THE EFFECT OF SEED APPLIED AND ROOT-APPLIED GROWTH REGULATORS ON THE GERMINATION AND GROWTH OF MUSKMELON

by

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INTRODUCTION

Uniform stand establishment is an important critera for successful vegetable crop production. In the case of muskmelon production the grower must depend on successful germination and transplanting to contribute to a profit. It is known that germination of muskmelon seed is inhibited at temperatures less than 20°C (Nelson and Sharples, 1980a). Methods which would promote earlier germination would reduce energy costs, and allow earlier establishment of plants for a realization of a potentially higher profit from the sale of early season melons. In addition to being sensitive to suboptimal temperatures, muskmelon seedlings are extremely sensitive to transplant conditions. Development of cultural methods which may overcome transplant shock would provide obvious benefits from increased yield.

Reviews have noted that germination of seeds inhibited by low temperatures can be overcome through the applications of growth regulators or hormones such as gibberellins, cytokinins, and ethylene (Jones and Stoddart, 1977; Thomas, 1977; Ketring, 1977). These hormones apparently overcome inhibiting mechanisms allowing enzymatic activity which alters the hormonal balance in favor of germination (Nikolaeva, 1977).

This study will investigate the hypothesis that favorable concentrations of various growth hormones or regulators will enhance germination of muskmelon seeds at suboptimal temperatures.

Why are muskmelons sensitive to transplanting? Under transplant conditions the root systems of seedlings may be introduced into an adverse environment, as well as being subjected to damage during handling procedures. Although many factors are probably involved in transplant shock it may be that the large leaf area and warm growing conditions, which increase

physiological processes, place an excessive demand on root systems.

While studies are limited, root application of growth hormones and regulators have been used successfully in both field and greenhouse tests on transplanted crops (Arteca, 1982; Patil, et. al., 1981). Additionally, the introduction of starch polymer acrylates (Hydro-gels) may allow increases in growth because a favorable environment is maintained during transplanting. Thus root application of growth regulators dissolved in a starch polymer acrylate may show promise for increasing the growth, survival, and yield of transplanted muskmelons.

This study will also test the hypothesis that growth of aerial portions of muskmelon seedlings will respond favorably to root application of growth regulators and hormones. If response is improved, this may indicate a possible method of reducing transplant shock in the field.

LITERATURE REVIEW: Hormones and Seed Germination

Germination involves reestablishing metabolic events, which were temporarily suspended in dormancy, that will ultimately lead to the formation of a seedling. DNA replication and transcription are stimulated resulting in cell division and protein synthesis. Respiration increases while substrates in the embryonic axis and cotyledons are degenerated providing energy for synthesis of new proteins from starches, lipids, and amino acids (Jann and Amen, 1977). There are numerous factors which will stimulate a seed to complete germination (radicle protrusion) including chilling, light, temperature, or scarification. Muskmelon seeds are considered quiescent since they will germinate under favorable environmental conditions; namely, warm temperatures (greater than 24°C) as well as adequate water. The use of plant hormones and growth regulators as agents of overcoming germination inhibition is well documented.

GIBBERELLIN

Applications of 1.0 mM $GA_{4/7}$ and GA_3 stimulated low temperature (15°C) germination of pepper seeds (Nelson & Sharples, 1980b); however, Watkins and Cantliffe (1983) found that $GA_{4/7}$ was slightly more effective in stimulating pepper seed germination than GA_3 at concentrations of 10-1000 ppm. $GA_{4/7}$ significantly increased low temperature germination percentages of sugar beet seed only after 3 weeks of incubation (Nelson & Sharples, 1980b). At 12-15°C, germination rates of seed treated with combinations of $GA_{4/7}$, kinetin and ethephon were not different from $GA_{4/7}$ treatments alone. The effect of $GA_{4/7}$ as well as combinations of treatments on tomatoes depended on

the variety in question. However, while $GA_{4/7}$ did increase germination rates over untreated controls, they were not different than seeds treated with acetone, which was the infusion technique used for the treatments. Treatment of cucumber seeds, at 12°C, with $GA_{4/7}$, GA_3 and combinations of $GA_{4/7}$ with kinetin and ethephon all significantly increased germination over the control. By the end of 14 days of incubation, seeds treated with $GA_{4/7}$ and GA_3 were 92% and 71% germinated, respectively. Only 6% of the controls had germinated during this same time period (Nelson and Sharples, 1980a). Similar, but less dramatic effects, were observed with $GA_{4/7}$ treatments of muskmelon and watermelon. The infusion agent, acetone, appeared to inhibit germination. Other species of seed, the germination of which has been increased by GA's, include Primula sp. (Miller and Holcomb, 1982), sunflower (Kummar and Sastry, 1974), and Apium graveolens (Palevitch and Thomas, 1974).

In addition to promoting earlier germination, Coronel (1982) found that pepper seed germination was more uniform when GA_3 was applied in aerated water columns compared to untreated seeds in aerated water columns.

The mechanism of GA action in seed germination is still elusive. Many studies have been published demonstrating GA action on events occurring during germination, but a direct relation between GA actions and germination has yet to be found. GA has been shown to stimulate activities of enzymes which would be necessary for breakdown of cotyledonary reserves into sugars, which are energy sources for embryonic growth (Jones and Stoddart, 1977). Evenson and Loy (1978) demonstrated that GA increased catalase activity (an indicator enzyme for lipid degradation) in watermelon embryos. Invertase activity (responsible for converting sucrose to glucose) in embryonic axes was also increased due to GA treatments. No net effect on protein break-

down in the embryonic axes could be determined as GA had no effect on the amino acid pool size in either the cotyledons or the axes. Although GA_3 stimulation, via the embryonic axis, of α-amylase synthesis is well known in cereal seeds (Jacobsen et. al., 1983), the same effect is not consistently found among dicot species. Metivier and Paulilo (1980) found that GA_3 did not promote amylase or protease activity regardless of the presence of the embryonic axis, which is considered by some researchers the source of stimulation of hydrolytic enzyme activity (Jones and Stoddart, 1977) involved in germination. Other researchers (Bewley and Black, 1982 ; Evenson and Loy, 1978) have been unable to directly link GA stimulated enzyme activity with germination triggering mechanisms.

Besides possibly increasing enzyme activity, GA is also suspected of regulating respiration in germination. Jones and Stoddart (1977) cite studies which demonstrate GA control of the pentose phosphate (PP) pathway during germination. However, Bewley and Black (1982), in a more recent review, find evidence of GA control of the PP pathway unconvincing because of methods used to obtain the data.

At low temperatures Watkins, et. al. (1983), found that respiration of pepper seed was reduced, and increased oxygen concentrations did not increase either respiration or germination. Additionally, the authors found that applications of $GA_{4/7}$ increased germination at low temperatures regardless of oxygen concentrations, and respiration rates increased only after radicle emergence. The authors concluded that at suboptimal temperatures GA does not control germination by regulating respiration rates but by some other triggering mechanism yet to be identified.

There has been some question if GA actually is involved in the germination events or only after germination processes have started. Galli, et. al. (1975) found GA_3 promoted germination by cell elongation of Haplopappus gracilis as well as stimulated the resumption of DNA synthesis. Watkins and Cantliffe (1983) showed that AMO-1618, a GA synthesis inhibitor, delayed germination of pepper seed, and GA_3 overcame this delay. Coronel and Motes (1982) found that germination rates of pepper seeds treated with GA_3 were the same as those seeds receiving a 24 hour treatment of distilled water in an aerated column. Watkins and Cantliffe (1983) concluded that GA synthesis may not be a factor contributing to events associated with germination, but may be necessary for the completion of germination resulting in radicle extension.

CYTOKININS

Cytokinins have been widely implicated as a "permissive" hormone in germination events (Thomas, 1977; Bewley and Black, 1982). Where germination has been prevented by various inhibitors (chilling, high temperatures, ABA), it has been demonstrated that cytokinins can overcome this inhibition (Khan, 1971). Webb and Wareing (1972) demonstrated that kinetin applications overcame seed dormancy in Acer psuedoplatanus, whereas GA_3 had no effect breaking dormancy. Both kinetin and benzylaminopurine (BA) promoted germination of dormant peanut seeds as well as those inhibited by ABA (Ketring and Morgan, 1971; 1972). BA enhanced germination of developing sunflower seeds better than GA. However, ethrel was more effective in promoting the highest percent germination (Kummar and Krishna Sastry, 1974). Both BA and ethrel overcame ABA inhibition of germination. Germination of lettuce seed under high temperature conditions was promoted by both BA and

kinetin (Pietraface and Blaydes, 1981; Rao, et. al., 1975). Additionally, Rao, et. al. found synergism with kinetin and ethrel in overcoming ABA inhibition of germination. Combinations of BA and GA enhanced celery seed germination under high night temperatures. BA treatments alone enhanced germination but not as well as treatments with GA (Palevitch and Thomas, 1974).

