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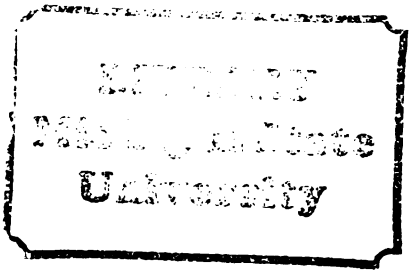

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EVALUATION OF CHEMICALS FOR FLORAL INDUCTION AND STALK ELONGATION IN SUGARBEET (BETA VULGARIS L.)

presented by

Martin D. Mahoney

has been accepted towards fulfillment
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Major professor

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EVALUATION OF CHEMICALS FOR FLORAL INDUCTION AND STALK ELONGATION IN SUGARBEET (*BETA VULGARIS* L.)

By

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ABSTRACT

EVALUATION OF CHEMICALS FOR FLORAL INDUCTION AND STALK ELONGATION IN SUGARBEET (Beta Vulgaris L.)

by

Martin D. Mahoney

Floral induction and stalk elongation of sugarbeet (Beta vulgaris L.) were evaluated after applications of gibberellic acid (GA_3) at various photoperiods. Combination of GA_3 with plant hormones or hormone-like chemicals ethephon [(2-chloroethyl)phosphonic acid], 2,4-D[2,4-dichlorophenoxy)-acetic acid], NAA(α-napthaleneacetic acid), and kinetin (6-furfurylaminopurine), and herbicides reported to alter plant lipid metabolism were also evaluated. GA_3 applications in combination with photoperiods of 18/6, 24/0 hr (day/night) or 14/10 hr plus a 2-hr nightbreak substantially increased flowering over the untreated controls. Growth chamber, greenhouse or field application of GA_3 in combination with ethephon, 2,4-D, kinetin, members of thiocarbamate, acetanilide, and benzoic acid herbicide classes, naptalam (N-1-naphthylphtahalamic acid), TCA (trichloracetic

acid), ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-S-benzofuranyl methanesulfonate), dalapon (2,2-dichloropropionic acid), and glyphosate [N-(phosphonomethyl) glycine] resulted in a synergistic increase in stalk elongation, but not floral induction. Uptake of ^{14}C-GA_3 by sugarbeet foliage was not increased by pretreatment with alachlor (an acetanilide herbicide) and did not explain that interaction.

GA_3 may substitute for the cold temperature when foliar applications of GA_3 induce biennials to flower. A shift in the fatty acid composition of membranes may be involved. Low temperature, alachlor, GA_3, and GA_3 plus alachlor decreased the saturated and increased the unsaturated fatty acid composition of both mitachondrial and plasmalemma membrane fractions. The unsaturated fatty acid content of plasmalemma membranes of annual and florally induced biennial sugarbeets increased with time, whereas the mitochondria fraction showed no change. In non-induced plants, there was a shift toward greater fatty acid unsaturation in mitochondria but not plasmalemma membranes. Fall applications of alachlor and vernolate which produce similar effects as low temperature on plant cell membranes increased the survival rate of sugarbeets in one study, which suggests that these materials may aid in the cold hardening of plants. In a second study, sugarbeet survival in the absence of chemical treatments was too high to adequately assess the chemical affects.

To my wife Chris and my sons Michael and Ryan

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INTRODUCTION[1/]

Considerable research has been directed toward elucidation of the mechanisms involved in floral induction of seed plants and has been summarized in several reviews (6, 13, 14, 24, 25). Unfortunately, there have been no major advances in recent years in determining the underlying biochemical and physiological mechanisms involved in floral induction (24).

Flower initiation in plants represents the transition from vegetative growth to reproductive development. The flowering process encompasses several steps. It often starts with perception of an environmental stimulus (temperature, daylength), followed by changes in the shoot apex, and terminates in the appearance of flower or inflorescence primordia (24).

Two environmental parameters of prime and specific importance in floral initiation are daylength (photoperiod) and low temperature (vernalization). Photoperiodic plants require a specific daylength, (usually called inductive), and they fall into two main response types, long-day plants and short-day plants (7). The long-day types require exposure to photoperiods longer than a critical daylength

in order to undergo rapid floral induction whereas short-day plants require a photoperiod shorter than a critical daylength. There is generally an overlap in daylength necessary for floral induction of long- and short-day types of different species (14). The number of days necessary for photoinduction varies widely with species, and long- and short-day plants can be facultative or obligate with respect to their photoperiodic requirements and generally have to reach a certain age requirement for flowering (14).

Two additional but apparently much less frequent photoperiodic response types are characterized by a dual daylength requirement; the long-short-day and the short-long-day plants (18, 22). A fifth group or response type to photoperiod are those plants which will flower irrespective of daylength and are called day-neutral plants.

The major site of photoperiodic perception has been shown to be the leaves in both long- and short-day plants (12). In several cases, if only a single leaf perceived the inductive photoperiod flowering will be initiated.

The promotion of floral induction by low temperature is a process known as vernalization and is generally associated with plants in nature that have to pass through a winter period before they are capable of flowering (14). Winter annuals and biennials are plants that have this cold requirement, the latter being of a facultative nature

for winter annuals but obligate for biennials (23).

Plants with a cold requirement for flowering or promotion of flowering have a subsequent requirement for higher temperatures and in most cases, also for long-days before flower primodia are formed. In such plants, the cold period is of a strictly inductive character with the actual initiation of floral parts occurring in warmer temperatures and long-days (23). Winter annuals such as fall or winter cultivars of Triticum aestivum L. and other cereals have a facultative requirement for both cold temperatures and long-day as they will flower in the absence of these conditions although at a considerably slower rate (14). On the other hand, biennials such as sugarbeets (Beta vulgaris L.) have an obligate requirement for both cold temperatures and long-days.

Other characteristics of the low temperature effect include site of perception at the shoot tip, an optimum temperature generally in the range from 0 to 10 C, depending on species, and reversal of the inductive effect by high (greater than 15 C) temperatures (23). However, after a plant has been subjected to low temperatures for long periods of time (several weeks), the vernalized state is said to be stabilized and will not be reversed by high temperatures.

Since perception of photoinduction occurs in the leaf but the response occurs in the shoot apex, a

"communication" is clearly taking place. This fact has given rise to the flower-hormone (florigen) concept as proposed by Chailakhyan (2). This gained further support from grafting experiments which showed that a receptor plant, maintained under non-inductive conditions would flower when a florally induced donor was grafter to it (9, 20). It has further been proposed that plants which require vernalization produce an additional hormone-like substance (vernalin) and that the presence of this substance is necessary for the formation of florigen (14), the ultimate factor required for flowering in cold requiring plants. Despite a considerable amount of research, these substances unfortunately have never been isolated and the biochemical processes involved have not been elucidated.

As mentioned above, most sugarbeet cultivars are biennial plants with an obligate or qualitative requirement for cold temperatures for floral induction. The effective range of low temperature is from 2.75 to 10 C, with an optimum of 4.4 C and these temperatures have to be applied for approximately 1 to 2 months, followed by a requirement for warmer temperatures (21 to 27 C) and long-days (14 to 16-hr photoperiod) (15, 19, 21). This property of biennial sugarbeet poses a problem for the plant breeder because of the length of time required to obtain seed under natural conditions. Thus, if sugarbeets could be induced to flower in one growing season, it would facilitate sugarbeet improvement through accelerated

breeding programs (11).

It was recently observed that young biennial sugarbeets were induced to flower within 30 days in a particular growth chamber (11). This phenomenon lasted for a period of approximately 2 years but could not be reproduced afterwards. Examination of records of photoperiod and temperature indicated that climatic induction of these plants was precluded. Although the cause of floral induction in this chamber was not readily apparent, a chemical induction may have been involved.

Gibberellic acid (GA_3) can induce floral initiation without any cold treatment in several biennials but not in beet (1). Sugarbeet was induced to flower by GA_3, only under partial photothermal induction (continuous illumination with temperatures of 7 to 8 C for 43 days)(8). It is thus apparent that GA_3 does not substitute completely for the cold temperature requirement in sugarbeet.

Cold temperatures cause a shift in the fatty acid content of plant cell membranes toward greater unsaturation (10). The herbicides diethatyl [N-(chloroacetyl)-N-(2,6-diethylphenyl)glycine] and vernolate (S-propyl dipropylthiocarbamate) have been shown to have an effect similiar to that of low temperature on plant cell membranes under warm temperature (30 C), causing a shift to a higher percentage of unsaturated fatty acids (17). Since these herbicides have been shown to substitute for the low temperature effect on membranes, the possibility exists

that these chemicals plus GA_3 might substitute completely for the cold temperature induction necessary for flowering in sugarbeets.

The herbicide EPTC(S-ethyl dipropylthiocarbamate) (a vernolate analog) interacts antagonistically with GA_3 in corn (Zea mays L.) (4). GA_3 has also been shown to interact with other plant hormones on various processes including flowering in plants other than sugarbeet (3, 5, 16).

The objectives of this investigation were to evaluate the effect of GA_3 and GA_{4+7} in combination with photoperiod, other plant hormones, and herbicides that alter lipid metabolism on floral induction and stalk elongation in sugarbeet and to determine the basis for any observed interaction.

1/ Chapter 1, 2, 3 and 4 will be submitted to Agronomy Journal, Weed Science, Plant Physiology, and Journal of the American Society of Sugar Beet Technologists, respectively, for publication. Discrepencies between chapters with respect to format and listing of units occurs because of different requirements for each journal.

CHAPTER I

Evaluation of Hormonal and Environmental Influence on Floral Induction and Stalk Elongation in Sugarbeet (Beta vulgaris L.)

ABSTRACT

If biennial sugarbeet (Beta vulgaris L.) could be induced to flower in the first year, it would facilitate sugarbeet improvement through acelerated breeding programs. Gibberellic acid (GA_3) applications to the foliage of 2 to 4-week-old 'EL44', 'US H20' and 'FC701/5' sugarbeets receiving various photoperiods or in combination with the chemicals ethephon (2-chloroethyl)phosphonic acid, 2,4-D(2,4-dichlorophenoxy)acetic acid), NAA (naphthalene-acetic acid), kinetin (6-furfurylaminopurine), diethatyl (N-chloroacetyl-N-(2,6-deithylphenyl)glycine) and EPTC (S-ethyl-N,N-dipropylthiocarbamate) were evaluated. GA_3 application in combination with photoperiods of 18/6, 24/0 hr (day/night), or 14/10 hr plus a 2-hr nightbreak substantially increased flowering over the untreated controls. GA_3 application in combination with ethephon, 2,4-D or kinetin resulted in a significant increase in stalk elongation over either chemical alone, indicating a synergistic interaction.

An understanding of the control of the biochemical and physiological mechansims involved in floral induction of plants presents many opportunities. Among, these are increasing yield, quality and/or harvestability of crops and an increase in the number of generations of seed in a relatively short period of time for the purpose of crop inprovement. Crop improvement through accelerated breeding programs would be particularly useful for biennial crop species such as celery (Apium graveolens L.), carrots (Daucus carota L.), beets (Beta vulgaris L.) and members of the genus Brassica.

Control of flowering in biennial sugarbeet would be beneficial in two ways. Inhibition of flowering in sugarbeet would allow control of these plants where they are growing as weeds in a sugarbeet crop grown for sugar (Arnold, 1980). This problem is widespread throughout the sugarbeet growing regions in Europe and is starting to become a problem in certain sugarbeet growing regions of the United States. A second benefit from the control of flowering in sugarbeet would be to induce these plants to behave as flowering annuals for the improvement of the crop through accelerated breeding programs (Hogaboam, 1982).

Although sugarbeet is predominately a biennial species, annual types do exist. These flower under long days, but do not require a cold induction period; whereas,

the biennial types require a cold induction period (2.75 C to 10 C with an optimum of 4.4 C for approximately one to two months) followed by a requirement for warmer temperatures (21 to 27 C) and long days (14 to 16-hr photoperiod) (Pack, 1925; Shaw, 1917; Stout, 1946). Biennial sugarbeet lines and cultivars exhibit a bolting tendency which is dependent on temperature, day length and length of the photothermal inductive periods as described above. It was found that without any cold temperature exposure, a certain strain of sugarbeet could be induced to flower at 23 C by use of continuous high-intensity illumination (Steinberg and Garner, 1936). Under 18-hr photoperiods, flowering did not occur at 23 C but was induced if the temperature was lowered to 16 or 18 C. Other research has shown that biennial sugarbeet will flower under 14-hr fluorescent plus continuous incandescent illimination (Hogaboam, 1982), eliminating the need for continuous high-intensity lighting. Combinations of cool temperature and incandescent light have also been shown to shorten the time to flower in sugarbeets. Thus, it appears that the effects of light and temperature are complementary on sugarbeet floral induction.

Adjusting environmental parameters to induce flowering in sugarbeets does not lend itself well to field production of seed in one growing season which would be necessary for accelerated improvement of this crop. However, recent

research indicated that it was possible to induce biennial sugarbeet to flower within one growing season (Hogaboam, 1982). Sugarbeets grown in a particular growth chamber flowered within 30 days following seeding and this phenomenon occurred in several experiments over a period of more than two years. This chamber was set at a non-inductive 14/10 hr light/dark period with 22/14 C day/night temperature. Careful examination of records of photoperiod and temperature conditions did not provide any indication that the sugarbeets were climatically induced to flower in this chamber. Additional experiments in this chamber revealed that not all lines evaluated, flowered, while in others, almost all of the plants were induced. Another experiment also indicated that this induction occurred within 2 months from the time of seeding. Duplicate sets of experiments done in another chamber under the same set of environmental conditions did not induce flowering. Although it was not readily apparent what caused the flowering response, this observation holds out hope that a purely chemical induction might be possible. The possibility of ozone or freon leaks within the chamber suggests examination of these agents for their potential for floral induction.

After it had been demonstrated that gibberellic acid (GA_3) acted as a growth promoting substance, it was found that it can cause seed stalk development in plants

including sugarbeets (Brian et al., 1954; Marth et al., 1956) and hasten the reproductive development of sugarbeet seedlings (Gaskill, 1957). These results offer a potential for field production of sugarbeet seed under non-inductive climatic conditions. However, GA fulfilled only part of the requirements for flowering as it was effective only when applied under continuous incandescent light and a temperature of approximately 8 C. Other research substantiated these results in that a non-bolting sugarbeet line would flower under continuous light plus GA_3 (Stout, 1959) and a biennial variety of intermediate bolting tendency flowered under an 18 but not a 9 hr photoperiod in combination with gibberellin treatment (Snyder and Wittwer, 1959). However, the minimal day length needed for flower induction by GA was not determined, and GA_3 was the only gibberellin tested. In addition, the optimum stage of GA application to sugarbeet was not established in these studies.

In recent literature, it has been shown that GA interacts with other chemicals in plants. Gibberellins and N^6-benzyladenine were shown to have synergistic effect on flowering in Chrysanthemum morifolium cv. Pink Champagne (Pharis, 1972). Gibberellin also interacted synergistically with kinetin in purple nutsedge (Chetram and Bendixen, 1974) and with ethylene to reverse induced dormancy in lettuce seed (Dunlap and Morgan, 1977). Antagonistic interactions of GA with herbicides in barley endosperm (Devlin and Cunningham, 1970), Avena seedlings

(Chang et al., 1975) and corn (Harvey et al., 1975; Donald, 1977) have been reported.

