	6	26	16	18	14
1	5	11	16	10	11	4
2	None	2	27	14	17	2
2	3	2	31	24	22	12
2	4	14	23	5	21	1
2	5	5	25	6	7	-4
3	None	0	20	15	17	7
3	4	10	28	26	15	12
3	5	-8	18	20	15	2
4	None	11	14	0	8	6
4	5	-7	25	14	15	7
5	None	0	12	14	13	11

Position versus Percent Retardation

Linear [y]	*
Quadratic	*
Cubic	*

[z] Applications were made when the stem segments were 0.5 cm in length.

[y] Significance at P = .05 (*), P = .01 (**), P = .001 (***), not significant (N.S.)

Table 5. Percent retardation of Chrysanthemum morifolium cv. 'Circus' stem segments resulting from single and multiple applications of 292 ug of daminozide delivered as three 75ul droplets applied to the leaf immediately below the stem segment when it was 0.5 cm in length.

Segment [z]		Percent Retardation				
		Stem Segment Number				
First Application	Second Application	1	2	3	4	5
		'Circus'				
1	None	-7	15	6	-2	5
1	2	-3	25	6	3	11
1	3	6	25	19	10	6
1	4	-9	5	1	6	2
1	5	-6	26	15	5	7
2	None	6	28	7	2	21
2	3	-12	16	10	14	12
2	4	5	24	13	16	18
2	5	-3	25	25	15	23
3	None	4	15	11	-1	2
3	4	3	3	17	12	16
3	5	-14	0	8	5	18
4	0	-10	11	7	9	23
4	5	-6	6	3	7	23
5	None	0	12	17	20	37

Position versus Percent Retardation

Linear [y]	***
Quadratic	***
Cubic	***

[z] Applications were made when the stem segments were 0.5 cm in length.

[y] Significance at P = .05 (*), P = .01 (**), P = .001 (***), not significant (N.S.)

Figure 1. Diagram identifying stem segment partitioning of the second lateral shoot. The first and sixth stem segments are single internodes. The remaining segments are composed of two adjacent internodes. Experimental plants consisted of three shoots. One daughter shoot is shown.

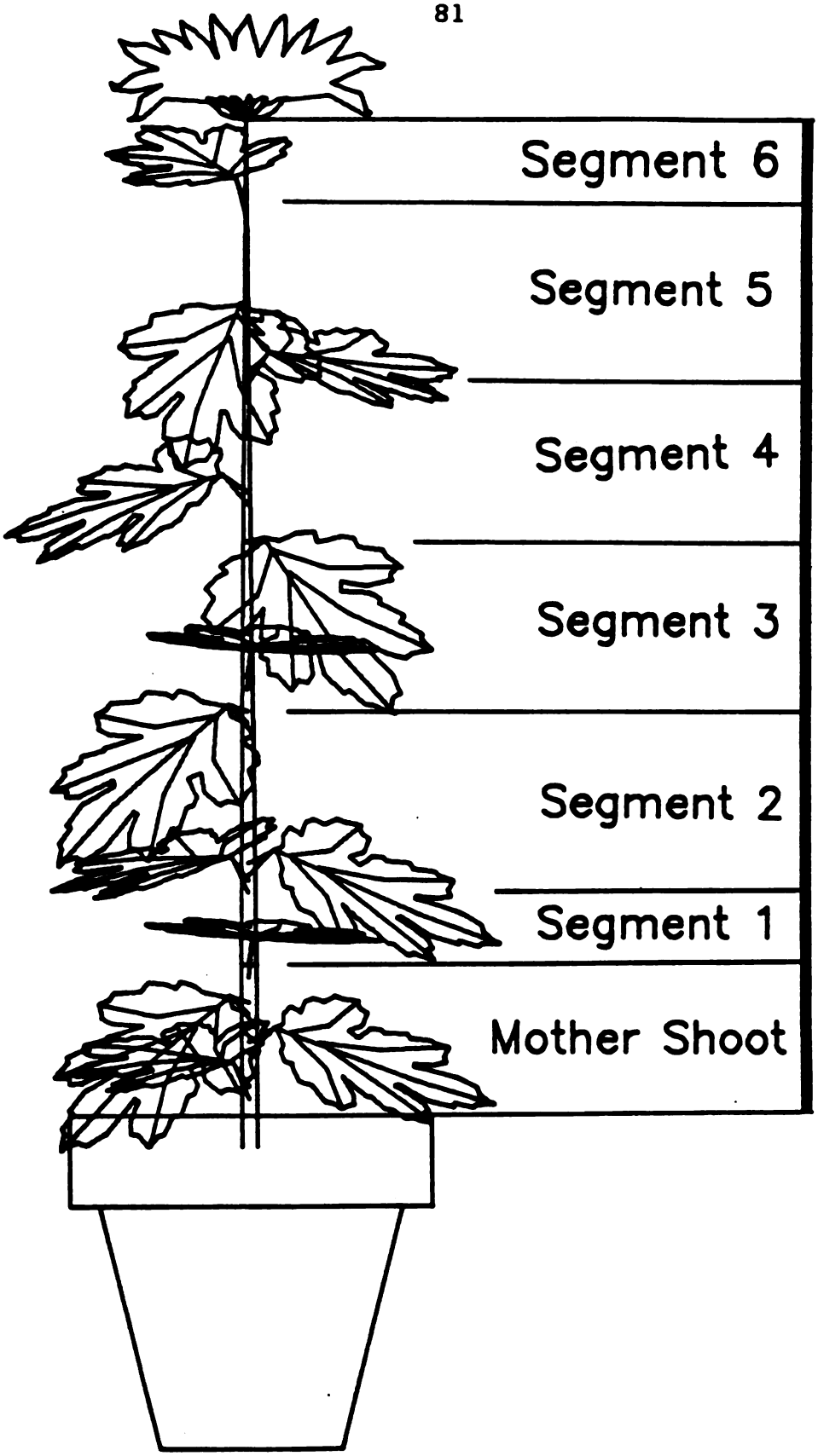

Segment 6
Segment 5
Segment 4
Segment 3
Segment 2
Segment 1
Mother Shoot

Figure 2. Effect of the time of a daminozide application on final second lateral shoot length of BGA (a) and Circus (b). Plants were sprayed with a 2500 mg l^{-1} solution of daminozide till 'runoff'.

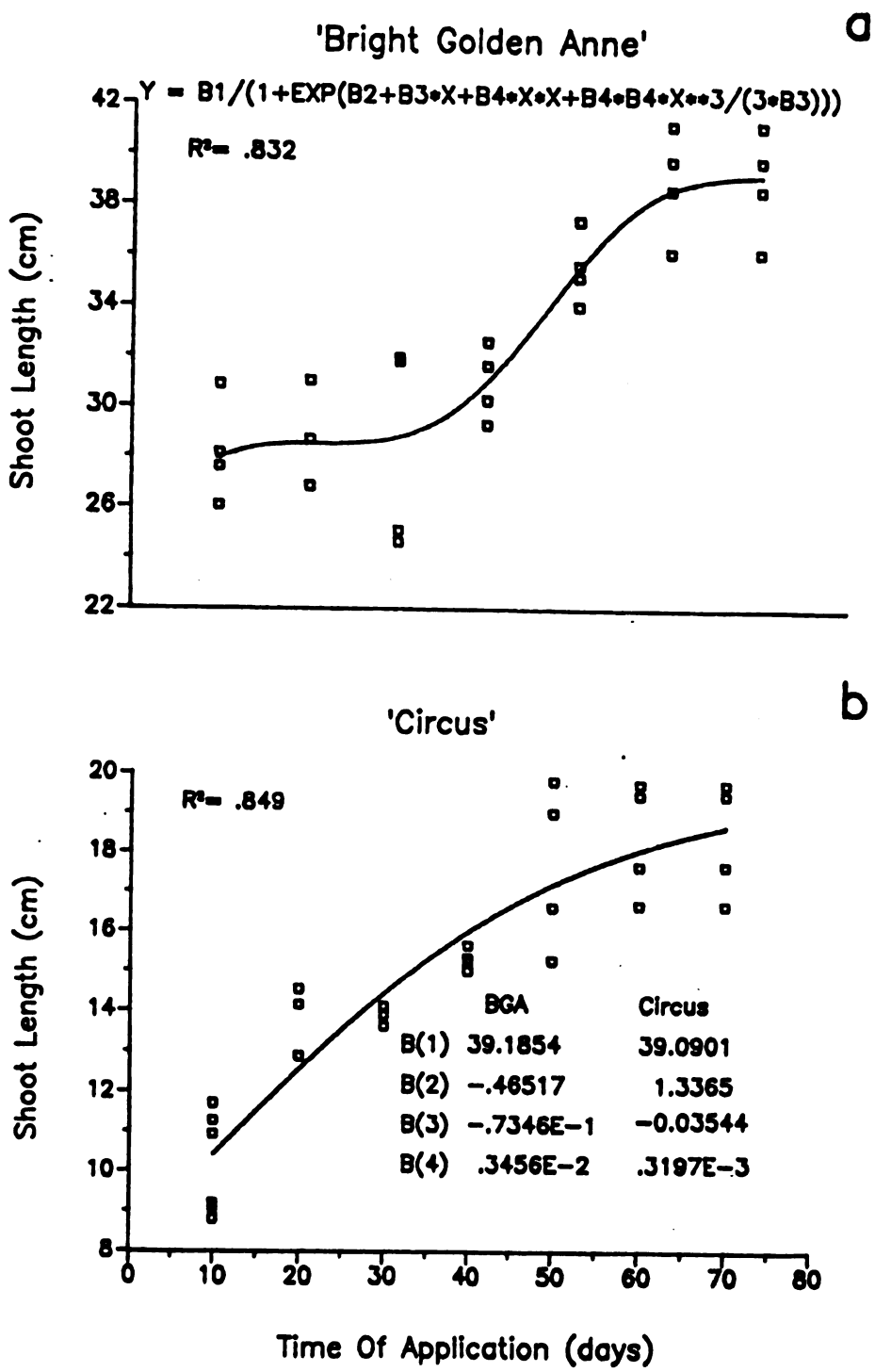
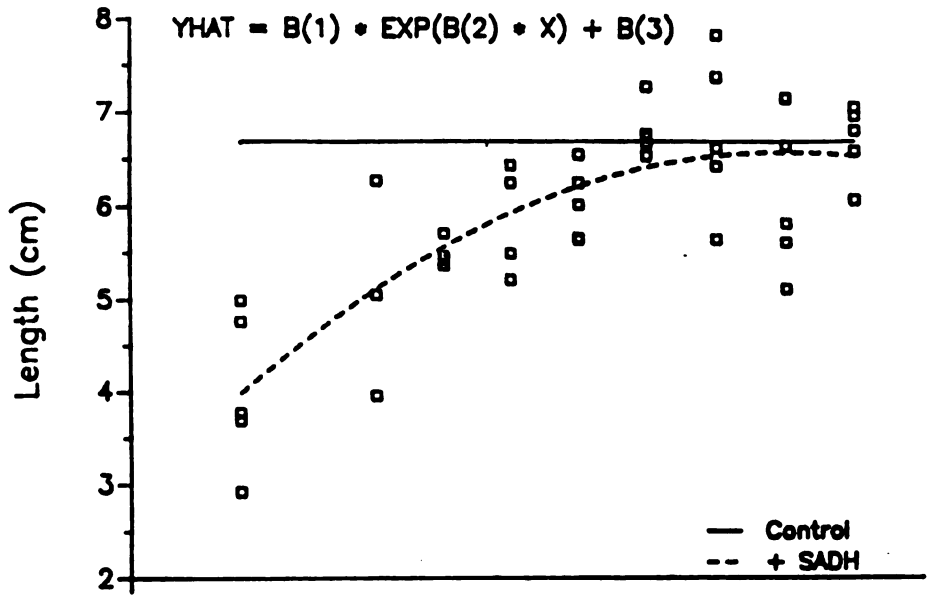

a
'Bright Golden Anne'
Y = B1/(1+EXP(B2+B3*X+B4*X*X+B4*B4*X**3/(3*B3)))
R²= .832
Shoot Length (cm)
42
38
34
30
26
22
b
'Circus'
R²= .849
Shoot Length (cm)
20
18
16
14
12
10
8
0
10
20
30
40
50
60
70
80
BGA
Circus
B(1) 39.1854
39.0901
B(2) -.46517
1.3365
B(3) -.7346E-1
-0.03544
B(4) .3456E-2
.3197E-3
Time Of Application (days)

Figure 3. Effect of the time of a daminozide application on the final stem segment length of BGA (a) and Circus (b). The leaf at the base of the second stem segment recieved an application of 292 ug of daminozide delivered as five 25 ul droplets of a 2333 mg l^{-1} solution.

a

'Bright Golden Anne'



b

'Circus'