Kinetin was not found to stimulate low temperature germination of tomato, pepper, or sugar beet seeds (Nelson and Sharples, 1980b). However, combination of $GA_{4/7}$, kinetin and ethrel did induce earlier low temperature germination of tomato seeds over the control by 35%. Similar results in a later study were found with cucurbit seeds (Nelson and Sharples, 1980a). Also, muskmelon seeds were not stimulated by any treatment to germinate as early as cucumber seeds. Lagarda and Martin (1983) found that 10 ppm ABA could inhibit germination of olive seed, and neither applications of $GA_{4/7}$ or BA could overcome inhibition regardless of ABA presence.

Although no biochemical mechanism for cytokinin action has been elucidated, a review by Thomas (1977) cites evidence that a cytokinin/inhibitor complex controls synthesis of proteins via control of mRNA. Studies show that cytokinin can overcome ABA induced inhibition of RNA polymerase whereas GA cannot. However, two reviews (Thomas, 1977; Bewley and Black, 1982) reported that some dormant (because of unfavorable environment or ABA applications) species of seeds will only germinate with combinations of cytokinins and GA. Treatments of GA or cytokinins alone were not as effective in breaking dormany.

Both Rao, et. al. (1975) and Klyacho, et. al. (1982) demonstrated that kinetin and BA enhanced polyribosome formation in lettuce seed and

pumpkin cotyledons. This observation led Klyachko et. al. to conclude that this event was the primary action initiating protein synthesis.

Among the proteins synthesized by the seed are enzymes such as α-amylase and protease, both of which breakdwon cotyledonary reserves for transport to the embryo where the products are used for growth. Gepstein and Ilan (1980) found that both kinetin and zeatin, and not GA or IAA, can replace the effect of the embryonic axis, thus inducing protease activity of bean cotyledons.

Conversely, watermelon cotyledons showed no response in proteolytic activity after BA treatments (Fantelli, et. al. 1983). However, the authors also noted that electron microscope photos showed a marked increase in the breakdown of protein bodies of BA treated cotyledons. They concluded that possibly the BA enhanced proteases which caused this breakdown were inaccessible with their detection techniques. On the other hand, increased amylolytic and proteolytic activities in bean cotyledons (either detached or attached to the embryo axis) were detected as a result of BA applications (Metivier and Paulilo, 1980). Additionally, the authors detected an amylase inhibitor in the axis which could explain why high BA concentrations did not increase amylase activity in embryonated cotyledons but did do so in detached cotyledons.

Another possible control on cytokinin activity is its conversion from an active form to an inactive form such as a ribonucleotide once inside the seed (Pietraface and Blaydes, 1981; Thomas, 1977). Pietraface and Blaydes found that nucleotide forms of BA increased in lettuce seed up to 2 hours after incubation and then decreased the next 6 hours.

During this same 6 hour period the active ribonucliotide form of BA increased. These shifts in metabolits coincide with the commencement of cell division during germination. However, Bewley and Black (1982) have noted that effects of cytokinins are observed several hours after incubation. Thus, no definitive relationship between action of ctyokinins and germination events has been established.

ETHYLENE

Ethylene, as well as cytokinins and gibberellins, have proven to have an equally important role in seed germination. Although ethylene is noted as being a growth inhibitor and ripening agent among other things, its role in seed germination is also substantial.

Ketring and Morgan (1971) showed enhanced germination of dormant peanut seeds with treatments of BA, kinetin, and 2-chloro-ethylphosphonic acid (CEPA, Ethrel, or Ethephon). This germination response to treatments was positively correlated to enhanced ethylene production in the seeds. Seeds showing little germination response had a correspondingly low ethylene production. CO_2 production was also stimulated by the treatments, but this only occurred after radicle and hypocotyl elongation. While ABA inhibited germination of peanut seeds, both kinetin and ethylene overcame this effect. The fact that kinetin could reserse the effects of ABA was correlated again with stimulated ethylene production (Ketring and Morgan, 1972).

GA applications which stimulated lettuce seed germination were also found to stimulate ethylene production in the germinating seeds. Using heat treatments to selectively inactivate internal synthesis of ethylene, Stewart and Freebairn (1969) found no germination, as well as no ethylene synthesis, regardless of GA application. However, when 100 ppm ethylene was

applied germination rates of 81 to 87% occurred despite heat treatments.

Even though ethylene and ethylene generating regulators can stimulate early germination of seeds, combinations of kinetin and ethrel were found to have an even greater effect on germination of lettuce seed at high temperatures (Rao, et. al. 1975). While ethrel alone could not overcome the inhibitory effects of ABA, kinetin and ethrel in the presence of ABA did improve germination of 32%.

Ethephon promoted low temperature germination of cucumber seeds only after fourteen days of incubation, but the final germianion was still higher than that of the control (Nelson and Sharples, 1980a). On the other hand, combinations of ethephone with $GA_{4/7}$ and kinetin promoted germination by the seventh day of incubation. By the fourteenth day, all of the combinations with ethephon had resulted in germation rates of 82 to 95%. Ethephon had no effect on early germination of muskmelons at low temperatures. Only ethephon in combination with $GA_{4/7}$ improved final germination to any degree.

Stimulation of early germination of tomato seeds by ethephon at low temperatures was variety dependent (Nelson and Sharples, 1980b). Where early germination had occurred within a variety, both ethephon and a combination of ethephone, kinetin, and $GA_{4/7}$ were equally effective. However, no differences between treatments and controls were detected by final germination counts. Applications of ethephon alone to pepper and sugar beet seeds at low temperatures had no effect on early or final germination percentages. Only kinetin, $GA_{4/7}$, and ethephone together improved germination to any extent.

The effects of ethylene at the biochemical level are still sketchy and incomplete. Ketring and Morgan (1972) have postulated a role for ethylene in an inhibitor/stimulatr complex in peanut seeds. They found that after-ripening influenced seed germination and ethylene production. As after-

ripening progressed, germination rates increased as well as ethylene production. BA was found to overcome ABA inhibition and promote CO_2 and ethylene evoluation. Furthermore, it was demonstrated that ethylene production was not a post dormancy event as was CO_2 evolution. Ethylene in concentrations of 8.5, μl/l to 50, μl/l overcame ABA inhibited germination. Seeds which lost dormancy naturally exhibited a concomitant rise in ethylene production up to an internal concentration of 0.3 to 0.2 μl/l after 48 hours incubation at which point germination occurred.

Where seeds whose germination is dependent on the action of phytochrome, ethylene has an inhibitory effect (Suzuki and Taylorson, 1981). Ethylene of 1, ul/liter inhibited germination of Potentilla norvegica until the gas was evacuated and the seeds were exposed to red light then germination occurred. Irradiations with far red light did not increase germination. This indicated to the authors that ethylene interferred with action of Pfr (far red phytochrome) which does promote germination.

Since activation of protein synthesis requires initiation of polyribosome formation, Rao, et. al. (1975) studied the effects of growth regulators on this formation. Ethrel and its combination with kinetin increased the number of polyribosomes (with ethrel and kinetin acting synergistically) of lettuce seed after 24 hours incubation. However, this phenomena could only be correlated with cotyledon expansion and not with germination rates which ranged from 96-98% between treatments. Ketring (1977), however, cites studies with peanut seeds which showed increases in RNA synthesis with ethylene stimulated germination of seeds treated with ABA. However, this response was not found later in the afterripening process.

As mentioned previously, ethylene did enhance CO_2 evoluation but this occurred only after radicle elongation. Thus breaking of dormancy or

stimulation of germination by ethylene was not related to its effect on respiration (Ketring, 1971). However, Ketring (1977) cited several studies demonstrating that ethylene increased hydrolysis of ATP implicating a role for ethylene which apparently provides greater amounts of energy needed to increase germination.

The action of ethylene on the activities of hydrolytic enzymes is contradictory; it sometimes promotes and other times inhibits activities of enzymes such as α-amylase (Ketring, 1977). Eastwell and Spencer (1982) showed that barley aleurone layers treated with ethylene and GA_3 initially increased amylase activity compared to GA_3 treatments alone. However, after 48 hours this activity decreased compared to GA_3 controls. At the same time proteolytic activity began to rise suggesting the breakdown of α-amylase Furthermore, protease inhibitors (preventing the breakdown of α-amylase) tended to cause an increase in α-amylase activity in aleurone layers subjected to ethylene. The authors concluded that protease activity may interfere with the observed effect of ethylene on α-amylase activity.

No correlation or investigation was made to relate ethylene applications to increases in protease activity. Hydrolytic activity occurs as a post germinative event; ethylene perhaps only serves to hasten germination processes.

MATERIALS AND METHODS: Seed Germination Studies

Cantaloupe seeds (cv. Mainstream) were soaked in beakers for 16 hours, at room temperature, in acetone containing one of the following treatments:

Growth Regulator/Hormone (mM)			
GA_3 [1]	$GA_{4/7}$ [2]	BA [3]	Ethephon [4]
1.0	1.0	.05	.30
0.5	0.5	.025	.15
0.1	0.1	.01	.075

Controls consisted of both acetone and water containing no regulators. Each petri dish contained 12 seeds. After 16 hours the seeds were dried for 24 hours. Then the seeds were placed in petri dishes containing filter paper moistened with distilled water. The treatments were duplicated so that one set was placed in a dark room at 23-24.4°C and the other set was placed in a dark room at 16°C. Germination, determined as radicals protruded, was counted daily up to 28 days (for cold temperature treatment).

