ONION DORMANCY IN RELATION TO TEMPERATURE, APPLIED GROWTH SUBSTANCES AND AN ENDOGENOUS GROWTH INHIBITOR

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ABSTRACT

ONION DORMANCY IN RELATION TO TEMPERATURE, APPLIED GROWTH SUBSTANCES AND AN ENDOGENOUS GROWTH INHIBITOR

By

Sauveur Mahotiere

The relationship between growth and dormancy characteristics for two varieties of onions, the level of an endogenous inhibitor present during the dormancy of these varieties, the effect of wounding and oxygen treatment on dormancy release and the effect of temperature and chemicals in promoting growth of shoots excised from dormant bulbs were investigated.

For field studies, 'MSU 4535', a genotype with a long dormant period matured later than 'Downing's Yellow Globe' with an intermediate dormant period. The initial inhibitor content of 'MSU 4535' was higher than that of 'Downing's Yellow Globe', and it declined in both cultivars during storage. The inhibitor resembled abscisic acid (ABA) in several respects but was not ABA itself. Dormancy release in onion is temperature-dependent with 7.5-12.5°C being generally more effective than the range of 0-5° and 20-30°C. Dormancy was more intense in large bulbs than in medium or small ones. Transverse wounding was effective in breaking dormancy in onion but increasing the oxygen

tension was not. Gibberellic acid (GA_3 and $GA_{4/7}$), ethephon, and naphtaleneacetic acid (NAA) had no effect but kinetin did break dormancy. Shoot apices excised from dormant bulbs responded to temperature in a manner similar to the entire bulb but a much shorter exposure was required. Kinetin and sucrose were very effective in promoting growth of the shoot apices when applied alone but had no effect in combination with temperature. ABA applied before or after temperature treatment nullified the temperature effect. 4-Hydroxy-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidine carboxylate (Amo 1618) was effective in reducing growth of the excised onion shoot apices only if applied prior to temperature treatment. Sucrose fully overcame the effect of Amo 1618 but not that of ABA. Kinetin slightly counteracted the effect of ABA and Amo 1618. Low temperatures (0-5°C) were more effective in increasing reducing sugars in onion shoots than high temperatures (10-30°C). ABA and Amo 1618 nullified this effect.

ONION DORMANCY IN RELATION TO TEMPERATURE, APPLIED GROWTH SUBSTANCES AND AN ENDOGENOUS GROWTH INHIBITOR

By

Sauveur Mahotiere

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In memory of my

daughter, Cassandre.

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INTRODUCTION

The onset of dormancy in onion bulbs is associated with senescence of the leaves. Dormancy is important from many aspects. The shelf-life of the bulbs depends upon the degree of dormancy of the bulb which, in turn, is affected by cultivar, and by cultural and storage practices. In addition, dormancy permits the survival of the species under adverse conditions. Dormancy may govern the production of both seeds and bulbs. The time of flower and bulb formation is controlled by the degree of dormancy of the bulbs and the temperature to which they are exposed during storage. Onion bulb dormancy is thus of interest to farmers, retailers, consumers, physiologists and breeders.

For these reasons, a good understanding of the dormancy phenomenon in this horticultural commodity is both interesting and useful.

In this thesis, I have investigated the following:

1. Effects of temperature and growth substances in breaking dormancy of whole bulbs or isolated shoot apices.
2. The levels of an endogenous inhibitor during the dormancy of onion bulbs.

LITERATURE REVIEW

The Concept of Rest and Dormancy

Wareing and Saunders (136) define dormancy as a state in which growth is temporarily suspended. This phenomenon is caused either by external or internal conditions. To avoid confusion between the two states of growth suspension, some authors (74,120,132) term the former "dormancy" and the latter "rest". Vegis (132) presented an extensive review of rest and dormancy with temperature models for phases of rest. He distinguished three phases:

1. Early rest or predormancy.
2. Middle rest or true dormancy.
3. The state of potential maximum growth activity or post dormancy.

Buch and Smith (21), however, used the terms rest and dormancy interchangeably and questioned the real meaning of the above distinction. Emilson (45), in his study on the potato, also makes the distinction between rest and dormancy, but notes that dormancy includes and may coincide with the rest period.

Both states of growth suspension have been demonstrated in onion bulbs (59,61,125,135). Wareing's and Saunders'

definition (136) fits this study which will be concerned with internal conditions controlling true dormancy or rest in onion bulbs. The general term of dormancy, however, will be used.

Onion Bulb Formation and Induction of Dormancy

Anatomical Changes

Before the onset of bulbing, the formation of leaf blades is inhibited and bladeless sheaths are produced. These, along with the sheaths of previously formed bladed leaves, increase in thickness, resulting in swelling of the bulb (1). This swelling occurs by increase in cell size and development of intercellular spaces without cell division (50,123). During bulbing, the apparently mature (vacuolated) cells of the innermost leaves swell laterally and become isodiametric (30,108). During the differentiation into sheath and blade, a rapid increase in the diameter of the growing region occurs (54). Thus, the base of the newly formed leaf is pushed farther and farther away from the center. At the same time, the leaf blade and the sheath continue to increase in thickness so that by the time the younger leaf is differentiated, no more periclinal walls are formed. Further increase in the thickness of the sheath to form the fleshy bulb-scale is due to the formation and enlargement of intercellular spaces and to the growth of the cells.

Hormonal Changes Associated With Photoperiodism

The increase in cell size during bulbing suggests that auxins are involved in the bulbing process (30). The investigations of Leopold (78) indicated that the auxin content of plants varied with the photoperiod under which the plants were grown. Cook (34) studied the effect of daylength on the auxin content of 8 different plant species including Zinnia and Xanthium, and found that the plants grown under long days contained more auxins and were considerably larger than those grown under short day conditions. Clark and Heath (31) observed a rapid increase of IAA following the transfer of young seedling onions from short days to long days. The maximum auxin content occurred after 3-5 long days, thereafter a sharp decrease was observed. Tsukamoto (130), noted a remarkable decrease in the auxin activity from the end of the bulb-forming period to the lifting period.

Considering the bulbing ratio (i.e.,

$$\left(\frac{\text{maximum diameter of the base}}{\text{minimum diameter of the neck}}\right),$$

Clark and Heath (31) observed that a measurable increase in the bulbing ratio occurred 14 days after the long-day treatment, at which time the level of auxin in the long-day treated plants had fallen to a value below that of the control. Thus, IAA apparently initiated the bulbing process but continuation of bulbing was independent of the IAA level (30). Gibberellins may also be involved in photoperiodism. Cleland and Zeevaart

(33) found that the total amount of extractable GA in Silene plants was 25-75% higher under long days than under short days (46). These findings support Digby and Wareing's (39) observation that Betula leaves exposed to long days contained more GA than those exposed to short days. However, despite the fact that onion is a long day plant, Tsukamoto (130) demonstrated that the GA level was lower in onion plants grown under long days than under short days.

The above discussion suggests that bulb formation in onion is induced under long day conditions. Magruder and Allard (84), using 18 of the most important varieties of onions grown in the United States, found that increasing the length of the photoperiod hastened the maturity of all the varieties studied.

One of the factors controlling the induction of dormancy in woody plants is daylength. In the majority of the species so far studied, long days promote vegetative growth and short days bring about the cessation of growth and the formation of resting buds (135). According to Wareing (133), dormancy in Acer pseudoplatanus is controlled by the daylength to which the mature leaves are exposed. There exists, however, some evidence that in certain plant species dormancy is induced by long photoperiods. Lunularia cruciata and onion fall into this category (50,131).

Hemberg (51,52) appears to have first suggested that inhibitors are involved in bud dormancy. In several woody

species, higher amounts of growth inhibitors have been extracted from leaves and buds under short days than under long day conditions (90,99,100). However, in the case of Lunularia cruciata, more inhibitors were accumulated under long days than under short days (131). Tsukamoto's study (130) also demonstrated that storage leaves and inner leaves of onion contained more inhibitors under long days than under short days.

Exogenous Control of Dormancy in Onion

Temperature and Relative Humidity

When dormancy is broken, the primordial bladed leaves at the center of the onion bulb may emerge as sprouts (1,58). Under conditions of high relative humidity, the non-dormant bulb may root (143) and in that case, root emergence precedes sprout emergence (1,61). Removal of the roots can delay sprouting (1).

From a commercial standpoint, a sprouted and/or rooted onion is undesirable. Thus, keeping the onion in a dormant or nearly dormant condition has been the goal of many researchers. Their investigations have been aimed at observing the sprouting and rooting tendency of the onion bulbs at different temperatures after a certain period of time in storage. Jones (61) planted bulbs which were periodically removed from storage and demonstrated that they sprouted and rooted more

quickly, the longer they were held in storage. The most critical factors during storage were temperature and relative humidity. If bulbs were not removed from storage, sprouting was usually greater at 10°C (50°F), than at 5°C (41°F) and or at 0°C (32°F). Jones (62) stored bulbs for 4 months at 5 temperatures ranging from 3.7 to 30°C. Sprouting during storage was progressively greater with increasing temperature up to 16 to 20°C and then decreased at 30°C. Thompson (126), however, observed less sprouting in storage at 15.5°-21.1°C (60-70°F) than at either 5 or 10°C (41 or 50°F). Wright et al. (143) stored bulbs at 0, 5 and 10°C under controlled humidity, and found that sprouting increased with increasing temperature but was little affected by humidity. The effectiveness of intermittent temperature in breaking dormancy of onion bulbs was reported by Boswell (15) who observed that storage at 0°C followed by 10°C caused more rapid growth than storage at 10°C for the entire period. Rooting was greatly favored by high humidities but little affected by temperature. More recently, Abdalla and Mann (1) demonstrated the presence of a rest period in onion which after harvest disappears at all storage temperatures, the intermediate temperatures being in general more effective than the extremes. However, some investigators have reported that extreme temperatures (30-40°C) were more effective than intermediate or lower temperatures in breaking dormancy in onion (1,82). Exposure of onion bulbs to freezing

temperature resulted in decay upon subsequent exposure to high temperature (14). Quick vacuum cooling was also unsuccessful in breaking dormancy in onion (60).

Controlled Atmosphere Storage

Controlled atmosphere storage was shown to be a good way of controlling onion dormancy. Chawan and Pflug (35) working with Downing Yellow Globe and Abundance onions concluded that controlled atmosphere storage in general improved the appearance and keeping quality of onions. The best storage conditions were found to be 10% CO_2 and 3% O_2 or 5% CO_2 and 5% O_2 at 5°C.

Choice of Variety

Magruder et al. (85) on the basis of two years of tests in seven areas of the United States, grouped onion varieties according to their suitability for storage. Despite great variability in the production, maturity, time between harvest and storage, and conditions of storage, there was a remarkable agreement in storage rating quality within varieties. Among varieties, however, there was great variation in storage life and generally those varieties which rooted and sprouted quickly were the poorest keepers. Bulbs stored at high temperatures lost marketability faster than those stored at low temperatures.

Chemical and Physical Treatments

Besides environmental control and the choice of varieties, chemicals have been tested as a way of controlling dormancy.

Maleic hydrazide applied before harvest retards sprouting and improves the marketability of the product (57,58,59,141).

So far, use of chemicals for prolonging dormancy has been more successful than for shortening it. Boswell (14) exposed onion bulbs to an atmosphere saturated with ether at room temperature but failed to break rest.

Contrary to its effects on sprouting of gladiolus corms, ethylene chlorhydrin has not given any positive result in onions. Loomis (82) suggested that the effect of ethylene chlorhydrin in breaking the rest period may be associated with hydrolysis of starch reserves, and that this and similar compounds will not be effective in plant organs which contain little or no starch such as most bulbs.

The application of exogenous gibberellic acid induces bud-break in a number of woody species (135) and its effectiveness in shortening the rest period of potato tubers is well documented (81,105,106). Thomas (125), however, could not break dormancy in onion bulbs with GA_3, $GA_{4/7}$ or naphthyleneacetic acid (NAA). However, transverse and longitudinal wounding was very effective in promoting both sprout and root elongation (14,82).

Anatomical and Biochemical Changes During the Breaking of Onion Bulb Dormancy

Visible sprout and/or root formation are sometimes considered as unique indicators of the termination of the rest

period. Such an approach may be misleading, for, the invisible anatomical and physiological changes leading to the sprouting and rooting phenomena may be of importance for the understanding of dormancy and for its control. According to Chouard (28), all organs or their primordia are present during dormancy but either do not grow or grow slowly. He also noted that the frequency of mitosis decreased with the inhibition of growth. Abdalla and Mann (1) found that in onion the sprouts resulted from the elongation of leaves which were formed before harvest, suggesting that sprouting was independent of leaf initiation during storage. The same investigators (1) observed mitotic figures in the shoot apex from twenty days before harvest until fifty days after storage at 0, 15, and 30°C. Mitotic activity decreased during storage while the cells had the capability to enlarge. They concluded that sprouting was independent of cell division but was associated with cell enlargement in the inner leaves.

Color changes have been observed during and after the rest period in the shoot apices of onion bulbs. During rest, the apices are white but they turn yellow or green when rest is broken (58). The response of the onion bulb to storage suggests some biochemical changes (125). Bennett (9), studying the level of total, reducing and non-reducing sugars under 0, 5 and 15°C storage temperature, could not show any significant change in the soluble sugar level. With increasing temperature, however, the level of reducing sugars was lower and that of

non-reducing sugars higher. With eleven temperatures ranging from 0 to 40°C, the same conclusion was reached, i.e., low temperature favored accumulation of reducing sugars. Also, the aminonitrogen content of onion bulbs stored at higher temperatures was generally higher (144).

Recently, Poovaiah et al. (102), studying peroxidase activity in onion bulbs with long vs short dormant periods, found that peroxidase activity was higher in the short dormant than in the long dormant bulbs.

Virtually all processes connected with growth, development and metabolism in plants are governed one way or another by hormones (71). There is evidence that bulbs do not escape this rule. In fact, a possible relationship between temperature treatment and the level of gibberellin-like activity has been reported in tulips. Exposure to 5°C for 9 weeks resulted in an increase in the activity of both free and bound extractable gibberellins (4). The occurrence of gibberellin-like substances in onion in free and bound form has been reported, but their presence was not associated with any physiological process (5). Thomas (125), however, studying the GA level of Rijnsburger onions stored at 5°C, observed a decrease in gibberellin activity before sprouting and an increase as sprouting commenced. Tsukamoto (130) and Kato (66) obtained similar results by storing onions at room temperature, i.e., the gibberellin-like substance decreased after harvest and increased gradually until the period prior to sprout leaf elongation.

Abscisic acid accumulation has been correlated with induction of rest period in buds of woody plants (44,134,80). Its level has been reported to fluctuate during the rest period, suggesting that ABA alone does not account for the rest phenomenon (36). Sondheimer et al. (117), however, found that the most striking effect of chilling on Fraxinus seeds was a decrease in ABA level. Regarding onion bulb dormancy, few studies exist concerning the role of inhibitors in controlling the rest period. An accumulation of inhibitor tentatively identified as abscisic acid in onion bulbs was observed shortly before harvest (130). Interestingly, this inhibitor increased immediately after digging and then decreased during storage at room temperature. Thomas (125), working with long and short dormant types of onions stored at 5°C also observed a decrease in the inhibitor level at the end of dormancy. Furthermore, the long-dormant type contained more of this inhibitor than the short dormant type. He did not decisively identify the inhibitory substance. Fresh juice extracted from dormant onion applied to young seedling onions inhibited their growth and this inhibition declined progressively with time. Here again, this inhibitory substance has not been identified (67). A substance inhibiting seed germination and identified as allyl sulphide was present in large quantities in dormant onions and in lesser amounts in sprouting bulbs (68). In a previous study, however, the same investigators (66) found that the concentration of some inhibitory substances in dormant onion showed an opposite trend.

In summary, the existing literature provides the following information: Onion bulbs exhibit both rest and dormancy. The bulbing process results from cell enlargement and is under photoperiodic and hormonal control. The end of the bulbing process, which coincides with the induction of rest, is associated with low levels of auxins and gibberellins and a high level of inhibitors.

Dormancy of onions is affected by genotype, temperature, relative humidity and CO_2 and O_2 levels. Maleic hydrazide prolongs dormancy, while GA, IAA, ethylene chlorhydrin and ether vapor have been ineffective in breaking it. The breaking of dormancy is associated with cell enlargement, and with yellowing and greening of the shoot apices, increases in reducing sugar, peroxidase activity and gibberellin-like substances, and a decrease in inhibitors.

