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HORMONES, PHOTOPERIOD AND VERNALIZATION IN THE CONTROL OF FLOWERING IN *BRASSICA*

by

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ABSTRACT

Stem elongation and flowering in *Brassica* can be controlled by manipulations of temperature, light (quality and photoperiod) and by application of plant growth regulators. Gibberellins (GAs) are an important part of the signal transduction pathway of stem elongation and floral induction. Application of GAs to *Brassica* spp. showed variable results on flowering and stem elongation depending upon GA structure, the species and cultivar. The biennial, cold-requiring cultivar, cv. WW1033 flowered without vernalization in response to applied GA_5 and 2,2-diMe GA_4, and in response to continuous light. Differences in timing of the appearance of flowers occurs between vernalized (prior to stem elongation) and GA-treated plants (after stem elongation). Thus, GAs may be involved in induction of both flowering and stem bolting but the physiology of the two separate processes in *Brassica* is not yet fully understood. An end of day far-red light (FR) treatment results in increased elongation (hypocotyl and epicotyl) in annual and biennial cultivars of *Brassica*. The FR is also florigenic in the biennial

cultivar under non-inductive long-day, warm conditions. Red light (R) results in a variable promotion of elongation which is cultivar and tissue specific. Stem elongation becomes minimal as the quality of light nears a 1:1 balance of R:FR. The elongation of hypocotyls and epicotyls induced by R and FR could be explained by increases in levels of endogenous GAs (active per se, or precursors to the active GAs). For *B. napus* cv. WW1033, the photoperiod prior to vernalization has no effect on the timing or rate of flowering following vernalization. However, this cultivar is an obligate long-day (LD) plant with regards to photoperiod following vernalization (i.e., short-day (SD) post-vernalization resulted in no internode elongation or flowering). The increases in stem elongation under LD post-vernalization conditions can also be explained by increases in GA levels. Finally, SD-grown, vernalized plants are capable of elongating and flowering when transferred to LD conditions, indicating that the vernalized state continues to be stable under SD, warm conditions. The de-vernalization that occurs with rapid warming under LD allows for slight internode elongation, no flowering, and relatively low GA levels.

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A special thanks to: Stania and Yaro for their very large basement and for turning me onto wine (Chateau Bearspaw), David Pearce for his calm analysis of what I screwed up, Siew Hwee and Tino, for keeping this place anything but dull (and quiet), Cindy for her analysis of the situation (No! its wrong), Ilabanta for his encouragement and Ed for his no-nonsense approach to everything and Dr. Stewart Rood for getting me started on this road .

To Dick, for his exceptional supervisory skills between early May and late December, 1:30 pm to 9:00 pm, except during hunting, fishing, hiking and horse trips or when there is a conference anywhere in the world. Jokes aside, I couldn't have asked for a better supervisor; who else would have let me get away with what I did, but still teach me things. Thanks for putting out all the fires.

To David Reid for putting up with me in RPP's absence (see above), his calming influence and work in high speed sampling in winter alpine tree physiology.

To all my family for the years of having to endure the question:
When is your [son (-in-law), brother (-in-law), uncle] going to be finished school?
Thanks for all the encouragement and help, Mom, Dad, Cam and Shelley.

Finally, to my wife Valerie, she has put up with me through the best (Oz) and worst (candidacy) and still married me, Thanks for all your love and support.

Thank You All,

Dedication

I am not sure that god always knows

who his great men are:

He is so very careless of what happens

to them while they live.

Mary Austin

On behalf of the Botany division of the University of Calgary,

I would like to dedicate this thesis to the memory of

Dr. Chuxing Sheng.

A good friend and scientist.

We will all miss him.

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ABBREVIATIONS

°C = degrees centigrade

ABA = abscisic acid

BA = ^{6}N-benzyladenine

BSTFA = bis-(trimethyl silyl)-trifluoacetamide

CCC=chloroethyltrimethyl-ammonium chloride

CR(P) = cold requiring (plant)

cv(s). = cultivar(s)

DN = day neutral

D.W. = dry weight

EtOAc = ethyl acetate

EtOH = ethanol

h= hour

FR = far-red light

GAs = gibberellins

$GA_{\#}$ = gibberellin #

GC-MS-(SIM)= gas chromatography-mass spectroscopy-(selected ion monitoring)

HPLC = high performance liquid chromatography

HOAc = formic acid (formate)

IAA = indole-3-acetic acid

KRI = Kovats retention indices

LD(P) = long day (plant) (14h or >)

LLD = 24h light

MTBSTFA=N-methyl-N-t-butyldimethyl-silyl trifluoroacetate

Me = Methyl

MeOH = Methanol

PAR = photosynthetic active radiation

PGR(s) = plant growth regulator(s)

PVPP = polyvinylpolypyrrolidone

R = red light

ref = reference

Rt = retention time

SD(P) = short day (plant)(8h)

SNK = Student-Newman-Keul's

spp = species

TBO = toluidine blue-0

TMSC = trimethylchlorosilane

WT = wild type

CHAPTER ONE

To succeed in science, you must avoid dumb people (JD Watson, 1993).

GENERAL INTRODUCTION

Canola or high quality oilseed rape (*Brassica napus* and *B. rapa* (syn. *B. rapa*)) has attracted interest in recent years because the oil contains high levels of mono-unsaturated fatty acids (Taylor *et al.*, 1990). The distinct, yellow-flowered plant has not only become more popular on the Canadian prairies and dinner tables but also in the number of publications in the recent plant science journals. Canola has been used to help unravel the questions of: gibberellin (GA) biosynthetic pathways (Rood *et al.*, 1990b), developmental pathways of flowering (Mandel *et al.*, 1991) and more applied uses such as the production of high quality pharmaceuticals through transgenic technology (van Rooijen and Moloney, 1995 and ref. cited within). The large number of varieties and mutants available (Rood *et al.*, 1989 and 1990a) make canola an ideal research tool.

BOTANY OF CANOLA

The name 'canola' is designated for varieties of oilseed rape which have less than 2% erucic acid in the oil and less than 30 µmole of glucosinolate per

gram in the meal (Kneen, 1992). In just 24 years, the production of canola has increased 700-fold to where it now ranks second in Canadian production only to wheat (More, 1992). At present, *B. napus* and *B. rapa* are grown in equal amounts in Canada (Bunting, 1986), but cultivars used in both species are almost exclusively spring-sown varieties (seeded in late spring and harvested in autumn of the same year), termed summer annuals. Winter, cold-requiring (CR) or biennial varieties of *Brassica* are normally seeded in late autumn, over-winter as a tight rosette and are harvested by early summer. Winter varieties are more common in Europe and the northwestern United States where winter conditions are more temperate and allow better survival of the seedling over the winter months. The variable conditions (cold followed by warm chinook winds) of Western Canadian winters sometimes cause the axis of the rosette to bolt slightly which makes it susceptible to a later winter kill (Mandel, personal observations). Nonetheless, interest is maintained in winter canola as it consistently out-yields spring varieties by 20% or more (Bunting, 1984). Finally, the two species of canola quality *Brassica* used in Canada are *B. napus* (Argentine origin) and *B. rapa* (Polish origin). *Brassica napus* is the result of a cross between *B. rapa* and *B. oleracea* (Bunting, 1986) and species have annual and biennial varieties.

One of the more successful "accidents" with winter canola occurred on the farm of Don Opp, South East of Claresholm, Alberta. Working with Keith Topinka (Farming For the Future (FFF)) on a project to determine if planting density could affect over-winter survival, an accident occurred. One of the test fields was seeded with a winter canola to summer canola seed ratio of 10:1. With a long fall, the winter canola germinated and remained as a rosette while the annual canola

bolted and flowered in early October. Although questioned by more conservative farm neighbours, the "accident" continued. During the winter, snow accumulated and was trapped in the summer canola, resulting in a good winter cover. In spring the winter canola recovered, bolted and flowered, resulting in an acceptable yield, while winter kill and patchy survival of the other test fields (lacking the accidentally-seeded summer canola to create and maintain a good winter snow cover) resulted in poor yields. Although promise was shown in winter canola, funding for the project was reduced and these results were not followed up (Mandel, R., 1989 contract work with FFF). This "accident", along with my agricultural interests has kept me working with canola and has developed into my PhD project.

GIBBERELLIN IN CANOLA

Since the late 1980s, a great deal of research has gone into canola, and much has revolved around flowering and bolting which are linked to GAs. Early work by Rood *et al.*, (1987) looked at the levels of GA_1 during bolting in *Brassica* and showed that GA_1 was the effector of elongation in *Brassica*. Mandel *et al.*, (1991) showed a relationship between applied GAs and flowering in annual and biennial *Brassica* spp. grown under non-inductive conditions (short-day (SD) or non-vernalized). Zanewich and Rood (1995) showed that the levels of GAs increased following vernalization and speculate that GAs serve as likely intermediaries between cold treatment and subsequent growth and development. The involvement of GAs in growth and development of *Brassica* led to my study of its relationship to floral induction, photoperiod perception and vernalization.

GIBBERELLIN BIOSYNTHESIS

The early steps leading to GA_{12}-aldehyde (the first compound possessing the *ent*-Gibbane structure) are shown in Figure 1.1. Acetyl units from acetyl CoA form mevalonic acid which combine and cyclize to rings to eventually form kaurene. Kaurene undergoes sequential oxidation of carbon-19 followed by contraction of the B-ring to form GA_{12}-aldehyde. From GA_{12}-aldehyde there are a number of parallel pathways, differing mainly in the point of initial hydroxylation and oxidation steps. It seems likely that the early-13-hydroxylation and early-non-hydroxylation pathways of GA biosynthesis (Figures 1.2, 1.3) are operative in summer annual canola cultivars (Zanewich and Rood, 1993, Rood *et al.*, 1987, Hedden *et al.*, 1989). Therefore, I propose to examine changes in endogenous levels of those GAs within these pathways that show significant modification with vernalization and other flower promoting treatments. Additionally, certain environmental treatments, such as light quality, which modifies early vegetative shoot growth will be assessed for their effect on endogenous GA levels. Analysis of the levels of GAs and their precursors in the above pathways will be accomplished by GC-MS-SIM using [$^2H_2$] labelled internal standards which have been synthesised by Prof. L.N. Mander (Research School of Chemistry, Australian National University).

VERNALIZATION

Vernalization is a term given to the onset of floral induction and development in plants that results from a low temperature treatment (the optimum temperatures for vernalization vary between species but range between -3°C and +5°C for

Brassica)(Lang, 1965). Low temperature in this range can also trigger dormancy break in certain seeds and in buds. A more specific definition of vernalization is:

> *The perception and transduction of the inductive effect of low temperature that by itself (generally) does not evoke flowering, but which allows the effect (floral evocation) to be expressed following vernalization*
> (paraphrased from Metzger, 1988a)

Although this definition may be interpreted as circular in its logic, it is a simple definition for a complex process. Coining of the term vernalization has been attributed to a number of different individuals. It had been know for centuries that cold temperatures were required by some plants to promote flowering but Gassner, in 1918, definitively established this by experimentation (Bernier *et al.*, 1981). In a review by Evans (1969), he notes that the word "vernalization" is an English translation of "yarovisation" (in some references the y is substituted for j), meaning "springization". The search for the elusive "vernalin", like "florigen", to date has been unsuccessful. The early work with vernalization was extensively reviewed by Lang (1965). A small number of species, including *B. napus* and *B. oleracea* (cited in Lang, 1965), showed that the vernalization response was graft transmissible, that is, a cold-requiring (CR) plant (recipient) could be induced to flower if a previously vernalized part (donor) was grafted to it. Although it was assumed that if the vernalized state was graft transmissible, it would be extractable from vernalized tissue. Yet the causal agent for flowering that is produced after the required cold period still remains unidentified.

However, there is the work by the Metzger group (Hazebroek *et al.*, 1993, Hazebroek and Metzger, 1990a) on *Thlaspi*, implicating early steps in the

biosynthesis of precursors of GAs (i.e., metabolism and turnover of *ent*-kaurenoic acid) as being responsible for blocking GA biosynthesis in non-vernalized rosette plants. This also implies that the florigenic signal following vernalization is a GA, a concept that is consistent with the fact that applied GAs can cause flowering in some unvernalized plants, though not all CR species. Based on this, a major thrust of my research has been to examine several varieties of cold-requiring plants (CRP) of canola which differ in their response to applied GAs and to length of cold. This may determine which steps (if any) in GA biosynthesis may be blocked in non-vernalized, non-bolting and/or non-flowering plants. Metzger (1988b) also showed that the shoot tip, not the leaves, is the site of cold perception, thus confirming the extensive older literature (Lang, 1965; Vince-Prue, 1975). Therefore, the shoot tip (apical meristem and subtending tissue (sub-apical)) will be analysed for changes in endogenous GA and their kaurenoid precursors.

STEM ELONGATION AND FLOWERING

It is difficult to separate the developmental processes of stem elongation (bolting) and flowering. In *Brassica spp.*, like most rosette species such as *Thlaspi* (Metzger 1990b) and *Spinacia* (Zeevaart, 1971), elongation always follows floral development except when PGRs are exogenously applied.

The involvement of GAs in stem elongation has been proposed for many years (Phinney, 1985), but it has only been recently, through the use of dwarf mutants, that GAs role in stem elongation was definitively shown (Phinney, 1985). The role of GAs in floral initiation has yet to be fully established; however, it has been postulated that they have a central role (Pharis and King, 1985).

Internode elongation is minimal for all CRP rosette species until after they are vernalized. Following the end of the thermo-inductive cold treatment, internode elongation is observable within 7 to 10 days in *B. napus* and 7 to 8 days in *Thlaspi arvense*. Elongation occurs in internodes between intermediate-aged leaves. Since bolting in stems of rosette species is caused by increased GA levels and GA biosynthesis (Metzger, 1990b, and ref. cited therein), such a localization of response indicates that an ability to respond to a GA (e.g., "sensitivity") may be dependent upon the developmental stage of cells in the internode regions (Metzger and Dusbabek, 1991). In *T. arvense*, bolting can be used as a visual marker for thermo-induction (Metzger, 1990a) and floral development coincidental with bolting in *Thlaspi*, whether due to thermo-induction or in response to GAs applied to non-induced plants (Metzger and Dusbabek, 1991). Interestingly, many CR *Brassica spp.*, when maintained in warm LD, may bolt in response to a variety of exogenously applied GAs. However, only a selected few varieties will flower, and these only in response to application of very specific GAs or GA derivatives. The lack of an easily identifiable marker for flowering (such as bolting *per se* in *T. arvense*) in *Brassica spp.* makes identification of floral-induced plants more difficult in CR *Brassica; h*owever, the bolting response may still be a useful tool for determining and separating putative role(s) of GAs in the very different processes of induction of flowering and bolting.

The requirement for vernalization prior to floral evocation is species specific, and can even be accomplished with imbibed seeds for some species (*B. rapa* and *B. juncea* (cited in Vince-Prue, 1975)) although in other species, including *B. napus*, only seedlings can be vernalized (cited in Vince-Prue, 1975). Also, the

minimum required age of the seedling at the time of vernalization is variety specific (cited in Vince-Prue, 1975).

The use of annual and biennial *Brassica spp.* for the study of floral induction is a logical choice in that it provides an ability to separate flowering from bolting (in many other rosette species this is not possible). The separation of these two responses may indicate that different hormones (possibly different GAs) may be required for each response. Results to date indicate that bolting of CR canola can be obtained in the absence of flowering, but (usually) not the reverse. A possible explanation is that while a specific GA may be highly florigenic, it may also subsequently be metabolized to a more bolting-promotive form (i.e., GA_5, GA_5-GA_3). Exogenous applications of hormones to elicit a response is thus not in itself sufficient to answer the important questions of floral induction since "naturally induced" plants (induced by vernalization for biennials, or by LD for annuals) usually show floral bud development prior to bolting. Yet, in experiments where exogenous GA applications result in flowering of CR canola, the development of floral buds generally follows bolting (Mandel *et al.*, unpublished). Determinations of changes in endogenous hormone levels during various stages of floral induction may therefore be a means of determining which endogenous hormone(s) is (are) required to mimic the natural inductive system more closely.

The elongating stem, pod and seed tissues of *Brassica* spp. contain high levels of endogenous GAs which can originate from at least two biosynthetic pathways, the early C13-hydroxylation pathway (Figure 1.2)(Rood *et al.*, 1987) and early-non-hydroxylation pathway (Figure 1.3)(Hedden *et al.*, 1989). Steady-state levels of certain of these GAs are known to increase during flowering under

inductive LD (Rood *et al.*, 1987, 1989, Suge and Takahashi, 1982) or after vernalization (Metzger, 1985, Suge, 1970, Zanewich, 1993). Pearce *et al.*, (unpublished results) also showed that apex tissue [cv. Westar] not only contained increased levels of abscisic acid (ABA) and GA_4, GA_{19} and GA_{20} and decreased levels of indole-3-acetic acid (IAA), but also a putative oligosaccharin which is 10-fold higher in LD (e.g. 28h continuous light) than in 8h short-day (SD).

Exogenous application of various GAs will promote stem elongation (Mandel *et al.*, 1991, Rood *et al.*, 1989, Talon and Zeevaart, 1990) and flowering in many rosette CRP or LDP held under non-inductive conditions (reviewed in Lang, 1965 and Zeevaart, 1971 and 1983) and also in numerous other LDP maintained under non-inductive SD (Lang, 1965, Zeevaart, 1971). Many annual *Brassica* spp. respond very well to exogenous applications of GAs under non-inductive photoperiods by bolting and induction of flowering (Lang, 1965). Some species of CR (biennial) *B. napus* have been induced to flower with exogenous GA_3 under warm, LD conditions (Chailakhyan, 1957; Lang, 1957 and reviewed by Zeevaart, 1983), but CR canola varieties (*B. napus* and *B. rapa*) have to date been unresponsive in flowering in response to GA_1, GA_3, GA_4, GA_7, GA_9 and GA_{20} under LD, warm conditions (Mandel *et al.*, 1991). The combined use of annual and biennial *Brassica* species may therefore be a useful tool for better understanding the putative role of GAs in vernalization and photoperiodic induction of both bolting and flowering.

LIGHT: DURATION AND COMPOSITION

Reid *et al.*, (1991) reviewed environmental factors affecting plant growth and development with particular attention being paid to light and its relationship to plant hormones. Suge and Takahashi (1982) showed the importance of photoperiod in the induction of stem elongation and flowering in Chinese cabbage (*B. rapa* var. pekinensis). As was shown 13 years earlier for *A. thaliana* (Napp-Zinn, 1969), flowering in Chinese cabbage could be promoted by continuous light (LLD), even in the absence of vernalization. However, doses of GA_3 as high as 2.2 mg per plant were unable to promote flowering in short day (SD), non-vernalized plants (Suge and Takahashi, 1982). Lang (1965) and Zeevaart (1983) have also reviewed a number of reports in which exogenous GAs (usually GA_3) could induce flowering in many annual LD rosette plants maintained under SD, and also in some non-vernalized, cold-requiring plants.

Not only can the duration of light be a trigger for flowering, the composition of light appears to be an important control of floral induction/evocation. Thus, the ratio of red light (R)(655 to 675 nm) to far-red light (FR)(725 to 735 nm) is a very significant factor in controlling flowering of both LDP and SDP (Evans *et al.*, 1993a, Martinez-Zapater and Somerville, 1990, Vince-Prue, 1975 and ref. cited therein). In LDP, a high R:FR ratio has little or no effect, or may even delay flowering (cited in Vince-Prue, 1975). In contrast, a low R:FR ratio accelerates flowering in many LD species (e.g., sugar beet and *Lolium*, (Lane *et al.*, 1965) wheat (Friend *et al.*, 1963)). Lockhart (1964) proposed that FR light stimulated the (phytochrome-mediated) conversion of an inactive GA to an active form in the internode, resulting in elongation. Beall (1985) showed that *Phaseolus* plants grown in normal PAR

supplemented with FR increased GA metabolism and levels of endogenous GA_1. In a follow-up study, Beall *et al.*, (1996) showed that white light supplemented with FR resulted in internode elongation. Upon microscopic observations, it was found that the cell length component had a lesser effect on the elongation than did cell division. As well, an increase in [^{3}H] thymidine labelled nuclei was observed in FR-supplemented plants. Beall *et al.*, (1996) showed GA_1 and GA_{20} levels had increased nearly two-fold in far-red supplemented plants. Investigations into whether floral promotion induced by changes in the composition of light (R:FR) is hormone (GA)-mediated should further advance an understanding of the physiology of flowering. Thus, I have used FR light as a tool in my research on both summer annual and CR cultivars of canola.

According to Napp-Zinn (1985) photoperiod/light composition and vernalization are interrelated processes. In CR strains of *Arabidopsis,* the LD requirement (which is phytochrome-mediated) can be substituted by vernalization or FR light under SD warm conditions (Bagnall, 1993). The use of such strains and mutants to determine if changes in hormone levels (GAs) are consistent between vernalization as a flowering inductive treatment and FR-induced floral promotion, should further our understanding of the events leading up to floral evocation.

There are also "tools" other than photoperiod, temperature and light quality. Thus, the use of GA biosynthesis inhibitors (such as the acylcyclohexanediones and CCC (Figure. 1.5)), which have specific sites of action, may aid in the understanding the roles of GAs in flowering induction and flower development. Evans *et al.*, (1993b) have show that an acylcyclohexanedione application to plants given one partially inductive LD causes significantly enhanced flowering in *Lolium*,

as does FR-enrichment. Speculatively, the acylcyclohexanedione may block 3β-hydroxylation of GA_{20} (Figure 1.2) and the conversion of GA_5 to GA_3 (see Figure 1.3), thereby increasing GA_5 levels and synergistically enhancing the partial induction of the one LD. It is relevant here that GA_5 is highly florigenic not only for *Lolium*, but also when applied exogenously to warm LD-grown *B. napus* cv. WW1033.

RATIONALE OF RESEARCH

With additional knowledge on floral induction of annual varieties and vernalization (low temperature leading to flowering) as it relates to bolting and floral induction in biennial varieties, it may be possible to develop planting schedules or techniques (e.g., plant hormone treatments) that will make biennial canola a viable crop in Canada.

Finally, there is also a practical rationale for this type of research, namely that the greater our understanding of the physiology of flowering and how the environmental triggers act in floral induction, the more likely it is that we will be able to control flowering for practical purposes. In agriculture, the goal is to increase crop yield and with a better understanding of the physiological requirements for flowering we may indeed be able to develop varieties (and/or treatments to existing varieties) which will maximize return to the farmer.

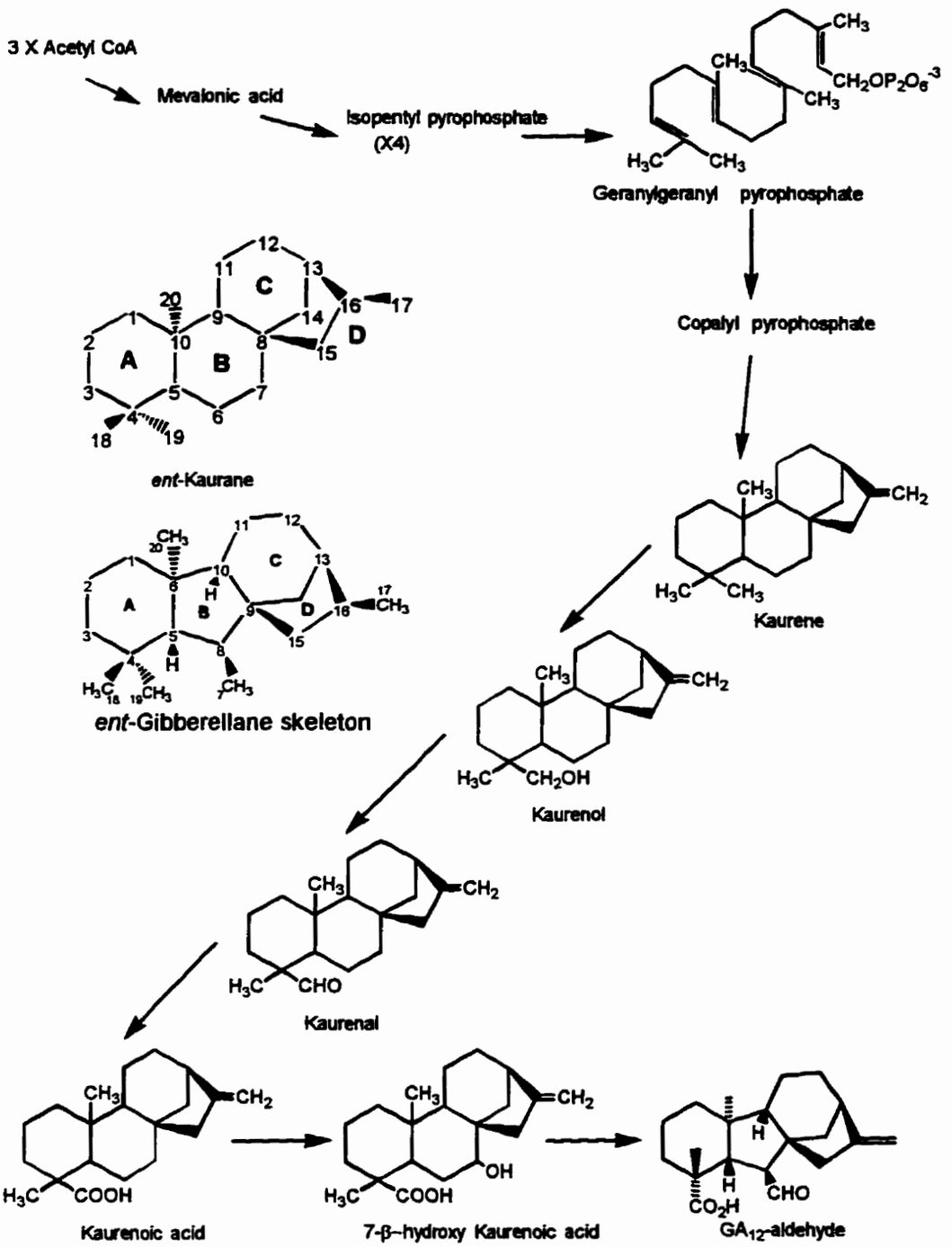


Figure 1.1. The *ent*-gibbane and *ent*-kaurane structures and the proposed biosynthetic pathway from acetyl-CoA to GA_{12}-aldehyde.

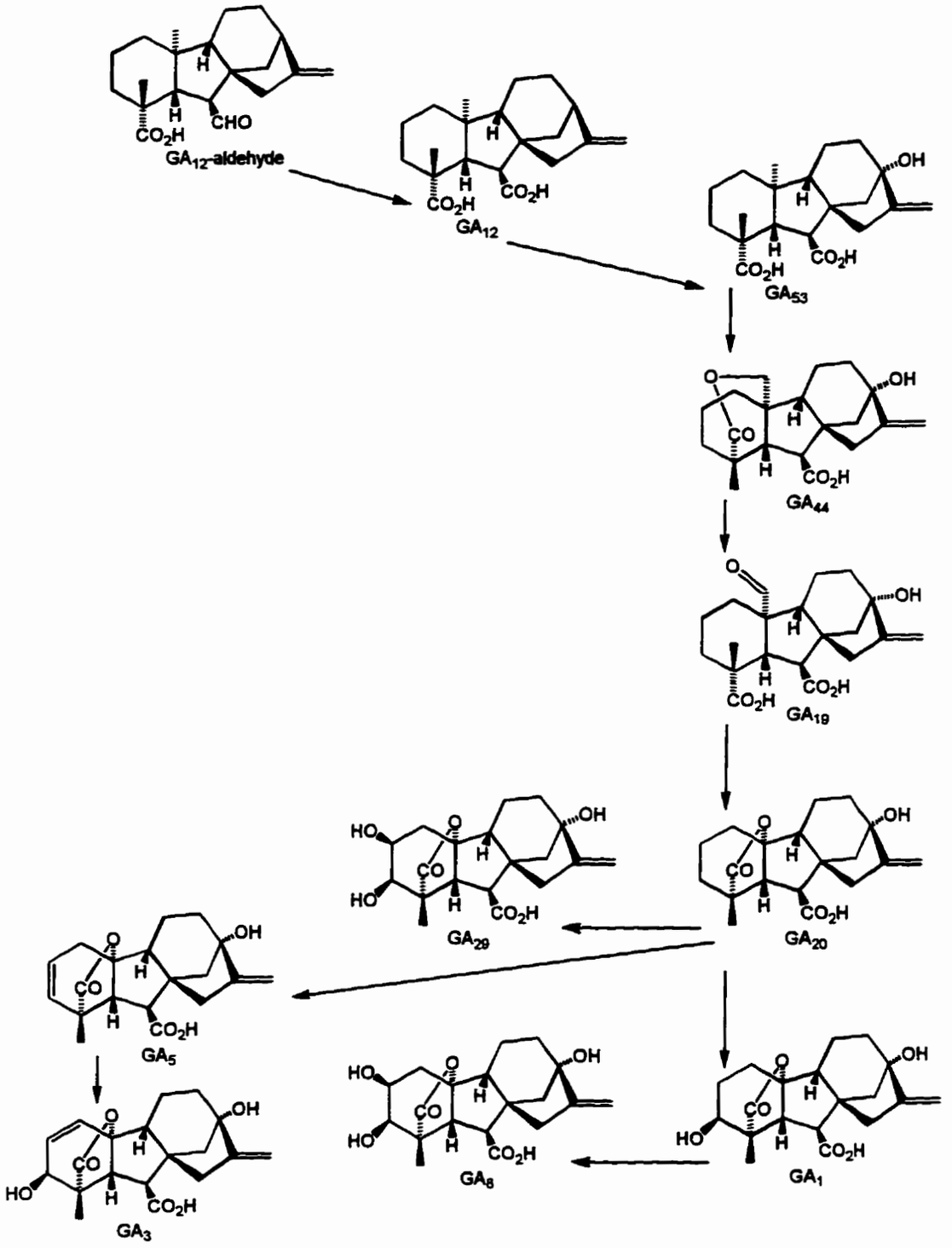


Figure 1.2. The proposed C13-hydroxylation GA biosynthetic pathway.

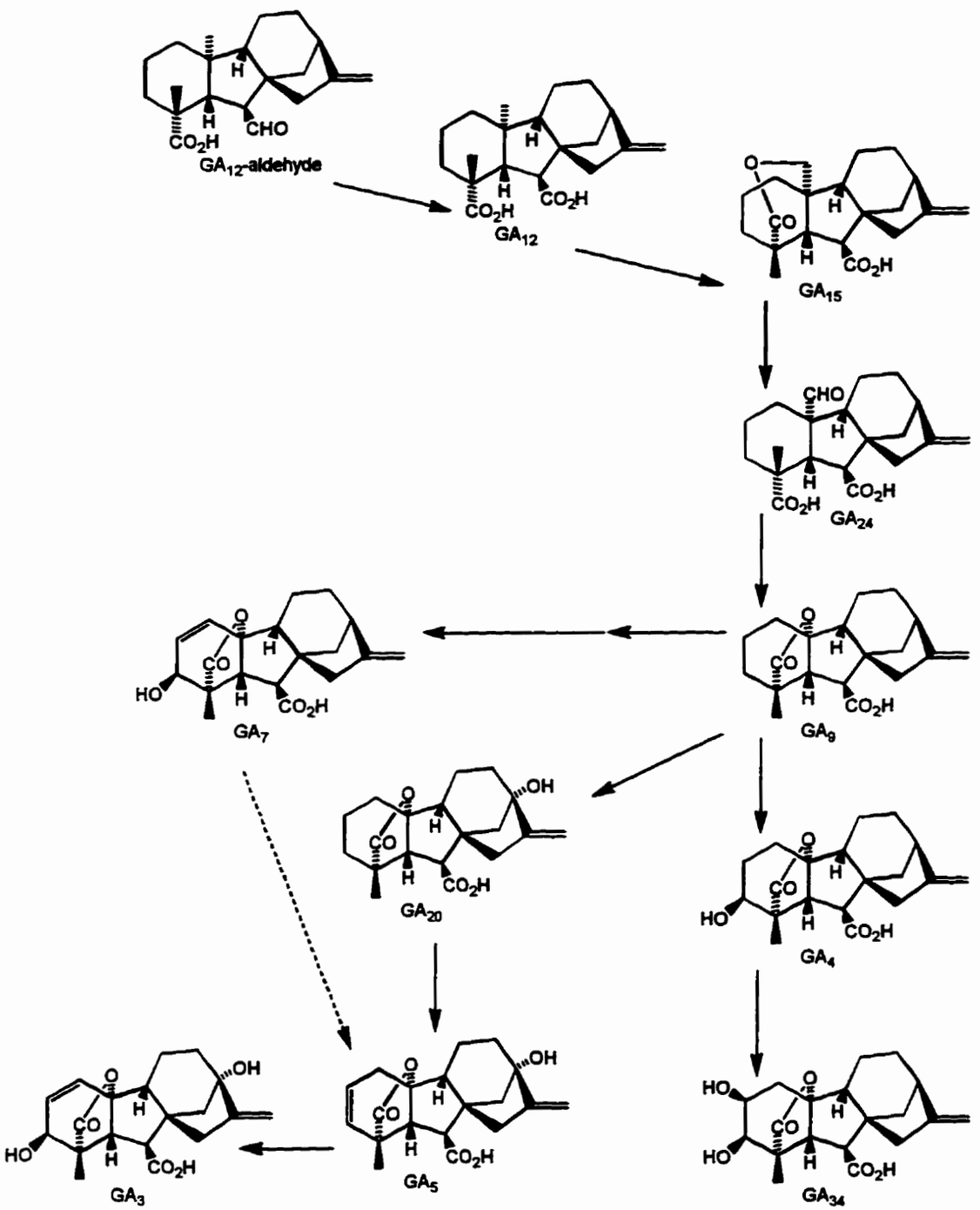
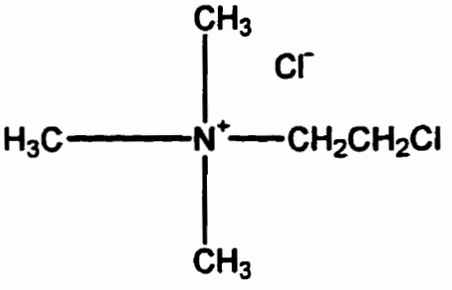
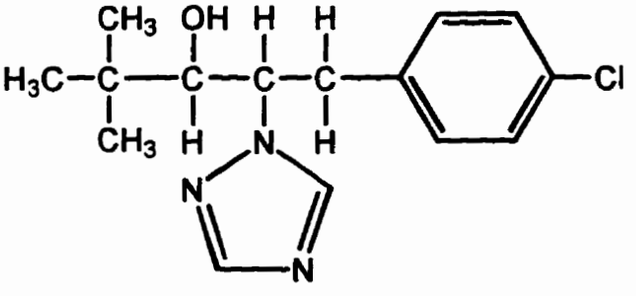


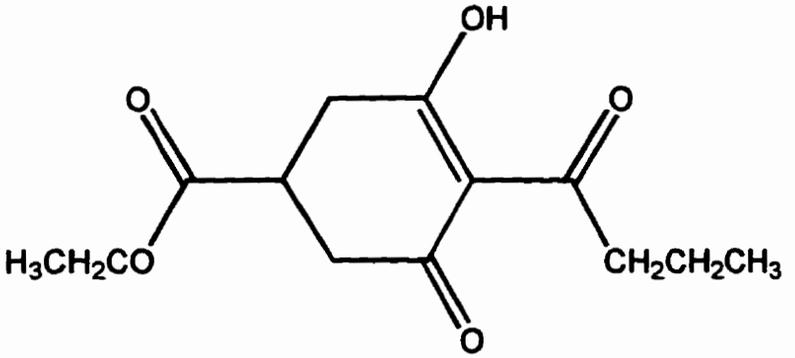
Figure 1.3. The proposed non-hydroxylation GA biosynthetic pathway.



Chlormequat chloride (CCC)



Paclobutrazol (PP333)



An Acylcyclohexanedione (Lab 198 999)

Figure 1.4. Structures of plant growth regulators applied to *Brassica* spp.

CHAPTER TWO

To make a huge success,

a scientist has to be prepared to get into deep trouble (JD Watson, 1993).

GENERAL MATERIAL AND METHODS

Plant material for this project was grown in four different locations over a six year period. Initial experiments were carried out at the University of Lethbridge greenhouse and later moved to the University of Calgary greenhouses and growth chambers. The Agriculture Canada Lethbridge Research Station was also utilized for both greenhouse and growth chamber experiments. Finally the CSIRO Phytotron in Canberra, Australia was used for experiments involving rigorous control of temperature and light condition.

GROWTH CONDITIONS.

A. Greenhouse

The University of Calgary greenhouses (lat. 51°05' N) was used to grow plant tissue, relying upon natural daylight, supplemented with metal-halide lamps (both to increase the PAR and to extend the photoperiod during periods of shortened day length). Canola seeds (the cultivar used for each experiment will

be stated in the material and methods section of each subsequent chapter) were planted four per pot and allowed to germinate and grow for seven days in plastic pots (25 cm dia.) in a peat moss : vermiculite : perlite medium (2:1:1). Plants were thinned to one seedling per pot on day 7 to 10 post emergence to ensure uniformity and offset germination rate variation. Greenhouse temperatures and humidity fluctuated with external conditions and for that reason no plants were grown during the extreme heat of the summer (late June to mid August). During cooler seasons the greenhouse was maintained at a minimum of 18°C. Pots were watered daily to near field capacity; and 0.3 g of 28-14-14 (N-P-K) fertilizer, plus micronutrients were added weekly. Control of insects and mites was accomplished as required using a range of commercial chemicals (Pentac^{RT}, Safers Insecticidal soap^{RT}, Pirimor^{RT}).

Conditions at the Lethbridge greenhouses (University and Agriculture Canada (latitude 49°65' N)) were similar except the Lethbridge greenhouses used high pressure sodium vapour lamps to supplement and extend the photoperiod. Temperature control was more uniform at the Agriculture Canada greenhouses, therefore, experiments could be conducted during the summer months.