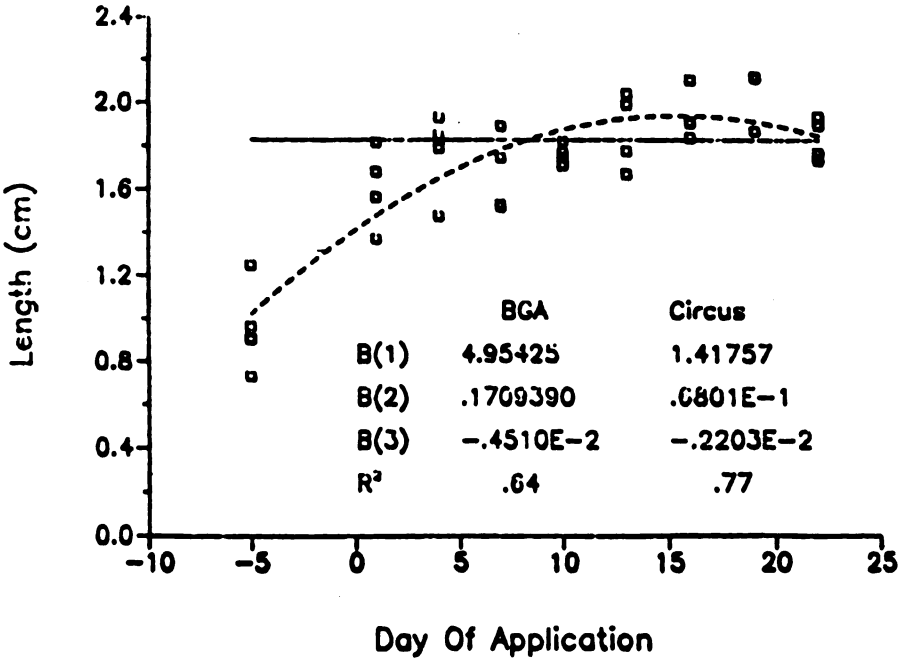
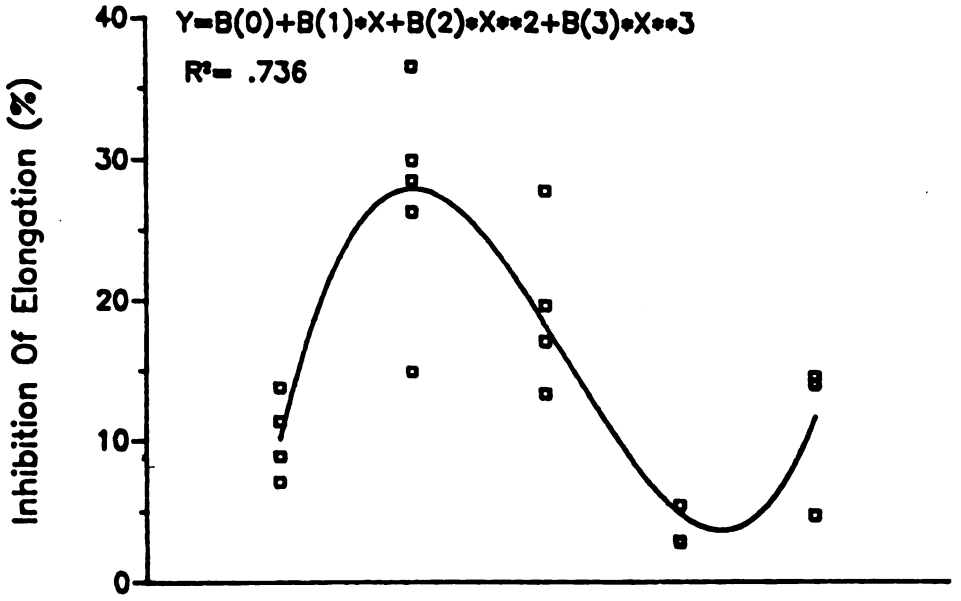


Figure 4. Effect of stem segment position of BGA (a) and Circus (b) on the response of that segment to a 292 ug daminozide application delivered as three 25 ul droplets of a 7500 mg l^{-1} solution.

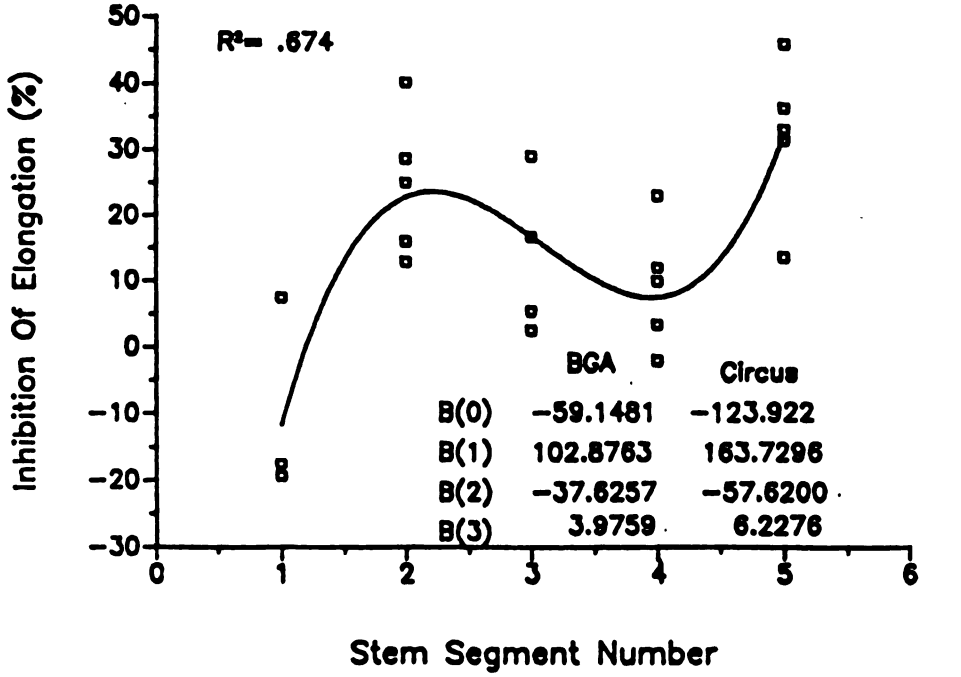
a

'Bright Golden Anne'



b

'Circus'



Section II

The Influence of Day Temperature and Photosynthetic Photon Flux on the Response of *Chrysanthemum morifolium* Ramat. cvs. 'Bright Golden Anne' and 'Circus' to Daminozide

The influence of day temperature and photosynthetic photon flux on the response of *Chrysanthemum morifolium* Ramat. cvs. Bright Golden Anne' and Circus' to daminozide.

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Abstract

The Influence of Day Temperature and Photosynthetic Photon Flux on the Response of *Chrysanthemum morifolium* Ramat. cvs. 'Bright Golden Anne'and 'Circus' to daminozide

Retardation of final shoot length by a 5000 mg l^{-1} daminozide application to 'Bright Golden Anne' and 'Circus' decreased 76 and 83 percent as day temperature (DT) increased from 10 to 26°C. Increasing DT from 26 to 30°C reversed this trend and increased daminozide retardation of final shoot length.

Photosynthetic photon flux (PPF) did not influence plant response to daminozide at low retardant concentrations. At high daminozide concentrations (>2500 mg $^{-1}$) retardation of elongation increased 10 to 17 percent as PPF increased from 200 umol s^{-1} m^{-2} to 600 umol s^{-1} m^{-2}.

Three methods were evaluated for quantifying the response of shoot elongation over time to DT and daminozide concentration. Method I and III were of the form f(x) * g(x) = retarded elongation: where g(x) = an inhibitor function, and f(x) defined the rate of shoot elonga-

tion (Method I) or absolute shoot length over time (Method III). Method II predicted gompertz function parameter estimates through multilinear regression; when evaluated within a gompertz function absolute shoot elongation over time was predicted. Method II had the greatest potential for modeling stem elongation as influenced by daminozide

Introduction

The chemical butanedioic acid mono (2,2-dimethyl hydrazide) (daminozide) has been found to reduce internode elongation in a number of plant species (1,3,4,14,16,17). Once applied to a sensitive plant species, stem elongation is depressed for some period of time after which elongation may resume at a rate similar to control plants (6,19). Studies of daminozide persistence within the plant have indicated that breakdown is very slow and is negligible during the life of most herbaceous plants (5,19). Zeevaart attributed the loss in response to daminozide over time to be caused by daminozide dilution within the plant tissue during growth to some concentration below which no retardation occurred (19).

The length of time from a growth retardant application to the resumption of unretarded elongation is influenced by the environment (3,6,13,17). Reductions in effectiveness

associated with variations in the environment have been noted with the response of Chrysanthemum to daminozide (9). The extent to which either temperature or PPF determine the response of a plant to an daminozide application has not been quantified.

The results presented in this paper quantify the influence of both PPF and day temperature (DT) on the duration of daminozide induced retardation of stem elongation in Chrysanthemum morifolium. Research by Karlson and Heins (11) indicated that there was no significant interaction between PPF and daminozide effectiveness, therefore, this factor was not studied.

The ultimate goal of this research is to predict daminozide effectiveness within Chrysanthemum morifolium over time under a variety of environmental conditions. The ability to predict daminozide effectiveness would enable daminozide reapplication prior to the loss of effectiveness of a previous application if additional height control is necessary.

Materials and Methods

General Procedures: Rooted cuttings of Chrysanthemum morifolium 'Bright Golden Anne' (BGA) and 'Circus' were planted in a commercial potting mix (sphagnum peat, vermiculite, perlite) in 10.2 cm plastic pots. The cul-

tivars 'Bright Golden Anne' and 'Circus' were were chosen because of their commercial significance and to compare responses to daminozide of cultivars with different flower forms: decorative and daisy. All plants were placed in controlled environment chambers under a photosynthetic photon flux (PPF) of 325 umol s^{-1} m^{-2} delivered with cool white fluorescent lamps for 16 hour d^{-1}. Day and night temperatures were maintained at 20°C and 16°C, respectively. Plants were fertilized at each irrigation with 200 mg l^{-1} of N and K. After 7 days, the apical meristem of the plants were removed and the photoperiod was reduced to 8 hours d^{-1} to induce flowering. Twelve days after the initiation of short days, lateral breaks other than the apical 3 of each plant were removed. daminozide was applied as a foliar application to the whole plant 15 days after the start of short days.

All treatments were applied to both 'Bright Gloden Anne'(BGA) and 'Circus' and data were collected on total shoot length every 4 days with a Maxcal 'Max-15' digital caliper.

Individual Experimental Procedures

Day Temperature Experiment: Twenty plants of each cultivar were placed in 5 controlled environment chambers. At the initiation of short days the day temperature (DT) in each of

the chambers was changed to either 10°, 18°, 22°, 26°, or 30°C.

The plants within each DT treatment were separated into four groups of five plants each. Each group received a foliar application of daminozide with a concentration of either 0, 1250, 2500, or 5000 mg l^{-1}. The daminozide was delivered as a foliar application until 'runoff'. This resulted in a dose applied to the plants of 0.0, 5.63, 11.25, or 22.50 mg, respectively.

Final shoot length data were analyzed at the cessation of elongation of the second lateral shoot as a split-plot statistical design with DT as the main plot and daminozide concentrations as the subplots. The experiment consisted of twenty treatments with 5 replicates per treatment.

Photosynthetic Photon Flux Experiment: Twenty plants of each cultivar were placed in 4 controlled environment chambers. At the initiation of short days (8 hours) the PPF within each environmental chamber was changed to either 50, 200, 400, or 600 umol s^{-1} m^{-2}. Plants within each PPF treatment were separated into 4 groups of 5 plants each. Each group received an application of daminozide with a concentration of either 0, 1250, 2500, or 5000 mg l^{-1}. The average dose applied with each application was the same as in the DT experiment.

Final shoot length data were analyzed as a split-plot

statistical design with PPF as the main plot and daminozide concentrations as the subplots. Twenty treatment combinations were used with 5 replicates per treatment.

Data Analysis Of Elongation

Technique I (Figure 1): A nonlinear regression function was selected as a means of quantifying shoot elongation over time for three reasons: 1) nonlinear regression functions are based on the premise that growth which has yet to occur is in some way related to the growth which has previously occurred (this would be expected with most eucaryotic organisms where growth rate is often influenced by an organism's size) 2) nonlinear function parameter estimates can have real biological meaning, i.e. when significant they may represent the rate of growth, initial length, or the asymptote of a fit data set 3) nonlinear functions are capable of approaching an asymptote. Based on the characteristics of the stem elongation over time rate , i.e. nonsymeteric point of inflection and a slow approach in final shoot length (FSL), the gompertz function (10) was fit to the shoot length data (Figure 2). The gompertz function takes the form of:

$$\text{Shoot Length} = ae^{-be^{-cT}}$$

Where a = asymptote (FSL)

ae^{-b} = initial stem length
c = rate constant
T = time
(ln b)/c = inflection point (IF)
a/(ln IF) = mean absolute extension rate (MAER)

Parameter estimates were calculated from the data for the gompertz function for elongation of unretarded shoots as DT or PPF varied using the software program 'Plotit'. First derivatives for each function were calculated at 5 day intervals and were submitted to multilinear regression analysis as the dependent variable with time and DT or time and PPF level as the independent variables. The 'All possible subsets' subroutine of the BMDP statistical software package (7) was used to determine the best possible prediction function based on r^2. Maximum likelihood parameter estimates were calculated from the data for the gompertz function using 'Plotit' for daminozide retarded elongation after day fifteen. First derivatives for each function were calculated and a multiplication factor, i.e. a number which when multiplied by the rate of growth of untreated plants yielded the rate of retarded elongation, was calculated at five day intervals. The predicted multiplication factors were submitted to multilinear regression analysis with time, daminozide concentration, and day temperature or PPF as the independent variables. The 'All possible subsets' subroutine of the BMDP statistical software package (7) was used to determine the best possible prediction function

based on r^2.

Technique II (Figure 3): The gompertz function was fit to shoot elongation data over time of both untreated and daminozide treated plants from day 0 to 60, and day of application ,15, to day 60, respectively (Figure 3). All parameter estimates were tested for significance using the Student's t value. If the parameters within a model were not significant, the FSL (or asymptote) was weighted and the data were resubmitted to nonlinear regression analysis. The maximum number of times that this procedure was employed was twice. The 'a' parameter estimate was then submitted to multilinear regression analysis as the dependent variable with DT and daminozide concentration as the independent variables. The 'All possible subsets' subroutine of the BMDP statistical software package was used to determine the best possible prediction function based on r^2.

The 'b' and 'c' parameters were predicted in a similar fashion to the 'a' parameter with the exception that the 'a' parameter was used, in addition to DT and daminozide concentration, as an independent variable.

Prediction of daminozide retarded elongation on an absolute length basis was accomplished by using the predicted absolute length of untreated plants prior to the day of an daminozide application (day 15) and the predicted retarded absolute shoot length after day 15.

Technique III (Figure 4): The gompertz function was fit to shoot elongation data over time for both untreated plants and daminozide treated plants as described for Method II. However, rather than predicting daminozide retarded growth through prediction of parameter estimates, absolute shoot length was predicted through multiplication by an inhibitor function (Figure 4). The inhibitor function was derived by dividing the predicted retarded absolute shoot length by the untreated shoot length and submitting these data to the 'All possible subsets' subroutine of the BMDP statistical software package as the dependent variable and DT, daminozide concentration, and time as the independent variables.

Results and Discussion

Shoot Length Versus Time: Final lateral shoot length (FSL) increased 135% for both BGA and Circus as DT increased from 10 to 26°C, then decreased as DT increased from 26 to 30°C (Table 1 and 2, Figure 2). Of the change in BGA shoot length, 87 percent of the increase in shoot length was associated with an increase in DT from 10 to 18°C. With Circus only 42% of the increase in shoot length occurred as DT increased from 10 to 18°C; 93 percent of the increase in shoot length occurred with an increase in DT from 18 to

26°C. As DT was further increased from 26 to 30C shoot length decreased 22% for Circus, but remained unchanged for BGA.

As DT increased from 10 to 26°C the mean absolute stem extension rate (MAER) increased and the inflection point of the fit gompertz function occured earlier; this indicated that the time at which shoots reached their final length occurred earlier, and the rate at which they approached their final length increased (Table 1 and 2). As DT further increased from 26 to 30°C, the time at which shoots attained their final length was delayed and the MAER decreased.