In this study, the objectives were to determine relative effectiveness of two gibberellins, critical day length plus GA, freon, ozone, GA in combination with other hormones and selected herbicides on flowering. The influence of leaf removal on GA action in sugarbeet was also of interest.

MATERIALS AND METHODS

Plant Material, Growth Conditions, Experimental Design. The following procedures were used unless specified otherwise.

Five to ten sugarbeet seeds, selected from lines 'EL44' (intermediate bolting tendency) and 'FC701/5' (high bolting tendency) and cultivar 'US H20' (intermediate bolting tendency) were placed 2.5 cm deep in a commercial potting mixture (Metro-Mix 300) in 948 ml styrofoam cups. Prior to chemical treatment, the seedlings were thinned to one plant per pot of a uniform size. All formulated chemicals (GA_3 formulation was Pro Gibb) were diluted in tap water on a mg/l basis and applied to the foliage until runoff using a model No. 152 DeVilbiss atomizer with 0.35 to 0.70 kg/cm^2 pressure. During the growth period, all pots received 100 ml of a 5000 mg/l 20:20:20 NPK fertilizer

solution once every 2 weeks. After cessation of stalk elongation (4-6 weeks after the last chemical application) stalk height was measured and flowering data recorded. All experiments were done in the growth chamber, which had fluorescent and incandescent lamps or in the greenhouse under natural or supplementary (16/8 hr, day/night) lighting from fluorescent or sodium vapor lamps. The growth chambers were set at a 14/10 hr, 22/14 C light-temperature regime with a photosynthetic photon flux density (PPFD) that ranged from 300 to 400 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Greenhouse temperatures ranged from 16 to 29 C from late fall through early spring and 20 to 35 C from late spring through early fall. The PPFD under fluorescent and sodium vapor lamps in the greenhouse was 150 and 300 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and that under natural lighting was 750 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ during the summer months and 300 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ during the winter months. All pots were placed in their respective growth environments (greenhouse or growth chamber) under conditions listed above immediately after seeding.

The experimental design was a randomized complete block with three to five replications. The data were analyzed using a two-way factorial analysis of variance when two treatment factors were used and means were compared by Duncan's multiple range test. All experiments were repeated to confirm results.

Comparison of GA_3 and GA_{4+7}. GA_3 and GA_{4+7} (Pro Gibb 47 from Abbott Laboratories) were applied to 3-week-old

'US H20' sugarbeet seedlings at 0, 1, 20, 100, 500 and 2500 mg/l either once or eight times over 3 weeks. The experiment was done in the greenhouse under natural lighting from July 25 through October 18, 1980.

Critical Photoperiod in Combination with GA_3. GA_3 was applied to the foliage of 'FC701/5' sugarbeets in growth chambers at 0, 200, 250, 500 and 1000 mg/l under a 14/10 hr photoperiod; 0 and 250 mg/l under a 16/8 hr photoperiod; 0, 500 and 1000 mg/l under 18/6 and 14/10 (fluorescent) plus 24/0 (incandescent) hr photoperiods. GA_3 was also applied at 0, 500, 1000, and 5000 mg/l to plants grown under a 14/10 hr photoperiod with a 2 hr or 20 minute night break with incandescent lighting in the middle of the dark phase.

Effect of Freon and Ozone on Sugarbeets. Ozone was generated by passing a stream of air (20 cc/min) along an ultraviolet light within a sealed container. The generated ozone was forced into a sealed growth chamber containing 'US H20' or 'FC701/5' sugarbeets. The plants were grown under sodium vapor lamps with a PPFD of 450 $\mu mol \cdot m^{-2} \cdot sec^{-1}$.

In another experiment, freon (freon 12 by Dupont) was applied to sugarbeets grown in this chamber. This material was forced into the chamber as a gas at 3 cc/min from a 13.6 kg cylinder used for recharging refrigeration units. Plants were examined for flowering and/or stalk height 8 to 10 weeks after seeding.

Leaf Removal in Combination with GA_3. GA_3 was applied

to the foliage of 2 to 3-week-old 'US H20' and 'FC701/5' sugarbeet seedlings at 0, 50, 200, 500, 1000 and 2500 mg/l in the growth chamber or greenhouse. In the growth chamber experiment, the chemical was applied in 7 weekly applications. Prior to the fourth application, all except the four youngest visible leaves were removed from the stem. The photoperiod was 16/8 hr (day/night) with the temperatures at the usual settings. In the greenhouse experiment (under sodium vapor lamps from September 7 through December 21, 1981) GA_3 was applied in six weekly applications with various combinations of leaf removal. In one set of plants, all but the youngest four leaves were removed throughout the duration of the experiment. In three other sets, leaves were removed as follows:

Set 2 - All leaves except the four youngest leaves once prior to the fourth application.

Set 3 - As for set 2 except leaves were removed once prior to the first application.

Set 4 - The four youngest leaves were removed once prior to the fourth chemical application.

Combinations of GA_3 with Ethephon [(2-Chloroethyl)-phosphonic acid]. Combinations [GA_3 and ethephon solutions mixed together (tank-mixed)] of GA_3 at 0, 50, 250, 500, 1000, 3300 and 5000 mg/l with ethephon (Ethrel) at 0, 1, 2, 5, 10, 20 and 100 mg/l were applied to the foliage of 2 to 3-week-old 'FC701/5' or 'US H20' sugarbeet seedlings grown in greenhouse or growth chamber. In the greenhouse

experiments, the chemicals were applied once or the applications were repeated four times at weekly intervels. The plants were grown under natural lighting from June 17 through September 4, 1980. In the growth chamber, the chemical combinations were applied as repeated applications three times per week for 2 weeks to 'FC701/5' sugarbeets and in four weekly applications to 'US H20'sugarbeets.

Combinations of GA_3 with Auxin-Type Chemicals. Combinations (tank-mixed or sequential) of GA_3 at 0, 10, 50, 200, 500, 1000 and 2500 mg/l with 2,4-D (2,4-(dichlorophenoxy)-acetic acid) at 0, 0.125, 0.25, 0.50, 1.0, 2.5, 5 and 25 mg/l were applied to the foliage of 3 to 4-week-old 'FC701/5' or 'US H20'sugarbeet seedlings. The tank-mixed combinations were applied in three weekly applications to plants grown in the greenhouse from November 15, 1980 through February 4, 1981. For the sequential applications, GA_3 was applied at four weekly intervals. Two weeks following the last GA_3 application, 2,4-D was applied in three weekly applications. One of the sequentially applied chemical experiments was done in the greenhouse under sodium vapor lamps and included NAA (α-naphthaleneacetic acid)/GA_3 combinations. The other experiment was done in the growth chamber.

Combination of GA with Kinetin (6-furfurylaminopurine). GA_3 and GA_{4+7}(2% solution from Abbott Laboratories) were applied at 0, 1000 and 5000 mg/l in combination (tank-mixed) with kinetin at 0, 0.01 and 1.0 mg/l on 3-week-old

'US H20' and 'EL44' sugarbeets grown in the greenhouse under natural lighting from October 30, 1979 through January 4, 1980. The form of kinetin used was Cytex which is a seaweed extract containing 100 ppm kinetin.

Combinations of GA with Diethatyl [N-Chloroacetyl-N-(2,6-diethylphenyl) glycine ethyl ester] and EPTC (S-ethyl-N,N-dipropyl thiocarbamate). GA_3 and GA_{4+7} (2% solution) were applied at 0, 1000 and 5000 mg/l in combination (tank-mixed) with the herbicides diethatyl (Antor 4E) and EPTC (Eptam 7E) at 0, 10 and 100 mg/l on 3-week-old 'US H20' and 'EL44' sugarbeet seedlings grown under natural lighting in the greenhouse from October 30, 1979 through January 4, 1980.

RESULTS AND DISCUSSION

Comparison of GA and Critical Photoperiod in Combination with GA_3. GA was found to be most effective for inducing stalk elongation in sugarbeets when given as repeated applications to the foliage of young sugarbeet plants (Table 1). GA induced stalk elongation but not flowering under non-inductive environments with no differences between GA_3 and GA_{4+7}. Biennial sugarbeets have been induced to flower under continuous (24 hr) incandescent light (Gaskill, 1952). By increasing the length of the photoperiod in increments from 14/10 to 24/0 day/night with light from incandescent plus supplemental

fluorescent lamps flowering occurred only at the 24/0 day/night photoperiod in the absence of GA_3 (Table 2). When GA_3 was applied to plants growing under various photoperiods, flowering was enhanced with a minimum day length of 16 hr for the response to occur. Flowering was also enhanced with GA_3 applications when sugarbeets received a 2 hr nightbreak in the middle of the dark period of the 14/10 hr, day/night, cycle. Flowering was not enhanced when the plants were given a 20 minute nightbreak.

Leaf Removal in Combination with GA_3. Non-induced leaves have been reported to produce an inhibitory effect on flowering (Lang, 1965). GA_3 applications to sugarbeet seedlings which had mature or young leaves removed, resulted in no flower initiation and a generally negative effect on stalk elongation (Tables 3 and 4). Only one treatment (GA_3 at 200 mg/l on 'FC701/5') produced a positive effect when leaves were removed (Table 3).

Combinations of GA_3 with Ethephon. Ethylene has been reported to induce flowering in members of the Bromeliaceae (Zeevaart, 1978) and to interact synergistically with GA_3 on lettuce seed germination (Dunlap and Morgan, 1977). Ethephon alone or in combination with GA_3 did not induce flowering in sugarbeets under various environmental conditions (Tables 5, 6, 7). However, a synergistic interaction was observed as GA_3/ethephon combinations resulted in greater stalk height than either chemical alone.

Combinations of GA_3 with Auxin-Type Chemicals. Auxin-type materials are not generally associated with floral induction (Zeevaart, 1978) but have been shown to interact with GA_3 for increased hypocotyl elongation in cucumber (Cucumis sativus L. cv. National Pickling) (Kazama and Kasuni, 1974), the strongest effect being obtained when GA_3 was given to the plant material as a pretreatment. Combinations of 2,4-D with GA_3 at a given combination of rates (tank-mixed or sequential) resulted in a synergistic interaction in the form of a significant increase in stalk height over that of either chemical alone (Tables 8 and 9). In these and similar experiments, 2,4-D did not induce flowering alone or in combination with GA_3, except for one experiment (Table 9). However, it should be noted that sugarbeets growing in this chamber were subjected to 2 weeks of 3 C daytime temperatures due to a malfunction of the heating system. The cool temperature in combination with the chemical treatments may have been responsible for the induction. NAA did not appear to enhance the GA effect on stalk elongation nor did it have any effect on flowering.

Combinations of GA with Kinetin. Cytokinins have been shown to interact with GA to induce flowering in chrysanthemum and other plant responses (Pharis, 1972, Chetram and Bendixen, 1974). Kinetin in combination with GA_3 or GA_{4+7} did not induce flowering after single applications (Table 10). However, kinetin interacted synergistically with GA_{4+7} for increased stalk enlongation. Combinations with GA_3 resulted in an antagonistic interaction, indicating that the rates

of kinetin may be too high.

Combinations of GA with Diethatyl and EPTC. Combinations of GA_3 and several herbicides have produced varied interactions in plants as described previously (Devlin and Cunningham, 1970; Chang et al., 1975; Donald, 1977). The interactions were of an antagonistic nature and the work was done on monocotyledons. Single applications of GA in combination with diethatyl or EPTC did not induce flowering or produce a significant interaction for increased stalk elongation (Table 11). However, rates of EPTC may have been too high as decreasing stalk heights were obtained with high rates of GA for 'US H20' and all GA rates in 'EL44' sugarbeets. This indicates that lower EPTC rates might produce a synergistic interaction. Diethatyl/GA combinations resulted in a trend toward increased stalk height with increasing rates of the herbicide in 'US H20'. The same effect was observed in 'EL44' with low rates of GA plus the herbicide, but diethatyl plus high GA rates resulted in a trend toward decreasing stalk height.

Ozone and freon gas applied to sugarbeets did not induce flowering or stalk elongation. Ozone appeared to injure the plants slightly.

CONCLUSION

Repeated applications of GA_3 induced greater stalk elongation than single applications, were found to produce a response equal to GA_{4+7}, and neither chemical induced flowering under non-inductive photoperiod. GA_3 induced or enhanced flowering as day length increased, but only induced stalk elongation in plants with certain leaves removed. GA_3 in combination with other hormones resulted in a significant increase in stalk height but did not induce flowering.

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Table 1. Effect of GA_3 and GA_{4+7} applied once or as repeated applications on stalk elongation in 3-week-old 'US H20' sugarbeets.

Treatment Rate	Single Application		Repeated Application	
	GA_3	GA_{4+7}	GA_3	GA_{4+7}
(mg/l)	(mm)			
0	4 A*	-	22 A	-
1	11 A	5 A	22 A	29 A
20	12 A	6 A	47 A	50 A
100	24 A	18 A	110 B	143 B
500	44 AB	33 A	241 C	287 C
2500	48 A	47 A	279 C	246 C

*Treatment means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2. Effect of GA_3 on percent of plants that flowered when applied to 3-week-old 'FC701/5' sugarbeets grown under various photoperiods.

Treatment	Rate	Photoperiod (Day/Night in Hours)					
		14/10	14/10[†]	14/10[‡]	16/8	18/6	24/0
	(mg/l)	(%)					
GA_3	0	0	0	20	0	0	40
GA_3	200	0	-	-	-	-	-
GA_3	250	0	-	-	-	-	-
GA_3	500	0	30	-	-	60	100
GA_3	1000	0	60	20	-	60	100
GA_3	2500	-	-	-	43	-	-
GA_3	5000	-	40	40	-	-	-

[†]Photoperiod received a 2 hour nightbreak in the middle of dark period.

[‡]Photoperiod received a 20 minute nightbreak in the middle of the dark period.

Table 3. Effect of GA_3 in combination with leaf removal on stalk elongation in 'FC701/5' or 'US H20' sugarbeets grown in a growth chamber.

Treatment	Rate	FC701/5 Leaf Removal†		US H20 Leaf Removal	
		-	+	-	+
	(mg/l)	(mm)			
GA_3	0	19.2 A*	28.2 A	6.6 A	15.2 AB
GA_3	50	99.4 AB	79.4 A	76.2 BC	47.6 AB
GA_3	200	220.2 B	360.0 C	126.4 CD	131.8 CD
GA_3	500	525.8 E	446.0 CDE	216.8 E	179.2 DE
GA_3	1000	383.8 CD	494.2 DE	211.0 E	167.0 DE

*Means within varieties followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

† = leaves not removed; + = leaves removed.

Table 4. Effect of GA_3 in combination with various sequences of leaf removal on stalk elongation in 'US H20' sugarbeets grown in the greenhouse.

Chemical	Rate	Leaf Removal†				
		-	Bottom C	Bottom-1	Bottom-4	Top
	(mg/l)	(mm)				
GA_3	0	6.0 A*	0.0 A	0.0 A	4.0 A	8.0 A
GA_3	200	59.6 ABC	28.8 AB	59.8 ABC	41.0 ABC	77.2 A-D
GA_3	500	219.2 D	163.8 BCD	163.2 BCD	150.4 A-D	184.0 CD
GA_3	1000	573.6 FG	459.8 EFG	437.0 EF	386.8 E	470.8 EFG
GA_3	2500	585.4 G	375.2 E	447.4 EFG	400.2 E	399.6 E

† = no leaves removed; bottom-C = all except top four leaves removed throughout experiment; bottom-1 = all except top four leaves removed once after first application; bottom-4 = all except top four leaves removed once after fourth application; top = top four leaves removed once after fourth application.