Additionally, radical lengths were measured for 3 days after germination. The experiment was replicated three times. Data were analyzed by SAS computer analysis system which produced ANOVA tables and Duncan's multipe range test at the 5% level.

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[1] $Gibberellin_3$

[2] $Gibberellins_{4/7}$

[3] 6-benzyl aminopurine

[4] (2-chloroethyl) phosphonic acid

RESULTS AND DISCUSSION: Seed Germination Studies

Table 1 shows germination percentages (done on arcsin square root transformations) of seeds incubated at temperatures of 24°C. At high temperatures all treatments either did as well or worse than the water control in promoting either early or uniform germination. By day eight, when most of the seeds had germinated, no treatment had improved final germination compared to the water control. Since no significant differences were found between the acetone and water controls, and since all regulators were infused using acetone, the results of regulator treatments will be compared to the acetone controls. Although none of the treatments was significantly different from acetone by day 2 of incubation, treatments of $GA_{4/7}$ and ethephon tended to exhibit higher germination percentages than either BA or GA_3. Germination ranged from 83% to 96% for $GA_{4/7}$ treatments, and 90% to 99% for ethephon treatments. The two highest concentrations of BA (0.05 mM and 0.025 mM) and GA_3 (1.0 mM and 0.5) were similar in trends showing the lowest germination percentages ranging from 59% to 64% on day 2. By day 3, no significant differences were detected between treatments. However, it is interesting to note that by the third day all BA treatments had increased germination up to 93% to 100%. On the eigth day, only 1.0 mM GA_3 significantly inhibited germination compared to acetone. Germination of 1.0 mM GA_3 was 20% less than the acetone control.

Table 1. Effects of growth regulators at different concentrations on germination of muskmelon at 24°C.[1]

Hormone/ Regulator	Concentration (mM)	Germination (%) Days After Incubation 2	3	8
GA_3	1.0	61 c[2]	73 c	78 c
GA_3	0.5	62 c	74 bc	84 bc
GA_3	0.01	78 abc	87 abc	87 bc
$GA_{4/7}$	1.0	90 abc	92 abc	100 a
$GA_{4/7}$	0.5	96 ab	97 ab	97 ab
$GA_{4/7}$	0.01	83 abc	99 a	100 a
Ethephon	0.30	99 a	100 a	100 a
Ethephon	0.15	96 ab	96 abc	96 ab
Ethephon	0.075	90 abc	93 abc	98 ab
BA	0.05	59 c	94 abc	96 ab
BA	0.025	64 c	93 abc	93 abc
BA	0.01	67 bc	100 a	100 a
Acetone		78 abc	96 abc	98 ab
Water		99 a	100 a	100 a

[1]Each value represents means of 3 replications containing 12 seeds each.

[2]Mean separation by Duncan's Multiple range test at 5% level, done on arcsin square root transformations of data.

Although the acetone treatments were not significantly different from the water control, the fact that only 78% of seeds had germinated by day 2; indicates inhibition by this treatment. This may explain why the treatments of BA and GA_3 did not show uniform or higher germination by day 2; the acetone may interact with the regulators imposing inhibition. On the other had, GA_3 itself would have inhibited germination as indicated by the germination rates on day 8.

Table 2 presents the germination data for seeds incubated at 16°C. Germination was late (not starting until the second week) as well as very non-uniform. The data were summarized for two dates; 18 and 28 days the final day of germination counts. At the low temperatures, several differences in germination occurred due to treatment and type of regulator, as well as concentration . $GA_{4/7}$ at 0.05 mM promoted germination up to 91% by day 18, whereas only 6% of the acetone control were germinated by this time.

$GA_{4/7}$ at 1.0 and 0.1 mM and ethephon at 0.30 and 0.15 mM were similar in effect promoting germination ranging from 70 to 75%. The germination effects of $GA_{4/7}$ and ethephon at low temperatures are generally similar to their effects on seed germination at 24°C. The lowest concentration of ethephon and BA at 0.05 and 0.25 mM behaved similarly exhibiting fairly low germinations of 38%, 47% and 30%, respectively. There were no significant differences between the acetone control and any GA_3 treatment or BA at 0.01 mM. By day 28, the trends in treatment effects had changed

Table 2. Effects of growth regulators at different concentrations on germination of muskmelon at 16°C.[1]

Hormone/ Regulator	Concentration (mM)	Germination % Days After Incubation 18	28
GA_3	1.0	6 g[1]	8 f
GA_3	0.5	16 efg	33 de
GA_3	0.1	7 g	27 def
$GA_{4/7}$	1.0	75 ab	78 ab
$GA_{4/7}$	0.5	91 a	94 a
$GA_{4/7}$	0.1	75 ab	81 ab
Ethephon	.30	70 abc	84 ab
Ethephon	0.15	71 abc	89 a
Ethephon	0.075	38 de	53 cd
BA	0.05	47 cd	61 bc
BA	0.025	30 def	38 cd
BA	0.01	13 fg	27 def
Acetone		6 g	11 ef
Water		4 g	10 f

[1]Each value represents means of 3 replications containing 12 seeds each.

[2]Means separated by Duncan's Multiple Range test at 5% level, done on arsin square root transformations of data.

very little. All the $GA_{4/7}$ treatments and 0.30 and 0.15 mM ethephon had enhanced final germination (78 to 94%) compared to the control in which only 11% had germinated. Again, all the GA_3 treatments had not improved germination to any extent when compared to either the acetone or water controls. The BA (0.05 and 0.25 mM) treatments fared only slightly better than GA_3 but not as well as either the $GA_{4/7}$ or 0.30 and 0.15 mm ethephon treatments. The acetone treatments did not appear to inhibit low temperature germination as it appeared to do at the higher temperature.

As an estimate of seedling vigor, radicle lengths were determined daily up to 3 days after germination (Tables 3 and 4). Although many of the treatments promoted early germination at low temperatures, growth of the radicles was severely inhibited (Table 3), with no radicle elongating mroe than 30.0 mm. In some cases, radicles were distorted as well. Significant differences in growth were found only on the first day of measurement, and the water control showed at least inhibition with average radicle lengths of 0.198 cm. While none of the other treatments was signficantly different from each other, the highest concentration of $GA_{4/7}$ (1.0 mM) and ethephon (0.30 mM) and the acetone control did as well statistically as the water control. Radicle lengths were 0.173, 0.176, and 0.171 mM for 1.0 mM $GA_{4/7}$, 0.30 mM ethephon, and acetone respectively. It might be possible that acetone, as well as cold temperatures, inhibit radicle growth even though this is not reflected in the

Table 3. Effects of growth regulators at different concentrations on radicle length at 16°C.

Hormone/ Regulator	Concentration (mM)	Radicle Length (cm) Days After Germination 1	2	3
GA_3	1.0	0.152 b[2]	0.180	0.190
GA_3	0.5	0.159 b	0.178	0.189
GA_3	0.1	0.160 b	0.166	0.178
$GA_{4/7}$	1.0	0.173 ab	0.199	0.234
$GA_{4/7}$	0.5	0.161 b	0.192	0.212
$GA_{4/7}$	0.1	0.163 b	0.171	0.217
Ethephon	0.30	0.176 ab	0.194	0.227
Ethephon	0.15	0.164 b	0.184	0.205
Ethephon	0.075	0.163 b	0.177	0.198
BA	0.05	0.157 b	0.168	0.175
BA	0.025	0.160 b	0.178	0.201
BA	0.01	0.154 b	0.163	0.168
Acetone		0.171 ab	0.183	0.206
Water		0.198 a	0.238	0.289
			NS[3]	NS

[1]Each value represents mean of 3 replications containing 12 seeds each.

[2]Mean separation by Duncan's Multiple Range Test at 5% level.

[3]NS - means not significant from another based on F test.

statistical analysis. No significant differences in lengths were found between treatments by day 2 or 3.

On day 3, the least inhibition found among the treatments, excluding water, was with seeds treated with either $GA_{4/7}$ or ethephon. Treatments of either GA_3 or BA tended to inhibit radicle elongation to the greatest extent.

A toxic effect appeared to manifest itself in extreme cotyledon expansion and distortion of radicles. It was noted that when germination did occur, the seed coats were, in many cases, broken by expanding cotyledons rather than radicle elongation. Compared to the acetone or water control, the cotyledons of seeds treated with the highest concentration of any regulator tended to be extremely enlarged. Abnormal radicle growth, resulting in a twisted appearance, was identified primarily with treatments of 0.30 mM ethephon and 0.5 and 0.1 mM $GA_{4/7}$. This effect of twisting in the ethephon treatments usually decreased with decreasing concentration.

The effects of growth regulators on radicle elongation at 24°C (Table 4) are substantially different than at 16°C. By the first day after germination, radicle elongation was greatest with $GA_{4/7}$ treatments as well as the two controls. $GA_{4/7}$ at 0.5 mM exhibited the greatest growth, 2.13 cm, while the acetone controls had an average length of 1.8 cm. Radicle growth was inhibited by other treatments, particularly those including ethephon at all three concentrations, 0.25 and 0.5 mM BA, and 1.0 mM GA_3.