MATERIAL AND METHODS

Field Study of Growth and Maturation

The growth and bulbing of cv Downing's Yellow Globe onions were compared with that of cv MSU 4535 to determine if a correlation existed between these characteristics and storageability. MSU 4535 is an inbred line of long dormancy, while Downing's Yellow Globe has an intermediate dormant period. Seeds were sown at the MSU Muck Farm, May 29, 1970. Forty plants of each variety were randomly selected and staked 5 weeks after emergence. Diameters of the neck and the base were measured then and at weekly intervals thereafter until the onset of senescence. The Downing's Yellow Globe variety started maturing about one week before MSU 4535, but for convenience, both were harvested on October 16, when the tops had dried and fallen. The bulbs were cured in ventilated crates held in a workroom at ambient temperature for two weeks at which time they were cleaned and sorted for storage experiments.

In 1971, the varieties Abundance, Downing's Yellow Globe, and Spartan Banner, short, intermediate and long dormant type respectively, were grown. The seed was planted May 5 and the

bulbs were harvested on October 11, according to the same criteria as the previous year. The order of maturing from earliest to latest was Abundance, Downing's Yellow Globe and Spartan Banner. The curing procedure was the same as previously described except that forced air was blown through the crates to hasten drying and reduce losses from decay.

Constant temperature rooms were used for storage.

Effects of Temperature During Storage of Whole Bulbs

Continuous Temperature

The first year, the sprouting response of Downing's Yellow Globe and MSU 4535 onions to storage at 0, 10 and 20°C was investigated. The bulbs were put in crates and placed in controlled temperature rooms. The relative humidity was not controlled. Random samples of 40 uniform bulbs were removed after 2, 4, 8 and 16 weeks and 20 were planted with their bases in water at 20°C. The remainder was put in paper bags and kept in dry air at the same temperature. The controls were either held with their bases in water or they were put in paper bags at 20°C from the beginning. Percentages of bulbs rooting and sprouting were recorded weekly. In this study sprouting and rooting are defined as the appearance of leaves and roots at the top and the base of the bulbs, respectively.

When a long time was required for rooting and sprouting, immersion of the bases in water often resulted in rotting. However, as dormancy ended, root initiation occurred in a matter of days. Also, because Downing's Yellow Globe and MSU 4535 have relatively short dormant periods, sprouting sometimes occurred in samples stored for a long period of time at higher temperatures. Furthermore, a 20-bulb sample did not permit detection of small differences between treatments.

For these reasons, some modifications were adopted during the second year. Spartan Banner onions, a relatively long dormant type, were used in 1971. Bulb samples were removed from the storage rooms after 8 and 16 weeks at 0, 5, 10, 20 and 30°C. Three or four replicates of 20 bulbs each were used. Moist peat moss was substituted for water and the dry treatment was omitted. The rooting and sprouting data were recorded daily until 50 percent of the bulbs had rooted and sprouted.

To determine variability due to size, the bulbs were classified as follows:

Small (4-5 cm in diameter)
Medium (7-8 cm in diameter)
Large (10-11 cm in diameter)

These bulbs were stored at 10°C for 8 weeks and then planted as usual. The controls were planted immediately after curing.

Effects of 0, 5, 10, 20 and 30°C on root development of Spartan Banner were also studied. Bulbs were stored for 8 weeks and planted at 20°C in moist peat moss. Four replicates of 10 bulbs each were used. Ten days after planting, the roots

were carefully removed, washed, and dried with filter paper. Their length and weight were measured.

Effect of Storage Temperature on Growth of Apices in situ

During the first year, six sound and uniform MSU 4535 and Downing's Yellow Globe onion bulbs were removed from storage after 5 and 8 weeks at 0, 10 and 20°C. At 10 and 20°C, sprouting occurred in the Downing's Yellow Globe bulbs and not enough bulbs were available for removal after 8 weeks. The scales were removed to expose the shoot apex (see Figure 1-A), the shoot apices were then measured to assess the effect of storage temperature on their elongation inside the bulb. During the second year, a similar experiment was performed with Spartan Banner, using temperatures of 0, 10 and 30°C for 8 weeks. In both cases, apices excised from unchilled bulbs were used as controls. The apices, after measurement, were grown according to the procedure described later (p. 21).

Effects of Intermittent Temperature, Root Removal and Substitution by Kinetin on Sprouting

These experiments were performed to answer the following questions: 1) Is continuous chilling necessary to break dormancy in onion? 2) Does rooting govern sprouting and if so, how? Abundance and Spartan Banner bulbs were used.

Figure 1. Photograph of excised shoot apices of dormant Spartan Onion.

A. Shoot apices held at 10°C for 96 hours.

B. Shoot apices held at 10°C for 96 hours, then grown in dark at 20°C in moist sand for 96 hours.

Distance between bars = 1 inch.


ONION SHOOT
PREPARATIONS
INITIAL LENGTH

A


ONION SHOOT
PREPARATIONS
FINAL LENGTH

B

Treatment for Abundance

1 - Control, not stored
2 - 8 weeks at 10°C
3 - 8 weeks at 10°C, 2 weeks at 30°C
4 - 3 weeks at 10°C, 2 weeks at 30°C, 3 weeks at 10°C
5 - 8 weeks at 10°C, then derotted daily after planting.

Treatment for Spartan Banner

1 - Control, not stored
2 - 8 weeks at 0°C
3 - 8 weeks at 0°C, injected with half a milliliter of water and derooted daily
4 - 8 weeks at 0°C, injected with half a milliliter of 100 ppm kinetin solution and derooted daily.

After removal from storage, bulbs were planted in moist peat moss at 20°C and examined daily until 50% had sprouted.

Effect of Wounding and Oxygen Treatment of Unchilled Bulbs on Sprouting and Rooting

According to some authors (109,113), dormancy in seeds is related to oxygen deficiency. The effectiveness of wounding in promoting sprouting has been attributed to release of inhibitory gases (14) and oxygen penetration to the growing point (82). These experiments were designed to test this hypothesis on onion bulbs. The sprouting and rooting procedure as well as the number of bulbs used was the same as described previously, and MSU 4535 and Abundance were used. Two kinds of wounding were tested: transverse and longitudinal. In the first case, one fourth of the upper portion of the bulb was removed and

in the second, the same proportion was removed from one side. Care was taken to avoid damage of the base and the inner shoots. The wounded bulbs were placed in water at 20°C. Intact bulbs served as controls. To test the oxygen tension, intact bulbs were exposed to a flow of 100 percent of oxygen for 48 hours at the rate of 472 cubic centimeters per minute. The controls and the oxygen-treated bulbs were placed in water at 20°C.

Growth of Excised Apices

Effects of Temperature Upon Subsequent Growth of Excised Shoot Apices

After assessing the effect of storage temperature upon the growth of the apices *in situ* (p. 17), these excised shoot apices were washed 3 times with distilled water and then planted in moist sand in the dark at 20°C. Shoot length was measured after 24, 48, 72, 96 and 120 hours. The controls were shoot apices excised from dormant bulbs kept at ambient temperature. In 1970, only six MSU 4535 and Downing's Yellow Globe shoots were used per treatment for lack of materials. In the 1971 Spartan Banner experiments, four replicates of five apices each were used per treatment and the shoot apices were grown in moist sand for 96 hours (see Figure 1-B, page 19).

Effect of Cutting Bulb Prior to Low Temperature Treatment Upon Subsequent Growth of Excised Apices

Greenhouse-grown Spartan Banner bulbs were used in the first experiment. Intact bulbs and bulbs with the upper half removed were exposed to 10°C for 24, 48 and 72 hours. Excised apices were also included. The shoot apices were then excised from the treated bulbs, washed with distilled water and planted as previously described. After 96 hours, the final length was recorded. A second experiment was performed according to the above procedure. However, the Spartan Banner bulbs used were grown in the field, and five replicates of 5 shoots each were used. The data were analyzed by using analysis of variance and Duncan's multiple range test (41).

Effect of Temperature and Time of Exposure Upon Subsequent Growth of Isolated Apices

Excised shoot apices were washed and exposed to 0, 5, 10, 12.5 and 20°C for 96 hours, and then treated as above.

Effect of High Temperature in Blocking the Response to Low Temperature

The excised shoots were submitted to the following treatments:

1 - Controls, no treatment

2 - 10°C for 96 hours

3 - 10°C for 96 hours, 30°C for 24 hours or 20°C for 24 hours

4 - 10°C for 96 hours, 30°C or 20°C for 24 hours, 10°C for 48 hours

5 - 10°C for 48 hours

6 - 20°C for 96 hours

The apices were then grown as previously described.

Effects of Chemical Treatment

The following chemicals were used either alone or in combination: sucrose (10 percent); ethephon (100 ppm); kinetin (100 ppm); GA_3 or $GA_{4/7}$ (100 ppm); ABA (1 ppm); Amo 1618 (100 ppm) and IAA (1 or 10 ppm). Distilled water served as a control. The possible antagonistic effects of sucrose and kinetin on ABA or Amo 1618 treatment were also investigated.

The apices were soaked in the test solution for 10 hours. They were then either planted directly at room temperature or exposed to 10°C for 96 hours and then planted at room temperature.

Effects of Temperature, ABA, and Amo 1618 on Levels of Reducing Sugars in Onion Shoots

Onion shoot apices (var. Spartan Banner) were exposed to 0, 5, 10, 20 or 30°C for 96 hours. Additional apices were

dipped into solutions of ABA (1 ppm), or Amo 1618 (100 ppm) for 10 hours before exposure to 10°C for 96 hours. Exposure to temperature was carried out in the dark to prevent development of chlorophyll. For extraction of reducing sugars the method of McCready et al. (87) was followed with some modifications. One gram of the treated material was cut into small pieces and ground in a mortar with 4 ml of hot 80 percent ethanol. The mortar was rinsed twice with 2 ml of hot 80 percent ethanol. The extract was transferred to a centrifuge tube, stirred, allowed to stand for 20 minutes, and then centrifuged for 10 minutes at 10,000 rpm. The supernatant solution was then decanted into a flask.

Reducing sugars were determined by the Nelson Method (32,89). Aliquots of 0.2 ml of the supernatant solution in a test tube was mixed with 1 ml of Nelson's reagent, prepared just before use by mixing 25 ml of solution A consisting of:

12.5 grams of Na_2CO_3
12.5 grams of potassium sodium tartrate
10.0 grams of $NaHCO_3$
100.0 grams of Na_2SO_4
500.0 ml of distilled water

with 1 ml of solution B consisting of:

7.5 grams of $CuSO_4.5H_2O$
50.0 ml of distilled water
1 drop of concentrated H_2SO_4

The tubes were placed in a boiling water bath for exactly 20 minutes and cooled by plunging them immediately in a cold

water bath. One ml of arsenomolybdate reagent was added to each test tube and color was developed by shaking vigorously. The volume was adjusted to 10 ml with distilled water and read at 540 nm in a colorimeter.

Preparation of arsenomolybdate reagent:

Solution A

25 grams of $(NH_4)_6\ MO_7O_{24}\cdot 4H_2O$
450 ml of distilled water
21 ml of concentrated H_2SO_4

Solution B

3 grams of $Na_2HA_sO_4\cdot 7H_2O$
25 ml of distilled water.

Solutions A and B were mixed and stored together at 37°C for 24 hours during which time the reagent turned yellow. A freshly prepared solution of glucose (100 mg/l) was used as a standard. Four replicates were run for each treatment. The data were statistically analyzed and Duncan's new multiple range test was used for comparing the treatment means (41).

Level of Acidic Inhibitor in Onion Shoots During Storage

Extraction

The samples were extracted and the acid fraction prepared according to the procedure used by Aung and De Hertogh(4) for the extraction of gibberellins. Shoot apices excised from MSU 4535 and Downing's Yellow Globe bulbs were removed from

storage after 2, 4, 8 and 16 weeks at 10°C or 16 weeks at 0°C. After 8 weeks at 10°C, all the Downing's Yellow Globe bulbs had sprouted, so no data are presented for Downing's Yellow Globe stored at 10°C for 16 weeks.

After removal from storage, the onions were frozen and kept at -18°C until extracted. The bulbs were allowed to thaw for 4 hours. The shoots were then excised, frozen in liquid nitrogen, homogenized with absolute methanol in a Waring blendor, and extracted with constant shaking for 48 hours at room temperature. The extractions were run in duplicate for each treatment. After filtration through double Whatman paper No. 3 and Celite 535, the methanolic extracts were concentrated in vacuo at 40°C. The dry residues were resuspended in 0.33 M KH_2PO_4 buffer at pH 8 and petroleum ether. The aqueous phase was partioned 5 times with fresh petroleum ether and the petroleum phase similarly partitioned 5 times with fresh buffer. The petroleum ether phase was discarded. The aqueous phase was further partitioned 3 times with ethyl acetate, the latter being discarded. The pH of the aqueous layer was adjusted to 2.5 with HCl, and extracted 5 times with ethyl acetate. After the excess water had been frozen out, the ethyl acetate fraction was evaporated to dryness in vacuo and the residue resuspended in absolute ethanol. This ethanolic solution constituted the free acidic fraction and was stored at 5°C until used.

Chromatography

Duplicate aliquots of 10 grams of the acidic fraction were evaporated *in vacuo*, dissolved in 0.2 ml of absolute ethanol, and streaked on 100 micron thick silica gel thin-layer chromatograms (Eastman Kodak Co.). Each aliquot was applied to a section of a plate 6 cm wide, leaving a third section for use as a blank control. The plates were developed (ascending) to a height of 15 cm in isopropanol:ammonium hydroxide:water (10:1:1, v/v). The developing tank was lined with filter paper and equilibrated for 48 hours before use. Four chromatograms (i.e., 2 per extraction), were developed for each treatment. After drying, the chromatograms were cut into ten 1.5 cm sections representing 1 Rf unit, and eluted with absolute ethanol for 12 hours. The eluates were evaporated to dryness *in vacuo*, and aliquots were removed for assay with wheat coleoptile sections.

Once the zone of inhibition had been established by bioassay, eluates from the active region of the chromatogram were combined, and aliquots representing 2 gram-equivalents fresh weight of tissue were removed. Relative levels of inhibitors were determined by assaying these aliquots with wheat coleoptile sections.

Wheat Coleoptile Bioassay

The general procedure followed was that described by Nitsch and Nitsch (91). Genesee wheat seeds were used throughout the experiment. The seeds were germinated in the dark at

25°C. When the coleoptiles were 2.5-3.0 cm long, 4-mm sections were cut 3 mm from the tip under green light. Five sections were placed in a test tube containing 0.3 ml of 2 percent sucrose, 10^{-2}M phosphate and 5 x 10^{-3}M citrate buffer (pH 5.0). A clinostat prevented geotropic curvature. A microscope provided with a micrometer eyepiece was used to measure the sections after 24 hours incubation in the dark.

Characterization of the Inhibitor

Thin Layer and Column Chromatography

An aliquot of the free acidic fraction (p. 27), equivalent to 8 grams of onion shoot, was chromatographed on silica gel thin-layer plates with fluorescent indicator. Four different solvent systems were used:

1 - Isopropanol:ammonia: water (10:1:1 v/v/v)
2 - Benzene:acetic acid:water (8:3:5 v/v/v)
3 - 1-Butanol:n-propanol:ammonia:water (2:6:1:2 v/v/v/v)
4 - 1-Butanol:acetic acid:water (40:11:29 v/v/v)

For further identification, onion extract and synthetic ABA were separately chromatographed in solvent 1. Standard ABA was located by examining the plates under ultraviolet light, and the corresponding zone on the extract plate was scraped and methylated with diazald(N-methyl-N-nitroso-p-toluene-sulfonamide) according to Schlenk and Gellerman (112) as modified by Powell (103). Diethylether was substituted for the methylene chloride. The methylation procedure involved:

1 - Dissolving the sample in a 1:9 methanol:ether solution.

2 - Generating diazomethane from a test tube containing 1.5 ml of carbitol(2-(2-ethoxy-ethoxy)ethanol), 1.0 ml of 60% potassium hydroxide, and 1.5 ml of a saturated solution of diazald(N-methyl-N-nitroso-p-toluenesulfonamide) in ether into the sample tube in a stream of nitrogen saturated with ether.