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 5. Effect of single applications of GA_3/ethephon combinations on stalk elongation in 2 to 3-week-old 'US H20' sugarbeets grown in the greenhouse.

Treatment	Rate	GA_3 (mg/l) 0	1000	3300	5000
	(mg/l)	(mm)			
Ethephon	0	0 A*	36.2 AB	85.6 C-F	78.2 CDE
Ethephon	1	0 A	33.4 AB	64.5 BC	140.2 H
Ethephon	5	0 A	36.4 AB	90.6 C-G	106.4 E-H
Ethephon	10	3.4 A	35.4 A	65.2 BCD	124.0 GH
Ethephon	50	0 A	27.6 A	85.6 C-F	100.2 D-G
Ethephon	100	0 A	115.6 FGH	-	-

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 6. Effect of repeated applications of GA_3/ethephon combinations on stalk elongation in 2 to 3-week-old 'US H20'sugarbeets grown in the greenhouse.

Treatment	Rate	GA_3 (mg/l) 0	50	500	1000
	(mg/l)	(mm)			
Ethephon	0	43.2 A*	212.2 BC	291.4 DEF	367.8 G
Ethephon	1.0	26.8 A	256.4 CDE	358.0 FG	297.6 D-G
Ethephon	2.0	30.6 A	251.0 CDE	263.4 CDE	341.6 FG
Ethephon	5.0	27.8 A	215.6 BC	305.2 D-G	320.4 EFG
Ethephon	20	22.0 A	232.4 CD	296.4 D-G	323.4 EFG
Ethephon	100	29.6 A	153.8 B	194.6 BC	-

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 7. Effect of repeated applications of GA_3/ethephon combinations on stalk elongation in 2 to 3-week-old 'FC701/5' and 'US H20' sugarbeets grown in a growth chamber.

		FC701/5		US H20			
		GA_3 (mg/l)		GA_3 (mg/l)			
Treatment	Rate	0	250	0	50	250	500
	(mg/l)	(mm)					
Ethephon	0	0 A*	238.0 B	3.8 A	66.3 BC	139.0 DE	130.5 DE
Ethephon	1	0 A	281.5 B	-	-	-	-
Ethephon	2	0 A	233.3 B	4.0 A	68.8 C	102.8 CD	118.8 DE
Ethephon	10	-	-	5.0 A	106.5 CD	145.3 DE	157.5 E
Ethephon	20	-	-	6.3 A	116.3 DE	151.8 F	199.8 F

*Means within sugarbeet line or cultivar followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 8. Effect of repeated applications of GA3/2,4-D combinations on stalk height in 3 to 4-week-old 'FC701/5' or 'US H20' sugarbeets grown in the greenhouse.

Treatment	Rate	FC701/5			US H20		
		GA_3 (mg/l)			GA_3 (mg/l)		
		0	500	2500	0	500	2500
	(mg/l)	(mm)			(mm)		
2,4-D	0	17.2 ABC*	87.4 DEF	88.8 DEF	17.4 ABC	47.2 CDE	67.0 DEF
2,4-D	0.125	9.0 AB	58.0 CD	154.4 H-K	11.8 AB	58.4 DEF	130.0 I
2,4-D	0.25	8.6 AB	55.0 BCD	158.2 I-K	3.8 AB	73.4 DEF	90.8 FGH
2,4-D	0.50	10.8 AB	78.2 DE	107.8 E-H	9.4 AB	48.0 CDE	116.8 HI
2,4-D	1.0	9.2 AB	55.0 BCD	183.0 KL	10.6 AB	68.8 DEF	112.8 GHI
2,4-D	2.5	10.4 AB	187.8 KL	207.6 L	17.0 ABC	60.0 DEF	-

*Means within sugarbeet line or cultivar followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 9. Effect of repeated applications of GA_3 in combination with sequentially applied 2,4-D on stalk elongation and flowering in 3 to 4-week-old 'US H20' sugarbeets grown in the growth chamber or greenhouse.

Treatment	Rate	Growth Conditions	GA_3 (mg/l) 0	10	50	200	1000
	(mg/l)		(mm)				
2,4-D	0	Growth Chamber	0 A*	0 A	0 A	15.8 AB	75 B†
2,4-D	1	Growth Chamber	0 A	0 A	4.4 A	30.6 AB	73.4 B†
2,4-D	5	Growth Chamber	0 A	0 A	7.6 A	22.0 AB	37.8 AB
2,4-D	25	Growth Chamber	0 A	3.4 A	18.4 AB	18.2 AB	193.0 C††
2,4-D	0	Greenhouse	4.0 A	3.2 A	0 A	27.2 AB	91.6 C
2,4-D	1	Greenhouse	0 A	7.2 AB	0 A	40.6 B	127.4 D
2,4-D	5	Greenhouse	0 A	0 A	8.4 AB	18.0 AB	100.6 CD
2,4-D	25	Greenhouse	0 A	5 A	15.6 AB	28.4 AB	112.0 CD

†One out of five sugarbeets flowered.

††Four out of five sugarbeets flowered.

*Means within growth condition followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 10. Effect of single applications of GA/Kinetin combinations on stalk elongation in 3-week-old 'US H20' and 'EL44'sugarbeets in the greenhouse.

		US H20					EL44				
		GA_3 (mg/l)			GA_4A_7 (mg/l)		GA_3 (mg/l)			GA_4A_7 (mg/l)	
Treatment	Rate	0	1000	5000	1000	5000	0	1000	5000	1000	5000
	(mg/l)	(mm)									
Kinetin	0	1.0 A*	26.3 A-D	38.3 CD	18.3 ABC	29.0 A-D	3.3 A	8.0 AB	62.3 D	16.0 AB	32.7 A-D
Kinetin	0.01	14.7 ABC	16.7 ABC	34.7 CD	18.0 ABC	33.7 BCD	-	-	-	-	-
Kinetin	0.1	5.3 ABC	10.7 ABC	28.3 A-D	10.3 ABC	50.0 D	9.6 AB	39.7 BCD	27.7 ABC	20.0 AB	92.0 E
Kinetin	1.0	-	-	-	-	-	3.3 A	16.0 AB	56.7 CD	24.3 ABC	36.7 A-D

*Means within sugarbeet line or cultivar followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 11. Effect of GA in combination with EPTC and diethatyl on stalk elong

Table 11. Effect of GA in combination with EPTC and diethatyl on stalk elongation in 3-week-old 'US H20' or 'EL44' sugarbeets grown in the greenhouse.

		US H20					EL44				
		GA_3 (mg/l)			GA_4A_7 (mg/l)		GA_3 (mg/l)			GA_4A_7 (mg/l)	
Treatment	Rate	0	1000	5000	1000	5000	0	1000	5000	1000	5000
	(mg/l)	(mm)					(mm)				
EPTC	0	1.0 A*	26.3 AB	38.3 B	18.3 AB	29.0 AB	3.3 A	8.0 A	62.3 B	16.0 A	32.7 AB
EPTC	10	8.0 A	11.0 AB	28.0 AB	25.3 AB	31.3 AB	7.0 A	25.7 A	61.0 B	25.7 A	32.0 AB
EPTC	100	4.3 A	25.0 AB	20.7 AB	28.7 AB	23.7 AB	5.3 A	18.7 A	32.0 AB	21.0 A	-
Diethatyl	0	1.0 A	26.3 ABC	38.3 BC	18.3 ABC	29.0 ABC	3.3 A	8.0 AB	62.3 D	16.0 AB	32.7 A-D
Diethatyl	10	17.6 ABC	11.7 AB	41.3 BC	28.0 ABC	27.3 ABC	3.0 A	20.3 AB	56.7 CD	24.3 AB	37.7 BCD
Diethatyl	100	4.7 A	41.7 BC	48.7 C	23.7 ABC	43.3 BC	3.3 A	24.7 AB	31.0 ABC	23.3 AB	30.3 ABC

*Means within each line or cultivar for each herbicide followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

CHAPTER 2

Influence of Herbicides Which Alter Plant Lipid Metabolism on the Action of GA in sugarbeet (Beta vulgaris L.)

ABSTRACT

Application of GA in combination with herbicides reported to alter plant lipid metabolism were evaluated for their effect on stalk elongation and floral initiation in sugarbeets (Beta vulgaris L.). Combinations of GA with members of thiocarbamate, acetanilide, and benzoic acid classes of herbicides, naptalam (N-1-naphthylphthalamic acid), TCA (trichloroacetic acid), ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-S-benzofuranyl methanesulfonate), dalapon (2,2-dichloropropionic acid) and glyphosate [N-(phosphonomethyl)-glycine] resulted in a synergistic increase in stalk elongation . Flowering was induced by GA_3 alone and in combination with alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide] and EPTC (S-ethyl dipropylthiocarbamate) only, in one field experiment, in 5-15% of 'FC701/5' sugarbeets. Uptake of ^{14}C-GA_3 by sugarbeet foliage was not increased by pretreatment with alachlor, and thus was not the basis for the observed interaction.

INTRODUCTION

Floral induction of biennial sugarbeet in one growing season would facilitate sugarbeet improvement through accelerated breeding programs (8). Biennial sugarbeets require a cold induction period (2.75 to 10 C with an optimum of 4.4 C for approximately 1 to 2 months) followed by a requirement for warmer temperatures (21 to 27 C) and long days (14 to 16 hr photoperiod) (12,14,15).

Recently, it was observed that young biennial sugarbeets were induced to flower within 30 days from seeding in a particular growth chamber (8). Examination of records of photoperiod and temperature conditions precluded environmental induction of sugarbeet flowering in this chamber. This phenomenon occurred in several subsequent experiments over a period of approximately 2 years. Although the cause of the floral induction was not readily apparent, a chemical induction may have been involved.

Gibberellic acid (GA_3) can induce floral initiation without the cold treatment in the biennials Hyoscyamus niger (10), carrot (Daucas carota L.), celery (Apium graveolens L.) but not beet (2). However, sugarbeet was induced to flower if GA_3 was applied under a partial photothermal induction (continuous illumination and temperature of 7 to 8 C for 43 days) (6). GA_3 did not cause floral initiation without

the cold treatment in sugarbeets. Wheatly and Johnson (15) were unable to induce flowering with GA_3 applications to April planted sugarbeets.

Cold temperatures cause a shift in the fatty acid content of plant cell membranes toward greater unsaturation (7). The herbicides diethatyl [N-(chloroacetyl)-N-(2,6-diethylphenyl)glycine] and vernolate (S-propyl diphropylthiocarbamate) act similiarly as the low temperature effect on plant cell membranes under warm temperature (30 C) causing a shift to more unsaturated fatty acids (13).

EPTC (S-ethyl dipropylthiocarbamate) and BASF 13-338 [4-chloro-S(dimethylamino)-2-phenyl-3(2H) pyridazinone] induce a shift to more saturated fatty acids in soybean (Glycine max (L.) merr.) and wheat (Triticum aestevum L.) respectively (17, 18). EPTC, TCA, and ethofumesate have been shown to reduce epicuticular wax deposition in cabbage (Brassica oleracea L.) (6, 9, 11).

Since diethatyl and vernolate have been shown to substitute for the low temperature effect on membranes, there exists the possibility that these herbicides could also mimic the low temperature effect on other plant processes. Thus it might be possible to substitute completely for the cold temperature induction necessary for flowering in sugarbeets with combinations of these herbicides and GA.

The objectives of this study were to evaluate the effect of combinations of GA with diethatyl and vernolate on floral induction and stalk elongation in sugarbeet. Since EPTC

(a vernolate analog) and other herbicides also affected other aspects of plant lipid metabolism, they were also evaluated in combination with GA for their effect on floral induction and stalk elongation in sugarbeet.

MATERIALS AND METHODS

Plant material, growth conditions, experimental design.

Five to ten sugarbeet seeds, selected from lines 'EL40' (low bolting tendency), 'EL44' (intermediate bolting tendency) and 'FC701/5' (high bolting tendency) and cultivar 'US H20' (intermediate bolting tendency), were placed 2.5 cm deep in a commercial potting mixture (Metro-Mix 300) in 948 ml styrofoam cups. Prior to chemical treatment, the seedlings were thinned to one plant of a uniform size per pot. All formulated chemicals [GA_3(Pro Gibb formulation) and herbicides (Table 1)] were diluted in tap water on a mg/L basis and applied in combination [solutions of herbicide and GA_3 mixed together] to the foliage until runoff using a model No. 152 DeVilbiss automizer with 0.35 to 0.70 kg/cm^2 pressure. During the growth period all pots received 100 ml of a 5000 mg/L 20:20:20 N-P-K fertilizer solution once every 2 weeks. After cessation of stalk elongation (4-6 weeks after the last chemical application) stalk height was measured and flowering data recorded. The experiments were done in a growth chamber, which had fluorescent and incandescent lamps; in a greenhouse under natural or natural plus supplemental (16/8 hr day/night)

lighting from fluorescent or sodium vapor lamps; or in pots grown outdoors. The growth chambers were set at a 14/10 hr, 22/14 C day/night light-temperature regime with a photosynthetic photon flux density (PPFD) that ranged from 300 to 400 μmole · m^{-2} · sec^{-1}. Greenhouse temperatures ranged from 16 to 29 C from late fall through early spring and 20 to 35 C from late spring through early fall. The PPFD under fluorescent and sodium vapor lamps in the greenhouse was 150 and 300 μmol · m^{-2} · sec^{-1}, respectively, and that under natural lighting was 750 μmol · m^{-2} · sec^{-1} during the summer months and 300 μmol · m^{-2} · sec^{-1} during the winter months. All pots were placed in their respective growth environments (greenhouse, growth chamber, or outdoors) under the conditions listed above immediately after seeding.

The experimental design was a randomized complete block with three to five replications. The data were analyzed using a two or three-way factorial analysis of variance for two or three treatment factors, respectively, and means were compared by Duncan's multiple range test. All experiments were repeated to confirm results.

Chloroacetanilides applied in combination with GA. In the first greenhouse experiment (from January 3 through February 20, 1980, under supplementary fluorescent lighting) diethatyl solutions (made from Antor 4E) were applied once at 0, 5, 100, and 200 mg/L in combination with GA_3 and GA_{4+7} (2% solution from Abbott Laboratories) at 0, 500, 1500 and 5000 mg/L to the foliage of 2-week-old 'US H20',

'EL40' and 'EL44' sugarbeet seedlings. In two subsequent greenhouse experiments (from January 31 through March 15, 1980 and April 8 through June 12, 1980, under natural lighting), other members of the chloroacetanilides [alachlor (Lasso 4E), metolachlor (Dual 8E) and acetochlor (5E formulation)] and diethatyl were applied once at rates ranging from 0.5 to 500 mg/L in combination with GA_3 and GA_{4+7} at 0 and 5000 mg/L to the foliage of 2-week-old 'US H20' and 'EL44' sugarbeets.