B. Growth Chambers

University of Calgary and Agriculture Canada (Lethbridge) growth chambers were equipped with combinations of GroLux™ (Sylvania™) fluorescent or cool white fluorescent tubes, with and without supplement of incandescent bulbs and/or quartz halogen bulbs. Light intensity and composition were monitored using a LiCor™ model 1700 scanning spectroradiometer or equivalent. The ratio of R:FR

light levels was modified by adding either 100W incandescent bulbs or 500W quartz halogen floodlights fitted with FR (750 nm) filters (Carolina Biological Supply Company, 68-6810). A continuous flow water chamber (3 cm deep plastic chamber) was used for heat control. Photoperiod, light intensity and temperature were varied depending upon experimental design and plant variety and will be detailed in the material and methods section of each subsequent chapter.

C. Canberra, A.C.T., Australia

The Black Mountain Commonwealth Science and Industry Research Organization (CSIRO) "Ceres" phytotron in Canberra (Lat. 35°19' S) was used between Nov. 1, 1993 and April 30, 1994. Seeds were sown in 10 cm pots in a 1:1 mixture of vermiculite:perlite. Pots were watered twice daily, with Hoaglands (#2) nutrient solution in the a.m. and demineralized water in the p.m. Light intensity and composition was measured using a Macom SR3000 spectroradiometer.

i. Natural light conditions

Greenhouse conditions used natural daylight, supplemented from 4:00 a.m. to 8:00 p.m. with 150W 'Computalux' reflector light, giving 60µmole m^{-2} sec^{-1} at plant level. The minimum relative humidity was 40% and wind speed of 0.6 m sec^{-1} and CO_2 exchange rate of 0.24 m^3 sec^{-1} during the day and 0.024 m^3 sec^{-1} at night.

ii. B and C unit conditions of the phytotron

Any changes in photoperiod from the above were accomplished using naturally lit B (large) and C (small) units (designed and built by CSIRO)(Figure 2-1a

and b). The phytotron B and C units are computer-controlled growth chambers which not only have the benefit of using natural light, but also the ability to control photoperiod by computer-controlled shutter mechanisms. Extension and/or manipulation of photoperiods was accomplished using these computer-controlled incandescent or high BCJ (Far-red) lights. This allowed for experiments to be designed with differing photoperiods but with only slight differences in PAR.

Temperature and humidity were maintained as in the greenhouses, wind speed was approx. 0.6 m sec^{-1} downward through the canopy and CO_2 exchange rates were 0.03 m^3 sec^{-1} in B units and 0.01 m^3 sec^{-1} in C units.

iii. Artificial light conditions.

Artificially lit chambers were maintained at a day temperature of 21°C and a night temperature of 16°C. A range of light sources were used including:

- 1) Philips™ TLMF 140/33 RS fluorescent tubes, with or without four 150W incandescent lights (to extend photoperiod or manipulate R:FR ratio).
- 2) Combinations of metal arc lamps (Siemans Power Star™ HQ1T WD 400W), 150W incandescent, 400W Quartz halogen lamps and BCJ Far-red enriched 150W bulbs.

The use of these light combinations were varied depending upon the desired spectral composition. Wind speed was approx. 0.6 m sec^{-1} downward through the canopy and a CO_2 exchange rate of 0.03 m^3 sec^{-1}. Plants were adjusted so that they received 200 µmole m^{-2} sec^{-1} of light at plant level.

iv. Vernalization Conditions

Plants were grown within B units in the greenhouse with a 16h photoperiod (LD (8h natural light supplemented with 8h low intensity incandescent light)) or 8h photoperiod (SD). At 4 weeks post-emergence, plants were transferred to the cold chamber (4°C day and 3°C night temperature, with 50µmole m^{-2} sec^{-1} of light at plant level). Plants were maintained under these conditions for 8 weeks with tissue for hormone analysis being harvested at intervals during and after the vernalization period. Following vernalization, plants were returned to B units through a stepwise warming (5°C every 3 days), or returned to warm LD conditions immediately (to look at the effects of de-vernalization)). Photoperiod treatments were as follows:

Pre-vernalization	Vernalization	Post-vernalization
LD	------>SD	---------->SD
		---------->LD
SD	------> SD	---------->SD
		---------->LD

MEASUREMENTS AND OBSERVATIONS

Measurements of stem height to the apex, and plant growth stage (Harper and Berkenkamp, 1975) were recorded on a weekly basis, or more often during periods of rapid growth. Other measurements and observations, such as hypocotyl and internode lengths, leaf area and shoot dry weight were also carried out in specific experiments. Flowering was assessed both visually and by dissection.

PLANT GROWTH REGULATOR (PGR) APPLICATIONS

Hormone solutions were usually applied exogenously to the apex in 10 µl of 70% ethanol. The concentrations and types of applied PGRs varied between experiments, depending upon pretreatment (i.e. acylcyclohexanediones or CCC), photoperiod, species and cultivar. The CRP canola treated with Cycocel Extra (CCC) received 1 ml per plant of 1% active ingredient solution diluted to 10 ml and applied as a foliar spray. Other PGRs (such as acylcyclohexanediones) were applied by spray in accordance with the manufacturer's recommended rates.

HORMONE EXTRACTION AND PURIFICATION

Tissue for hormone analysis was frozen in liquid N_2 followed by lyophilization. It was then extracted utilizing the methods of Koshioka *et al.*, (1983) with some modification. Lyophilized tissue which had been stored at -70°C was ground with $N_{2(liq)}$ in a mortar with a pestle and extracted 4 times in 80% aqueous MeOH. Alternatively, it was ground in 80% aqueous MeOH at ca. 3° to 7°C using a Brinkmann polytron™. Prior to filtration of the ground tissue, stable isotope [$^2H_2$]-labelled internal standards, including some or all of the following were added: GA precursors (*ent*-kaurene, *ent*-kaurenol, *ent*-kaurenal, *ent*-kaurenoic acid, 7ß-hydroxy-*ent*-kaurenoic acid), GA_{12} aldehyde and GA_{12}; and GAs from the early non-hydroxylation biosynthesis pathway (GA_{15}, GA_{24}, GA_9, GA_4 and GA_7); GAs from the early C13-hydroxylation pathway (GA_{44}, GA_{53} and GA_{19}); GAs which could originate from either pathway (GA_{20}, GA_3, GA_1, GA_8 and GA_{29}). As well, [$^2H_6$] ABA and [$^{13}C_6$] IAA and 8000 Bq each of [^{3}H] GA_1, GA_4 and GA_9 were also added.

Deuterated and tritiated standards used in this study were obtained from the

following source: [1,2(n)-^{3}H]GA_1, Amersham, 37.7 Ci/mmole;

[1,2(n)-^{3}H]GA_4, Amersham, 32.2 Ci/mmole;

[1,2(n)-^{3}H]GA_9, Yokota *et al.*, (1976), 37.7 Ci/mmole)

all [$^2H_2$] GA standards, Mander (ANU)

	% purity
GA_1	98.21
GA_3	98.01
GA_4	97.7
GA_7	assumed 100%
GA_8	97.5
GA_9	assumed 100%
GA_{19}	91.9
GA_{20}	98.3
GA_{44}	assumed 100%
GA_{53}	assumed 100%
Kaurenoids	assumed 100%
ABA	83.7
IAA	90.6

The MeOH extract was filtered under vacuum through Whatman #1 paper and then subjected to one or more of the following purification procedures:

- (1) reduced to the aqueous phase under vacuum, pH adjusted to 3.0 and partitioned against 3 volumes of EtOAc (saturated with 1% formate)
- (2) dried to minimal volumes under vacuum (aqueous), applied to celite powder and dried under a stream of air, then loaded onto a short silica gel partition column (5g silica in a 12mm diameter column) and eluted stepwise with a mixture of EtOAc, *n*-hexane (5:95, 25:75, 50:50, 75:25, 95:5) and finally with MeOH (Koshioka *et al.*, 1983)
- (3) applied directly to a preconditioned (rinsed with 100% MeOH then

80%MeOH) C_{18} preparatory column (Koshioka *et al.*, 1983) and eluted with a range of MeOH plus water solutions

- (4) reduced to the aqueous phase under vacuum, pH adjusted to 8.0, partitioned against 3 volumes of diethyl ether (water saturated)(hormones remain in aqueous phase), then slurried with PVPP and filtered

Which method of initial purification I used depended upon the amount of tissue extracted and the relative "dirtiness" of the initial extract. Aliquots were taken for liquid scintillation spectrometry to determine the recovery of the radioactive internal standard prior to taking the samples to dryness.

Various fractions from (1) to (4) above, which contained the compounds of interest, were chromatographed on a 10-73% MeOH gradient-eluted reversed-phase μ-Bondpack C_{18} HPLC column (Waters Assoc.)(Koshioka *et al.*, 1983), at a flow rate of 2 ml per min. The elution pattern of GAs using this column is generally on the basis of polarity (degree of hydroxylation and hydroxyl location). Figure 2.2 shows the Rt of some of the key GAs and other hormones using the 10 to 73% MeOH gradient. Using a combination of high specific activity [^{3}H] GA_1, GA_4, GA_9, IAA and ABA as internal standards (added at the time of initial extraction) and a range of [^{3}H] external standards, regions of the HPLC eluate were prepared directly for GC-MS, or taken to bioassay (see below) or bulked for further Nucleosil [$N(CH_3)_2$] HPLC purification. Additional amounts of high specific activity [^{3}H] GA_1, GA_4 and GA_9 were added to the samples prior to any subsequent Nucleosil HPLC(see below).

Bulked fractions from C_{18} HPLC were chromatographed on the Nucleosil

HPLC using an isocratic gradient of 99.9% MeOH and 0.1% acetic acid at a flow rate of 1 ml per min (Takahashi *et al.*, 1986). Two minute fractions were collected and aliquots taken to locate the [^{3}H] standard by liquid scintillation spectrometry. The HPLC fractions were then assessed for sheath elongation activity using a dwarf rice bioassay (see below).

BIOASSAY

Oryza sativa L., cv. Tan-ginbozu seeds were soaked for 24h in a 1 ppm solution of technical grade Uniconizole (S-3307)(Nishijima *et al.*, 1989) at room temperature, followed by a further 24h soaking in running water at 32°C. Germinated seeds were planted in 1% water agar media and incubated for 48h at 32°C, near 100% humidity and continuous light. Aliquots of the fraction after C_{18} and Nucleosil HPLC were generally 1/50 and 1/100. These were applied to the growing leaf of the rice seedling in 1.0 and 0.5 µl microdrops respectively, of 95% EtOH. The seedlings were then incubated for a further 72h at 32°C. Following the 72h incubation period the elongation of the second leaf sheath was measured, giving a semi-quantitative measure of the bioactive GAs present in a sample. Although relative levels of GA bioactivity may be determined via use of GA_3 standards on the rice, the bioassay was generally used only to locate bioactive GA fractions (i.e. endogenous GAs plus the added [$^2H_2$] GA internal standards).

EXTRACTION OF KAURENOIDS

Approximately 500mg D.W. of upper stem (apex and 1cm of subtending tissue) was ground in 50ml of 100% MeOH using a Polytron™ and stirred for 5h at

4°C before being filtered through a Büchner funnel with a Whatman #1 filter. The filtrate was stored at -20°C and the powder re-extracted for 8h in 30 ml of 100% MeOH. The filtrates were combined, and after adding 25ng of [$^2H_2$] internal standards of kaurene, kaurenal, kaurenol, kaurenoic acid and 7β-OH kaurenoic acid, were taken to dryness *in vacuo* at 35°C ensuring that excessive heat or vacuum was not applied to avoid sublimation, especially of the kaurene.

A dry silica gel column (2g) was assembled using a 10ml glass syringe with glass wool on top and bottom. The column was washed with 2 volumes of 5ml of *n*-hexane by forcing through the column with air pressure. Samples were then loaded from the flask onto the column with 5ml of hexane, forced through the column and collected. The flask was washed twice more with 5ml hexane and fractions collected. (fractions 1-3)

The column was then eluted sequentially with 5ml of each of the following solutions three times and fractions were collected separately:

2.5%	EtOAc / 97.5% hexane + 1% HOAc	(fractions 4-6)
5%	EtOAc / 95% hexane + 1% HOAc	(fractions 7-9)
15%	EtOAc / 85% hexane + 1% HOAc	(fractions 10-12)
50%	EtOAc / 50% hexane + 1% HOAc	(fractions 13-15)
100%	EtOAc + 1% HOAc	(fractions 16-18)

Low specific activity radioactive standards of 7β-OH kaurenoic acid, kaurenoic acid, kaurene and GA_{12}-aldehyde were run as external standards to determine relative retention times on silica columns and HPLC columns.

Fractions containing kaurene (fractions 1 to 3 above) were then run on an isocratic C_{18} reversed phase HPLC (100% MeOH with 2mM HOAc) at 1ml/min. Fractions were collected and with the aid of external standards, the fraction containing kaurene was dried with a gentle stream of N_2, then dissolved in EtOAc and injected into the GC-MS.

Fractions containing kaurenal (fraction 6 above) were dissolved in 5ml of aqueous 80% MeOH and passed through a preconditioned C_{18} Sep-Pak column. This column was further eluted with two more 5ml volumes of aqueous 80% MeOH and collected as 3 separate fractions, with the majority of kaurenal eluting in fraction 2. Samples were then taken to dryness, dissolved in the initial solvent, and injected onto a C_{18} HPLC eluted with a gradient of 80% MeOH + 2mM HOAc to 100% MeOH 2mM HOAc over 20min at 1%/min. Fractions were collected at one minute intervals and the fraction containing kaurenal was injected into the GC-MS.

Fractions containing kaurenol, kaurenoic acid and 7β-OH kaurenoic acid (fractions 8 to 11 above) were processed as above for kaurenal. Following the first HPLC, fractions were collected, methylated and run on a second C_{18} HPLC as the methyl ester prior to injection on GC-MS.

GC-MS-SIM ANALYSIS

Biologically active peaks from the C_{18} or Nucleosil HPLC fractions located by either internal or external radioactive standards, were analyzed using GC-MS-SIM. Fractions for GC-MS were transferred into 1ml Reacti-vials® and dried in a gentle flow of N_2 at room temperature. Fractions were then dissolved in 10 µl of MeOH and 100 µl of ethereal diazomethane (CH_2N_2), sealed and allowed to react

for 1h at room temperature. The samples were dried again with a gentle stream of N_2 and re-methylated with 50 µl of diazomethane. The methylated samples were dried as above and silylated with 50 µl bis-(trimethyl-silyl)-trifluoacetamide [BSTFA] with 1% TMCS (trimethylchlorosilane) (Pierce Chem. Co.). Derivatization was carried out at 70°C for 30 min. Samples were allowed to cool, as above and after dissolving in a small volume of *n*-hexane, samples were reduced to approximately 5 µl with N_2, with 1 µl being injected into a Hewlett Packard model #5890 Series II gas chromatograph fitted with a DB1-15N column linked to a Hewlett Packard model #5790A mass spectrometer. The oven temperature was programmed to increase from 60°C to 195°C @ 15°C/min, then from 195°C to 275°C @ 5°C/min. Data analysis was via the integration functions of the Hewlett Packard 5970 MS ChemStation (windows based software). Endogenous ABA was analyzed with the same GC-MS equipment using a DB-1701 column after methylation, which was achieved by adding 100 µl of diazomethane and reacting for 1h at room temperature. The GC oven temperature program was 40°C to 180°C @ 20°/min, then 180°C to 250°C at 5°/min.

Identification of GAs was based on comparisons of Kovats Retention Indices (KRIs) with published data (Gaskin and MacMillan, 1991) and with authentic standards. The levels of endogenous GAs were calculated by isotope dilution using the most abundant peak area ratio monitored for the particular GA (usually the molecular ion pair). The isotopic dilution method uses the equation described by Fujioka *et al.*, (1986):

ng of GA = S/100[(BCE /(D-FC))-A]

S = ng deuterated standard added at extraction

A = % unlabelled molecules in the internal standard

B = % labelled molecules in the standard

C = measured intensity of unlabelled GA

D = measured intensity of M+ of completely labelled GA

E = a factor calculated from the relative intensities of the ions in the M+ cluster of unlabelled GA and the amount of partly labelled GA relative to fully labelled GA in the internal standard

F = the intensity of the ion of m/z equivalent to M+ of the fully labelled GA in the M+ cluster of the unlabelled relative to the intensity of M+.

HISTOLOGY

Plants were grown in CSIRO Phytotron in Canberra under five different light conditions for seven days, prior to harvesting for hormone and histological analysis. Hypocotyls were removed using a sharp razor blade and immediately placed in glass scintillation vials containing FAA fixative (EtOH : acetic acid : formaldehyde : water, 50 : 5 : 3.7 : 41.3). The tissue/fixative was vacuum infiltrated for 15 minutes to remove air spaces, then shipped to Calgary for subsequent analysis.

Old fixative was then removed and fresh FAA added to the tissue, which was stored for seven days prior to a dehydration series. Tissue was divided into 1 cm segments, with the top, middle and bottom of the hypocotyl being processed separately. Dehydration was carried out by removing the FAA and replacing it with 50% TBA (*tert*-butyl alcohol in distilled water), in which it was incubated for 24h.

The series continued with 24h incubations in 60%, 70%, 80%, 90% and 95% EtOH plus 0.1% Eosin Y. Three changes of 100% EtOH were carried out the following day. Clearing was carried out using a xylene series (25%, 50%, 75% xylene in EtOH) and finally three washes with 100% xylene (each was incubated for 24h).

Paraffin wax (Paraplast Plus^{RT}) was heated (molten) to 60°C, as were the sample vials containing xylene. Approximately one half of the xylene was removed, replaced with molten wax, mixed by swirling and incubated at 60°C for at least 4h. This process was repeated three times. All the wax/xylene solution was then removed and replaced with molten wax 5 times.

Paper index cards were used to form a 2 cm x 4 cm open top boat which was placed on a hotplate (65°C). Tissue and wax was poured into the boat and arranged with a warm dissecting needle. Wax was added to a depth of at least 1cm and allowed to harden. Wax blocks were removed from the boats, trimmed with a hot knife and affixed to a microtome plate with hot wax. Serial sections (approx. 7µm thick) were formed in ribbons placed on slides and stained with safranin O, basic fuchsin and crystal violet (0.5%, 0.2%, 0.2%) and then counterstained with fast green.

Medial longitudinal sections were identified and the length of cells in the first cortical layer was determined using a digitizing video camera attached to a compound microscope. Cell length was determined using the Java^{RT} software.

Figure 2.1. Operation of B-units for control of environmental conditions at the CSIRO Phytotron. (top) open (bottom) closed.

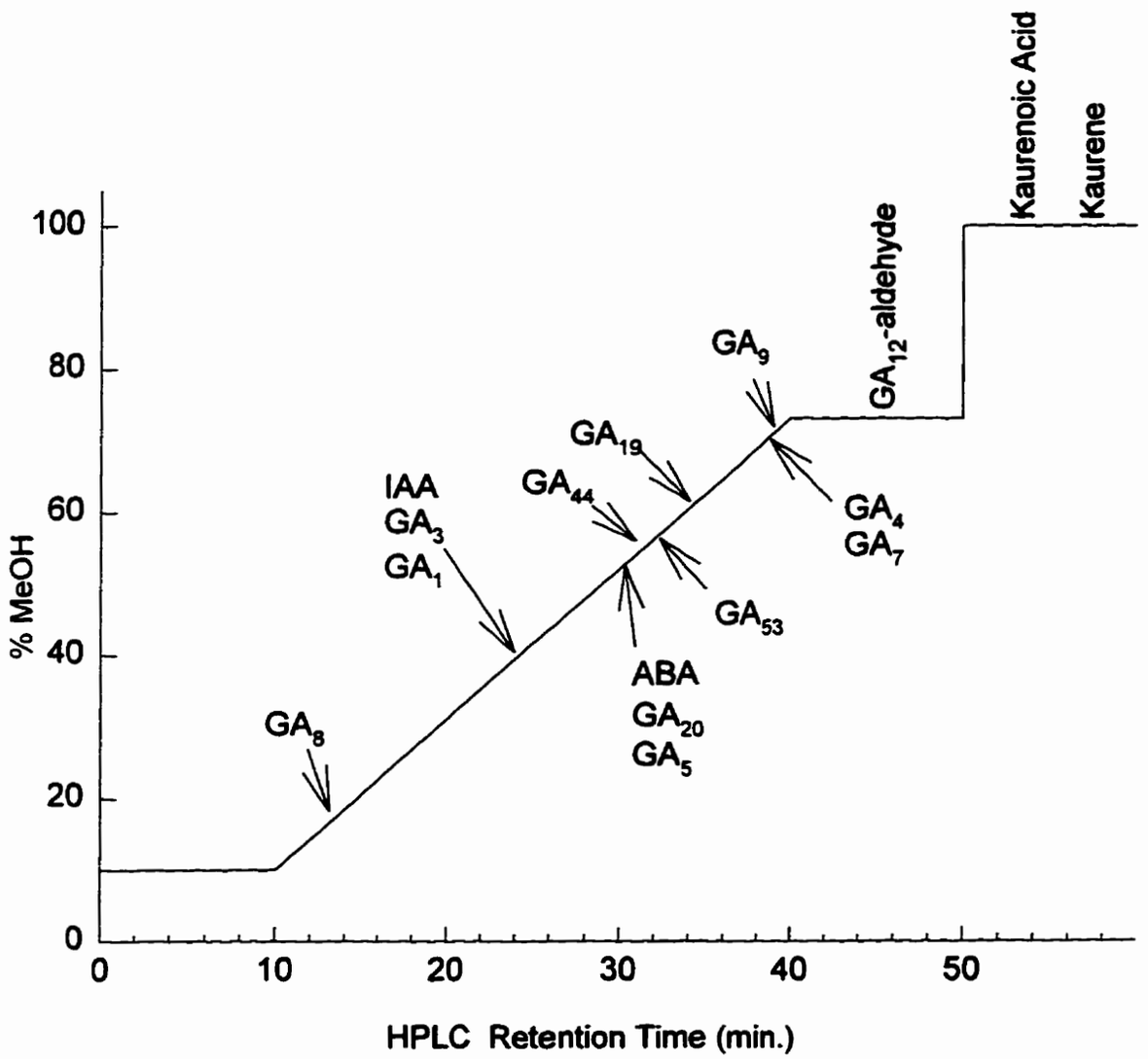


Figure 2.2. Retention times (Rt) of characteristic plant hormones on a 10-73% aqueous MeOH HPLC gradient using a reversed-phase μ-Bondpack C_{18} HPLC column (Waters Assoc.) with a 2 ml / minute flow rate.

CHAPTER THREE

Be sure you always have someone up your sleeve who will save you when you find yourself in deep s--- (JD Watson, 1993).

EXOGENOUS HORMONE APPLICATION

INTRODUCTION

Use of the biennial *Brassica* (syn. cold-requiring and winter canola) system as an experimental material allows one to discriminate between the physiology of bolting and floral initiation in cold-requiring biennials, especially in regard to the putative role of endogenous GAs in each of the two processes. Additionally, exogenously applied PGRs (GAs and CCC) to both annual and biennial cultivars may also help in our understanding of the involvement of different GA structures in bolting and flowering.

Brassica spp, contain relatively high levels of endogenous GAs and the levels of some GAs increase during flowering under inductive LD photoperiods (Rood *et al.*, 1987, 1989, Suge and Takahashi, 1982), or after vernalization (Zanewich, 1993, Metzger, 1988, Suge, 1970). As discussed earlier, the exogenous application of certain GAs can induce bolting (Mandel *et al.*, 1991) and/or flowering (Mandel *et al.*, 1991; reviewed by Lang, 1965; Zeevaart, 1971, 1973) under non-inductive growth conditions.

Flowering of the LDP *Lolium temulentum* (a grass) by GAs under non-inductive SD was promoted by applied Gas. The presence of a double bond and the number and location of hydroxyl groups is important for enhancing florigenicity of the GA molecule (Evans, 1990, Pharis *et al.*, 1989). Gibberellin A_1, a known effector of shoot elongation, had only a low florigenic effect on *L. temulentum*, as did GA_4 (Evans, 1969, Pharis *et al.*, 1987, Evans *et al.*, 1990). However, the GA analogue, 2,2-diMe GA_4, was highly florigenic and promotive of stem elongation in *L. temulentum* (Evans, 1990, Pharis *et al.*, 1989 and 1987). Such different physiological functions for different GAs, based upon structural differences of the GAs, may allow for a separation of the roles of various GAs in floral induction, as opposed to stem elongation (bolting).

Of the more than 100 known natural GAs and numerous GA derivatives, only a small number are bioactive *per se* in growth assays and for floral induction (Phinney, 1984; Pharis and King, 1985; Evans *et al.*, 1990). The florigenic qualities of GAs have been extensively examined over the years (see reviews by Zeevaart, 1983; Pharis and King, 1985). For example, GAs promote flowering in several plant systems: GA_4/GA_7 in Pinaceae (Pharis *et al.*, 1987); GA_5 in *Chrysanthemum* (Pharis, 1972) and *Hydrophyllus* (Lona and Fioretti, 1962) and GA_3, GA_{32} and 2,2-dimethyl GA_4 in *Lolium* (Pharis *et al.*, 1987). Recently, Evans *et al.*, (1990 and 1993) related GA structure to highly active in floral induction (presence of an A-ring double bond, location and numbers of hydroxyl groups in C- and D-rings) verses stem elongation (presence of C-3β hydroxyl group).

The plant growth retardant CCC (action of which blocks *ent*-kaurene biosynthesis (Hedden, 1990)) was also used to study the possible role of GAs in

flowering as was done earlier by Evans (1969) and Suge (1982). Reid and Crozier (1972) showed that levels of GA-like substances increased following application of CCC. While CCC can inhibit flowering in SD, LD, and cold-requiring plants (cited in Pharis and King, 1985) it has also been reported to promote flowering in woody angiosperms (cited in Pharis and King, 1985) and interacts with GA_3 in a synergistic fashion in flowering of *Lolium* (Evans, 1969). It is this "floral promotive" ability of CCC, that led me to test the compound, with and without applications of various GAs on winter canola.

According to Bernier *et al.*, (1981), cytokinins are a class of compounds known to be involved in the control of flowering. Exogenous application may either be promotive or inhibitory to floral initiation depending upon the timing, concentration applied or condition of the plant. According to Bernier *et al.*, (1990) the cytokinin-induced inhibition of flowering in *B. rapa* reported by Krekul and Seidlová (1977; cited in Bernier *et al.*,1990) was due to the use of supra optimal concentrations of the hormone. Pharis (1972) showed that combinations of GA_5 and ^{6}N-benzyl adenine (BA) were synergistic in promoting-flowering of *Chrysanthemum*. One of the goals of this chapter is to determine if there is a synergistic relationship between applied GAs and BA in *Brassica* spp.

Time to flowering is a major consideration in the choice of canola varieties grown by farmers in northern agricultural areas. Early frost damage to maturing seeds results in lower yield and reduced seed quality, therefore, it is a major economic concern. Hastening of flowering in canola varieties has been carried out by breeding programs and hastening of maturation (desiccation) by herbicide applications such as Roundup™ and 2,4-dichlorophenoxyacetic acid (2,4-D). The

possibility that flowering might be hastened by applications of a natural compound such as one of the GAs was one of the goals of this project.

The use of exogenous PGRs has long been the starting point in helping to determine what their effects and possible roles are in plant development. Exogenous applications of GAs known to be native to *Brassica*, as well other GAs shown to promote elongation or be highly florigenic in other plant systems, were thus investigated under non-inductive growth conditions.

MATERIAL AND METHODS

Plants were grown either under greenhouse conditions or in growth chambers as stated in the general material and methods (chapter 2). Growth chambers were equipped with Gro Lux™ fluorescent tubes (R:FR light was balanced at 1.35 and a PAR of 250 $\mu E\ m^{-2}s^{-1}$). In experiments carried out under greenhouse conditions aqueous solutions of PGRs in 0.1% Tween 20 (v/v) surfactant were applied as 1ml per plant foliar sprays starting with plants aged 28 days. Applications were made three times per week for 3 weeks. In growth chamber experiments, PGRs were solubilized in 10 to 25 µl 95% EtOH (depending on the rate and solubility of the PGR) and pipetted onto the apex of plants. Measurements of the stem height and growth stage (according to Harper and Berkenkamp, 1975) were recorded on a weekly basis following application of PGRs.

Data were analyzed for significance using the Student-Newman-Keul's test. Data groups which differ from one another with $\alpha=0.05$ were assigned different letters. Error bars represent 95% confidence intervals and the percent of plants induced to flower by the final week of the study were indicated in brackets.

RESULTS

Biennial CR Brassica under Greenhouse conditions

Preliminary experiments carried out at the University of Lethbridge were used to identify cultivars of cold-requiring *Brassica* which would be used in subsequent studies. Specifically, I wanted a cultivar which could be induced to flower in the absence of vernalization, so I screened both *B. napus* and *B. rapa* cultivars by applying a range of GAs and GA analogues, including GA_1, GA_3, GA_4, GA_5, GA_7, GA_8, GA_9, GA_{19}, GA_{20} and 2,2-dimethyl GA_4 (as well as several other synthetic GA analogues produced by L.N. Mander and shown to be active in other plant systems (Evans *et al.*, 1993a)). Figure 3.1 to 3.4 illustrate the effects of 100µg applications of GA_1, GA_3 and 2,2-dimethyl GA_4 to plants of two cultivars, *B. rapa* [cv. Arktus (fig 3.1) cv. PER (fig 3.2)] and *B. napus* [cv. Glacier (fig 3.3), cv. WW1033 (fig 3.4)]. All GAs applied to *B. napus* and *B. rapa* significantly ($P \leq 0.05$) promoted stem elongation (bolting). Other cultivars were tested, but data are not shown (the four figures presented are representative of their responses). Three GAs, GA_1, GA_3 and 2,2-dimethyl GA_4 are acting as a "classical" elongation hormone (Lang, 1965). Gibberellin A_1 has been shown to be the major GA that is likely responsible for stem elongation in *Brassica* (Rood *et al.*, 1989), but 2,2-dimethyl GA_4 gave the highest rate of stem elongation of the GAs tested. In all cultivars tested, the primary effect of applications of GA_1, GA_3 and 2,2-dimethyl GA_4 was to induce rapid stem elongation within three weeks of initial application. In all cultivars tested, the highest growth promotion resulted from 2,2-dimethyl GA_4 applications. Responses with GA_1 and GA_3 were less pronounced but did not differ significantly except for cv. Artkus. Only in *B. napus* cv. WW1033 was one of the

GAs, 2,2-dimethyl GA_4, able to induce flowering in a small number of plants. The GA_4-C13-acetate tested on *B. rapa* cv. Arktus was neither growth nor floral promotive, possible because the plants were unable to metabolize the C13-acetate to yield a C13-hydroxyl (i.e. GA_1).

Biennial Cold-Requiring Brassica grown under controlled environmental conditions

All subsequent work with CRP of *Brassica* was carried out in growth chambers. Figure 3.5 shows the effect of 300µg applications of GA_3, 2,2-dimethyl GA_4, and GA_5 on stem elongation and flowering compared with those of the vernalized control plants of WW1033 grown under 16h 24°C day, 8h 18°C night conditions. It is evident that 2,2-dimethyl GA_4 application results in stem elongation very close to that of the vernalized controls, but is unable to completely replace the florigenic effect of vernalization. Application of GA_5 resulted in more florigenic activity than 2,2-dimethyl GA_4 but GA_5 was less effective in promote elongation. Applications of GA_3 gave stem elongation but no flowering.

Day zero for stem length measurements for vernalized control plants was defined as the day they were transferred from the cold chamber to LD warm. A difference in the timing of floral bud initiation was observed between GA-treated plants and the vernalized controls. Elongation of plants treated with GAs was followed by floral bud development, whereas floral buds were present on vernalized plants prior to stem bolting. This observation indicates that there is a difference between floral initiation due to exogenous GA application and natural vernalization (i.e. the cold-induced florigen cannot promote elongation at vernalizing temperatures). As a follow up, GA treated plants were dissected one

and two weeks post GA application, but no floral buds were apparent.

The effects of applications of increasing amounts of GA_3, GA_5 and 2,2-dimethyl GA_4 on stem elongation relative to controls and the percent flowering are shown in Figure 3.6. Increasing the amount of 2,2-dimethyl GA_4 increased the percentage of plants flowering and also resulted in a dramatic increase in stem elongation. Increasing amounts of GA_5 application also resulted in increased flowering, but with only minimal increases in elongation. High levels of GA_3 (2000µg/plant) were unable to promote flowering but did result in increased stem elongation.

Biennial CRP Brassica grown in 11h SD, warm

Cold-requiring plants of *B. napus* cv. WW1033 , when maintained under an 11h SD, significantly elongated their stems in response to the applications of 300 µg of 2,2-dimethyl GA_4 or GA_1 (most promotive, elongation was 2-fold greater than controls) and GA_5 (elongation was 1.5-fold greater than controls)(Figure 3.7). Under an 11h SD, GA applications were unable to promote flowering in cv. WW1033. All other varieties of *B. napus* and *B. rapa* responded in a similar manner to exogenous GAs applied under SD warm (i.e. significant stem bolting, but no flowering).

Biennial Cold-Requiring Plants of Brassica Grown in Continuous Light (LLD)

The use of LLD elicits very different flowering responses for *B. napus* than for *B. rapa* (Figure 3.8). *Brassica napus* cv. WW1033 bolts and flowers under LLD without GA application at four weeks post-emergence, although applied GAs do

not hasten the days to flowering or increase stem elongation at four weeks post-application (Figure 3.8). *Brassica napus* cv. Glacier remains as a rosette without flowering and without GA treatment under LLD, but when 2,2-dimethyl GA_4 or GA_5 is applied, both flowering and bolting occur. Plants of non-GA treated *B. rapa* cv. Rapido also remain as a rosette under LLD though cv. Rapido plants treated with 2,2-dimethyl GA_4 or GA_5 bolt under LLD, but fail to flower (Figure 3.8).

Plant Growth Retardant Application to B. napus cv. WW1033

Figure 3.9 shows the effect of the two known inhibitors of GA biosynthesis, CCC and PP333 on the stem length of plants of *B. napus* cv. WW1033 grown under LD warm conditions. During the first two weeks following application, PP333 treatment gave a greater reduction in growth than CCC relative to the untreated controls. However, after two weeks, stem elongation of PP333-treated plants mirrored that of controls, whereas CCC-treated plants remained stunted for the duration of the experiment. Therefore, CCC was used in subsequent experiments to look at the effects of CCC and GAs on flowering

Non-vernalized biennial Brassica naps cv. WW1033 grown in 16h LD, warm+ CCC

Application of CCC to GA_5-treated plants, reduced stem elongation by approximately 50%, but the percentage of plants that flowered increased from 88% to 100% (Figure 3.10). For 2,2-dimethyl GA_4 treated plants, CCC also reduced stem elongation and increased flowering (from 75% to 84%)(Figure 3.10). Surprisingly, stem elongation of controls is also decreased by CCC but the plants do not flower (Figure 3.10). Similar bolting responses were obtained for CCC-

treated *B. napus* cv. Glacier and *B. rapa* cv. Rapido (i.e. CCC-treatment reduced GA-induced stem elongation). However, even doses of GA_5 and 2,2-dimethyl GA_4 as high as 1 mg per plant did not cause flowering (data not shown).

Summer Annual Brassica napus cv. Westar in 11h SD

Under our growth chamber light conditions, within eight weeks all plants (including controls) of *B. napus* cv. Westar form a tight raceme, but there is no stem elongation or elongation of the floral axis in 11h SD. Application of 2,2-dimethyl GA_4 or GA_5 will, however, promote stem elongation (Table 3.1) and anthesis, but the floral axis (raceme) still does not elongate. Within 48h of transfer to a 22h LD, however, floral axis elongation and anthesis occur for all plants in all treatments (Table 3.1), as does stem elongation. Within seven days under 22h LD all plants resemble the 22h LD controls with respect to anthesis and raceme elongation, although stem elongation of controls transferred from 11h to 22h is much less than 22h controls (Table 3.1).

Biennial Brassica napus cv. WW1033 grown in 16h LD, GA + BA

The effects of exogenous applications of GA_5, BA and combinations of both on floral initiation (measured four weeks post-application) is shown in Table 3.2. At 33 µg per plant GA_5 alone was not florigenic but in combination with a 10ml spray of a 100 mg/L BA solution, 20% of treated plants flowered. Flowering in plants treated with 100 µg GA_5 increased from 20% (GA_5 alone) to 100% with GA_5 plus BA at 33 mg/L. Application of BA alone was ineffective in floral induction and no control plants flowered, even by eight weeks post-application.

Hastening of flowering of annual Brassica napus cv. Westar

The exogenous application of 2,2-dimethyl GA_4 increased the percentage of flowering plants from 0% (control) to 80% by two weeks post-application and from 67% (control) to 100% at week three (Figure 3.11). Application of GA_3, however, did not hasten flowering of cv. Westar (Figure 3.11). Stem elongation was promoted equally well by 2,2-dimethyl GA_4 and GA_3 relative to controls (Figure 3.11).