3 - Stopping the reaction upon appearance of a yellow color in the sample tube.

4 - Evaporating the methanol:ether solution of the sample tube under a stream of nitrogen.

Both the methylated extract and methylated synthetic ABA were chromatographed on silica gel thin-layer plates in solvent 1. The location of synthetic Me-ABA was detected under UV light. Eluates of this region were bioassayed with wheat coleoptile sections. Non-methylated ABA was chromatographed as a control.

Synthetic ABA and the acidic fraction were also chromatographhd on charcoal-celite (1:2) columns (charcoal:Darco G-60: Celite-535:Johns-Manville, Lompac, California. One hundred fifty ml of 60 percent acetone in water was used for elution. Ten-ml fractions were collected, and evaporated under reduced pressure at 40°C, and the residues were assayed with wheat coleoptile sections.

Gas-liquid Chromatography and Mass Spectrometry

Eluate from the zone of the highest biological activity (Rf 0.4-0.7) on TLC silica gel plates developed in solvent 1, and the active tubes (4 to 10) from the charcoal celite column were methylated as previously described.

Methylated samples were dissolved in chloroform before injection into the Packard 7300 series gas-liquid chromatograph with flame ionization or electron capture detector. One g -equivalent of tissue and 1 ng authentic ABA were injected.

The samples were then dissolved in hexane and analyzed in LKB 9000 Gas Chromatograph-Mass Spectrometer whose conditions are also described in Table 1.

Table 1. Conditions of gas chromatography and mass spectrometry used in the characterization of the onion inhibitor.

Instrument:	Packard 7000 Series	Packard 7000 Series	LKB 9000 Gas Chromatograph-Mass Spectrometer
Column	6 ft U-shaped glass column 2 mm i.d.	6 ft U-shaped glass column 2 mm i.d.	4 ft coiled glass column 2 mm i.d.
Solid support	Gas-Chrom-Q (60/80 mesh)	Gas-Chrom-Q (60/80 mesh)	Supeleo-port (80/100)
Liquid phase	3% DC - 200 (12,500 cstk)	3% DC - 200 (12,500 cstk)	3% SE - 30
Detector	Electron capture (15 m Ci Ni^{63} at 5v)	Flame ionization	Total ion current
Gas flow H_2		40 ml/min	
N_2	75 ml/min	40 ml/min	
Air		400 ml/min	
He			30 ml/min
Temperature			
Inlet:	250°C	250°C	220°C
Column:	210°C	210°C	200°C
Detector:	280°C	280°C	290°C

RESULTS AND DISCUSSION

Field Studies of Growth and Maturation

The time of occurrence of maximum linear growth coincides with that for maximum bulbing index for each variety (Figure 2, Table 2). Senescence begins simultaneously for varieties and for linear growth and bulbing index. These changes occurred in Downing's Yellow Globe about one week earlier than in MSU 4535. MSU 2935, a long dormant line, originally was included in this study. Unfortunately, the season was too short and this variety was not harvested because of immaturity at harvest time. The growth curve for this variety (data not shown) was a straight line.

No comparison of the growth of Abundance, Downing's Yellow Globe, and Spartan Banner was made in summer 1971. However, Abundance was first to mature, followed by Downing's Yellow Globe. At the time of harvest, when frosts were imminent, 50 percent of the Spartan Banner tops were still green, necessitating careful selection of bulbs with dry tops.

Good keeping onions thus appear to have a longer growing period than poor keeping varieties. Tronickova's (128,129) data supports this conclusion. Abdalla and Mann (1) observed

Figure 2. Bulb development of Downing's Yellow Globe and MSU 4535 as measured by the diameter of the leaf base. Measurements were started 38 days after sowing. Each point is the average of 40 plants.

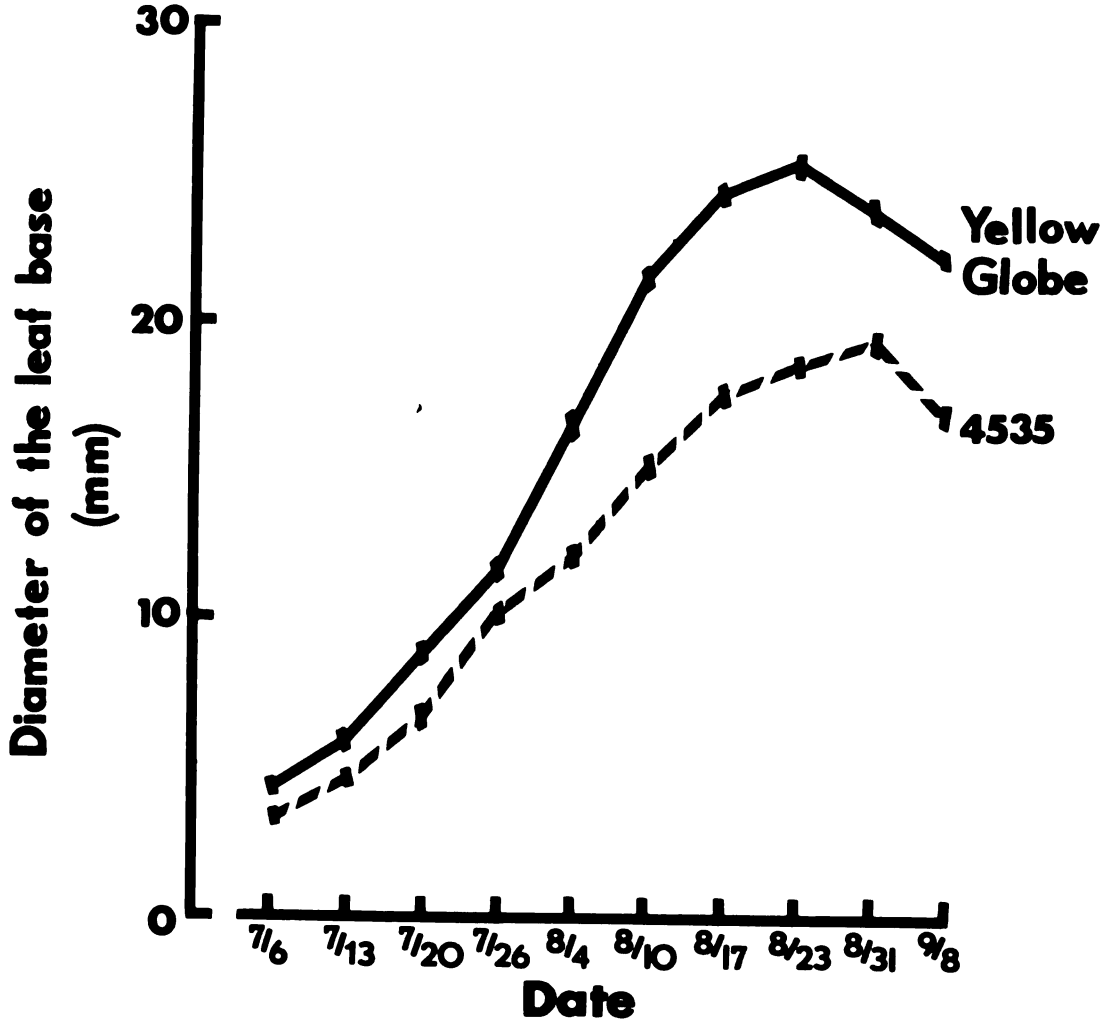
30
20
10
0
Diameter of the leaf base (mm)
Yellow Globe
4535
7/6
7/13
7/20
7/26
8/4
8/10
8/17
8/23
8/31
9/8
Date

Figure 2

Table 2. Bulbing indices of Downing's Yellow Globe and MSU 4535 during development, 1970.

Variety	Date						
	7/16	8/4	8/10	8/17	8/23	8/31	9/8
Downing's Yellow Globe	1.15	1.45	1.72	1.80	2.00	1.95	1.84
MSU 4535	1.12	1.30	1.43	1.70	1.80	1.85	1.63

*Bulbing index: Ratio of maximum diameter of the base to minimum diameter of the neck (31). Each figure is the mean of 40 plants.

that under California conditions, the variety "Excel", a short dormant type, matured one month earlier than Australian Brown 5, a long dormant one.

Dormancy in onion is obviously genetically controlled. Correlations between growth rate and keeping quality are possibly due to the synthesis of some substances during the growing period which accumulate in the bulbs and prolong the dormant period. Kato's (65) data showing that the auxin content increased earlier in an early variety than in a mid-season variety are also a plausible explanation.

Effect of Temperature During Storage of Whole Bulbs

Continuous Temperature

The sprouting response of Downing's Yellow Globe and MSU 4535 held at 2 temperatures are compared in Figure 3. Downing's Yellow Globe sprouted at a faster rate than MSU 4535. Under dry conditions, 10°C appeared to be more effective than 0°C in breaking dormancy in MSU 4535, while for Downing's Yellow Globe 0°C was more effective. Considerable rotting and deterioration occurred in the bulbs placed in water after storage, thus the data are not reported. In general, 10°C was more effective in breaking dormancy in MSU 4535 bulbs placed in water than either 0 or 20°C. Conversely 0°C was more effective for Downing's Yellow Globe while 10 and 20°C tended to delay sprouting.

Figure 3. Effect of storage temperatures (0-10°C) on subsequent sprouting of MSU 4535 and Downing's Yellow Globe onion bulbs exposed to 20°C in dry air. The controls were put to sprout in dry air immediately after curing. Incomplete graphs indicate that bulbs had sprouted in storage before transfer to 20°C. Each point is based on a 20-bulb sample.

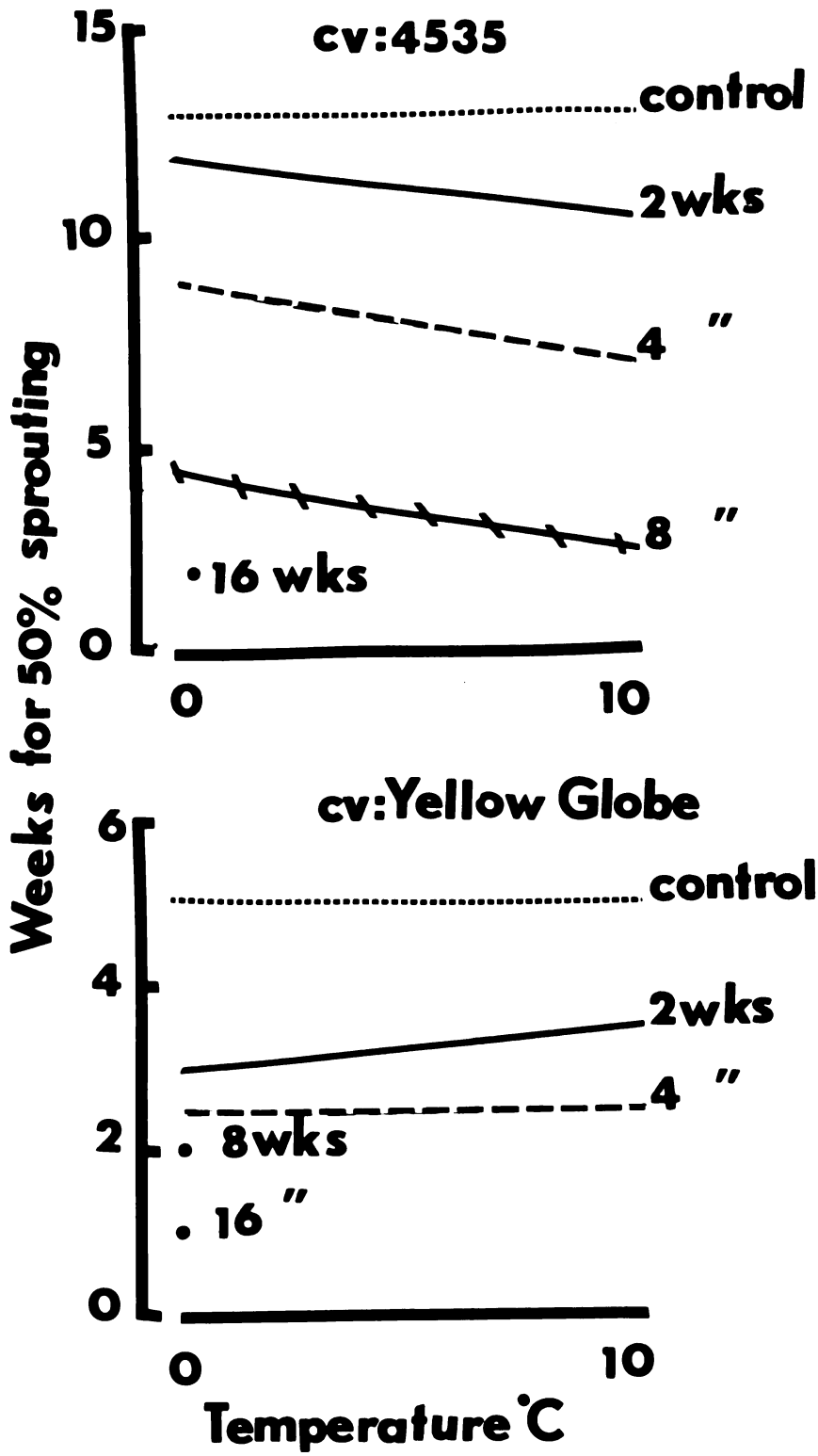
cv:4535
control
2wks
4 "
8 "
16 wks
15
10
5
0
0
10
cv:Yellow Globe
control
2wks
4 "
8wks
16 "
6
4
2
0
0
10
Weeks for 50% sprouting
Temperature °C

Figure 3

A similar experiment with Spartan Banner was undertaken in 1971. In contrast to the results obtained with MSU 4535 and Downing's Yellow Globe, all temperatures were effective in promoting sprouting. After 16 weeks, the most rapid sprouting occurred in bulbs stored at 20°C, the slowest in those stored at 0°C (Figure 4).

Root formation as well as root development in response to temperature showed the same trends as sprouting (Figures 5 and 6) but the differences between temperatures were less marked. Early sprouting was associated with better root development at 20 and 30°C.

The smaller the bulbs, the more quickly they sprouted and rooted when transferred to moist conditions at 20°C (Figures 7 and 8).

Regardless of variety and temperature, increasing the storage period reduced the time required for rooting and sprouting. However, differences were observed in sprouting of the varieties. Dormancy in onion is therefore a hereditary property, with the beginning, duration and the end of rest genetically controlled.

Magruder et al. (85), reported differences in keeping quality among onion varieties. Abdalla and Mann (1) found that Australian Brown 5 and Excel responded differently to identical temperature. Although the internal conditions of the onion bulbs at the time of storage as well as the duration of

Figure 4. Effect of storage temperatures (0-30°C) on subsequent sprouting of Spartan Banner onion bulbs exposed at 20°C with their base in moist peat moss. Each point is the average of 3 replicates of 20 bulbs each. The controls were planted in the moist peat moss immediately after curing. Vertical bars = Standard error.

