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Emergence at High Temperature and Seedling Growth Following Pretreatment of Lettuce Seeds with Fusicoccin and Other Growth Regulators

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Abstract. **The rate and total emergence of lettuce (*Lactuca sativa* L. 'Empire') seedlings incubated at 33°C for 10 hr, alternating with 23° for 14 hr, was markedly increased by seed treatment with 0.5 mM fusicoccin (FC). Neither gibberellic acid (GA) nor kinetin (K) were effective in improving emergence when used alone. The combination of FC with GA or K appeared to give a synergistic enhancement of emergence rate. The radicle elongation of seedlings was reduced by seed treatment with FC, K, or combinations of FC, GA, and K in tests at 20°. The greatest reductions were caused by combinations that included FC. The inhibiting effect of FC on radicle growth was reduced by using a concentration of 0.05 mM. Treatment with 0.05 mM FC resulted in slower emergence but gave the same total emergence as 0.5 mM FC at high temperature. Both FC and GA stimulated hypocotyl elongation.**

Permeation of growth regulators into lettuce seeds via acetone has been shown to be an effective method of overcoming the effects of high temperature stress on germination and emergence. Fusicoccin, a diterpine glucoside, has been especially effective in promoting germination and emergence at supraoptimal temperatures (1). This compound promotes the germination of seeds of both light-sensitive and nonlight-sensitive cultivars (1). In addition, FC stimulates hypocotyl elongation without causing spindly growth (1).

The stimulating effect of GA or K on lettuce seed performance has been demonstrated (1, 2, 19). However, GA has caused excessive hypocotyl elongation and etiolated seedlings (1, 10). Kinetin treatment results in normal hypocotyl growth but has been shown to reduce radicle growth (1, 9–11). When GA and K are combined, a synergistic enhancement of germination at high temperature has been reported (1, 2).

Although the action of FC alone on seed germination has been much studied, little is known about the effect of this compound when used in combination with other growth regulators. The combination of FC and GA has produced an additive increase in the germination rate of sugarbeet seeds at low temperature (15). An analogue of FC, cotylenin E, has been shown to interact synergistically with GA, K, and ethephon in promoting germination (12). An objective of this study was to determine the effect of FC alone and in combination with GA and/or K on lettuce seed performance at supraoptimal temperatures.

Our preliminary tests indicated that FC was associated with reduced radicle growth in lettuce seedlings. This study assessed the effect of FC and other growth regulators on seedling radicle and hypocotyl elongation.

Materials and Methods

Seeds of 'Empire', 'Coolguard', and 'Vanguard 75' lettuce were stored in sealed containers at 3°C before being used. Seeds were soaked for 4 hr in redistilled acetone alone or acetone containing 0.5 mM FC, 1.0 mM GA (gibberellin A_3), and 0.5 mM K alone or in various combinations. A preliminary test indicated that a 4-hr permeation time was as effective as 12 hr when treating seeds with FC. The effect of FC on seedling emergence and growth characteristics was evaluated further using concentrations of 0 (acetone check), 0.005, 0.05, and 0.5 mM. After treatment, seeds were air-dried at 25° for 24 hr and stored at 3°.

Emergence tests consisted fo 20 seeds per treatment planted 4 mm deep into screened (1.6 mm) and uniformly mixed loam soil. Flats containing soil and planted seeds were enclosed in polyethylene bags into which water was added to bring the soil to 22% moisture. Bags were sealed, and flats were incubated in the dark at 33°C for 10 hr, alternating with 23° for 14 hr. Each treatment was replicated 4 times. Seedlings were considered emerged when at least 1 cm high. Arcsin transformations were made on emergence data before analysis of variance.