These results suggest the optimal DT for stem elongation in Chrysanthemum morifolium cvs. BGA and Circus occurs between 26 and 30°C when the night temperature is 16°C (Figure 5). 'Circus' responded to an increase or decrease in DT about the optimal DT more strongly than BGA. BGA appeared to have a slightly higher optimal DT (28-29)°C than Circus (26-27)°C. The existence of an optimal temperature is expected since an increase in DT must eventually result in denaturation of proteins and a subsequent decrease in final shoot length and increase the rate of respiration to a level which may limit elongation through a reduction in the total carbohydrates available for elongation (18). Went reported optimal day temperatures for stem elongation for a number of plant species including Pisum, Achillea,

Cypridedium,and Cymbidium (18).

Karlsson and Heins (12) suggested that shoot length in BGA increased linearly as DT increased from 10 to 30°C. Since no experimental treatments above 26°C DT had night temperatures below 20°C in their work, the lack of an optimal or maximum stem length as DT increased appeared to be a result of a delay in flower initiation and an increase in node number. Internode length did not decrease in their work at temperatures above 26°C.

Daminozide decreased the 'a' parameter of the gompertz function and the absolute stem extension rate in all DT treatments, i.e. FSL decreased and the time when the FSL was reached was delayed as evidenced by the delay in the day the inflection point occurred (Fig. 6, Tables 1 and 2). Overall daminozide reduction of FSL increased under conditions which were not optimal for elongation, i.e. DT of 10 and 30°C (Figure 7). At optimal DT, i.e. 26 to 29°C the response to an daminozide application was limited indicating that the daminozide may be diluted more rapidly at these temperatures due to increased stem growth and loose effectiveness more rapidly than at less than optimal temperatures (Fig. 7).

In general, BGA was more responsive to daminozide applications than Circus with temperatures below 26°C. Circus was more responsive to daminozide applications with day temperatures above 26°C (18) (Table 1 and 2)

As DT increased, daminozide response decreased. A 50 percent reduction in daminozide effectiveness was seen as DT was increased from 10 to 30°C with a 5000 mg l^{-1} application (Figure 8). Schonherr and Bukovac determined that an increase in temperature from 20-25°C to 25-30°C increased daminozide penetration into Phaseolus vulgaris leaves by 48% (15). This increase in permeability was believed to occur through an increase in flexibility of the cutin matrix through dissociation of weak bonds within the membrane. Results presented in our research suggest that some internal factor is influencing plant response to daminozide near a day temperature of 26°C which resulted in a overall reduction in response despite of the presumed high absorbance of daminozide through the leaf cuticle due to the relatively high day temperature.

Applications of daminozide increased the length of time which a shoot elongated as determined from the delay in the point of inflection (Tables 1 and 2) of the fitted growth functions. Previous studies on the influence of daminozide on cell elongation and division in Chrysanthemum indicated that daminozide increased cell number within an internode, but decreased cell elongation to such an extent that overall internode length was reduced (8). Normal shoot development through both cell division and elongation is hypothesized to result in a dilution of daminozide below some critical con-

centration needed for retardation. It is suggested that those cells which had formed as a result of daminozide stimulated division which had not yet expanded prior to a loss of responsiveness, develop normally after daminozide is no longer effective. This cell elongation results in an extended period of elongation late in development. The magnitude and duration of the extended period was enhanced with nonoptimal day temperatures.

Photosynthetic Photon Flux: Shoot length was not influenced by an increase in PPF (Fig. 9). The reduction in shoot length associated with an daminozide application was PPF dependent. daminozide effectiveness decreased as PPF increased from 50 to 400 umol s^{-1} m^{-2}, then increased as PPF increased from 400 to 600 umol s^{-1} m^{-2}.

II Rate Of Elongation

Shoots of BGA plants had a higher rate of elongation than shoots of Circus for all DT treatments except at 26°C where the maximum rate of elongation for Circus was similar to that of BGA (1.01 cm day^{-1} vs. 1.08 cm day^{-1}) (Figure 10).

Mean stem elongation rate increased as DT increased from 10 to 26°C. Circus exhibited a greater change in the rate of elongation than BGA as DT increased above 22°C

(Table 1 and 2). An increase in the DT from 22 to 26°C resulted in a 45 percent increase in the maximum rate of elongation for Circus as compared to only a 20 percent increase with BGA. Further increasing DT from 26 to 30°C decreased the maximum rate of elongation by 33 and 3 percent for Circus and BGA, respectively.

The day when the maximum rate of elongation occurred (inflection point) was cultivar and DT dependent (Tables 1 and 2). With a 26°C DT, the maximum rate of elongation occurred on day 15 and 20 for Circus and BGA, respectively. As the DT either increased or decreased from 26°C, the day when the maximum rate of elongation occurred was delayed. The maximum delay under these experimental conditions was greater with BGA (14 days at 10°C) than Circus (8 days at 10°C).

The length of time a shoot elongated was shortest with a DT of 26°C (Fig. 10). As temperatures deviated from 26°C the length of time which a shoot elongated increased. Shoots from BGA plants elongated 6 to 21 days longer than shoots of Circus plants for all DT treatments.

Daminozide Induced Retardation: Application of daminozide resulted in a discontinuity in the rate of shoot elongation at the time of an application (Fig. 11). Following an daminozide application, three distinct post application phases of elongation were evident: 1) elongation from

the time of an application to the start of a brief burst in elongation (Phase I), 2) the brief burst in elongation (Phase II), 3) and elongation following Phase II (Phase III) (Fig. 11).

The nature of Phase II made the development of a single function to describe postapplication elongation difficult. Since the overall influence of Phase II on the absolute length was not great, it was not included in the model. When Phase II was not included it was possible to develop a single function which described the percent retardation of both Phase I and III as they were influenced by DT over time.

Day Temperature Experiment The relationship between time and percent retardation due to an daminozide application was quadratic irrespective of DT (Figure 12). The percent retardation initially increased at high daminozide concentrations for about ten days after an application (from day 15 to 25) then decreased to day 55. The magnitude of the quadratic response increased as daminozide concentration increased and was greater with BGA than Circus.

Percent retardation decreased as DT increased from 10 to 26°C irrespective of time then increased as DT increased from 26 to 30°C at high daminozide concentrations (Figure 8). The response to daminozide was linear and increasing as daminozide concentration increased irrespective of DT.

An increase in DT from 10 to 26°C decreased the time that an daminozide application was useful in retarding elongation. Increasing DT from 26 to 30°C increased the time which an application was effective (Figure 13).

Photosynthetic Photon Flux Experiment A change in PPF did not influence percent retardation of elongation at low concentrations of daminozide. As the concentration of daminozide increased percent retardation of elongation was greater at 100 umol s^{-1} m^{-2} than at 500 umol s^{-1} m^{-2} (Figure 14). With BGA, no difference in the percent retardation was seen between different PPF levels until concentrations of daminozide were above 2500 mg l^{-1}.

Model Evaluation: Retardation of stem elongation by daminozide is an episodic event, i.e. stem elongation is influenced by some stimulus which varies in magnitude with time. One form a model describing an episodic event may take is:

$$\text{Shoot length} = f(x)*g(x)$$

Where: f(x)= unretarded elongation
g(x)= some multiplication factor which when multiplied by f(x) yields the retarded absolute shoot length, or shoot elongation rate.

Method I and II (Figures 2 and 3) are based on this form where g(x)= function defining the retardation of the

rate of elongation by daminozide (Table 3) and retardation of absolute shoot length over time (Table 4), respectively. The use of an inhibitor function is attractive in that, the influence of a stimulus such as DT, daminozide concentration, or time on the response of a plant to a growth inhibitor can be studied independently. Models which are composed of a single function quantifying absolute shoot elongation over time as influenced by one or more stimuli may be more difficult to interpet, since the kinetics of the elongation response, as one or more stimuli change, is inherent within the function.

Model I (Figure 2 and 15) is based on the prediction of growth rate over time (Table 5). The benefit of this type of system is the increased sensitivity to small changes in elongation thereby gaining a better insight into the kinetics of the loss of daminozide response. The limiting factor with this type of model is the flexibility required by the predicted function which is not necessarily possible when multilinear regression techniques are used. This problem is compounded by two successive multilinear regressions on a given function, and multiplication of two functions, thus compounding any previous errors in prediction. As a result this method was not found to be acceptable as a means for predicting daminozide retarded elongation over time.

Method I was found to yield acceptable results when a variation of this method was used to quantify the influence of daminozide on Chrysanthemum morifolium shoot mass over time; where g(x) was derived from a function previously used to quantify retardation of an exponentially growing bacteria culture by tetracycline (6). Leith and Reynolds also used a variation of Method I to quantify the influence of a short term ozone exposure on plant dry weight (11).

Method II (Figure 3 and 15) uses multilinear regression of parameter estimates resulting from gompertz functions fit to elongation data at different DT and daminozide concentrations to predict gompertz parameter estimates under a variety of DT and daminozide concentrations (Table 6). The resulting parameter estimates are then used to predict elongation over time through a second gompertz function. The gompertz function was chosen for two reasons: 1) the function fit the data well 2) there were indications that nonlinearity of the data/model relationship was low, i.e. low iteration count to convergence and significant parameter estimates. This suggested that the parameter estimates were not variable and that interpetations may be made from the parameter estimates with confidence .

The benefits of Method II are twofold: 1) only one step involving multilinear regresssion is used which does not involve division of the dependent variable prior to mul-

tilinear regression analysis 2) the resulting model may be interpeted through parameter estimation evaluation.

The success of Method II depends on the validity of the initial parameter estimates calculated from the raw data. Small errors in the initial parameter estimates may result in poor prediction of absolute shoot length, especially if they occur in the 'b' and 'c' parameters, or exponentials. Despite the potential for error, method II predicted final shoot length within 5% of the raw data values.

Method III (Figure 4 and 15) is similar to Method I with the exception, that absolute lengths are used instead of elongation rates. Since Method III regressed absolute shoot lengths and not elongation rate it was not as sensitive to small changes as Method I, however, it still contains an retardation multiplier (Table 4) which aids in evaluation of daminozide effectiveness kinetics as influenced by time, daminozide, and DT. Disadvantages of this method are similar to those of Method I.

Each method may lend itself to multiple application studies. Method I and III would be the easiest to adapt where g(x) may be multiplied by a second g(x) function for each subsequent application. Each of these functions would dimish in magnitude since daminozide has not been found to be additive in its reduction of elongation with multiple applications (8). Method II could be adapted by developing

sets of gompertz functions as time of application, DT, and daminozide concentration vary. A multilinear function could then be used to predict the final parameter estimates.

The three modeling methods has specific benefits which make each one more appropriate for specific types of episodic studies than the others:

Method I) Studies where all factors are held constant and the stimulus is varied, i.e. application of a retardant, change in temperature, water stress. This is particularly useful when recovery kinetics are of interest.

Method II) Methods where changes in plant response to a single stimulus at different physiological stages in plant developement are of interest.

Method III) Studies where the influence of multiple stimuli on a plant sytem at different times in plant development are of interest.

Conclusions

1) An optimal DT ranging from 26 to 30°C existed for stem elongation on Chrysanthemum morifolium with a night temperature of 16°C. As DT deviated from this optima, final shoot length was reduced, and the time at which shoots reached their final length occurred later.

2) An application of daminozide reduced final shoot length and delayed the time when a shoot reached its final length.

3) Plant response to an daminozide application was quadratic at high daminozide concentrations. As DT increased from 10 to 30°C. The minimum retardation of stem elongation associated with a 5000 mg l^{-1} application of daminozide occurred at 26°C. At low daminozide concentrations, the percent retardation of stem elongation was not influenced by DT.

4) Retardation of stem elongation increased linearly as daminozide concentration increased from 0 to 5000 mg $^{-1}$.

5) PPF had little effect on plant response to a daminozide application except at high PPF levels (500 umol s^{-1} m^{-2}), where PPF reduced daminozide response slightly.

6) Three methods for modeling the influence of time, DT, and daminozide concentration are presented for both BGA and Circus. Method II showed the greatest potential for quantifying episodic processes involving a growth retardant application.

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Table 1. Effect of day temperature (DT) and daminozide concentration on maximum likelihood parameter estimates of final shoot length (a), initial length determinant (b), and mean relative stem extension rate (c), of gompertz functions representing Chrysnathemum morifolium cv. 'Bright Golden Anne' lateral shoot elongation over time. The influence of DT and daminozide on the mean absolute stem extension rate (MAER) and inflection point (IP) are also presented.

Day Temperature[z]	daminozide conc.[y]	Final Shoot Length[u]	Parameter a	b	c	MAER[x]	IP[w]
10	0000	11.68	-	-	-	-	-
	1250	9.41	-	-	-	-	-
	2500	8.26	-	-	-	-	-
	5000	6.16	-	-	-	-	-
18	0000	21.66	25.47	7.05	.085	.404	23
	1250	19.67	21.58	6.07	.076	.332	24
	2500	18.52	20.96	5.32	.073	.332	23
	5000	14.79	17.70	4.20	.061	.272	24
22	0000	24.12	26.81	7.30	.085	.425	23
	1250	22.51	25.76	5.08	.071	.408	23
	2500	20.15	23.57	4.27	.065	.393	22
	5000	17.05	21.64	3.44	.051	.332	24
26	0000	31.10	32.22	7.97	.106	.596	20
	1250	29.61	31.03	6.50	.095	.575	20
	2500	28.55	30.39	5.83	.087	.563	20
	5000	27.43	29.72	5.77	.088	.550	20
30	0000	30.87	31.17	4.18	.059	.479	24
	1250	27.22	28.97	3.80	.053	.426	25
	2500	27.77	29.07	3.80	.053	.427	25
	5000	24.07	27.98	3.52	.044	.354	29

[z] day temperature (°C)
[y] daminozide concentration is in mg a.i. daminozide l^{-1}
[x] 60 days divided by total increase in length (a)(cm day^{-1}), IF*e
[w] calculated as ln (b)/ (c) in days.
[u] second lateral shoot length on day 60 (cm)

Table 2. Effect of day temperature (DT) and daminozide concentration on maximum likelihood parameter estimates of final shoot length (a), initial length determinant (b), mean relative stem extension rate (c), of gompertz functions representing Chrysanthemum morifolium cv. 'Circus' lateral shoot elongation over time. The influence of DT and daminozide on the mean absolute stem extension rate (MAER) and inflection point (IP) are also presented.