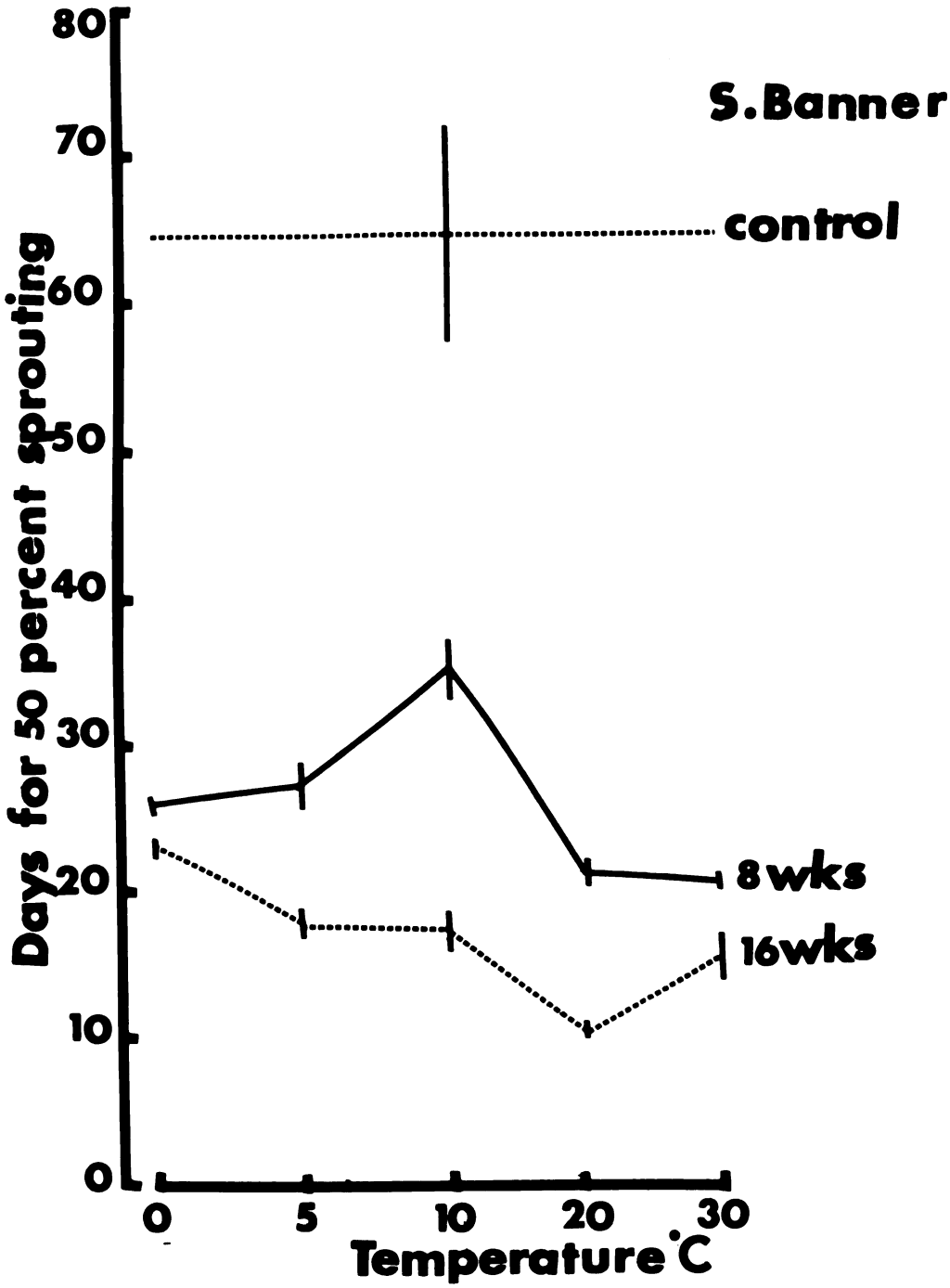
S.Banner
control
8wks
16wks
Days for 50 percent sprouting
80
70
60
50
40
30
20
10
0
0
5
10
20
30
Temperature °C

Figure 4

Figure 5. Effect of storage temperatures (0-30°C) on rooting of Spartan Banner onion bulbs planted in moist peat moss at 20°C after storage for 8 and 16 weeks. The controls were planted immediately after curing. Vertical bars = Standard error.

Figure 6. Root growth and development of Spartan Banner onion bulbs planted in moist peat moss at 20°C after 8 weeks of temperature treatment (0-30°C). Root measurement was made 10 days after planting. Vertical bars = Standard error.

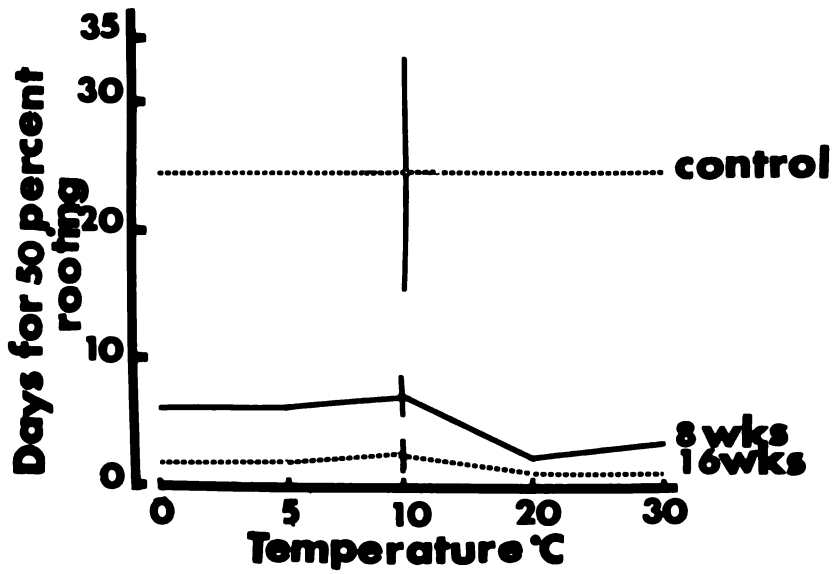

Figure 5

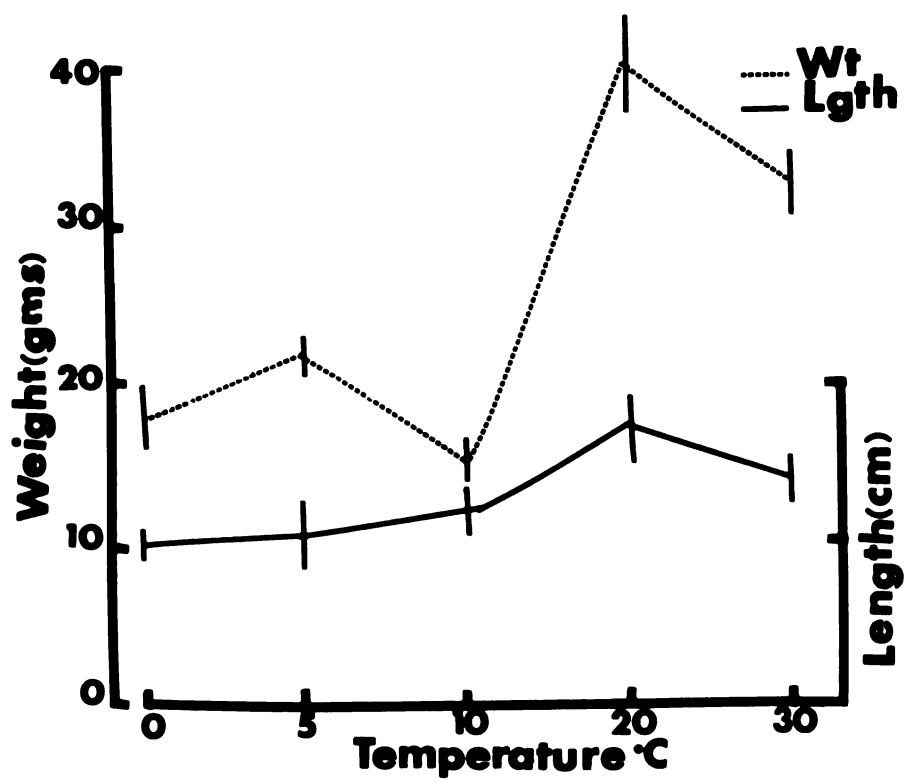

Figure 6

Figure 7. Effect of size on sprouting of Spartan Banner onion bulbs planted in moist peat moss at 20°C after storage for 8 weeks at 10°C. The controls were planted immediately after curing.

A = Small size bulbs (4-5 cm diam.)
B = Medium size (7-8 cm diam.)
C = Large size (10-11 cm diam.)
Vertical bars = Standard error.

Figure 8. Effect of size on rooting of Spartan Banner onion bulbs planted in moist peat moss at 20°C after storage for 8 weeks at 10°C. The controls (before storage) were planted immediately after curing.

A = Small size bulbs (4-5 cm diam.)
B = Medium size (7-8 cm diam.)
C = Large size (10-11 cm diam.)
Vertical bars = Standard error.

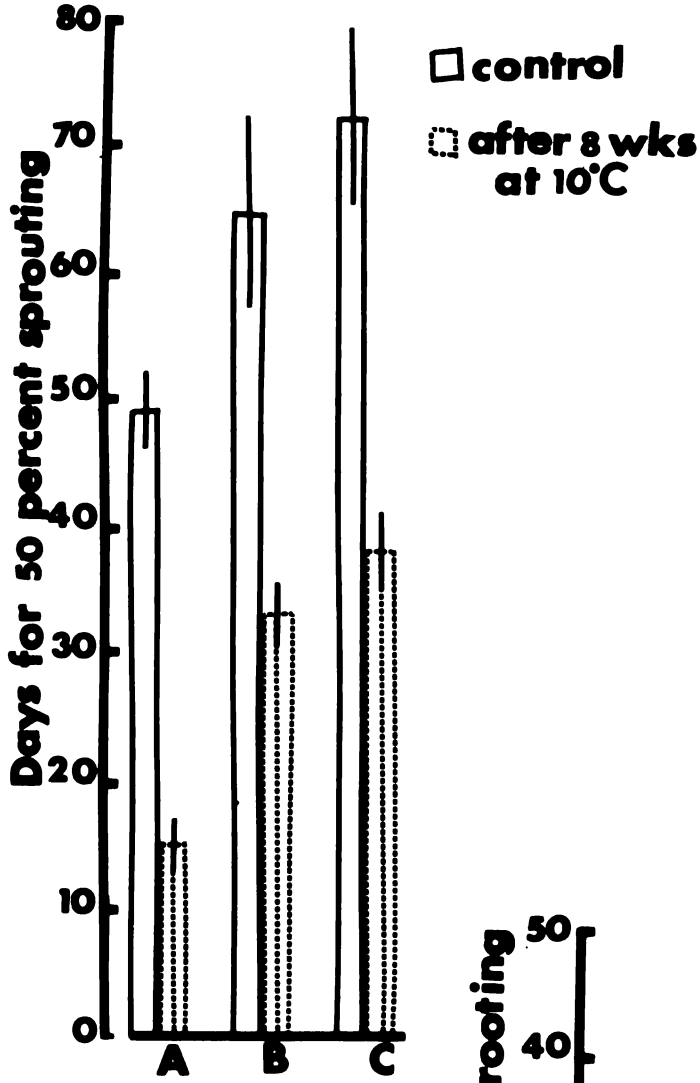

Figure 7

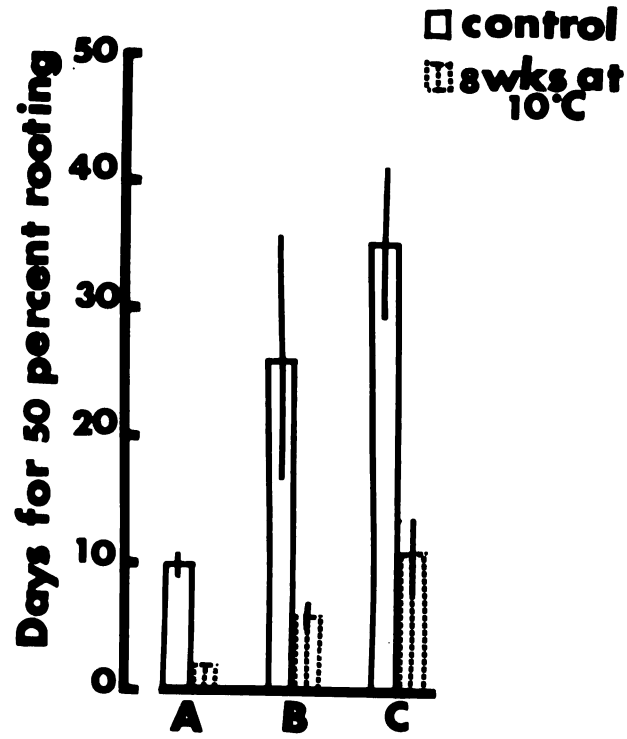

Figure 8

the storage are important, most of the varieties have specific temperature requirements to break their dormancy.

The size of onion bulbs affects flowering and seed production. The plants developing from small bulbs tend to remain vegetative while plants from medium and large size bulbs are more inclined to produce flowers and seeds (2,3,88,115). Small size bulbs also produce flowers earlier than large bulbs (3). Size may also affect dormancy. Small excised plugs from potato tubers sprout earlier than large ones (107). Possibly the lower resistance of small bulbs to temperature change accounts for this observation. Aura's finding (6) that small onion bulbs required a shorter heat treatment to prevent flowering than did large ones supports this hypothesis.

Rooting precedes sprouting. Since it appears to have a major effect on sprout emergence, temperature may modify onion shoot dormancy through its effect on root dormancy. The data in Figures 5 and 8 show that there is root dormancy in the onion which disappears with time. This dormancy, in general, is affected similarly to shoot dormancy by temperature except that it is broken earlier. The effect of storage temperature on root growth as expressed by length and weight (Figures 6 vs 4) again suggests that temperature influences onion shoot dormancy through its effect on the root, in the sense that whenever root initiation and growth are promoted, shoot growth is also enhanced. Conversely, frequent observations revealed that in general unsound bulbs may sprout without

forming roots and did not subsequently root. Apparently, the growing sprout depletesthe food reserves at the expense of the root primordia. Lathrop and Mecklenburg's (75) data indicating in Taxus sp that with the increase in shoot growth after dormancy there is a concomittant decrease in root regeneration potential, supports this hypothesis.

Effects of Intermittent Temperature, Root Removal and Treatment with Kinetin on Sprouting

Table 3 shows the effects of intermittent temperature and root removal on the subsequent sprouting of Abundance onions. The controls were placed in the rooting medium without any prior temperature treatment. High temperature (30°C) delayed sprouting or induced secondary dormancy when applied after a low temperature treatment. However, the effects of high temperature were completely offset by an additional 3 weeks of 10°C. Rooting showed the same trend as sprouting. Root removal delayed sprouting. A similar effect is shown in Table 4 for root removal, but the effect of the root could be partially restored by kinetin application.

Abdalla (1) also showed that root removal could delay sprouting of onion. Shoot elongation may be dependent upon the supply of water and nutrient elements from the roots. In our experiment, however, the derooted onion bases were in direct contact with the moist medium, so water deficiency probably cannot account for the result. Nutrient supply probably

Table 3. Effect of intermittent warm-cold temperature on sprouting and rooting, and the effect of root removal on sprouting of Abundance onion bulbs planted at 20°C in moist peat moss (days to 50% sprouting or rooting).

Treatment	Sprouting	Rooting
Control, not stored	35.2 ± 2.69	13.7 ± 6.14
8 wks at 10°C	17.3 ± 2.07	3.00 ± 0.82
8 wks at 10°C; 2 wks at 30°C	30.4 ± 2.85	15.5 ± 0.97
3 wks at 10°C; 2 wks at 30°C; 3 wks at 10°C	8.66 ± 1.69	2.00 ± 0.00
8 wks at 10°C, then derooted daily.	23.67 ± 1.67	

Table 4. Effect of root removal and kinetin treatment on sprouting of Spartan Banner onion bulbs planted at 20°C in moist peat moss (days to 50% sprouting).

Treatment	Sprouting
Control, not stored	32.70 ± 2.51
8 weeks at 0°C	12.50 ± 1.03
8 weeks at 0°C; injected with water and derooted daily	21.50 ± 1.07
8 weeks at 0°C; injected with kinetin and derooted daily	16.50 ± 1.00

is not a critical factor because in the early stage of shoot growth, the fleshy scales surrounding the shoot apices can provide the necessary nutrients. Thus the root may be providing something that neither the medium nor the scales can provide.

The fact that kinetin could partially substitute for the roots suggests that the latter provide growth substances. Went (139) hypothesized that a specific shoot factor, caulocaline, was synthesized in the roots. There is now direct and indirect evidence that the roots are the source of cytokinin for the aerial parts of many plant species (22,63,83,70,114). A similar situation may exist in onion, but further study is needed to characterize this hormonal substance.

Effect of Wounding and Oxygen Treatment of Unchilled Bulbs on Sprouting and Rooting

While transverse wounding was more effective in promoting sprouting of both cultivars than was longitudinal wounding, the latter promoted rooting while the former did not (Table 5). Oxygen application did not alleviate onion dormancy; it even prolonged it in Abundance. Thus, lack of oxygen cannot be responsible for dormancy in onion. A similar conclusion was reached for Phacelia tanacetifolia seeds (25) and for potato (111). Consequently, some other explanations should be offered for the wounding effect.

Table 5. Effect of wounding and 100% oxygen on sprout and root emergence of MSU 4535 and Abundance onion bulbs. (Weeks for 50% sprouting and rooting.)