For the determination of seedling growth characteristics, treated seeds were germinated on slanted media in covered acrylic boxes as described by Sharples and Kuehl (17). In slant boxes, the developing radicles and hypocotyls elongated generally parallel, allowing measurements without disturbing the seedlings. Each treatment consisted of 20 seeds replicated 4 times. Boxes were sealed and seeds germinated in the dark at 20°C or 25°. Near optimal temperatures were used, because higher temperatures can induce thermoinhibition, particularly of untreated seed. Measurements of radicle growth were made every 12 hr from 12 through 96 hr after seeds were incubated. Hypocotyl measurements were made 48, 60, and 72 hr after seeds were incubated. Seeds that did not germinate and seedlings that died during the test period were discarded and not included in measurements.

Results

Effect of FC and other growth regulators on emergence from soil. The rate and total emergence of 'Empire' lettuce seeds at high temperature was increased markedly by FC (Table 1). Neither GA nor K were effective in improving emergence when used alone. When FC was combined with GA or K, an apparent synergistic enhancement of emergence rate was obtained. The combination of FC + GA + K was superior on day 2 to FC and FC + K, but not FC + GA. On subsequent days, there were no differences in emergence among treatments that in-

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Table 1. Effect of FC, GA, or K alone or in combination permeated into 'Empire' lettuce seeds on seedling emergence from soil at high temperature.

	Emergence(%)			
	Days after planting[z]			
Treatment	2	3	4	7
Untreated[y]	0 d[x]	0 c	0 c	0 c
FC	34 c	76 a	84 a	90 a
GA	0 d	2 c	3 c	3 c
K	0 d	1 c	2 c	8 c
FC + GA	56 a	85 a	89 a	93 a
FC + K	44 b	83 a	90 a	84 a
GA + K	1 d	11 b	32 b	54 b
FC + GA + K	54 a	81 a	91 a	94 a

[z]Incubation temperature was 10 hr at 33°C alternating with 14 hr at 23°.
[y]Acetone check was no different than untreated check.
[x]Mean separation in columns by Duncan's multiple range test, P = 5%. Values are the means of 4 consecutive tests.

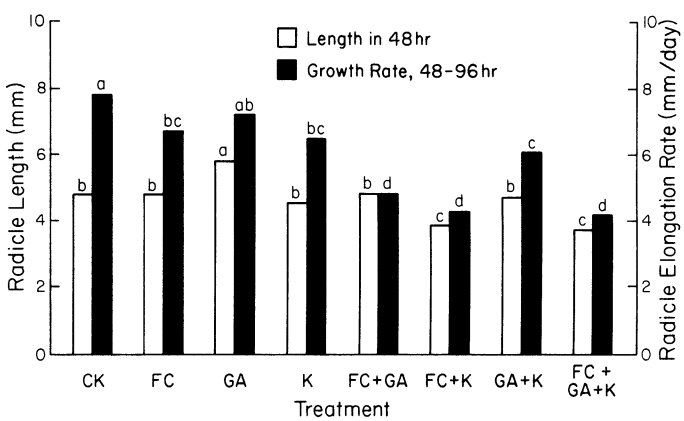

Fig. 1. Effect of 'Empire' lettuce seed pretreatment with FC, GA, and K alone or in combination on seedling radicle length after incubation for 48 hr and radicle elongation rate for the period 48 to 96 hr after incubation began. Incubation was at a continuous 20°C. CK = untreated control. Mean separation within each growth characteristic by lower case letters according to Duncan's multiple range test, 5% level.

cluded FC. The GA + K combination appeared to have a synergistic effect on emergence after day 2 but was inferior to FC treatments. Atypical emergence, in which the hypocotyl emerged but not the cotyledons, was observed in 5% of seeds treated with FC + GA + K.

Effect of FC and other growth regulators on radicle growth. Permeation of lettuce seeds with FC or K had no effect on radicle length after 48 hr incubation, while GA increased length (Fig. 1). The combinations of FC + K and FC + GA + K reduced initial radicle growth.