Day Temp.[z]	Daminozide Conc.[y]	Final Shoot Length[u]	Parameter a	Parameter b	Parameter c	MAER[x]	IF[w]
10	0000	7.24	9.59	4.36	.063	.152	23
	1250	6.29	7.86	4.50	.061	.116	25
	2500	6.33	10.03	3.37	.040	.122	30
	5000	5.75	8.26	2.92	.039	.113	27
18	0000	10.27	11.00	5.91	.087	.203	20
	1250	9.27	10.92	3.95	.059	.173	23
	2500	8.78	10.28	3.62	.061	.180	21
	5000	8.01	9.50	3.01	.056	.176	20
22	0000	11.83	13.84	4.99	.077	.242	21
	1250	9.41	9.95	6.63	.095	.191	19
	2500	8.93	9.76	4.47	.084	.199	18
	5000	8.21	9.92	3.49	.063	.183	20
26	0000	16.98	20.00	7.72	.138	.487	15
	1250	16.02	16.62	9.72	.155	.405	15
	2500	16.21	16.81	9.02	.150	.410	15
	5000	16.43	16.99	7.44	.136	.414	15
30	0000	13.15	13.32	6.80	.098	.247	20
	1250	11.92	13.50	5.06	.080	.250	20
	2500	10.99	11.59	4.55	.083	.236	18
	5000	9.64	10.29	4.88	.082	.197	19

[z] day temperature (°C)
[y] daminozide concentration is in mg a.i. daminozide l^{-1}
[x] 60 days divided by total increase in length (a) (cm day^{-1}), IF*e
[w] calculated as ln (b)/ (c) in days
[u] second lateral shoot length on day 60 (cm)

Table 3. Regression coefficients predicting the multiplication factor as influenced by day temperature (DT), time, and daminozide concentration (CN).

Term	Regression Coefficients	
	BGA	Circus
Time	-.041602100	-
$Time^2$	.000884756	-
$Time^5$	-.1416873E-08	-
CN	-	-.0004559180
CN*Time	-.00000897330	.0000224419
$CN*Time^2$	.1609652E-06	-.2031000E-06
CN^3*Time	-	-.3661670E-12
CN^3*Time^3	-	.3812351E-16
CN*DT	-	-.0000170769
$CN*DT^4$	-	.3245306E-09
CN^2*DT	-	.6064439E-08
CN^2*DT^3	-	-.3531014E-11
DT^3*Time	.2802678E-06	-
DT^3*Time^3	-.8882487E-10	-
Intercept	1.34911000	1.0004200
R^2	.82	.54

Table 4. Multilinear regression coefficients predicting gompertz function parameters as influenced by day temperature (DT), daminozide concentration, and the 'a' parameter.

Term	Regression Coefficients a	b	c
	'Bright Golden Anne'		
a	-	.5218310000	.00769129000
DT	2.562130	-	-
DT^3	-.00102908	-.0002768460	-.00000331345
CN	-.00088206	.0020788200	.00006669700
DT*CN	-	-	-.00000509957
DT^2*CN	-	-.0000114014	.9766194E-07
DT^3*CN	-	.3098457E-06	-
Intercept	-17.35120000	-4.3764200000	-.08681720000
R^2	.87	.92	.95
	'Circus'		
a	-	.534140	.0100378
DT	-9.08978000	-	-
DT^2	.53045800	-	-
DT^3	-.00913851	-	-
DT*CN	-.00001915	-	-
Intercept	55.67280000	-1.11064	-.0345133
R^2	.88	.73	.85

Table 5. Multilinear regression function parameters which describe the influence of day temperature and time on the rate of growth of Chrysanthemum morifolium cvs. 'Bright Golden Anne' and 'Circus'.

Term	Regression Coefficient	
	BGA	Circus
DT	-	-.0438070
DT^6	-	.1348027E-07
DT^7	-	-.4205546E-09
Time	-.0381609	-
$Time^2$	.000898661	-
$Time^7$	-.960873200E-13	-
$DT*Time$	.005842440	.00274723
$DT*Time^2$	-.000129120	-.0000694193
$DT*Time^5$	-	.1190032E-09
$DT*Time^6$	.8495166E-12	-
DT^2*Time^4	.1755890E-09	-
DT^4*Time^7	-.3257841E-18	-
DT^5*Time	-.2715808E-08	-
DT^5*Time^2	.4956677E-10	-
Intercept	-.0196249	.4320320
R^2	.91	.58

Table 6. Multilinear regression coefficients which predict g(x), or the inhibition function, as influenced by DT daminozide concentration, and time.

	Regression Coefficients	
Term	BGA	Circus
DT	-.78582200	-
DT^2	.00177616	-
Time	-.01597210	-
DT*Time	.00123335	.000510675
DT^3*Time	-.7940874E-06	-
CN*Time	-.00000428564	-.00000131498
CN*$Time^2$	.4914954E-07	.1657185E-07
CN*DT	.8276570E-06	-.00000123030
CN^3*DT	.1662337E-13	-
Intercept	1.77103	.956785
R^2	.92	.78

Figure 1. Schematic diagram representing the procedures followed for prediction of daminozide retardation of shoot elongation of Chrysanthemum morifolium for Method I.

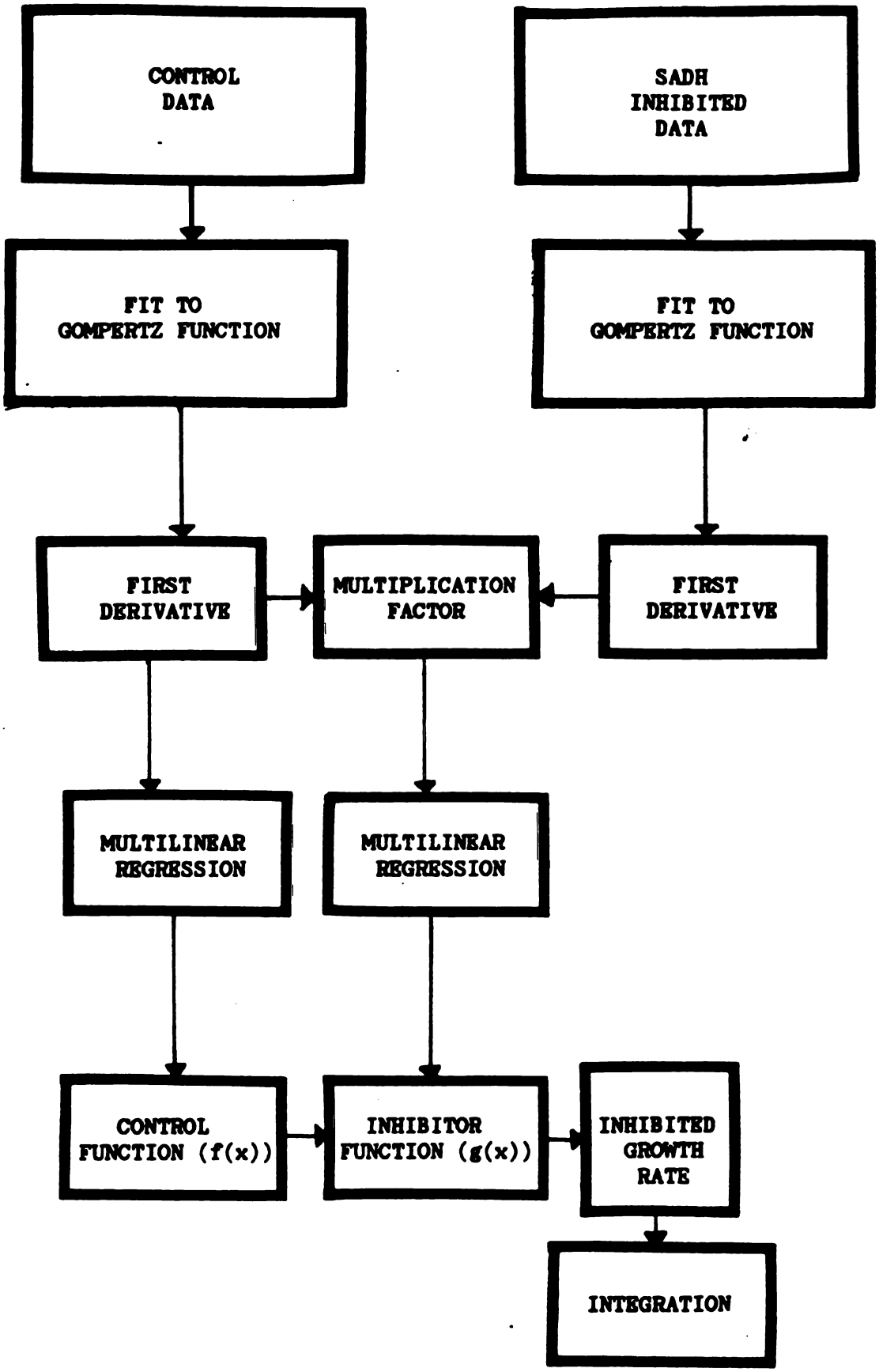
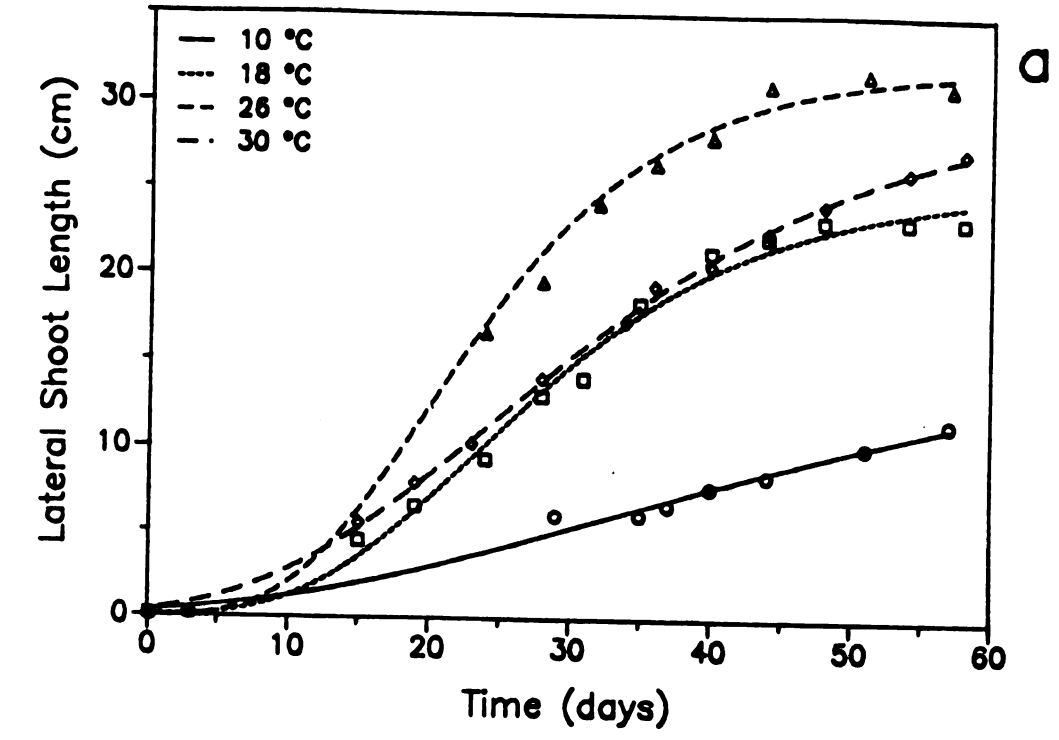
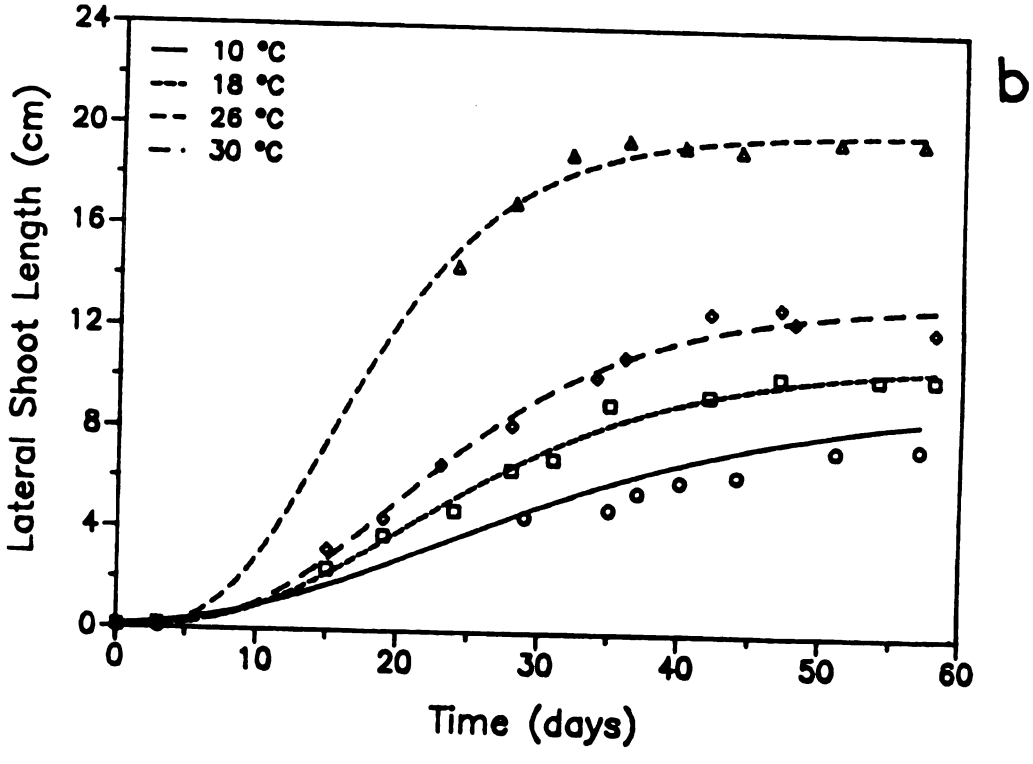

CONTROL DATA
SADH INHIBITED DATA
FIT TO GOMPERTZ FUNCTION
FIT TO GOMPERTZ FUNCTION
FIRST DERIVATIVE
MULTIPLICATION FACTOR
FIRST DERIVATIVE
MULTILINEAR REGRESSION
MULTILINEAR REGRESSION
CONTROL FUNCTION (f(x))
INHIBITOR FUNCTION (g(x))
INHIBITED GROWTH RATE
INTEGRATION

Figure 2. Influence of day temperature on second lateral shoot elongation of Chrysanthemum morifolium Ramat. 'Bright Golden Anne' (a), and 'Circus' (b). Lines represent gompertz function estimates (Tables 1 and 2) fit to each data set.