Treatment	MSU 4535		Abundance	
	Sprouting	Rooting	Sprouting	Rooting
Control	11	5	5	2
Transverse wounding	5	4	3	2
Longitudinal wounding	9	3	3	1
Oxygen	10	5	6	3

Wounding of plant organs leads to numerous metabolic changes, including an increase in respiration (11,119), renewed capacity for protein synthesis (42,43), increase in mitochondrial number (77), RNA synthesis (76) and phospholipid synthesis (121). The onset of renewed growth may be related to changes in hormonal balance (16,56,116). Slicing of apple resulted in an increase of ethylene synthesis (79). Similar changes probably occur in onion following wounding.

Growth of Excised Apices

Effect of Storage Temperature on Growth of Apices in situ

Growth of shoot apices during storage of whole bulbs was greatest at 10°, least at 0°, and intermediate at 20 or 30°C, for all cultivars (Figure 9). Response of Downing's Yellow Globe is omitted as it was identical with that of MSU 4535.

Abdalla and Mann (1), performing a similar experiment with the cultivar Excel found that 0 and 30°C retarded growth of the intact shoot. Periodic observation of sprout leaf elongation inside the bulbs can be a fairly accurate way to ascertain the degree of dormancy without waiting weeks or months for visible sprouting. These results confirm that at intermediate temperatures of 10-20°C onion bulbs tend to sprout in storage at a higher rate than at low (0°) or high temperature (30°C).

Figure 9. Effect of storage temperature on growth of intact inner sprout leaves of MSU 4535 and Spartan Banner onion. Each point is the mean of 6 sprout leaves excised from 6 uniform bulbs for MSU 4535 and 20 for Spartan Banner.

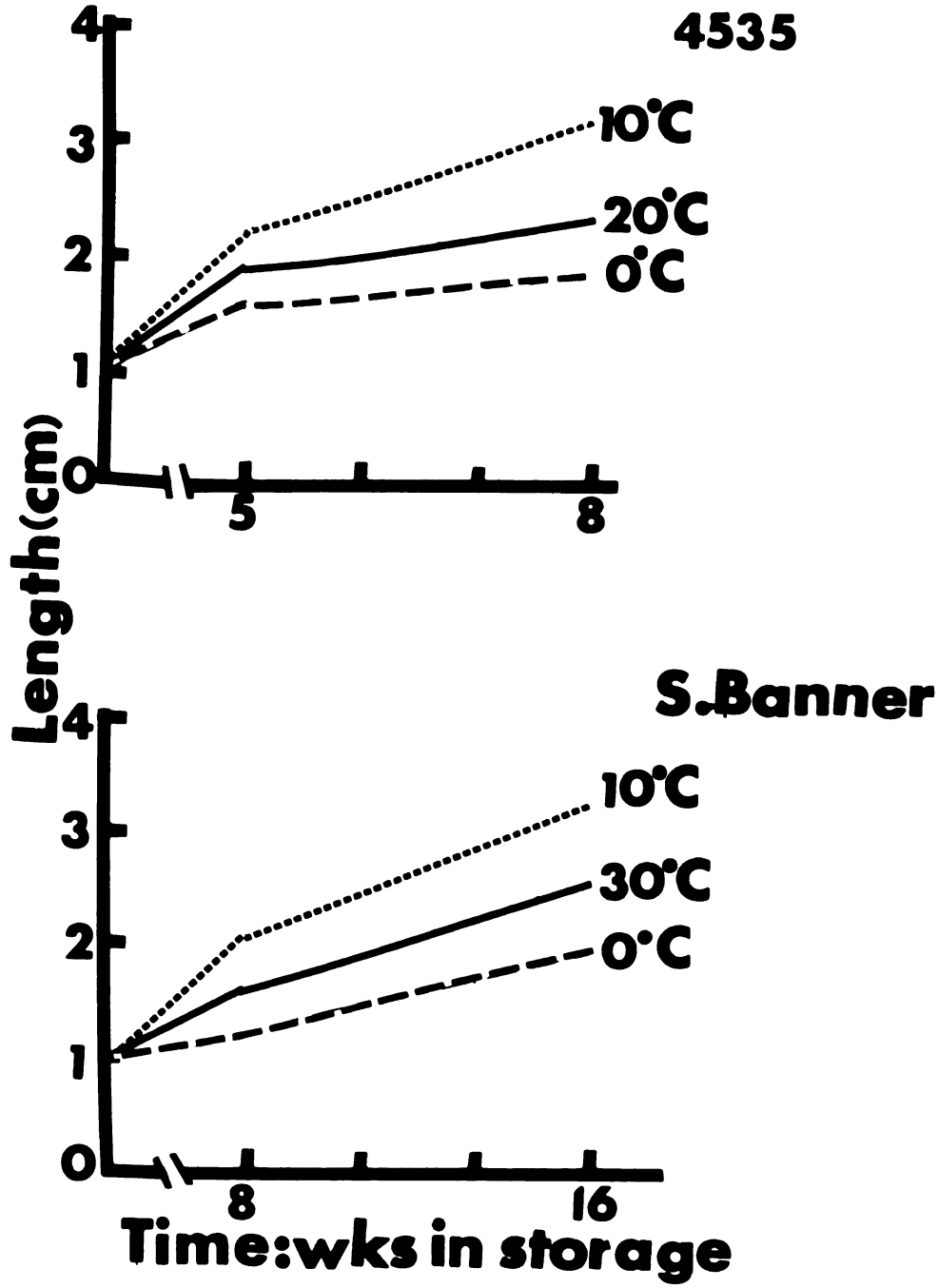
4535
10°C
20°C
0°C
4
3
2
1
0
5
8
Length(cm)
S.Banner
10°C
30°C
0°C
4
3
2
1
0
8
16
Time:wks in storage

Figure 9

Growth of Excised Apices

Effective chemical treatment of onion bulbs is difficult because of the protected position of the apices, i.e., the potential sprout leaves. In addition, one bulb can contain up to 3-6 shoots, making accurate injection of solutions difficult. Furthermore, variability exists in the degree of dormancy in the same lot of bulbs, requiring the use of many onions to reduce experimental error. Since color change of the shoots is a fairly accurate index of dormancy (58), the use of excised shoots allows selection of uniform material. Although shoot preparation requires some skill and is time consuming, response to treatment can be obtained more quickly than with entire bulbs. Finally, this system in many instances constitutes a check for the response of whole bulb to some treatments.

Effect of Storage Temperature Upon Subsequent Growth of Excised Apices

Five weeks of storage markedly promoted growth of sprout leaves following their excision from the bulbs (Figure 10). Temperatures of 0 and 10°C were equally effective on Downing's Yellow Globe while 20°C was much less effective. Results were similar for MSU 4535 except that 10°C was only slightly more effective than 20°C. Eight weeks at 0°C produced maximal growth in both cultivars.

Figure 10. Elongation of excised sprout leaves as influenced by previous storage temperature. Excised sprout leaves were planted in moist sand in the dark at 20°C and growth measurement was recorded at different periods. Each point represents the average of 6 excised sprout leaves.

A = MSU 4535
B = Downing's Yellow Globe

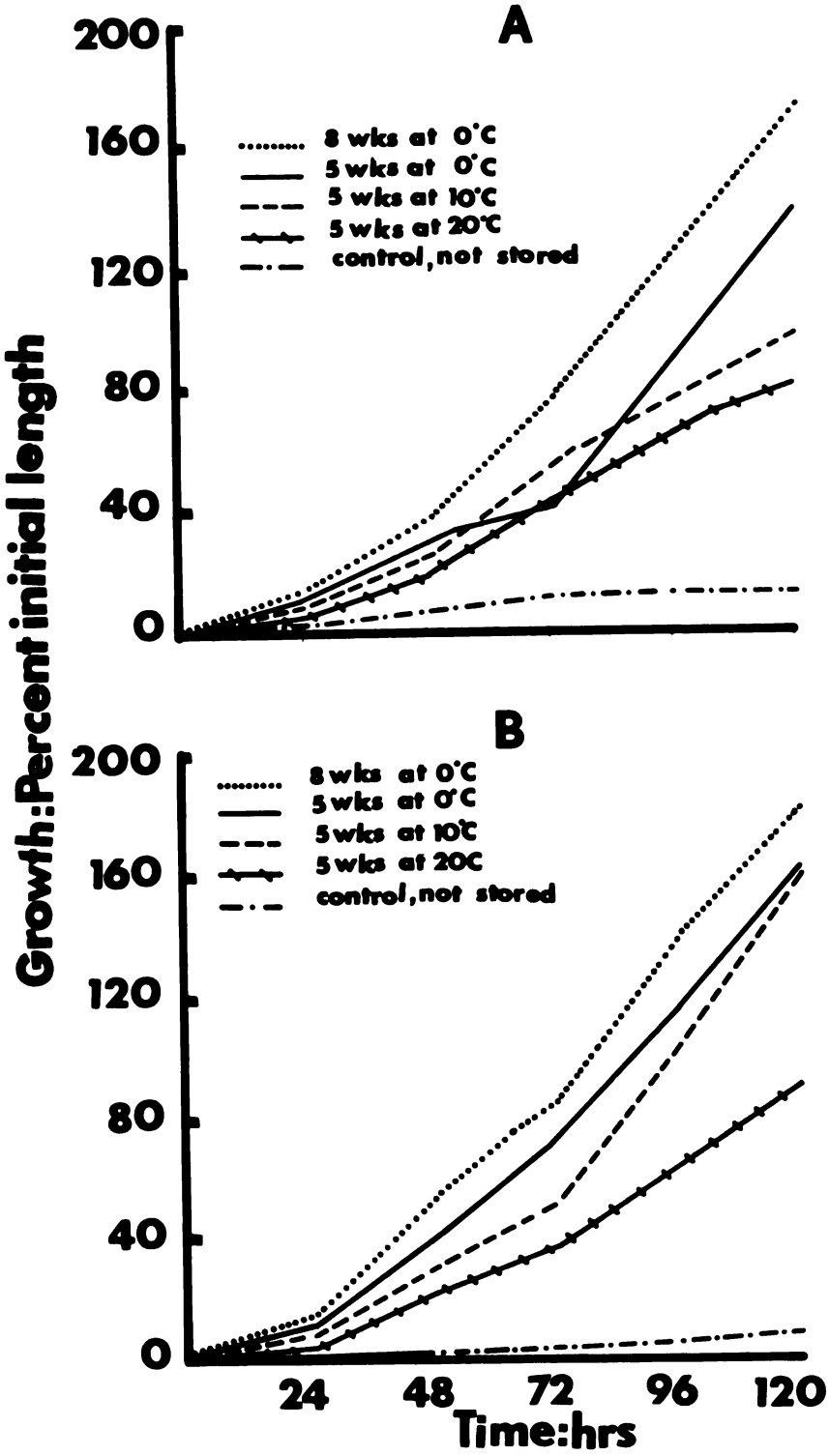
A
200
160
120
80
40
0
8 wks at 0°C
5 wks at 0°C
5 wks at 10°C
5 wks at 20°C
control, not stored
B
200
160
120
80
40
0
8 wks at 0°C
5 wks at 0°C
5 wks at 10°C
5 wks at 20C
control, not stored
Growth: Percent initial length
24
48
72
96
120
Time: hrs

Figure 10

Data for Spartan Banner (Figure 11) show similar trends. After 8 weeks storage, apices from bulbs stored at 0°C grew the most, while 30 and 10°C were much less effective. After 16 weeks storage, however, 0 and 30°C were equally effective.

Growth of shoots *in situ* was least at 0° (Figure 9). Yet this storage temperature was most effective in promoting subsequent growth of excised shoots at 20°C. Thus, 0°C is probably effective for storage of onions, not because it keeps them dormant, but because it prevents or retards shoot growth. A similar result is reported by Boswell (15) with bulbs planted in the field. Bulbs stored at 0°C produced more vigorous plants than those stored at higher temperatures. The efficiency of the 0°C treatment in promoting subsequent growth of the excised shoots may be attributed to lower utilization of food reserves, during the low temperature treatment as a result of reduced respiration. These materials were, therefore, available when the excised shoots were placed at 20°C.

Effect of Cutting Bulbs Prior to Temperature Treatment Upon Subsequent Growth of Excised Apices

Shoot apices directly exposed to the low temperature treatment grew more when planted in moist sand at 20°C than those excised from similarly treated half-cut or intact bulbs (Tables 6 and 7). Although the growth of the shoots excised from the half-cut *vs* intact bulbs in experiment 2 did not differ significantly, the former did grow more. Thus the

Figure 11. Elongation of sprout leaves excised from Spartan Banner onion bulbs after exposure to various storage temperatures for 8 and 16 weeks. The sprout leaves were planted in moist sand for 96 hours in the dark at 20°C. Each point is the average of 20 sprout leaves. Vertical bars = Standard error.

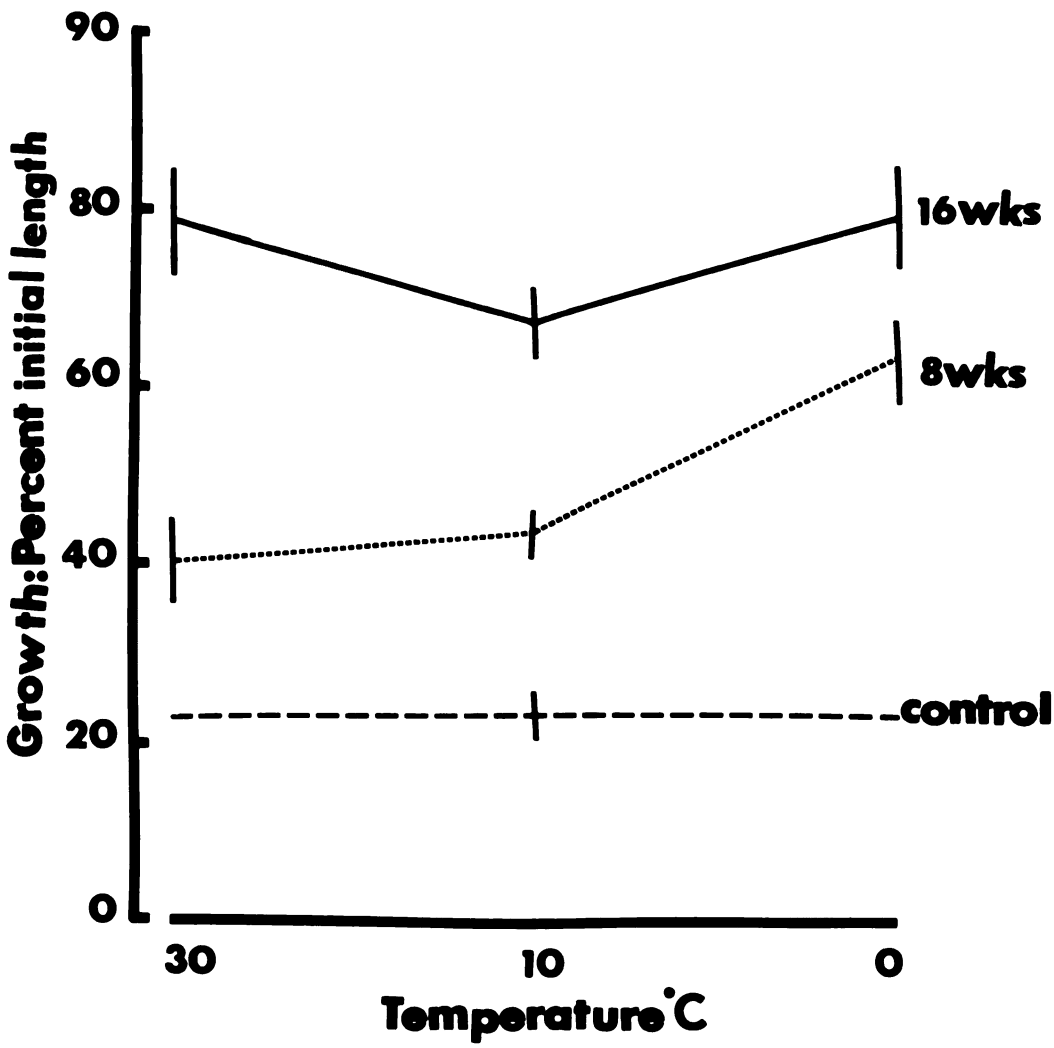
90
80
60
40
20
0
Growth: Percent initial length
16wks
8wks
control
30
10
0
Temperature °C

Figure 11

Table 6. Effect of exposure of onion shoots, half-cut bulbs and whole bulbs to temperature (10°C) for different period of time on the subsequent growth of excised shoots planted in moist sand in the dark at 20°C for 96 hours (cv Spartan Banner grown in greenhouse). Growth: percent increase over initial length.