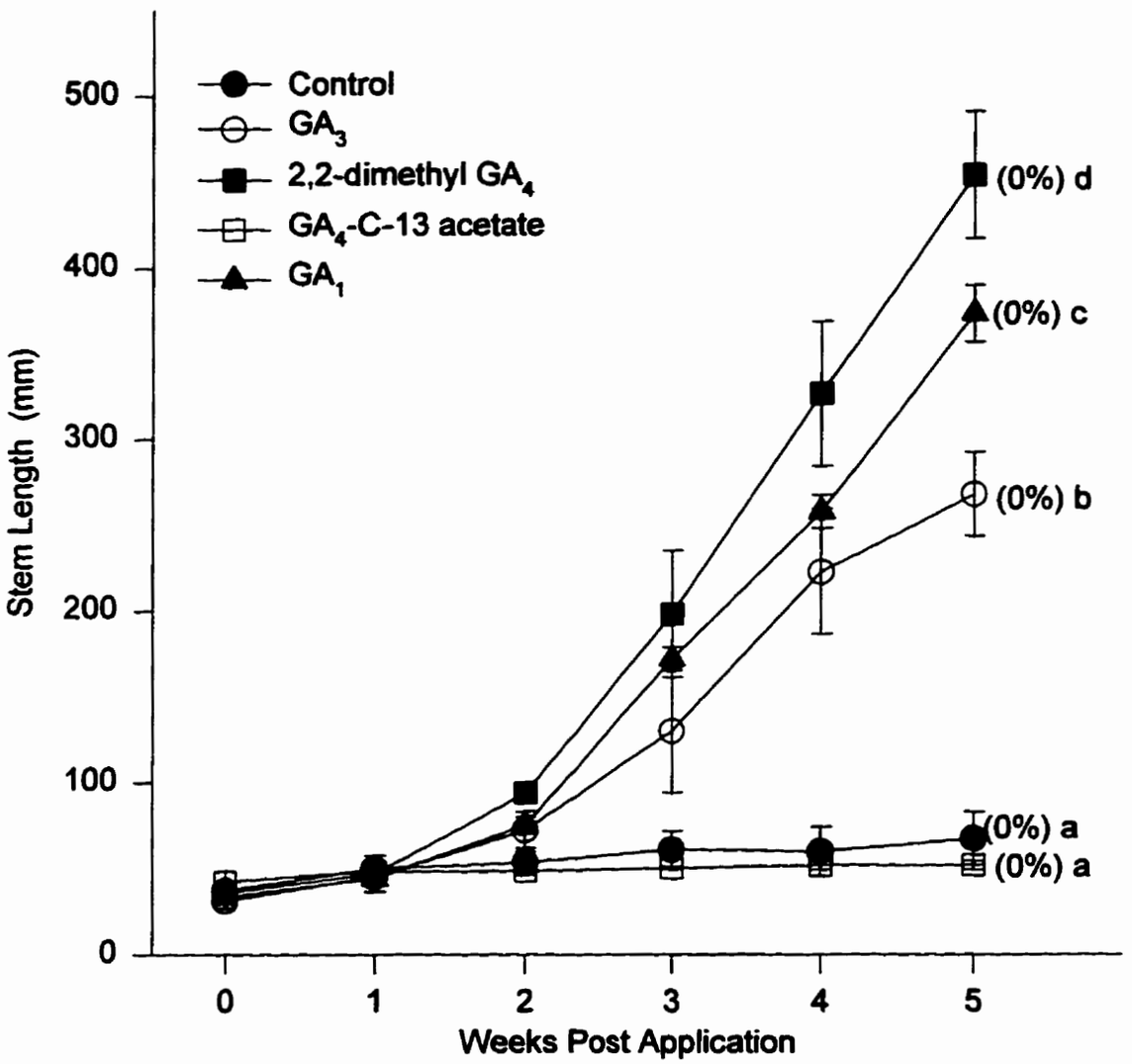


Figure 3.1. The effect of 100 µg applications (3 times application of 33.3 µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_1 , GA_3, 2,2-diMe GA_4 and GA_4-C13-acetate on the stem length of *B. rapa* cv. Arktus. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

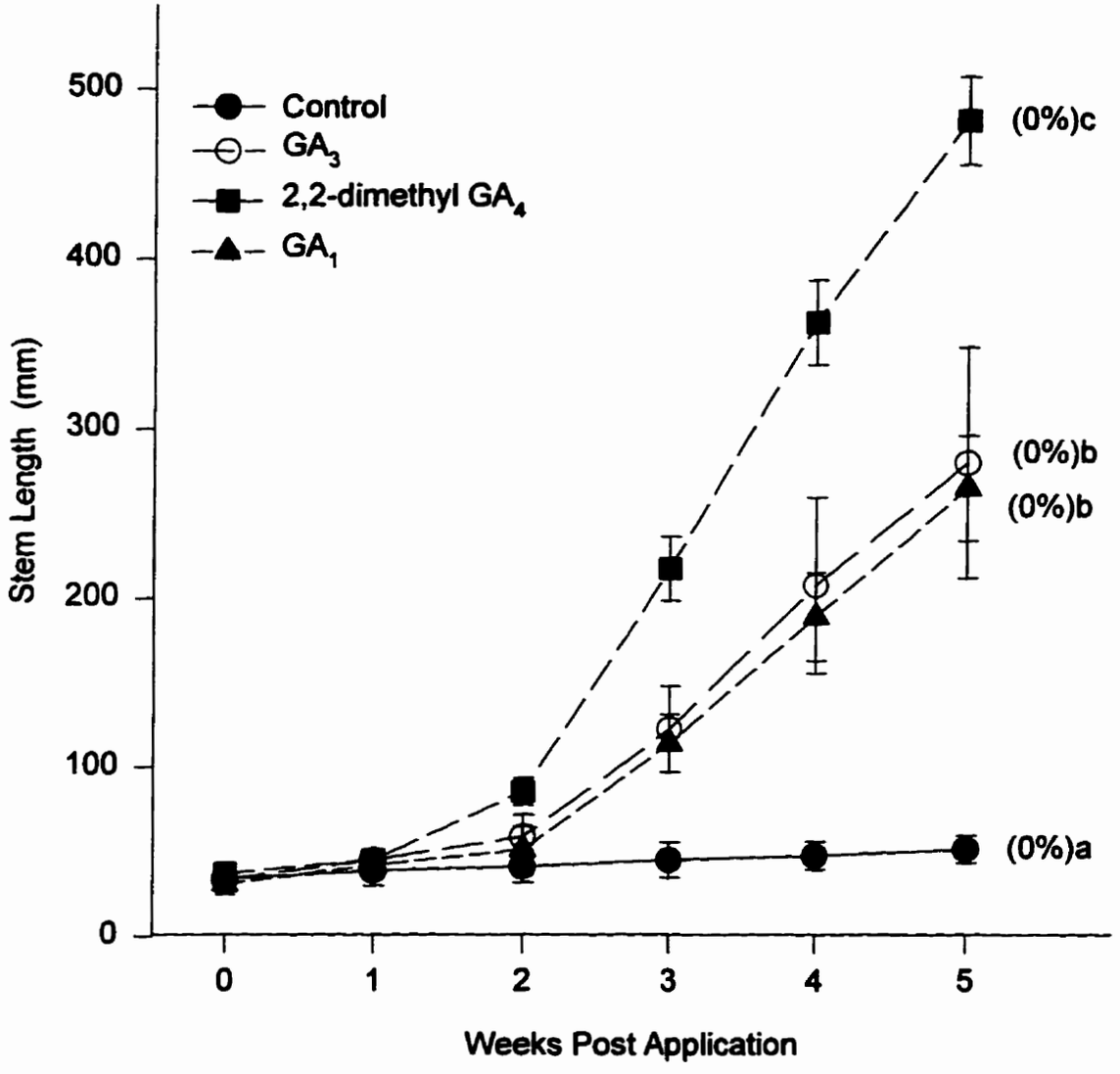


Figure 3.2. The effect of 100 µg applications (3 times application of 33.3 µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_1 , GA_3 and 2,2-diMe GA_4 on the stem length of *B. rapa* cv. PER. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

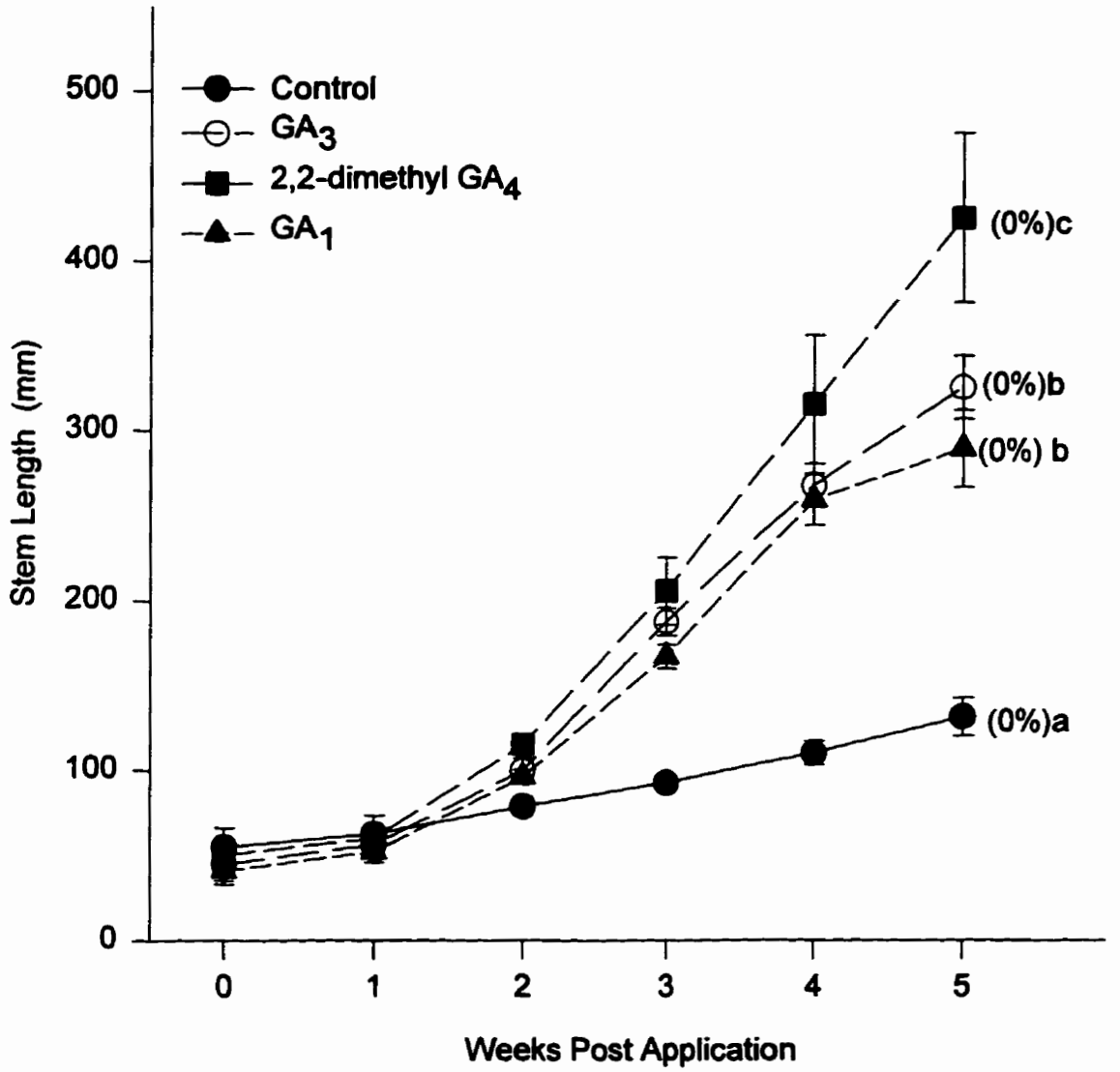


Figure 3.3. The effect of 100 µg applications (3 times application of 33.3 µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_1 , GA_3 and 2,2-diMe GA_4 on the stem length of *B. napus* cv. Glacier. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

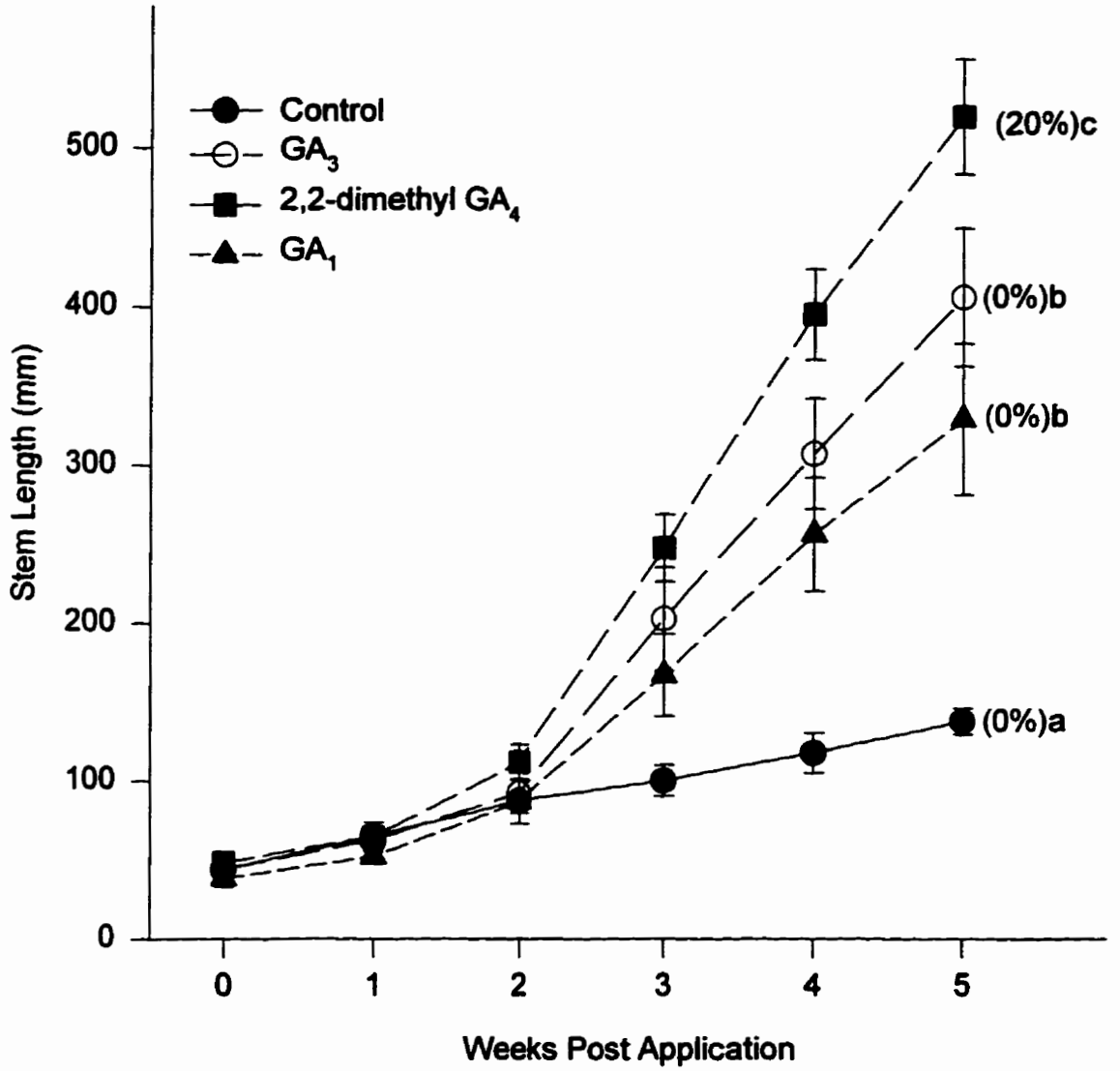


Figure 3.4. The effect of 100 µg applications (3 times application of 33.3 µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_1 , GA_3 and 2,2-diMe GA_4 on the stem length of *B. napus* cv. WW1033. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

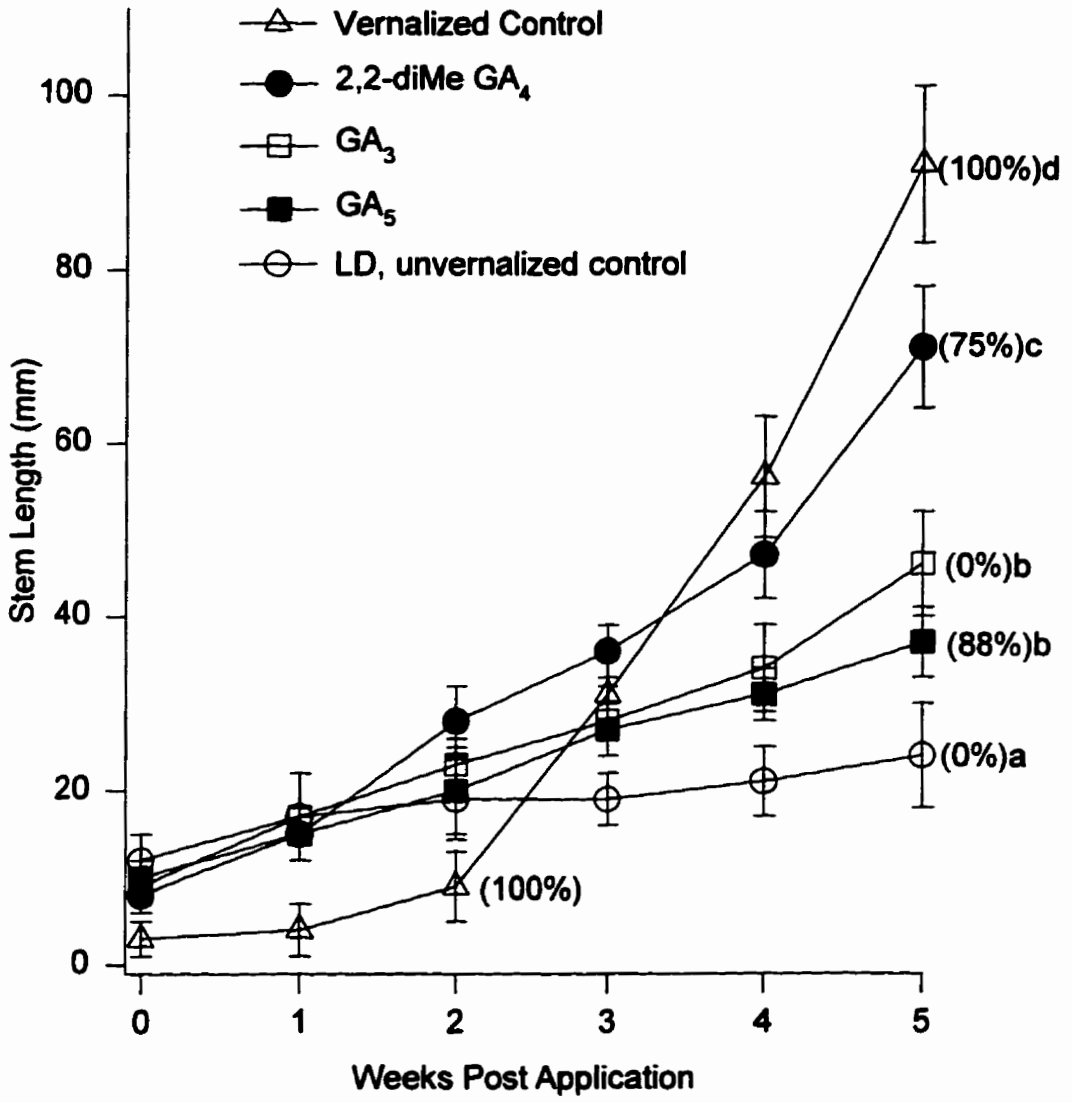


Figure 3.5. The effects of vernalization (SD, low temperature followed by LD warm) and of 300 µg applications (3 times application of 100µg in 50µl 95% EtOH beginning 4 weeks post-emergence) of GA_1 , GA_3 and 2,2-diMe GA_4 on the stem length of *B. napus* cv. WW1033. The GA-treated plants receive no cold, but were grown under LD. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

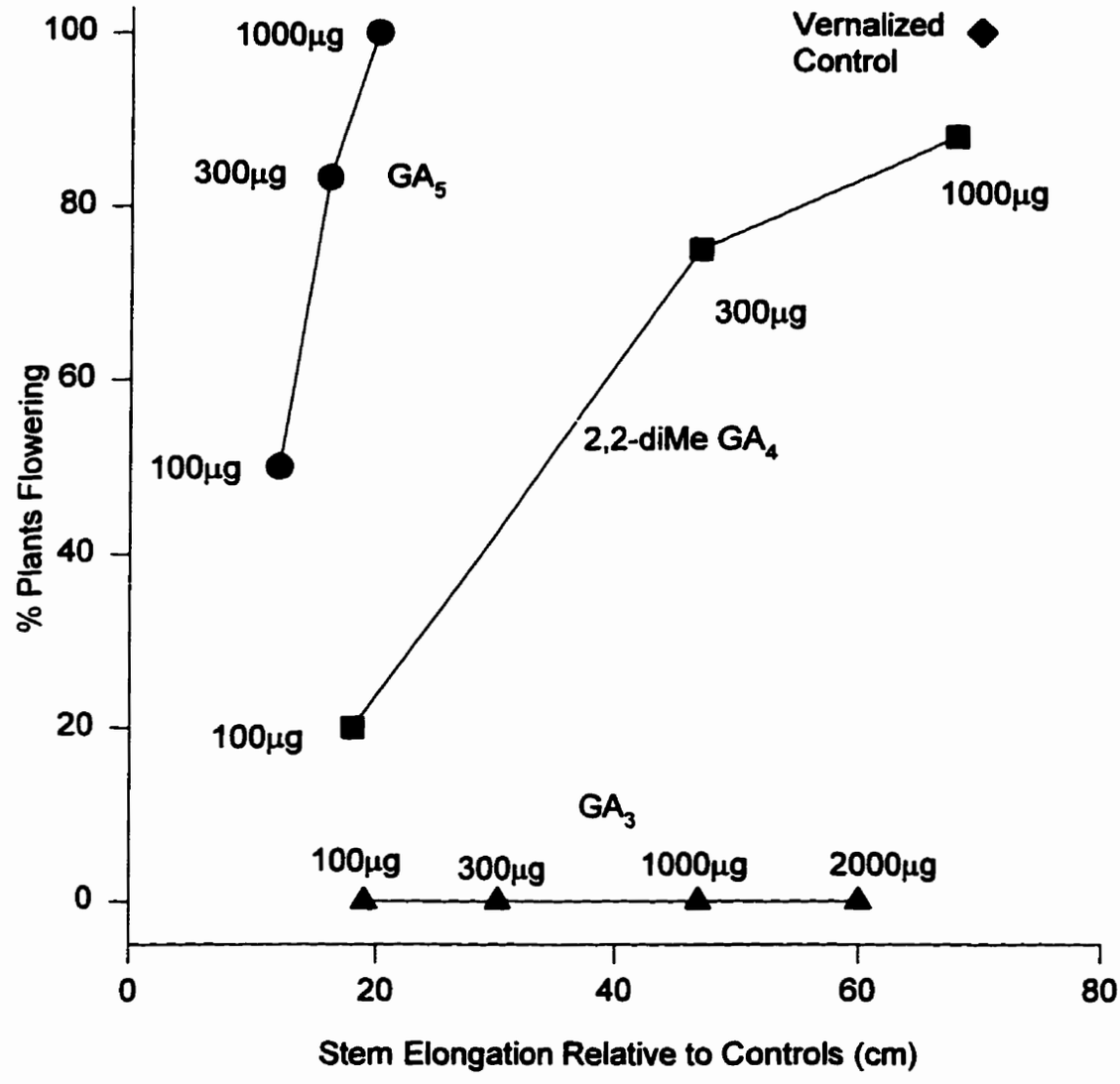


Figure 3.6. The effect of applications of GA_3, 2,2-diMe GA_4 and GA_5 and vernalization (3° SD, followed by LD warm) on the % of plants flowering and stem elongation of *B. napus* cv. WW1033, 5 weeks post application.

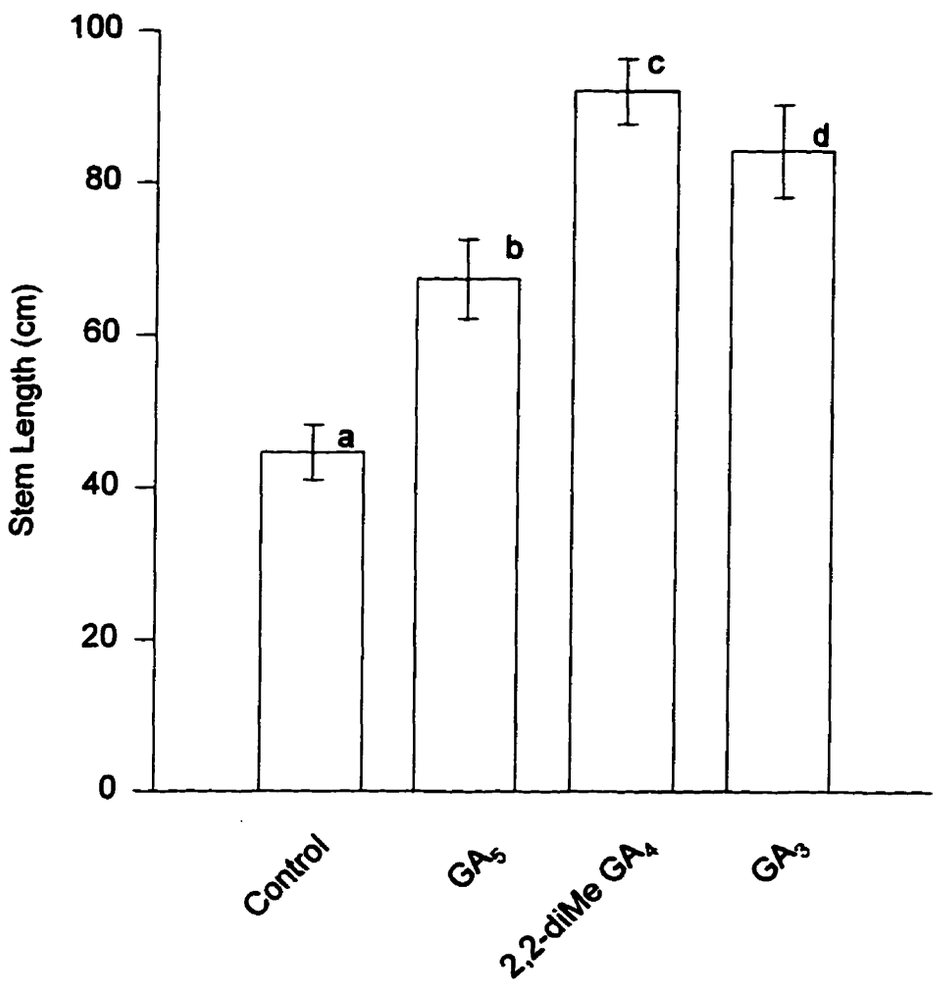


Figure 3.7. The effect of 300µg applications (3 times 100µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_3, 2,2-diMe GA_4 and GA_5 on the stem length of *B. napus* cv. WW1033, 5 weeks post application under warm conditions at 11h SD photoperiod. (error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

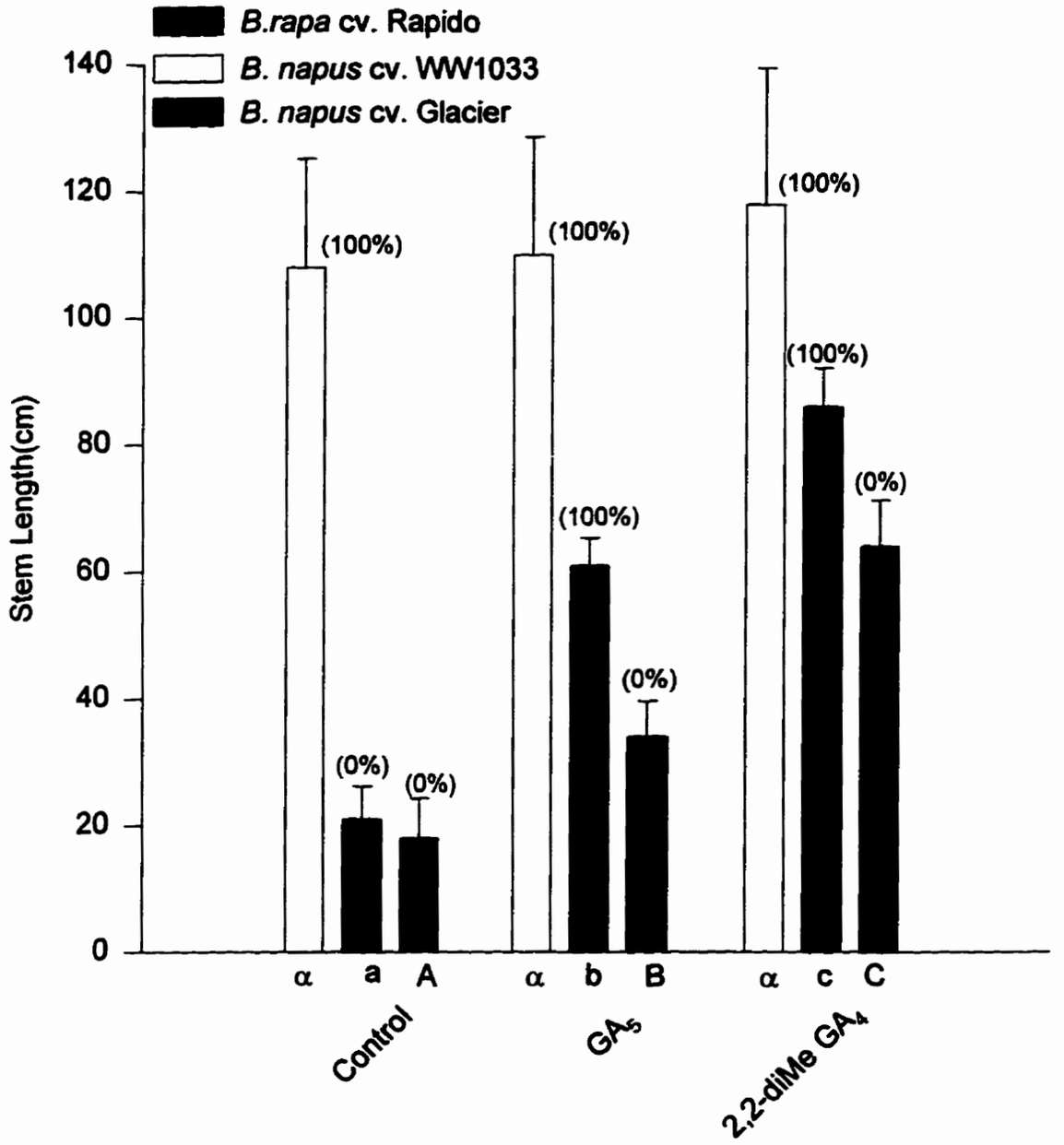


Figure 3.8. The effect of 300µg applications of each of (3 times 100µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_3, 2,2-diMe GA_4 and GA_5 on the stem length of *B. napus* cv. WW1033, 5 weeks post application under warm conditions under continuous light (LLD). (error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

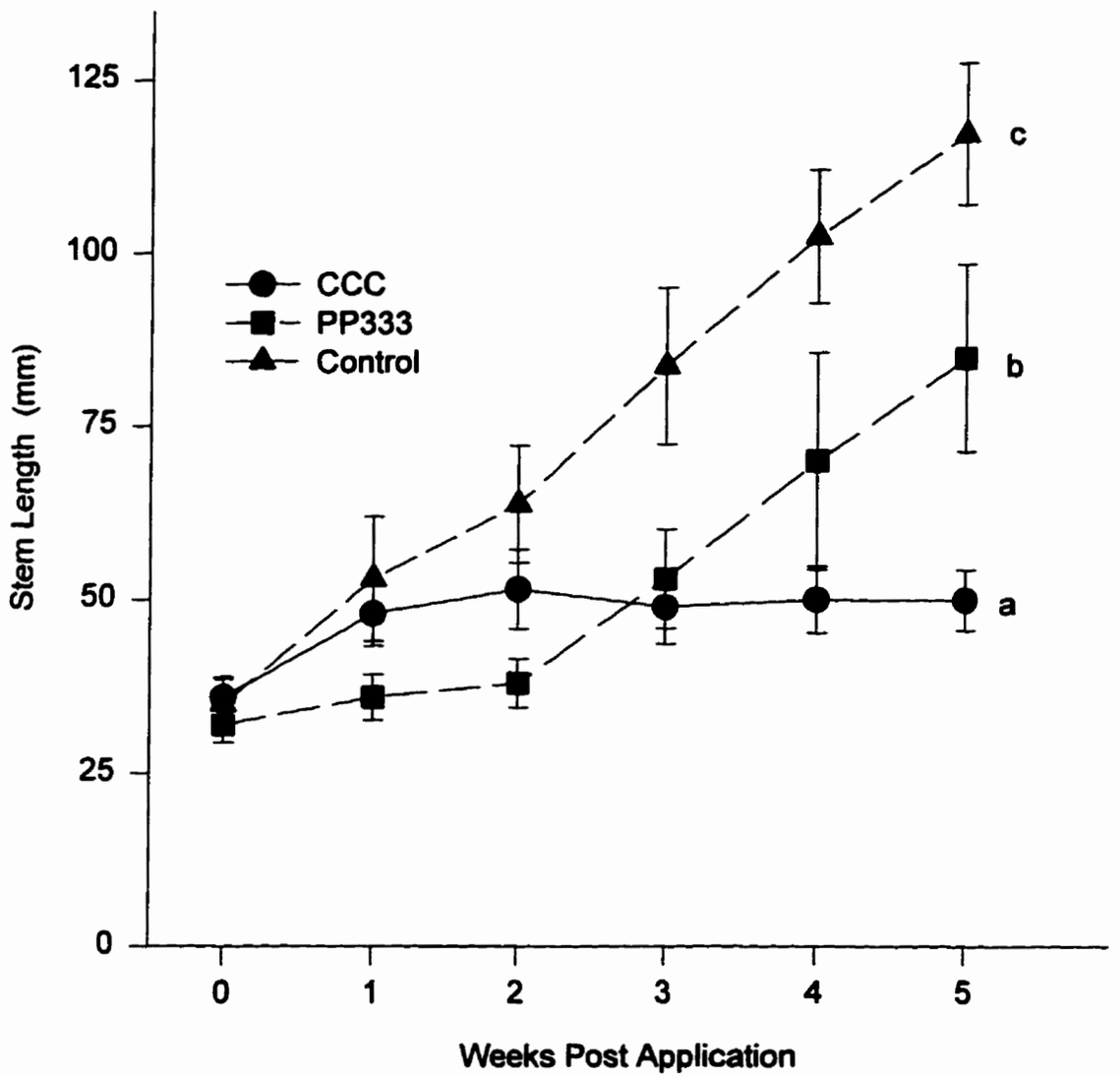


Figure 3.9. The effect of 1ml of a 2% active ingredient solution of CCC and PP333 applied as a foliar spray on the stem length of *B. napus* cv. WW1033, 5 weeks post application under warm conditions at 16h LD photoperiod. (error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

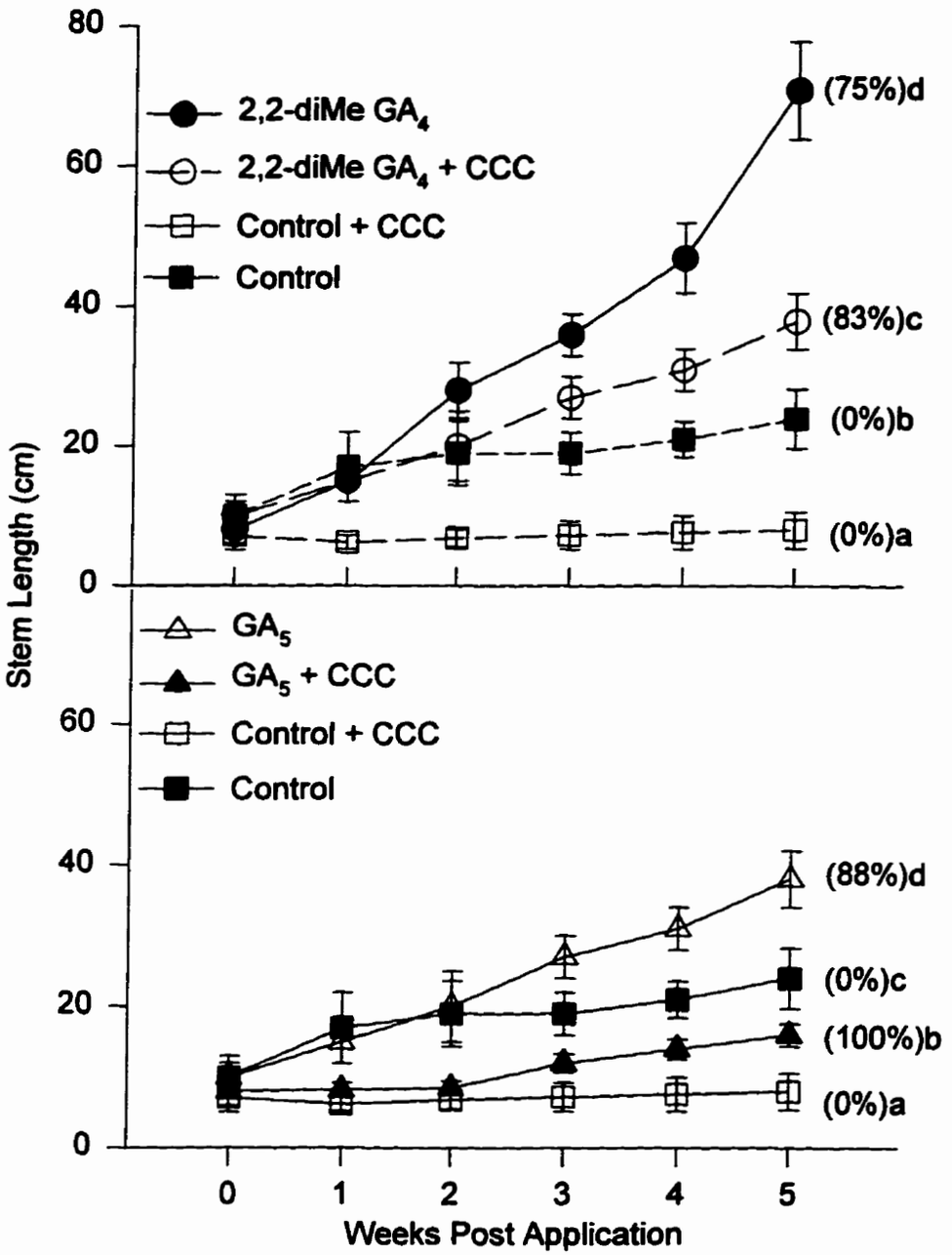


Figure 3.10. The effect of 1ml of a 2% active ingredient solution of CCC applied3 weeks post emergence and 300µg applications (3 times 100µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of 2,2-diMe GA_4 and GA_5 on the stem length of *B. napus* cv. WW1033, 5 weeks post application under warm conditions at 16h LD photoperiod. (error bars represent 95% confidence intervals, letters represent SNK groupings at week 5).