10 °C
18 °C
26 °C
30 °C
Lateral Shoot Length (cm)
Time (days)
0
10
20
30
40
50
60

a


10 °C
18 °C
26 °C
30 °C
Lateral Shoot Length (cm)
Time (days)
0
4
8
12
16
20
24
10
20
30
40
50
60

b

Figure 3. Schematic diagram representing the procedures followed for prediction of daminozide retardation of shoot elongation of Chrysanthemum morifolium for Method II.

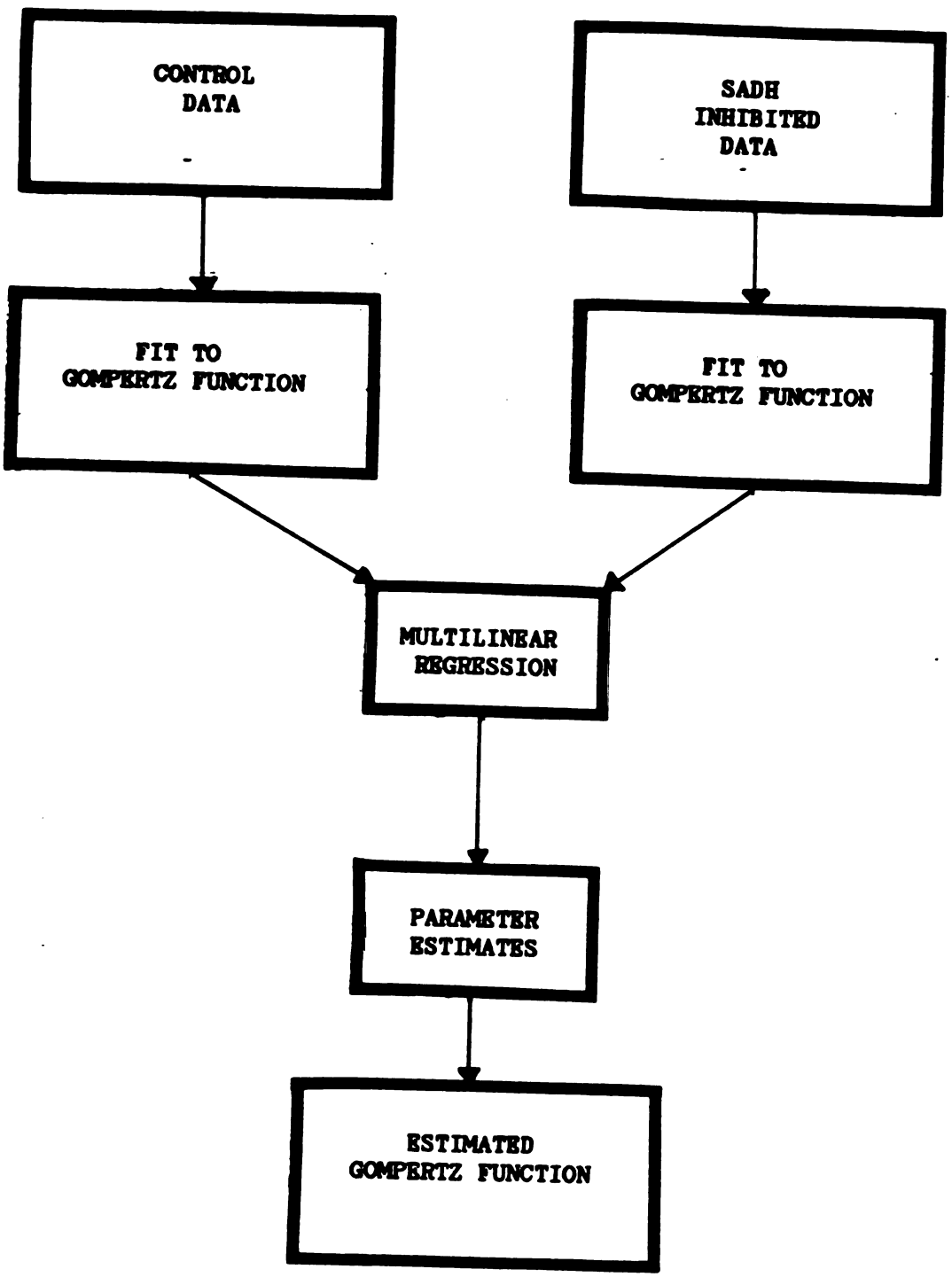

CONTROL DATA
SADH INHIBITED DATA
FIT TO GOMPERTZ FUNCTION
FIT TO GOMPERTZ FUNCTION
MULTILINEAR REGRESSION
PARAMETER ESTIMATES
ESTIMATED GOMPERTZ FUNCTION

Figure 4. Schematic diagram representing the procedures followed for prediction of daminozide retardation of shoot elongation of Chrysanthemum morifolium for Method III.

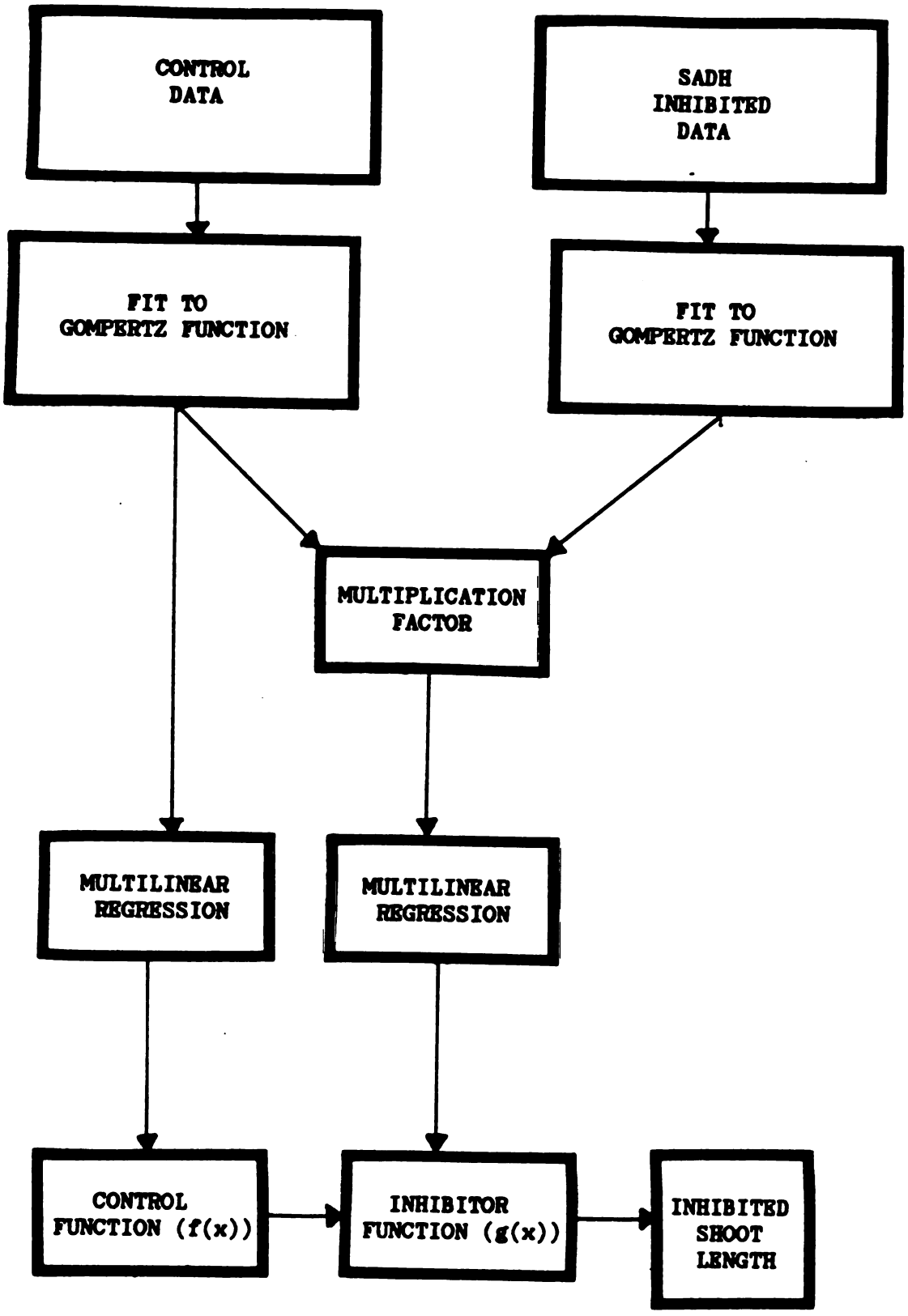

CONTROL DATA
SADH INHIBITED DATA
FIT TO GOMPERTZ FUNCTION
FIT TO GOMPERTZ FUNCTION
MULTIPLICATION FACTOR
MULTILINEAR REGRESSION
MULTILINEAR REGRESSION
CONTROL FUNCTION (f(x))
INHIBITOR FUNCTION (g(x))
INHIBITED SHOOT LENGTH

Figure 5. Response surface calculated from method II indicating the absolute shoot length of Chrysanthemum morifolium 'Bright Golden Anne' as influenced by time and day temperature.

Figure 6. The influence of a daminozide application on day 15 after the start of short days on second lateral shoot elongation over time on Chrysanthemum morifolium cv. 'Bright Golden Anne'.

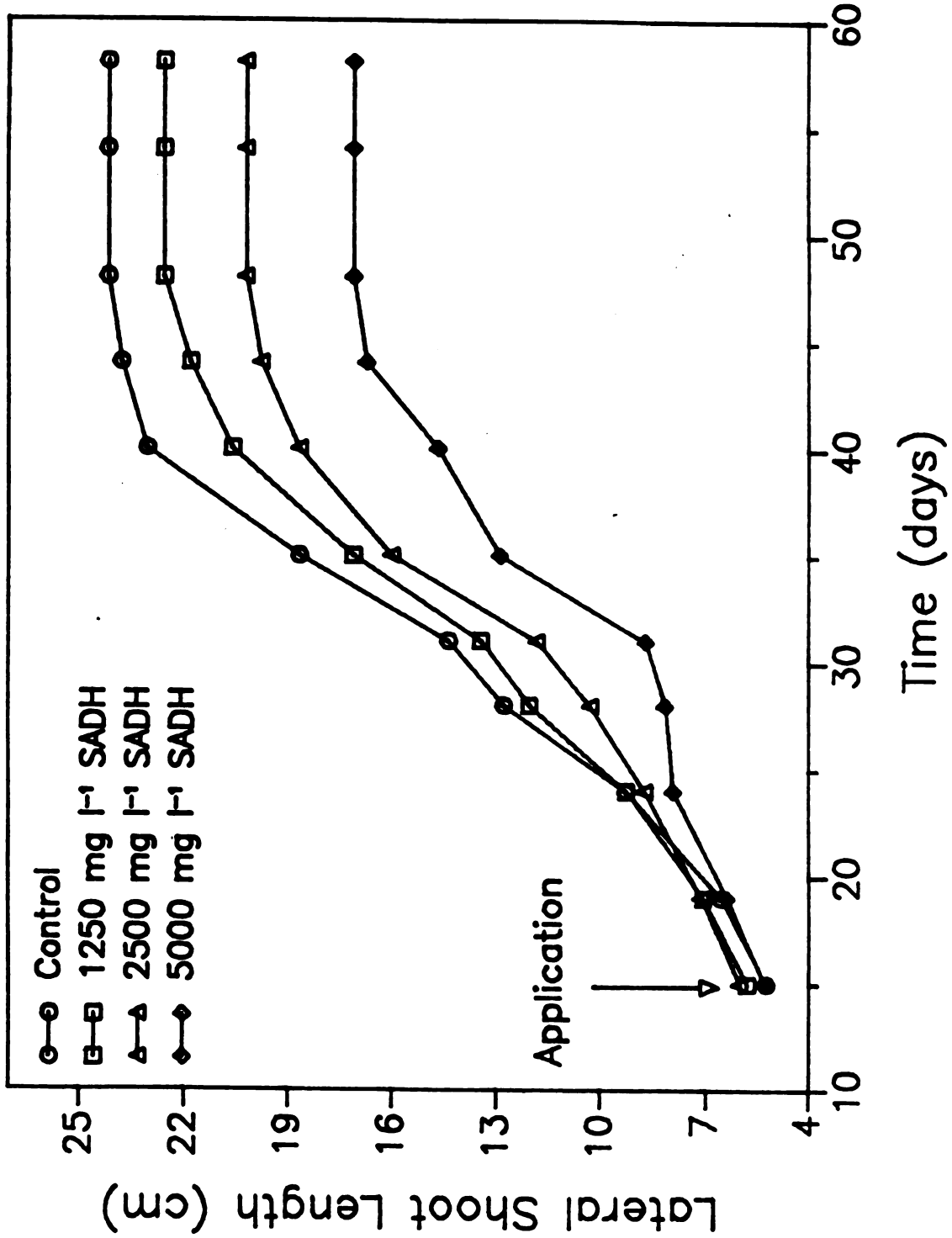
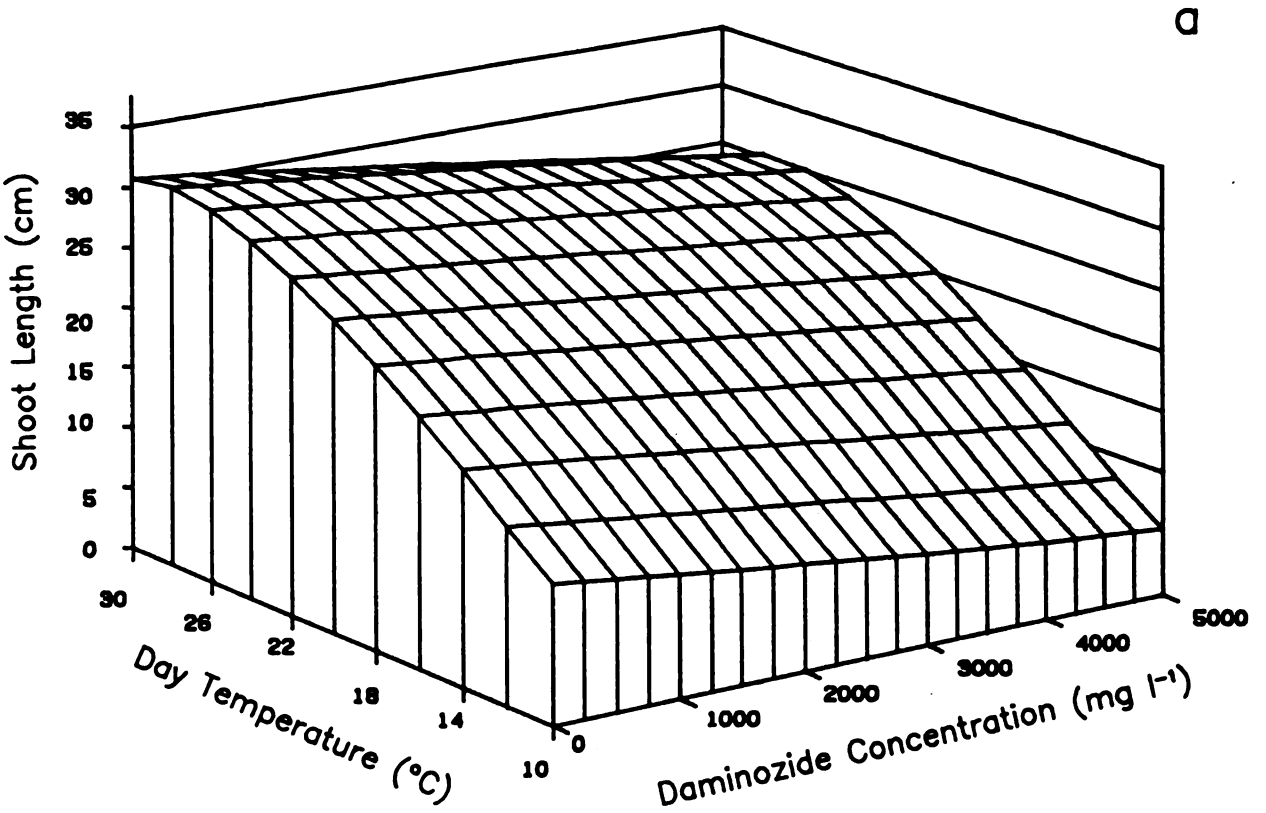