Table 4. Effects of growth regulators at various concentrations on radicle length at 24°C.[1]

Hormone/ Regulator	Concentration (mM)	Radicle Length (cm) Days After Germination 1	2	3
GA_3	1.0	1.24 f[2]	2.38 e	3.27 e
GA_3	0.5	1.74 cde	2.91 cd	3.64 de
GA_3	0.1	1.82 bcd	3.14 bc	3.88 cd
$GA_{4/7}$	1.0	2.01 abc	3.43 b	4.23 bc
$GA_{4/7}$	0.5	2.13 a	4.0 a	4.95 a
$GA_{4/7}$	0.1	2.05 ab	3.46 b	4.28 bc
Ethephon	0.30	1.21 f	1.58 g	1.74 i
Ethephon	0.15	1.24 f	1.74 fg	1.96 i
Ethephon	0.075	1.31 f	1.81 fg	2.07 hi
BA	0.05	1.50 ef	2.12 ef	2.49 gh
BA	0.025	1.48 ef	2.30 e	2.73 fg
BA	0.01	1.61 de	2.53 de	3.16 ef
Acetone		1.86 abcd	3.15 bc	3.91 bcd
Water		2.10 a	3.46 b	4.39 b

[1]Each value represents the means of 3 replications containing 12 seeds each.

[2]Mean separation by Duncan's test at 5% level.

The radicles of seeds receiving these treatments were, on the average, 71% as long as the radicles of the acetone control.

By the second day, 0.5 mM $GA_{4/7}$ had promoted elongation 27% over the control. The trends observed on the first day generally still persisted by the second day. Day 3 data show a clear trend of growth regulator and concentration effects on radicle growth. $GA_{4/7}$ at 0.5 mM still promoted the greatest radicle growth at 4.95 cm. The acetone control was similar in effect to 0.1 and 1.0 mM $GA_{4/7}$ with lengths ranging from 3.91 to 4.28 cm. All three ethephon treatments inhibited elongation more than any other treatment. The three growth regulators which inhibited growth can be ranked in order from greatest inhibition to the least: ethephon, BA, GA_3. With all three of these regulators, radicle inhibition was the greatest at the highest concentration and the effect decreased with decreasing concentration. For instance, radicle lengths for treatments of 1.0, 0.5, and 0.1 mM GA_3 were 3.83, 3.63, and 3.27 cm, respectively.

Although ethephon treatments did not inhibit germination, radicle growth was severely stunted, producing twisted radicles with poor root hair formation. Hypocotyls of seedlings were distored by severe thickening and stunting of growth. The effects of BA on radicles were similar to those of ethephon as well as producing abnormally shaped cotyledons. Typically, the cotyledons of BA treated seeds had curled edges. These abnormal effects on the seedlings were not observed when GA_3 treatments were used. However, $GA_{4/7}$ tended to produced seedlings with straight hypocotyls, while the controls typically had crooked hypocotyls.

DISCUSSION

At temperatures of 24℃ no treatment promoted early germination compared to the control. Treatments of $GA_{4/7}$ or ethephon promoted germination similar to the control, while GA_3 and BA appeared to inhibit early germination. By the day of final germination counts, 1.0 mM GA_3 still inhibited germination while no differences were found between the other treatments. Although the acetone treatment was not significantly different from water alone, after 2 days of incubation germination was lower than that of seeds treated with water. It could be possible that acetone, used as the infusing agent, induced inhibition when combined with the regulator treatments. Perhaps acetone actually desiccated the seeds since they were dried for 24 hours following treatment before being placed in petri dishes. By the third day though, acetone treated seeds apparently overcame this inhibiting effect since the difference between them and the control was only 4%. Simultaneously, BA treatments also had increased germination to within 7% of the control by day 3.

Despite the fact that ethephon did not inhibit germination, it apparently produced a toxic effect to the extent that radicle growth was severely limited with this treatment. Perhaps ethephon promoted cell division, but not elongation, as apparently was the case in $GA_{4/7}$ treatments where both radicle elongation as well as uninhibited germination occurred.

At temperatures of 16°C, 1.0, 0.5, and 0.1 mM $GA_{4/7}$ and 0.30 and 0.15 mM ethephon substantially promoted early germination after two weeks of incubation. This trend continued on through the final day of germination counts.

For the most part, despite treatment, germination was atypical in that the cotyledons expanded to burst the seed coat and not the radicle. Nelson and Sharples (1980a and 1980b) also noted 5-10% atypical germination (cotyledons emerging first) with GA_3 and $GA_{4/7}$ only. No other mention of treatment side effects was made in any other study. Regardless of treatment, radicle elongation was severely inhibited by the lower temperature. Growth regulator treatments tended to enhance this inhibition somewhat since the water controls promoted the greatest elongation by the first day after germination. Yet, this inhibition was slight as there were no differences in radicle growth by day 2 or 3. At 16°C, (Nelson and Sharples (1980a) observed acetone inhibition of muskmelon germination to an extent even greater than that of this study. However, enhanced germination was noted when acetone was applied to cucumber seed. Seeds of pepper, tomato, and sugar beet treated with acetone did not exhibit any germination inhibition (Nelson and Sharples, 1980b) at low temperatures.

$GA_{4/7}$ has been shown to stimulate early, low temperature germination in other studies as well as this study (Nelson & Sharples, 1980a and 1980B; Watkins and Cantliffe, 1983). Additionally, in agreement with this study, $GA_{4/7}$ treatments still resulted in higher germination by the final day of germination counts for both muskmelon and cucumber seeds (Nelson and Sharples, 1980a). On the other hand, no differences in total germination were found between $GA_{4/7}$ treatments and the control for either pepper, tomato, or sugar beet seeds (Nelson & Sharples, 1980 b; Watkins and Cantliffe, 1983).

While Nelson and Sharples (1980a) did not observe that ethephon stimulated early or more germination of muskmelon seed, they did observe this with cucumber seeds using concentrations greater than those of this study. The

authors also found that tomato seeds at low temperatures were stimulated by ethephon to germinate, but pepper and sugar beet seed germination were no different than the controls (Nelson and Sharples, 1980b).

The results of this study agreed with Nelson and Sharples (1980a and 1980b) in that neither cytokinins or GA_3 promoted early low temperature germination of warm season crops. However, Watkins and Cantliffe (1983) observed early germination of pepper seeds with GA_3 concentrations as high as 50 to 1000 pppm. But no differences were detected in the total germination counts.

At low temperatures, $GA_{4/7}$ and ethephon apparently play an important role in hastening some portion of the germinative process. It is unknown, though, whether this effect is pre-germinative or post-germinative in nature. For example, inhibition of water may stimulate the germinative process and the presence of a regulator such as ethephon or $GA_{4/7}$ simply aids to hasten the process. Watkins and Cantliffe (1983) showed that AMO-1618, an inhibitor of GA synthesis, did delay germination but did not stop the process (addition of GA_3 overcame this delay, though). This indicated to the authors that GA may not be necessary for initiation of germination but was needed in later processes to allow for radicle protrusion.

The present study indicates that despite the promotive effects of $GA_{4/7}$ and, to some extent, ethephon on low temperature germination, these treatments could still not overcome an unknown mechanism of low temperature inhibition to allow for normal radicle growth. No such radicle inhibition was apparent at high temperatures for treatments of $GA_{4/7}$ or controls.

CONCLUSION

The results of this study suggest that no treatment applied at low temperatures to promote early germination can be recommended. While all three concentrations of $GA_{4/7}$ and 0.30 mM and 0.15 mM ethephon did enhance germination, growth of the seedling was abnormal either due to cold temperature inhibition or toxicity induced by the regulator itself. Toxicity was indicated by abnormal seedling growth at 24°C in response to applications of ethephon, BA, or GA_3.

Although acetone inhibition was insignificant at either 16°C or 24°C, it is unknown if this solvent could have interacted with the regulators (ethephon, BA, and GA_3) to inhibit both germination and radicle elongation observed at 24°C.

LITERATURE REVIEW: Hormone Applications to Plants

Plant Response to ABA

Abscisic acid (ABA) is present throughout the plant and has numerous physiological effects on growth. It is associated with dormancy induction, promotion of senescence, and appears to interact with growth promotive hormones to inhibit growth (Weaver, 1972). Additionally, during periods of water stress endogenous ABA levels increase in the plant thus promoting stomatal closure and reducing transpiration (Moore, 1979).

Reports of the effects of root applied ABA on shoot growth are relatively limited. Most studies of root applied ABA have emphasized root growth responses. Hydroponically grown cauliflower plants which were root treated with ABA increased in root to shoot dry weight ratios (Watts, et. al., 1981; Biddington, 1982). Also, root branching was increased as well as root hair number and length. Root extension was variable between experiments (Biddington, 1982). Lateral root formation and root elongation were stimulated in tomato and peas, respectively (Torrey, 1976).

