HYPOBARIC STORAGE OF CELERY AND CAULIFLOWER SEEDLING TRANSPLANT

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ABSTRACT

HYPOBARIC STORAGE OF CELERY AND CAULIFLOWER SEEDLING TRANSPLANTS

By

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Storage of transplants requiring vernalization at temperatures below 10°C induces flowering. Hypobaric ventilation during storage at 0°C of celery and cauliflower seedling transplants delayed floral initiation. Storage for 2 weeks at 0°C at 25 mm-Hg prevented bolting of celery (Utah 52-70) upon outplanting. Bolting became prevalent as the storage duration was increased to 6 weeks particularly in air at atmospheric pressure. Buttoning of two cauliflower varieties (Clou and Self-Blanche) stored for 4 weeks at 0°C at 25 mm-Hg was also prevented. Partial Inhibition (Clou 62% and Self-Blanche 98%) was achieved by storage for 4 weeks at 150 mm-Hg. Hypobaric storage for 4 weeks decreased the survival percentage of celery to 96.9% and cauliflower to 91.7%. The loss of plants due to bolting and buttoning accounted for the majority of yield decline. There appeared to be a slight but direct deliterious effect of hypobaric storage which contributed to decreased yields. It was not determined if the effect of hypobaric storage on delaying floral initiation was due to enhanced diffusion of volatiles from the plants or to the low P_{O_2} (ca. 0.5%) at 25 mm-Hg total air pressure.

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By

Douglas Joseph Jardine

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I dedicate this work to my grandfather

Raymond "Jim" Grobbel

who sparked my interest in the field of

agriculture so many years ago

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INTRODUCTION

The cost of raising celery transplants in greenhouses is becoming increasingly expensive with energy and labor costs continuing to rise. Growers may save both time and money by using plants grown out-of-doors in California if seedlings could be raised and shipped to Michigan at a reasonable cost. This would also free greenhouse space for other valuable commodities such as bedding plants. Air freight is expensive and shipping by land has its problems. Temperatures during transport and storage warm enough to prevent seedstalk formation (e.g. $10^{o}C$) cause excessive deterioration of the tissue during extended shipment periods. Temperatures low enough to maintain the plants in good condition (e.g. $0^{o}C$) cause vernalization and thus the plants form seedstalks during the first season which makes them unmarketable.

Hypobaric storage is a relatively new process which has increasingly diverse applications. The equipment is in development for transporting perishable commodities over long distances under hypobaric conditions. The purpose of this experiment was to determine if hypobaric ventilation during storage would inhibit floral initiation of celery transplants held at sufficiently low temperatures to cause vernalization for up to 6 weeks. Cauliflower, a crop which responds to cold in a manner similar to celery, was used to determine if the principle might be generally applicable to other crops.

LITERATURE REVIEW

CELERY

Celery, Apium graveolens L., is second only to lettuce in importance as a salad crop in value and popularity. A large part of the celery crop grown in the United States is consumed raw, but considerable quantities are processed in vegetable juices, soups, stews and other cooked products.

Celery is native to marshy lowlands and, according to Sturtevant (63), its habitat extends from Sweden southward to Egypt, and in Asia even to the mountains of India. It grows wild in Tierra del Fuego, California and New Zealand. The wild plant was probably first used for medicinal purposes hundreds of years before it was used for food.

Although celery is a biennial plant, it is grown and cropped as an annual. Celery plants, for an early crop in regions of short growing seasons, are started in greenhouses, hotbeds or coldframes. The late crop in such regions, and most of the celery grown in mild climates as in California and Florida, is started in outdoor seedbeds. However, direct seeding in the field is practiced in the coastal region of California where the crop is grown to maturity under ideal climatic conditions.

When seedlings are raised in greenhouses, two methods for starting them may be used. The time of sowing is determined largely by the time the crop is desired for use. The first method requires that seed be

drilled in rows or broadcast into flats or seedbeds. After 4 or 5 weeks, they are set into the soil of the greenhouse or flats, spacing them 40 X 40 or 50 X 50 mm. Ten to 12 weeks usually elapse between sowing and field transplanting. In the second method, plants are transplanted directly from the seedbed to the field at 8 to 10 weeks.

Storage of seedlings most often consists of leaving them in the seedbed or flats and retarding the growth rate by withholding water. Pulled plants are generally not stored for more than a day or two and this usually occurs when bad weather halts planting. Storage temperatures of 0 to 10°C can be used for 1 to 2 days. Periods longer than 2 days may cause the plants to bolt (66) while temperatures above 10°C will cause excessive deterioration.

Bolting, or premature seeding as it is sometimes known, is the development of flowers and seeds during the first year of growth by a biennial plant. This is the result of vernalization during the early stages of plant growth and development. Vernalization is the promotion of flower formation by a period of low temperature and was observed and described by several authors as early as the middle of the 19th century. Lang (40) in a review in the Encyclopedia of Plant Physiology, pays great tribute to the work of Gassner (29) and compares it to the pioneering work of Garner and Allard (28) on photoperiodism. Vernalization is unusual among biological phenomena in that its relation to temperature is the reverse of that which holds for chemical reactions in general (48).

Plants other than biennials often require a period of cold treatment during their development before they can flower. These include so-called winter annuals such as winter cereals and a certain number of

perennials. Many biennials, (e.g. cabbage), will remain in the vegetative state for a period of years if protected from cold in winter (40). Chailakhyan (15) points out that only temperate plants that undergo winter conditions can be expected to have a vernalization requirement, and these are likely to be long day plants.