The radicle elongation rate for the period 48 to 96 hr after incubation began was reduced significantly when seeds were pretreated with FC, K, or combinations of FC, GA, and K (Fig. 1). The greatest reductions in growth were caused by those combinations that included FC. 'Vanguard 75' and 'Coolguard' lettuce were affected similarly by FC (data not shown). GA alone had no effect on elongation rate and did not overcome the retarding effect of FC.

Effect of FC and other growth regulators on hypocotyl growth. Both FC and GA stimulated hypocotyl elongation during the first 48 hr of incubation (Fig. 2). The promoting effects of FC and GA were similar. When the 2 compounds were combined, the enhancement of growth was greater than when either was used alone. Treatment of seeds with FC and/or GA resulted in seedlings with spindly hypocotyls.

The promoting effect of FC and GA on hypocotyl growth was evident also during the 48- to 96-hr period after incubation began (Fig. 2). The combination of FC + GA was superior to GA alone, but not to FC. Kinetin alone and combinations of FC + K or GA + K were not significantly different than the control.

Effect of FC concentration on seedling emergence and radicle and hypocotyl elongation. The 0.5-mM concentration of FC resulted in the highest rate of emergence (Fig. 3). Total emergence was the same for 0.5 and 0.05 mM FC treatments, but emergence was slower at the lower concentration. FC at the 0.005-mM concentration had no effect on emergence.

All concentrations of FC increased initial growth of radicles. After 24 hr of incubation, radicles were 5.10, 5.18, and 4.37 mm long for seeds treated with 0.005, 0.05, and 0.5 mM FC,

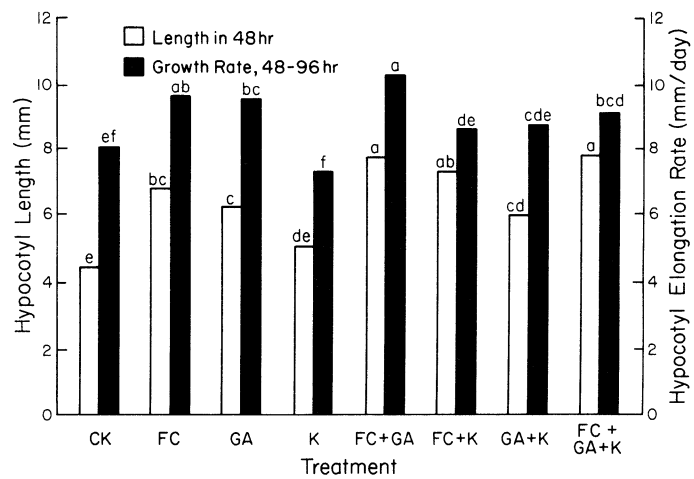

Fig. 2. Effect of 'Empire' lettuce seed pretreatment with FC, GA, and K alone or in combination on seedling hypocotyl length after incubation for 48 hr and hypocotyl elongation rate for the period 48 to 96 hr after incubation began. Incubation was at a continuous 20°C. CK = untreated control. Mean separation within each growth characteristic by lower case letters according to Duncan's multiple range test, 5% level.

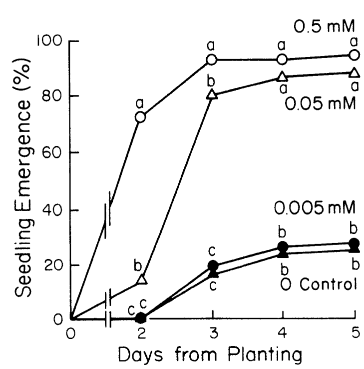

Fig. 3. Effect of 'Empire' lettuce seed pretreatment with various concentrations of FC on seedling emergence at high temperature. Incubation temperature was 10 hr at 33°C alternating with 14 hr at 23°. Mean separation at each date by lower case letters according to Duncan's multiple range test, 5% level.

respectively, compared to 3.76 mm for untreated seeds. However, radicle elongation was inhibited substantially by 0.5 mM FC during the 24- to 60-hr period of incubation (Fig. 4). The 0.05-mM treatment reduced radicle elongation during the 24- to 36-hr period, but not thereafter. After 96 hr of incubation, radicle length was reduced 26% by 0.5 mM FC compared to 4% by 0.05 mM FC (data not shown).