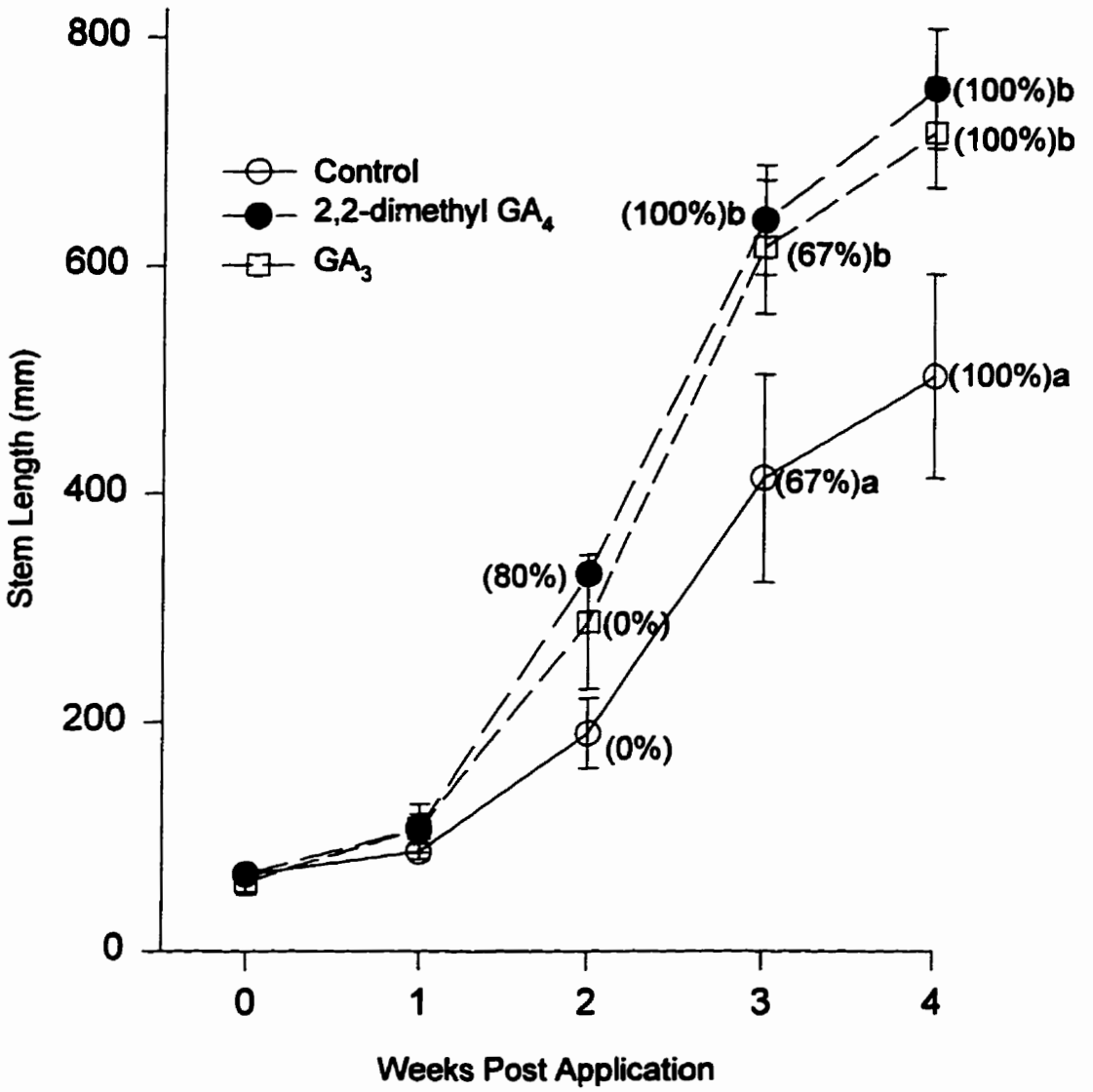


Figure 3.11. The effect of 300µg applications (3 times application of 100µg in 50 µl 95% EtOH beginning 4 weeks post-emergence) of GA_3 and 2,2-diMe GA_4 on the stem length and % of flowering plants for *B. napus* cv. Westar grown under 16h LD, warm conditions. (Numbers in brackets represent % plants flowering, error bars represent 95% confidence intervals, letters represent SNK groupings at week 3).

Table 3-1. The effect of photoperiod and exogenous GA application on the floral stage of the summer annual *B. napus* cv. Westar grown under various photoperiods at 24°C in growth chambers.

Photoperiod	Treatment	Elongation (cm)	Flowering %	Flowering Stage
11h	control	42	100	3.2
	GA_5	71	100	4.1*
	2,2-dimethyl GA_4	89	100	4.1*
11h → 22h	control	72	100	4.4
	GA_5	106	100	4.4
	2,2-dimethyl GA_4	110	100	4.4
22h	control	119	100	4.4

* Anthesis without raceme axis elongation

Table 3.2. The effect of GA_5, ^{6}N-benzyladenine (BA) and GA_5 + BA on the flowering of *B. napus* cv. WW1033 grown under LD warm greenhouse conditions. (BA applications were made 3 times/week for one week to 3 week post-emergence plants, GA applications were made 4 weeks post-emergence).

Treatment	PGR Application Rate	% Flowering plants four weeks post-application of PGRs
GA_5	33µg	0%
	100µg	20%
	200µg	100%
	300µg	100%
BA	33mg/L	0%
	100mg/L	0%
	330mg/L	0%
	1000mg/L	0%
GA_5 + BA	33µg + 10mg/L	0%
	33µg + 33mg/L	0%
	33µg + 100mg/L	20%
	100µg + 10mg/L	0%
	100µg + 33mg/L	100%
	100µg + 100mg/L	100%
Control		0%

DISCUSSION

Stem bolting in all of the biennial CRP cultivars, *B. napus* cv. WW1033, and Glacier and *B. rapa* cv. Rapido and Arktus, was significantly ($P \leq 0.05$) promoted in non-vernalized plants, grown under warm LD conditions, by the exogenous application of GA_3, GA_1, GA_5 and 2,2-diMe GA_4. Floral induction was also observed under these non-inductive conditions but only for one cultivar, WW1033 and only in response to two GAs, GA_5 and 2,2-diMe GA_4. At rates as low as 100 µg/plant; GA_5 was approximately two-fold more florigenic than 2,2-diMe GA_4. The use of exogenously applied GA_3 to promote stem elongation has been well documented (Zeevaart, 1983, Suge and Takahashi, 1982, Lang, 1965), but there are conflicting results on its ability to promote of flowering. In my study GA_3 could not induce flowering even at rates of 2000 µg/plant. Zanewich and Rood (1993) similarly were unable to promote flowering in *B. napus* cv. Crystal with a range of exogenous GAs(GA_3, GA_1, GA_5 and 2,2-diMe GA_4). However, Suge and Takahashi (1982) did induce flowering with GA_3 in Chinese cabbage (*B. rapa*), which is also a CRP.

It appears that the ability to promote stem elongation with exogenous GAs under non-floral inductive conditions is common to many plant species (reviewed by Pharis and King, 1985), However, the promotion of flowering is more specific, both with regards to GA structure and plant species. In fact, even cultivars within species respond differently. A partial answer to this may be that the modern cultivars have been extensively bred to change key characteristics, and that in

selections for these characteristics changes may have occurred in their responses to GAs. For example, changes may have occurred through these breeding programs in the timing window for optimal response, or in the ability to metabolize or catabolize GAs. Further work utilizing the very interesting differential responses between cultivars should provide a much better understanding of floral induction.

Total amounts of GAs applied per plant were as high as 2 mg of GA_3 and 1 mg of 2,2-dimethyl GA_4 and GA_5. Although this level of application may seem extremely high, 800 µg per plant of GA_4 or GA_7 was required to promote significant stem elongation and flowering in *B. rapa* (Suge, 1982). These high levels may be required to overcome rapid GA conjugation, deactivation and/or other biochemical blocks that may be present in the absence of apex vernalization (Suge, 1982).

Also, only a small portion of applied GA is likely to be absorbed. For example, young rice plants took up only 2.5% of the applied [^3H] and [$^2H_2$] GA_{20} (100ng/plant) was extracted from the tissue and only 0.35% of that was converted to GA_1, the "effector" of sheath elongation and an immediate metabolic product of GA_{20} (Takagi *et al.*,1994). Thus, the uptake of exogenously applied GAs by *Brassica* spp. may be extremely low, and if catabolism is high, and conversion to "effector" low, then these factors mac explain why initial applications must be at such high doses.

In many rosette LDP, photoperiod is a major factor determining whether an exogenously applied GAs will promote floral induction. To a lesser degree, photoperiod also influences GA-induced stem elongation. According to Lang

(1965), a strong relationship exists between vernalization and photoperiod. Under a non-inductive SD, GA_3 often promotes stem elongation and flower formation for many species (Lang, 1965, Zeevaart, 1978). However, GA_3 promoted stem elongation without flowering in vernalized or non-vernalized CRP *B. rapa* (Chinese cabbage) plants kept under SD conditions (Suge, 1982). In another CRP *B. napus* (cultivar unreported) flowering was also induced under LD by GA_3 in non-vernalized plants (cited in Lang, 1965). Interestingly, under conditions of continuous light (LLD), both stem elongation and flowering occurred, even without the use of exogenous GAs in non-vernalized Chinese cabbage (Suge, 1982). Also, the application of the GA biosynthesis inhibitor, ancymidol, could inhibit bolting but not flowering under LLD (Suge, 1982). These findings suggest that C-3β,13-dihydroxylated GAs (such as GA_1 and GA_3) are likely responsible for stem elongation, but not for flower induction in many *Brassica* species and cultivars. They also suggest that GA (and florigen) synthesis *in vivo* is under photoperiodic control. oAccording to Metzger (1990b) the lack of success in inducing flowering in biennial CRP *B. napus* with exogenous GAs may be due to use of the incorrect GA (non-florigenic) and/or improper timing of applications.

My findings on *B. napus* are in agreement with observations in *Lolium temulentum*, a LD-requiring grass that flowers when exposed to a single LD. In *Lolium* there is a structural hierarchy in efficacy of floral induction by different GAs (Evans, 1990, Pharis *et al.*, 1989 and 1987). Floral induction by GAs under non-inductive SD was enhanced by hydroxylation at C-12ß, -13 and/or -15 (Evans,

1990, Pharis *et al.*, 1989). A double bond in ring A (either C-1,2 or C-2,3) appreciably enhanced flowering, especially when one or more hydroxyl groups at C-12, -13, or -15 were present (Evans *et al.*, 1990). However, a C-3ß hydroxyl group, while promoting stem elongation, tended to diminish florigenicity (Evans *et al.*, 1990). A fully saturated ring A (eg. GA_1, GA_4 and GA_{20}) very much reduced flowering relative to the ring A double bond analogues, GA_3, GA_7, GA_5, respectively (Evans *et al.*, 1990), yet GA_1, GA_4 and GA_{20} were promotive of stem elongation (Evans *et al.*, 1990). The structural specifications for high florigenicity by a GA in *Lolium* may in part explain why GA_5 was so effective in eliciting flowering in non-vernalized biennial *B. napus* cv. WW1033.

The principal native early C13-hydroxylation pathway GAs in *Brassica* includes GA_1, GA_{19} and GA_{20} (Rood *et al.*, 1987, Hedden, 1990). MacMillan (1990) indicates that in higher plants (maize, specifically) there is a conversion of GA_{20} to GA_5 and further to GA_3. Gibberellin A_3 is also found in bolting stems of *Brassica* (Rood, 1987), although GA_5 has yet to be characterized from *Brassica*.

The GA analogue, 2,2-diMe GA_4, was also highly florigenic in non-vernalized CRP biennial *Brassica* as well as being promotive of stem elongation. The 2,2-diMe GA_4 also promoted both stem elongation and flowering in *L. temulentum* to a much greater degree than did GA_3 (Pharis *et al.*,1987).

The possible reasons for the unusual activity of 2,2-diMe GA_4 may include, steric effects such as flattening of the A ring, (Beale and MacMillan, 1982), or protection against 2ß-hydroxylation, which is known to result in loss of bioactivity

(Hoad *et al.*, 1982). Such different physiological functions for different GAs, based upon structural differences, may allow for a separation of the roles of various GAs in floral induction, as opposed to stem elongation.

The plant growth retardant CCC has been used to study the possible role of GAs in flowering. The application of CCC has also been shown to interact with GA_3 in a synergistic fashion in fruit set and berry enlargement (Rappaport, 1980), and flowering (Evans, 1969) and can induce flowering in some woody angiosperms (cited in Pharis and King, 1988) and CCC can also inhibit flowering (SD, LD, and CRP)(cited in Pharis and King, 1985). Like many other growth retardants, it blocks GA biosynthesis, acting at the *ent*-kaurene synthesis step (Hedden, 1990). The application of CCC alone to biennial *B. napus* inhibited stem elongation to approximate 50% of the height of control plants but flowering was unaffected. Even though levels of free endogenous GAs have not been determined for CCC-treated biennial *B. napus*, this decrease in stem elongation is likely attributable to a decrease in the levels of endogenous vegetative-growth promotive GAs (e.g., GA_1 and/or GA_3).

Application of CCC may modify GA biosynthesis in other ways (Hedden 1990), such as enhancing the conjugation of some GAs in the biosynthetic pathway (Snir, 1975). This may explain how flowering due to GA_5 and 2,2-dimethyl GA_4 was synergized by CCC.

The biennial CRP *B. napus* cv. WW1033, when grown in 11h, SD, non-vernalized conditions bolted in response to a wide range of GA structures including

GA_3 but did not flower in response to GA_3 as was observed in Chinese cabbage by Suge and Takahashi (1982). In 16h warm LD, GA_5 and 2,2-dimethyl GA_4 induced both stem elongation and flowering in non-vernalized plants of cv. WW1033, whereas GA_1, GA_3, GA_4, GA_7, GA_9, GA_{19}, GA_{20}, GA_4-C13-acetate and 15-OH GA_1 (Mandel and Rood, data not presented) promoted only stem elongation (non-vernalized WW1033 controls plants maintained in warm 16h LD did not bolt or flower). However, under LLD both stem elongation and flowering occurred with cv. WW1033, even without GA applications. The mechanism by which LLD can substitute for vernalization remains unknown. However, the differential effect of GA_5 and 2,2-dimethyl GA_4 (floral promotive) relative to GA_1 and GA_4 (non-floral promotive) under 16h LD is reminiscent of the differential efficacy shown for these same GAs in *Lolium* (Evans *et al.*, 1990). Thus, in CRP of *Brassica*, GAs with certain structural characteristics (A-ring didehydro or C-2β-dimethyl, and C ring with one or more hydroxyls) interact positively with LD to replace the low temperature requirement. Lang (1965) also noted the interrelationship between photoperiod and vernalization, and for *Arabidopsis* cultivar variation in the ability of photoperiod to substitute for vernalization (Table 3.1:, Napp-Zinn, 1969).

The induction of floral raceme formation for the summer annual cv. Westar under an 11h photoperiod was unexpected. In past experiments with cv. Westar, a photoperiod greater than 13h was always required to promote flowering. Thus, I was unable to determine whether this novel result was due to a new seed batch of cv. Westar having been selected for use under a shorter day length, or whether

some environmental factor, such as FR-enrichment in new growth chamber lights, might have caused this difference. Raceme formation without further floral development also shows that photoperiod (or other factors, such as FR-enrichment) was sufficient to induce floral bud initiation but a further increase in day length was required to trigger the next stages of inflorescence development. Exogenous application of GA_5 and 2,2-dimethyl GA_4 could substitute for a longer daylength for obtaining anthesis in cv. Westar, but not for the elongation of the raceme axis, indicating that flower development may be controlled by different GAs than those responsible for bolting (i.e. speculatively, florigenic and flower bud-opening GAs are promoted by 11h days, but bolting GAs requiring a longer day length). What may be required then, is the combination of a highly florigenic GA (e.g., GA_5) plus an elongation-promoting GA (e.g., GA_1) to mimic the effect of the 20h photoperiod which caused proper development of the inflorescence and its flowers.

The floral promotive effect of cytokinins was reviewed by Bernier *et al.*, (1981). Even though flowering in some SD, LD and DN species was promoted by exogenous cytokinin applications, flowering was inhibited by cytokinins in many of SD, LD and all CR species (including *B. rapa*). This contradictory evidence (cytokinins being promotive and inhibitory) may be due to the concentration, timing, site and mode of application, or the presence or absence of other factors (e.g., GAs or carbohydrates) (Bernier *et al.*, 1981). Pharis (1972) observed a synergistic effect of cytokinins (BA) and GAs (especially GA_5) in the promotion of flowering in

Chrysanthemum morifolium and flowering could even be promoted by applications of BA alone. Applications of BA alone could not promote flowering in *B. napus* cv. WW1033, although quite low levels of BA could synergize the GA According to Bernier *et al.*, (1981), the role of cytokinins in floral initiation involves an early mitotic stimulation and synchronization of development in the meristem region. This could prepare the meristem region for increased efficiency in sensing the inductive GA, which in turn may explain the reduced levels of GAs required to induce flowering in the presence of BA.

SUMMARY

The exogenous application of GAs to *Brassica* spp. showed very different results on flowering vis-à-vis stem elongation depending upon species, cultivar and GA structure. In general, growth-active GAs (as defined by their ability to cause leaf sheath elongation in the dwarf rice bioassay) result in stem elongation in all *Brassica* cultivars tested. Additionally, one CRP cultivar, *B. napus* cv. WW1033 flowered under a number of experimental conditions including the exogenous application of GA_5 and 2,2-diMe GA_4 as well as with continuous light (LLD). The differing responses of several related cultivars of *Brassica* to the same treatments illustrates the complexities of floral initiation. Differences in the timing of inflorescence appearance between vernalized (prior to stem elongation) and GA-treated plants (after stem elongation), together with results of application of GAs of varied structure under non-vernalized conditions, indicates that GAs are likely involved in both processes (flowering and bolting) in *Brassica*.

CHAPTER FOUR

Never do anything that bores you (JD Watson, 1993).

THE EFFECT OF LIGHT QUALITY ON SHOOT GROWTH AND GA LEVELS

INTRODUCTION

The use of controlled environment conditions to grow plants allows for stringent control of environmental factors. However, a downside of this type of control is that the simulation of natural growth conditions is not ideal, with light quality being the major concern. Plants have evolved to maximize their growth and development in a wide range of sunlight conditions, including shorter plants growing under taller plants. In studying plants grown under artificial light sources, we must remember that at least some of the observed growth characteristics may result from abnormal light conditions. Thus, in designing experiments using artificial light, care must be taken to ensure that light quality is monitored and maintained.

The means by which plants sense the quality of red and far-red light is via the phytochrome family of photoreceptors. Red (655 to 665 nm) and far-red (725 to 735 nm) light cause a structural change in the biliprotein, phytochrome, which

leads to differing physiological response. According to Smith (1982 and 1995) the major functions of phytochrome in higher plants are:

- to detect photoperiod length
- to detect light quality (level of shading by other plants)
- to detect presence of light (de-etiolation response)

Sharrock and Quail (1989) isolated and characterized five phytochrome genes from *Arabidopsis* and work reviewed in Smith (1995) (using mutants and transgenic plants) has attempted to assign roles to individual members of the phytochrome family. Extensive work has been carried out with phytochrome mutants (the majority with *Arabidopsis*) to determine the several functions of members of the phytochrome family (Zagotta *et al.*, 1996; Reed *et al.*, 1994; references cited in Smith, 1995). Much of this work deals with the inhibition of hypocotyl elongation of dark-grown seedlings by light (i.e. de-etiolated). According to Smith (1995), phytochrome A mediates the high-irradiance far-red response and phytochrome B is responsible for the classical low irradiance red\far-red reversible responses.

The relationship between phytochrome and physiological action has been the subject of speculation involving many responses including: flowering (Vince-Prue, 1983); stem and hypocotyl elongation [in pea, (Kende and Lang, 1964), bean, (Lockhart, 1964) and *Sinapis alba,* (Morgan *et al.*, 1980)] and photoperiod perception (reviewed extensively in Vince-Prue 1975, Smith, 1995). However, although evidence for the connection is extensive, it is not yet definitive.

The involvement of phytochrome in the detection of photoperiod length by plants was first indicated by light break experiments. In a classic experiment, Hamner and Bonner (1938) began to unravel the complexities of photoperiod responses using the SD plant *Xanthium strumarium*. Flowering of SD plants exposed to an inductive long night could be inhibited by a short exposure to red light. This inhibition could be reversed by subsequent exposure to FR light (see historical review in Smith and Whitelam, 1990). This led to the theory that phytochrome undergoes a reversion from Pfr to Pr during the long night with flowering being induced if a critical level of Pr is reached. Red light during the night break re-establishes the previous level of Pfr, thereby inhibiting flowering. Far-red light given at the end of a day, or during a night break, changes the Pfr to Pr and flowering ensues. Therefore, it is the ability of phytochrome to interchange between its two states in response to light and the night reversion, that allows for a plant to determine the length of the photoperiod.

A second function of phytochrome is to determine the level of shading by other plants (Smith, 1995). Most plant species must have sufficient light to mature. Thus, they must avoid shading (except shade species). Unobstructed sunlight has a R:FR ratio of near 1, but light filtered through dense vegetation can be as low as 0.2 (Holms and Smith, 1977). The overtopping vegetation removes much of the photosynthetic active blue and red light leaving, after transmission, a higher proportion of FR (Smith, 1995). It is the high level of FR, acting through phytochrome that triggers the increased elongation of the shaded plant.

The ability of a plant to sense light is essential for the transition from germination to photo-autotrophic growth (Smith and Morgan, 1983). Etiolated seedlings, upon emergence from the soil, change their growth characteristics. They become green, have decreased elongation and increased leaf area. This transition is phytochrome-mediated (Smith, 1995).

Many of the functions of phytochrome have a relationship with GAs (i.e. phytochrome-mediated flowering and elongation in many species can be enhanced by exogenous GA applications (Mandel *et al.*, 1991)) and/or plants have been shown to have increased GA levels during flowering or elongation (Metzger, 1985). In this chapter, three extensive experiments were carried out:

- To assess the effects of light quality on hypocotyl elongation and GA levels of young seedlings (seven days post-emergence) of the summer annual *B. napus* cv. Westar and determine whether elongation responses were due to changes in cell elongation or in cell number
- To assess the effects of light quality on epicotyl length and GA levels of *B. napus* cv. Westar plants (45 days post-emergence)
- To assess the effects of light quality on epicotyl length and GA levels of *B. napus* cv. WW1033, a CRP (biennial cultivar) plants (42 days post-emergence)

With a goal of investigating whether elongation growth changes caused by different light qualities can be related to the levels of GAs present in their elongating tissue.

MATERIAL AND METHODS

Summer annual *B. napus* cv. Westar and CRP biennial cv. WW1033 were grown in the Canberra Black Mountain Phytotron, as noted earlier in the general material and methods chapter. Light intensity in the growth chambers was controlled at or near 375 µmole photon $m^{-2}s^{-1}$ during the 10h growth period, and somewhat less than 25 µmole photon $m^{-2}s^{-1}$ during the 4h extension phase. Plants grown in natural light conditions were maintained such that plants received 10h of high intensity sunlight followed by 4h of low intensity light.

Plants used to determine the effects of light quality during germination were sown in growth media, either in the greenhouse or under growth chamber conditions. Plants germinated in greenhouse conditions were transferred to growth chambers after emergence but did receive an 8h of exposure to natural high intensity sunlight.

After 7 days some of the annual (cv. Westar) plants were harvested for histology and hormone measurements of the elongated hypocotyl. Others were maintained to determine growth characteristics and harvested for analysis of stem tissue at day 45 post-emergence. In a parallel study, plants of the biennial cv. WW1033 were grown until day 42 post-emergence before harvesting for GA analysis.

RESULTS

The spectral composition of light used to grow plants is shown in Figure 4.1. Fluorescent tubes show an increase in energy over the natural sunlight (open greenhouse) in the green to orange portion of the spectrum (550 to 650 nm), a slight decrease in blue region (450 to 550 nm) and a dramatic decrease in to far-red region (725 to 735 nm). Metal halide bulbs show an energy profile that is higher than natural sunlight in all regions except the FR.

Spectra of supplemental lights are shown in Figure 4.2. Incandescent bulbs result in an appreciable increase in both the FR region (725 to 735 nm) and R regions (655-665 nm). The BCJ bulbs have an increase in the FR region, but little increase in the R region. Table 4.1 thus shows a comparison of light intensity in each growth condition as well as the R:FR ratios. The BCJ bulbs give off the lowest R:FR, although incandescent bulbs yield nearly twice the intensity of R. Fluorescent tubes also give off large amounts of R, but emit only small amounts of FR. Natural light has nearly equal levels of R:FR. The metal halide bulbs most closely mimic natural sunlight, although with a slight increase in the level of R.

Daylight extension, with narrow wavelength ranges of light, establishes a phytochrome condition within the plant's leaves based on the wavelength of light that the plant was exposed to just prior to the dark period. That is, daylength extension with R results in Pfr form phytochrome and extension with FR results in the Pr form.

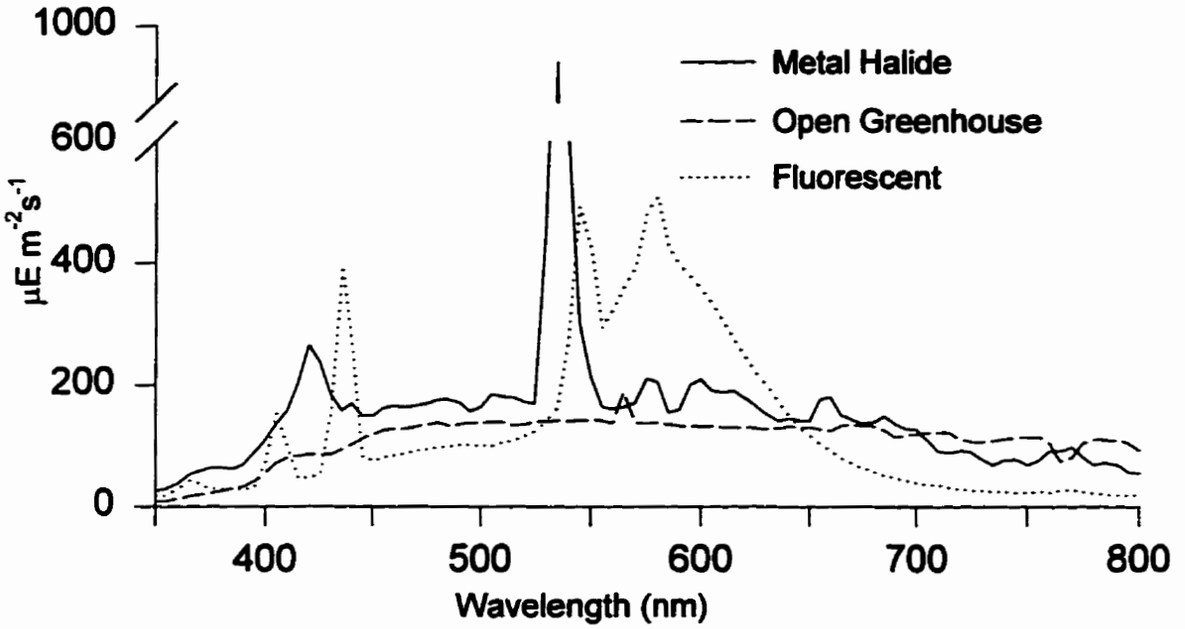


Figure 4.1. Spectra of light from metal halide bulbs, fluorescent tubes and natural sunlight under greenhouse conditions.

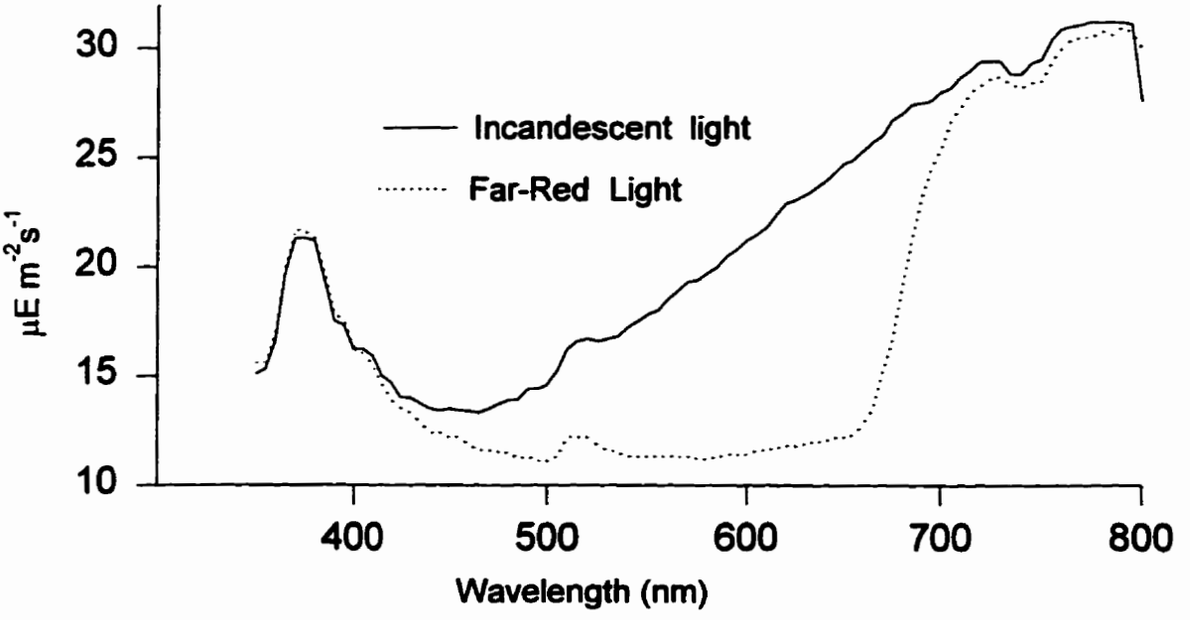


Figure 4.2. Spectra of light from incandescent and BCJ (far-red) bulbs used for photoperiod extension.

Seven day post-emergence *B. napus* cv. Westar Hypocotyl Growth

Growth Effects

Figure 4.3 and 4.4 shows the relative hypocotyl length of seven day post-emergence *B. napus* cv. Westar grown under five different light conditions. The smallest hypocotyl elongation was observed under greenhouse conditions (natural daylight; R:FR ratios almost balanced). Elongation of hypocotyls of plants grown in growth chambers increased with an increase in R (metal halide and fluorescent sources) but it also increased with decreased R (incandescent and far-red supplemented sources). This trend is borne out when final hypocotyl length is plotted against the R:FR ratios (Figure 4.5). The least hypocotyl elongation occurs with a R:FR ratios near 1 and elongation increases as the R:FR increases or decreases. The trend is significant at $P \leq 0.02$.

Hormone Levels

Plant hormone levels seven day post-emergence hypocotyls of *B. napus* cv. Westar of measured for plants grown under the five light treatments (Table 4.2)..

The early 13-hydroxylated GAs were the most abundant, with levels of GA_{20}, the immediate precursor and of GA_1, and GA_8 its immediate deactivation product, being the most plentiful. Levels of ABA and IAA were also determined, but there was no obvious relationship between levels of these hormones and the various growth parameters. Gibberellin A_5 and A_6 were not detected in any of the tissue analysed ($[^2H_2]$ GA_5 and $[^2H_2]$ GA_6).

To determine if the levels of GAs detected could account for the hypocotyl

elongation seen, a series of first order regression analysis of GA levels and hypocotyl length were carried out for plants grown under five light conditions (various R:FR ratios), 14h photoperiod and warm conditions (except metal halide (10h)). Figure 4.6 shows that the positive trend between GA_1 levels and hypocotyl elongation is not significant at $P \leq 0.05$)(critical $r^2=0.613$). Other GAs, ABA and IAA also showed no significant relationship between hormone levels and hypocotyl elongation when analysed individually.

To determine if levels of certain GAs, when grouped were correlated with hypocotyl elongation, a series of regression analyses of grouped GA levels with elongation were accomplished. Table 4.3 shows levels of the likely "per se active" GAs found within hypocotyl tissue (i.e. $GA_1+GA_3+GA_4+GA_7$) and Figure 4.7 shows the relationship between their sum and hypocotyl length (significant at $P \leq 0.02$). Table 4.4 shows levels of various GAs of the 13-hydroxylation pathway relative to their "light quality" environment. Figure 4.8 shows the relationship between the total of the 13-hydroxylation pathway GA levels (i.e. $GA_{19}+GA_{20}+GA_1+GA_8$) and hypocotyl elongation and Figure 4.9 shows the relationship without GA_8. The positive relationship shown between each of these summations (Figures 4.7, 4.8 and 4.9) and hypocotyl length are all highly significant ($P \leq 0.02$).

Other growth parameters of the seven day post-emergence seedlings were also measured and the relationship between these parameters and GA levels investigated. The length of hypocotyl cortical cells was used to determine if the increase in hypocotyl length was a result of cell elongation or division. Figure 4.10

thus shows the mean cortical cell length. The calculated number of cells per hypocotyl (i.e. hypocotyl length÷cell length)(Table 4.5) was used to determine the changes in cell length and cell number relative to those found in the shortest hypocotyls (i.e. for plants grown under open greenhouse conditions). Thus, the increase in hypocotyl length of metal halide-grown plants is due mainly to an increase in cell number. Incandescent extension of metal halide lights results in a further increase in cell number, with only a slight increase in cell length. Fluorescent light results in a nearly equal increase in cell length and cell number (nearly 3-fold), whereas FR extension of the metal halide light nearly doubles the cell numbers and also gives a 3.4-fold increase in cell length.

To determine if there is a relationship between cortical cell length and GA level, first order regressions were carried out. Figure 4.11 shows that the positive trend between GA_1 levels and cortical cell length is not significant at $P \leq 0.05$. The positive trend between the sum of levels of the bioactive GAs (Figure 4.12) is also not significant at $P \leq 0.05$. However, the relationship between the sum of the GA levels of the early C13-hydroxylation pathway and cortical cell length is highly significant ($P \leq 0.01$)(Figure 4.13) and is also significant for GAs of the early C13-hydroxylation pathway without GA_8 ($P \leq 0.05$)(Figure 4.14).

Figure 4.15 shows the effect of light treatments on the stem dry weight, but there was no significant relationship between stem dry weight and GA levels by regression analysis (data not shown).

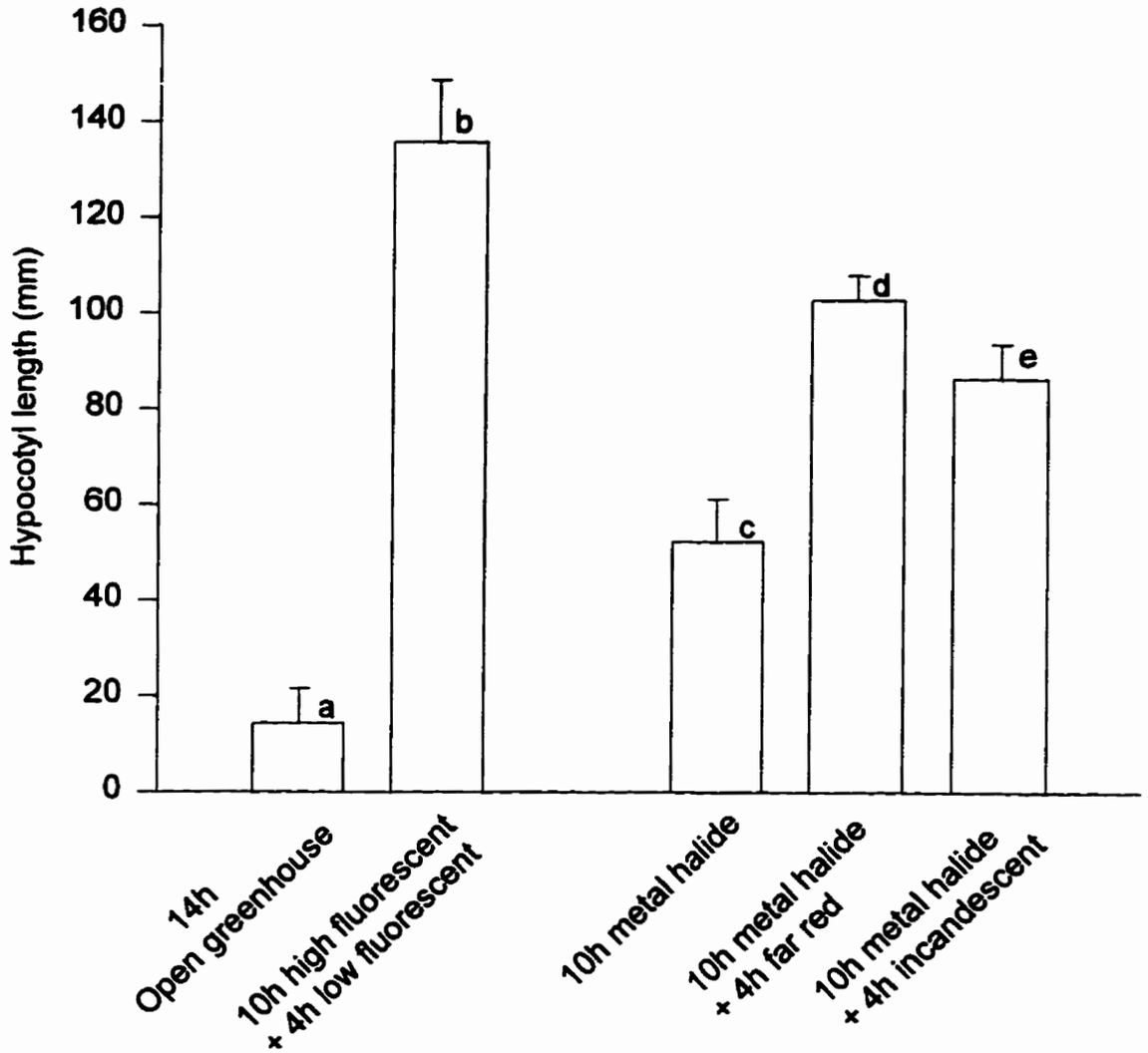


Figure 4.3. The effect of light treatments on hypocotyl length at seven days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

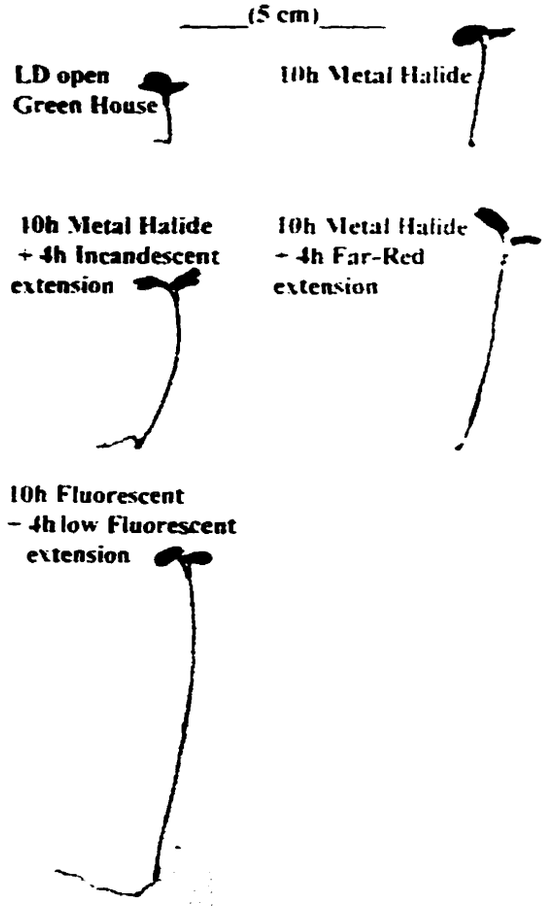


Figure 4.4. The effect of light treatments on hypocotyl length at seven days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. Details of light treatments are given in Table 4.1.