Gregory and Purvis (32) demonstrated that vernalization is an aerobic process and does not occur when plants or seeds are cooled in an atmosphere of nitrogen. In the same work, they reported data indicating that vernalization is a cumulative process. Sarkar (55) as reported by Purvis (48), indicates that plants with an obligate cold requirement are not as a rule sensitive to thermoinduction before they have made some minimum amount of growth. Purvis (48) reports that just as photoperiodic reactions begin from the transformation of photosynthetic products i.e., sugars and other reduced products, thermoperiodic reactions also begin with sugars stored in seeds or plant tissue. It has been suggested that the "cold requirement" represents a deficit of some precursor which is requisite for the photoperiodic response. In plants with little or no cold requirement, this precursor is already present, or is readily formed at normal temperatures (48). Denffer (21), as reported by Lang (40), proposed that the role of temperature was to prevent the formation of an inhibitor of flowering. Melchers (44), as reported by Chailakhyan (15), offers the possibility of a chemical known as vernalin which is produced under conditions of low temperature and high sugar. It should be noted that none of these precursors, inhibitors or chemicals have ever been isolated.

The bolting of celery is a problem which has continually plagued growers and, in some years, caused the loss of entire crops. Bailey

(5) was one of the first to make mention of this problem. Walker (69) offered the suggestion that perhaps bolting resulted when plants remained in the seedbed too long. Reid (49), Whipple (70) and Abell (2) also addressed themselves to the problem but were unable to identify the cause of this phenomenon. Some of their suggestions included checking growth of plants due to freezing, drying and other conditions retarding growth development, poor seed and starting plants too early in the winter.

Thompson (64) was the first to make a systematic study of the problem of bolting. The conclusion of his first experiments was that the length of day controlled seedstalk development since the earlier he planted his seeds, the more susceptible they were to bolting. However, his results may have been confounded since cooler temperatures were also associated with earlier seeding. Starring (59) suggested that cool temperatures seemed to cause a fairly uniform premature seedstalk formation. After further study, Thompson (65) reported that relatively low temperatures (4-10^{o}C) for two weeks or longer would likely result in premature seeding. He also found that a temperature of 10^{o}C would not induce seeding after two weeks but would likely induce it after a month or more. In further studies, Thompson (66) observed that a period as short as 2 days at 4^{o}C appreciably promoted seedstalk development and that alternating between low, medium or high temperature did not nullify the effect of the low temperature treatment.

Pawar and Thompson (45) demonstrated that plants of ages 14 days and 160 days subsequently flowered when subjected to the same treatment. Plants of different size, but of the same age, were equally affected by low temperature exposure.

Platenius (46) was the first to work on the physiology of seedstalk

development in celery. He observed that the percentage of sugar increased in plants at 4 to 10°C as compared to those kept at 20 to 25°C and that these levels could be reduced by placing the plants at a high temperature. "Bolters" could be produced from young plants provided they received a low temperature treatment. High sugar containing plants grown at medium temperatures did not bolt indicating that low temperature is more important than carbohydrate accumulation. He concluded that the ultimate cause of seedstalk formation may be the localized accumulation of certain chemical compounds in the meristematic tissues, possibly not involving more than a few cells.

Curtis and Chang (19), in a clever experiment, placed rubber coils around the meristematic tissue of young celery plants. When the plants were placed in a warm environment with cold water passing through the coils, floral initiation occurred. When the plants were placed in a cold environment with warm water moving through the coils, initiation of flowering did not occur.

Wittwer, Coulter and Carolus (73), Coulter (18) and Clark and Wittwer (16) studied the effects of growth regulators on seedstalk development in lettuce and celery. 2,4-dichlorophenoxyacetic acid, triiodobenzoic acid and napthalene acetic acid treatments all hastened seedstalk development. However, treatment of celery with α-o-chlorophenoxypropionic acid at 100 ppm prevented seedstalk development.

Emsweller (26) demonstrated that it is possible to secure strains of celery that will not bolt to seed under conditions that cause a very high degree of bolting in other strains. He also demonstrated that it is possible to isolate lines so strongly annual that they produce some plants with seedstalks even when grown under the most favorable

conditions for vegetative growth. A general statement on varieties would indicate that most self-blanching or yellow varieties bolt readily while the green varieties, which are most commonly grown, bolt less readily. Bouwkamp and Honma (7) using the reciprocal cross of inbred yellow and green lines, demonstrated that the easy bolting response in celery is controlled by a dominant major gene (Vr). Their test for homogeneity indicated that this character was genetically independent from other varietal characteristics.

Though the vernalization and flowering of celery in the first season is a problem for many growers, it is of great benefit to producers of seed. Hanisova and Krekule (33) have described methods whereby low temperatures are used to induce bolting in order to increase the speed of production. Their methods allow the production of seed on a yearly basis for all but the most bolt resistant varieties. It also gives a more uniform seedstalk development.

CAULIFLOWER

Cauliflower, (Brassica oleracea var. botrytis L.), is grown for its white, tender head or curd, formed by shortened flower parts. Cauliflower was first cultivated in Europe but was in common use over 2000 years ago. Like celery, the cultivated cauliflower is a biennial which is cropped as an annual. In the production of the early or spring crop, considerable loss occasionally results from the occurence of buttoned, or "prematurely headed" plants. These plants are characterized by small exposed heads appearing shortly after setting plants out in the field while normal plants of the same age appear to be entirely vegetative.

Carew and Thompson (14) determined that these "vegetative" plants actually form curds at the same time but this is obscured by heavy vegetative cover. They claim that it is rather misleading to use the term premature heading. Buttoned plants have no market value, and in years when the condition is severe, may cause considerable economic loss.

Early references to the problem of buttoning of cauliflower consist mostly of statements based on observations rather than on research. Bailey (4) and Brill (8) attributed buttoning to factors which slowed the growth rate. Judson (38) attributed buttoning to stunting of plants by keeping them in the seedbed too long. Jones and Ernst (37) agreed with Judson and also suggested that extremely low temperatures while the plant is young may cause it to button.

In the past 50 years, researchers have attributed buttoning to a number of factors including soil nitrogen (50) and the C:N ratio of the plant (31), transplant age and size (41), temperature (1, 57), variety and light (57, 71).