Applications have also inhibited growth of either excised or intact roots. Philipson and Couts (1979) found that not only did ABA inhibit root growth, but also induced dormancy of Sitka spruce. Several examples of growth inhibition are cited by Torrey (1976).

The inhibitory effects of ABA on leaf growth are well known. Injections of ABA at the shoot base of Triticum aestivum reduced leaf area (by both cell division and elongation), total number of epidermal cells, and number of stomata Quarrie and Jones, 1977). Treated plants also produced fewer spikelets per head than controls.

The effect of ABA, regardless of method of application, on stomatal resistance and transpiration is well documented. Applications of ABA in irrigation water reduced transpiration three to ten minutes after application (Zeevaart, 1979). This response was readily reversible. Injections of ABA into Triticum aestivum increased and maintained stomatal resistance for three days (Quarrie and Jones, 1977). Sprayed applications of ABA also had similar results (Kirkham, 1983).

The mechanism of action of ABA is presently unknown. However, some biochemical effects of ABA have been elicited. One such effect is the alteration of the plasma membrane of cells, which affects stomatal behavior. A pH gradient change apparently allows the diffusion of ABA out of the chloroplast, a major storage organ of ABA, into the cytoplasm where it can act on the membranes of guard cells (Hartung, et. al. 1983). The alteration of the plasma membrane allows the extrusion of potassium ions and a subsequent loss of cell turgor occurs (Moore, 1979). In addition, an ABA carrier, driven by a pH gradient, has been detected in the elongating portion of roots (Rubery and Astle, 1983).

Another biochemical effect of ABA is its inhibition of RNA and protein synthesis. ABA antagonizes growth promoting hormones, as is also the case in the inhibition by ABA of GA induced enzyme synthesis in the aluerone layer of barley (Moore, 1979).

Plant Response to Cytokinins

Cytokinins, largely produced in root tips of plants (Torrey, 1976), are generally regarded as growth promoters, stimulating cell division. However, like other plant hormones, cytokinins induce a variety of responses including delaying senscence, promoting lateral bud development, increased

leaf expansion, and chloroplast development (Salisbury and Ross, 1978; Sharif and Dale, 1980; Fasulo, 1980).

Kinetin applications in hydroponic solutions and root dipping in treated hydrogels produced an increase in photosynthetic rates, relative growth rate, total leaf area, and total dry weight of tomato plants (Arteca, 1982 and Dong and Arteca, 1982). Dong and Arteca (1982) also found a significant decrease in leaf resistance due to kinetin treatments. Additionally, photosynthetic rates increased with increased treatment time, but this effect became reduced over a period of eight days to where treatments differed very little from the control. Continuous application of kinetin actually reduced photosynthetic rates (Dong and Arteca, 1982). Similarly, tiller bud weights of barley were increased by application of cytokinins (Sharif and Dale, 1980). Associating cytokinin supply with nutrient supply, the authors found that bud weights were increased even further with combined cytokinin and nutrient treatments.

Both root and shoot apex application of 6-benzladenine (BA) to watermelon seedlings induced hypocotyl elongation as well as increased cell size but not cell number (Loy, 1980). Fresh and dry weight of the hypocotyls and cotyledons also increased due to BA treatments, whereas root weights were decreased. Root weight reduction due to cytokinin application have been noted in other studies also (Stenlid, 1982; Sharif and Dale, 1980). Similar to other studies mentioned, most effects were strongest during the first 48-60 hours after treatment.

The precise mechanism of action of cytokinins is currently unknown. However, the fact that cytokinins occur in RNA and affect protein synthesis leads one to suspect translational control (Moore, 1979; Galston et. al., 1980). Yet the role of free cytokinins in plant growth cannot

be overlooked. No strong evidence shows that exogenously applied hormones are always incorporated into RNA (Galston, et. al. 1980; Stoddart and Wareing, 1970; Gwodz and Wozny, 1983).

Kinetin increased the activity of ribulose-1, 5-diphosphate carboxylase (RuDPC) in the leaves of Phaseolus vulgaris, but the synthesis of plastic ribosomal RNA or protein I fraction were not increased (Stoddart and Wareing, 1970). Likewise, BA applications have increased the activity of RuDPC and tRNA synthetases in the plastids of Curcurbita pepo cotyledons (Klyachko and Parthier, 1980).

In review cytokinin activity, evidence of ribosomal and protein interactions with cytokinins has been found (Moore, 1979). Klyachko and Parthier (1980) found that in Curcurbita cotyledons kinetin can counteract the effect of myomycin, which inhibits plastid development by preventing protein synthesis in the 70s ribosomes. Kinetin treated cucumber cotyledons showed an increase in polyribosome formation. Transcription inhibitors did not affect kinetin promoted polyribosome formation. However, aurin tricarboxylic acid (ATA), an inhibitor of protein-synthesis initiation which blocks ribosomal binding to mRNA, also reduced the effect of kinetin on polyribosome formation (Gwozdz and Wozny, 1983).

Reddy, et. al. (1982) reported a protein in barley embryos that binds with various cytokinins. However, a direct association of a cytokinin binding protein with plant growth respons has not yet been shown.

Plant Responses to Gibberellins

Gibberllins (GA) are regarded as growth promoters stimulating both cell division and elongation. They are produced in the roots as well as in other plant organs. The inhibition of shoot gorwth and reduced active GA content has been shown to coincide with the removal of root tips (Torrey,

1976). Also, the root application of (2-chloroethyl) trimethyl ammonium chloride (CCC), a possible inhibitor of GA synthesis, tends to reduce GA content in the xylem sap (Torrey, 1976).

The use of GA is widespread in horticultural science including: breaking of seed dormancy, promoting flowering, size control of Thompson seedless grapes, and delaying senes-ence in citrus fruit (Salisbury and Ross, 1978). While foliar applications of GA are quite prevalent, root-application studies are rather limited. Bare root applications of GA (50-250 ppm) to roots of transplanted Solanum melongena produced increased plant height and leaf number (Patil, et. al., 1981). Additionally, GA treatments induced earlier flowering as well as increased the final yields. The authors attribute the yield increase to an increase in photosynthetic area.

At more physiologically relevant levels (0.014-1.4 μM), hydroponic applications of GA_3 increased photosynthetic rates of tomato plants (Arteca and Dong, 1981). In short dip studies (1 to 12 hrs.) photosynthetic rates increased 25%-35% in the first 24 hours after treatment. However, after nine days, this effect became reduced to where no difference could be detected between treatments and control. Low light intensities also enhanced the effect of GA, which increased photosynthetic rates by 90%. GA treatments under high light intensities only increased photosynthesis by 40$. Since photosynthetic rates increased within two hours after treatment, the authors concluded that cell elongation, which would require more time to respond to GA, was not the only factor contributing to increased photosynthesis (Arteca and Dong, 1981).

In a later paper (Dong and Arteca, 1982), GA treatments were found also to increase relative growth rate and total dry weight. However, GA_3

had no effect on leaf resistance. Conversely, Arteca (1982) found that GA applied in a starch polymer acrylate gel to the roots of tomato transplants had no effect on relative growth weight, total leaf area, or dry weight after three weeks of growth.

Salisbury and Ross (1978) have outlined three different phenomena occurring together which result in a plant's response to gibberellins. First, gibberellins can promote cell division in the shoot apex. Essential to cell division is RNA synthesis, of which two facets have been investigated by Tomi, et. al. (1983a; 1983b). The authors observed that the template activity of chromatin of GA_3 treated pea seedlings was increased 1.5 times compared to the control. Additionally, the amounts of RNA polymerase, introdued in vitro, attaching to the chromatin was increased by GA_3. They concluded that GA_3 treatments increase the number of initiation sites available for transcription as well as enhancing enzyme attachment to chromatin (Tomi, et. al., 1983a). Further studies (Tomie, et. al., 1983b) showed that GA_3 also enhanced the activities of RNA polymerase I and II.

A second event associated with responses to gibberellins is the hydrolysis of starch and other reserve carbohydrates into glucose and fructose (Salisbury and Ross, 1978). Cell growth is stimulated by respiration of hexoses providing energy for growth processes. However, an equally important effect of GA-induced hydrolysis is to decrease the water potential in the cell, thereby allowing water movement into the cell resulting in increased size. A review by Jones (1983) pointed out that this phenomenon was evident for cucumber hypocotyls, but not for lettuce hypocotyls. In the case of lettuce hypocotyls, GA induced cell elongation by altering the yielding properties of the cell wall.

This cell wall alteration is the third event which occurs in response to GA treatment, as pointed out by Salisbury and Ross (1978). No mechanism has been elucidated as to how GA promotes cell wall extension. Jones (1983) points out studies which indicate that cell growth due to GA treatment is in response to synthesis of new cell wall material. Studies conducted by Jones (1983) indicate that calcium ions may regulate cell wall extension during GA treatments. Movement of calcium ions into the protoplast would reduce calcium ion concentration in the cell wall, thus enhancing the loosening of the cell wall.