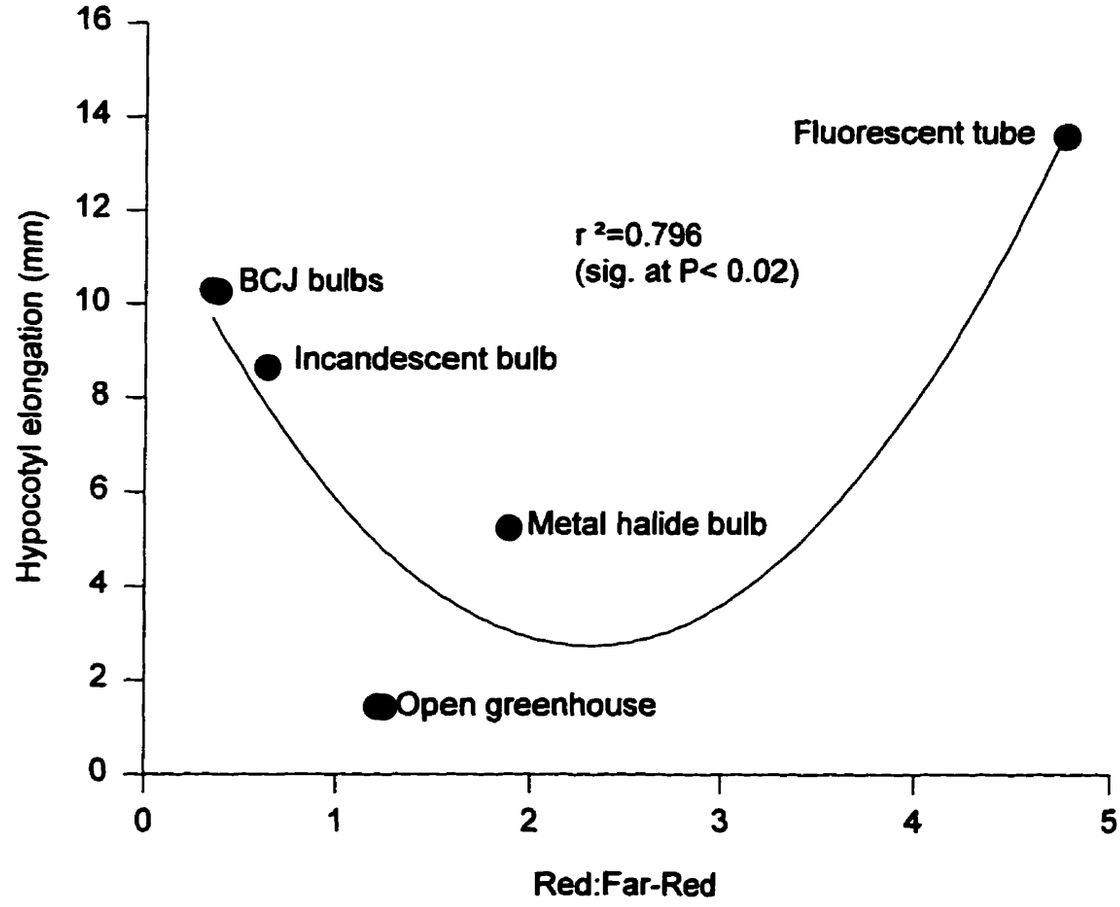


Figure 4.5. The relationship between R:FR ratios in light given during 4h photoperiod extension on hypocotyl elongation at day seven post-emergence for *B. napus* cv. Westar seedlings.

Table 4.1. The red to far-red ratios (R:FR) and photosynthetically active radiation (PAR) of light used to grow seedlings of *Brassica napus* cv. Westar.

	10h Metal Halide	4h Far-Red extension	4h Incandescent extension	Fluorescent 10h high	Fluorescent +4h low extension	Greenhouse 10h high	Greenhouse +4h low extension
PAR $\mu E\ m^{-2}sec^{-1}$	388	1.06	14.9	375	22.1	1400	220
R:FR ratios	1.62	0.35	0.64	4.77	4.65	1.22	1.19

Table 4.2 Hypocotyl length and levels of plant hormones found in hypocotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 7 days post emergence. Values are ng GA, IAA and ABA / g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)) ND= non-detected levels.

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10h Metal Halide	14h Open Greenhouse
Hypocotyl Length (cm)	10.28 ±.52	8.64 ±1.01	13.58 ±1.29	5.23 ±.89	1.43 ±.72
GA_1	16.35 ±1.61	17.31 ±2.00	12.34 ±2.04	10.89 ±.89	5.71 ±.50
GA_3	5.89 ±.43	9.52 ±1.22	7.62 ±1.61	7.72 ±.49	6.41 ±1.84
GA_4	5.28 ±1.31	4.04 ±.46	11.40 ±1.14	5.90 ±1.55	8.38 ±.42
GA_5, GA_6	ND	ND	ND	ND	ND
GA_7	6.82 ±1.07	7.77 ±.44	12.18 ±.89	8.51 ±1.24	6.71 ±.82
GA_8	37.40 ±5.57	15.90 ±2.41	39.44 ±5.17	17.70 ±5.89	16.75 ±7.99
GA_9	3.90 ±1.27	5.29 ±.98	5.76 ±1.95	5.21 ±1.24	4.39 ±1.04
GA_{20}	4.15 ±.57	5.23 ±.59	7.57 ±.13	6.38 ±.43	3.99 ±.78
GA_{19}	21.30 ±2.86	15.31 ±1.82	20.50 ±2.66	11.85 ±.74	7.65 ±.50
ABA	98.74 ±4.68	78.18 ±6.63	77.01 ±8.43	75.00 ±9.51	70.40 ±6.21
IAA	331.10 ±113.78	274.25 ±117.25	727.35 ±114.08	622.72 ±60.89	1188.96 ±113.52

Table 4.3.Hypocotyl length and levels of bioactive GAs found in hypocotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 7 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10h Metal Halide	14h Open Greenhouse
Hypocotyl Length (cm)	10.28 ±.52	8.64 ±1.01	13.58 ±1.29	5.23 ±.89	1.43 ±.72
GA_1	16.35 ±1.61	17.31 ±2.00	12.34 ±2.04	10.89 ±.89	5.71 ±.50
GA_3	5.89 ±.43	9.52 ±1.22	7.62 ±1.61	7.72 ±.49	6.41 ±1.84
GA_4	5.28 ±1.31	4.04 ±.46	11.40 ±1.14	5.90 ±1.55	8.38 ±.42
GA_7	6.82 ±1.07	7.77 ±.44	12.18 ±.89	8.51 ±1.24	6.71 ±.82
Total Active GAs	34.24	38.64	46.54	33.04	26.76

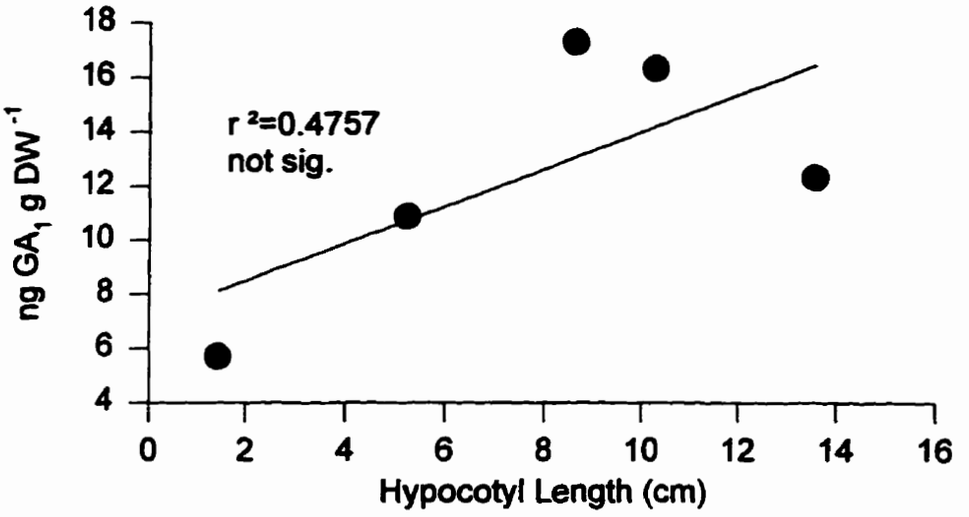


Figure 4.6. Regression of GA_1 levels extracted from hypocotyls and hypocotyl length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the mean of three GA_1 levels. Details of light treatments are given in Table 4.1.

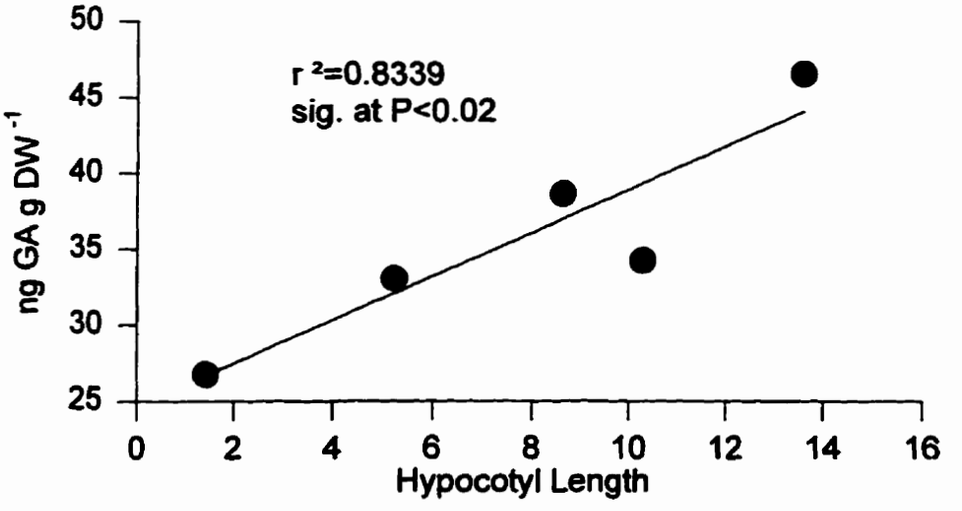


Figure 4.7. Regression of the total level of bioactive GAs (GA_1+GA_3+GA_4+GA_7) extracted from hypocotyls and hypocotyl length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

Table 4.4 Hypocotyl length and levels of GAs from the early-C13-hydroxylation pathway found in hypocotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 7 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10h Metal Halide	14h Open Greenhouse
Hypocotyl Length (cm)	10.28 ±.52	8.64 ±1.01	13.58 ±1.29	5.23 ±.89	1.43 ±.72
GA_1	16.35 ±1.61	17.31 ±2.00	12.34 ±2.04	10.89 ±.89	5.71 ±.50
GA_8	37.40 ±5.57	15.90 ±2.41	39.44 ±5.17	17.70 ±5.89	16.75 ±7.99
GA_{20}	4.15 ±.57	5.23 ±.59	7.57 ±.13	6.38 ±.43	3.99 ±.78
GA_{19}	21.30 ±2.86	15.31 ±1.82	20.50 ±2.66	11.85 ±.74	7.65 ±.50
Total GA ng/g D.W.	79.15	53.75	79.85	46.82	34.1

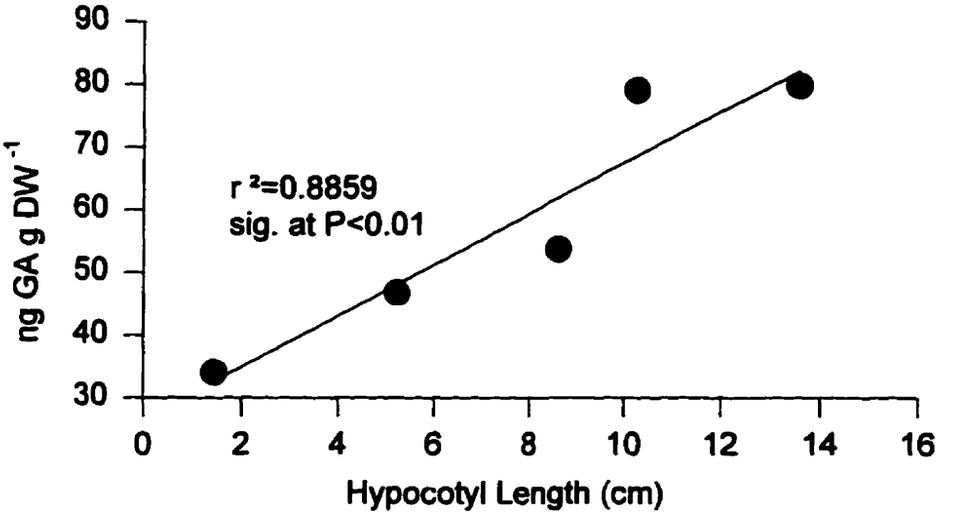


Figure 4.8. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}+GA_8) extracted from hypocotyls and hypocotyl length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

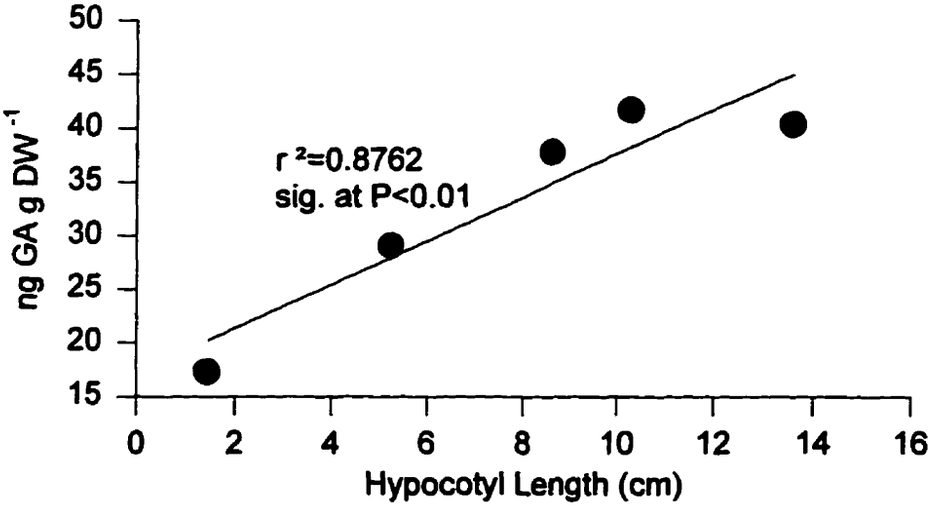


Figure 4.9. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}) extracted from hypocotyls and hypocotyl length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

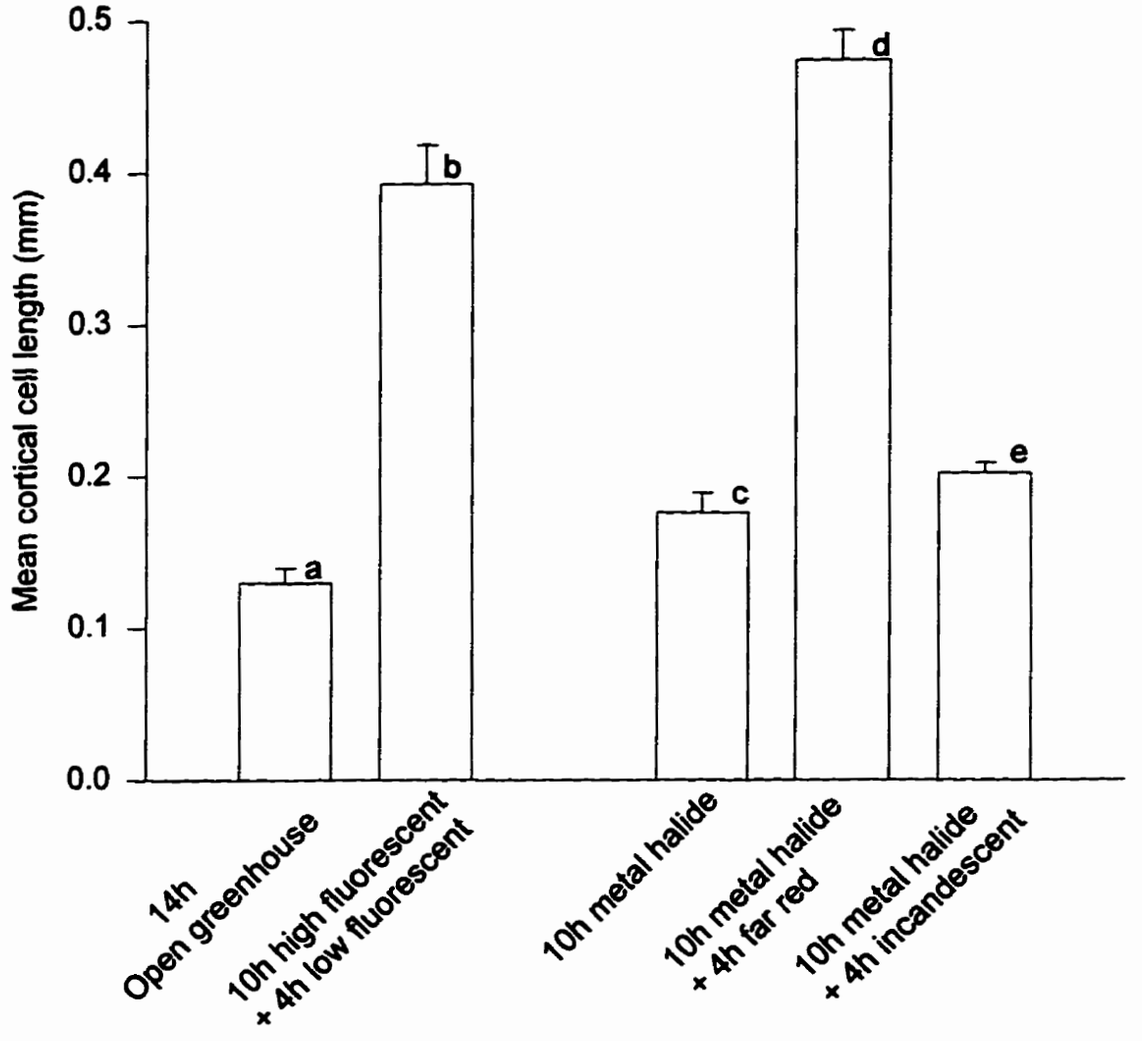


Figure 4.10. The effect of light treatments on hypocotyl cortical cell length at seven days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

Table 4.5 Hypocotyl length, hypocotyl cortical cell length and hypocotyl cell number for seedlings of *B. napus* cv. Westar grown under various light conditions seven days post-emergence. ± 95% confidence levels, letters represent SNK groupings ($P \leq .05$)). Details of light treatments are given in Table 4.1.

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10h Metal Halide	14h Open Greenhouse
Hypocotyl Length (cm)	10.28 ±.52	8.64 ±1.01	13.58 ±1.29	5.23 ±.89	1.43 ±.72
Cortical Cell Length (mm)	0.455	0.194	0.385	0.17	0.134
calc. # cells in total hypocotyl length	22.1	43.2	34.12	28.27	11.44
relative increase in cell length	3.4	1.45	2.87	1.27	1
relative increase in cell number	1.93	3.78	2.98	2.47	1

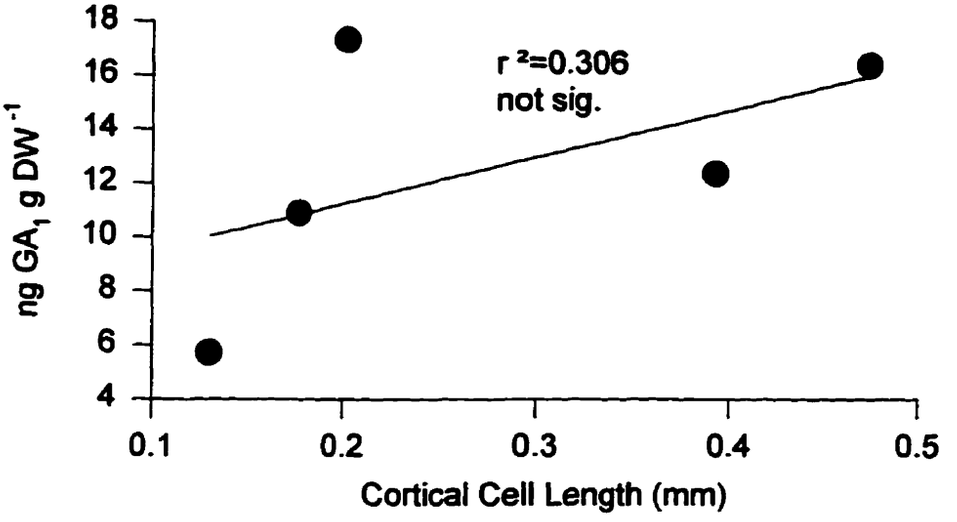


Figure 4.11. Regression of GA_1 levels extracted from hypocotyls and hypocotyl cortical cell length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the mean of three GA_1 levels. Details of light treatments are given in Table 4.1.

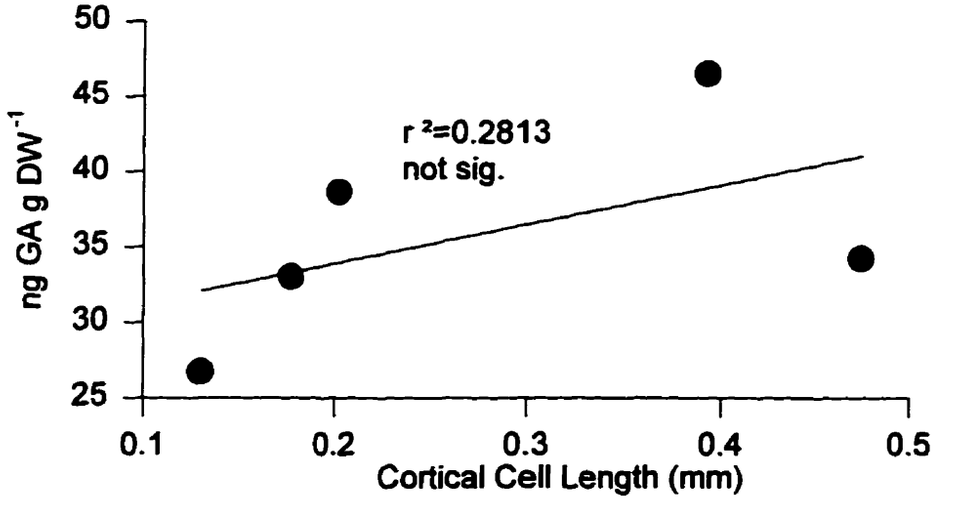


Figure 4.12. Regression of the total level of bioactive GAs (GA_1+GA_3+GA_4+GA_7) extracted from hypocotyls and hypocotyl cortical cell length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

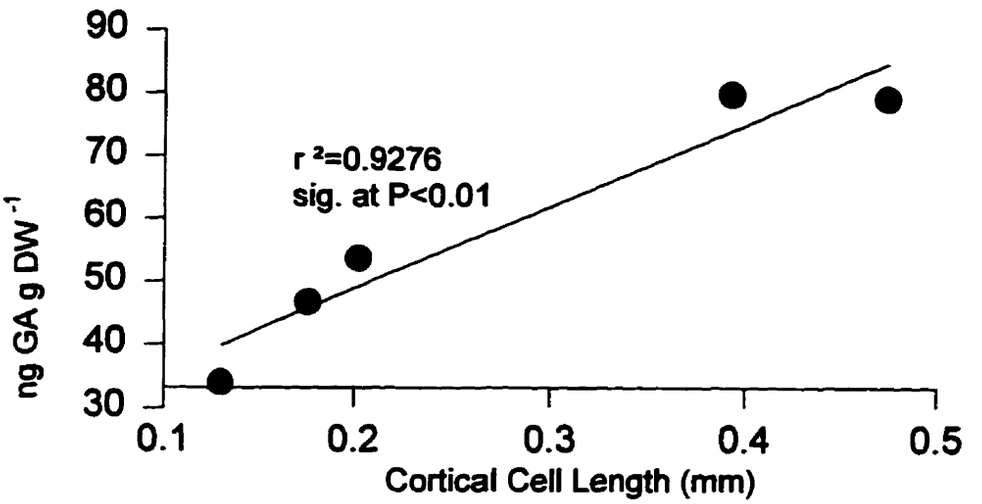


Figure 4.13. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}+GA_8) extracted from hypocotyls and hypocotyl cortical cell length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

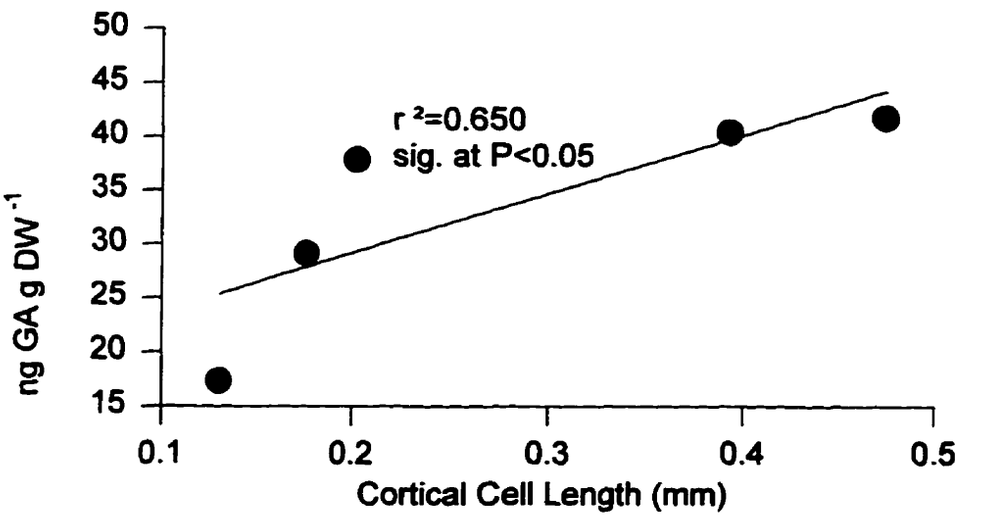


Figure 4.14. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}) extracted from hypocotyls and hypocotyl cortical cell length for seven day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

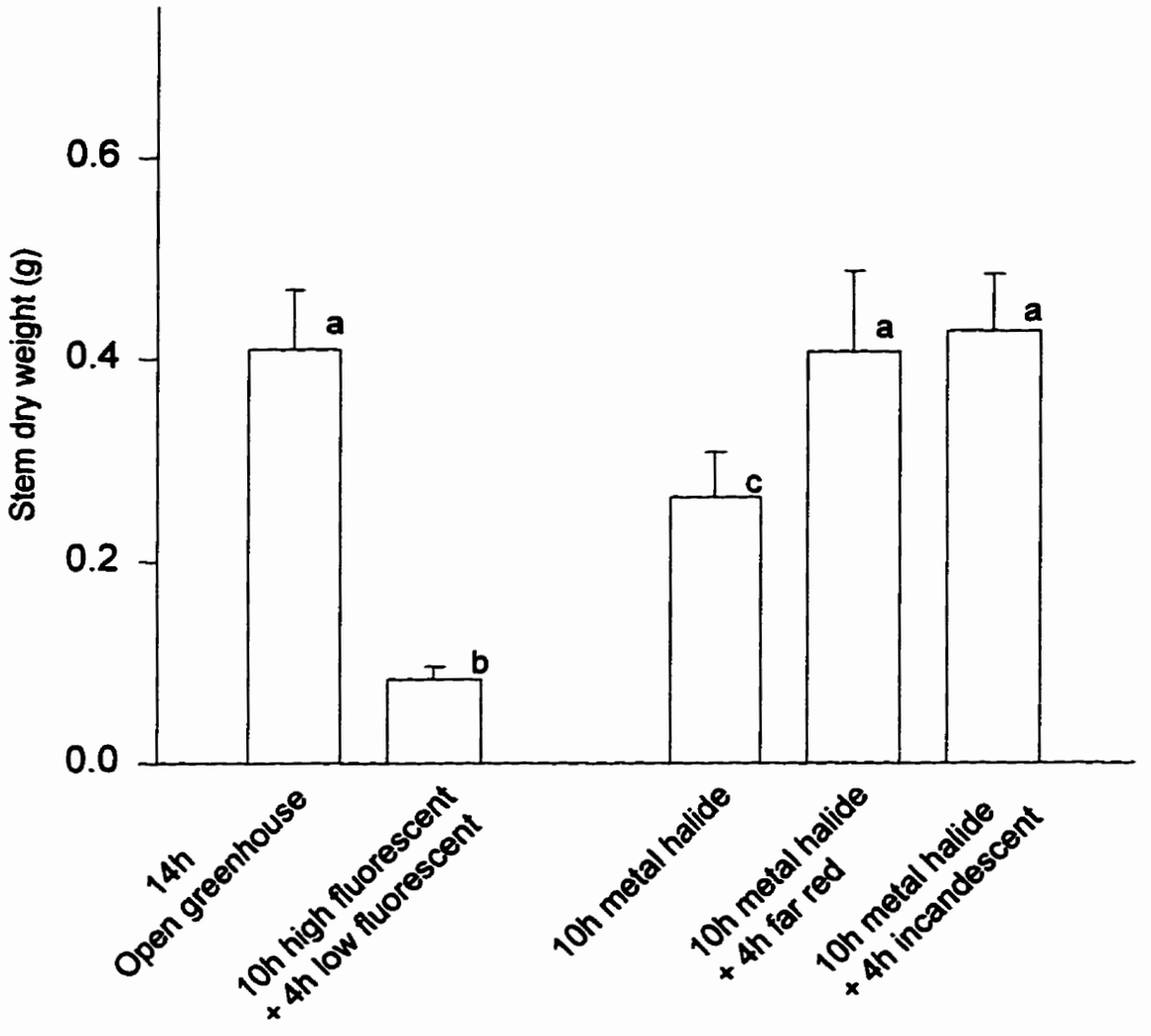


Figure 4.15. The effect of light treatments on stem dry weight at seven days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

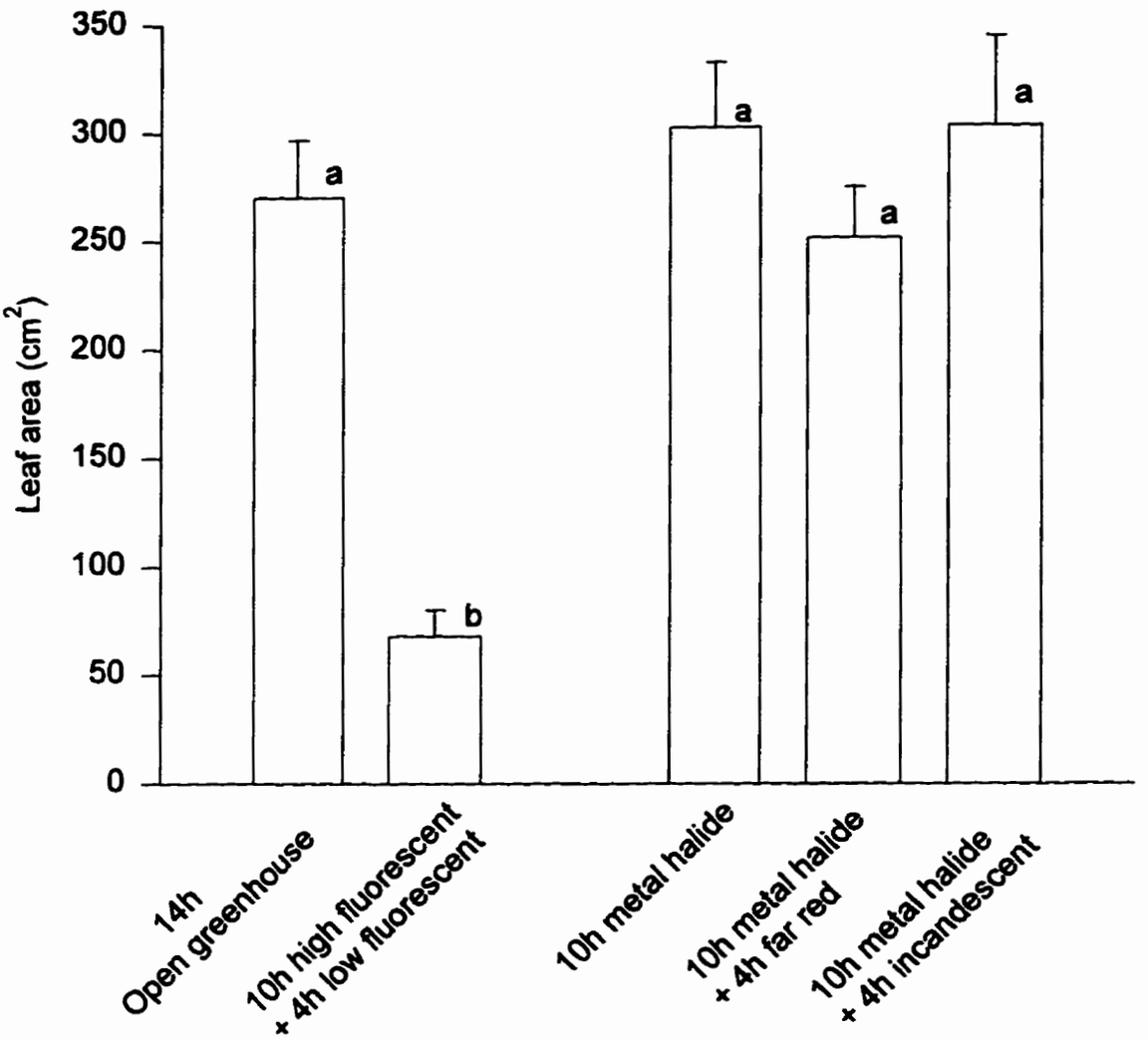


Figure 4.16. The effect of light treatments on leaf area at seven days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

45 day post-emergence *B. napus* cv. Westar Epicotyls

Growth Effects

Figure 4.17 shows the height (epicotyl length) of 45 day post-emergence *B. napus* cv. Westar grown under five different light conditions. Until day 7, most of the stem elongation was the result of hypocotyl elongation (Figure 4.18), but beyond 7 days hypocotyl elongation ceased and epicotyl elongation dominated. Epicotyl elongation of fluorescent, greenhouse and metal halide light grown plants (R:FR =4.77, 1.22 and 1.62 respectfully) showed little increase relative to other light conditions. However, FR induced elongation was evident under metal halide conditions supplemented with incandescent light (R:FR= 0.64)(3-fold increase over non-supplemented, metal halide only) and further with far-red supplementation (R:FR= .35)(4.5 fold increase over non-supplemented, metal halide only). This increased growth was due to increased internode elongation as the number of internodes remained relatively constant (data not presented).

Figure 4.18 shows the effect of light quality differences on early hypocotyl and epicotyl elongation. The left column of Figure 4.18 shows hypocotyl length and height to apex (epicotyl length is the area between the two lines) of plants germinated in natural sunlight and then transferred to growth chambers (which provide light of different qualities). The right column shows hypocotyl length and height to apex for plants germinated and grown in growth chambers. In all cases, the increased height to apex for growth chamber-germinated plants over those of greenhouse-germinated plants can be accounted for by hypocotyl elongation.

Hence, there is no significant difference between the two germination locations for epicotyl lengths.

Hormone Levels

Plant hormone levels measured for epicotyl tissue grown for 45 day post-emergence *B. napus* cv. Westar in the five light treatments are shown (Table 4.6).