The first investigation devoted solely to the problem of buttoning was conducted by Robbins, Nightingale and Schermerhorn in 1930 (50). They observed that plants with a limited supply of nitrogen in the nutrient solution buttoned while those with adequate nitrogen did not. Further investigation showed that this condition was associated with a high percentage of carbohydrates and a low percentage of assimilated nitrogen in the plant. They observed 37% more carbohydrate on a dry weight basis in stems and petioles, 188% more in blades and 16% more in the roots of the buttoned plant as opposed to the vigorously growing vegetative plant. At the same time, the concentration of assimilated nitrogen was much higher in the vigorous vegetative plant with 5% more

in roots, 104% more in stems and petioles and 163% more in blades. Carew and Thompson (14) also observed that plants grown in a soil with a low nitrogen content and poor early season growing conditions buttoned more than plants with an adequate nitrogen supply.

Fontes and Ozbun (27) reported that treatments promoting carbohydrate accumulation i.e. low temperature, promoted flowering in broccoli whereas prevention of carbohydrate accumulation by SADH, high temperature, juvenile plants or leaf pruning reduced flowering. Grainger (31) suggested that a high C:N ratio must be attained to initiate flowering. Sadik and Ozbun (52) demonstrated that flowering of cauliflower would be attenuated by reducing carbohydrate synthesis during cold treatment or by depleting carbohydrates after a cold treatment. They suggested, that ignoring other factors, high carbohydrate levels accompany or precede flowering.

Transplant age and size is also an important consideration. Loomis (41) observed that the larger the plant at transplanting, the greater the severity of the growth check, and this resulted in more buttoning in cauliflower. Gilbert (30) and Jensma (36) pointed out the need for young plants and good growing conditions. Sadik (51) and Aamlid (1) determined that 16-20 leaves must be formed before the curd is initiated. Wiebe (71) on the other hand, claims the juvenile stage ends at 4-8 leaves. In work with Chinese cabbage, Eguchi, Matsamura and Koyama (25) reported that old seedlings were more sensitive to cold temperature than young seedlings in relation to seedstalk formation. Skapski and Oyer (58) actually defined cold sensitivity in terms of plant size; plants with stem diameters of approximately 5 mm and a fresh weight greater than 5 grams had a greater tendency to button than smaller ones.

Cobbet (17) suggested and Aamlid (1) demonstrated that a high temperature would inhibit curd formation. Skapski (57) reported that low temperature initiated curds while high temperature kept plants in their vegetative state. Wiebe (72) reported that the highest temperature which would induce curd formation was 23°C for the variety 'Aristokrat'. Sadik (51), using two varieties of cauliflower, demonstrated that one variety, February-Early March, required a cold treatment for curd initiation while the other, Snowball M, required no cold treatment for flowering. In further testing the vernalization requirement, Wiebe (71) found that subjecting seeds to low temperatures had no affect on the curd initiation of cold requiring varieties. Heide, Junttilla and Samuelson (34) reported that low temperature during the seed development phase of red beet plants increased the bolting susceptibility of the progeny. Salter and Ward (53) concur with Skapski (57) that cold temperature treatments may be applied to fall varieties to yield a more uniform maturity.

Skapski and Oyer (58) and Skapski (57) have observed that buttoning is a varietal characteristic with early season varieties being much more susceptible than later types. Wiebe (71) also suggests that light has no affect on the plant before vernalization and only a slight affect afterwards, namely that low light intensity causes a slight delay in maturity.

Low temperature appears to be the key cause of both bolting in celery and buttoning in cauliflower. Low temperatures however, are required to maintain the young plants in a viable condition until outplanting. Hypobaric storage (12) is a process under development for the handling and storage of various types of perishable commodities

including rooted and unrooted cuttings (9). The idea was conceived that hypobaric storage of celery and cauliflower plants may attenuate the effect of low temperature on floral initiation and became the subject of this thesis. Since hypobaric storage is a new concept, certain principles that require elaboration follow.

HYPOBARIC STORAGE CONCEPTS

In principle, a commodity is maintained at its normal cold storage temperature in a chamber kept at some desired subatmospheric pressure while it is continuously ventilated with fresh humid air by using a vacuum pump to keep the storage pressure constant. The first recorded use of reduced atmospheric pressure for the purpose of prolonging the storage life of vegetative material was that of Stoddard and Hummel in 1957 (61). Using a modified kitchen refrigerator, they demonstrated that various products, e.g. lettuce, celery and green beans, could be kept from 3 to 6 days longer when the pressure was reduced from ambient atmospheric pressure to approximately 660 mm-Hg. Burg and Burg (10) observed that fruit kept better in a partial vacuum than at normal atmospheric pressure at an equivalent P_{O_2} because ethylene was kept at hyponormal levels since its diffusivity was increased as the atmospheric pressure was decreased. The principle involved was shown (11) to be an application of Ficks law of diffusion:

$$ds/dt = \frac{-x\ A'\ D(C_{in} - C_{out})}{T}$$

where ds/dt is the rate of gas transport, x is the fraction of the surface area through which gas exchange occurs, A' is the surface area

of the fruit, C_{in} and C_{out} are the concentrations of gas within and outside the fruit, D is the diffusion coefficient and T is the effective thickness of the barrier to diffusion.

If the commodity is in equilibrium with its environment, the rate of gas evolution equals the rate of synthesis in the tissue. Accordingly, Ficks law states that the outward diffusion rate is determined by the product of the surface area perforated by air-filled spaces times the gas concentration gradient, times the diffusion coefficient, divided by the thickness of the barrier limiting gas exchange. The diffusion coefficient D is inversely related to the absolute air pressure provided the rate limiting step for gas exchange occurs in an air phase:

$$D = D_o(T/T_o)^m(P_o/P)$$

where D is the diffusivity of the gas in air at absolute temperature T and pressure P, D_o is the diffusivity at 273°K and 760 mm-Hg and (m) is a constant with a value between 1.5 and 2 depending on the gas mixture. Throughout the temperature range used for storage, the temperature correction is insignificant, and therefore the diffusivity is inversely related to absolute pressure (11).