Experiment 1*	Time of Exposure (hrs)			
Treatment	0	24	48	72
Excised shoots	40.2±5.6	76.03±3.01	90.5±3.20	101.00±2.70
Half-cut bulbs		55.50±2.30	61.25±1.75	70.80±3.00
Whole bulbs		40.55±4.00	45.23±2.8	46.70±3.60

*Means and standard error for 10 shoots.

Table 7. Effect of exposure of onion shoots, half-cut bulbs and whole bulbs to 10°C for 96 hours on the subsequent growth of excised shoots planted in moist sand in the dark at 20°C for 96 hours (cv Spartan Banner grown in the field). Growth: percent increase over initial length.

Experiment 2*	Treatment		
Control, not chilled	Half-cut bulb	Whole bulb	Excised shoot
20.50^{a}	40.57^{b}	34.22^{b}	49.00^{d}

*Means followed by the same letter are not significant at the 5% level.

onion scales may play a role in dormancy. However, the shoot apex appears to be the primary receptor of the temperature stimulus.

Effect of Temperature and Length of Exposure Upon Subsequent Growth of Isolated Apices

The period of exposure, as well as temperature, affect the subsequent growth of the shoots (see Figure 12). The increasing order of effectiveness, whatever the period of exposure, is 0, 5 and 10°C. Response at each temperature increased with increasing time of exposure. Two additional experiments were designed to determine the optimum temperature (Table 8). In experiment 1, growth was maximal at 5 and 10°C, intermediate at 0°, and minimal at 20°C. The second experiment indicated that the temperature range 7.5-12.5°C was much more effective than the control and 20°C.

By comparison of these data with those for apices *in situ* (Figure 9), the effectiveness of 10°C and the ineffectiveness of 20°C are again confirmed. A contradiction, however, arises from comparison with Figures 10 and 11. In Table 8, 0° is less effective than 10°C, while in Figures 10 and 11 it is more effective. However, the data in Figures 10 and 11 were obtained with shoot apices excised from bulbs subjected to temperature treatment for 5 to 16 weeks while those in Table 8 are for shoot apices exposed for a short period of time. Thus the environmental conditions of the apices were not the same.

Figure 12. Effect of temperatures and time of exposure on subsequent growth of onion shoot planted in moist sand at 20°C in the dark for 96 hours. Each point is the average of 10 apices (cv Spartan Banner).

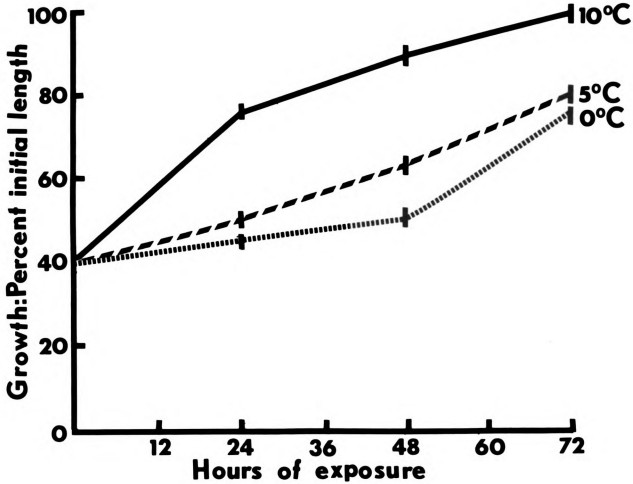
100
80
60
40
20
0
Growth:Percent initial length
12
24
36
48
60
72
Hours of exposure
10°C
5°C
0°C

Figure 12

Table 8. Effectiveness of different temperatures in stimulating growth (percent of initial length) of excised onion shoot (cv Spartan Banner) after 96 hours of exposure. Shoots planted in moist sand in dark at 20°C for 96 hours.

	Temperature (°C)**					
	Control	0	5	7.5	12.5	20
Experiment 1	27.15[a]	49.33[b]	62.51[c]	---	67.25[c]	22.42[a]
Experiment 2	14.32	---	---	40.17	42.00[b]	19.30[a]

**Means followed by the same letter are not significantly different at the 5% level.

*The controls were excised immediately, before planting.

In excised apices, the synthesis of materials necessary for growth apparently occurs at a faster rate at 10°C than at 0 or 5°C, but since the exposure is brief their utilization *in situ* is limited. Thus they are available for growth when the shoots are transferred to a suitable environment. During long periods of storage, on the other hand, the turnover rate of the synthesized material *in situ* would be higher. Shoot apices excised from bulbs previously stored at 20°C were partially depleted of reserves and consequently the growth is less.

Comparison of the temperature responses of shoot apices with those of entire bulbs (Table 8 *vs* Figures 3 and 4) shows that, in general, intact bulbs and shoot apices respond similarly to temperature except that the shoot apices are less responsive to high temperature (20°C). In both cases, 10°C appears to be most effective in promoting growth. However, 20°C is also effective for intact Spartan Banner bulbs (Figure 4).

The possible physiological importance of the scales should not be minimized. Besides the reserve material that they provide for the growth of the shoots, they probably participate in the dormancy-breaking process, perhaps by serving as insulation.

Effect of High Temperature in Blocking the Response to Low Temperature

The effect of intermittent temperature treatments on the subsequent growth of the shoot apices is illustrated in Table 9.

Table 9. Effect of high temperature (30°C) in inducing secondary dormancy in onion shoots (cv Spartan Banner) and its reversal by subsequent low temperature treatment (10°C). Growth is expressed as percent increase over initial length after 96 hours in moist sand in dark at 20°C.

Treatment	Growth*	
	Experiment 1	Experiment 2
Control, shoot not stored	20.15[a]	20.35[a]
96 hours at 10°C	39.78[b]	44.92[b]
96 hours at 10°C+ 24 hours at 30°C	16.25[a]	---
96 hours at 10°C+ 24 hours at 20°C	55.20[c]	---
96 hours at 10°C 24 hours at 30°C 48 hours at 10°C	---	38.47[b]

*Means followed by the same letter are not significantly different at the 5% level.

Exposure to a temperature of 30°C following the low temperature treatment induced secondary dormancy of the shoot apieces (Experiment 1), but this secondary dormancy was easily reversed by a relatively short exposure to low temperature (Experiment 2). Exposure to 20°C following the low temperature treatment was promotive. Comparison with the response of intact bulbs (Table 3) shows a striking similarity.

The cumulative effect of the low temperature treatment is illustrated in Table 10. Again the data indicate that low temperature need not be applied continuously to be effective, provided that the high temperature is not high enough to cause injury. In fact, interruption of the low temperature treatment by a period at 20°C resulted in some promotion of growth. Response increased with length of exposure to low temperature. Thus low temperature treatment appears to favor synthesis of growth promoters in the tissue. Increasing the exposure increases the accumulation of material, stimulates subsequent growth when placed at 20°C. The failure of rapid vacuum cooling, as practiced by Jaffe and Isenberg (60), to elicit significant growth supports this hypothesis.

In the intermittent temperature experiment, again the shoots behave the same way as the whole bulbs. Boden (12) found that alternating temperature promoted germination of dormant Eucalyptus pauciflora seeds. Some evidence for an antagonistic effect of high temperature on peach bud dormancy has been reported (10,96,138). However, high temperature

Table 10. Cumulative effect of low temperature treatment (10°C) in stimulating growth of excised onion shoots (cv Abundance) after planting in moist sand in dark at 20°C. Growth is expressed as percent increase over initial length.*

Treatment	Growth
Control	24.23[a]
96 hours at 10°C	45.49[bc]
48 hours at 10°C- 24 hours at 20°C- 48 hours at 10°C	51.27[c]
48 hours at 10°C	39.15[b]
96 hours at 20°C	18.36[a]

*Means followed by the same letter are not significantly different at the 5% level.

(30°C) is effective in inducing secondary dormancy of onion only when applied after a low temperature treatment (Table 9). A temperature of 20°C, although ineffective by itself, promotes growth when applied either after or between the low temperature treatment. This finding does not agree with the statement of Vegis (132) that "even a moderate rise in temperature at an early stage of post-dormancy can induce secondary dormancy" (p. 191).

Effects of Chemical Treatment

A preliminary experiment with intact bulbs indicated no effect of NAA, ethephon or GA_3 on Spartan Banner or Abundance, whereas kinetin hastened sprouting in both.

The effect of kinetin, IAA, GA_3, $GA_{4/7}$, and ethephon on excised onion shoots are presented in Table 11. Kinetin was again the only chemical effective in promoting growth. Ethephon and IAA were somewhat inhibitory, while GA_3 and $GA_{4/7}$ had no effect.

The effects of IAA and low temperature in a factorial experiment are shown in Figure 13. At a concentration of 1 ppm, IAA inhibited growth of nonchilled apices but promoted growth of chilled apices. Interaction was highly significant.

To determine if Amo 1618 would interfere with the response to chilling, this chemical was applied either before or after a low temperature treatment (Table 12). The former treatment markedly reduced shoot growth in comparison with the chilled control while the after-storage treatment had no effect.

Table 11. Effect of growth regulators on growth of excised onion shoots (cv Spartan Banner). Shoots were not chilled. Growth: percent initial length.*

Treatment	Growth
Water Control	18.64[a]
Kinetin, 100 ppm	37.13[b]
IAA, 10 ppm	17.19[a]
GA_3, 1000 ppm	18.83[a]
$GA_{4/7}$ 1000 ppm	18.67[a]
Ethephon, 100 ppm	14.83[a]

*Means with the same letter are not significantly different at the 5% level.

Figure 13. Effect of IAA and chilling on growth of shoots (Spartan Banner). Chilled shoots were held at 10°C for 96 hours. IAA (1 ppm) was applied before chilling. The main effect and chilling x IAA are significant at the 1% level. IAA is not significant at either 1% or 5% level.

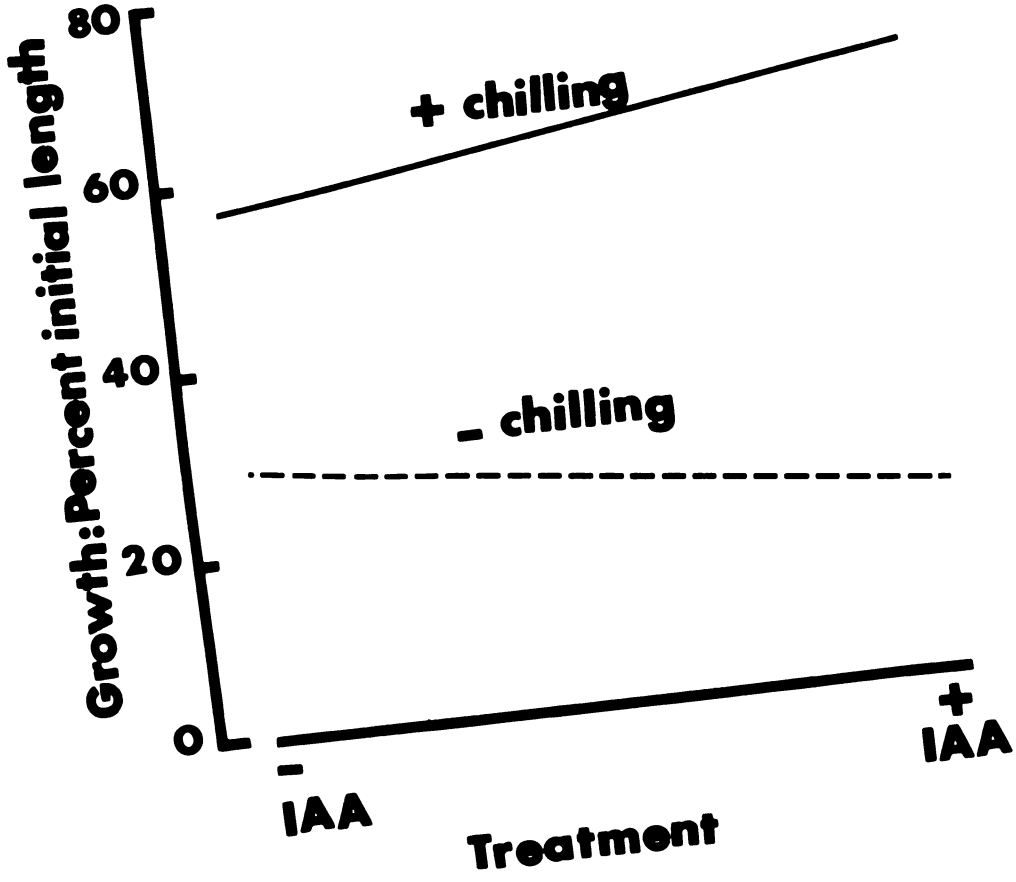
80
60
40
20
0
Growth: Percent initial length
+ chilling
- chilling
-
IAA
+
IAA
Treatment

Figure 13

Table 12. Effects of Amo 1618 and chilling on the growth of excised onion shoots (cv Abundance). Amo 1618 was applied before and after chilling. Growth: percent initial length.*

Treatment	Growth
Water control	14.40[a]
96 hours at 10°C	42.54[c]
Amo 1618, then 96 hours at 10°C	26.50[b]
96 hours at 10°C Amo 1618 after-	44.34[c]

*Means followed by the same letter are not significantly different at the 5% level.

These results suggest that gibberellins were synthesized during the chilling treatment.

In preliminary experiments, sucrose was more effective than either glucose or fructose in promoting growth of non-chilled excised apices. Sucrose was as effective as chilling (Table 13) in promoting growth of non-chilled onion shoots but had little effect on chilled shoots. Amo 1618 was not tested on non-chilled shoots, but it inhibited growth of chilled shoots. However, the synergistic effect of sucrose plus Amo 1618 was greater than that of sucrose alone (Table 13).

The effects of kinetin (Figure 14) were similar to those of sucrose (Table 13) in that both promoted growth of non-chilled shoots, but had no effect on chilled shoots. Kinetin greatly overcame the inhibitory effect of Amo 1618 of non-chilled shoots but was only partially effective on chilled shoots. Sucrose was not tested in combination with Amo 1618 on non-chilled shoots but it completely reversed the effect of the latter on chilled shoots, the response being even greater than that obtained with sucrose alone (Table 13).

Contrary to the results with Amo 1618, ABA was effective in inhibiting growth even when applied after temperature treatment. Sucrose was without effect on ABA action, and kinetin was only slightly effective in overcoming the ABA effect (Table 14).

Table 13. Effect of sucrose, Amo 1618 and chilling on growth of excised onion shoots (cv Downing's Yellow Globe). Growth: percent initial length.*

Treatment	Growth
Water control	20.35[a]
Sucrose, 10%	38.99[b]
96 hours at 10°C	39.18[b]
Sucrose - 96 hours at 10°C	44.55[b]
Amo 1618 - 96 hours at 10°C	19.75[a]
Amo 1618 - Sucrose 96 hours at 10°C	54.77[c]

*Means followed by the same letter are not significantly different at the 5% level.

Figure 14. Effect of kinetin and Amo 1618 with and without chilling on growth of onion shoots (cv Spartan Banner). Chilled shoots were held at 10°C for 96 hours. Kinetin and Amo 1618 were applied before chilling. The main effects (chilling, kinetin, Amo 1618) as well as the interactions (chilling x Amo 1618, kinetin x Amo 1618, chilling x kinetin, chilling x kinetin x Amo 1618) are significant at the 5% level.