As with seven day old hypocotyl elongation and hormone levels in the hypocotyls, the early 13-hydroxylated GAs were the most abundant in epicotyl tissue, with GA_1, GA_{20} and GA_8 being the most plentiful (Table 4.6). Levels of ABA and IAA were also determined, but there was no obvious relationship between levels of these hormones and the growth parameters measured. Gibberellin A_5 and A_6 were not detected in any of the tissue analysed (deuterated internal standards were recovered).

Epicotyl length showed a positive and significant relationship with GA_1 levels ($P \leq 0.02$) (Figure 4.19), but trends between hormone levels and epicotyl length for other GAs, although positive, were not significant. Groupings of GAs were then made, as was done for hypocotyls. The sum of the biologically active GAs being (GA_1, GA_3, GA_4 and GA_7) compared with epicotyl length and the light quality environment (Table 4.7). Using regression analysis, it was found that the relationship between the sum of these active GAs and epicotyl length was not significant ($P \leq 0.05$)(Figure 4.20). The sum of GA levels from the 13-hydroxylation pathway and epicotyl length are shown in Table 4.8. Regression analysis showed that the sum of the 13-hydroxyl pathway GAs and epicotyl length show a positive

trend but is not significant. However, when GA_8, the inactive catabolite of GA_1, was removed from the total a positive and significant relationship was seen between GA levels and epicotyl length ($P \leq 0.02$)(Figure 4.22).

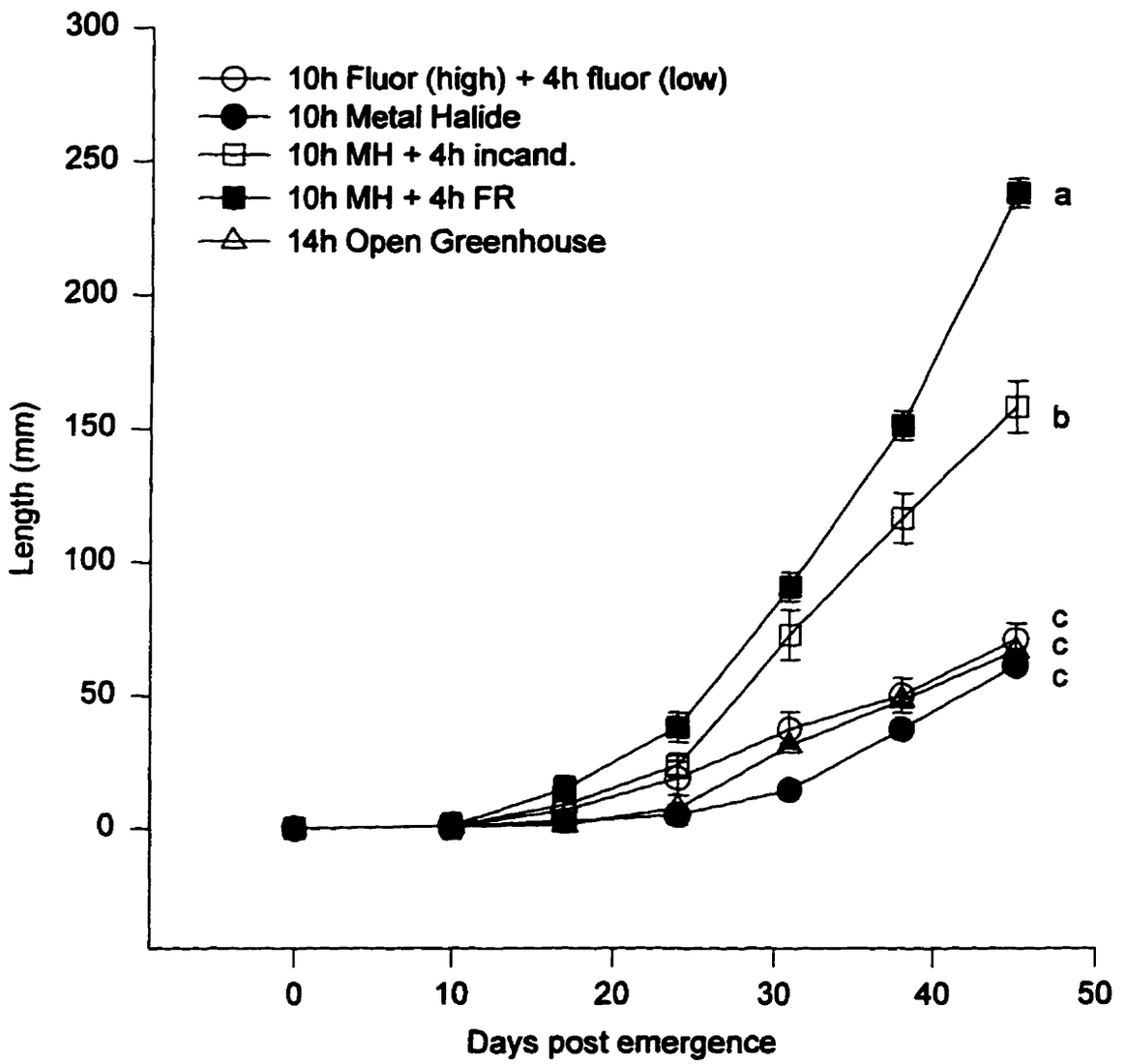


Figure 4.17. The effect of light treatments on epicotyl (stem) length at 45 days post-emergence for *B. napus* cv. Westar seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

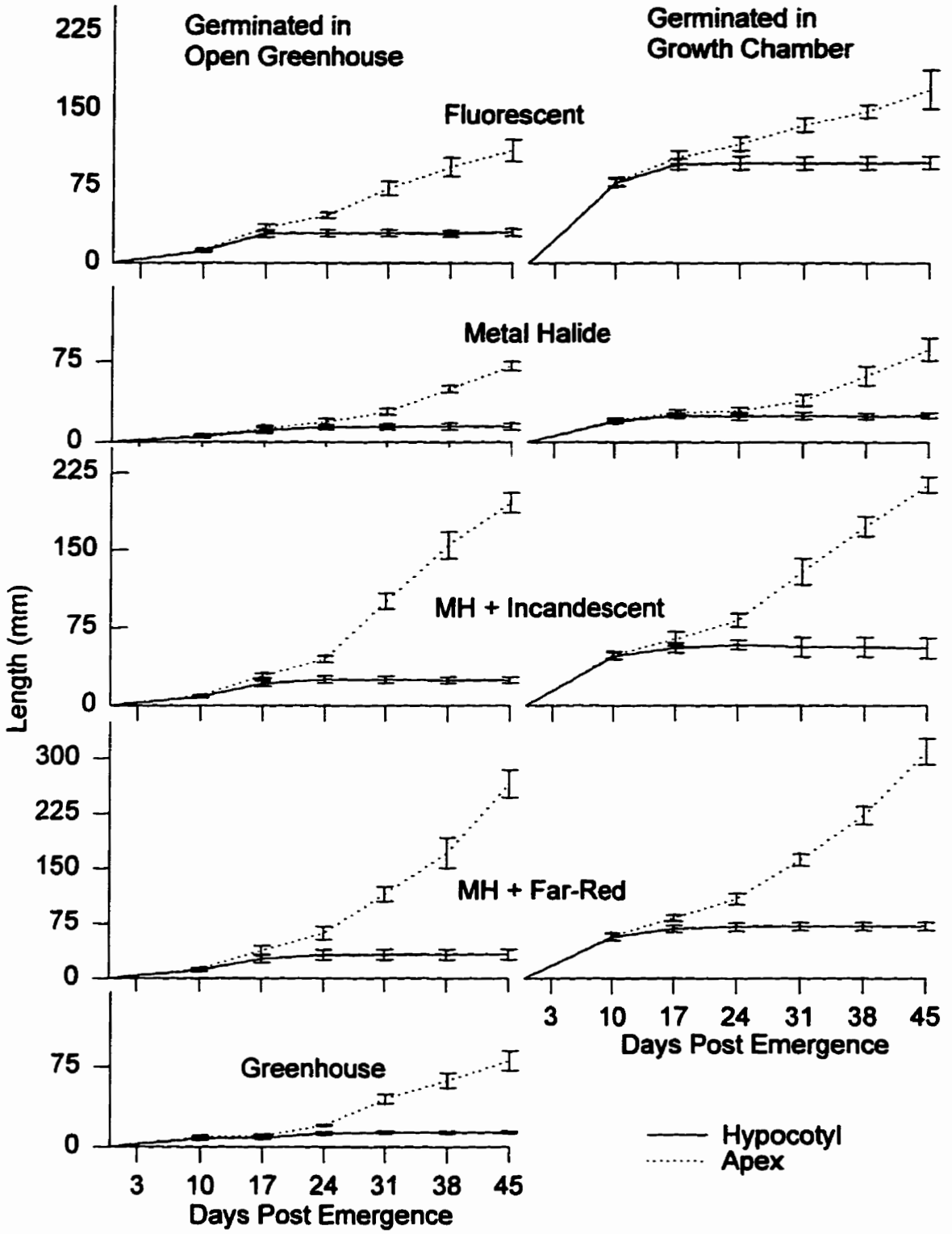


Figure 4.18. The effect of germination light quality and growth condition (under various light conditions) on hypocotyl and epicotyl length of *B. napus* Westar.

Table 4.6 Epicotyl length and levels of plant hormones found in epicotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 45 days post emergence. Values are ng GA, IAA and ABA / g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)) ND= non-detected levels.

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + low fluoro	10h Metal Halide	14h Open Greenhouse
Epicotyl Length (mm)	238 ±5.38	158.4 ±9.73	71 ±6.06	61.4 ±2.28	66.75 ±0.74
GA_1	16.31 ±2.67a	13.20 ±1.62b	10.69 ±0.83b	6.02 ±2.20c	4.81 ±1.06c
GA_3	6.61 ±.86	5.24 ±.77	5.79 ±.92	9.74 ±1.22	7.42 ±1.71
GA_4	8.35 ±1.29	8.82 ±1.67	6.94 ±1.80	9.56 ±2.23	7.26 ±1.34
GA_5, GA_6	ND	ND	ND	ND	ND
GA_7	4.89 ±.94	5.83 ±.74	5.21 ±.46	6.85 ±.94	7.30 ±.96
GA_8	20.88 ±6.30	30.11 ±6.36	27.70 ±4.94	35.19 ±7.91	8.54 ±4.06
GA_9	5.39 ±.84	3.37 ±.56	9.27 ±1.14	8.11 ±.88	5.11 ± 1.83
GA_{20}	3.58 ±.17	3.56 ±.34	4.41 ±1.27	3.28 ±.58	3.66 ±.62
GA_{19}	16.11 ±6.36	14.69 ±3.55	14.86 ±4.03	13.46 ±4.80	15.32 ±6.52
ABA	52.21 ±5.77	61.54 ±9.16	48.73 ±3.79	53.84 ±9.43	61.02 ±9.13
IAA	308.15 ±33.46a	364.15 ±36.37b	673.02 ±47.44c	204.03 ±34.97d	250.86 ±53.23d

Table 4.7. Epicotyl length and levels of bioactive GAs found in epicotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 7 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + low fluoro	10h Metal Halide	14h Open Greenhouse
Epicotyl Length (mm)	238 ±5.38	158.4 ±9.73	71 ±6.06	61.4 ±2.28	66.75 ±0.74
GA_1	16.31 ±2.67a	13.20 ±1.62b	10.69 ±0.83b	6.02 ±2.20c	4.81 ±1.06c
GA_3	6.61 ±.86	5.24 ±.77	5.79 ±.92	9.74 ±1.22	7.42 ±1.71
GA_4	8.35 ±1.29	8.82 ±1.67	6.94 ±1.80	9.56 ±2.23	7.26 ±1.34
GA_7	4.89 ±.94	5.83 ±.74	5.21 ±.46	6.85 ±.94	7.30 ±.96
Total GA ng/g D.W.	36.07	31.27	28.63	30	26.79

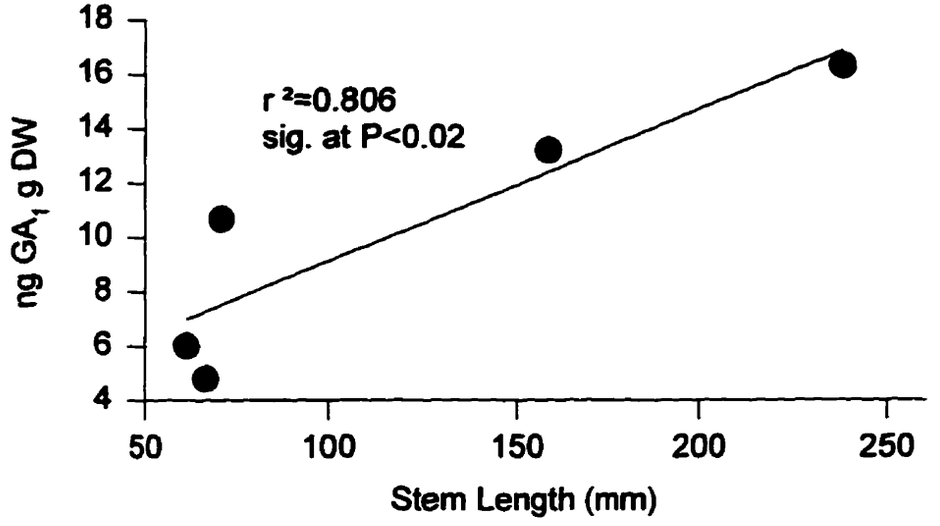


Figure 4.19. Regression of GA_1 levels extracted from epicotyls and epicotyl length for 45 day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the mean of three GA_1 levels. Details of light treatments are given in Table 4.1.

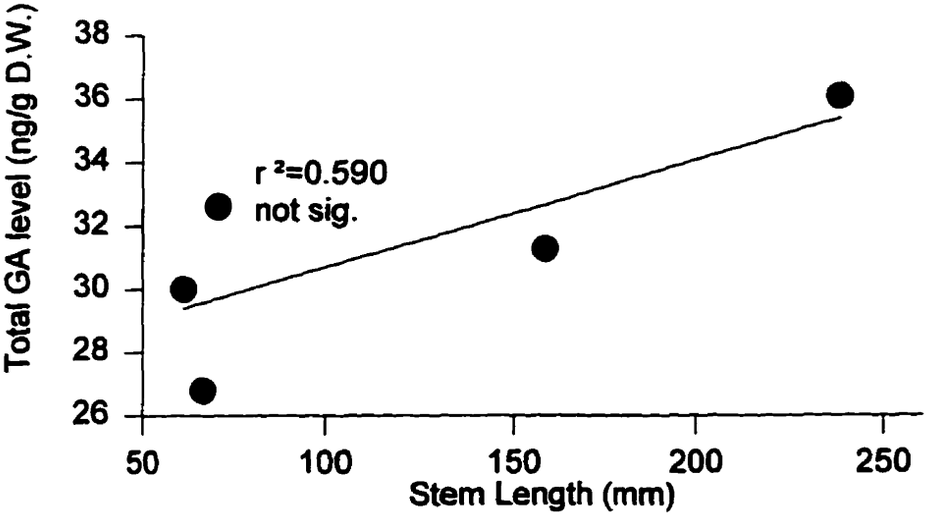


Figure 4.20. Regression of the total level of bioactive GAs (GA_1+GA_3+GA_4+GA_7) extracted from epicotyls and epicotyl length for 45 day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

Table 4.8 Epicotyl length and levels of GAs from the early-C13-hydroxylation pathway found in epicotyls of plants of *B. napus* cv. Westar grown under various light conditions measured and harvested at 45 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + low fluoro	10h Metal Halide	14h Open Greenhouse
Epicotyl Length (mm)	238 ±5.38	158.4 ±9.73	71 ±6.06	61.4 ±2.28	66.75 ±0.74
GA_1	16.31 ±2.67a	13.20 ±1.62b	10.69 ±0.83b	6.02 ±2.20c	4.81 ±1.06c
GA_8	20.88 ±6.30	30.11 ±6.36	27.70 ±4.94	35.19 ±7.91	8.54 ±4.06
GA_{20}	3.58 ±.17	3.56 ±.34	4.41 ±1.27	3.28 ±.58	3.66 ±.62
GA_{19}	16.11 ±6.36	14.69 ±3.55	14.86 ±4.03	13.46 ±4.80	15.32 ±6.52
Total GA ng/g D.W.	56.88	61.56	57.66	57.95	32.33

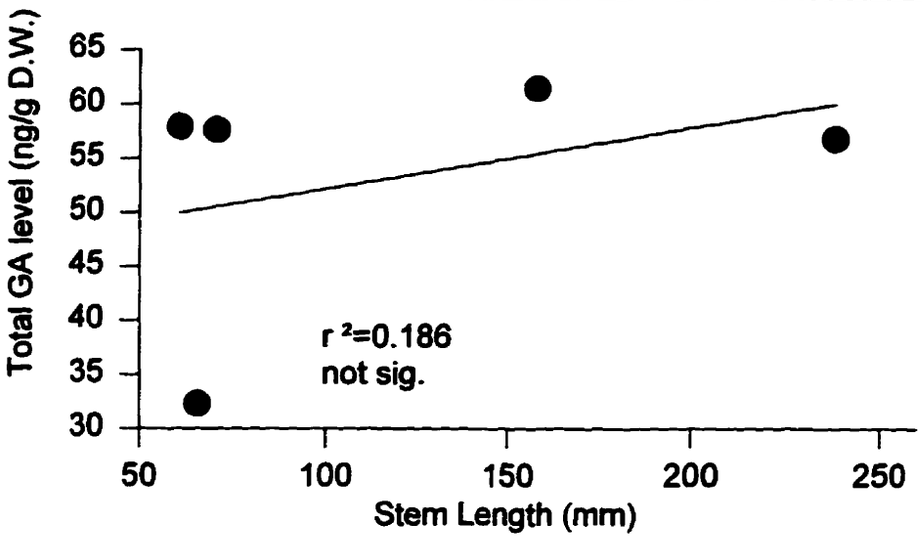


Figure 4.21. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}+GA_8) extracted from epicotyls and epicotyl length for 45 day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

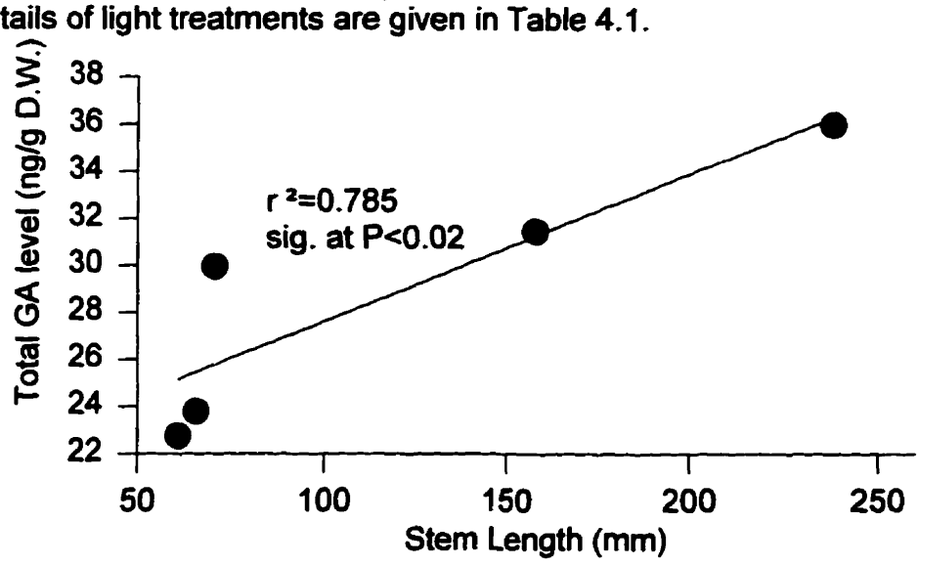


Figure 4.22. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}) extracted from epicotyls and epicotyl length for 45 day post-emergence *B. napus* cv. Westar grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

42 day post-emergence *B. napus* cv. WW1033 Stems

Growth Effects

Figure 4.23 shows the epicotyl length for plants of *B. napus* cv. WW1033 grown under five different light conditions. Tissue was harvested 42 day post-emergence. Until day 7 most of the stem growth was due to hypocotyl elongation (data not presented), after day 7, hypocotyl elongation ceased and epicotyl elongation dominated. Stem elongation of greenhouse- and metal halide-grown plants (R:FR 1.22 and 1.62 respectfully) showed little increase relative to the other light conditions. Rapid elongation through day 7 occurred when R was increased with fluorescent bulbs (R:FR= 4.77)(all growth accounted for by hypocotyl elongation). However, subsequent epicotyl elongation under this high red light did not differ significantly from growth induced by FR supplemented metal halide-grown plants. The light induced elongation was also evident in metal halide conditions supplemented with incandescent light (R:FR= 0.64)(2.4 fold increase over non-supplemented) and additional elongation occurred with FR supplementation (R:FR= .35) and high R (R:FR= 4.77)(3-fold increase over non-supplemented). This increase was due to increased internode elongation as the number of internodes remained relatively constant (data not presented).

Under a warm 10h photoperiod, *B. napus* cv. WW1033 was induced to flowering by an end-of-day extension (4h) with FR. A end-of-day extension with R or natural sunlight was ineffective at inducing flowering.

Hormone Levels

Plant hormone levels in 42 day post-emergence stem tissue of *B. napus* cv. WW1033 measured for plants growth under the five light treatments are in Table 4.9. As in the previous experiments, the early 13-hydroxylated pathway GAs predominated, with GA_1, GA_{20} and GA_8 being the most plentiful. As before, no endogenous GA_5 or GA_6 was detected and levels of ABA and IAA did not show a significant relationship with stem length.

Figure 4.24 shows a positive relationship between GA_1 and stem elongation, but significance occurred only at $P>0.10$. No other GAs showed a more significant relationship (data not presented).

Table 4.10 shows the sum of the biologically active GAs in epicotyl tissue of cv. WW1033 ($GA_1+GA_3+GA_4+GA_7$) and Figure 4.25 shows the significant relationship ($P\leq0.05$) between this sum of active GA levels and stem length. The sum of the GAs of the early-13-hydroxylation pathway is given in Table 4.11 and their regression analysis with epicotyl length is shown in Figure 4.26 and is not significant ($P>0.1$). However, when values for GA_8, the biologically inactive catabolite of GA_1 are deducted, a significant relationship ($P\leq0.05$) is shown (Figure 4.27).

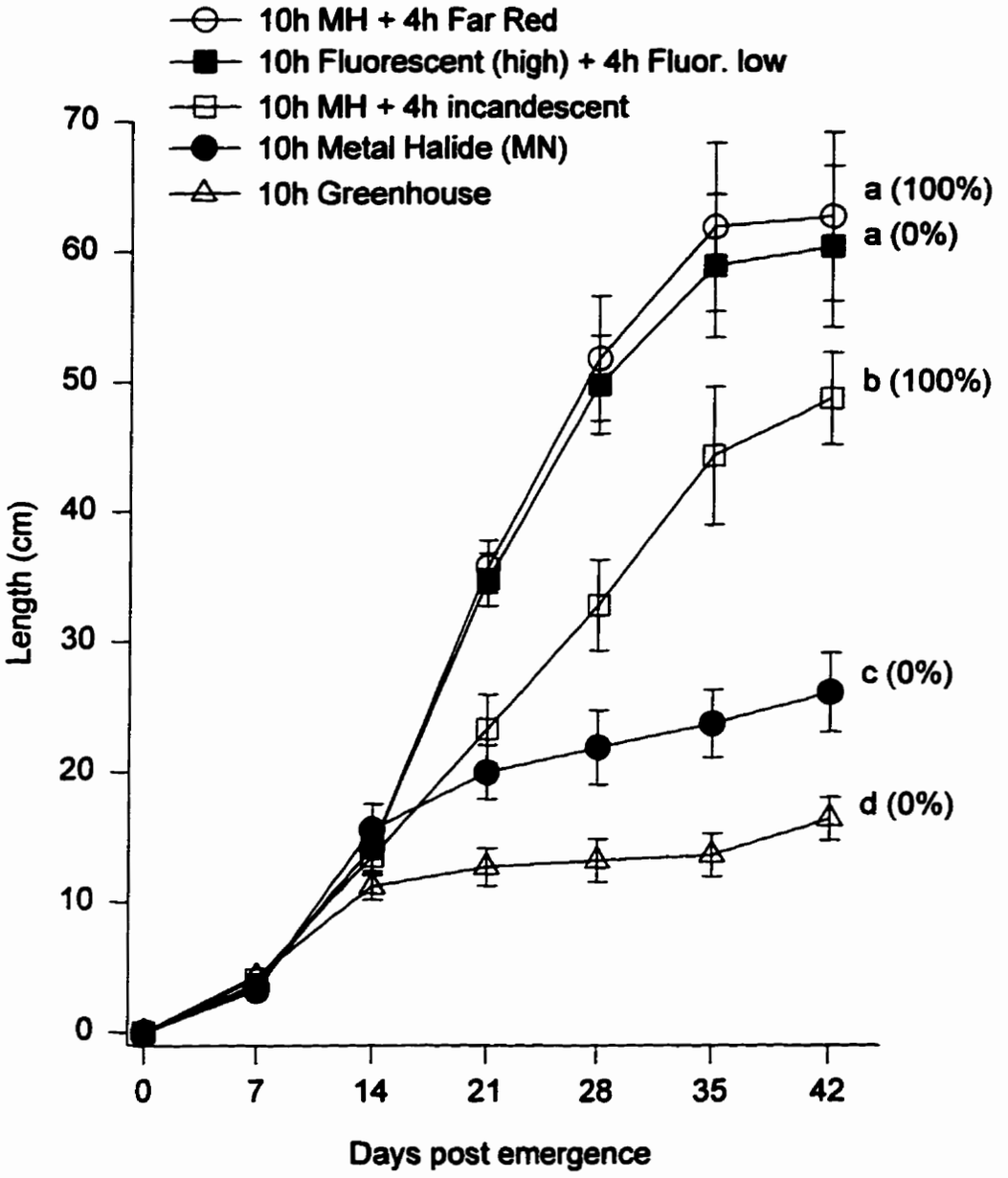


Figure 4.23. The effect of light treatments on epicotyl length at 42 days post-emergence for *B. napus* cv. WW1033 seedlings grown under various light quality regimes and warm conditions. (error bars represent 95% confidence levels, letters represent SNK groupings). Details of light treatments are given in Table 4.1.

Table 4.9. Epicotyl length and levels of plant hormones found in epicotyls of plants of *B. napus* cv. WW1033 grown under various light conditions measured and harvested at 42 days post emergence. Values are ng GA, IAA and ABA / g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)) ND= non-detected levels.

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10hMetal Halide	14h Open Greenhouse
Epicotyl Length (cm)	60.7 ±6.22a	48.7 ±3.54b	60.4 ±6.50a	26.1 ±3.04c	18.4 ±1.66d
GA_1	21.73 ±1.22	19.35 ±2.02	11.03 ±1.19	8.20 ±1.03	8.71 ±1.28
GA_3	3.51 ±.62	4.13 ±1.19	8.95 ±1.13	6.74 ±2.79	3.86 ±1.57
GA_4	3.76 ±.35	5.39 ±1.38	6.84 ±1.42	8.59 ±1.17	6.70 ±.37
GA_5, GA_6	ND	ND	ND	ND	ND
GA_7	6.27 ±2.30	4.47 ±2.29	4.52 ±.83	6.12 ±.65	4.58 ±.95
GA_8	24.33 ±2.24	12.30 ±4.12	34.31 ±6.91	20.04 ±6.28	40.11 ±5.02
GA_9	3.26 ±2.66	3.40 ±.64	4.99 ±1.03	4.26 ±1.63	4.93 ±1.46
GA_{20}	5.22 ±1.07	3.44 ±.48	5.78 ±1.08	5.70 ±1.07	3.44 ±.10
GA_{19}	28.01 ±6.24	13.73 ±1.36	21.98 ±4.29	10.21 ±.24	19.53 ±3.82
ABA	45.02 ±3.81	53.44 ±12.18	61.02 ±9.13	58.71 ±11.62	51.85 ±9.18
IAA	611.04 ±81.31	408.06 ±68.53	861.33 ±129.0	557.71 ±68.10	492.75 ±54.17

Table 4.10. Epicotyl length and levels of bioactive GAs found in epicotyls of plants of *B. napus* cv. WW1033 grown under various light conditions measured and harvested at 42 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + 4h low fluoro	10h Metal Halide	14h Open Greenhouse
Epicotyl Length (cm)	60.7 ±6.22a	48.7 ±3.54b	60.4 ±6.50a	26.1 ±3.04c	18.4 ±1.66d
GA_1	21.73 ±1.22	19.35 ±2.02	11.03 ±1.19	8.20 ±1.03	8.71 ±1.28
GA_3	3.51 ±.62	4.13 ±1.19	8.95 ±1.13	6.74 ±2.79	3.86 ±1.57
GA_4	3.76 ±.35	5.39 ±1.38	6.84 ±1.42	8.59 ±1.17	6.70 ±.37
GA_7	6.27 ±2.30	4.47 ±2.29	4.52 ±.83	6.12 ±.65	4.58 ±.95
Total GA ng/g D.W.	35.27	33.34	31.34	29.65	23.85

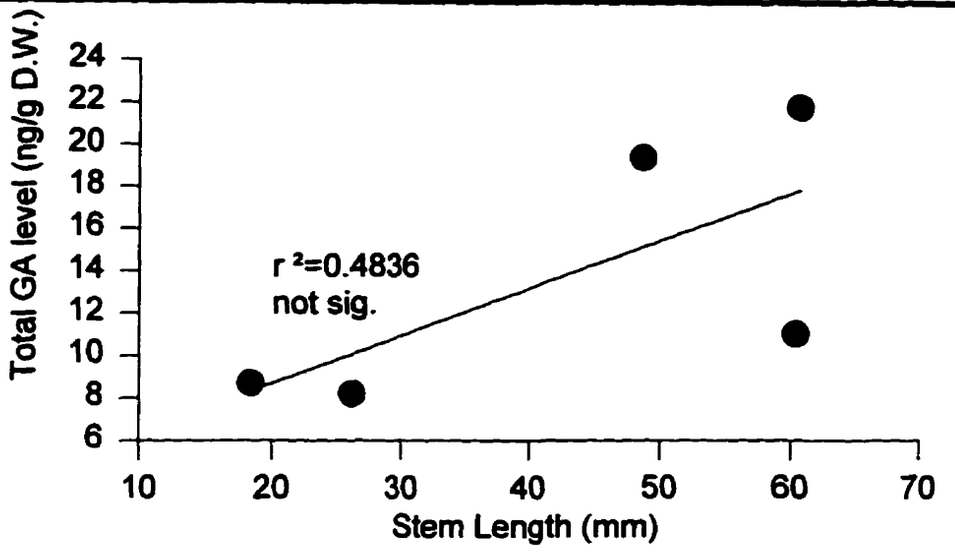


Figure 4.24. Regression of GA_1 levels extracted from epicotyls and epicotyl length for 42 day post-emergence *B. napus* cv. WW1033 grown under five different light regimes and warm conditions. Each value represents the mean of three GA_1 levels. Details of light treatments are given in Table 4.1.

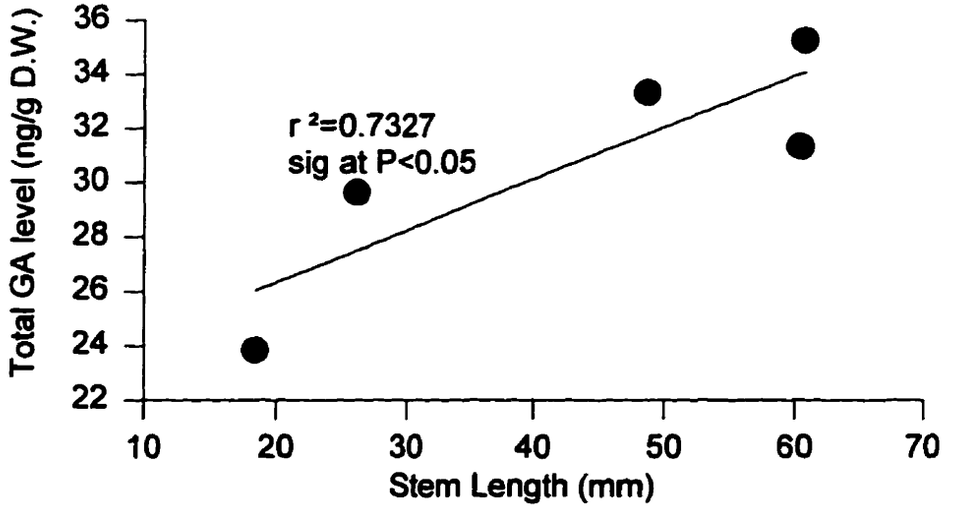


Figure 4.25. Regression of the total level of bioactive GAs (GA_1+GA_3+GA_4+GA_7) extracted from epicotyls and epicotyl length for 42 day post-emergence *B. napus* cv. WW1033 grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

Table 4.11. Epicotyl length and levels of GAs from the early-C13-hydroxylation pathway found in epicotyls of plants of *B. napus* cv. WW1033 grown under various light conditions measured and harvested at 42 days post emergence. Values are ng GA/g D.W. (Numbers represent the means of three replicates ± 95% confidence levels; letters represent SNK groupings ($P \leq .05$)).

	10h Metal Halide + 4h Far Red	10h Metal Halide + 4h Incandescent	10h Fluorescent + low fluoro	10h Metal Halide	14h Open Greenhouse
Epicotyl Length (cm)	60.7 ±6.22a	48.7 ±3.54b	60.4 ±6.50a	26.1 ±3.04c	18.4 ±1.66d
GA_1	21.73 ±1.22	19.35 ±2.02	11.03 ±1.19	8.20 ±1.03	8.71 ±1.28
GA_8	24.33 ±2.24	12.30 ±4.12	34.31 ±6.91	20.04 ±6.28	40.11 ±5.02
GA_{20}	5.22 ±1.07	3.44 ±.48	5.78 ±1.08	5.70 ±1.07	3.44 ±.10
GA_{19}	28.01 ±6.24	13.73 ±1.36	21.98 ±4.29	10.21 ±.24	19.53 ±3.82
Total GA ng/g D.W.	81.29	48.82	73.9	44.15	71.79

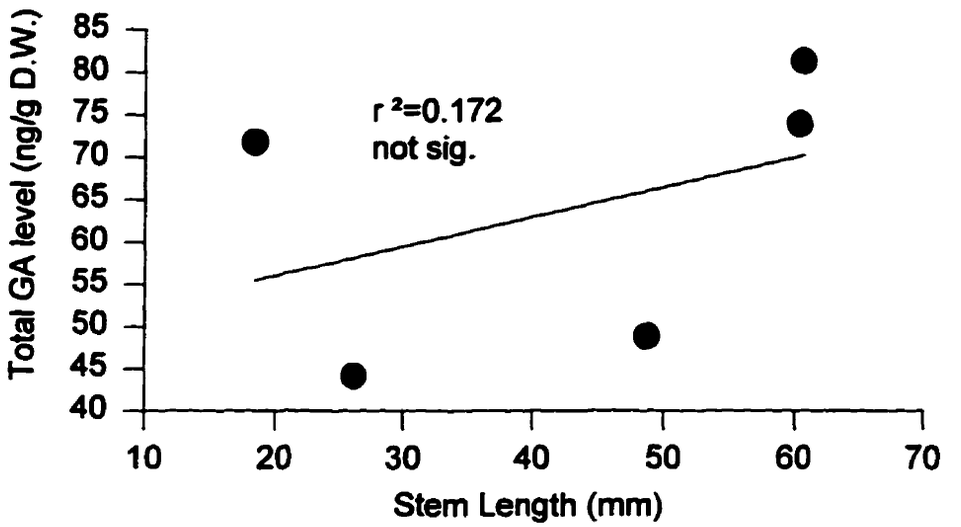


Figure 4.26. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}+GA_8) extracted from epicotyls and epicotyl length for 42 day post-emergence *B. napus* cv. WW1033 grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

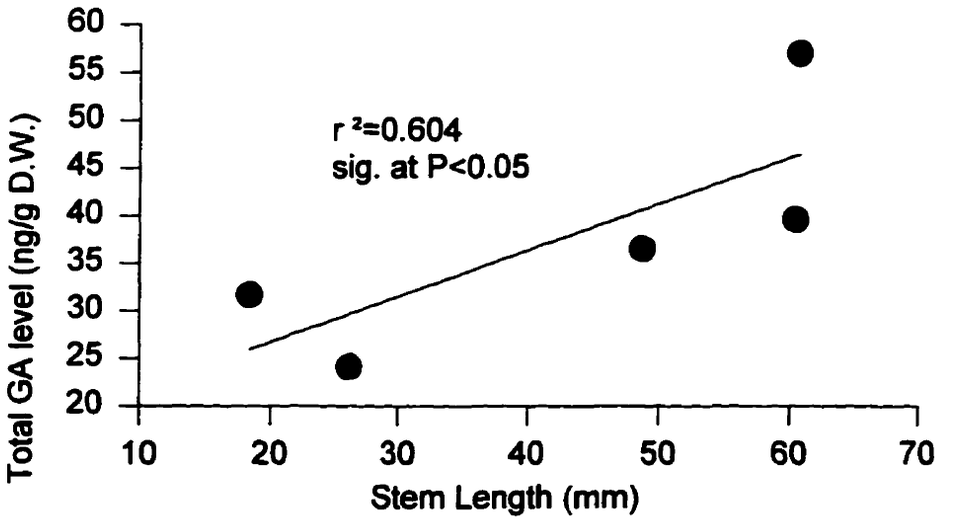


Figure 4.27. Regression of the total level of GAs from the C13-hydroxylation pathway (GA_1+GA_{19}+GA_{20}) extracted from epicotyls and epicotyl length for 42 day post-emergence *B. napus* cv. WW1033 grown under five different light regimes and warm conditions. Each value represents the sum of the mean of three GA levels. Details of light treatments are given in Table 4.1.