Arteca and Dong (1981) cite studies which show that photosynthetic rates, increased by GA_3 treatments, were soley due to an elongation response of the cell. However, in their own studies, since photosynthesis of tomato leaves increased within two hours after treatment, the authors concluded that cell elongation would take too long to account for these increases. Stoddart and Wareing (1970) showed that GA_3 treatments increased the activity of ribulose-1, 5-diphosphate carboxylase, the enzyme responsible for catalyzing the reduction of CO_2 in the dark cycle of photosynthesis.

METHODS AND MATERIALS: Root Dip Studies

Muskmelon seeds (cv. Mainstream) were planted in moistened "Jiffy-7" peat pellets, allowed to germinate and grow in a growth chamber (32°C, 16 hr. day) for 9-10 days. Upon the expansion of the first true leaf, the root ball of each seedling was given one of the following treatments:

ABA (M)[1]	GA_3 (ppm)[2]	BA (ppm)[3]
10^{-4}	5	5×10^{-3}
10^{-5}	50	5×10^{-4}
10^{-6}	500	5×10^{-5}

Furthermore, two methods of application were used. The first method involved application of hormones in distilled water. The second method was application of hormones in the starch polymer acrylate gel, Terra-Sorb[4]. Control plants received either a gel or water treatment with no hormones.

The plants were treated for six hours in the growth chamber and transplanted to 15.2 cm clay pots containing 'Jiffy-Mix'. The transplants then received no water for 24 hours.

The plants were grown for three weeks under greenhouse conditions (fan and pad cooled). Each treatment was repeated three times in the experiment. The experiment was repeated three times so time was considered a blocking factor.

[1]Abscisic acid

[2]Gibberellin$_3$

[3]6-benzylaminopurine

[4]Terra-sorb is the registered trademark of a gelatinized starch-hydrolyzed polyacrylonitrile graft copolymer using potassium hydroxide distributed by Industrial Services International, Inc., Bradenton, FL.

One experiment (or block) was planted at a time at approximately ten day intervals. The studies lasted from August 12 to September 17, 1983.

Measurements were taken 2, 4, 7, 14, and 21 days after treatment. Variables measured included total leaf area, number of leaves, and total stem length. Total leaf area was determined by measuring the length and width of each leaf on days 2-14. On day 21 total leaf area was determined with a 'Li-Cor' leaf area meter (model 3100). Total stem length was determined with a caliper ruler.

On day 21 the plants were harvested and dried in an oven at 100°C for 24 hours for determination of final dry weight. Data were analyzed using SAS to generate an analysis of variance table. Treatment means were separated by Duncan's Multiple Range Test at the 5% level.

RESULTS - Root Dip Experiments

Treatments applied in water were analyzed separately and are consequently discussed separately. Upon studying interactions, it was determined that treatments applied with gels elicited interactions over time (that is, treatments behaved differently between blocks). Treatments applied with water did not show similar interactions. No other significant interactions were detected. F tests for the following interactions were conducted: Gel and no-gel; gel, no-gel, and treatment (Hormone and Concentration); treatment and block; gel, no-gel, and block.

Root Dip Experiment - Water Applications

Four days after treatment, 10^{-4} and 10^{-5} M ABA treatments significantly inhibited production of new leaves by 40%, when compared to the control (Table 5). With the exception of 10^{-4} and 10^{-5} M ABA, no other treatments were different than the control on day 4 and this same trend continued through the seventh day. However, after a week, the ABA-induced inhibition became reduced to only 27% and 11% of the control for 10^{-4} and 10^{-5} M, respectively. In fact, by day 14 no significant differences were observed between any treatment. Yet by day 21, a promotive effect was detected in plants receiving treatments of 500 ppm GA_3, 5 x 10^{-5} ppm BA, and 10^{-6} M ABA. All three of these treatments had approximately 8% more leaves than the control.

Total leaf area is presented in Table 6. By day 2, 500 ppm GA_3 promoted the greatest increase in leaf area and was 35% greater than the control. The three concentrations of BA, 5 x 10^{-3}, 5 x 10^{-4}, and 5 x 10^{-5} ppm all did as well as 500 ppm GA_3, but they were not statistically different from the control of 14.70 cm^2. ABA at 10^{-4} M on day 2 inhibited increases of leaf area being only 20% of the control. Lower concentrations of either ABA or GA_3 also were not statistically different from the control.

With the exception of 10^{-4} M ABA, none of the other treatments was different from the control on days 4-14. No differences between treatments were detected by day 21. Strong inhibition of leaf growth (in area) was still observed with 10^{-4}M ABA, with leaves having an area 36%, 29% and 21% of the control on days 4, 14, and 21, respectively.

After day 4, the leaf area of the 10^{-4} M ABA treatment began to decrease steadily until there were no differences between it and the control.

Table 5. Total number of leaves per plant as influenced by growth regulators at various concentrations.

Growth Regulator (applied in water)	Concentrations (ppm)	Days After Transplanting 4	7	14	21
		Number of Leaves Per Plant[1]			
		[2]			
GA_3	5	1.56 a	2.89 a	8.56 ab	17.2 bc
	50	1.56 a	3.0 a	9.11 a	18.8 ab
	500	1.60 a	3.0 a	9.30 a	19.2 a
BA	5×10^{-3}	1.56 a	3.0 a	9.22 a	18.7 ab
	5×10^{-4}	1.78 a	3.0 a	9.22 a	17.7 abc
	5×10^{-5}	1.75 a	3.0 a	9.37 a	19.1 a
ABA	10^{-4} M	1.0 b	2.11 c	7.89 b	16.6 c
	10^{-5} M	1.0 b	2.56 b	8.44 ab	17.8 abc
	10^{-6} M	1.44 a	2.89 a	9.11 a	19.1 a
Control	Water	1.67 a	2.89 a	8.67 ab	17.7 bc

[1] Treatment values are the average of 9 plants over 3 replications.

[2] Means separated by Duncans Multiple Range test at 5% level.

Table 6. Total leaf area per plant as influenced by growth regulators at various concentrations.

Growth Regulator (applied in water)	Concentration (ppm)	Days After Transplanting 2	4	7	14	21
		Total Leaf Area Per Plant (cm^2)[1]				
		[2]				
GA_3	5	15.84 b	39.00 a	130.17 ab	799.11 a	1312.8
	50	15.79 b	40.85 a	138.17 a	887.78 a	1445.1
	500	19.81 a	46.25 a	142.00 a	827.00 a	1355.2
BA	5×10^{-3}	17.14 ab	44.44 a	147.14 a	896.00 a	1422.6
	5×10^{-4}	17.41 ab	43.22 a	137.64 a	856.22 a	1301.3
	5×10^{-5}	16.65 ab	43.66 a	140.51 a	872.75 a	1594.1
ABA	10^{-4} M	11.77 c	24.80 c	95.51 c	678.78 b	1267.4
	10^{-5} M	14.86 b	30.27 bc	119.04 b	781.00 a	1373.8
	10^{-6} M	16.50 b	40.53 a	129.28 ab	840. 11a	1522.4
Control	Water	14.70 b	38.56 ab	134.98 ab	863.67 a	1409.6
						NS[3]

[1] Treatment values are the average of 9 plants over 3 replications.

[2] Means separated by Duncan's Multiple Range Test at 5% level.

[3] Means are not significant at the 5% level.

A similar trend was also observed with the 500 ppm GA_3 treatment. However, the greatest difference from the control occurred on day 2 with 500 ppm GA_3, with which leaves had a 35% greater leaf area. This difference decreased to 20% and 5% by days 4 and 7, respectively, but these differences were not significant. After day 7, the control actually exhibited greater leaf area than 500 ppm GA_3.

Table 7 presents data of total stem length of plants receiving growth regulator treatments in water. As with the results on leaf area, 500 ppm GA_3 promoted the greatest stem elongation while 10^{-4} ABA inhibited it to the greatest extent. GA_3 at 500 ppm promoted stem elongation 22%, 17% and 12% greater than the control on days 2, 4, and 7, respectively. All of the other GA_3, as well as the BA treatments, were not significantly different from either 500 ppm GA_3 or the control. By the fourteenth day, 500 ppm GA_3 no longer promoted stem elongation greater than the control.

At 10^{-4} M, ABA inhibited elongation by 17%, 40% and 29%, of the control on days 2, 4, and 7, respectively. Other ABA treatments of 10^{-5}M or 10^{-6}M were not different from the control.

Again, similar to trends observed with the leaf area measurements, differences between the control and both 500 ppm GA_3 and 104^{-4}M ABA tended to decrease over time. However, this inhibitory effect of 10^{-4}M ABA was not fully manifested until the fourth day while the effects of 500 ppm GA_3 were greatest on day 2. No significant differences between the control and other treatments were observed by day 14, and by day 21 no significant differences were detected between any treatments.

Table 7. Total stem length per plant as influenced by growth regulators at various concentrations.