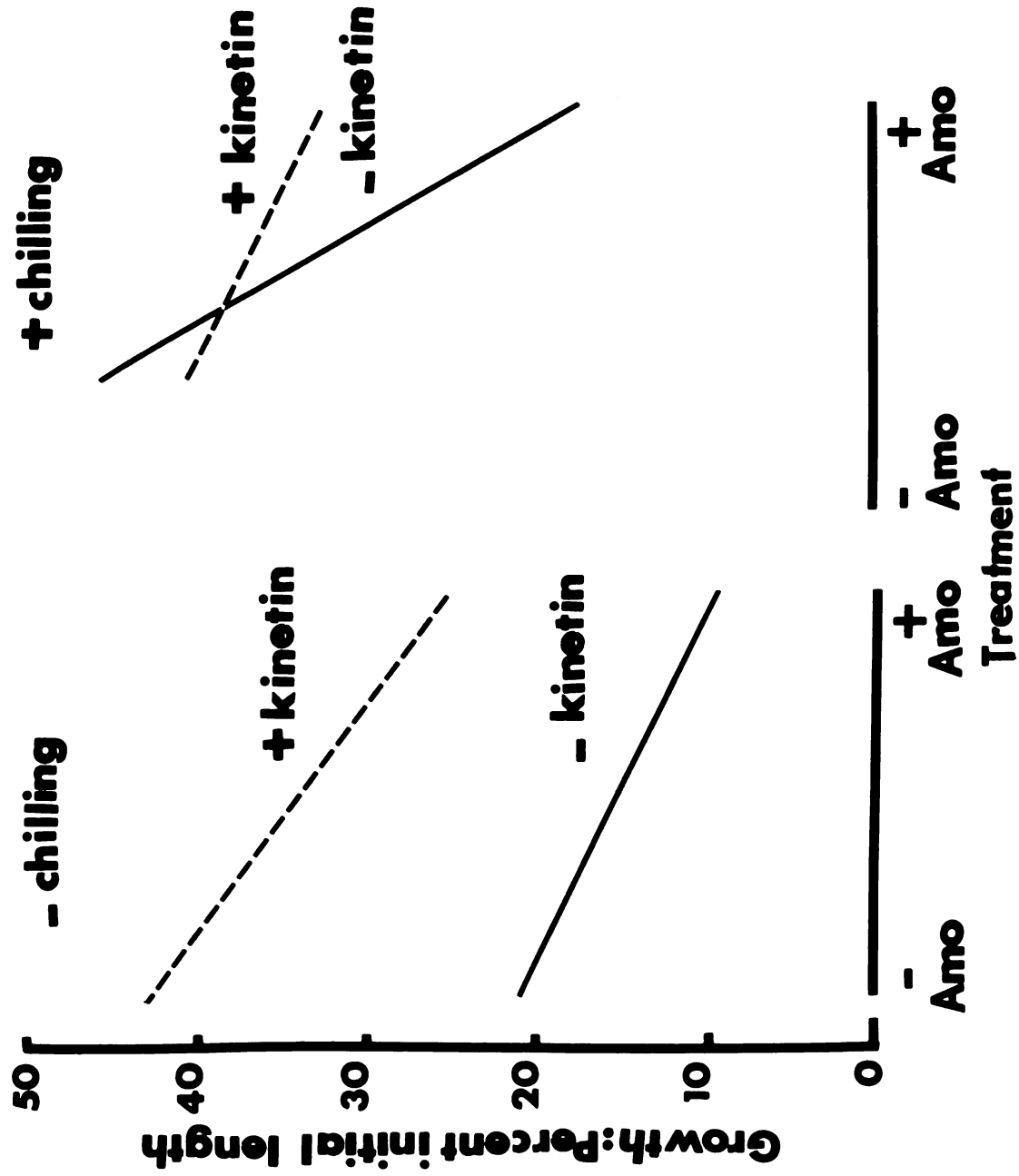
+chilling
+ kinetin
- kinetin
-chilling
+kinetin
- kinetin
Growth: Percent initial length
50
40
30
20
10
0
- Amo
+ Amo
- Amo
+ Amo
Treatment

Figure 14

Table 14. Effect of ABA, kinetin + ABA, sucrose + ABA with and without chilling on growth of excised shoots (cv Spartan Banner). Growth: percent initial length*

Chemical treatment**	Not chilled	Chilled
None	16.03[ab]	37.62[c]
ABA, 1 ppm before chilling	9.01[a]	7.69[a]
After chilling	---	11.15[ab]
Kinetin, 100 ppm	40.23[c]	---
ABA + kinetin	13.99[ab]	18.35[b]
ABA + sucrose, 10%	7.12[a]	7.28[a]

*Means followed by th- same letter are not significantly different at the 5% level.

**Applied before chilling except where otherwise noted.

The interaction of auxin with chilling (Figure 13) deserves some consideration, for similar results were obtained by Guthrie with potato tubers (49). Evidence exists that auxin action depends on the presence of gibberallins (92). Thus, IAA may synergise with gibberellins, synthesized during the low temperature treatment.

Kinetin breaks dormancy of buds and seeds of some species (48,53,72,140), but its mechanism of action is at present unknown. It reportedly promotes nucleic acid and protein synthesis (93), but more critical studies suggest that its effects are due to suppression of protein degradation (73,122). Cytokinins may also affect carbohydrate metabolism in plants (13), thus making available reserve materials for growth. Similar processes may occur in onion shoots treated with kinetin. The finding that sucrose could substitute for the low temperature treatment in promoting growth (Table 13) seems to support this hypothesis. In addition, the finding that kinetin can remove the block to growth imposed by inhibitors and increase some promoters (27,53) introduces a new dimension in the interpretation of the role of this hormone in the dormancy release process.

Gibberellic acid breaks dormancy in buds and seeds (40, 48,86,99,104). However, it fails to do so in onions (55,125). Many cases, in fact, are known where GA_3 fails to break dormancy (17,18,137). However, this failure does not exclude the participation of gibberellins in the dormancy breaking process,

for they may act synergistically with other hormones. Furthermore, some three dozen different gibberellins are now known (37), and each one may have a specific physiological role. Thus, gibberellins other than GA_3 and $GA_{4/7}$ may be involved in the breaking of dormancy in onion.

Ethylene, a fruit-ripening hormone (19,20,104), is now generally accepted as a plant hormone ranking in importance with the auxins, gibberellins and cytokinins as a controlling factor in many physiological processes, including sprouting of corms (122) and germination of some species of seeds (8, 127). However, ethephon, an ethylene-releasing compound (35), did not break dormancy in onion. Ethylene chlorhydrin does not break dormancy in onion, and the failure has been attributed to insufficient starch (82). Whether or not the presence of critical levels of starch in the tissue is a sine qua non for the effectiveness of ethylene remains to be determined experimentally. However, its efficiency in the case of potato and gladiolus corms (38,82) seems to support such a hypothesis.

Perhaps onion dormancy may be partially controlled by carbohydrate level, which is, in turn dependent upon hormonally regulated enzymatic activity. Promotive effect of sucrose on growth of tomato roots and seed germination has been reported (26,124).

The growth retardants CCC and Amo 1618 are both potent inhibitors of GA biosynthesis and most of their physiological

effects are attributed to this property (7,18,23,69,94). In some cases, chilling predisposes a tissue to synthesize gibberellins but actual synthesis takes place after transfer to a higher temperature (110). That Amo 1618 nullified the chilling effect when applied before but not after temperature treatment suggests that a growth promoter is synthesized during the low temperature treatment and exerts its effect when the tissue is transferred to conditions suitable for growth. This hypothesis is supported by Thomas' finding that CCC had no effect on sprouting of onion previously subjected to low temperature (125).

The fact that sucrose is more effective than kinetin (Table 13 vs Figure 14) in overcoming the effect of Amo 1618 is another indication of possible involvement of gibberellins. GA has been reported to promote synthesis and/or release of hydrolytic enzymes leading to the degradation of starch in certain seeds (29,97,101). Abscisic acid inhibits shoot growth whether applied before or after chilling (Table 14). Kinetin only partially overcomes the ABA effect while sucrose is ineffective. Thus the modes of action of Amo 1618 and ABA appear to differ. The failure of kinetin to completely overcome the ABA effect may indicate that either the concentration of ABA applied was too high or the penetration of the kinetin was insufficient. Similar results were obtained when duckweed cultures (Lemna minor) were grown in media containing ABA and the cytokinin, benzyladenine (95).

Effects of Temperature, ABA, and Amo 1618 on the Levels of Reducing Sugars in Onion Shoots

The levels of reducing sugars in the shoots were inversely proportional to the temperature of incubation (Table 15). At 30°C, a net loss occurred in comparison with the control. Both Amo 1618 and ABA prevented the rise in reducing sugar at 10°C (Table 16). High temperature is known to enhance utilization of reserve materials. In Phacelia tanacetifolia seeds the respiratory quotient was very high at 28°C, a temperature at which little or no germination occurred (25). Whether the decrease in reducing sugars in onion is due to a faster rate of utilization or a reduced rate of synthesis remains to be determined. Other studies on onion show an accumulation of reducing sugars at low temperature (9,144). Recently, Fontes and Ozbun (47) and Kacperska-Palacz and Wcislinka (64), working with broccoli and rape plants, demonstrated low temperature enhancement of reducing sugar level.

The fact that both Amo 1618 and ABA prevent the increase in reducing sugars during chilling is indirect evidence that these chemicals block the synthesis or activation of some hydrolytic enzymes which in turn may be under hormonal control. This hypothesis is supported by the ability of CCC to prevent accumulation of reducing sugars in rape leaves during chilling (64).

Table 15. Reducing sugar levels (percent fresh weight) in Spartan Banner onion shoots after 96 hours of exposure to 5 different temperatures.*

Temperature Treatment (°C)					
None	30°	20°	10°	5°	0°
14.04[b]	11.30[a]	15.48[b]	19.71[c]	22.50[d]	25.86[e]

*Means followed by the same letter are not significantly different at the 5% level.

Table 16. Reducing sugar levels (percent fresh weight) in Spartan Banner onion shoots as affected by temperature, Amo 1618 (100 ppm) + temperature and ABA (1 ppm) + temperature. ABA and Amo 1618 were applied before exposure of onions shoots to 10°C for 96 hours.

	Chemical treatment		
Chilling (hr)	Control	Amo 1618	ABA
0	13.85[a]	---	---
96	20.77[b]	12.88[a]	13.64[a]

*Means followed by the same letter are not significantly different at the 5% level.

Levels of Acidic Inhibitor in Shoots During Storage

The levels of inhibitor in the acidic fraction of shoot extracts, as measured by wheat coleoptile bioassay are shown in Figures 15 and 16. Three facts emerge from these data:

1. Inhibitor content was higher in MSU 4535 than in Downing's Yellow Globe, which are long and intermediate dormant types, respectively.
2. The inhibitor decreased during storage at 10°C and at 0°C, but never completely disappeared. Comparison of Figures 15 and 16 vs Figure 3 shows a fairly good parallel between the degree of dormancy and the inhibitor level.
3. Growth promoters were observed in some samples, but their levels could not be related to dormancy.

Thus, a true inverse correlation appears to exist between the level of inhibitor and the degree of dormancy in onion. However, the following questions remain:

a) Are the results valid? In the bioassay used, the possibility exists that promoters having the same Rf as the inhibitor could have been responsible for the results obtained. This study has demonstrated the effectiveness of kinetin as a substitute for low temperature in breaking dormancy in entire onion bulbs and in shoot apices. The demonstration by Staden et al. (118) of a zeatin-like compound in stratified Acer saccharum seeds lends support to a role of

Figure 15. Elongation response of Genesee wheat coleoptile sections to eluates from thin layer chromatograms of the acid fraction of onion shoot apices excised from whole bulbs before and after storage. Each chromatogram is the average of 4 replicates of 10 gram-equivalents of shoot.

A = MSU 4535

B = Downing's Yellow Globe

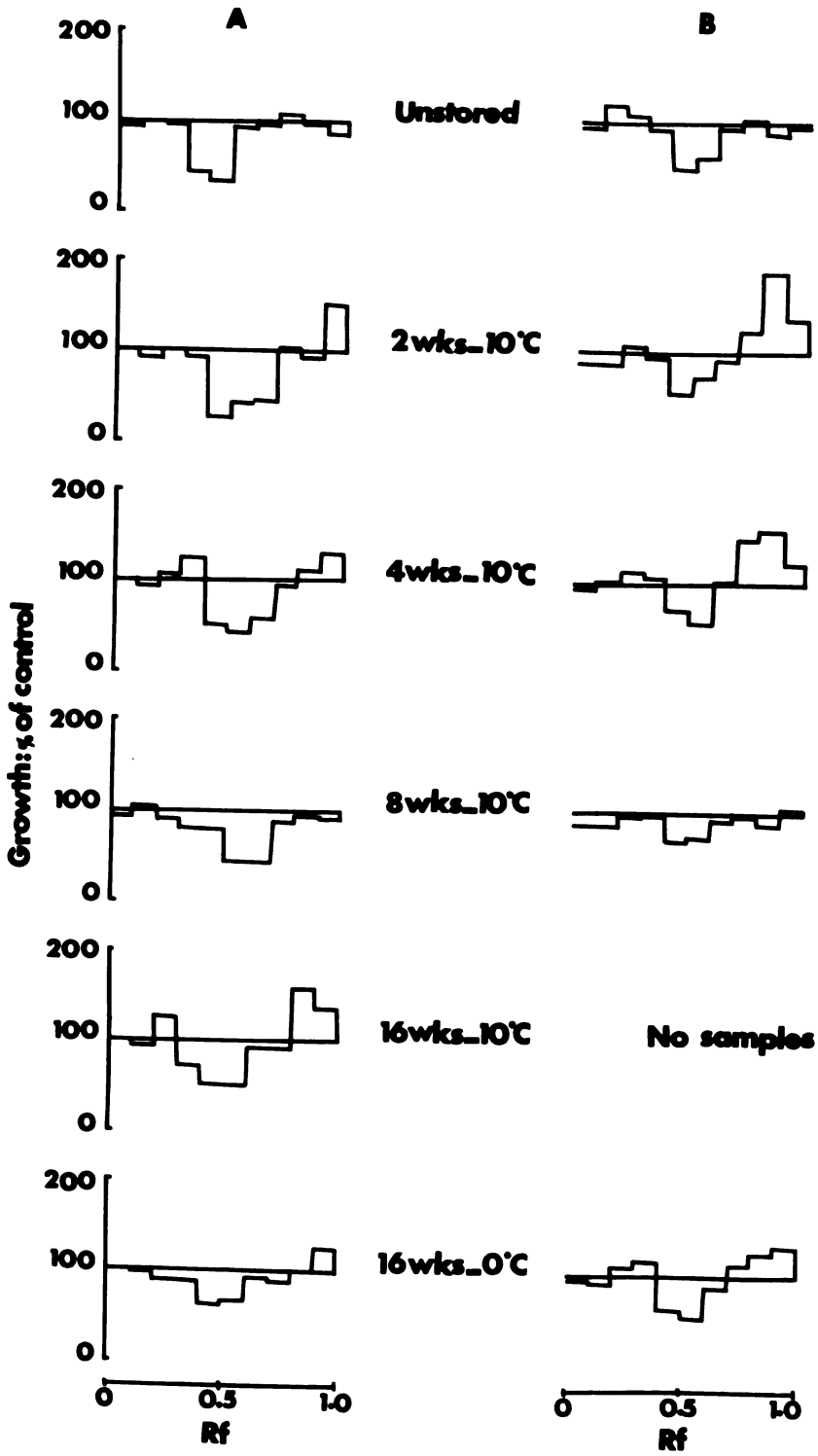
A
B
200
100
0
Unstored
2wks_10°C
4wks_10°C
8wks_10°C
16wks_10°C
16wks_0°C
No samples
Growth: % of control
0
0.5
1.0
Rf

Figure 15

Figure 16. Elongation response of Genesee wheat coleoptile sections to eluates from thin layer chromatograms of the acid fractions of shoot apices. Each point is the average of 4 replicates of 2 gram-equivalents of shoot each.