FC stimulated early growth of hypocotyls at all concentrations. After 48 hr of incubation, hypocotyls of seedlings from FC-treated seeds were 21% longer than those from untreated seeds (data not shown). Treatments of 0.5 and 0.05 mM FC resulted in higher elongation rates than untreated control during the 48- to 60-hr period of incubation (Fig. 5). During the 60- to 72-hr period, only the highest concentration increased the hypocotyl growth rate.

Discussion

This study provides additional evidence that FC is an effective growth regulator for alleviating the effects of high temperature on lettuce emergence. Combinations of FC and GA or K had an apparent synergistic effect on emergence rate but did not improve final emergence. The advantage of combinations of FC with other growth regulators over FC alone might have been greater had higher incubation temperatures been tested. FC apparently promotes rapid germination by stimulating early cell elongation and thus rapid protrusion of the radicle from the seed coat (7). Haber and Luippold (8) found that at 30°C lettuce seeds underwent cell division but not cell expansion and thus did not germinate. The stimulation by FC of germination of 'Grand Rapids' seeds kept in darkness was shown to be correlated with a stimulation of elongation of the embryonic axes (3).

Radicle elongation of the cultivars tested was inhibited markedly by seed treatment with 0.5 mM K and 0.5 mM FC. The inhibiting effect of K on lettuce radicle growth has been reported previously (1, 9–11). Conversely, FC has been reported to have a promoting effect on root elongation in lettuce (18) and other crop plants (6, 13, 16). Under high-temperature stress, FC apparently promotes germination by stimulating cell enlargement and initial growth of the radicle. In this study, FC began to inhibit growth sometime after the protrusion of the radicle from the seed. Dalassandro and Borraccino (5) found that FC-treated *Vicia faba* roots grew more than untreated roots because cell elongation was stimulated; however, FC markedly inhibited cell division. It is possible that although FC may promote cell elongation and early radicle growth in lettuce, at high concentrations it also may inhibit cell division to an extent that eventually reduces root growth.

The undesirable effect of FC on radicle growth can be minimized by using a lower concentration without greatly reducing its effectiveness in promoting high-temperature emergence. A FC concentration of 0.05 mM had only a slight inhibitory effect on radicle growth and resulted in the same total emergence at 0.5 mM.

Both FC and GA stimulated hypocotyl elongation through 96 hr. The combination of the 2 compounds was generally superior to either used alone. GA has previously been shown to stimulate lettuce hypocotyl growth (1, 11). The effect of FC appears to be one of promoting cell enlargement (4, 14). GA promotes cell elongation and cell division (7).

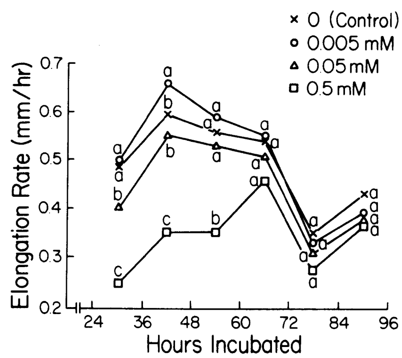

Fig. 4. Effect of 'Empire' lettuce seed pretreatment with various concentrations of FC on radicle elongation for the 24–96-hr period after incubation began. Elongation rates were determined for each 12-hr period beginning with the 24–36-hr period after incubation began. Incubation was at a continuous 25°C. Mean separation within each 12-hr period by lower case letters according to Duncan's multiple range test, 5% level.