Lateral Shoot Length (cm)
25
22
19
16
13
10
7
4
10
20
30
40
50
60
Time (days)
Control
1250 mg l⁻¹ SADH
2500 mg l⁻¹ SADH
5000 mg l⁻¹ SADH
Application

Figure 7. Response surfaces calculayted from method II describing Chrysanthemum morifolium cvs. 'Bright Golden Anne' (a) and 'Circus' (b) final shoot length as influenced by daminozide concentration, and day temperature.


a
Shoot Length (cm)
35
30
25
20
15
10
5
0
30
26
22
18
14
10
Day Temperature (°C)
0
1000
2000
3000
4000
5000
Daminozide Concentration (mg l⁻¹)

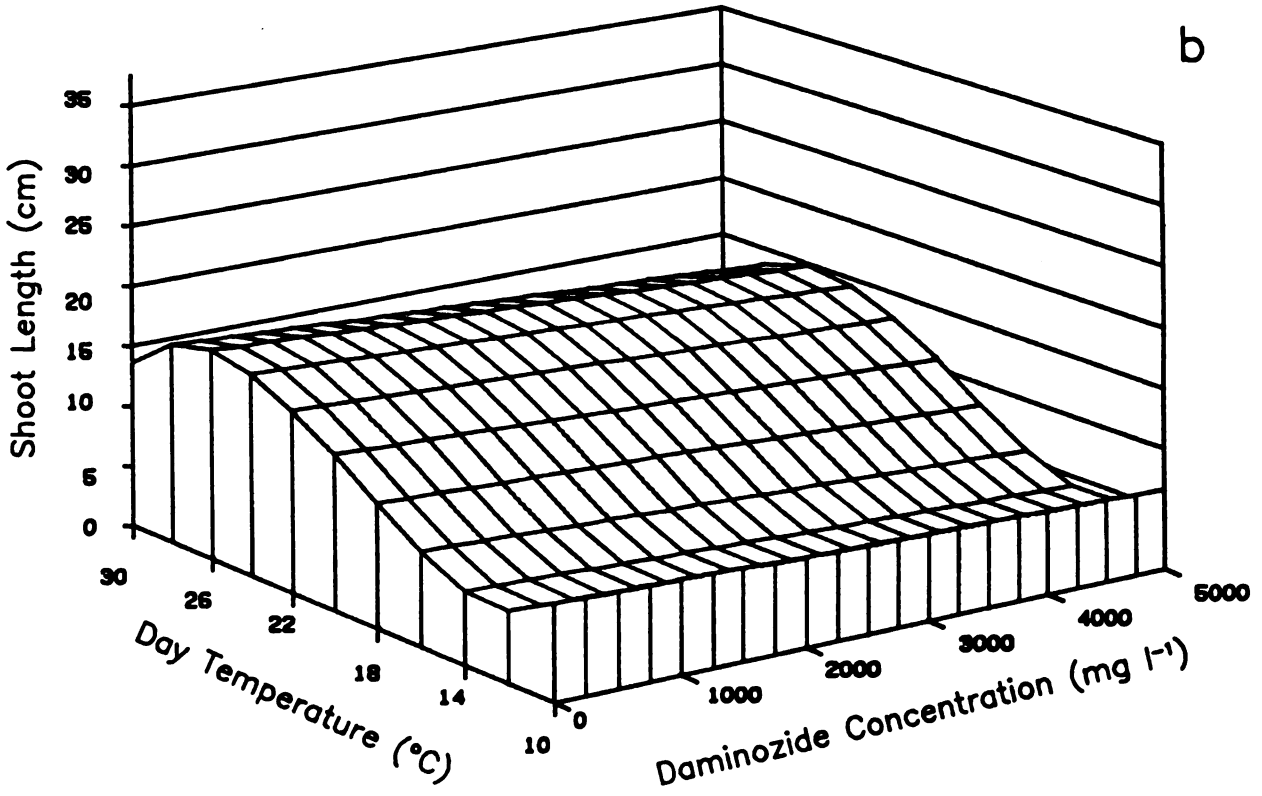

b
Shoot Length (cm)
35
30
25
20
15
10
5
0
30
26
22
18
14
10
Day Temperature (°C)
0
1000
2000
3000
4000
5000
Daminozide Concentration (mg l⁻¹)

Figure 8. Response surface calculated from method II describing the influence of daminozide concentration and day temperature on percent inhibition of shoot length resulting from a daminozide application 15 days after the start of short days to Chrysanthemum morifolium cv. 'Bright Golden Anne'.

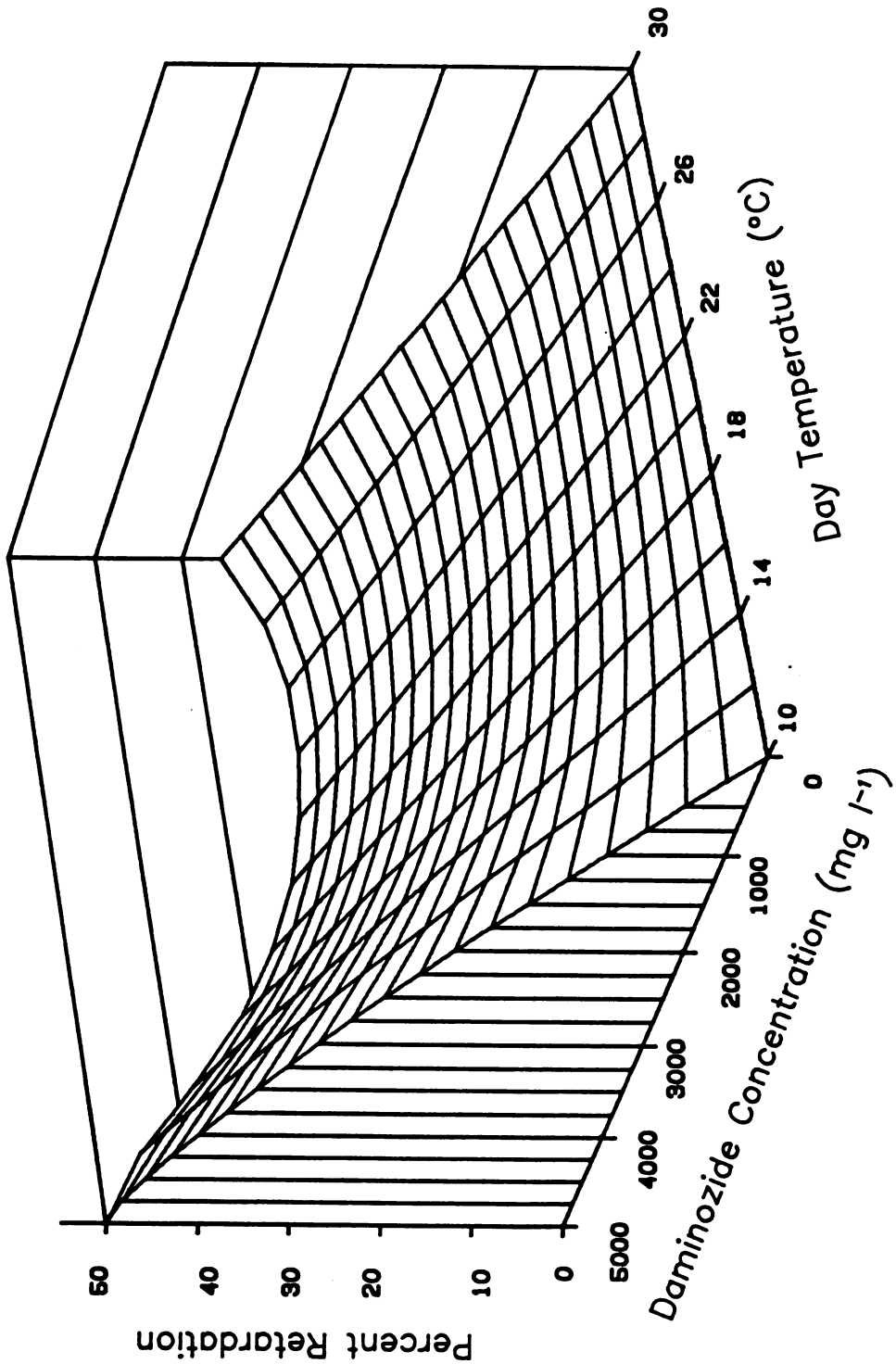
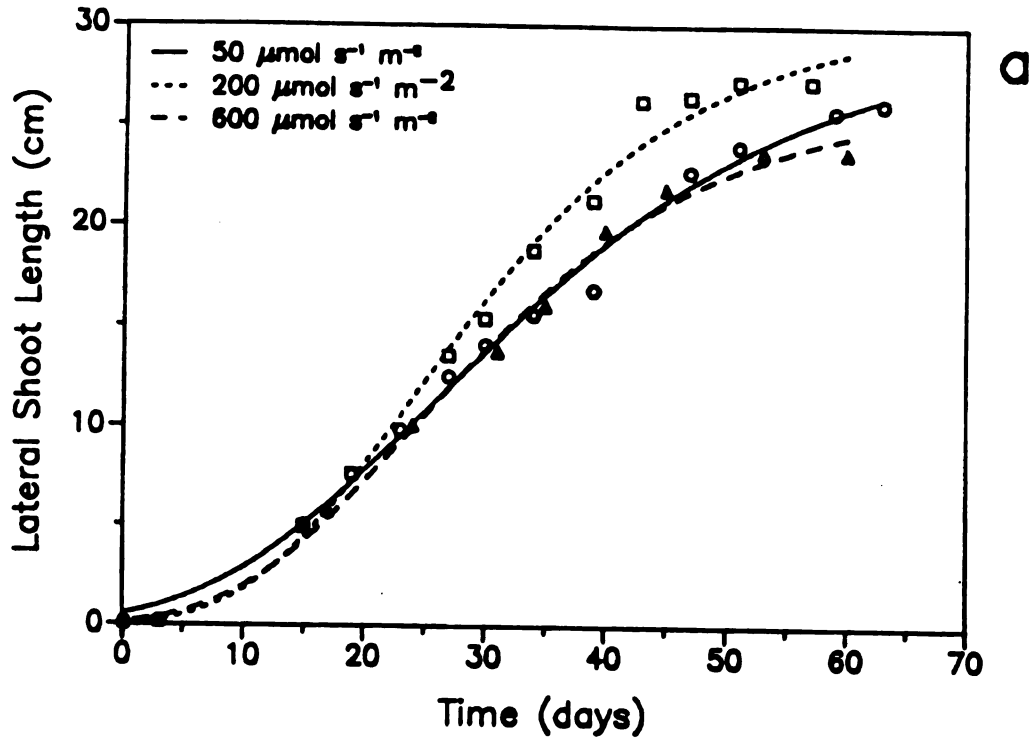
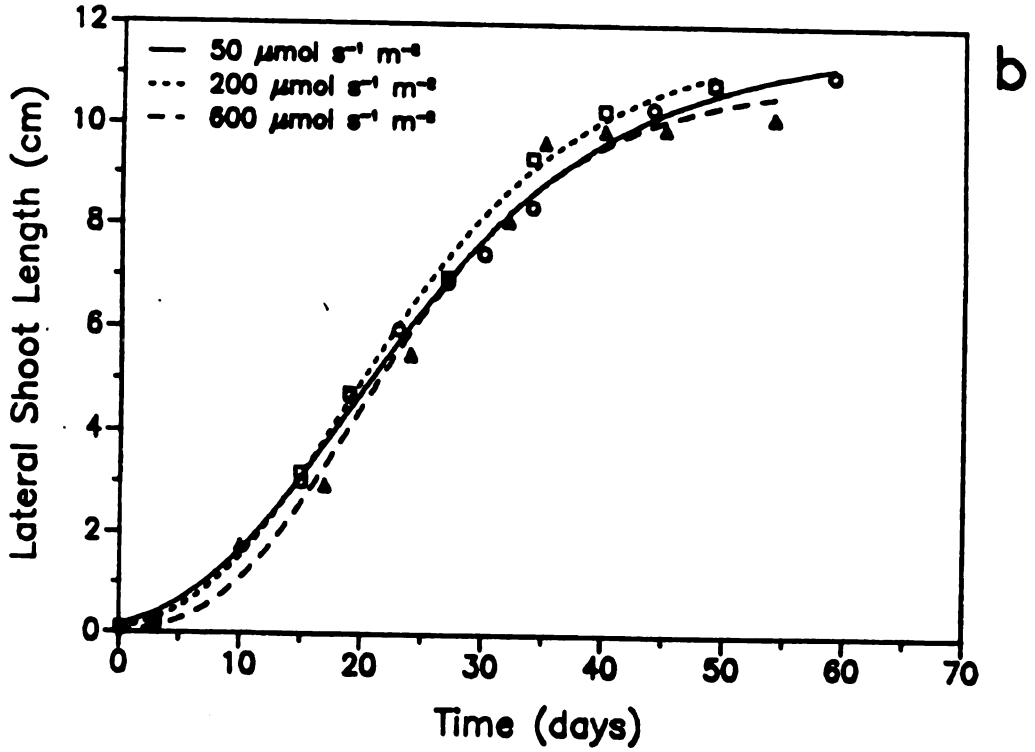

Percent Retardation
50
40
30
20
10
0
Daminozide Concentration (mg l-1)
5000
4000
3000
2000
1000
0
Day Temperature (°C)
10
14
18
22
26
30

Figure 9. The influence of photosynthetic photon flux on shoot elongation over time of Chrysanthemum morifolium cvs. 'Bright Golden Anne'(a) and 'Circus'(b). Lines represent gompertz function estimates (Tables 1 and 2) fit to each data set.