DISCUSSION

There is no doubt that the quality of light received by a plant affects its growth patterns. Elongation of hypocotyls and stems is under the control of light quality in a number of species (pea, Kende and Lang, 1964; bean, Lockhart, 1964; *Sinapis alba*, Morgan *et al.*, 1980). In some cases elongation has been related to increases in FR. Plants that grow in the shade of other plants and are etiolated because of this, are exposed to increased levels of FR and decreased levels of R (Casel and Smith, 1989). In an extensive review by Smith *et al.*, (1990), they conclude that there are two ways in which plants have adapted to changes in the composition of their normal light. First is the tolerance of shade (i.e. shade plants). This is one strategy by which plants have adapted to growth in light conditions with high FR. These plants do not elongate their stems or hypocotyls in response to shade (high FR)(Casel and Smith, 1989). The second strategy is that of shade avoidance, in which plants rapidly elongate, thereby growing into a more favourable light condition.

Seven day post-emergence *B. napus* cv. Westar hypocotyls

Growth Characteristics

Brassica appears to follow the shade avoidance strategy when light enriched in FR is given. However, it also shows an elongation response (hypocotyls) to high levels of R (Figures 4.3 and 4.4), which is surprising. Plants grown under open greenhouse conditions show normal de-etiolated growth characteristics, with very

short hypocotyls. As Figure 4.5 shows, any change (from normal sunlight) in the R:FR ratio results in an increase in hypocotyl elongation. Although the elongation due to increased FR has been reported previously (Beall *et al.*, 1996, López-Juez *et al.*, 1995, Kende and Lang, 1964, Lockhart, 1964, Morgan *et al.*, 1980), most reports show an inhibition of elongation with increased R (Kigel and Cosgrove, 1991). López-Juez *et al.*, (1995) working with cucumber mutants *lh* (deficient in B-like phytochrome), did show that white light (higher in R) resulted in more hypocotyl elongation in response to exogenously applied GA_4-induced in the *lh* mutant than in wild type (WT) seedlings. However, when white light was supplemented with FR, the WT hypocotyls responded equally to GA_4 applications. Hence, the hypocotyls of *Brassica* appear to act as if it is both phytochrome B deficient and normal (responds to FR). Beall *et al.*, (1996) looked at the effect of FR on stem elongation of beans and found that a R:FR ratio of 0.41 resulted in an internode elongation of as much as 3-fold over controls (R:FR=3.1). Although stems (epicotyls) and hypocotyls differ in the magnitude of response, they may be reacting in a manner similar to increased FR. The enhanced elongation of hypocotyls in response to high R (Figures 4.3, 4.4 and 4.5) is contrary to much of the published literature. The causal agent of hypocotyl elongation in dark-grown lettuce has been shown to be GA_1 (Toyomasu *et al.*, 1992). Their work looked at levels of GAs in rapidly elongating dark-etiolated hypocotyls relative to white light-grown hypocotyls, and found that the levels of GA_1 decreased in the de-etiolated light-grown hypocotyls. In plants grown in the dark, phytochrome exists mainly in

the Pr form. Phytochrome in dark-grown plants is thus in the same form (Pr) as FR grown plants (Smith, 1995; Smith and Whitelam, 1990), therefore, the increase in hypocotyl length can be explained by increased GA levels. Red light has the effect of reducing the elongation of dark-grown pea hypocotyls (Behringer and Davies, 1993, reviewed in Smith, 1995; Smith and Whitelam, 1990), yet exposure of light grown *Brassica* hypocotyls to high R results in elongation. Although the role of phytochrome in R-induced hypocotyl elongation is unknown, the effect of R certainly results in increased levels of bioactive GAs (Figures 4.7, 4.8 and 4.9).

Hormone levels

Table 4.2 shows levels of GAs in hypocotyls of plants grown under various light conditions. As seen in Figure 4.6, hypocotyl elongation of *Brassica* cannot be explained by the level of GA_1 alone. Although GA_1 is the major active GA in *Brassica* (Rood *et al.*, 1989), my harvest times show levels of this active GA present at only one point in time. The early-C13-hydroxylation pathway contains other GAs that lead to, or are products of GA_1. Thus, there is some logic to examining the increased growth in relation to more than one GA. Table 4.4 and Figure 4.8 show that the summed levels of several GAs from this pathway (i.e. GA_{19}, GA_{20}, GA_1 and GA_8), have a highly significant relationship with hypocotyl elongation. The rationale for including both precursors and deactivation products of GA_1 is that it should show a wider window of time as a reflection of GA activity. For example, the inclusion of GA_8 may indicate how much GA_1 has recently been

deactivated, and the inclusion of GA_{19} and GA_{20} represent precursor GAs, soon to be converted to GA_1. One can also sum all bioactive GAs. Thus, levels of $GA_1+GA_3+GA_4+GA_7$ also show a positive and significant relationship with hypocotyl length (Table 4.3 and Figure 4.7). Toyomasu *et al.*, (1992) suggested that high GA levels were responsible for the rapid hypocotyl elongation in dark-grown lettuce, and that this increase in GA levels was likely due to light conditions. My results with *Brassica* hypocotyls confirm this conclusion. Lockhart (1964) also speculated that FR-induced stem elongation resulted from increased levels of GAs. Although this study, along with Beall *et al.*, (1996), shows that GA levels change in response to FR light, work by Reed *et al.*, (1996) with phytochrome B mutants of *Arabidopsis* suggests that while GAs are required for hypocotyl elongation, the action of phytochrome B is not to increase GA levels in the seedling, but rather to decrease the responsiveness of the seedling to GAs. Reid *et al* (1990) working with pea and López-Juez *et al.*, (1995) with cucumber, showed no increase in endogenous GA levels in response to FR light. Hence, the increase in levels of GAs in response to increased R and FR may be a species specific response. Also, these conflicting findings show that there is a range of responses to light quality changes that may be tissue, timing or species specific.

The reasons why the increased elongation due to very high R:FR ratios has not been reported previously could be due to the exceptionally high level of R produced by the CSIRO fluorescent tubes. Interestingly, the high R-induced elongation is organ specific, only the hypocotyl, and not stems (epicotyls) of annual

B. napus cv. Westar responds in this manner (Figure 4.18).

Hypocotyl elongation may be due to two growth parameters, increased cell number and/or increased cell length. Figure 4.10 shows the effect of light treatments on cortical cell length and Table 4.5 summarizes of the effects of light on cell number and cell length. Thus, small increases in R cause elongation mainly by increasing the cell number, whereas large increases in R result in elongation due to both increased number and length of cells. The FR-induced hypocotyl elongation is due mainly to increased cell length and to a lesser degree (lesser than R), an increase in cell number. There is no significant relationship between cortical cell length and GA_1 levels (Figure 4.11) or to the sum of the levels of the bioactive GAs (Figure 4.12). However, there is a positive and significant relationship between cortical cell length and the sum of GAs from the early-C13-hydroxylation pathway (Figures 4.13 and 4.14). This leads to the theory that GAs from the early-C13-hydroxylation pathway may be the primary causal factor involved in both FR- and high R-mediated hypocotyl elongation in *B. napus*. Beall *et al.* (1996) and Nagatani *et al.* (1990) also showed that FR promotes stem (epicotyl) elongation by increasing both cell number and length. In early work by Arney and Mancinelli (1966) and Feucht and Watson (1958), it was shown that the elongation of internodes by exogenous application of GA_3 was due to both increases in cell number and cell length. However, Metzger (1988a), working with *Thlaspi arvense* petioles and Weller *et al.*, (1994) with pea epicotyls found that elongation resulting from applied GA_3 was due to increased cell division, whereas,

day-light extension by FR resulted in elongation by increased cell length. This indicates that there could be different causal mechanisms for the two cell responses that make up elongation. Speculatively, different tissues or species may utilize different GAs for cell division versus cell elongation. Thus, the roles of GAs in light-mediated growth is still open to interpretation and further work is required.

Stem dry weight (Figure 4.15) and leaf area (Figure 4.16) show similar trends in that high levels of R inhibit shoot development. However, there is no significant relationship between either stem dry weight or leaf area and endogenous GA levels. The very small leaf area (cotyledons) and extremely elongated hypocotyls of R-grown plants (R:FR; 4:1) is similar to very etiolated characteristics seen in dark-grown plants. Beall (1985) indicated an increase in leaf area under increased FR working with bean, yet Holms and Smith (1977), with *Chenopodium* and *Cucurbita*, found reduced leaf areas. It therefore appears that the response of leaf area to FR is species specific. Decreases in leaf area and stem dry weight have not been reported previously for high R-conditions and further experimentation is required to determine whether this is a species specific response or is found in other members of *Brassica*.

Epicotyl (Stem) Elongation in *B. napus* cv. Wester (annual)

Growth Characteristics

One of the main functions of phytochrome is to regulate the transition from germination to photo-autotrophic growth (Smith and Morgan, 1983). Prior to

emergence, seedlings are etiolated. Some of the characteristics include: undeveloped leaves; rapid stem elongation; a hooked plumula and the absence of chlorophyll. When exposed to white light, de-etiolation ensues (greening caused by chlorophyll synthesis), slowing of stem growth, opening of the hook and development of leaves, Smith and Morgan, 1983).

My results indicate that there is no significant relationship between epicotyl elongation and the quality of light at germination (Figure 4.18). Hence, the increase in apex height (total plant height) at day seven is due to a very enhanced hypocotyl elongation. Increased epicotyl elongation is more responsive to FR than is hypocotyl elongation and the opposite trend is evident for R. While elongation of both hypocotyls and epicotyls to FR has been extensively studied as it relates to shade avoidance (Beall, 1996; Davis and Simmons, 1994; Buck and Vince-Prue, 1985; Vince-Prue, 1977; reviewed by Smith, 1982 and 1995), the elongation I obtained with very high levels of R appears to be novel. However, this very high red light (R:FR ratio ≈4) may not have been studied by others due to it being such an unnatural condition, or it may be *Brassica* specific.

In early work with light quality and GAs, Lockhart (1964) showed that internode elongation of pea was increased by FR only when the distal leaf was intact but was insensitive to FR when this leaf was removed. Internode elongation could be regained by exogenous application of GA_3, even with the distal leaf removed, therefore, he theorized that leaves produce a GA precursor that moves to the region of elongation and can be activated by FR or prevented by R.

Although this theory was plausible, Kende and Lang (1964) showed that levels of bioactive GA-like substances did not change in light- and dark-grown pea seedlings and they believed that light decreased the sensitivity to GAs. In a more recent study in pea, Ross and Reid (1991) showed that light appears to act almost entirely by altering the plant's responsiveness to GA_1. They state that this highlights the need to consider both GA level and tissue responsiveness.

Vince-Prue (1977) showed that stems of the LDP *Fuchisa hybrida* exhibit two types of light responses. In early internodes FR promoted elongation when given as an end of day supplement and the growth response was R reversible. Subsequent internodes, however, elongated in response to both R and FR (or combinations). Buck and Vince-Prue (1985) attempted to determine which light response was most responsible for internode elongation. They found that internodes already beginning to elongate were dependent upon R:FR ratios (FR increased elongation) and not photoperiod length. However, in later elongating internodes, photoperiod length was more growth-promotive than high FR. Some plant organs may thus have a specific program of when they will respond to a specific environmental condition. In *Brassica* this program could, speculatively, involve elongation to any changed light quality from that of unfiltered sunlight (R:FR≈1.0).

Hormone Levels in the Epicotyls

According to Phinney (1985), GAs are thought to regulate internode

elongation and therefore shoot growth. Rood *et al.*, (1989, 1990b) and Hedden *et al.*, (1989) showed that GA_1 was the major GA present and was the GA most likely responsible for stem (epicotyl) elongation in *Brassica*. As well, Rood *et al.*, (1987) and Hedden *et al.*, (1989) showed that the early C13-hydroxylation pathway predominated in *Brassica*. I have also shown that the early-C13-hydroxylation pathway is active in *Brassica* stems (epicotyls), and that there is a positive and often significant relationship between GA levels and stem elongation. Specifically, the levels of endogenous GA_1 are positively and significantly correlated with epicotyl elongation (Figure 4.19). This was not the case with hypocotyl elongation under the same light conditions (Figure 4.5). The summed levels of late step GAs in the C13-hydroxylation pathway also show a positive and significant correlation with epicotyl elongation but only if GA_8 levels are excluded (Figures 4.21 and 4.22). Hence, catabolic history, the conversion of GA_1 to GA_8 involving deactivation by 2β-hydroxylation (Hedden and Crocker, 1991), detracts from the correlationfor the epicotyls, but not the hypocotyls. As discussed earlier, summing the levels of GAs in a specific pathway can provide information on amounts of precursor GAs that are destined to become the active form (GA_1 and/or GA_3). *Brassica* is known to contain at least five GAs which are likely to be biologically active per se (GA_3, GA_4, GA_7 and GA_1)(Rood *et al.*, 1987) and GA_{85} (Sheng *et al.*, 1992), and the sum of GA_1, GA_3, GA_4 and GA_7 also showed a positive and significant correlation with epicotyl length (Figure 4.20). Levels of GA_{85} were not measured due to a lack of suitable internal standard. This indicates that although GA_1 has been reported as

the "effector" of stem elongation in *Brassica*, (Rood *et al.*, 1987, and Hedden *et al.*, 1989), it is likely that other C-3β-hydroxylated GAs are involved as "causal effectors".

Epicotyl Elongation of *B. napus* cv. WW1033 (biennial)

Growth Characteristics

Growth resulting from changes in light quality differs between the annual cultivar Westar and the biennial cultivar WW1033. This is expected as WW1033 has many characteristics which distinguish it from all other biennial cultivars tested. Most of these characteristics involve flowering responses, but it should be remembered that stem elongation and flowering are linked in *Brassica*.

High levels of R and FR also result in increased epicotyl elongation in CRP (Figure 4.23). Although the response to FR is well documented (Kende and Lang, 1964; Lockhart, 1964; Morgan *et al.*, 1980; Casel and Smith, 1989; review by Smith *et al.*, 1990), the literature is limited on the growth-promotive effects of R. Yet Buck and Vince-Prue (1985) did show an internode elongation effect when R was given as a 16h photoperiod extension, (that said, 1h end-of-day treatment with R was inhibitory of internode elongation). Increased FR resulted in flowering as well as epicotyl elongation under non-vernalized conditions (data not presented). However, high R only caused elongation (no flowering)(data not presented). More work with this very rich R source needs to be accomplished.

Hormone Levels

Although no literature could be found dealing with the R-induced epicotyl elongation, this R-induced growth could be explained by my analysis of GA levels in the elongating tissue. While levels of GA_1 alone or the sum of levels of GA_1+GA_8+ GA_{19}+GA_{20} did not show a significant relationship with epicotyl length (Figures 4.24 and 4.26), the summed levels of GA_1 and its immediate precursors (GA_{19} and GA_{20}) did (Figure 4.27). Also, the summed levels of bioactive GAs, GA_1+GA_3+GA_4+GA_7 showed positive and significant relationships (Figures 4.25).

SUMMARY

Far-red light results in increased elongation (both hypocotyl and epicotyl) in both annual and biennial, CRP cultivars of *Brassica*. Far-red light is also florigenic in the biennial cv. WW1033 under non-inductive, warm conditions. Very high R ratios result in variable elongation which is cultivar and tissue specific. Red light causes hypocotyl elongation in the annual cv. Westar, but no significant increase in epicotyl elongation was seen. Yet, in the biennial CRP cv. WW1033, epicotyl elongation was observed with this unique R treatment. Elongation decreases as the quality of light nears a 1:1 balance of R:FR. In all cases the elongation of hypocotyls or epicotyls could be correlated by levels of various GAs present in the tissue (either active C3β-hydroxylated GAs, or members of the active GA pathway).

CHAPTER 5

If you can't stand to be with your real peers,

get out of science (JD Watson, 1993).

Vernalization in *Brassica napus* cv. WW1033

INTRODUCTION

The economic advantages of winter (biennial) crops such as winter wheat (*Triticum aestivum*), over spring varieties is well known in agricultural areas. With the increased interest in canola, studies have been carried out to look at the logistics of winter canola in Alberta (Mandel *et al.*, 1989 unpublished Farming For the Future report). Early spring brings a rapid bolting (stem elongation) and flowering with harvesting by late July. The rapidly bolting stem of CRP cultivars becomes part of the reason why, to date, winter canola is not viable in Alberta. Local weather is very dynamic, and once the stem has started bolting, it is very susceptible to freezing damage. One possible means of increasing the CR plant's viability would be to delay or slow the bolting process by as little as a few weeks. In order to accomplish this, a more detailed understanding of the vernalization and bolting process is required.

Gibberellins have been linked to stem elongation and flowering in many species (reviewed in Pharis and King, 1985 and early work by Lang, 1965). As well the involvement of GAs has been proposed in vernalization (Lang, 1965 and reviewed in Vince-Prue, 1975). Also, Zanewich *et al.*, (1993) looked at the GA levels following vernalization in *B. napus* cv. Crystal and found an increase in all GAs examined, relative to non-vernalized controls.

The photoperiod requirement of plants with regards to flowering has been investigated by Zeevaart (1983) and reviewed by Lang (1965). Long day plants show a rapid stem elongation after photoperiod is increased (i.e. SD -> LD). From chapter three, it was shown that photoperiod could substitute for vernalization in *B. napus* cv. WW1033. This led me to pose two questions: What are the floral inductive photoperiod requirements of pre- and post-vernalized plants? And does the photoperiod requirement have a GA involvement?

Work with another CRP, *Thlaspi* has led to a proposal that a block in the biosynthetic pathway is under vernalization control (i.e. the oxidation of a kaurenoic acid (Hazebroek *et al.*, 1993 and Hazebroek and Metzger, 1990a)). This work involved feeds of labelled precursor and the accumulation of kaurenoic acid, which upon vernalization was further metabolized to C-13 desoxy GAs. The use of [$^2H_2$] labelled internal standards for use with GC-MS-SIM analysis would be a definitive test to determine the point of control. These standards have only recently been synthesised (by LN Mander) and will be used in a further study that will build upon this research.

The goal of this study was to identify the photoperiod requirements prior to and post-vernalization, and to measure the levels of GAs present in apex tissues (apex plus 2 cm region below apex) under inductive and non-inductive photoperiods. I will also study from a GA basis, the process of de-vernalization.

MATERIAL & METHODS

Plants of *B. napus* cv. WW1033 were grown in B units of the Phytotron in greenhouse conditions with a 16h photoperiod (LD (8h natural light supplemented with 8h low intensity incandescent light)) or under 8h photoperiod (SD). Plants were then transferred to the cold chamber (3°C, with 50µmole m^{-2} sec^{-1} of light at plant level) for 8 weeks. Plant tissue was harvested (2cm segment of apical region) at the beginning, mid-point (4 weeks) and end of vernalization period (8 weeks) as well as weekly following vernalization for 4 weeks. Following vernalization, plants were returned to the B units through a stepwise warming (5°C every 3 days or returned to warm conditions immediately (to look at the effects of de-vernalization)). Photoperiod treatments were as follows:

Pre-vernalization	Vernalization	Post-vernalization
LD	------>SD	---------->SD
		---------->LD
SD	------>SD	---------->SD
		---------->LD

Measurements of apex height, leaf number and plant growth stage (Harper and Berkenkamp, 1975) were recorded on a weekly basis, or during periods of rapid growth, more often. Other measurements and observations, such as hypocotyl, petiole and internode length, leaf area, shoot dry weight and leaf shape were carried out for specific experiments. Flowering was assessed both visually and by dissection.

RESULTS

Plants were grown under SD (8h) or LD (16h) prior to subsequent vernalization treatment under SD conditions. Upon return to warm conditions, one half of each set of plants was placed under LD and the other under SD. Within 14 days post-vernalization, 100% of LD post-vernalized plants showed visible flower bud racemes and had bolted, whereas 0% of the SD post-vernalization plants flowered or bolted for the duration of the experiment (10 weeks post-vernalization). The pre-vernalization photoperiod had no effect on the efficacy of the vernalization treatment, whereas there was an obligate LD requirement following vernalization for floral induction. Figure 5.1 shows the stem growth during vernalization and the effect of photoperiod on stem growth post-vernalization. Figure 5.2 shows plants two weeks post-vernalization and the non-vernalized control (four weeks under LD). The photograph (Figure 5.2) shows that there is little difference between the SD post-vernalization and the non vernalized plants. Figure 5.3 shows a closeup of the SD post-vernalization stem. The elongation of the SD post-vernalization plant (Figure 5.1 and 5.2) is due only to the increase in internode number, as there is no elongation of the internodes. The LD post-vernalization plants do not differ significantly in the number of internodes, but do differ in the length of internodes.

Figure 5.1 also shows the effect of de-vernalization (rapid warming) on stem growth and flowering of plants maintained under LD. Figure 5.4 shows that the increase in stem length of de-vernalized plants is due to internode elongation but the extent of internode elongation is not to the same extent as LD post-

vernalization plants.

In vernalized plants that failed to flower (SD post-vernalization and de-vernalized), applications of GA_5 (250 µg/plant) were able to induce flowering even when plants were maintained under SD conditions (SP post-vernalization only). At any time following vernalization, if plants maintained under SD were moved to LD, bolting and flowering occurred within two weeks. The bolting in GA-treated plants, as well as in transferred plants (SD to LD), occurred only on internodes four to eight and on subsequent internodes.

Hormone Levels

The levels of endogenous GAs present in stem tissue during SD vernalization, and under either SD or LD post-vernalization, are shown in Figures 5.3 to 5.9. In measurements of the levels of GA_1 (Figure 5.5), GA_8 (Figure 5.6), GA_{19} (Figure 5.7), GA_{20} (Figure 5.8) and GA_3 (Figure 5.9) there were significant differences ($P \leq 0.05$) between GA levels in plants maintained under LD and SD following vernalization. In measurements of the levels of GA_{44} (Figure 5.10) and GA_{53} (Figure 5.11), there were no significant differences ($P \leq 0.05$) between GA levels in LD and SD grown plants following vernalization, though there was a trend toward a slight increase. For all GAs measured there was no significant change in GA levels during vernalization nor did GA levels change in stem tissue of plants grown under SD following vernalization. Figure 5.12 shows the effect of photoperiod and de-vernalization on GA levels. Here, there is a trend for GA levels

in de-vernalized plants to be higher than those of SD post-vernalization plants (no bolting), but less than those of LD post-vernalization (bolting and flowering).

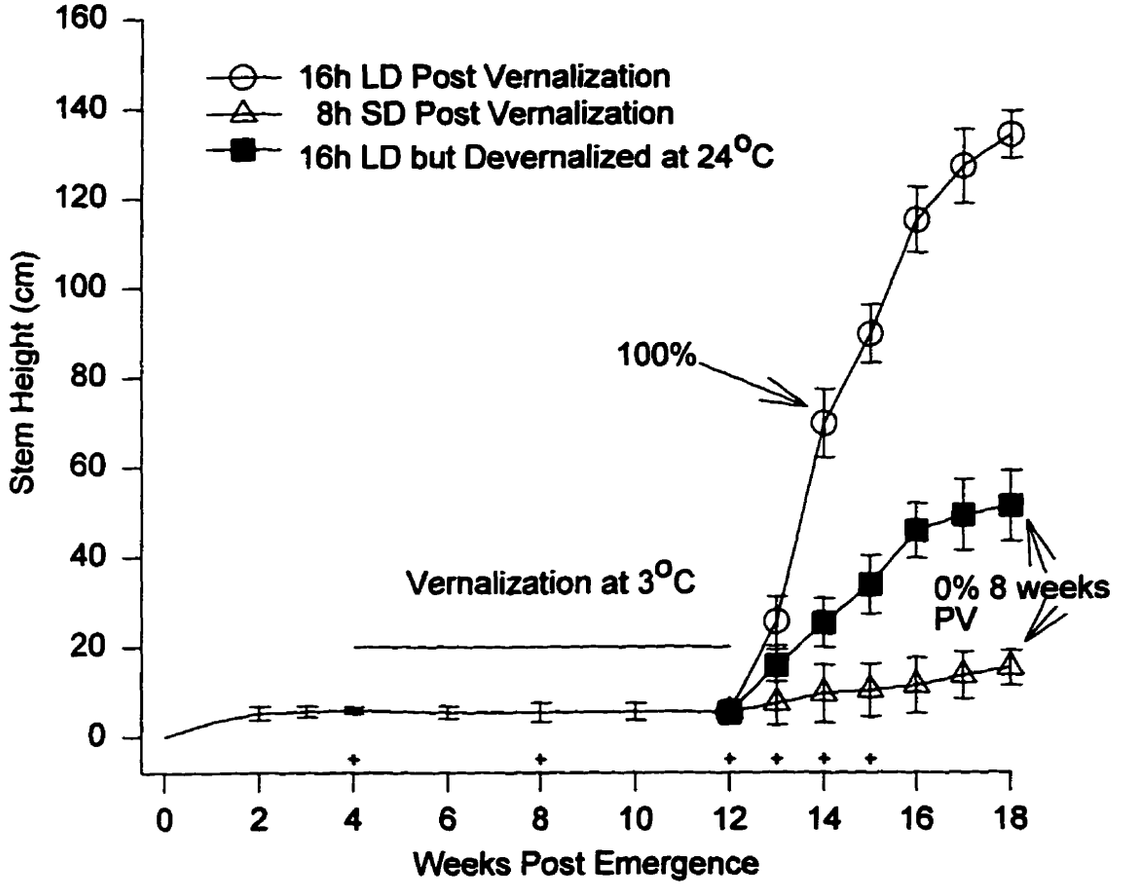


Figure 5.1. The effect of photoperiod following vernalization and rapid warming (de-vernalization) on height to apex of *B. napus* cv. WW1033. Percentage of flowering plants is indicated with arrows. (+ indicates dates of tissue harvest, error bars represent 95% confidence intervals). Vernalization was carried out at 3°C 8h SD, and plants were then transferred to 24°C conditions at 5°C intervals every 3 days. De-vernalization occurred by transferring plants from 3° to 24°C in one step.

Figure 5.2. The effect of post-vernalization photoperiod (2 weeks) on flowering and bolting of *B. napus* cv. WW1033. (left to right) LD post-vernalized; SD post-vernalized; and LD non-vernalized.

Figure 5.3. The effect of post-vernalization photoperiod (2 weeks) on flowering and bolting of *B. napus* cv. WW1033. Closeup of SD post-vernalized plant showing a lack of internode elongation.

Figure 5.4. The effect of de-vernalization following vernalization (2 weeks) on flowering and bolting of *B. napus* cv. WW1033.

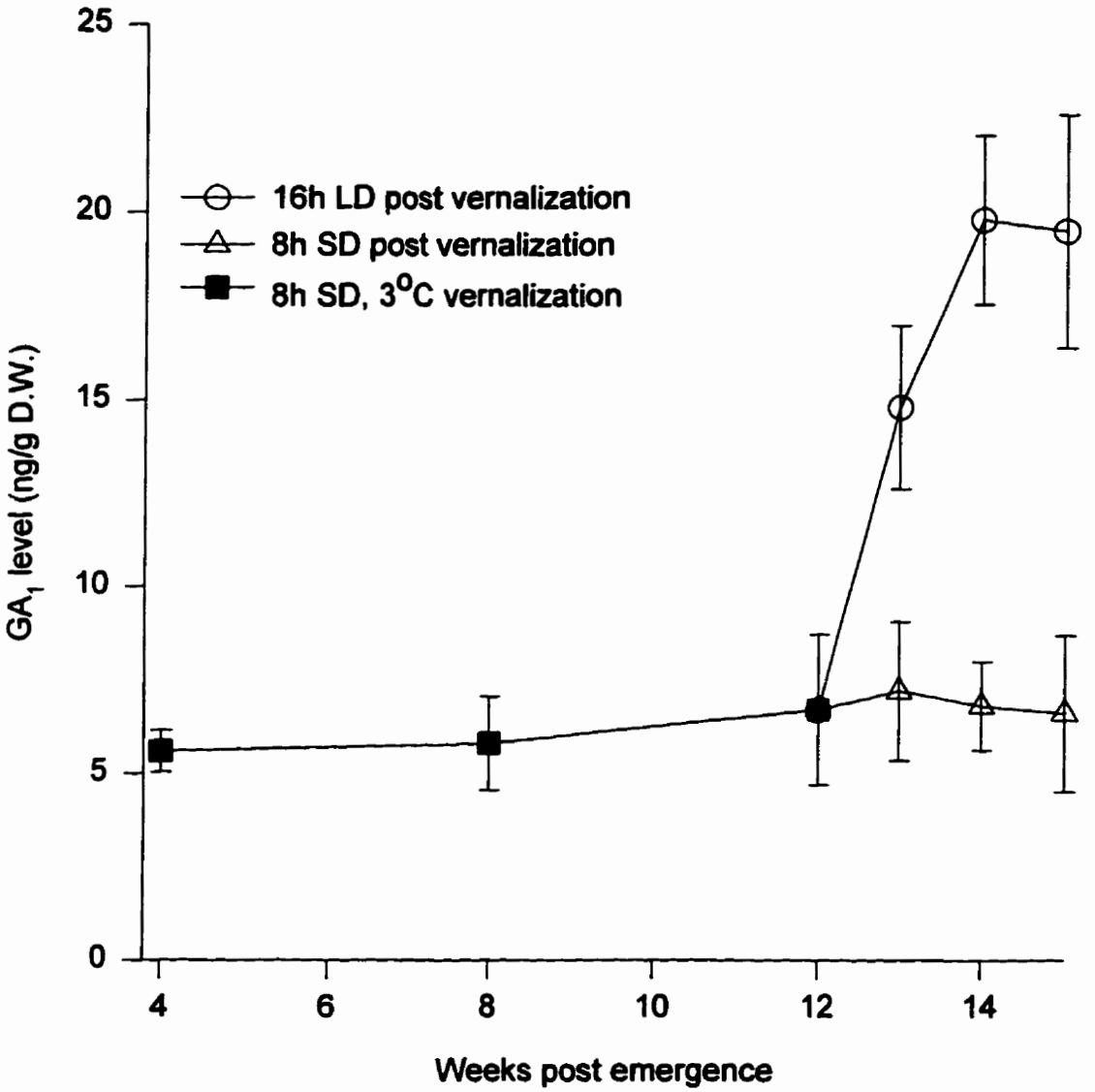


Figure 5.5. The effect of vernalization and post-vernalization photoperiods on GA_1 levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

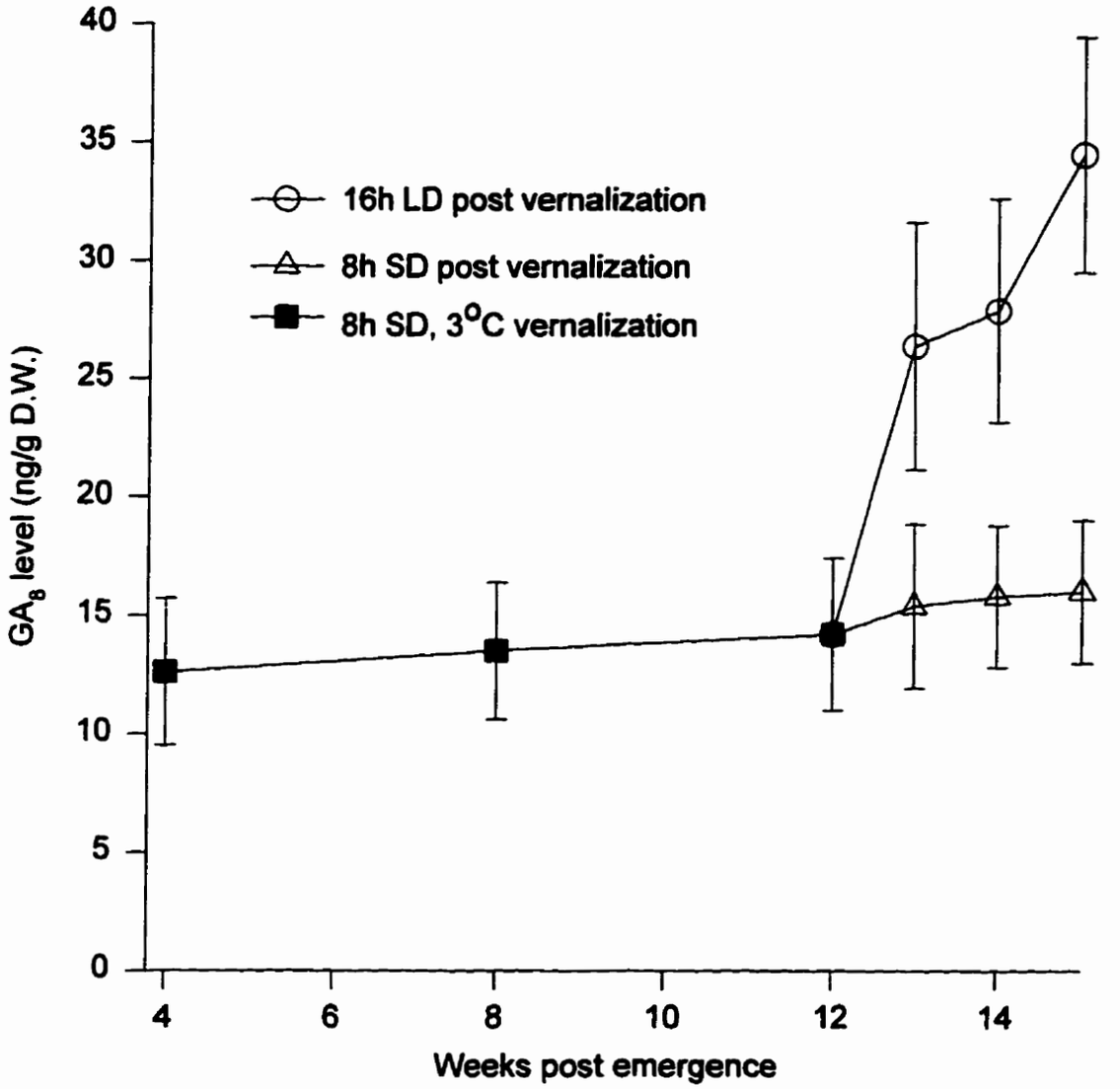


Figure 5.6. The effect of vernalization and post-vernalization photoperiods on GA_8 levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

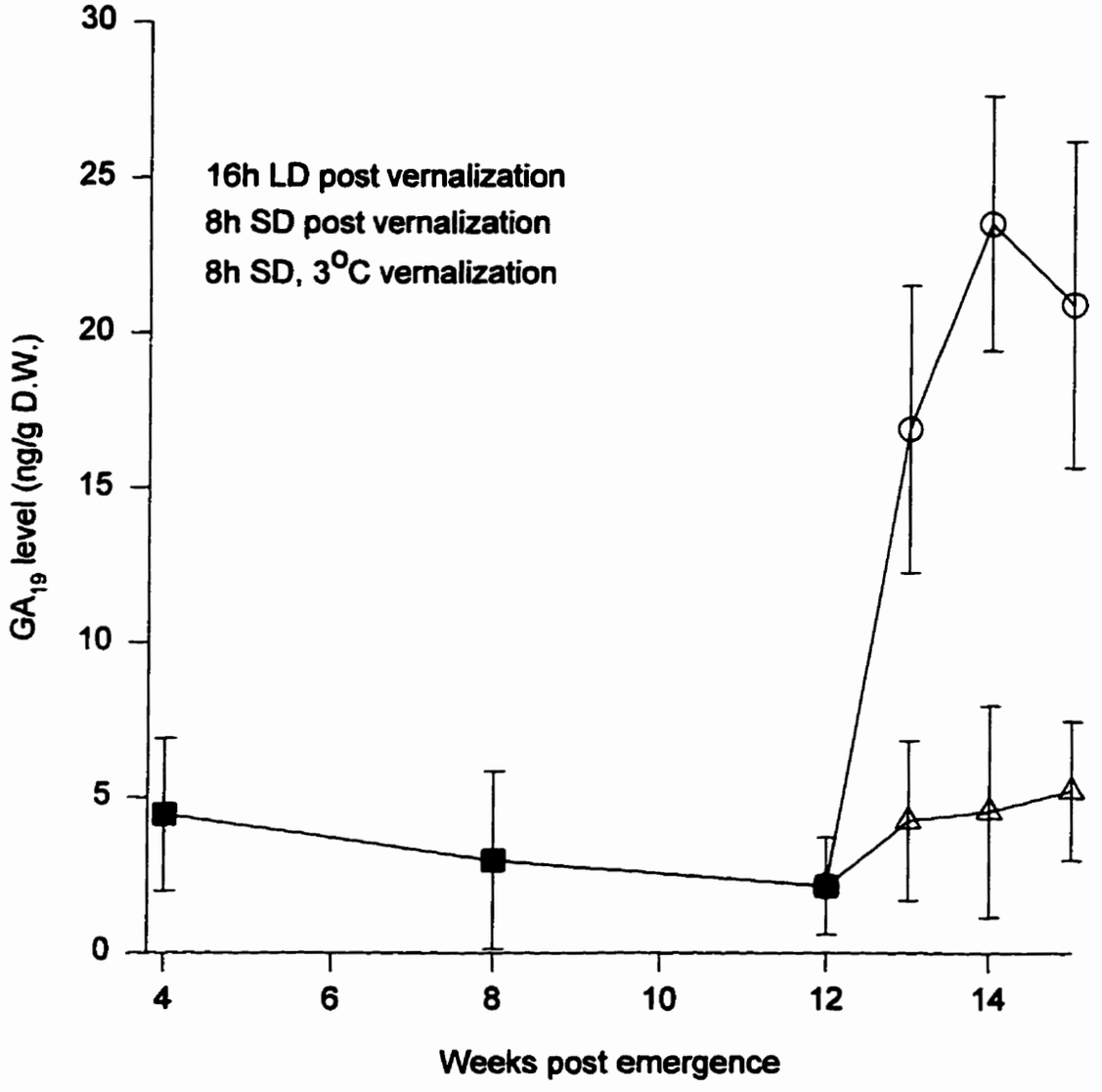


Figure 5.7. The effect of vernalization and post-vernalization photoperiods on GA_{19} levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