Thiocarbamate herbicides applied in combination with GA_3. Applications of GA_3 in combination with commercial formulations of four thiocarbamate herbicides [EPTC, vernolate, butylate + R-25788 (N,N-diallyldichloroacetamide) and cycloate] were made seven times over 3 weeks to the foliage of 3-week-old 'FC701/5' sugarbeet seedlings. The plants received GA_3 at 0 and 200 mg/L in combination with the thiocarbamates at 0, 0.25, 0.5 and 1.0 mg/L for the first six applications and ten times their respective rates for the last application. The experiment was done in the greenhouse under natural lighting from July 7 through October 6, 1980.

For the third experiment, combinations of EPTC and EPTC plus the antidote R-25788 (Eradicane 6.7E) at 0, 25 and 100 mg/L and 0+0, 25+2.1, and 100+8.3 mg/L, respectively, with GA_3 at 0 and 2000 mg/L were applied twice to the foliage of 3-week-old 'US H20' sugarbeet seedlings grown in the greenhouse under sodium vapor lamps.

Thiocarbamate and acetanilide herbicide combinations with GA_3 in outdoor experiments. GA_3 was applied at 0, 500, and 2500 mg/L in combination with EPTC at 0, 10 and 50 and alachlor at 0, 50, and 250 mg/L to the foliage of 2-week-old 'FC701/5', 'US H20' and 'EL44' sugarbeet seedlings. The plants were grown in a greenhouse potting mixture (sand:peat:clay, 1:1:1 by volume) and placed in the greenhouse under sodium vapor lamps. At the time of chemical treatment the plants were moved outdoors and received five weekly applications. The experiment was done from April 13 through July 14, 1981 and all treatments were replicated six times.

On May 1, 1981, a field experiment was initiated on a sandy loam soil. Prior to plowing, a 12-12-12 (N-P-K) fertilizer was applied at 448 kg/ha broadcast, followed by a 7-28-18 fertilizer with 2% Mn and 0.25% B applied at 140 kg/ha in the band at planting. 'EL40', 'EL44', 'FC701/5' and 'US H20' sugarbeets were seeded 5 cm apart in rows 0.7 m apart by 9.1 m in length. One row of each sugarbeet cultivar or line was randomized and planted within each plot. The plants were thinned to 15 cm 8 weeks after seeding and weeded throughout the growing period as necessary. GA_3 (Pro Gibb Plus from Abbott Laboratories) was applied at 0, 300, and 1200 mg/L in combination with EPTC at 0, 25, and 100 mg/L and alachlor at 0, 50, and 250 mg/L to the foliage of 4-week-old sugarbeet seedlings at the two to four leaf stage in seven

weekly applications. The chemicals were applied in water at 34 L/ha plus 0.25 (v/v) X-77 surfactant with a tractor-mounted plot sprayer for the first time. The other six applications were made in 73 L/ha plus 0.25% v/v X-77 surfactant with a knapsack sprayer, with pressure supplied from a cartridge of compressed CO_2. All treatments were replicated three times and flowering and stalk height data were collected 16 weeks after the first application.

Evaluation of glyphosate for flowering and stalk elongation. Glyphosate was applied to the foliage of clones from a selection of early bolting 'FC701/5' sugarbeets developed through tissue culture. The herbicide was applied once at 0, 1, 10, 100, and 1000 mg/L to the foliage of the sugarbeets in the six to eight leaf stage. The plants were grown in a chamber under 14 hr light from fluorescent plus continuous light from incandescent lamps.

In two subsequent experiments, glyphosate was applied from 0 to 100 mg/L in combination with GA_3 from 0 to 2000 mg/L and GA_{4+7} (Pro Gibb 47) from 0 to 500 mg/L to the foliage of 4-week-old 'FC701/5' seedlings. In the first experiment, the chemicals were applied twice, 11 days apart, to plants grown under sodium vapor lamps in the greenhouse. In the second experiment, the chemicals were applied six times over 5 weeks to plants grown in the greenhouse under natural lighting from July 22 through October 19, 1981.

GA combinations with naptalam. GA_3 and GA_{4+7} (2%

formulation) were applied from 0 to 5000 mg/L in combination with naptalam (Alanap 2E) from 0 to 20 mg/L to the foliage of 2-week-old 'US H20', 'EL40' and 'EL44' (experiment 1) and 4-week-old 'FC701/5' (experiment 2) seedlings. The treatment was made once in experiment 1 and in 3 weekly applications in the second experiment. Both experiments were done in the greenhouse under supplementary light from fluorescent lamps.

GA_3 combinations with benzoic acid-type chemicals. GA_3 was applied once at 0 and 5000 mg/L in combination with commercial formulations of benzoic acid-type chemicals [chloramben, dicamba, and TIBA (technical grade)] from 0 to 250 mg/L to the foliage of 2-week-old 'EL44' and 4-week old 'FC701/5' sugarbeet seedlings. These experiments were done in the greenhouse under natural lighting from January 31 through March 31, 1980, and August 13 through October 15, 1980, respectively.

GA_3 combinations with TCA, dalapon, ethofumesate, and pyrazon. GA_3 was applied at 0 and 1000 mg/L in combination with TCA (83% active ingredient) at 0, 100, 500, and 1000 mg/L; dalapon (Dowpon M) at 0, 10, 50, and 100 mg/L; ethofumesate (Nortron 1.5E) at 0, 5, 10, and 20 mg/L; and pyrazon (Pyramin 65 W) at 0, 10, 25, and 50 mg/L to the foliage of 3-week-old 'FC701/5' and 'US H20' sugarbeets. The treatments were made in four weekly applications, and the plants were grown in the greenhouse under sodium vapor lamps.

^{14}C-GA_3 uptake study. This experiment was done in the greenhouse under sodium vapor lamps with three replications per treatment. GA_3 was applied at 1000 mg/L in combination with alachlor at 0 and 50 mg/L to the foliage of 3-week-old 'FC701/5' seedlings. After these solutions were allowed to dry on the leaf, 0.2 µCi [1, 7, 12, 18-^{14}C] GA_3 (14 µCi/µmole) in 5 µl (10% isopropyl alcohol in water) was spotted on the center of the first true leaf of the sugarbeet seedling. Three and seven days after treatment, the plants were harvested. In one set of plants, the tip and base of the ^{14}C-GA_3 treated leaf (tip harvested 0.5 cm above and base harvested 0.5 cm below ^{14}C-GA_3 spot) and the youngest visible leaf were oxidized and analyzed for ^{14}C by liquid scintillation radioassay.

RESULTS AND DISCUSSION

Chloroacetanilides applied in combination with GA. Combinations of GA_3 with diethatyl, alachlor, acetochlor, and metolachlor resulted in a significant increase in stalk height over either chemical alone (Tables 2 and 3). This was found in all lines or cultivar tested except for GA_3 plus acetochlor on 'US H20' (Table 3). A significant increase in stalk height with GA_{4+7} was found only when applied in combination with diethatyl to 'EL40' and 'US H20' sugarbeets. These results indicate a synergistic interaction occurred between the herbicides and GA. The

interaction was dependent on chemical, chemical rate, and sugarbeet type used. Flowering was not induced by any of the treatments.

Thiocarbamate herbicides applied in combination with GA_3. Combinations of thiocarbamate herbicides (EPTC, vernolate, butylate plus R-25788, and cycloate) with GA_3 resulted in a significant increase in stalk height over either chemical alone (Table 4). Butylate plus the antidote R-25788 at the highest rate in combination with GA reduced the interaction to a non-significant level. Since R-25788 protects corn from butylate and EPTC (antagonistic response) injury (3), this may explain why the interaction between butylate and GA_3 was reduced. A second experiment lends support to this idea as R-25788 also reduced the interaction between EPTC and GA_3 at the low but not the high rates (Table 5). The inability of R-25788 to reverse the interaction between GA_3 and EPTC at 100 mg/L may be due to the high herbicide rate. None of the treatments induced flowering.

Thiocarbamate and acetanilide herbicides in combination with GA_3 in outdoor experiments. Various combinations of GA_3 with EPTC and alachlor resulted in a significant synergistic interaction for increased stalk height on plants grown in pots outdoors or in the field (Tables 6 and 7). Alachlor with GA_3 produced a significant increase in all sugarbeet types grown in pots and on 'FC701/5' and 'E144'

in the field. EPTC with GA_3 produced a significant increase in stalk height in 'FC701/5' and 'US H20' in pots and 'EL44' in the field. Flowering was observed on a few plants of 'FC701/5', in various treatments in the field experiment (Table 7).

Evaluation of glyphosate for flowering and stalk elongation. Glyphosate has been reported to inhibit meristems in several plant species (1). However, when applied to the foliage of a photoperiodically (24/0 day/night incandescent plus 14/10 day/night fluorescent) induced selection of 'FC701/5' sugarbeet clones, to determine if flowering could be inhibited, 1 and 10 mg/L enhanced flowering over the untreated control. Therefore, subsequent experiments were initiated to determine the effect of glyphosate in combination with GA_3 and GA_{4+7} on flowering and stalk elongation in sugarbeet. Combinations of glyphosate and GA_3 significantly increased stalk elongation (Table 8), but no flowering was induced by any of the treatments. Glyphosate plus 100 mg/L GA_{4+7} increased stalk length, but these results were not significantly different than those obtained with GA_{4+7} alone.

GA combinations with naptalam. Naptalam was evaluated with GA because of its classification as an amide, similar to the chloroacetanilides (1). Naptalam interacted synergistically with GA_{4+7} on all sugarbeet types tested (Tables 9 and 10). Combinations of GA_3 with increasing rates of naptalam increased stalk height, but these results

were not significantly different than those obtained with GA_3 alone. None of the treatments induced flowering.

GA_3 combinations with benzoic acid-type chemicals. GA_3 combinations with dicamba and chloramben resulted in a synergistic interaction as stalk height was significantly increased over that induced by either chemical alone in 'FC701/5' sugarbeets (Table 11). The chloramben rates used appeared to be too high for 'EL44' sugarbeets as a trend toward decreasing stalk height resulted. TIBA interacted with GA_3 for increased stalk height in 'EL44' but not in 'FC701/5' sugarbeets.

GA_3 combinations with TCA, dalapon, ethofumesate, and pyrazon. GA_3 also interacted synergistically with dalapon, TCA, ethofumesate, and pyrazon. Combinations with the first three herbicides resulted in significantly longer stalks than with GA_3 alone (Table 12). Although the differences were not statistically significant, GA_3 combinations with pyrazon resulted in increased stalk elongation over that obtained from GA_3 alone. The high degree of variability may be responsible for lack of significance between GA_3 and this combination. In a second experiment, GA_3/pyrazon combinations resulted in a significant increase in stalk height over GA_3 alone.

^{14}C-GA_3 uptake study. As mentioned above, several of the herbicides evaluated in these experiments have been reported to alter epicuticular wax deposition in plants. This might explain the synergistic interaction observed

with the GA, herbicide combinations. ^{14}C-GA_3 was applied to sugarbeet plants treated with alachlor and alachlor plus GA_3 to determine if the herbicide had any effect on GA_3 uptake. The results indicate that alachlor or alachlor plus GA_3 did not significantly alter ^{14}C-GA_3 uptake (Table 13). In fact it appears that alachlor produced a trend toward decreased uptake of ^{14}C-GA_3 into the treated leaf for both harvest times. Based on these results, it appears that the interaction between GA and the herbicides, may be taking place inside the plant.

CONCLUSION

GA exhibited a synergistic interaction in combination with several herbicides, which were reported to alter lipid metabolism, in the form of a significant increase in stalk height over that obtained with either chemical applied alone. The interaction was dependent on chemical, chemical rate, type of GA and sugarbeet line or cultivar.

Two models commonly used in reference to chemical interactions are additive and multiplicative types. Use of the F-test (as in this report) to determine if any interaction between two chemicals is significant only tests for additive responses, i.e. both chemicals competing with each other for a common site of action. A multiplicative model (Colby's (4) test for an interaction between chemicals) determines if the chemicals are

affecting different sites of action. Colby's test was used where the F-test showed a significant increase in stalk height over either chemical alone in this study. Results showed the measured value to be greater than the expected value in all cases, which indicates the synergistic interaction arises from chemicals affecting different sites of action.

None of the treatments induced the plants to flower except in the field experiment where some of the treatments (low rates of GA_3 plus alachlor and EPTC) induced several 'FC701/5' plants to flower.

Treatments with ^{14}C-GA_3 showed that alachlor did not result in increased movement of ^{14}C-GA_3 into the plant, a finding which indicates that the interaction between herbicides and GA may be taking place inside the plant.

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Table 1. List of herbicides evaluated in combination with GA on sugarbeets.

Common Name	Chemical Name
acetochlor	2-chloro-N(ethoxymethyl)-6'-ethyl-o-acetotoluidide
alachlor	2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide
butylate	S-ethyl diisobutylthiocarbamate
chloramben	3-amino-2,5-dichlorobenzoic acid
cycloate	S-ethyl N-ethylthiocyclohexanecarbamate
dalapon	2,2-dichloropropionic acid
dicamba	3,6-dichloro-o-anisic acid
diethatyl	N-(chloroacetyl)-N-(2,6-diethylphenyl) glycine
EPTC	S-ethyl dipropylthiocarbamate
ethofumesate	(±)-2-ethoxy-2,3-dihydro-3,3-dimethyl -5-benzofuranyl methanesulfonate
glyphosate	N-(phosphonomethyl)glycine
metolachlor	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide
naptalam	N-1-naphthylphthalamic acid
pyrazon	5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone
TCA	trichloroacetic acid
TIBA	triiodobenzoic acid
vernolate	S-propyl dipropylthiocarbamate

Table 2. Effect of single applications of GA, diethatyl combinations on stalk elongation in 2-week-old 'US H20', 'EL40' and 'EL44' sugarbeets grown in the greenhouse.[a]

		Diethatyl (mg/L)											
		US H20				EL40				EL44			
Treatment	Rate	0	50	100	200	0	50	100	200	0	50	100	200
	(mg/L)	(mm)											
GA_3	0	3 A	18 ABC	3 A	6 AB	0 A	11 ABC	5 AB	3 A	6 ABC	13 A-D	3 A	5 AB
GA_3	500	24 ABC	16 ABC	28 CDE	30 CDE	17 BCD	13 A-D	18 BCD	20 CDE	31 DEF	34 DEF	26 B-E	26 B-E
GA_3	1500	22 ABC	24 ABC	32 CDE	25 BCD	21 CDE	18 CD	21 CDE	22 CDE	34 DEF	40 EF	27 CDE	35 DEF
GA_3	5000	46 EF	45 DEF	53 F	125 G	24 CDE	26 DE	32 E	52 F	38 EF	42 EF	51 F	41 EF
GA_{4+7}	0	3 A	18 AB	3 A	6 A	0 A	11 ABC	5 AB	2 A	6 AB	13 ABC	3 A	5 AB
GA_{4+7}	500	15 AB	27 BC	18 AB	21 ABC	15 ABC	16 ABC	17 ABC	13 ABC	17 ABC	24 A-D	30 C-F	19 A-D
GA_{4+7}	1500	34 BCD	26 BC	33 BCD	39 CDE	18 ABC	20 BCD	27 B-E	41 DE	29 B-F	37 C-F	24 A-D	25 A-E
GA_{4+7}	5000	46 DEF	59 F	53 EF	62 F	28 CDE	27 B-E	30 CDE	45 E	52 FG	42 D-G	62 G	48 EFG

[a]Means within GA and cultivar or line followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 3. Effect of single applications of GA, acetanilide herbicide combinations on stalk elongation in 'US H20' and 'EL44' sugarbeets from two greenhouse experiments.[ab]