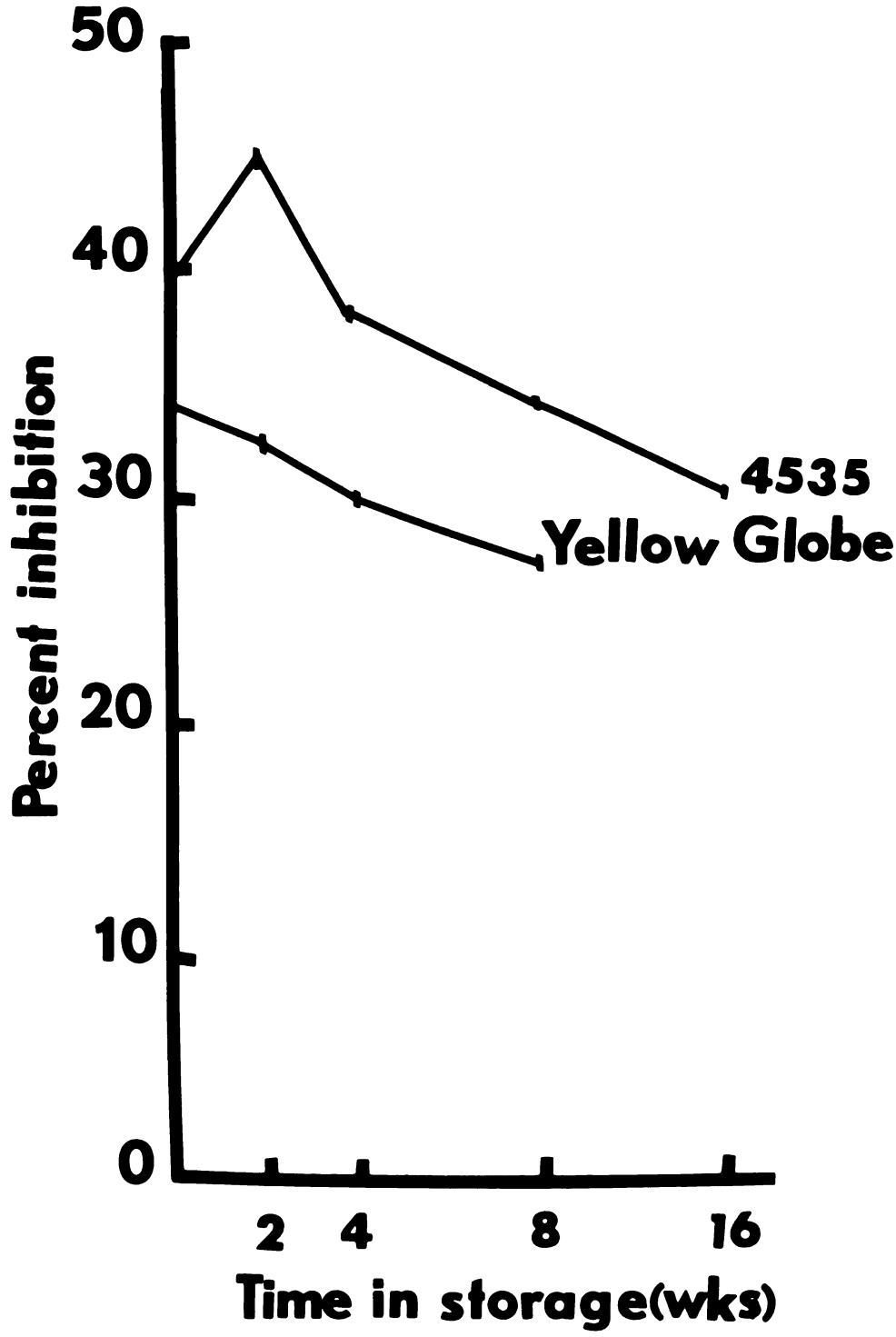
50
40
30
20
10
0
Percent inhibition
4535
Yellow Globe
2
4
8
16
Time in storage(wks)

Figure 16

endogenous cytokinins in the dormancy breaking process. Interestingly, the zeatin-like compound found by Staden et al. (118) had the same Rf value as abscisic acid (142) in the solvent used for paper chromatography. The possibility of an interplay between inhibitors and promoters should not be excluded. The data of Thomas (125) and Tsukamoto (130) as well as my own suggest this possibility.

b) Is the decrease observed in the inhibitor level the cause or the effect of dormancy release? The fact that the long dormant type contains more inhibitor than the intermediate one suggests a causal relationship.

Characterization of the Inhibitor

Thin layer chromatograms developed in isopropanol: ammonia:water (10:1:1 v/v/v) showed the Rf of the inhibitor (0.4-0.7) was identical with that of ABA. Identical Rf's were also observed in 3 additional solvent systems (Figure 17). When methylated samples of the inhibitor and ABA were chromatographed and bioassayed, activity was found at Rf 0.6 to 0.9 in both cases (Figure 18). Inhibitory activity was detected in fractions 5 to 11 following elution of both ABA and the acidic fraction from charcoal-celite columns (Figure 19).

Extracts were next subjected to gas liquid chromatography (GLC) and combined GLC-mass spectrometry (GLC-MS).

Figure 17. Response of wheat coleoptile sections to eluates of thin layer chromatograms of onion shoot extract (cv MSU 4535). The origins are on the left. The position of the synthetic ABA as observed under UV are indicated by vertical bar.

A = Isopropanol:Ammonia:Water 10:1:1 v/v/v
B = Benzene:Acetic acid:Water 8:3:5 v/v/v
C = 1-Butanol:n-propanol:Ammonia:Water 2:6:1:2 v/v/v/v
D = 1-Butanol:Acetic acid:Water 40:11:29 v/v/v

Figure 18. Response of wheat coleoptile sections to eluates from TLC of: A:ABA; B:methylated acid fraction of onion extract; C:methylated ABA. Plates developed in isopropanol:ammonia:water 10:1:1. Vertical bar indicates the position of authentic ABA under UV light.

Figure 19. Response of wheat coleoptile sections to eluates from charcoal-celite (1:2) columns of A) 8 ng of ABA and B) 20 gram-equivalents of the acidic fraction of an onion extract. Each column was eluted with 150 ml 60% acetone and 10 ml fractions were collected.

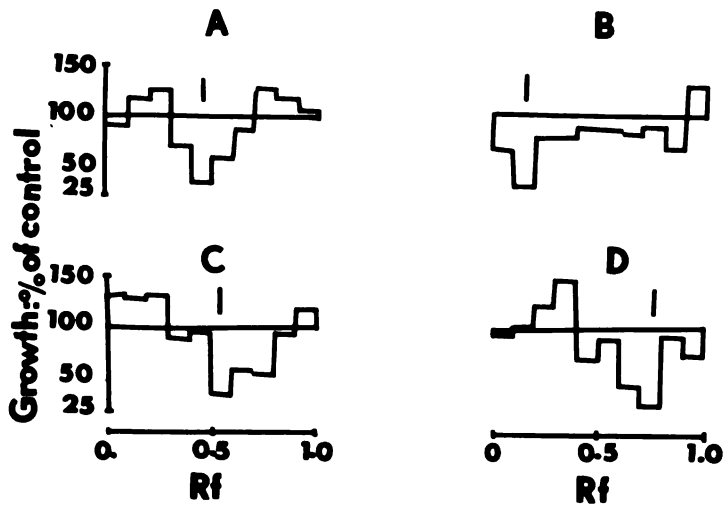

Figure 17

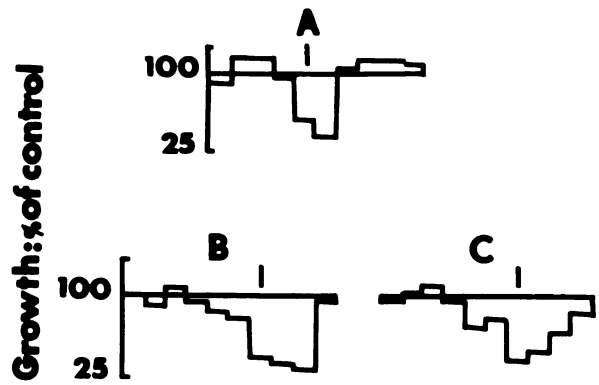

Figure 18

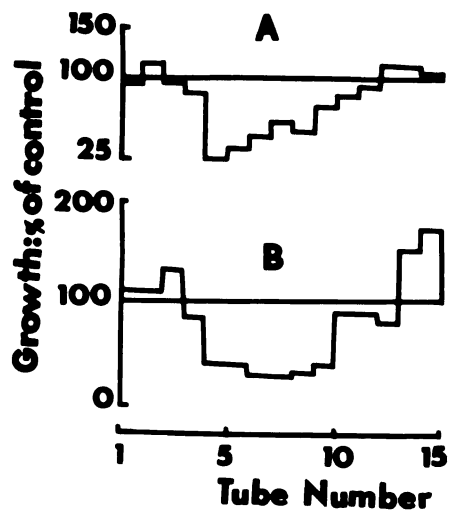

Figure 19

Aliquots of the crude acidic fraction were partially purified either by TLC in isopropanol:ammonia:water (10:1:1) (Sample A), or by column chromatography on charcoal:celite (1:2) (Sample B), as previously described, before methylation. Under the conditions used (Table 1) the retention time of authentic cis,trans-methyl abscisate was 2.0 minutes (Figure 20). A GLC trace of sample A following methylation (Figure 20) gave slight, if any, evidence of ABA, although one component of the extract had a retention time slightly greater than that of ABA. Sample B gave similar results, and no ABA was detectable in either sample when the electron capture detector was used in place of flame ionization (data not shown).

GLC-MS line diagrams of methyl ABA and the two major components of the methylated extract are shown in Figure 21. The identity of the latter could not be established.

Thus, the inhibitor observed in the onion extract resembled ABA chromatographically and biologically. However, no evidence could be obtained for the presence of ABA using gas chromatography or mass spectrometry.

Figure 20. Gas chromatographic lines of methylabscisate (left) and an acidic inhibitor from onion bulbs after thin layer chromatography and methylation (right). Conditions are given in Table 1. Quantities used were 1 ug Me-ABA and 1 gram-equivalent (fresh weight) of onion extract.

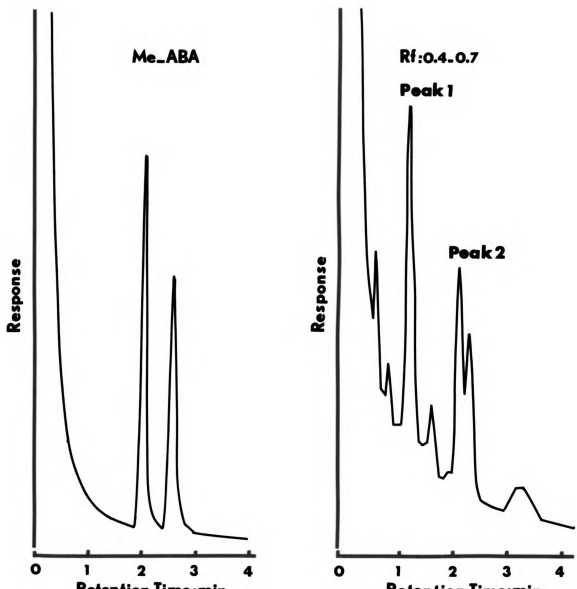
Me_ABA
Response
0
1
2
3
4
Retention Time:min.
Rf:0.4-0.7
Peak 1
Peak 2
Response
0
1
2
3
4
Retention Time:min.

Figure 20

Figure 21. Mass spectrograph line diagrams of authentic methylabscisate (top), of peak 1 (middle), and peak 2 (bottom) in methylated sample of inhibitory zone from thin layer chromatogram. (For retention time of peaks 1 and 2, see Figure 20).

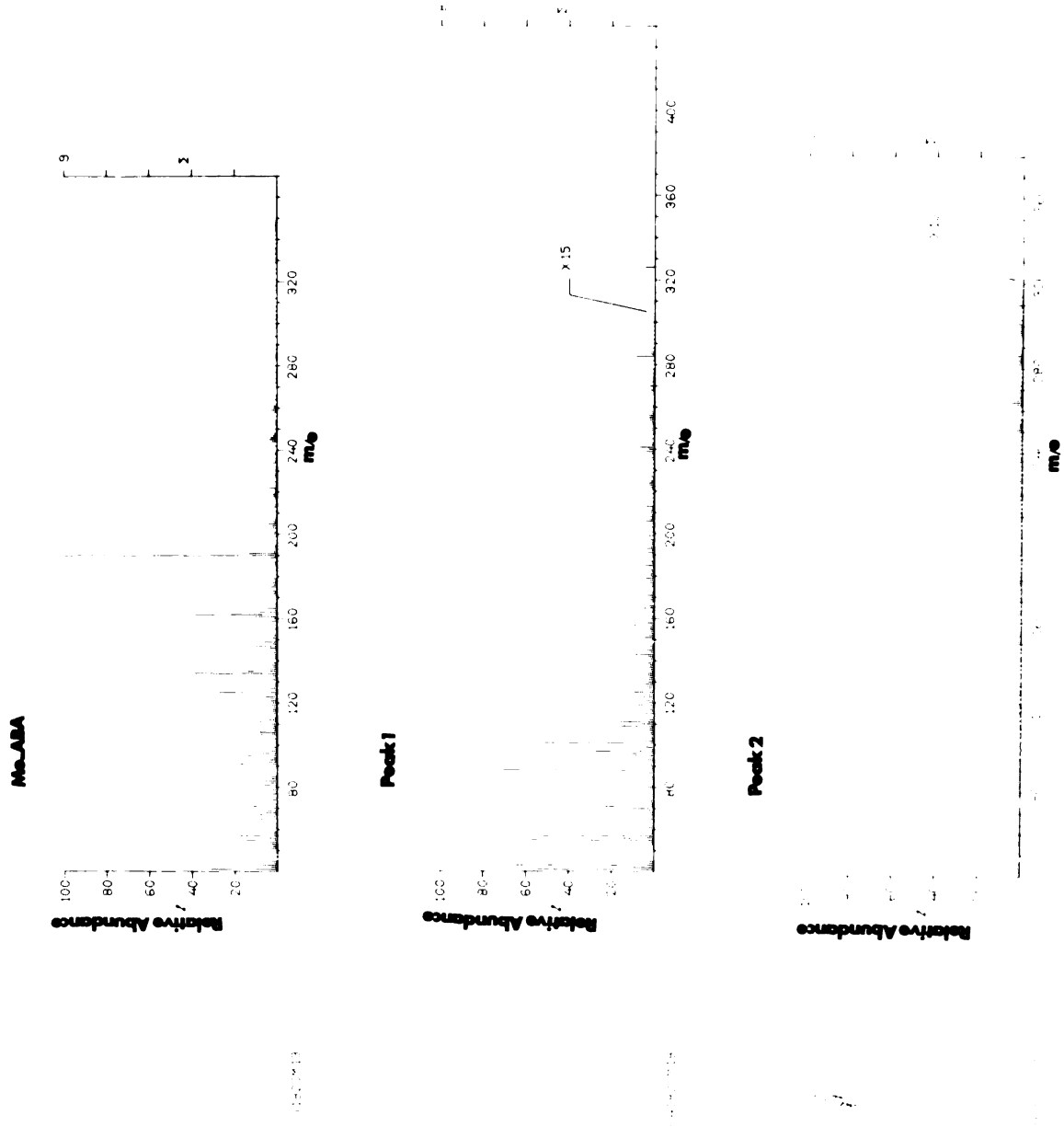

Figure 21

CONCLUSIONS

The following results were obtained in this thesis:

Onions having an intermediate or long dormant period matured later in the summer than those with a short one. Dormancy in onions declined with time. The change was genetically controlled, temperature-dependent with an intermediate temperature (5 to 15°C) being more effective than the extremes (0 or 30°C). Small onion bulbs rooted and sprouted faster than large ones. High temperature induced secondary dormancy in onion bulbs, but this dormancy was quickly overcome by subsequent low temperature treatment. Root dormancy in onion was weaker than shoot dormancy, and the latter appeared to depend on the former. Wounding the scales significantly reduced root and shoot dormancy, but oxygen treatment did not.

During storage, the shoot apices grew inside the bulbs. Intermediate temperatures (5-15°C) were more promotive than the extremes (0-20,30°C) in the onion varieties tested. Shoot apices excised from dormant bulbs responded to temperature and exogenous growth substances similarly to entire bulbs. Sucrose and kinetin were very effective in substituting for intermediate temperature treatment in the release of dormancy in onion shoots but no additive effect of these substances and

temperature could be obtained. Amo 1618 was very effective in nullifying the effect of intermediate temperature when applied prior to the temperature treatment. Applied after, it was without effect. Sucrose was very effective in overcoming the effect of Amo 1618 but kinetin was not. Abscisic acid restored dormancy in temperature-treated onion shoots and neither kinetin nor sucrose significantly alleviated this effect.

Temperature treatment increased the level of reducing sugars of onion shoots and lower temperature were more effective than higher temperature. Amo 1618 and ABA applied before the temperature treatment nullified the effect of low temperature on the accumulation of reducing sugars. The inhibitor level was higher in MSU 4535, a long dormant type, than in Downing's Yellow Globe, an intermediate dormant type, and it declined as dormancy was broken. Chromatographically and biologically, the inhibitor behaved like ABA, but gas liquid chromatography and mass spectrometry indicated that insufficient ABA was present to account for the biological activity.

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