The increase in the diffusivity of gases allows volatiles such as ethylene, acetaldehyde, carbon dioxide and farnesene to escape more easily while at the same time it allows oxygen to diffuse in more readily (11). Thus, oxygen concentrations which might ordinarily cause fermentation reactions to occur under normal atmospheric pressure, are often enough to sustain aerobic metabolism at greatly reduced pressures.

A low partial pressure of O_2 delays senescence and prolongs storage life by reducing the rate of metabolism as indicated by the low

respiration rate (6). Ethylene synthesis (42, 13, 20) and action (10, 56) are also diminished at low O_2 tensions. Ethylene above a certain threshold level accelerates the respiration rate and senescence of many fruits and vegetative tissue (39, 47, 10) and therefore shortens the storage life.

This system of storage, variously termed hypobaric storage, subatmospheric pressure storage, low pressure storage(LPS) or vacuum storage, can be used to extend the useful life of fresh fruits, vegetables, cut flowers, cuttings, potted plants, meat, poultry, fish, shrimp and other metabolically active matter. Burg and Burg (12) demonstrated that lowering the total pressure to 150 mm-Hg could extend the storage life of bananas by more than four times over those stored at normal pressure. Dilley (22) reported that hypobaric storage increased the retention of flesh firmness, reduced scald and increased the shelf life of several apple varieties. The loss of flesh firmness, pigmentation and total titratable acidity of apricots, peaches and pears was extended over a longer period of time by reducing atmospheric pressure to about 100 mm-Hg (54). It has been reported that the ripening of tomatoes may be delayed by atmospheric pressures in the 100 mm-Hg range (6, 67,60, 62, 74). The report by Stenvers and Bruinsma (60) indicates that the effect of LPS is not on the reduction of the ethylene concentration but rather on the low partial oxygen pressure. Bangerth (6) however, showed that ethylene removal was a key factor.

Bangerth (6) has reported that hypobaric storage delays the degradation of vitamin C in tomatoes, parsley, spinach, cress and radish. Bangerth (6) and Wu et al. (74) observed that low atmospheric pressures delay chlorophyll degradation, inhibit lycopene synthesis and prevent

the conversion of starch to sugar in tomato fruits. Wu et al. (74) observed an absence of aroma and flavor when tomatoes were initially removed from hypobaric conditions but after several hours the flavor and aroma returned to levels similar to fresh tomatoes. Burg (9) and Dilley, Carpenter and Burg (23) reported that the storage life of cut flowers, potted plants and cuttings could all be extended far beyond that observed with conventional storage. This extension of life has been attributed to the removal of ethylene by the system. Spore germination, mycelial growth and sporulation of tested fungi were inhibited by hypobaric conditions (3). An increase in inhibition was observed with a decrease in pressure demonstrating the same type of additive effect observed by Dilley et al. (23). Finally, Jadhau, Patil and Salunkhe (35) have observed that greening of Russet Burbank potato tubers can be completely inhibited at 126 mm-Hg and 80-85% relative humidity.

MATERIALS AND METHODS

Celery. Celery plants (Utah 52-70) were grown by Agricultural Systems of Union Carbide Corporation at Watsonville, California and shipped by air freight in polystyrene boxes or wooden crates to Lansing, Michigan on April 27, May 11, May 25 and June 8. The plants arrived within 36 hours and were received with wet ice still on the tops. The plants were stored overnight at 5°C before being placed under treatment. Plants in shipping containers which had 2 cm or more of roots in water from the melting ice were discarded. Similar procedures were followed for the remaining shipments with the exception of the May 25 shipment

in which case the roots were dry and were dipped briefly in water.

Each shipment consisted of 4 containers with approximately 1000 plants per container. The lots were divided in half and placed in 3 gallon plastic pails to accommodate the storage facilities. Storage was for 0, 2, 4 or 6 weeks in air under the following conditions: $0^{o}C$ at 25 mm-Hg, 0^{o} at 760 mm-Hg, 10^{o} at 25 mm-Hg and 10^{o} at 760 mm-Hg. At 25 mm-Hg the O_2 is 0.57 and 0.44% v/v respectively, at 0^{o} and $10^{o}C$ after correcting for the presence of water vapor.

The storage chambers consisted of two, 3 compartment retorts, each measuring 30 X 60 X 60 cm. The chambers were maintained in a temperature controlled room and hence were "jacket cooled". The chamber doors consisted of plexiglass plates fit against a silastic rubber gasket. A vacuum outlet from the chamber was connected to a vacuum pump. Fresh, ethylene-free air was admitted through a vacuum regulator (Matheson Model 49) at the desired flow rate and pressure. The air was bubbled through water at the operational pressure prior to entering the chamber. Humidification was accomplished in a 4 liter flask maintained 2 to $5^{o}C$ higher than the storage chamber temperature by placing the flask on a thermostatically controlled heating mat. A schematic of the system is shown in Figure 1. This system is similar to that described by Burg and Burg (12). A second set of chambers was also used with the only difference being an increased chamber volume.