		Study 1		Study 2					
		EL40		EL44			US H20		
		GA_3 (mg/L)		GA_3 (mg/L)		GA_{4+7} (mg/L)	GA_3 (mg/L)		GA_{4+7} (mg/L)
Treatment	Rate	0	5000	0	5000	5000	0	5000	5000
	(mg/L)	(mm)							
Alachlor	0	1 A	31 B	9 A	50 BCD	37 ABC	1 A	29 AB	14 A
Alachlor	1.0	7 A	38 B	-	-	-	-	-	-
Alachlor	10	0 A	44 B	10 A	75 DE	24 AB	3 A	47 BC	18 AB
Alachlor	50	-	-	11 A	70 CDE	26 AB	4 A	58 C	26 AB
Alachlor	100	7 A	40 B	10 A	97 E	39 ABC	6 A	93 D	22 AB
Acetochlor	0	1 A	31 ABC	9 A	50 CD	37 BC	1 A	29 CDE	14 ABC
Acetochlor	0.5	-	-	8 A	65 D	39 BC	5 AB	35 DE	23 B-E
Acetochlor	1.0	4 AB	66 D	-	-	-	-	-	-
Acetochlor	2.0	-	-	15 AB	30 ABC	30 ABC	5 AB	40 E	20 BCD
Acetochlor	10	3 AB	33 BC	9 A	89 E	35 BC	5 AB	35 DE	19 A-D
Acetochlor	100	2 AB	48 CD	-	-	-	-	-	-
Metolachlor	0	1 A	31 B	9 A	50 C	37 B	1 A	29 AB	14 AB
Metolachlor	0.5	-	-	13 A	53 C	45 BC	2 A	33 B	18 AB
Metolachlor	1.0	3 A	48 B	-	-	-	-	-	-
Metolachlor	2.0	-	-	7 A	60 C	-	3 A	63 C	-
Metolachlor	10	3 A	34 AB	-	-	-	-	-	-
Metolachlor	100	3 A	40 AB	-	-	-	-	-	-
Diethatyl	0	1 A	31 B	9 A	50 B	37 AB	1 A	29 DE	14 A-D
Diethatyl	100	6 A	28 B	10 A	46 B	33 AB	7 ABC	39 EF	24 CDE
Diethatyl	200	4 A	41 B	-	-	-	-	-	-
Diethatyl	250	-	-	13 A	31 AB	57 BC	6 ABC	19 A-D	70 G
Diethatyl	400	4 A	44 B	-	-	-	-	-	-
Diethatyl	500	-	-	13 A	78 C	55 BC	2 AB	48 F	22 B-E

[a]Means within herbicide, cultivar or line, and experiment followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]A line in place of data indicates the treatment was not made.

Table 4. Effect of repeated applications of GA_3, thiocarbamate herbicide combinations on stalk elongation in 3-week-old 'FC701/5' sugarbeet seedlings grown in the greenhouse.[a]

Treatment	Rate[b] (mg/L)	GA_3 (mg/L) 0	GA_3 (mg/L) 200
		mm	
Check	0	8 A	226 BC
EPTC	0.25	30 A	348 D
EPTC	0.5	18 A	531 F
EPTC	1.0	5 A	456 EF
Vernolate	0.25	4 A	457 EF
Vernolate	0.5	16 A	393 DE
Vernolate	1.0	15 A	366 DE
Butylate + R-25788	0.25 + 0.01	5 A	136 B
Butylate + R-25788	0.5 + 0.02	12 A	333 D
Butylate + R-25788	1.0 + 0.04	11 A	311 CD
Cycloate	0.25	20 A	158 B
Cycloate	0.5	6 A	156 B
Cycloate	1.0	9 A	373 DE

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Plants received 10 times the corresponding rate listed above for the last application.

Table 5. Effect of EPTC and EPTC plus R-25788 in combination with GA_3 on stalk elongation when applied to the foliage of 3-week-old 'US H20' sugarbeet seedlings grown in the greenhouse.[a]

Treatment	Rate (mg/L)	GA_3 (mg/L) 0	GA_3 (mg/L) 2000
		mm	
Check	0	0 A	58 B
EPTC	25	0 A	95 C
EPTC	100	0 A	105 C
EPTC + R-25788	25 + 2.1	0 A	41 B
EPTC + R-23788	100 + 8.3	0 A	100 C

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 6. Effect of repeated applications of GA_3 in combination with alachlor and EPTC on stalk elongation in 2-week-old 'FC701/5', 'US H20' and 'EL40' sugarbeet seedlings grown in pots outdoors.[a]

		GA_3 (mg/L)								
		FC701/5			US H20			EL40		
Treatment	Rate	0	500	2500	0	500	2500	0	500	2500
	(mg/L)	(mm)								
Check	0	32 A	95 AB	333 C	19 A	76 B	244 C	17 A	119 BC	181 CD
EPTC	10	23 A	85 AB	340 C	0 A	97 B	290 D	19 A	121 BC	217 D
EPTC	50	24 A	138 B	428 D	0 A	75 B	204 C	19 A	105 C	241 D
Alachlor	50	25 A	156 B	625 F	0 A	76 B	349 E	13 A	99 C	217 D
Alachlor	250	25 A	138 B	531 E	3 A	118 B	309 DE	14 A	117 BC	341 E

[a]Means within line or cultivar followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 7. Effect of repeated applications of GA_3 in combination with alachlor and EPTC on flowering and stalk elongation in 4-week-old 'FC701/5', 'US H20', 'EL40', and 'EL44' sugarbeets in the field.[a]

		GA_3 (mg/L)											
		FC701/5			US H20			EL40			EL44		
Treatment	Rate	0	300	1200	0	300	1200	0	300	1200	0	300	1200
	(mg/L)	(cm)											
Check	0	0 A	55 CD[c]	53 BCD	0 A	30 B	43 DE	0 A	19 B	21 B	0 A	20 BC	21 BC
EPTC	25	0 A	44 B	52 BCD	0 A	36 BCD	42 CDE	0 A	22 B	22 B	0 A	20 BC	23 CD
EPTC	100	0 A	48 BC[d]	60 DE	0 A	32 B	42 CDE	0 A	21 B	23 B	0 A	21 BC	27 D
Alachlor	50	0 A	43 B[b]	66 E[b]	0 A	32 B	45 E	0 A	19 B	25 B	0 A	18 BC	26 D
Alchlor	250	0 A	51 BCD[b]	66 E[b]	0 A	36 BC	44 DE	0 A	23 B	20 B	0 A	18 B	21 BC

[a]Means within line or cultivar followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Two plants (5%) flowered.

[c]Three plants (7.5%) flowered.

[d]Six plants (15%) flowered.

Table 8. Effect of repeated applications from two experiments of GA, glyphosate combinations on stalk elongation of 4-week-old 'FC701/5' sugarbeets grown in the greenhouse.[ab]

Treatment	Rate	Glyphosate (mg/L)								
		Experiment 1					Experiment 2			
		0	1.0	5.0	10	100	0	2.5	12.5	50
	(mg/L)	(mm)								
Check	0	0 A	0 A	0 A	0 A	0 A	28 A	35 A	31 A	35 AB
GA_3	5	-	-	-	-	-	95 A-D	49 ABC	42 AB	27 A
GA_3	25	-	-	-	-	-	69 ABC	68 ABC	45 AB	72 ABC
GA_3	100	-	-	-	-	-	112 A-E	99 A-D	168 D-H	217 GHI
GA_3	500	-	-	-	-	-	233 HI	201 F-I	214 GHI	347 J
GA_3	2000	58 BC	87 CD	111 D	52 B	76 BC	-	-	-	-
GA_{4+7}	5	-	-	-	-	-	93 A-D	53 ABC	54 ABC	42 AB
GA_{4+7}	25	-	-	-	-	-	60 ABC	69 ABC	73 ABC	56 ABC
GA_{4+7}	100	-	-	-	-	-	115 A-E	138 C-G	128 B-F	185 E-H
GA_{4+7}	500	-	-	-	-	-	355 J	278 IJ	313 J	328 J

[a]Means within experiment followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]A line in place of data indicates the treatment was not made.

Table 9. Effect of single applications of GA, naptalam combinations on stalk elongation in 2-week-old 'US H20','EL40' and 'EL44' sugarbeets grown in the greenhouse.[a]

Treatment	Rate	Naptalam (mg/L)											
		0	5	10	20	0	5	10	20	0	5	10	20
	(mg/L)	(mm)											
GA_3	0	3 A	1 A	3 A	3 A	0 A	4 ABC	2 AB	5 A-D	6 AB	6 AB	2 A	3 A
GA_3	500	24 ABC	22 ABC	24 ABC	42 BCD	17 A-E	21 C-F	22 DEF	19 A-E	31 BC	31 BC	38 CD	38 CD
GA_3	1500	22 ABC	24 ABC	19 AB	43 BCD	21 C-F	21 C-F	23 DEF	17 A-E	34 C	42 CD	45 CD	56 CD
GA_3	5000	46 BCD	48 CD	65 D	36 BC	24 EF	27 EF	37 F	38 F	38 CD	39 CD	57 CD	64 D
GA_{4+7}	0	3 A	1 A	3 A	3 A	0 A	4 ABC	2 AB	4 ABC	6 AB	6 AB	2 A	3 A
GA_{4+7}	500	15 AB	30 AB	25 AB	26 AB	15 BCD	18 CD	24 D	23 D	17 ABC	24 BCD	27 CDE	34 C-F
GA_{4+7}	1500	34 AB	28 AB	37 AB	28 AB	18 CD	28 DE	27 DE	26 DE	29 CDE	26 CDE	27 CDE	58 G
GA_{4+7}	5000	46 B	33 AB	92 C	81 C	28 DE	39 EF	46 F	48 F	52 FG	45 EFG	40 D-G	28 CDE

[a]Means within GA and cultivar or line followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 10. Effect of repeated applications of GA, naptalam combinations on stalk elongation in 4-week-old 'FC701/5' sugarbeets grown in the greenhouse.[a]

Treatment	Rate	Napatalam (mg/L)				
		0	0.5	1.0	2.5	5.0
	(mg/L)	(mm)				
Check	0	23 AB	22 AB	14 A	20 AB	10 A
GA_3	750	94 A-D	126 C-F	61 ABC	105 B-E	130 C-G
GA_3	3000	172 D-J	142 C-H	215 G-N	243 I-P	182 E-L
GA_3	5000	208 F-N	267 L-P	229 H-O	282 M-P	197 F-M
GA_{4+7}	900	205 F-M	175 D-J	157 D-I	193 F-L	179 D-K
GA_{4+7}	3300	251 J-P	221 H-O	223 H-O	303 OP	265 K-P
GA_{4+7}	5000	226 H-O	293 NOP	238 I-O	205 F-M	325 P

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 11. Effect of single applications of GA_3 in combination with benzoic acid-type chemicals on stalk elongation in 2-week-old 'EL44' and 4-week-old 'FC701/5' sugarbeet seedlings grown in the greenhouse.[ab]

		GA_3 (mg/L)			
		EL44 (Experiment 1)		FC701/5 (Experiment 2)	
Treatment	Rate	0	5000	0	5000
	(mg/L)	(mm)			
Chloramben	0	1 B	31 A	3 A	42 B
Chloramben	0.5	-	-	4 A	45 BCD
Chloramben	1.0	0 B	36 A	-	-
Chloramben	2.0	-	-	0 A	65 DE
Chloramben	5.0	-	-	3 A	42 B
Chloramben	10	3 B	26 A	-	-
Chloramben	100	4 B	23 A	-	-
Dicamba	0.25	-	-	4 A	47 BCD
Dicamba	1.0	-	-	0 A	63 CDE
Dicamba	2.5	-	-	3 A	77 E
TIBA	0	1 D	31 BC	-	-
TIBA	1	12 BCD	27 BCD	-	-
TIBA	10	0 D	34 AB	-	-
TIBA	50	-	-	4 A	41 B
TIBA	100	4 CD	59 A	3 A	37 B
TIBA	250	-	-	4 A	43 BC

[a]Means within experiment (and within herbicide for experiment 1) followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]A line in place of data in the table indicates the treatment was not made.

Table 12. Effect of repeated applications of GA_3 in combination with TCA, dalapon, ethefumesate and pyrazon on stalk elongation in 3-week-old 'FC701/5' and 'US H20' sugarbeets grown in the greenhouse.[a]

Treatment	Rate	GA_3 (mg/L)			
		FC701/5		US H20	
		0	1000	0	1000
	(mg/L)	(mm)			
Check	0	20 A	82 B-E	6 A	93 B-F
TCA	100	0 A	115 B-H	0 A	119 C-H
TCA	500	0 A	165 HI	0 A	64 B
TCA	1000	0 A	207 I	0 A	126 D-H
dalapon	10	0 A	144 FGH	0 A	85 B-E
dalapon	50	0 A	91 B-E	2 A	82 B-E
dalapon	100	0 A	73 BCD	0 A	75 BCD
ethofumesate	5	0 A	133 E-H	0 A	73 BCD
ethofumesate	10	0 A	123 D-H	0 A	79 BCD
ethofumesate	20	0 A	153 G H	0 A	97 B-F
pyrazon	10	0 A	135 E-H	0 A	69 BCD
pyrazon	25	0 A	115 B-H	0 A	92 B-F
pyrazon	50	17 A	125 D-H	5 A	102 B-G

[a] Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 13. Effect of alachlor in combination with GA_3 on the uptake of 0.2µCi ^{14}C-GA_3 (14µCi/µmole) in 3-week-old 'FC701/5' sugarbeet seedlings grown in the greenhouse.[a]

			^{14}C	
Treatment	Rate	Harvest Time	Treated leaf[b]	Newest leaf
	(mg/L)	(days)	(DPM/gm F. W.)	
GA_3	1000	3	9312 A	1066 A
Alachlor + GA_3	50 + 1000	3	2359 A	132 A
GA_3	1000	7	23,791 A	499 A
Alachlor + GA_3	50 + 1000	7	4678 A	1034 A

[a]Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Results from tip and base of treated leaf were combined.

CHAPTER 3

Influence of Low Temperature, GA_3, and Herbicide Combinations on Membrane Lipid Composition in Sugarbeets

ABSTRACT

Fatty acid composition of mitochondria and plasmalemma membranes of sugarbeet (Beta vulgaris L.) foliage was analyzed in annual, florally-induced biennial, and non-induced biennial plants which received treatment combinations of low temperature, gibberellic acid (GA_3), and the herbicide alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide). Low temperature decreased the saturated (palmitic and stearic acids) and increased the unsaturated (linoleic and linolenic acids) fatty acid composition of both membrane fractions in sugarbeet. Alachlor GA_3, and alachlor plus GA_3 induced an effect on membrane lipids similar to that of low temperature. Plasmalemma membranes of annual and florally induced biennial sugarbeets also exhibited a shift toward a higher percentage of unsaturated fatty acids over time whereas the mitochondria fraction showed no change. In non-induced biannial sugarbeet, the unsaturated fatty acids increased over time in the mitochondria membranes but not the plasmalemma.

Plant responses associated with low temperature include vernalization (low temperature promotion of flowering),

breaking of seed dormancy, breaking of winter dormancy in bulb of perennial woody plants, underground induction of storage organs such as tubers, and effects on vegetative form and growth of certain plants (14). Vernalization is important for the induction of flowering in winter annuals such as cereals, and biennials such as sugarbeets (Beta vulgaris L.). Gnerally, the cold requirement in cereals is quantitative, whereas in biennials it is considered qualitative or absolute (14, 16).