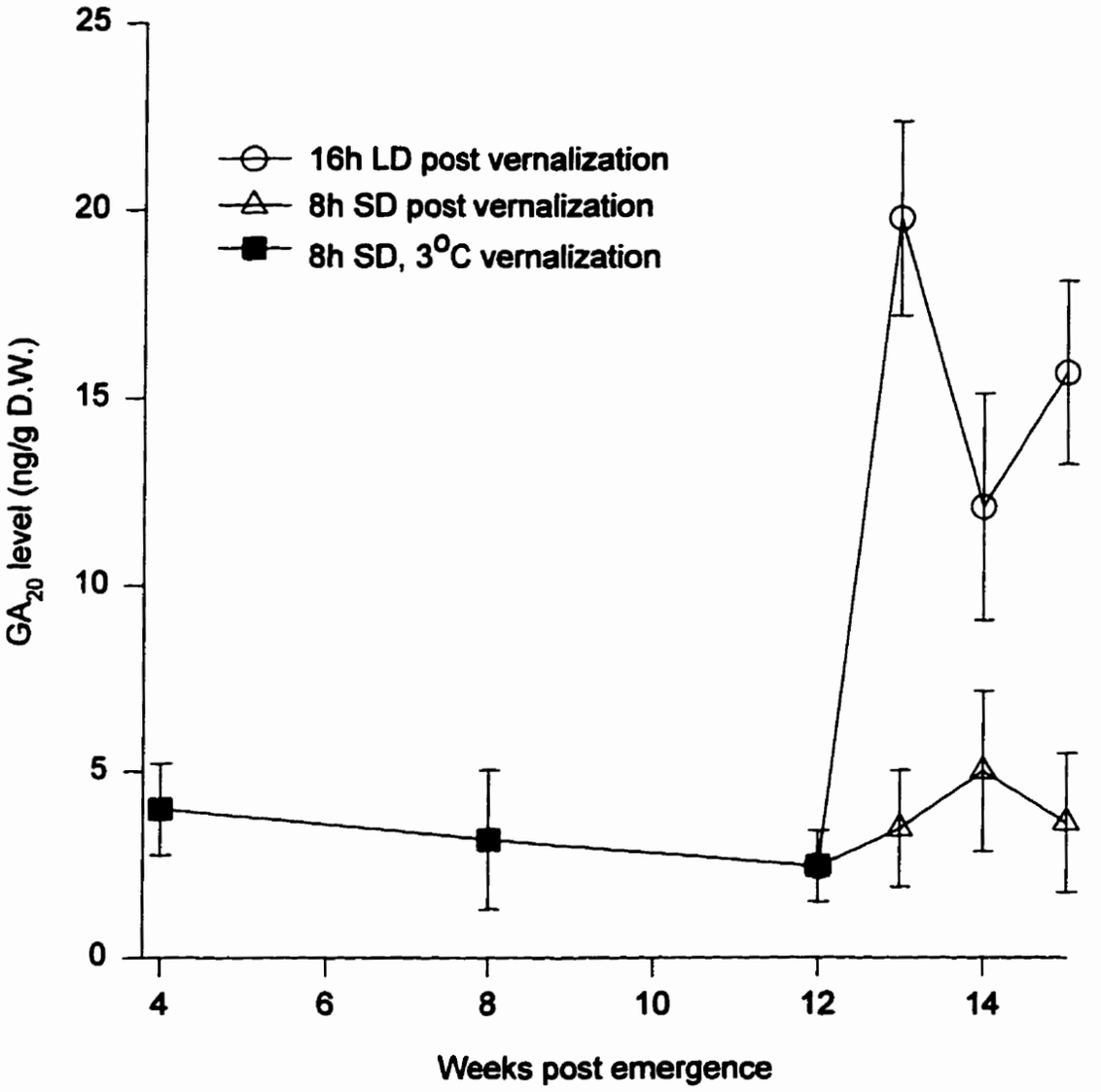


Figure 5.8. The effect of vernalization and post-vernalization photoperiods on GA_{20} levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

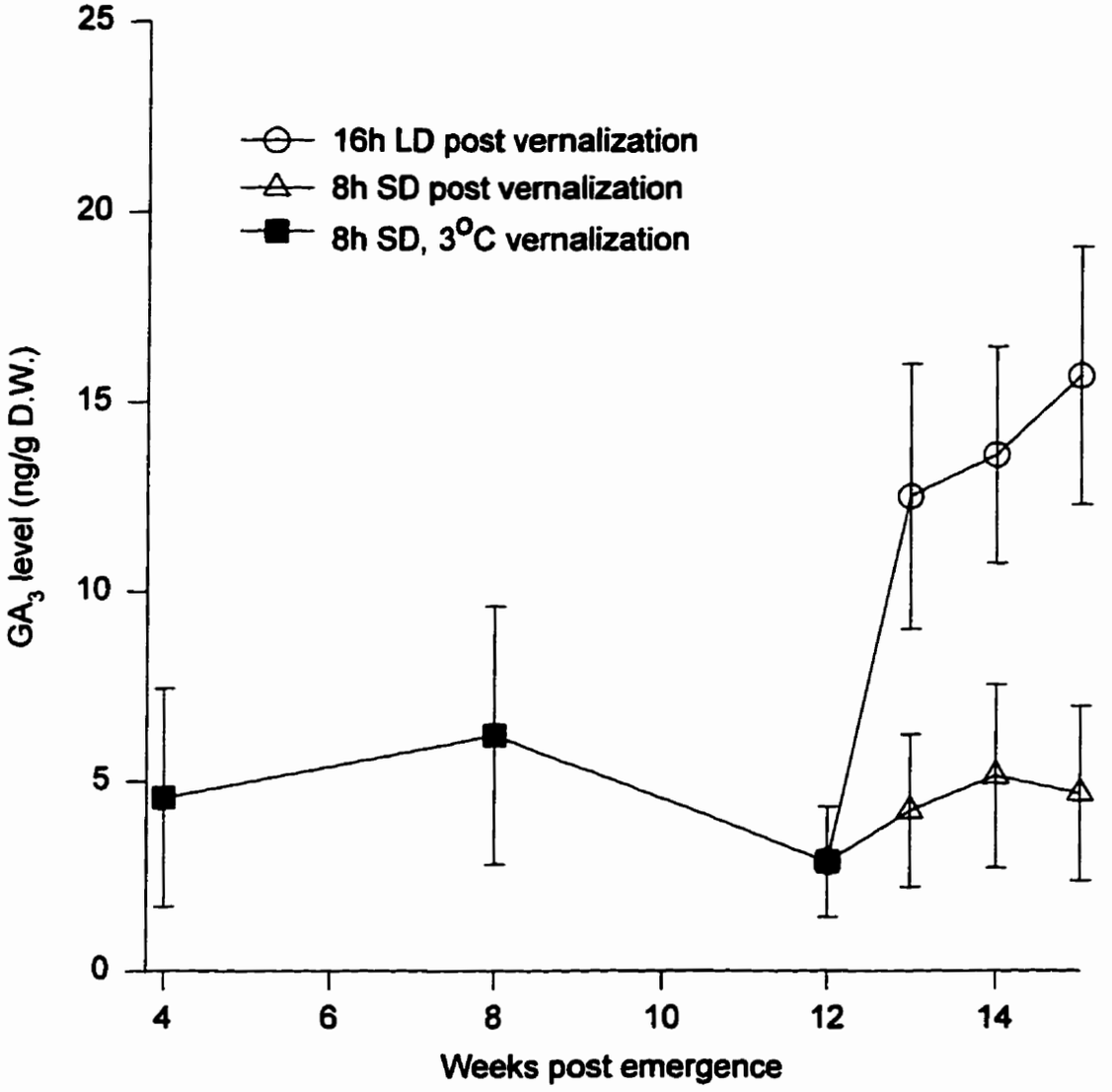


Figure 5.9. The effect of vernalization and post-vernalization photoperiods on GA_3 levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

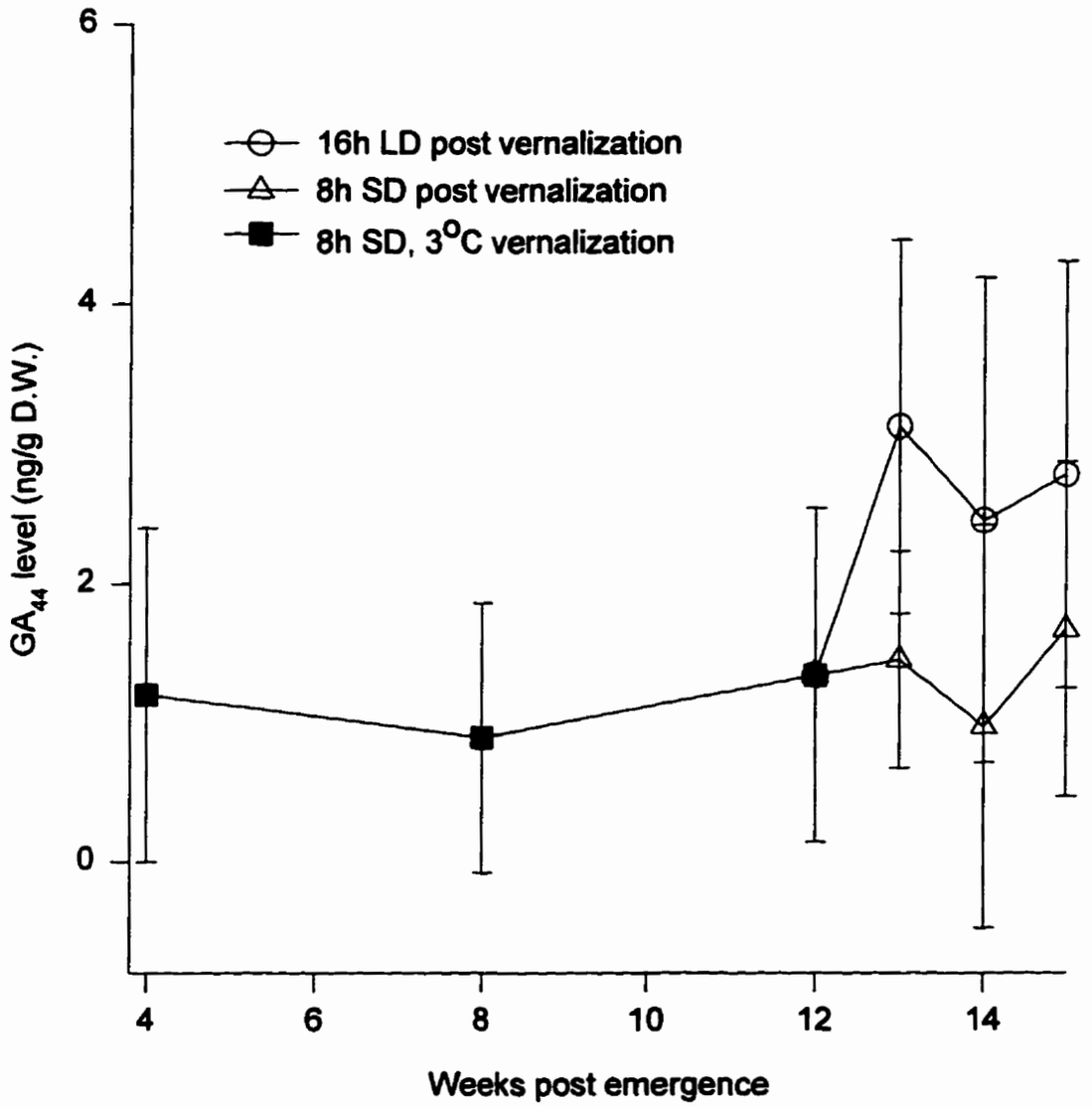


Figure 5.10. The effect of vernalization and post-vernalization photoperiods on GA_{44} levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

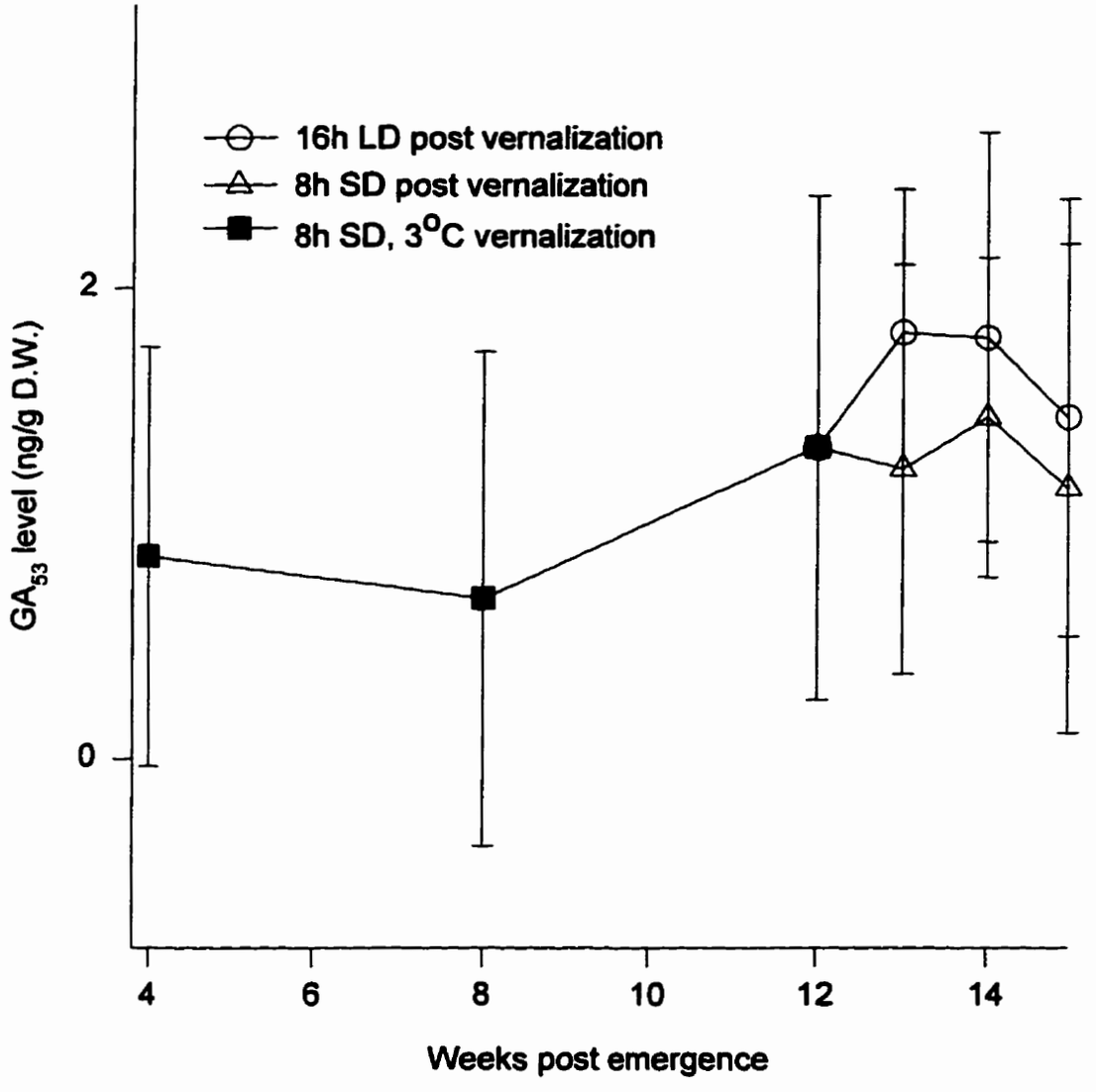


Figure 5.11. The effect of vernalization and post-vernalization photoperiods on GA_{53} levels in stem tissue of *B. napus* cv. WW1033. (mean of 3 samples, error bars represent 95% confidence levels)

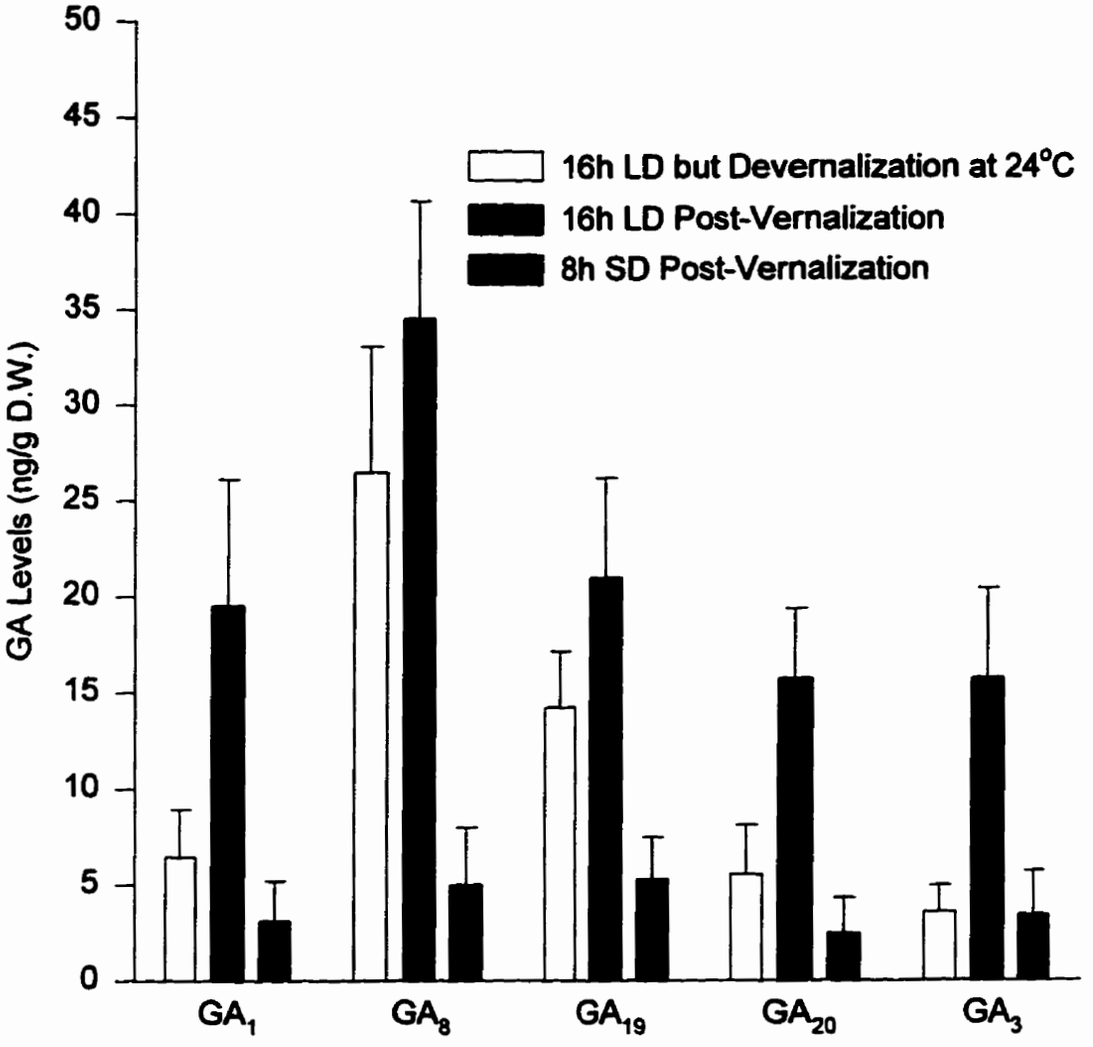


Figure 5.12. The effect of post-vernalization photoperiod (LD or SD) and rapid warming (de-vernalization) on GA levels in stem tissue of *B. napus* cv. WW1033 three weeks after removal from vernalizing temperature (mean of 3 samples, error bars represent 95% confidence levels).

DISCUSSION

The dependence of stem elongation (post-vernalization) on photoperiod length has been reviewed by Lang (1965). According to Lang's classifications, *B. napus* cv. WW1033 has a obligate vernalization and LD post-vernalization requirement. In the absence of vernalization, LD-grown plants do not elongate or bolt (data not shown), but following vernalization, elongation begins within one week if the plants are grown under LD conditions. No appreciable elongation occurs under SD warm post-vernalization conditions. This indicates that the control of bolting and flowering of biennial *Brassica* are under dual controls, both of which are obligate. This dual requirement may act as a tool to help separate the various effects that vernalization and photoperiod have in floral induction.

Successful vernalization is dependent upon proper environmental conditions following vernalization. Plants maintained under SD do not flower until they are transferred to LD conditions, and only the young internodes elongate (internodes formed under SD do not bolt). This indicates that the vernalized condition resides in the young, growing region. Metzger (1988b) showed that the shoot tip of *Thlaspi* was the site of cold perception. The vernalized state is stable under non-inductive photoperiods for at least 4 weeks (duration of experiment), only if the transition from cold to warm conditions was slow. De-vernalization (rapid warming) results in an inability to flower when plants are subsequently grown under an inductive photoperiod. There is however, slight stem elongation. The instability of the vernalized state could involve enzymes that are only functional under cool

conditions (Lang, 1965). The slight elongation of de-vernalized stems (Figure 5.4 and 5.12) can be explained by modest increases in endogenous GAs over those of SD post-vernalization, and less than those of LD post-vernalization. Thus, de-vernalization likely acts by keeping GA levels low (resulting in very little stem elongation) and possibly by reducing or eliminating the agent responsible for floral initiation.

Differences in the levels of GAs between vernalized and non-vernalized plants have been shown for *B. napus* cv. Crystal (Zanewich *et al.*, 1993) and in *Thlaspi* (Hazebroek and Metzger, 1990). Within days of vernalization, after transfer to LD warm, GA levels increase over those of non-vernalized controls. Within one week of the end of a vernalization treatment, plants grown under LD conditions increased their GA levels (GA_1, GA_8, GA_{19}, GA_{20}, GA_{44}, GA_{53} and GA_3) over those under SD conditions. The levels of endogenous GAs remained relatively constant during the vernalization process. This implies that both biosynthesis and metabolic deactivation remain constant or are slowed during the 3°C vernalization period. Although one of the blocks released by vernalization (i.e., a block in the kaurenoid pathway) has been proposed, it has yet to be definitively identified. Here, timing of tissue harvest is critical. Most vernalization-requiring species must be grown in cool conditions for at least two to three weeks (Vince-Prue, 1975), then moved to LD warm. In order to understand more fully the process of vernalization, it will be necessary to determine more precisely the timing of the events involved. From the present study, it is clear that GAs, although

involved in vernalization, are "downstream" in the process. It still has not been shown what triggers the production of GAs following vernalization (i.e. what step(s) is (are) actually blocked in non-vernalized plants.

According to Metzger and Zeevaart (1980), in spinach the conversion of GA_{19} to GA_{20} is under photoperiod control. There was an increase in GA_{20} with a decrease in GA_{19} levels following the transfer of plants from SD to LD. Similar findings have been reported with a number of rosette plants (*Silene*, Cleland and Zeevaart, 1970, *Agrostemma*, Jones and Zeevaart, 1980). In my study, levels of both GA_{19} and GA_{20} increase under LD following vernalization. This may be due to the removal of an earlier biosynthetic block which results in high levels of GA_{19} being maintained. Upon removal of the biosynthetic block when LD's are provided the high levels of GA_{19} are presumably rapidly metabolized to GA_{20}, GA_1 and GA_8.

My findings also show that once the vernalized state has been established, stem elongation of the plants is under GA-mediated, photoperiod control, similar to the situation in non-cold requiring summer annual varieties of *Brassica*. The two obligate requirements in biennial *Brassica* (low temperature vernalization and LD) are independent of one another with regards to timing. That is, the photoperiod requirement can be satisfied at any time following vernalization. If the mechanisms proposed for vernalization and photoperiodic induction of flowering are universal in plants, then cold treatment eliminates a block early in the kaurenoid pathway (Hazebroek and Metzger, 1990) and LDs remove a block ($GA_{19} \neq > GA_{20}$) later in the GA pathway (Metzger and Zeevaart, 1980).

As a follow-up to this study, levels of kaurenoid compounds in tissues analysed herein for GAs (Figures 5.5 to 5.11) will be determined by GC-MS-SIM using [$^2H_2$]-labelled quantitative internal standards. This will be accomplished for pre-, mid- and post-vernalized plants (both LD- and SD-grown) to determine if there is a metabolic block present in the kaurenoid pathway.

SUMMARY

Photoperiod prior to vernalization has no effect on the timing or rate of flowering following vernalization. *B. napus* cv. WW1033 plants are obligate LD plants following vernalization as SD post-vernalization results in no internode elongation and no flowering. The increase in stem elongation under LD post-vernalization conditions can be explained by increases in endogenous GA levels over those of SD post-vernalization grown plants. Plants maintained under short-day following vernalization are capable of elongating and flowering when transferred to LD conditions, indicating that the vernalized state is stable under SD conditions. De-vernalization causes slight internode elongation with no flowering, therefore, the vernalized state is temperature sensitive and is destroyed by rapid warming.

CHAPTER SIX

When you finally see the light at the end of the tunnel,

the roof usually collapses (author unknown).

GENERAL DISCUSSION

Because of their sessile nature, plants must be able to adapt to an ever-changing and often unpredictable environment. The sexually reproductive phase of a higher plant's life cycle, flowering, which results in seed production, is of intense interest to humans. A knowledge of the processes of floral induction, initiation and development is important to a number of industries including; agriculture, horticulture and biotechnology. Research into the physiology of flowering has been prolific but also inconclusive. Hypothetical compounds such as "florigen" and "vernalin" have been coined to explain what we have been unable to define.

Timing of the induction of flowering has been attributed to environmental cues including the photoperiod duration, quality of light and temperature. It is important for a plant to have tight control on the induction of flowering, especially in monocarpic species, where seed production may be poor if flowering is initiated

under less than ideal conditions.

In order for a plant to perceive the surrounding environment it must have a means of measuring and quantifying a particular condition. In the case of light, plants have evolved an elaborate family of photoreceptors (i.e. phytochromes) that are capable of determining the presence of light, its length (photoperiod) and its quality. Once a plant has perceived a flowering stimulus, there follows a series of signal-transduction steps leading to floral initiation and development.

The primary goal of my thesis research was to determine whether gibberellins are involved in the induction of flowering by light (both quality and photoperiod) and cold in *Brassica* species. The economic importance of *Brassica* (canola) has been stated earlier, but factors including: diversity of responses to environmental factors (temperature and light); relatively rapid responses to environmental factors; ease of cultivation and relatively large size of various organs, make canola an ideal research organism.

Plant Growth Regulators in Floral Induction

The exogenous application of growth-active GAs to *Brassica* plants grown under non-inductive conditions was in all cases able to promote stem elongation, and in a few cases was able to promote flowering. In fact, almost all GAs tested promoted stem elongation well over that of controls for annual cultivars of *Brassica* maintained under SD conditions, and also in biennial cultivars of *B. napus* and *B. rapa* maintained under both LD- and SD-warm conditions (i.e. non-vernalized). In

cases where GA application resulted in floral induction, stem elongation always preceded flowering, whereas the opposite is true in naturally induced plants.

The ability to flower in response to applied GAs and other PGRs under non-inductive conditions seemed to be both cultivar and GA specific. Cultivar WW1033 was the least intractable of the biennial cultivars, flowering in response to certain GAs, to vernalization and to continuous light. As noted in Evans *et al.* (1993a), structural modifications of the GA molecule are able to impart various levels of florigenicity, or promote elongation, with or without flowering. Although not known to be endogenous in *Brassica*, GA_5 and 2,2-diMe GA_4 were able to induce flowering in the biennial cv. WW1033 under non-inductive LD warm conditions. A positive relationship also exists between these GAs and the GA biosynthesis inhibitor CCC, the latter giving decreased stem elongation, but synergistically increased flowering. As well, a synergistic relationship exists between exogenously applied GAs and BA, with increased flowering occurring at lower GA levels when they are applied with BA.

The differential promotion of stem elongation and floral induction by exogenously applied GAs in *Brassica* spp., implies that natural induction may be under the control of different, but convergent signal transduction pathways, each interacting with different parts of, or different pathways in GA biosynthesis. The limited success in promoting flowering under non-inductive conditions (i.e. cultivar and GA specific responses), indicates that our understanding of the flowering process is still very preliminary. It is thus possible that floral induction is only

partially controlled by GAs, and that there are other promotive compounds which are not present, except following vernalization. Or, perhaps we have yet to identify the specific GA responsible for flowering due to its low levels or rapid metabolism. There is also the possibility that anti-florigenic molecules exist at higher levels under non-inductive conditions and/or that apex cells are non-responsive until low temperature is given. That is, the plant may not be sensitive to "florigen" or "vernalin" until after a period of low temperature.

Photoperiod Manipulation

Biennial *B. napus* cv. WW1033 plants could be induced to flower under non-inductive warm conditions if grown in continuous light (LLD). *Brassica* spp. are LD plants, but the substitution of the LLD for cold is unique to this cultivar, though not unknown for other Brassicaceae species. This indicates that LLD could be acting at the same point in the signal transduction pathway as vernalization. Lang (1965) also indicates that LLD can substitute for vernalization in several other CR species.

Changes to Light Quality

Brassica hypocotyls and epicotyls elongate in response to changes in the ratio of R:FR light from that of natural sunlight (R:FR=1). However, one cannot generalize between responses by hypocotyls, which cease to grow prior to epicotyl elongation, and responses by epicotyls. Also, the R:FR ratio to which seedlings are first exposed controls the elongation of the hypocotyl, even when the light

quality is subsequently changed. For the hypocotyl, a very increased R, or increased FR caused significant elongation, relative to LD greenhouse-grown plants. The levels of endogenous GAs (GA_1, GA_{20} and GA_{19}) were found to be significantly higher in hypocotyls exposed to elongation-promotive light sources (high R or High FR), relative to hypocotyls of those plants exposed to the less promotive light sources. In essence, levels of these three GAs were a significant variable with regard to elongation produced by light sources of different R:FR ratios. The increased hypocotyl elongation could be attributed to equal increases in both cell number and length when induced by R, but were attributed more to an increase in cell length under FR. The differences in cell number versus cell size indicate that R and FR light-induced hypocotyl elongation (which was significantly related to endogenous GA levels), may also involve other factors not identified in this study.

In the summer annual *B. napus* cv. Westar, when hypocotyl growth ceases, epicotyl elongation dominates. Increases in FR resulted in significant epicotyl elongation, but unlike the hypocotyl response, an increase in R did not promote epicotyl growth of the summer annual cultivar. There was a direct and significant relationship between epicotyl elongation and increased levels of certain endogenous GAs. For example, GA_1 or combinations of GA_1+GA_{20}+GA_{19} or a combination of GA_1+GA_3+GA_4+GA_7 were highly significantly correlated with epicotyl elongation. This may indicate that the signal transduction pathway from FR light to epicotyl elongation has, as a key intermediate step, increases in levels of

bioactive GAs and their precursors.

Although not vernalized, biennial plants of *B. napus* cv. WW1033 also elongate in response to changes in ratios of R:FR. Both R and FR cause increased hypocotyl and epicotyl elongationin biennial cv. WW1033, which can, as for the summer annual cultivars be related to the level of endogenous GAs (i.e. combinations of GA_1+GA_{20}+GA_{19} and GA_1+GA_3+GA_4+GA_7). The overall increase in plant height resulting from high ratios of R:FR for the CRP cv. WW1033 can be attributed solely to increased hypocotyl elongation, whereas the FR light results only in increased epicotyl elongation. This is quite different from the summer annual cultivars, where increases in hypocotyl growth could be gained from both R and FR, but only prior to epicotyl elongation.

These results mean that care must be taken in interpreting elongation responses since different qualities of light may have varied effects at different times of ontogeny or on different tissues.

Photoperiod and Vernalization

It was found that *B. napus* cv. WW1033 possesses an obligate requirement for LD with regard to flowering following vernalization. The photoperiod given prior to vernalization had no effect on the rate or time to flowering. The low temperature-induced vernalized state can be maintained under SD warm conditions, as cold-treated plants transferred from warm-SD to warm-LD flowered. Levels of key GAs (GA_1, GA_3, GA_8, GA_{19} and GA_{20}) all increase in the apical region

under LD post-vernalization conditions, but remain low under SD warm post-vernalization conditions. This indicates that although one of the proposed blocks in early GA biosynthesis may released by vernalization, there remains a second block that requires LD to be released.

The vernalized state is very sensitive to temperature following vernalization. Rapid warming (devernalization) results in the loss of the ability to flower, yet some stem elongation does occur. Levels of GAs following rapid warming (under LD) are appreciably lower than those of slowly warmed plants.

The importance of having a dual control on floral induction and initiation becomes apparent when one looks at the ecology and climate in which cold-requiring plants are native. Autumn temperatures may be low enough to vernalize plants, but the shortened photoperiod maintains plants in their vegetative state, even during temporary periods of warmth. Temperature alone is too unstable to be used as the key control point, whereas temperature combined with photoperiod allows for a more stringent control of flowering.

Further Experiments

From tissues grown and harvested in these experiments, levels of kaurenoid compounds present during and following vernalization will be measured. This may tell us definitively at which point in the early GA biosynthetic pathway the vernalization block lies (i.e. the block that is removed by low, vernalizing temperatures).

Finally, the use of the CR cultivar WW1033, a plant with multiple triggers for the initiation of flowering (GA treatment, cold, LD following cold and LLD)(Figure 6.1) gives us a very useful tool in understanding the several steps in the signal transduction pathway that leads to flowering on both the physiological and molecular levels.

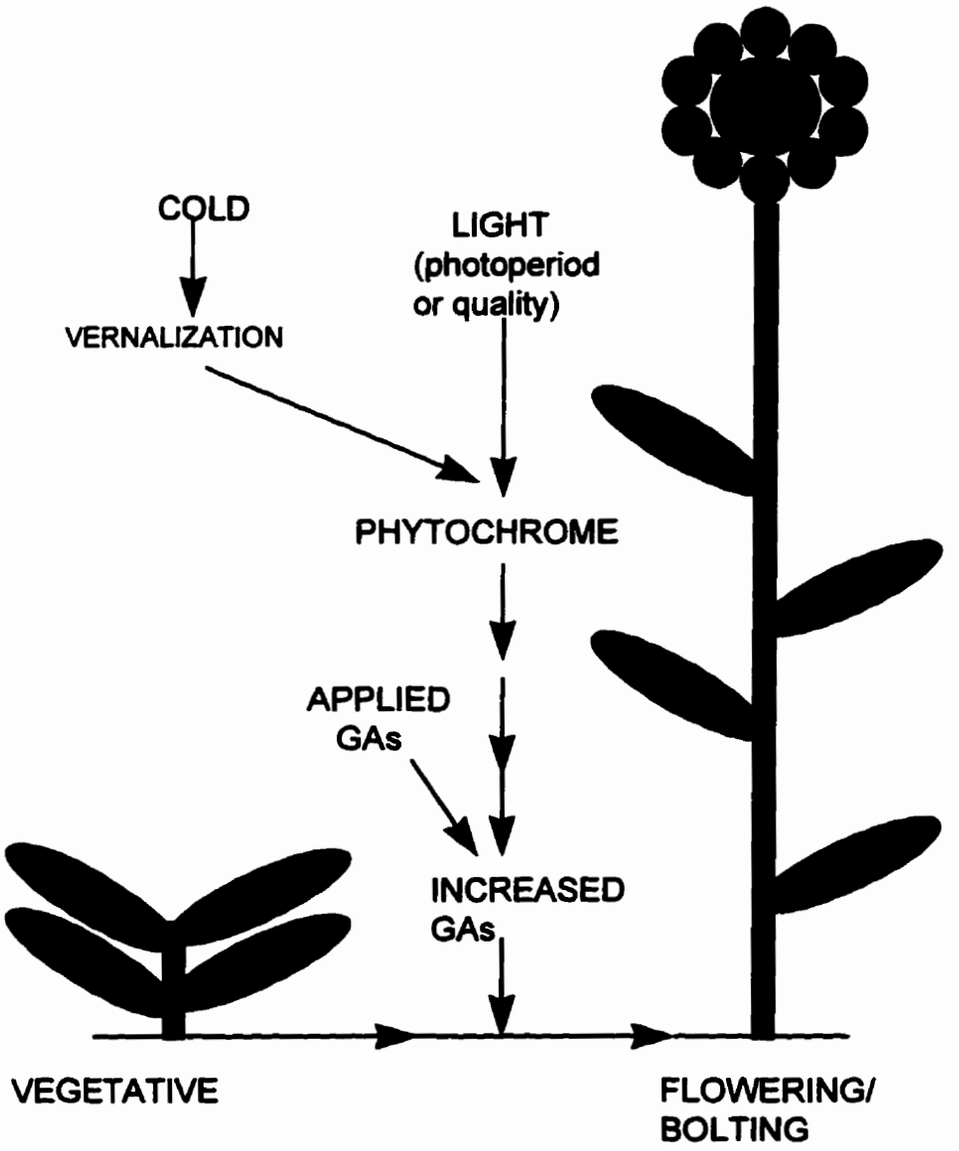


Figure 6.1. An overview of the interactions of cold, light and applied GAs on bolting and flowering in *Brassica*.

Conclusions

- Exogenous application of structurally specific GAs is able to induce flowering in the cold-requiring *Brassica napus* cv. WW1033 under non-vernalized warm long-day conditions.
- A synergistic relationship exists for the flowering response between floral promotive GAs and each of CCC and BA.
- The endogenous GAs involved in stem elongation differ in structure from those which are highly effective in inducing flowering, implying that stem bolting and floral induction are two independent processes.
- Continuous light can substitute for vernalization in *B. napus* cv. WW1033.
- Gibberellin levels of hypocotyls and epicotyls increase significantly in *B. napus* cv. Westar (annual) and cv. WW1033 (biennial) when exposed to varied light qualities (R:FR ratios) that result in enhanced levels of stem elongation.
- Gibberellin levels increase in the apical region of post-vernalized plants of *B. napus* cv. WW1033 under warm long-day but not under warm short-day conditions.
- Rapid warming following vernalization (de-vernalization) results in a loss of the vernalized state (no flowering) and reduced levels of GAs relative to gradually warmed LD plants.

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