Outplanting was made on June 11 at Hamilton, Michigan. The soil was a deep muck and weather conditions were sunny with temperatures of 22 to $27^{o}C$. When removed from storage, all plants held at $10^{o}C$ at 760 mm-Hg for 2, 4 and 6 weeks were badly deteriorated and decayed. Plants held at $10^{o}C$ at 25 mm-Hg for 6 weeks were also unplantable. The remaining

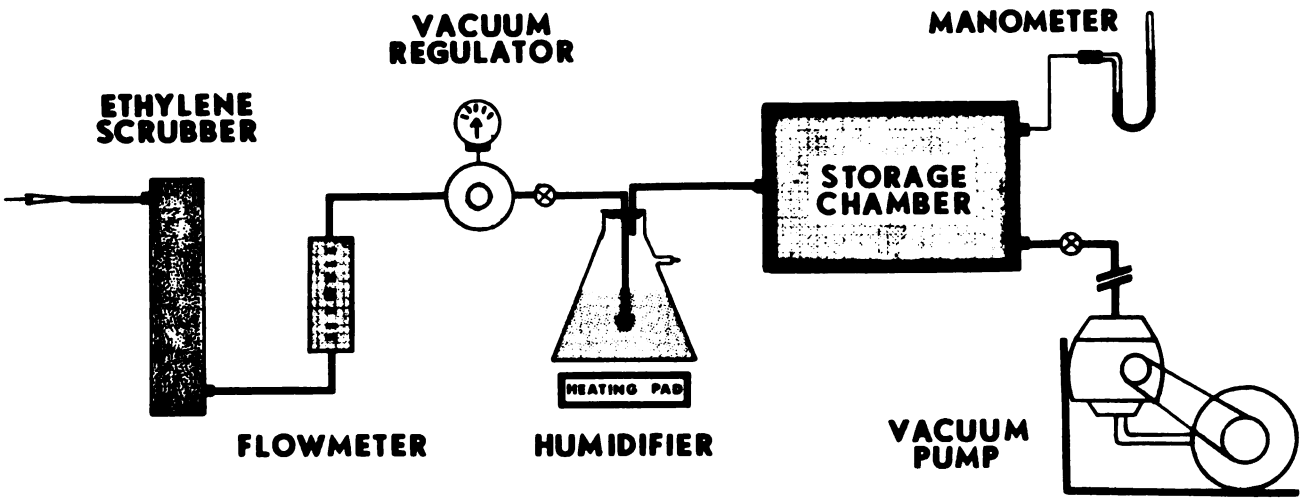

Figure 1. Schematic diagram of the hypobaric system used for the storage of transplants.

10^{o}C treatments, 25 and 760 mm-Hg stored for 2 and 4 weeks, though in poor condition, were planted but not included in any further experimental evaluation.

A two-row, self-propelled, Holland mechanical transplanter was used for outplanting. Each treatment had 4 replicates with all but 3 replicates containing 50 plants each. A shortage of plants required 2 replicates to contain 35 plants and one 40 plants. A completely randomized design was used. The cultivation, fertilization and spray program was carried out according to the standard commercial practices of Michigan. Growth ratings were taken on June 17, June 23, July 8 and August 12; a survival count was also taken on June 23. Subjective ratings were made on a scale of 0-9 where 0 was a dead plant and 9 was equal to the best of the cooperating growers plants already in the field. On August 8, an infestation of bacterial blight, Pseudomonas apii, occurred and an application of copper fungicide was made to correct the problem.

The plants were harvested on September 2, 81 days after outplanting. All harvesting was done by hand. Plants were divided into two groups; marketable and unmarketable. A plant was considered unmarketable if it had bolted or was suffering from aster yellow virus. The number of bolters and diseased plants were recorded and the plants discarded. The remaining plants were sorted into size classes of large, medium and small corresponding roughly to a 24, 30 or 36 count market pack. After sorting, the stalks were trimmed, counted and weighed. The trimmings for each plot were collected and weighed separately. In addition, 5 plants were selected at random from each plot for measurement of meristem length to assess incipient bolting.

Cauliflower. On July 26, cauliflower plants (40 days old) were harvested from a field seedbed in Richmond, Michigan. Two varieties, Self-Blanche from the Harris Seed Co. and Clou from the Stokes Seed Co., were used. A portion of the plants harvested the same day were planted immediately and served as controls. Each variety was considered a separate experiment and a split-split-plot design was employed with 3 replications per treatment and 20 plants per replicate. The soil was a Boyer sandy loam. On the day the controls were planted, soil moisture was low and the temperature was near 30°C. Planting was done with a two-row, Holland mechanical transplanter. Irrigation was applied immediately afterwards but some of the plants had already wilted beyond recovery.

The rest of the plants were transported to East Lansing and stored for 2 or 4 weeks at 0°C and either 25, 150 or 760 mm-Hg atmospheric pressure. The hypobaric system was that described previously but the containers employed were 46 liter milk cans modified for hypobaric use. At 2 and 4 weeks, plants were removed and outplanted at Richmond. The second and third plantings were made by hand because plants from the earlier setting made it difficult to get the planter into the plots. The plants were then grown utilizing standard commercial cultural practices.

Due to the prediction of severe frost, all plants were harvested on October 28. Buttoning was determined subjectively using descriptions from the literature (14) and the experience of the grower. Buttoned plants were counted and discarded. Curds, when present, were separated from the rest of the plant and weighed. Only an average weight of curd per plant was determined. After removal of the curd,

the fresh weight of the remaining above ground portion of the plants was taken. Since most of the non-buttoned plants had not yet formed a curd, this fresh weight was used as a basis for plant yield data. A survival count was also taken at this time.

RESULTS AND DISCUSSION

Celery. Recovery ratings were taken at 1, 2, 4 and 9 weeks (Table 1). After 1 week in the field, all plants were in fair condition but none were as good as those of the grower also planted by him on the same day which were used for reference in the ratings. In all cases, comparisons were made with plants which received no storage. At least two explanations are possible; first, the California plants were grown in sand and were transplanted into a muck soil. It is conceivable that such plants may require an adaptation period to adjust to a different soil pH, nutrient balance or water availability. Alternatively, and perhaps more likely, the California-grown plants, being considerabley larger than those normally transplanted in Michigan may have experienced more stress at outplanting. Dry soil conditions and warm, sunny weather likely put an increased stress on a root system already damaged by the original harvesting operation. Plants pulled from the seedbed have many of the fine root hairs responsible for the majority of water uptake by the plant damaged or removed. Since the California-grown plants had a larger above ground surface area, their water requirements would be greater than smaller plants. At later evaluations (4 and 9 weeks), the California-grown plants were similar to the Michigan-grown plants used for comparison. The capability of plants to reach normal

Table 1. Effect of Storage Pressure and Duration at 0°C on Recovery Ratings[z] for Celery Transplants at Various Intervals After Outplanting.