The cold requirement in sugarbeets impedes improvement of this crop through breeding programs due to the length of time needed between generations of seed (7). If this plant could be induced to flower in one growing season it would accelerate breeding programs.

It has been demonstrated that low temperatures have a distinctive effect on plant cell membranes (3, 6, 12). The saturated fatty acid [palmitic acid (16:0)] composition of membranes from plants growing under low or a shift from high to low temperatures decreased and the unsaturated fatty acids [linoleic (18:2) and linolenic (18:3)] increased. This was shown to occur in membranes of individual organelles such as mitochondria and plasmalemma and the type of shift appeared to be dependent on species and orgenelle examined (8, 12).

Giberellic acid (GA_3) has been shown to induce flowering in biennials such as Hyoscyamus niger (9), carrot (Daucus

carota L.), and species and cultivars of Brassica but not beet (2). It has been suggested GA_3 substituted for the cold requirement where flowering was induced but did not substitute for the obligate photoperiodic requirement in sugarbeets to induce flowering (16). GA induced flowering in sugarbeet only under partial photothermal induction conditions (continuous illimination and temperature of 7 to 8 C for 43 days) (5).

Recent research has shown that certain herbicides could substitute for the cold effect on plant cell membranes by increasing the percentage of unsaturated fatty acids in mitochondria and plasmalemma membranes of soybean (Glycine max (L.) Merr.) (13). Based on these results, herbicides that influence lipid metabolism were applied in combination with GA to the foliage of sugarbeet seedlings to determine if the obligate cold requirement could be replaced (10). The results indicated that several classes of herbicides interacted synergistically with GA to induce stalk elongation. However, none of the chemicals, alone or in combination with GA induced flowering in plants grown under non-inductive conditions.

The low temperature effect on sugarbeet plant cell membranes has not been documented. In addition, it has not been established that GA or herbicides mimic the low temperature effect on cell membranes of this plant. Therefore, the objective of this investigation was to determine the effect of low temperature, GA_3 and herbicides on cell membranes of

sugarbeet seedlings, and to compare these results to the fatty acid composition of membrane lipids in annual and florally induced biennial sugarbeet to determine whether induced changes were similiar to naturally occurring shifts.

MATERIALS AND METHODS

Experimental Conditions for Chemical and Temperature Treatment in Biennial Sugarbeet. Two sets of 948 ml styrofoam cups containing five to ten 'US H20' (intermediate bolting tendency) sugarbeet seeds planted 2.5 cm deep in a commercial potting mixture (Metro-Mix 300) were placed in two growth chambers, respectively. Environmental conditions within the chambers were 14/10-hr photoperiod with a day/night temperature of 22/14 C. The lamps were cool white fluorescent with supplemental incandescent and provided a photosynthetic photon flux density (PPFD) of 300 µmol · m^{-2} · sec^{-1}. Seventeen days after seeding, all plants were thinned to one plant per cup of a uniform size and the temperature in one chamber was reduced to 15/5 C (day/night) (Low temperature). Chemical treatments consisting of GA_3 (Pro Gibb) at 0 and 2500 mg/L in combination (solutions mixed together) with alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] (Lasso 4E) at 0 and 250 mg/L were mede twice over 3 days to the foliage of 17-day-old seedlings. One week after the initial chemical application, the chamber with the low temperature was changed back to 22/14 C (day/night). The plants were watered as needed and

fertilized once every 2 weeks with a 5000 mg/L solution containing a 20:20:20 (NPK) fertilizer. All treatments were replicated three or four times and the experiment was repeated to confirm results.

Experimental Conditions for Chemical Treatment of Biennial, Annual and Florally Induced Biennial Sugarbeets. An annual line and a biennial cultivar ('US H20') of sugarbeet were seeded in pots and maintained as described above with the exception of the following changes. Immediately after seeding, both sugarbeet types were placed in one chamber with a 16/8-hr photoperiod and a temperature of 22/14 C (day/night). In a second experiment, 2 sets of 'US H20' biennial sugarbeets were seeded and maintained as described above. One set was placed in a chamber with 14/10-hr photoperiod and 22/14 C, day/night temperature. The second set was placed in a chamber under inductive conditions for flowering (14 hr of light daily from fluorescent lamps plus 24 hr from incandescent lamps) (6). The temperature was 22 C for the 14 hr of illumination with fluorescent lighting and 14 C for the remaining 10 hr.

Seventeen days after seeding, two alachlor applications of 0 and 250 ml/L were made 3 days apart, to the foliage of the sugarbeets described above. Extra pots of sugarbeets for both experiments were seeded and remained in the growth chamber until the onset of flowering.

Membrane Separation. Plants were harvested at 0, 7 and 14 days following chemical treatment for fatty acid

analysis according to a slightly modified procedure described by Rivera and Penner (12). Plasmalemma and mitochondria membranes were obtained by homogenizing fresh foliage in a Virtis grinder for 90 sec in 30 ml of an ice-cold medium consisting of 0.26 M sucrose, 3 mM EDTA, 50 mM Tricine (N-Tris(hydroxy-methyl) methyl glycine), and 1% (w/v) BSA (Bovine Serum Albumin) (fatty acid free) (pH 7.8). The homogenate was strained through 4 layers of chessecloth and centrifuged at 13,000 g for 15 min at 2 C. The 13,000 g pellet containing mitochondria was resuspended in the homogenizing medium and centrifuged at 2500 g for 10 min to remove cell walls and other large cellular fragments. The resulting supernatant was then pelleted at 13,000 g for 15 min, the peliet rinsed and suspended in deionized H_2O, and repelleted at 13,000 g for an additional 15 min. The resulting mitochondria were then held at -15 C for further analysis. The supernatant containing plasmalemma from the original 13,000 g centrifugation was further centrifuged at 80,000 g for 30 min. The resulting pellet was then resuspended in 2 ml 20% (w/w) sucrose containing 1 mM $MgSO_4$ and 1 mM Tris-Mes (2-N-morpholinoethane sulphonic acid), pH 7.8. The suspension was layered onto a discontinuous sucrose gradient consisting of 28 ml of 45% (w/w) sucrose and 8 ml of 34% (w/w) sucrose. The sucrose solutions each contained 1 mM $MgSO_4$ and 1 mM Tris-Mes, pH 7.8. The gradient tubes were centrifuged for 2 hr at 95,000 g in a swinging bucket rotor (Beckman SW27 rotor). The plasmalemma

were obtained from the 34% to 45% interface, diluted in deionized H_2O, and pelleted at 80,000 g for 10 min. The plasmalemma samples were held at -15 C for further analysis.

Extraction and Analysis of Phospholipids. The frozen membrane samples were lyophilized and the lipids extracted according to a modified procedure of Folch et al. (4). Ten ml of $CHCl_3$-MeOH (2:1) (C/M) was added to the lyophilized tissue and the samples were shaken in a water bath at 33 C for 30 min. The extract was filtered (Whatman No. 4) into a second tube, and the residue re-extracted once with 5 ml C/M by shaking in a H_2O bath for 15 min. The filtered extracts were obtained and washed with 0.2 vols of 0.9% NaCl solution in a tube stirrer for 30 sec. After the mixture settled, the upper phase was discarded and the lower phase washed twice with 0.2 vols of $CHCl_3$-MeOH-H_2O (3:48:37 by volume) containing 0.9% NaCl. The lower phase containing the lipids was taken to dryness under N_2 at 33 C and the residue dissolved in 50 µl $CHCl_3$. The lipids were then applied to TLC plates (20 X 20 cm) precoated with 0.25 mm silica gel 60 F254. The phospholipids were separated from the remaining lipids in Me_2CO-MeCOOH-H_2O (100:2:1 by volume) and the phospholipid band selected on the basis of published R_f values (15) was scraped from the plates, extracted with 2 ml C/M followed by 1 ml MeOH and the resultant solution taken to dryness under N_2 at 33 C. Fatty acid methyl esters were prepared according to a modified procedure of Metcalfe et al. (11). 0.5 N methanolic

KOH (1 ml) was added to the dried sample followed by boiling for 5 min. After the tubes had cooled, 1 ml of 14% BF_3-MeOH was added and the samples boiled for an additional 2 min. One drop of saturated NaCl solution was then added and the methyl esters extracted 3 times with 1 ml hexane each. The extracts were combined, dried under N_2, and the residue was taken up in 50 µl acetone for GLC analysis. Fatty acid composition was determined by FID using a 1.83 m by 2 mm glass column packed with 12% stabilized DEGS on anakrom ABS and operated at 165 C with N_2 as the carrier gas. Peak identification and quantification was performed by comparison with authentic fatty acid methyl ester standards.

Data from the experiment with chemical and temperature treatment, comparison between annual and biennial sugarbeet, and comparison between induced and non-induced biennial sugarbeets were analyzed as a three-way factorial with a split plot, a two-way factorial with a split plot and a split split plot, respectively.

RESULTS AND DISCUSSION

Effects of Low Temperature, Alachlor and GA_3 in Biennial Sugarbeet. The five major fatty acids found in membrane fractions of sugarbeet foliage were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). Trace amounts of myristic (14:0) and palmitoleic (16:1) acids were occasionally observed.

Low temperature resulted in measureable changes in the fatty acid composition of both the mitochondria and plasmalemma membranes (Tables II through VI). The percentage of palmitic, stearic and oleic acids of the mitochondria membrane decreased with an increase in linoleic and linolenic acid composition after exposure to low temperature for 7 days. However, in the plasmalemma fraction, the palmitic and oleic acid composition decreased with an increase only in the percent of linoleic acid. This trend was observed as treatment temperature was increased. This relationship between temperature and fatty acid composition supports previous investigations concerning other plant species (1, 3, 8, 12, 15, 17).

Treatments of alachlor, GA_3, and GA_3 plus alachlor induced various shifts in the percent composition of palmitic, linoleic, and linolenic acid in the mitochondria and plasmalemma membranes (Tables II, V, and VI). Under low temperature GA_3 decreased the percent linolenic acid composition and GA_3 plus alachlor decreased the linolenic and increased the percent composition of palmitic acid in mitochondria membranes. Under warm temperature linoleic acid increased in the mitochondria fraction from all three chemical treatments. In the plasmalemma membrane fraction, only GA_3 plus alachlor increased the percent linolenic acid composition under low temperature. However, under warm temperature, alachlor and alachlor plus GA_3 increased the percent linolenic but decreased the percent composition of

palmitic acid. These results support those of Rivera and Penner demonstrating that membrane composition shifted toward a higher percentage of unsaturated fatty acids following herbicide treatment (13). Data from stalk height measurements 4 weeks after treatment taken on other plants that received these chemical and temperature treatments showed that alachlor GA_3 combinations produced a significant increase in stalk height (Table I), indicating that the GA_3 herbicide interaction took place in this experiment. However, it does not appear that results from the fatty acid analysis offer an explanation of this interaction in warm temperature treated plants. The chemical combination did not significantly increase the unsaturated fatty acid content compared to the increase induced by GA_3 or alachlor alone.

Fatty Acid Analysis of Annual, Biennial and Induced Biennial Sugarbeet. In these experiments, only trace amounts of stearic acid were obtained and will not be discussed. Percent fatty acid composition of mitochondria and plasmalemma membranes of biennial sugarbeets under non-inductive and inductive conditions for flowering was compared. In the mitochondria membranes of untreated, non-induced biennial, the percent palmitic and oleic acid composition decreased and the percent composition of linoleic acid increased with time (Tables VII through X). In untreated florally induced sugarbeet, only the percent composition of oleic acid decreased. In the plasmalemma fraction the

palmitic acid composition decreased in both non-induced and induced plants and only the percent linolenic acid composition of induced sugarbeet increased after 2 weeks (Tables VII and X). It should be noted that the percent linolenic acid composition of the non-induced plants increased after 7 days but decreased by 14 days, whereas the increase in the induced sugarbeet was consistent throughout the 2 week period (Table X). Thus, plants about to flower did not undergo an extensive shift toward unsaturation compared to plants exposed to low temperature.

Alachlor increased the degree of saturation in mitochendria membranes of non-induced sugarbeet as percent composition of linoleic acid increased and linolenic acid decreased after 14 days (Tables IX and X). In the plasmalemma of noninduced beets, alachlor increased the percent palmitic and linoleic but decreased the percent composition of oleic and linolenic acid after 7 days. However, after 14 days, only the percent palmitic acid was significantly different from the untreated control as the level of the others decreased. Alachlor also decreased the degree of unsaturation in induced sugarbeets as oleic acid increased and linoleic acid decreased. These results are in contrast to those reported earlier in this paper and by Rivera and Penner (13).

There were no changes in percentage of fatty acids in mitochondria membranes from untreated annual sugarbeet over time (Tables XI through XIV). However, the degree of

unsaturation increased in the biennial type as the percentage of palmitic acid decreased and linoleic acid increased (Tables XI and XIII). In the plasmalemma there were no changes in the fatty acid composition of biennial sugarbeet, whereas the percent palmitic acid composition decreased while the percent linolenic acid of the annual type increased (Tables XI and XIV). Alachlor had no effect on the fatty acid composition of either membrane fraction. These results are similiar to those obtained in the experiment comparing induced and non-induced biennial sugarbeet. The sugarbeets capable of flowering (photoperiodically induced biennial and the annual type) exhibited a shift toward a higher percentage of unsaturated fatty acids in the plasmalemma, but no shift was observed in the fatty acid composition of mitochondria membranes. Both the annual and induced biennial sugarbeets flowered 6 to 8 weeks after seeding.

CONCLUSION

Low temperature, alachlor and GA_3 alachlor combinations caused an increase in the percent of unsaturated fatty acids in mitochondria and/or plasmalemma membranes in sugarbeet. However, the alachlor and GA_3 combinations did not significantly increase the level of unsaturation over either chemical alone.

Flowering sugarbeets (photoperiodically-induced

biennial and the annual type) exhibited a shift toward a higher percentage of unsaturated fatty acids in the plasmalemma but not the mitochondria membrane. A shift to a greater percentage of unsaturated fatty acids in the mitochondria but not the plasmalemma membrane fractions occurred in non-induced biennial sugarbeets. Alachlor generally caused, a shift toward a larger percentage of saturated fatty acids.

Alachlor, GA_3 or alachlor plus GA_3 treatments did not completely substitute for the low temperature effect on membrane lipids, or produce effects on fatty acid composition similiar to those found in flowering sugarbeet.

A positive correlation was not apparent between changes in membrane fatty acid content in florally non-induced biennial sugarbeets treated with low temperature and/or chemical and changes in membrane fatty acid content of florally induced sugarbeets.