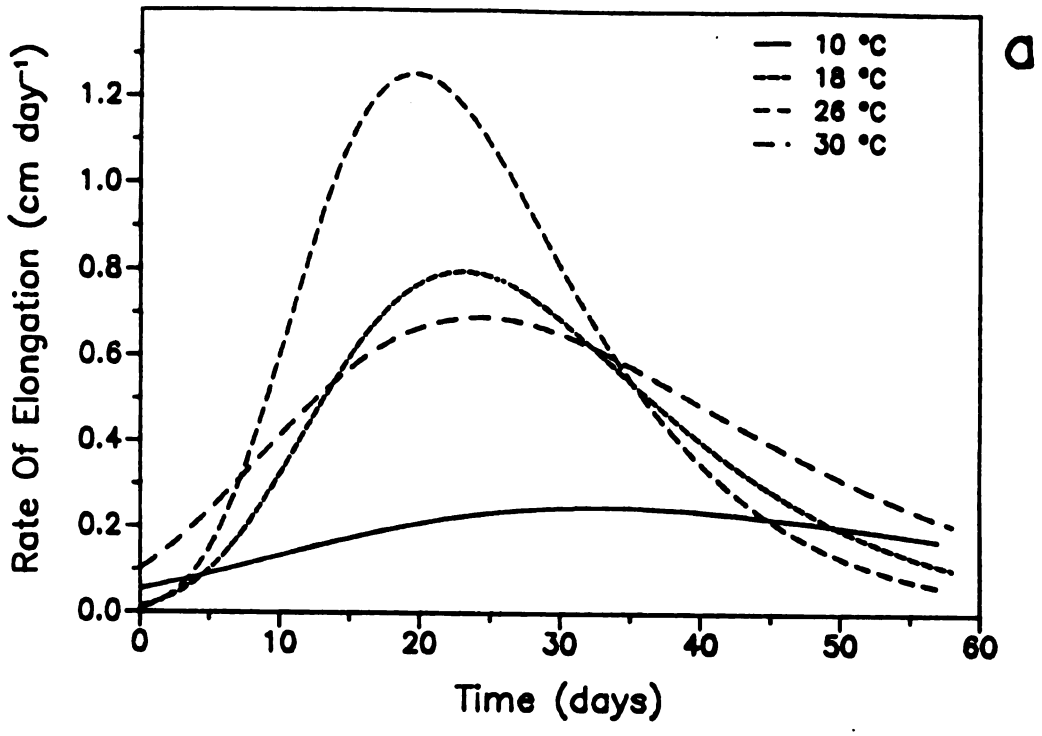

50 μmol s⁻¹ m⁻²
200 μmol s⁻¹ m⁻²
600 μmol s⁻¹ m⁻²
Lateral Shoot Length (cm)
Time (days)
0
10
20
30
40
50
60
70

a


50 μmol s⁻¹ m⁻²
200 μmol s⁻¹ m⁻²
600 μmol s⁻¹ m⁻²
Lateral Shoot Length (cm)
Time (days)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70

b

Figure 10. The influence of day temperature on the rate of shoot elongation of Chrysanthemum morifolium cvs. 'Bright Golden Anne'(a) and 'Circus'(b). Lines represent first derivatives of gompertz functions fit to shoot elongation data over time at each day temperature.


a
10 °C
18 °C
26 °C
30 °C
Rate Of Elongation (cm day⁻¹)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
10
20
30
40
50
60
Time (days)

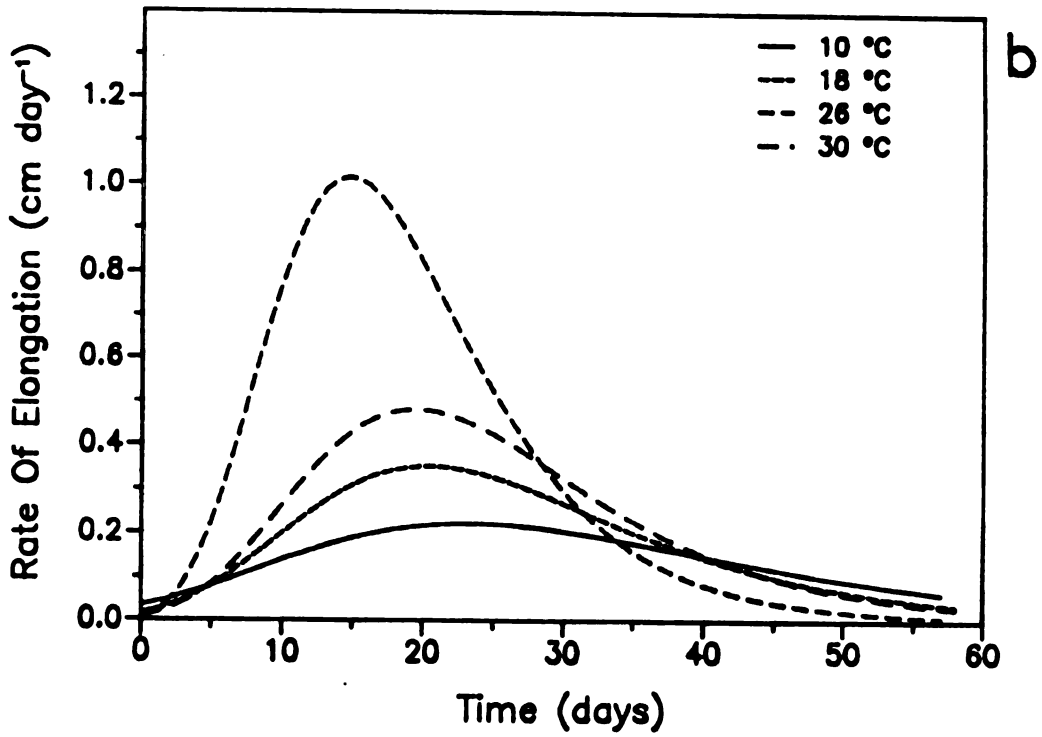

b
10 °C
18 °C
26 °C
30 °C
Rate Of Elongation (cm day⁻¹)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
10
20
30
40
50
60
Time (days)

Figure 11. Diagram identifying the three phases of daminozide influenced elongation following an application of daminozide 15 days after the start of short days to Chrysanthemum morifolium cv. 'Bright Golden Anne' grown with day and night temperatures of 30°C and 16°C, respectively. The solid line represents the first derivative of a gompertz function (Table 1) fit to shoot elongation over time. The segmented line represents the first derivative of retarded shoot elongation calculated at four day intervals.

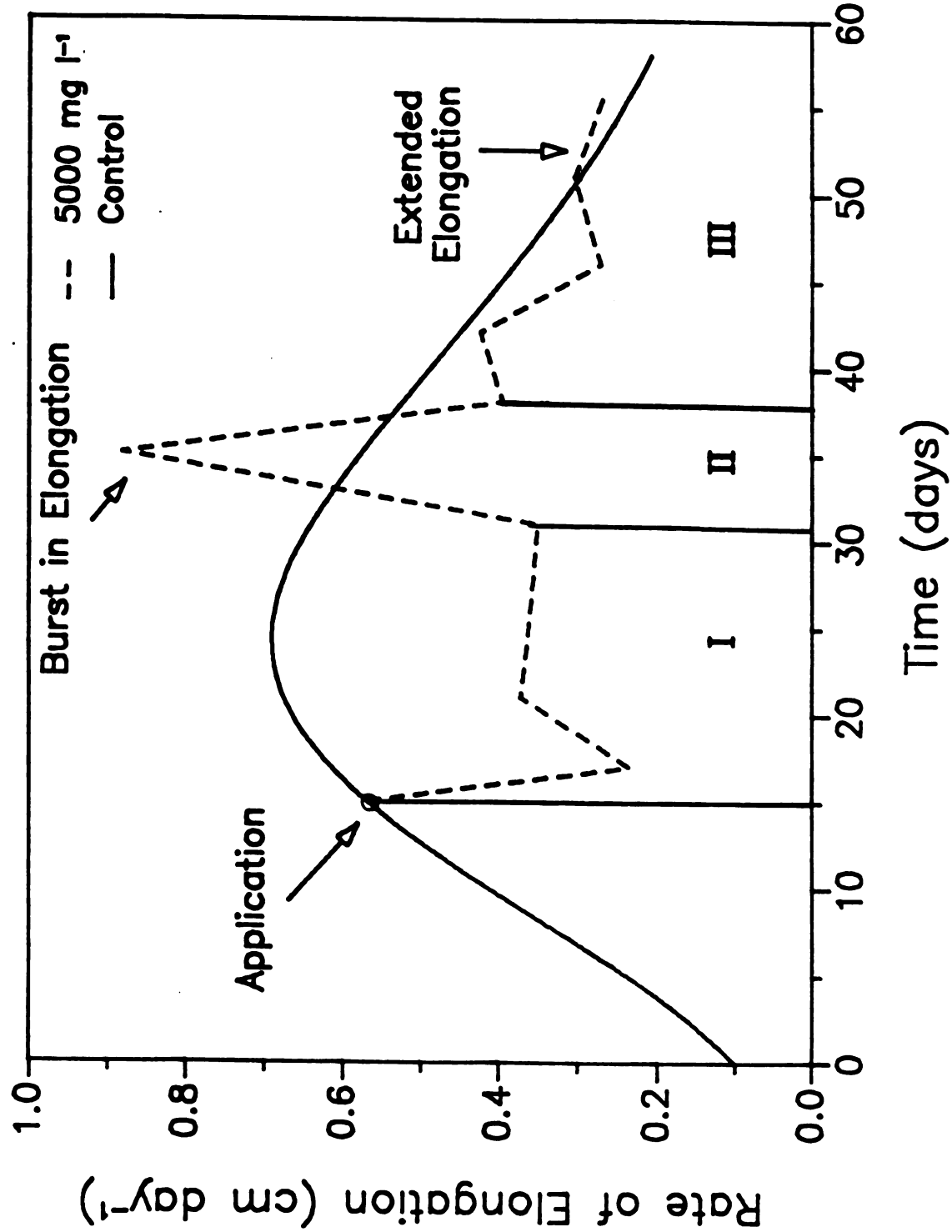

Rate of Elongation (cm day⁻¹)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Time (days)
Burst in Elongation
-- 5000 mg l⁻¹
— Control
Extended Elongation
Application
I
II
III

Figure 12. Response surface calculated from method I identifying the influence of time and daminozide concentration on the percent inhibition of shoot elongation resulting from an application of 2500 mg l^{-1} daminozide 15 days after the start of short days to Chrysanthemum morifolium cv. 'Bright Golden Anne'.

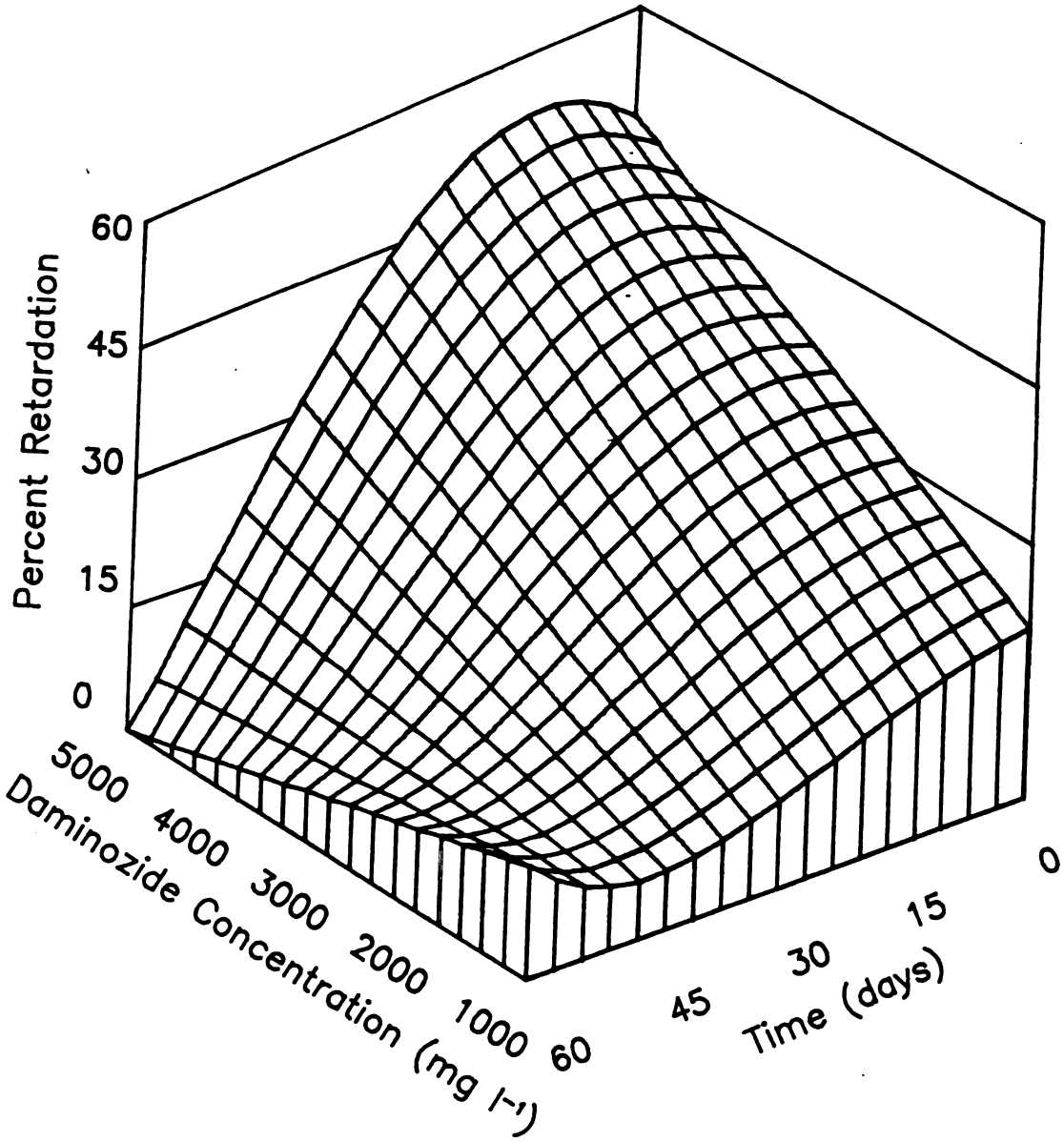

Percent Retardation
60
45
30
15
0
5000
4000
3000
2000
1000
Daminozide Concentration (mg l-1)
0
15
30
45
60
Time (days)

Figure 13. The influence of day temperature and time on the retardation of shoot elongation remaining following an application of 2500 mg l^{-1} daminozide 15 days after the start of short days to Chrysanthemum morifolium cv. 'Bright Golden Anne'as calculated from method II.