Pressure (mm-Hg)	Duration (weeks)	Date 6/17	6/23	7/8	8/12
25	0	7.25	7.50	8.50	8.75
25	2	7.75	7.75	8.50	9.00
25	4	6.25	6.25	7.50	9.00
25	6	4.75	5.50	6.75	8.50
760	0	7.00	7.50	8.25	9.00
760	2	6.50	7.25	7.50	8.75
760	4	7.00	7.00	8.50	9.00
760	6	5.00	6.50	7.75	9.00

[z] 1 = dead; 9 = best

maturity was apparently not hampered by either low temperature (0°C) or low pressure (25 mm-Hg) storage for at least 6 weeks.

Table 2 shows the percentage survival 2 weeks after planting and at harvest. Some plants originally counted as survivors on June 23 apparently did not survive. Analysis of the data revealed no real difference between any of the treatments however, there is a trend that may indicate that the percentage survival decreased as the duration of storage increased.

The crucial point of the experiment was to determine whether bolting could be inhibited by using hypobaric storage. The results are given in Figure 2 and Table 3. The controls (non-stored plants), as expected, had no bolters. After 2 weeks there were 1415 bolters per hectare from the 0°C at 760 mm-Hg treatment but no bolters were found among plants stored at 25 mm-Hg at the same temperature. The difference was not statistically significant but 1415 bolters per hectare is an unacceptable number with regards to grower profit. At 4 and 6 weeks there was a significant interaction between storage duration and pressure with regard to the number of bolters. The response to pressure therefore is dependent on the length of storage and vice versa.

Storage at 25 mm-Hg did not eliminate bolting over long storage periods at 0°C, but it did severely attenuate it. In looking for a plausible explanation for this phenomenon we must go back to the literature. As indicated in the review, Platenius (46) observed an increase in sugar accumulation in meristematic tissue of celery prior to seedstalk formation. Sadik (51) and Grainger (31) have indicated that this accumulation is necessary before floral initiation can occur. Wu et al. (74) have demonstrated that hypobaric storage of tomatoes

Table 2. Percentage Survival of Celery Transplants at Two Dates After Outplanting as Influenced by Storage Pressure and Duration at 0°C.

Pressure (mm-Hg)	Duration (weeks)	Date 6/23	Date 9/2
25	0	99.5[y]	95.5ab[z]
25	2	100	98.0ab
25	4	100	96.9a
25	6	97.5	93.7b
760	0	100	99.5a
760	2	99.5	98.4a
760	4	100	95.9ab
760	6	100	97.7ab

[z]Mean separation within columns by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

[y]Absence of letters indicates nonsignificance.