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Table I. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on Stalk Elongation in Sugarbeet 30 Days Following Treatment.[a]

		GA_3 (mg/L)			
		Cold		Warm	
Treatment	Rate	0	2500	0	2500
	(mg/L)	(mm)			
Alachlor	0	0 A	21.8 B	0 A	22.4 B
Alachlor	250	0 A	35.6 C	0 A	44.8 C

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table II. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on the Palmitic Acid (C16:0) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

		Palmitic Acid							
		Mitochondria				Plasmalemma			
		Days in cold		Days in warm		Days in cold		Days in warm	
Treatment	Rate	0	7	0	7	0	7	0	7
	(mg/L)	(%)							
GA_3	0	22.5 E	16.7 A	18.1 A-D	17.0 AB	55.2 F	38.5 BC	47.7 E	45.0 DE
GA_3	2500	-	19.6 A-E	-	18.2 A-D	-	39.2 BC	-	43.4 CDE
Alachlor	250	-	17.0 AB	-	20.5 B-E	-	40.9 CD	-	31.8 A
Alachlor + GA_3	250 + 2500	-	21.7 DE	-	16.9 AB	-	34.8 AB	-	31.6 A

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table III. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on the Stearic Acid (18:0) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

		Stearic Acid							
		Mitochondria				Plasmalemma			
		Days in cold		Days in warm		Days in cold		Days in warm	
Treatment	Rate	0	7	0	7	0	7	0	7
	(mg/L)	(%)							
GA_3	0	18.0 D	2.2 AB	10.4 C	6.9 BC	2.5 A	0.6 A	1.8 A	0.9 A
GA_3	2500	-	5.7 ABC	-	2.9 AB	-	0.9 A	-	0.2 A
Alachlor	250	-	2.7 AB	-	3.1 AB	-	2.1 A	-	0.0 A
Alachlor + GA_3	250 + 2500	-	7.3 BC	-	4.5 AB	-	0.6 A	-	0.8 A

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table IV. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on the Oleic Acid (C18:1) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

		Oleic Acid							
		Mitochondria				Plasmalemma			
		Days in cold		Days in warm		Days in cold		Days in warm	
Treatment	Rate	0	7	0	7	0	7	0	7
	(mg/L)	(%)							
GA_3	0	21.7 C	6.5 A	17.8 BC	12.2 AB	6.6 B	1.5 A	4.3 AB	1.9 A
GA_3	2500	-	8.4 A	-	5.2 A	-	1.7 A	-	2.2 A
Alachlor	250	-	6.4 A	-	5.9 A	-	4.5 AB	-	2.4 A
Alachlor + GA_3	250 + 2500	-	9.6 AB	-	6.5 A	-	1.2 A	-	2.5 A

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table V. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on the Linoleic Acid (18:2) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

		Linoleic Acid							
		Mitochondria				Plasmalemma			
		Days in cold		Days in warm		Days in cold		Days in warm	
Treatment	Rate	0	7	0	7	0	7	0	7
	(mg/L)	(%)							
GA_3	0	27.0 A	52.3 E	35.9 ABC	39.9 BCD	26.1 A	55.1 C	42.1 B	48.9 BC
GA_3	2500	-	49.6 E	-	50.0 E	-	51.1 BC	-	51.5 BC
Alachlor	250	-	46.2 DE	-	51.9 E	-	48.0 BC	-	55.2 C
Alachlor + GA_3	250 + 2500	-	43.6 CDE	-	48.8 E	-	53.2 BC	-	51.2 BC

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table VI. Effect of Cold Temperature, Alachlor, and GA_3 Combinations on the Linolenic Acid (C18:3) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

		Linolenic Acid							
		Mitchondria				Plasmalemma			
		Days in cold		Days in warm		Days in cold		Days in warm	
Treatment	Rate	0	7	0	7	0	7	0	7
	(mg/L)	(%)							
GA_3	0	10.8 A	22.4 DE	17.9 BC	24.1 C-F	8.6 BC	4.3 AB	4.1 AB	3.2 A
GA_3	2500	-	16.7 AB	-	24.7 DEF	-	6.9 ABC	-	2.8 A
Alachlor	250	-	27.7 EFG	-	18.7 BCD	-	4.1 AB	-	10.8 CD
Alachlor + GA_3	250 + 2500	-	17.9 BC	-	23.3 B-E	-	9.8 CD	-	13.9 D

[a]Means within membrane fraction followed by the same letter are not siginigicantly different at the 5% level according to Duncan's multiple range test.

Table VII. Effect of Alachlor in Combination with Non-inductive (14/10 hr day/night incandescent) and Inductive (24/0 hr day/night incandescent) Photoperiod on the Palmitic Acid (C16:0) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

			Palmitic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Photo-Period[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	N	24.7 C	21.9 ABC	21.0 AB	33.7 EF	22.6 AB	26.3 BC
Alachlor	250	N	-	22.7 BC	20.5 AB	-	34.1 F	20.5 A
Alachlor	0	I	21.8 ABC	21.0 AB	19.7 AB	31.5 DEF	28.1 CD	21.5 AB
Alachlor	250	I	-	19.4 A	20.2 AB	-	29.0 CDE	21.2 A

[a]Means within membrane fractions follwed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]N = Non-inductive, I = Inductive.

Table VIII. Effect of Alachlor in Combination with Non-indictive (14/10 hr day/night incandescent) and Inductive (24/0 hr day/night incandescent) Photoperiod on the Oleic Acid (C18:1) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

			Oleic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Photo-Period[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	N	4.8 B	4.8 B	2.5 A	2.4 AB	8.1 C	3.6 AB
Alachlor	250	N	-	4.0 B	2.0 A	-	5.0 B	2.2 A
Alachlor	0	I	3.7 B	4.1 B	2.3 A	3.3 AB	9.8 C	2.9 AB
Alachlor	250	I	-	4.5 B	2.2 A	-	12.5 D	9.3 C

[a]Means within membrane fractions follwed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]N = Non-inductive, I = Inductive

Table IX. Effect of Alachlor in Combination with Non-inductive (14/10 hr day/night incandescent) and Inductive (24/0 hr day/night incandescent) Photoperiod on the Linoleic Acid (C18:2) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

			Linoleic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Photo-Period[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	N	35.4 A	37.8 AB	45.2 C	47.8 ABC	39.5 A	45.5 ABC
Alachlor	250	N	-	37.7 AB	52.6 D	-	51.1 C	47.8 ABC
Alachlor	0	I	43.7 BC	43.1 BC	49.2 CD	48.7 BC	41.0 AB	49.7 C
Alachlor	250	I	-	44.0 BC	52.1 D	-	40.2 AB	41.0 AB

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]N = Non-inductive, I = Inductive

Table X. Effect of Alachlor in Combination with Non-inductive (14/10 hr day/night incandescent) and Inductive (24/0 hr day/night incandescent) Photoperiod on the Linolenic Acid (C18:3) Composition of Mitochondria and Plasmalemma Membranes in Biennial Sugarbeet.[a]

			Linolenic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Photo-Period[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	N	35.4 D	35.6 D	31.3 CD	16.1 AB	29.7 E	24.6 B-E
Alachlor	250	N	-	35.6 D	25.0 A	-	9.9 A	29.5 E
Alachlor	0	I	30.8 BCD	31.9 CD	29.4 ABC	16.5 AB	21.1 BCD	25.9 CDE
Alachlor	250	I	-	32.1 CD	25.6 AB	-	18.4 ABC	28.5 DE

[a]Means within membrane fraction follwed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]N = Non-inductive, I = Inductive

Table XI. Effect of Alachlor on the Palmitic Acid (C16:0) Composition of Mitochondria and Plasmalemma Membranes in Annual and Biennial Sugarbeet.[a]

			Palmatic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Type[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	Bi	29.5 D	26.1 CD	18.8 A	23.5 A	22.6 A	21.7 A
Alachlor	250	Bi	-	25.8 BCD	21.2 AB	-	21.3 A	23.2 A
Alachlor	0	An	25.3 BCD	24.1 BC	21.4 ABC	39.9 B	23.7 A	22.3 A
Alachlor	250	An	-	25.4 BCD	21.1 AB	-	23.7 A	21.9 A

[a]Means within fatty acid fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]Bi = Biennial sugarbeet, An = Annual sugarbeet.

Table XII. Effect of Alachlor on the Oleic Acid (C18:1) Composition of Mitochondria and Plasmalemma Membranes in Annual and Biennial Sugarbeet.[a]

			Oleic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Type[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	Bi	4.1 BC	3.5 ABC	2.9 ABC	3.6 A	2.3 A	2.9 A
Alachlor	250	Bi	-	2.8 ABC	2.6 AB	-	2.9 A	2.5 A
Alachlor	0	An	3.5 ABC	4.2 C	2.3 A	10.3 B	5.1 A	4.4 A
Alachlor	250	An	-	4.1 BC	2.3 A	-	6.0 A	3.7 A

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]Bi = Biennial, An = Annual

Table XIII. Effect of Alachlor on the Linoleic Acid (C18:2) Fraction of Mitochondria and Plasmalemma Membranes in Annual and Biennial Sugarbeet.[a]

			Linoleic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Type[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	Bi	43.1 A	46.4 AB	51.0 B	48.1 A	49.7 A	44.8 A
Alachlor	250	Bi	-	48.9 AB	48.4 AB	-	49.2 A	47.9 A
Alachlor	0	An	46.1 AB	49.8 B	51.1 B	42.1 A	44.1 A	42.1 A
Alachlor	250	An	-	49.3 B	50.7 B	-	39.4 A	47.3 A

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]Bi = Biennial, An = Annual.

Table XIV. Effect of Alachlor on the Linolenic Acid (C18:3) Fraction of Mitochondria and Plasmalemma Membranes in Annual and Biennial Sugarbeet.[a]

			Linolenic Acid					
			Mitochondria			Plasmalemma		
Treatment	Rate	Type[c]	0[b]	7	14	0	7	14
	(mg/L)		(%)					
Alachlor	0	Bi	23.1 ABC	24.0 A-D	27.3 CD	24.8 B	25.5 B	30.6 B
Alachlor	250	Bi	-	22.5 AB	27.7 D	-	26.6 B	26.4 B
Alachlor	0	An	25.1 A-D	22.0 AB	25.1 A-D	8.1 A	27.1 B	31.2 B
Alachlor	250	An	-	21.2 A	25.9 BCD	-	30.8 B	27.0 B

[a]Means within membrane fraction followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Time (days) following chemical treatment at which plants were harvested for membrane fatty acid analysis.

[c]Bi = Biennial, An = Annual.

CHAPTER 4

Effect of Herbicides That alter Plant Lipid Metabolism on Survival of Sugarbeet Seedlings.

ABSTRACT

Alachlor [2-chloro-2',6' diethyl-N-(methoxymethyl) acetanilide] and vernolate (S-propyl dipropylthiocarbamate) were evaluated for their effect on winter survival of sugarbeet seedlings in the field when applied in the fall to 2-month-old plants. Alachlor at 100 mg/l and vernolate at 50 mg/l increased in survival rate of 'US H20' in the first experiment. In the second experiment, the sugarbeet survival rate of the untreated controls was too high to adequately assess chemical effects. The high survival rate may have been caused by an unusually heavy snow-cover.

INTRODUCTION

If increased winter survival of sugarbeets could be acheived in the cold temperate climates of the Northern sugarbeet growing regions (such as Michigan), improvement of this crop could be facilitated through more efficient breeding programs producing seed in the north (4). Currently, the sugarbeets are removed from the field in the fall and the roots, with crown buds intact, are placed in a cold room (4 C) with the buds exposed to continuous illumination from incandescent lamps for 2 to 3 months. The following spring, the roots with the florally induced buds are replanted in the soil for purposes of flowering, cross pollination, and seed harvest. Although effective, this process reduces the amount of sugarbeet breeding research that can be accomplished.

Cold hardy temperate zone crop plants are able to withstand winter temperatures of -30 C or less, but in the spring and summer months, they are susceptible to cold and can be easily killed at temperatures near 0 C (12). Cold hardiness of these species is dependent on their genetically controlled acclimation to survive freezing temperatures and their ability to express this trait.

Prevailing ambient temperature appears to be the most important environmental parameter for imparting cold hardiness to cereals (7, 8). Low, above-freezing temperatures impart cold hardiness in the fall as most of these plants acclimate as temperatures gradually fall below 10 C (1), with optimal temperatures for cold acclimation near 3 C for cereals (7).

Stage of plant growth is important to acclimation and the maintainance of hardiness to cold temperature. It was found that winter wheat (Triticum aestivum L.), growing 11 weeks or more in the fall, prior to cessation of growth, suffered more winter injury than younger plants (10). The four to six leaf stage was the optimum stage for aquiring winter hardiness in this species.

There is considerable controversy on the biochemical and physiological processes involved in cold hardiness of higher plants (12). Much of this research has dealt with lipids in relation to the cold hardening phenomenon. Examining the fatty acid composition of plant cell membranes in cereals exposed to optimum temperatures for cold hardening resulted in an increase in the linolenic acid portion of lipids (2). Membrane lipids containing a higher percentage of unsaturated fatty acids have been shown to be more fluid at low temperatures, which would aid in maintaining membrane integrity at lower temperatures (6). However, additional research has shown that this shift toward greater fatty acid unsaturation in cereals may only be a

low-temperature response and not involved in cold acclimation, per se (3).

Chemical effects on membrane lipids and their relationship to cold hardening and chilling injury have also been investigated. Willemot (13) found that BASF 13-338 [4-chloro-5(dimethylamino)-2-phenyl-3(2H)-pyridazinone] inhibited linolenic acid accumulation and frost resistance in 12-day-old winter wheat plants. This chemical was also shown to affect cotton (Gossypium hirsutum L.) seedlings similiarly, ultimately leaving the plants more susceptible to chilling injury (11). Other research demonstrated that the herbicides diethatyl [N-(chloroacetyl)-N-(2,6 diethylphenyl)glycine] and vernolate (S-propyldipropylthiocarbamate) increased cold hardiness in soybean (Glycine max (L.) Merr.) and this result corresponded to an increase in the unsaturated fatty aicd content of plasmalemma membrane in the root (9). Research by Mahoney et al. (5) demonstrated that alachlor treatments to young sugarbeet foliage increased the unsaturated fatty acid content of mitochondria and plasmalemma membranes similar to a cold temperature treatment.

The objective of this investigation was to evaluate chemicals that increase the unsaturated fatty acid content of plant cell membranes for their potential to increase winter survival of sugarbeet seedlings in the field.

MATERIALS AND METHODS

Field experiments were conducted during the winter periods of 1980 - 1981 and 1981 - 1982, near East Lansing and Haslett, Michigan, respectively.

The first experiment was initiated on August 25, 1980 in a sandy loam soil. 'US H20' sugarbeet seeds were planted 2.5 cm deep and 5 cm apart in rows 0.3 m apart in plots 1.2 m wide by 1.2 m long. On October 27, 1980, the sugarbeets (in the 6 to 8 leaf stage) received foliar applications of solutions of alachlor (Lasso 4E) at 100 and 200 mg/L and vernolate (Vernam 7E) at 50 mg/L with 0.5% v/v Tween 20 surfactant. Applications were made with a hand-pump sprayer to the foliage until solution runoff occurred. All treatments were replicated three times. Two weeks following treatments, all plots were covered with 30 cm of wheat straw.

The second experiment was initiated on August 21, 1981 in a loamy sand soil. All plots contained one row each of three sugarbeet lines [G-O (Seed mixture produced at Sorenson in 1980), J-O (81B1-1) and I-O (50% each 81B2-00 and 81B5-00) types] planted 2.5 cm deep and 5 cm apart in rows 0.6 m apart and 4.3 m long. On October 28, 1981, the sugarbeets (in the 6 to 8 leaf stage) received foliar applications of

alachlor at 100 and 200 mg/l and vernolate at 25, 50 and 100 mg/l in 73 l/ha H_2O with 0.5% (v/v) X-77 surfactant. Treatments were made with a knap-sack sprayer, under 2.1 kg/cm^2 pressure supplied by a cartridge of compressed CO_2. Treatments were replicated four times and all sugarbeet lines were randomized within each plot. There were two sets of treatments for this experiment. One set received a cover of 30 cm of wheat straw 3 weeks after application, whereas the other received no cover.