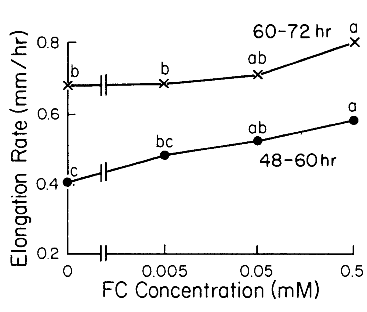

Fig. 5. Effect of 'Empire' lettuce seed pretreatment with various concentrations of FC on hypocotyl elongation rates for the 48–60-hr and 60–72-hr periods after incubation began. Incubation was at a continuous 25°C. Mean separation within each 12 hr period by lower case letters according to Duncan's multiple range test, 5% level.

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The Influence of Nitrate:Ammonium Ratios on Growth, Fruit Development, and Element Concentration in 'Floradel' Tomato Plants

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Additional index words. *Lycopersicon esculentum*, plant analysis

Abstract. **Tomato (*Lycopersicon esculentum* Mill. 'Floradel') plants were grown under greenhouse conditions in a modified Hoagland's solution to determine the influence of NO_3:NH_4 ratio (100:0, 75:25, 50:50, 25:75) on vegetative growth, fruit development, and tissue levels of N, P, K, Ca, and Mg at 3 stages of maturity. Vegetative growth prior to fruit set was increased significantly by adding 25% of the N as NH_4, although higher NH_4 ratios reduced vegetative growth. During flower and fruit development, the number of fruit formed with each flower cluster was not influenced by the NO_3:NO_4 ratio, although fruit weights were reduced significantly when NH_4 supplied any part of the N form. With each increment of NH_4 in the N ratio, tissue P increased whereas K, Ca, and Mg decreased. Kjeldahl N (less NO_3-N) in the vegetative tissue at all harvests increased with each increment of NH_4 in the N ratio. It is concluded that the use of Kjeldahl N as an indicator of the N status of the plant without consideration of the effect of N form on the percentage of N as well as the uptake and distribution of other essential elements could be misleading and potentially a misuse of this diagnostic tool.**

The influence of N form as either NO_3 or NH_4 on tomato growth has been investigated extensively with either young plants (in which the experiments were terminated before flowering) or initiated at flowering to determine the effect of N form on fruit yield. However, comparisons evaluating the influence of N form on vegetative growth and subsequent fruit development have not been delineated clearly. Generally, with NH_4 alone or as the primary source of N, vegetative growth of nonflowering tomato plants is adversely affected (11, 14), whereas combinations of NO_3 and NH_4 usually result in greater vegetative growth than when either N form is used alone (3, 9). The effect of N form on fruit yield has been consistent, with fruit yields being reduced when NH_4 is the primary source of N during fruit development (12–14). Thus, fertilizers containing both NO_3 and NH_4 generally are recommended for tomato production because vegetative growth is maximized, which is thought to increase fruit yield (3).

Since sufficiency levels for most of the essential elements have been established for each region or area of tomato production, growers can rely on plant analysis to determine if sufficient levels of the essential elements exist. Although the specific effects of N form on uptake and resulting tissue concentration of the major elements have been investigated (1, 8, 9, 11, 14), the effect of N form on elemental concentration in relation to vegetative growth and fruit development needs further clarification. Therefore, the objectives of this study were to determine the influence of NO_3:NH_4 ratios on the relationship between: a) vegetative growth and fruit development; and b) N, P, K, Ca, and Mg levels in the vegetative tissue and their relationship to vegetative growth and fruit development.

Materials and Methods

Tomatoes were grown in nutrient solution culture under greenhouse conditions (day/night temperature 32°/21°C, photoperiod average 13.4 hr). Seedlings were germinated in vermiculite, transplanted at the first true leaf stage into 14.5-liter vessels, and grown for 2 weeks in a half-strength modified Hoagland's solution prior to initiation of the N treatments.

Treatments consisted of 2 N sources, $Ca(NO_3)_2$ and/or

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