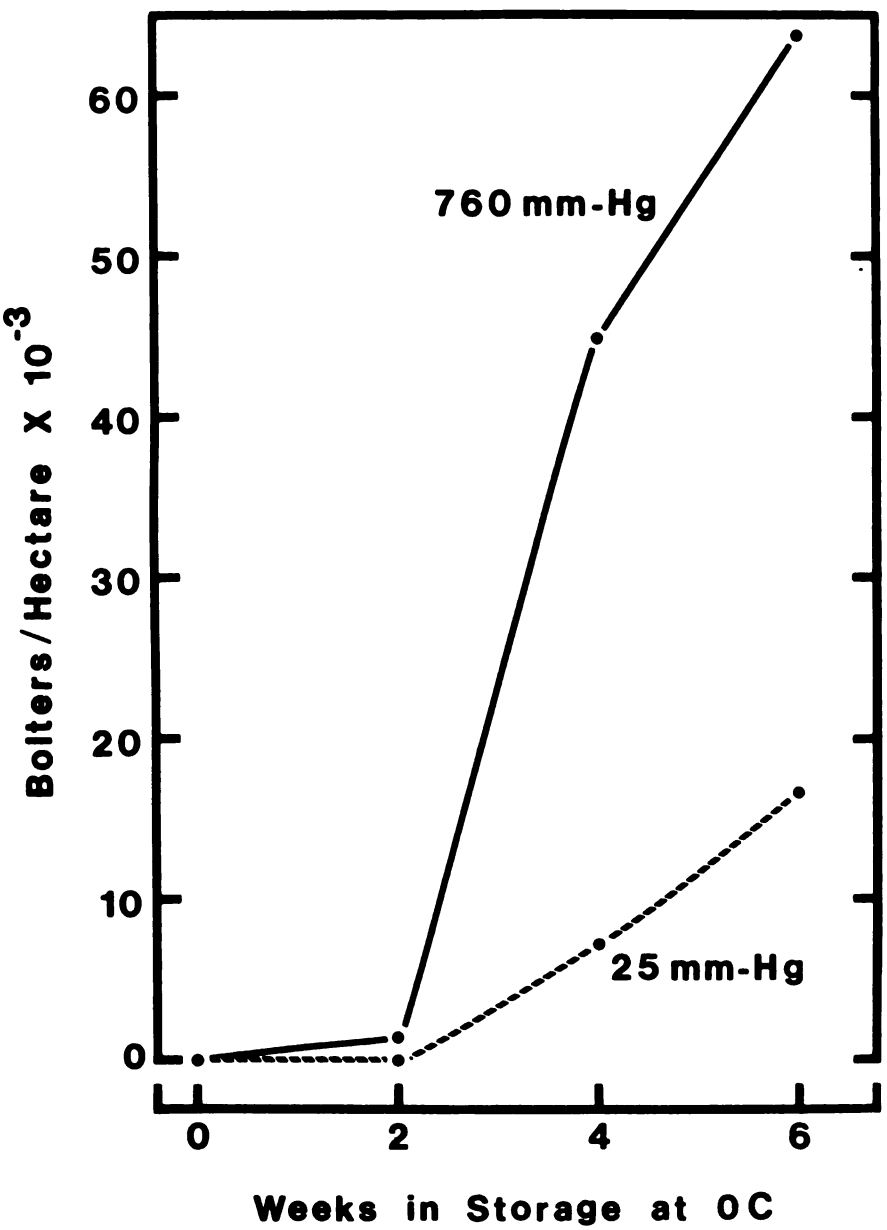

Figure 2. Effect of storage pressure and duration at $0^{o}C$ on floral initiation in celery.

Table 3. Effect of Storage Pressure and Duration at 0°C on Floral Initiation in Celery.

Pressure (mm-Hg)	Duration (weeks)	Bolters/Ha.
25	0	0a[z]
25	2	0a
25	4	7314a
25	6	16663b
760	0	0a
760	2	1415a
760	4	44981c
760	6	63846d

[z]Mean separation by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

reduced the rate of degradation of starches to sugar. If this inhibition of starch degradation occurs in celery also, it may offer an explanation for the reduced number of bolters at low pressure. Melchers (44) and others have postulated the presence of a substance known as vernalin which is produced under conditions of low temperature and high sugar. The chemical has never been identified but its action or synthesis, if it indeed exists, could be inhibited by low pressure either by reducing sugar accumulation or inhibiting some other metabolic process. It may also be possible that some volatile such as ethylene is removed which is necessary for vernalization to occur. The low oxygen tensions present under hypobaric conditions may offer the best explanation as to the nature of the inhibition of bolting under low pressure conditions. Gregory and Purvis (32) demonstrated that vernalization is an aerobic process. Under the low oxygen tension (0.57% v/v), the vernalization response may occur but at a highly reduced rate. This may explain the observation that bolting at 25 mm-Hg, though greatly attenuated, increases with time.

In constructing yield data, only marketable plants were used. Figure 3 indicates total marketable yield. After 2 weeks there was no significant decrease in yield between plants stored at 25 or 760 mm-Hg. At 4 and 6 weeks there was a drastic drop in yield per hectare at both pressures but the plants stored at the ambient pressure yielded significantly less than those stored at 25 mm-Hg. This drop in yield was due mostly to an increased number of bolters and fewer marketable plants per hectare (See Figure 4).

Maturity for market was based on stalk size. Consumer preference studies, based on sales, have indicated that the medium size stalk

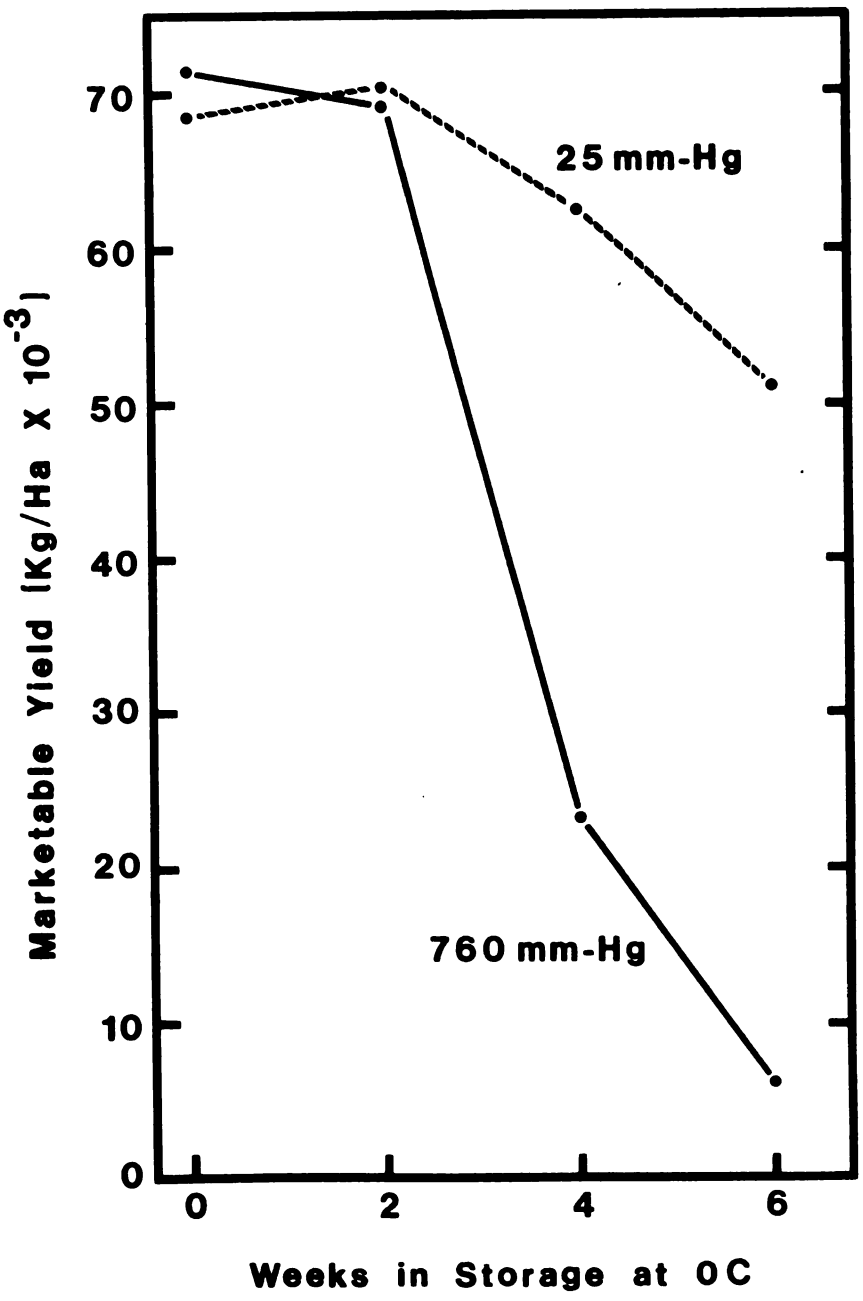

Figure 3. Effect of storage pressure and duration at 0°C on total yield of marketable celery. The yield does not include the weight of bolters nor trimmings.