Early the following spring (March 30 and April 16 for experiments one and two, respectively) the straw was removed from the plots, stand counts taken and flowering observed. The data were analyzed by analysis of variance and treatment means compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

In the first experiment, alachlor at 100 mg/l and vernolate at 50 mg/l applied to runoff, increased the number of sugarbeets that survived during the winter compared to the untreated control (Table 1). This indicates that a relationship may exist between herbicides that increase the fatty acid unsaturation and their ability to impart cold tolerance to plants as reported by Rivera (9). The percentage of plants that survived during the winter was not calculated because stand counts were not taken in the fall. Of the surviving plants approximately

80 percent from every treatment flowered indicating that the chemicals had no adverse affect on flowering.

In the second experiment, the chemical treatments had no effect on winter survival of sugarbeets (Table 2). It should be noted that approximately 100 percent of the plants without any cover and 80 percent of the plants with straw-cover survived the winter. Snowfall during the winters of 1980-1981 and 1981-1982 was 98 and 143 cm respectively. The larger amount of snow-cover during the second winter might explain the increased sugarbeet survival in this experiment. Because of the unexpectedly high survival rate chemical effects on winter survival could not be adequately assessed in this experiment. The lower survival rate of plants under the straw-cover than those with no cover was probably the result of rodent damage. As in experiment one, over 80 percent of the plants flowered in all treatments.

CONCLUSION

Treatments of alachlor at 100 mg/l and vernolate at 50 mg/l increased the number of 'US H20' sugarbeets that survived in the field during the winter. In the second experiment the survival rate of the untreated controls of covered and uncovered sugarbeets was too high to assess herbicide effects on survival. Approximately 80 percent of the sugarbeets flowered the following spring in all

treatments of both experiments.

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Table 1. Effect of Foliar Applications of Alachlor and Vernolate on the Survival of 'US H20' Sugarbeets in the Field During the Winter.[a]

Treatment	Rate	Number of Plants[b]
	(mg/l)	
Check	0	17 A
Alachlor	100	43 B
Alachlor	200	31 AB
Vernolate	50	44 B

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Average number of plants per plot for each treatment.

Table 2. Effect of Foliar Applications of Alachlor and Vernolate on the Survival of Three Sugarbeet Lines in the Field during the Winter.[a]

Treatment	Rate	Number of Plants[b]		
		G-O Type	J-O Type	I-O Type
	(mg/l)			
Check	0	24 A	19 A	25 A
Alachlor	100	25 A	25 A	28 A
Alachlor	200	24 A	29 A	25 A
Vernolate	25	24 A	26 A	28 A
Vernolate	50	31 A	21 A	23 A
Vernolate	100	25 A	24 A	23 A

[a]Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b]Average number of plants per plot for each treatment.

CHAPTER 5

SUMMARY

GA_3 in combination with photoperiods of 18/6, 24/0-hr (day/night), or 14/10-hr plus a 2-hr nightbreak substantially increased flowering compared to untreated controls.

Combinations of GA with the plant hormones on hormone-like materials ethephon, kinetin and 2,4-D; with the herbicides, reported to alter plant lipid metabolism. EPTC, cycloate, butylate plus R-25788, vernolate, diethatyl, alachlor, acetolhlor, metolachlor, TIBA, chloramben, dicamba, naptalam, TCA, ethofumesate, and dalapon, and with the herbicide glyphosate resulted in a synergistic increase in stalk elongation but no floral induction. Uptake of ^{14}C-GA_3 by sugarbeet foliage was not increased by pretreatment with alachlor and, apparently was not the basis for the observed interaction.

GA_3, alachlor, and alachlor plus GA_3 increased the percent unsaturated fatty acid composition of mitochondria and plasmalemma membranes similiar to cold temperature treatment.

The percent unsaturated fatty acid composition of plasmalemma membranes in annual and florally-induced biennial sugarbeets increased with time, whereas the mitochondria membranes showed no such change. In non-induced plants,

there was a shift toward a greater percentage of unsaturated fatty acids in mitochondria but not in plasmalemma membranes.

Fall applications of alachlor and vernolate increased the winter survival rate of sugarbeets in the field, suggesting that these materials may aid in cold hardening of sugarbeet.

APPENDIX I. Additional Data of GA/Plant Hormone Combinations not Presented in the Dissertation Text.

Table 1. Comparison between soil and foliar applied GA_3 for stalk elongation in 'US H20' sugarbeets grown in the greenhouse.

Treatment	Rate	Soil Application	Foliar Application
		(mm)	
GA_3	0	17.6 A	-
GA_3	1.12 kg/ha	22.4 A	-
GA_3	2.24 kg/ha	41.8 A	-
GA_3	4.48 kg/ha	28.2 A	-
GA_3	100 mg/l	-	140.6 B
GA_3	500 mg/l	-	237.4 C
GA_3	1000 mg/l	-	347.0 D

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2. Evaluation of GA_3 as a seed teratment on stalk elongation in 'EL44' and 'US H20' sugarbeets grown in the greenhouse.

Chemical	Rate	FC701/5	US H20
	(mg/l)	(mm)	
GA_3	0	17.4 A*	15.2 A
GA_3	10	14.8 A	16.4 A
GA_3	50	12.2 A	12.0 A
GA_3	100	13.8 A	13.2 A
GA_3	500	15.2 A	10.2 A
GA_3	1000	14.2 A	13.4 A
GA_3	5000	14.0 A	13.6 A

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 3. Effect of single applications of GA_3 on stalk elongation in 'EL44' sugarbeets at various stages of growth in the greenhouse.

GA_3 Rate	Age of Plant (Weeks from Seeding)					
	1	2	3	4	5	6
(mg/l)	(mm)					
0	7 STU*	6 STU	8 R-U	6 TU	0 U	0 U
100	8 R-U	13 N-U	16 K-U	16 K-U	17 J-U	15 M-U
250	9 Q-U	15 K-U	24 G-U	16 K-U	18 J-U	16 L-U
500	15 M-U	21 I-U	26 F-U	26 F-U	21 I-U	15 M-U
1000	13 O-U	26 F-U	48 B-K	36 E-T	37 D-T	42 C-P
1500	14 M-U	22 H-U	38 C-S	43 C-P	28 E-U	31 E-U
2000	12 P-U	37 D-T	35 E-T	54 B-H	35 E-T	49 B-J
2500	22 H-U	32 E-T	23 H-U	76 B	45 B-O	45 B-N
3000	19 J-U	41 C-Q	23 G-U	48 B-L	57 B-F	45 B-N
3300	24 G-U	39 C-R	30 E-U	46 B-O	108 A	46 B-M
4000	21 I-U	32 E-U	58 BCD	69 BC	45 B-O	40 C-Q
5000	21 I-U	52 B-I	59 B-E	59 B-E	55 B-G	75 B

*Treatment means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 4. Evaluation of single applications of formulated and unformulated GA_3 on stalk elongation in 3-week-old sugarbeets grown in the greenhouse.

		Experiment 1		Experiment 2		Experiment 3					
		EL44		FC701/5		FC701/5		US H20		EL44	
Treatment	Rate	U†	F‡	U	F	U	F	U	F	U	F
	(mg/l)	(mm)									
GA_3	0	3.8 AB*	1.0 A	10.0 A	16.2 A	8.6 A	6.0 A	8.4 A	8.0 A	8.0 A	11.0 A
GA_3	1000	5.4 BC	10.5 D	-	-	-	-	-	-	-	-
GA_3	2500	-	-	-	-	37.4 B	63.0 C	32.8 BC	22.4 AB	28.2 B	24.2 B
GA_3	3300	8.3 CD	15.7 E	-	-	-	-	-	-	-	-
GA_3	5000	9.9 D	15.8 E	101.6 B	131.8 B	-	-	-	-	-	-

†Unformulated GA_3

‡Formulated GA_3

*Means within varieties, and experiments followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 5. Evaluation of single applications of GA_3 on stalk elongation in 6-week-old 'EL44' sugarbeets when applied foliarly using three different techniques in the greenhouse.

Chemical	Rate	PIP GP†	ATM GP‡	ATM Total§
	(mg/l)	(mm)		
GA_3	0	1.0 A*	1.0 A	1.0 A
GA_3	1000	10.0 BC	7.2 B	11.0 BC
GA_3	3300	13.0 C	10.6 BC	18.4 D
GA_3	5000	12.6 C	12.2 C	19.0 D

†Solution applied to the growing point by pipette.

‡Solution applied to the growing point by atomization.

§Solution atomized over the entire plant.

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 6. Effect of repeated applications of GA_3 in combination with leaf removal on stalk elongation in 3-week-old greenhouse grown 'US H20' sugarbeets.

Chemical	Rate	Leaf Removal†	
		-	+
	(mg/l)	(mm)	
GA_3	0	25.0 A*	20.5 A
GA_3	50	22.2 A	20.4 A
GA_3	100	34.6 A	24.2 A
GA_3	200	35.8 A	22.6 A
GA_3	400	64.6 A	23.6 A
GA_3	800	53.6 A	37.4 A
GA_3	1600	91.4 A	63.8 A
GA_3	2500	206.2 BC	123.6 ABC
GA_3	3200	209.2 BC	116.2 AB
GA_3	5000	227.2 C	213.8 BC

*Means followed by the letter are not significantly different at the 5% level according to Duncan's multiple range test.

† = leaves not removed; + = leaves removed.

Table 7. Effect of single applications of GA_3 and GA_{4+7} in combination with ethephon on stalk elongation in 2-week-old 'US H20'and 'EL44' sugarbeet seedlings grown in the greenhouse.

		US H20			EL44		
		Ethephon (mg/l)			Ethephon (mg/l)		
Treatment	Rate	0	1.0	10	0	1.0	10
	(mg/l)	(mm)					
GA_3	0	1.0 A*	3.0 A	5.3 AB	3.3 A	7.3 ABC	4.0 AB
GA_3	1000	26.3 A-D	21.3 A-D	15.0 ABC	8.0 ABC	17.0 A-D	27.0 A-E
GA_3	5000	38.3 CD	43.7 D	26.3 A-D	62.3 F	66.7 F	34.0 CDE
GA_{4+7}	1000	18.3 A-D	15.3 ABC	21.0 A-D	16.0 A-D	26.3 A-E	20.3 A-E
GA_{4+7}	5000	29.0 A-D	32.7 BCD	43.0 BC	32.7 B-E	44.3 DEF	47.7 EF

*Means within varieties followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 8. Effect of GA_3 applied once in combination with ethephon on stalk elongation in 3-week-old 'US H20' and 'EL44' sugarbeets grown in the greenhouse.

		GA_3 (mg/l)							
		US H20				EL44			
Treatment	Rate	0	500	1600	5000	0	500	1600	5000
	(mg/l)	(mm)							
Ethephon	0	5.8 ABC*	22.0 A-D	96.4 E-I	188.2 LMN	0 A	26.8 A-F	34.6 B-G	86.2 JKL
Ethephon	5	0 A	24.4 A-D	72.8 C-H	148.6 I-M	0 A	17.0 ABC	56.0 F-I	66.6 H-K
Ethephon	10	3.4 AB	32.6 A-E	70.0 B-H	133.4 H-L	0 A	13.0 ABC	24.8 A-E	72.4 IJK
Ethephon	20	0 A	26.4 A-D	64.6 A-G	118.2 G-K	0 A	19.4 A-D	27.0 A-F	74.2 IJK

*Means within varieties not followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 9. Effect of single applications of GA_3 in combination with ethephon on stalk elongation in 4-week-old 'US H20' sugarbeets grown in greenhouse soil.

		GA_3 (mg/l)			
Treatment	Rate	0	3000	6000	10,000
	(mg/l)	(mm)			
Ethephon	0	14.0 A*	39.2 BCD	28.2 A-D	34.6 BCD
Ethephon	1	23.6 ABC	24.6 A-D	35.2 BCD	36.6 BCD
Ethephon	5	22.0 AB	40.0 CD	31.0 BCD	32.0 BCD
Ethephon	10	12.0 A	31.6 BCD	32.0 BCD	42.0 D
Ethephon	20	13.6 A	30.8 BCD	41.2 D	40.8 CD

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 10. Effect of GA_3 in combination with sequential applications of NAA and 2,4-D on stalk elongation in 3-week-old 'FC701/5' sugarbeets in the greenhouse.

Treatment	Rate	NAA (mg/l)			2,4-D (mg/l)		
		0	50	100	10	25	50
	(mg/l)	(mm)					
GA_3	0	14 A*	12 A	17 A	25 AB	22 AB	18 A
GA_3	50	26 ABC	35 ABC	30 ABC	31 ABC	49 A-E	26 ABC
GA_3	250	62 A-I	52 A-F	70 A-J	74 A-J	112 F-K	75 A-J
GA_3	1000	119 H-K	88 C-J	122 I-K	162 KL	99 D-J	129 JK

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 11. Effect of GA_3 in combination with sequentially applied 2,4-D on stalk elongation and flowering in 3-week-old 'FC701/5' and 'US H20' sugarbeets grown under greenhouse and growth chamber environment.

Treatment	Rate	Environment†	FC701 GA_3 (mg/l) 0	500	1000	2500	US H20 GA_3 (mg/l) 0	500	1000	2500
	(mg/l)		(mm)				(mm)			
2,4-D	0	GC	14.6 A*	64.6 ABC	86.8 BCD	154.4 EFG	0 A	141.0 BCD	164.4 CDE	153.8 CDE
2,4-D	2	GC	16.2 A	39.0 AB	126.4 C-F	151.6 D-G	0 A	65.2 AB	119.4 CD	228.2 EFG
2,4-D	10	GC	30.4 AB	117.8 C-F	204.2 GHI	240.8 HI	0 A	125.8 BCD	165.6 CDE	293.4 G
2,4-D	25	GC	19.4 A	89.0 B-E	179.0 FGH	260.2 I	0 A	61.0 AB	172.4 CDE	226.0 EFG
2,4-D	50	GC	19.4 A	135.8 DEF	214.8 GHI	347.6 J	0 A	102.2 BC	206.8 DEF	281.0 FG
2,4-D	0	GH	0 A	87.0 BC	141.1 CDE	250.0 FG	0 A	70.3 B	162.2 E-I	247.0 J
2,4-D	2	GH	0 A	81.3 BC	122.0 BCD	288.8 GH	0 A	98.2 BCD	126.7 B-E	196.6 F-J
2,4-D	10	GH	0 A	61.9 AB	139.9 CDE	308.2 GH	0 A	76.0 B	93.3 BCD	234.3 J
2,4-D	50	GH	0 A	104.8 BCD	163.4 DE	377.3 I	0 A	88.0 BC	150.0 D-H	206.1 HIJ
2,4-D	100	GH	7.5 A	132.1 CD	199.9 EF	321.6 HI	0 A	114.1 B-E	139.0 C-F	198.8 G-J
2,4-D	250	GH	8.8 A	132.6 CD	226.4 F	313.9 H	0 A	144.9 C-G	144.3 C-G	218.9 IJ

*Means within variety and within environment followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

†GC = Growth Chamber, GH = Greenhouse

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