Growth Regulator (applied in water)	Concentration (ppm)	Days After Transplanting				
		2	4	7	14	21
		Total Stem Length Per Plant (cm)[1]				
		[2]				
GA_3	5	1.81 ab	4.33 ab	9.87 abc	61.00 ab	171.8
	50	1.76 ab	4.31 ab	9.99 ab	68.97 a	215.2
	500	2.02 a	4.85 a	10.72 a	65.11 ab	224.2
BA	5×10^{-3}	1.84 ab	4.51 ab	10.51 ab	69.49 a	185.2
	5×10^{-4}	1.87 ab	4.34 ab	9.99 ab	69.00 a	186.3
	5×10^{-5}	1.81 ab	4.08 b	10.11 ab	69.69 a	218.6
ABA	10^{-4} M	1.38 d	2.47 c	6.79 d	56.02 b	187.6
	10^{-5} M	1.49 cd	4.07 b	8.93 c	61.07 ab	200.5
	10^{-6} M	1.82 ab	4.23 b	9.80 abc	69.33 a	231.0
Control	Water	1.66 bc	4.13 b	9.58 bc	65.44 ab	209.1
						NS[3]

[1]Treatment values are the average of 9 plants over 3 replications.

[2]Means separated by Duncan's Multiple Range Test at 5% level.

[3]Means are not significant at the 5% level.

Table 8. Total dry weight per plant, 3 weeks after transplanting, as influenced by growth regulators at various concentrations.

Growth Regulator (applied in water)	Concentration (ppm)	Dry Weight[1] (g)
		[2]
GA_3	5	6.19 a
	50	6.49 a
	500	6.36 a
BA	5×10^{-3}	7.02 a
	5×10^{-4}	6.17 a
	5×10^{-5}	6.81 a
ABA	10^{-4} M	5.12 b
	10^{-5} M	6.16 a
	10^{-6} M	6.54 a
Control	Water	6.54 a

[1] Treatment values are the average of 9 plants over 3 replications.

[2] Means separated by Duncan's Multiple Range Test at 5% level.

At the end of 21 days of growth, aerial portions of the plants were harvested for dry weight (Table 8). Only 10^{-4}M ABA was significantly different having a final dry weight of 5.12 g while the control weight was 6.54 g.

Root Dip Experiment - Gel Applications

Results of treatments applied with Terra-Sorb are presented in Tables 9-12. Significant differences between numbers of leaves (Table 9) for treatments were not detected until a week after treatment. By this time, only 10^{-4}M ABA differed from the control which, on the average, had one more leaf than 10^{-4}M ABA. By day 14, 10^{-4}M ABA treatments still inhibited leaf production when compared to the other treatments but not with the control; they tended to produce more leaves than the control. For example, on day 14, both 5 and 500 ppm GA_3 produced, on the average, one more leaf than the control.

While no significant differences were present on either days 4 or 21, analysis of variance procedures indicated that an interaction between block and treatment existed. This would indicate that the treatments were not consistent from one time (first block) to the next (second block). For instance, in block one, 10^{-5}M ABA had one leaf on day 4 while the control produced 1.3 leaves on the average. However, in the third block 10^{-5}M ABA, as well as the control, both had two leaves on day 4. On day 21, as another example, 10^{-5}M ABA produced 20 leaves in the second block while the control produced 17-18 leaves. On the other hand, in the third block, 10^{-5}M ABA produced only 16 leaves while the control produced 19 leaves.

Table 9. Total number of leaves per plant as influenced by growth regulators at various concentration.

Growth Regulator (applied in gel)	Concentration (ppm)	Days After Transplanting 4	7	14	21
		Number of Leaves Per Plant[1]			
			[2]		
GA_3	5	1.6	2.9 a	9.3 a	19.1
	50	1.3	2.7 a	8.4 a	17.7
	500	1.4	3.0 a	9.3 a	19.0
BA	5×10^{-3}	1.7	2.9 a	8.8 a	18.7
	5×10^{-4}	1.8	2.9 a	9.1 a	18.6
	5×10^{-5}	1.6	2.8 a	9.0 a	18.8
ABA	10^{-4} M	1.1	2.0 b	7.4 b	17.0
	10^{-5} M	1.4	2.7 a	9.0 a	18.1
	10^{-6} M	1.7	2.8 a	8.7 a	17.9
Control	Gel	1.4	2.9 a	8.3 ab	18.2
		NS[3]			NS

[1]Treatment values are the average of 9 plants over 3 replications.

[2]Means separated by Duncan's Multiple Range Test at 5% level.

[3]Means are not significant at the 5% level.

No pattern in treatment responses was apparent when the interactions were studied. This inconsistency most likely reflects the random manner with which the treated hydrogel contacted the root ball. Compared to dipping the root ball in water containing the treatment, dipping in hydrogel was less uniform.

Table 10 summarizes the total leaf area of the treatments applied with Terra-Sorb. The results are similar to those of treatments applied in water. GA_3 at 500 ppm and 10^{-5} M ABA showed the greatest increase in total area by the second day after treatment. Total leaf area per plant was 30% greater than the control for both these treatments. No other treatment was significantly different than the control on the second day. However, on the fourth day after treatment, 10^{-4} M ABA began to strongly inhibit total leaf area, which was 30% less than the control. Also, by the fourth day, 500 ppm GA_3 and 10^{-5} M ABA were no longer different than the control. After a week, the inhibitory effect of 10^{-4} M ABA still persisted, but the difference in total leaf area between it and the control decreased. Leaf area of 10^{-4} M ABA was 26% less than the control.

After two weeks, no significant differences were observed between the control and any of the treatments. By the third week no significant differences were detected at all. The lack of treatment differences after two to three weeks reflects the trend of decreasing effect, which the treatments had on the plants when compared ot the control. For example, 500 ppm GA_3 increased total leaf area over the control by 30%. However, this difference decreased progressively to 23%, 13%, 8% greater than the control on days 4, 7, and 14, respectively. Three weeks after treatment and with 500

Table 10. Total leaf area per plant as influenced by growth regulators at various concentrations.

Growth Regulator (applied in gel)	Concentration (ppm)	Days After Transplanting									
		2		4		7		14		21	
		Total Leaf Area Per Plant (cm^2)[1]									
		[2]									
GA_3	5	15.2	ab	39	ab	135	ab	850	a	1465	
	50	13.6	bc	33.5	bc	117	b	756	ab	1262	
	500	18.0	a	44.5	a	146	a	865	a	1392	
BA	5×10^{-3}	15.9	ab	41.2	ab	138	a	846	a	1347	
	5×10^{-4}	15.1	ab	42.9	ab	143	a	882	a	1392	
	5×10^{-5}	16.8	ab	39.4	ab	139	a	860	a	1398	
ABA	10^{-4} M	11.6	c	25.6	ab	95	c	692	b	1305	
	10^{-5} M	18.2	a	42.2	c	140	a	840	a	1394	
	10^{-6} M	17.1	ab	43.5	ab	140	a	870	a	1351	
Control	Gel	13.8	bc	36.1	ab	129	ab	804	ab	1448	
										NS[3]	

[1]Treatment values are the average of 9 plants over 3 replications.

[2]Means separated by Duncan's Multiple Range Test at the 5% level.

[3]Means are not significant at the 5% level.

ppm GA_3 total leaf area was 4% less than that of the control. ABA at 10^{-4} M showed similar trends, but its inhibition was greatest four days after treatment.

Interaction between block and treatment was significant for days 4, 14, and 21. For day 4, ABA and GA_3 interactions did not appear to be great. However, BA treatments showed little to no patter in response. Interactions on day 14 generally occurred in the third block for ABA, BA, and GA_3 treatments. On day 21, BA treatments showed the greatest interactions across all blocks. ABA and GA interactions were not as great and showed that 10^{-5} M ABA and 5 ppm GA did not cause any amount of interaction.

Total stem length per plant is presented in Table 11. No differences between control and treatments were detected until the fourth day after treatment. By this time, 500 ppm GA_3 increased stem lengths to 19% greater than the control, while 10^{-4} M ABA inhibited stem length by 24% less than the control. The same trend continued through the seventh day. Once again, two weeks after treatment, no significant differences were observed between the control and any of the treatments. Three weeks after treatment, like other variables measured, no significant differences were detected between any treatment means.

The same decreasing trend over time in differences observed in total leaf area between control and 500 ppm GA_3 and 10^{-4} M ABA was also detected in total stem length measurements. For 500 ppm GA the difference in total stem length from the control decreased gradually until its system length was 8% less than that of the control after 3 weeks. ABA at 10^{-4} had only 3% less total stem length than the control by the third week.

Significant interaction between treatment and block was only observed

Table 11. Total stem length per plant as influenced by growth regulators at various concentrations.

Growth Regulator (applied in gel)	Concentration (ppm)	Days After Transplanting 2		4		7		14		21
		Total Stem Length Per Plant (cm)[1]								
		[2]								
GA_3	5	1.7	ab	4.1	ab	10.1	ab	68.7	a	224
	50	1.6	bc	3.8	b	9.3	b	56.3	bc	180
	500	1.9	a	4.7	a	10.7	a	70.2	a	196
BA	5×10^{-3}	1.8	ab	4.5	ab	9.9	ab	67.2	ab	188
	5×10^{-4}	1.7	ab	4.1	ab	9.9	ab	70.1	a	198
	5×10^{-5}	1.8	ab	4.3	ab	9.9	ab	69.2	a	199
ABA	10^{-4} M	1.4	c	3.0	c	7.2	c	55.0	c	207
	10^{-5} M	1.8	ab	4.2	ab	10.3	ab	66.6	ab	198
	10^{-6} M	1.7	ab	4.4	ab	9.8	ab	67.2	ab	196
Control	Gel	1.6	abc	3.9	b	9.3	b	59.9	abc	213
										NS[3]

[1] Treatment values are the average of 9 plants over 3 replications.