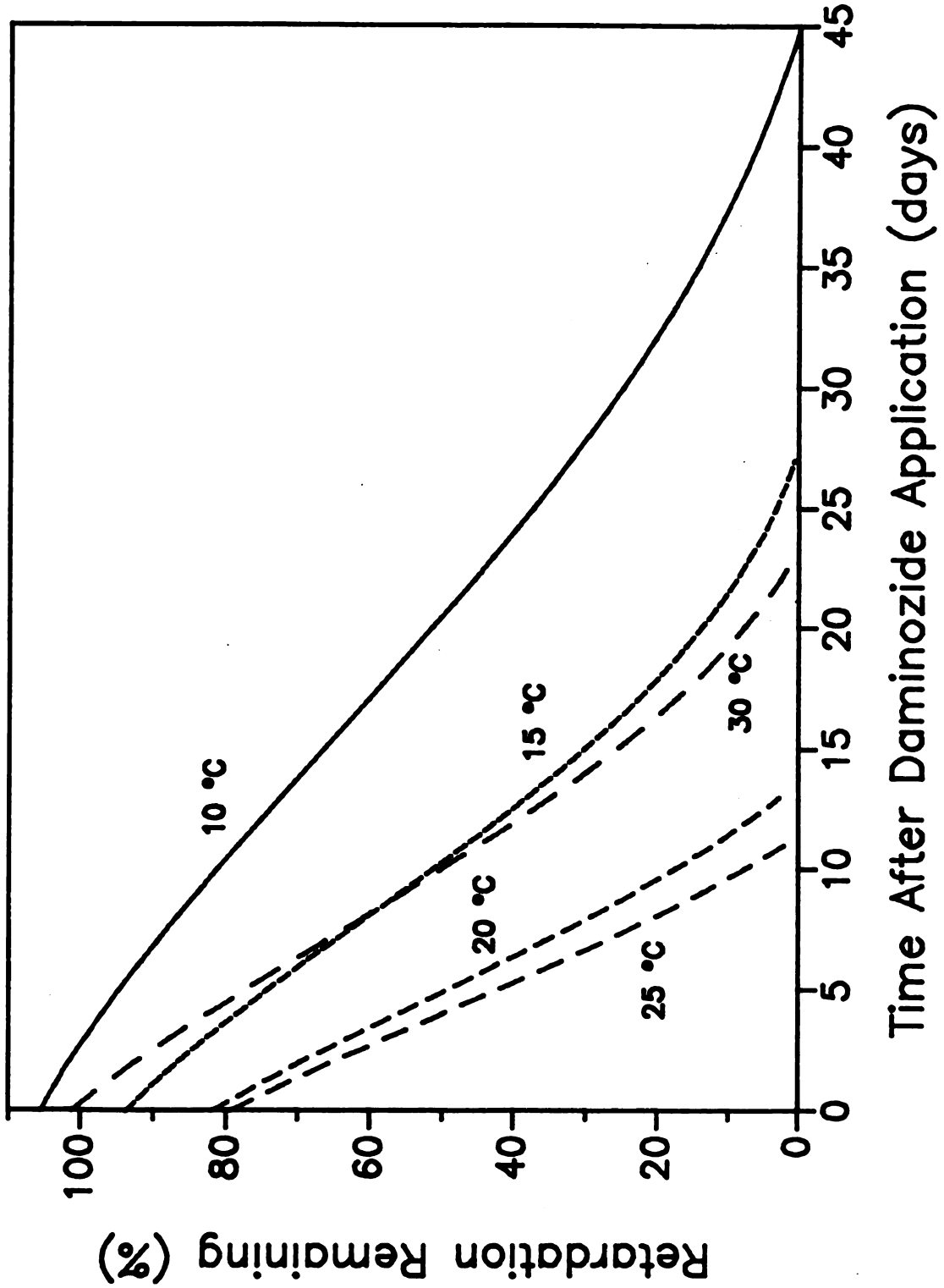
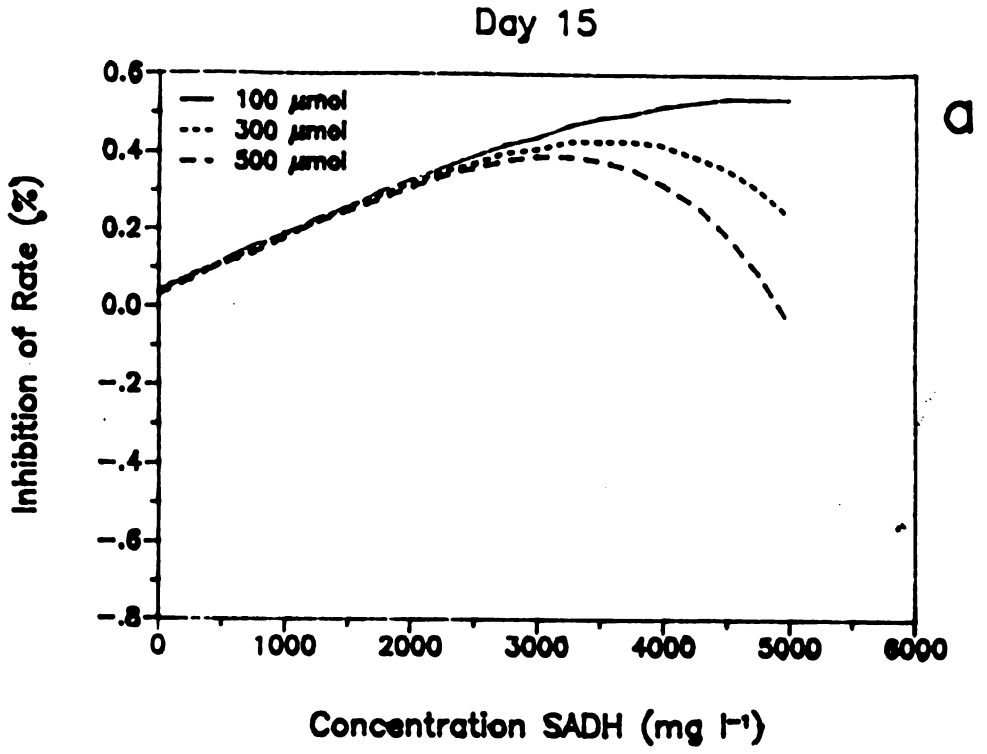

Retardation Remaining (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Time After Daminozide Application (days)
10 °C
15 °C
20 °C
25 °C
30 °C

Figure 14. The influence of photosynthetic photon flux and SADH concentration as calculated from method I on the percent inhibition resulting from and application of 2500 mg l^{-1} daminozide 15 days after the start of short days to Chrysanthemum morifolium cvs. 'Bright Golden Anne'(a) and 'Circus' (b).


Day 15
a
100 µmol
300 µmol
500 µmol
Inhibition of Rate (%)
0.6
0.4
0.2
0.0
-.2
-.4
-.6
-.8
0
1000
2000
3000
4000
5000
6000
Concentration SADH (mg l⁻¹)

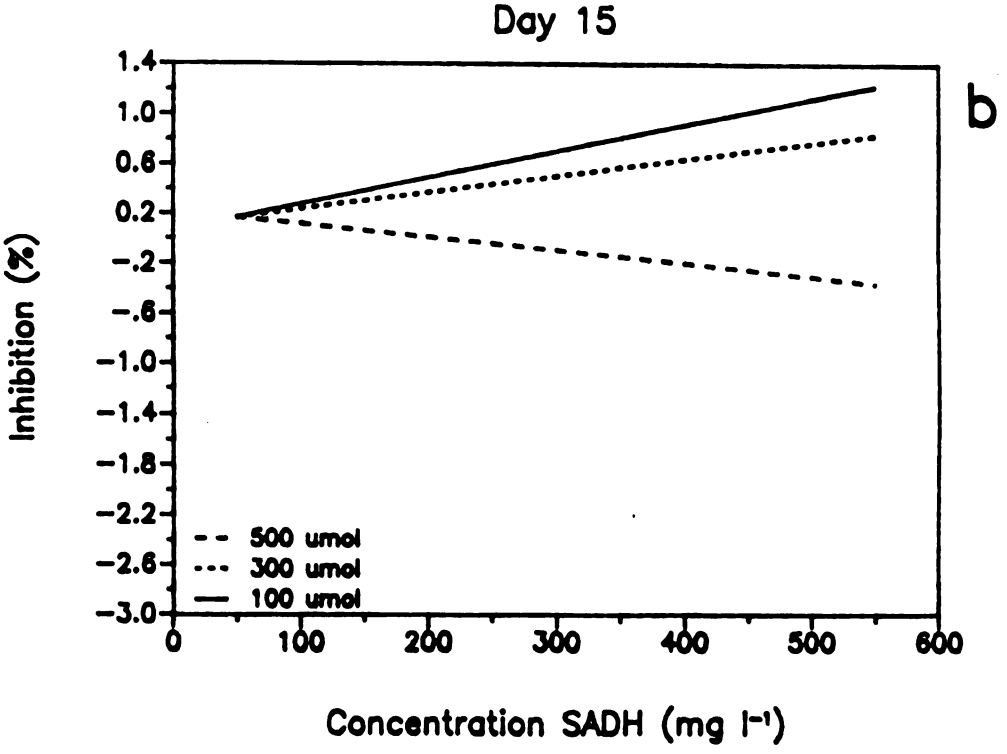
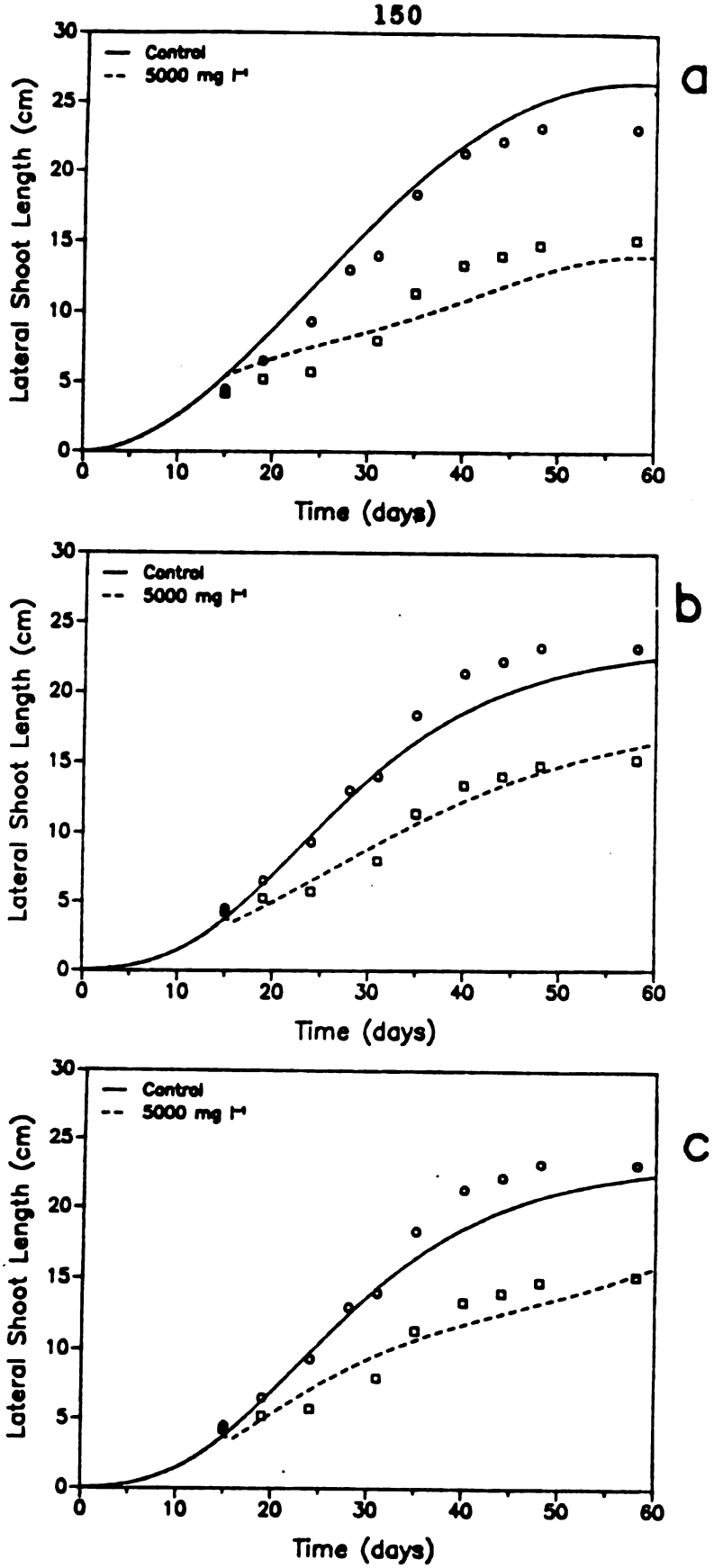

Day 15
b
500 umol
300 umol
100 umol
Inhibition (%)
1.4
1.0
0.6
0.2
-.2
-.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
0
100
200
300
400
500
600
Concentration SADH (mg l⁻¹)

Figure 15. Predicted versus actual response of Chrysanthemum morifolium cv. 'Bright Golden Anne' grown with 18°C day temperatures and an application of 5000 mg l^{-1} daminozide 15 days after the start of short days using Method I (a), Method II (b), and Method III (c).


Control
5000 mg l⁻¹
Lateral Shoot Length (cm)
Time (days)
a
b
c
0
5
10
15
20
25
30
0
10
20
30
40
50
60