[2] Means separated by Duncan's Multiple Range Test at the 5% level.

[3] Means are not significant at the 5% level.

on day 21, when no significant differences were detected between means. BA generally showed the greatest interaction across all blocks. The 10^{-5} M ABA and all GA_3 treatments appeared to show interactions in the third block.

No significant differences in total dry weight were found between treatments (Table 12) at the conclusion of the study. Again, interactions were detected between treatment and block. The 10^{-5} and 10^{-6} M ABA treatments responded differently in the third block when compared to the first two blocks. All three BA treatments also responded differently relative to the control in the third block. Interactions with the GA_3 treatments were generally created by 5 ppm GA_3 in the second block.

Table 12. Total dry weight per plant, 3 weeks after transplanting, as influenced by growth regulators at various concentrations.

Growth Regulator (applied in gel)	Concentration (ppm)	Dry Weight (g)
GA_3	5	6.54
	50	5.88
	500	6.76
BA	5×10^{-3}	6.30
	5×10^{-4}	6.86
	5×10^{-5}	6.82
ABA	10^{-4} M	5.93
	10^{-5} M	6.60
	10^{-6} M	6.21
Control	Gel	6.43
		NS[2/]

[1]Treatment values are the average of 9 plants over 3 replications.

[2]Means are not significant at the 5% level.

DISCUSSION

All variables measured on plants receiving treatments with the gel showed no differences between control and other treatments after fourteen days. However, for plants receiving treatments in water, only total leaf area and total stem length showed no significant differences between any treatments by the 21st day. In general, it appears that responses to treatments applied in water persisted slightly longer than those receiving gel applications. Of all the treatments, only 500 ppm GA and 10^{-4} M ABA tended to produce significant responses across all variables. Gel applications appeared to delay or show no response in number of leaves. Only 10^{-4} M ABA showed differences from the control and these differences only appeared on days 7 and 14. Water applications of 10^{-4} M ABA produced a response 4 days after treatment. On the other hand, 500 ppm GA_3 did not promote leaf production, significantly greater than the control, until day 21.

The response in terms of total leaf area was generally the same regardless of gel or water application. However, reduction of leaf area by 10^{-4} M ABA persisted slightly longer with the water application.

Gel applications delayed a response in total stem length until the fourth day after application, while responses to treatments in water were observed by the second day. Nevertheless, regardless of application, no differences between control and treatments were detected two weeks after treatment.

Generally, method of application did not influence the final dry weight, as no differences were observed except a reduction of weight of plants receiving 10^{-4} ABA with water.

Even though some responses to treatments applied in water persisted longer than those applied with gels, this does not appear meaningful when considering that the treatment effects generally became reduced over time. For instance, 500 ppm GA_3 had 8% more leaves than the control on day 21, but neither its final weight or total leaf area was significantly different from the control by this time.

The reduced effect of the treatment over time observed in this study is similar to other findings where short term applications of hormones were used. Dong and Arteca (1982) and Arteca and Dong (1981) found that photosynthetic rates of tomato plants were highest within the first 24 hours after kinetin or GA_3 treatment, but decreased rates occurred steadily over an eight-day period. Other physiological studies usually record responses to hormones only up to 48 hours after treatment (Loy, 1980) so a reduction of effect over time would not necessarily be detected.

Dong (1982) and Arteca and Dong (1981) applied GA_3 to tomato roots in a hydroponic solution and with a hydrophilic gel (the plants were then transplanted to soil). While growth rates increased with hydroponically applied GA_3, plants receiving a root dip of GA_3 with the gel did not exhibit increased growth 3 weeks after treatment. The lack of response to gel application was attributed to a GA concentration differential between the hydroponic solution and potting media. However, the results of this study do not support this hypothesis because most differences were detected within the first two to four days after treatment. A lack of growth response three weeks after treatment could be due to metabolism of the hormone or its conversion to an unavailable or inactive form.

The lack of differences between the control and some treatments (such as BA) could also be due to the fact that older plants may be less responsive to root treatments of this kind. Most cited studies which found a growth response used plants which were less than ten days old (Sharif and Dale, 1980; Arteca & Dong, 1981; Dong and Arteca, 1982). Finally, one could consider that the biochemical environment of the root ball in peat pellets may cause the hormone to become inactive.

The interactions between treatment and time observed with gel applications generally occurred on days when the treatment means were not significantly different from each other. No pattern in the nature of interactions could be detected. Perhaps, though, the persistence of the hormone in the gel may have been variable from one experiment or from even one plant, to the next. However, it is unknown how the hormone interacts with the gel.

Treatments of 500 ppm GA_3 show the most potential as a short term transplant aid for muskmelon. Besides increasing leaf area by cell elongation, studies show that GA_3 enhances photosynthesis, possibly by increasing RuDPC activity (Arteca and Dong, 1981; Stoddart and Wareing, 1970). Under non-stressful conditions (eg. no water or high temperature stress), GA_3 applications could perhaps increase the amount of photosynthates generated, thereby allowing for continued growth despite transplanting. However, field studies should be undertaken to evaluate the response of plants to treatment under various field conditions at transplanting. Also, the effects of GA_3 on photosynthesis should be further investigated to determine if the response to treatment is due solely to cell elongation and/or other factors such as increased chlorophyll content or increased enzyme activity in photosynthesis.

Although ABA, unlike GA_3, is a growth inhibitor of above-ground plant organs, its role of inducing a protection mechanism during stress (Salisbury & Ross, 1978), including drought, should not be overlooked. Other studies have shown enhanced root growth as a result of ABA treatments (Watt, et. al., 1981; Biddington and Dearman, 1982).

These findings emphasize the potential use of ABA or a synthetic analog as an antitranspirant for use under stressful transplanting conditions. Additionally, stimulation of root growth may be helpful when transplanting procedures incur root damage. Perhaps concentrations of less than 10^{-4} M should be used as this study points out a significant reduction of leaf number even at the end of two weeks, and total dry weight was reduced with water applications. Currently, though, the high cost of ABA prohibits any practical use of this hormone. However, it may be useful to pursue further studies under field transplanting conditions. Also, it would be helpful to determine other plant responses to ABA treatments such as stomatal resistance and photosynthetic rates.

CONCLUSIONS

The results of this study do not indicate any differences between application of treatment with either water or a hydrophilic gel. Generally, 500 ppm GA_3 increased total number of leaves, total leaf area, and total stem length per plant. However, these increases were only significant during the first week after treatment. Root applications of GA_3 or some other similar growth regulator may show potential as a transplant aid during planting. However, field investigators should be pursued in order to evaluate plant responses under various environmental conditions.

The use of 10^{-4} M ABA induced just the opposite effects of GA_3, reduced leaf number, leaf area, and stem length. Despite this though, ABA or a similar regulator may show potential as an antitranspirant to be applied under stressful environmental conditions at transplanting. The effects of 10^{-4} M ABA generally were also significant only through the first week after transplanting. However, lower rates would be recommended since the final dry weight was reduced using water applications. Again, field investigations should be conducted if these studies were to be further pursued. Also, the high cost of ABA may require that another similar regulator be used in these studies to make commercial use feasible.

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THE EFFECT OF SEED APPLIED AND ROOT-APPLIED GROWTH REGULATORS ON THE GERMINATION AND GROWTH OF MUSKMELON

by

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Muskmelon seeds were treated with acetone containing various concentrations of either gibberellin$_3$ (GA_3), gibberellin 4+7 ($GA_{4/7}$), 6-benzylaminopurine (BA), or 2-chloroethylphosphonic acid (ethephon). The seeds were then germinated at either 24°C or 16°C. At 24°C no treatment enhanced early germination compared to the control. In some cases, treatments of BA or ethephon were toxic as reflected by stunted radicle growth. At 16°C, 1.0, 0.5, and 0.1 mM $GA_{4/7}$ and 0.3 and 0.15 mM ethephon promoted earlier germination compared to the controls. However, regardless of treatment, low temperature of 16°C apparently inhibited seedling growth, since the radicle length did not excede 3 mm even 3 days after germination. Radicle growth at 24°C three days after germination ranged between 1.74 mm and 4.95 mm.

In a second experiment, the root balls of muskmelon seedlings were treated with various concentrations of either GA_3, BA, or abscisic acid (ABA). The treatments were applied wither with water or hydrophilic gel. There were no differences between method of application, although gel treatments tended to show treatment by block interactions. Generally, 500 ppm GA_3 increased total number of leaves, total leaf area, and total stem length per plant, while 10^{-4} M ABA reduced these variables. Most differences between treatments were detected within one week after treatment. However, by the second or third week there were generally no differences between treatments.