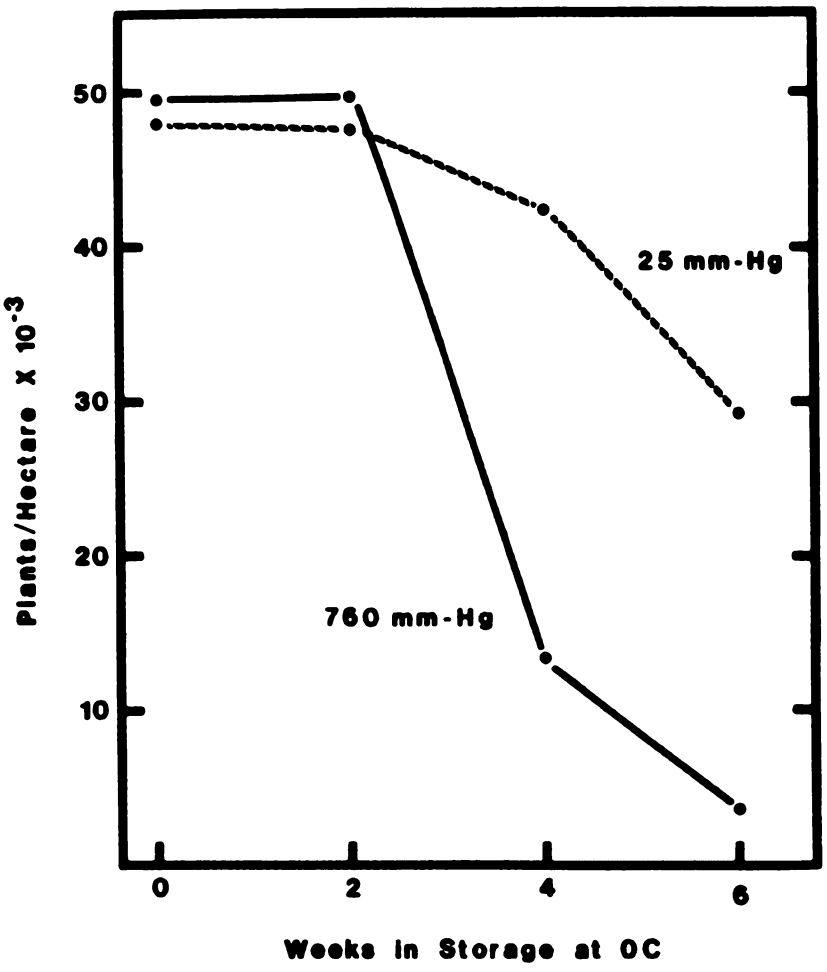

Figure 4. Effect of storage pressure and duration at 0°C on the total number of marketable plants per hectare.

(2½ dozen/crate) is most preferred followed by small and then large. Table 4 indicates that as storage time increased, the plants produced smaller stalks at both pressures. This may relate to the time of recovery in the field after storage; with longer recovery times delaying the time to reach market maturity. The danger of delaying harvest until the plants attain their maximum size is that seedstalks already initiated but not yet emerged, may elongate, making the plant unmarketable.

Figure 5 indicates the induction of floral initiation as measured by the length of the meristem when the plants were cut in half longitudinally. The only significant difference occurred at 4 weeks. The general trend however, was that those at the ambient pressure were more differentiated than those stored at low pressure and as such, would tend to bolt sooner. The drop in meristem length from 4 to 6 weeks at 760 mm-Hg may indicate genetic variability in the variety. With plants stored for 6 weeks, those most susceptible to bolting had already bolted by harvest time while those with some bolting resistance were in various stages of seedstalk elongation. In this treatment, the bolt resistant plants probably made up the majority of the sample and thus the sample was biased, accounting for the apparent reduction in floral initiation between 4 and 6 weeks of storage at 0^{o}C and 760 mm-Hg.

Cauliflower. Clou is considered an early season variety and Self-Blanche a fall variety. As such, Self-Blanche does not tolerate the heat of summer nearly as well as Clou. Table 5 indicates the percentage survival for both varieties after outplanting. Data for the controls demonstrate that Clou was more resistant to the adverse planting

Table 4. Celery Stalk Size[z] Distribution and Trimming Waste as Influenced by Storage Pressure and Duration at 0°C.

Pressure (mm-Hg)	Duration (weeks)	Percentage of Weight			
		Small	Medium	Large	Trimmings
25	0	6.6a[y]	43.5a	18.0cd	31.9[x]
25	2	10.5a	44.6a	11.8bc	33.1
25	4	8.6a	50.2a	6.2ab	35.0
25	6	23.3a	35.9a	6.3ab	34.5
760	0	6.5a	48.8a	12.3bc	32.4
760	2	9.1a	37.7a	21.9d	31.3
760	4	19.6a	47.5a	0.0a	32.9
760	6	47.0b	21.1b	0.0a	31.9

[z]Small, medium and large correspond to a 24, 30 and 36 count per crate.

[y]Mean separation within columns by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

[x]Absence of letters indicates nonsignificance.

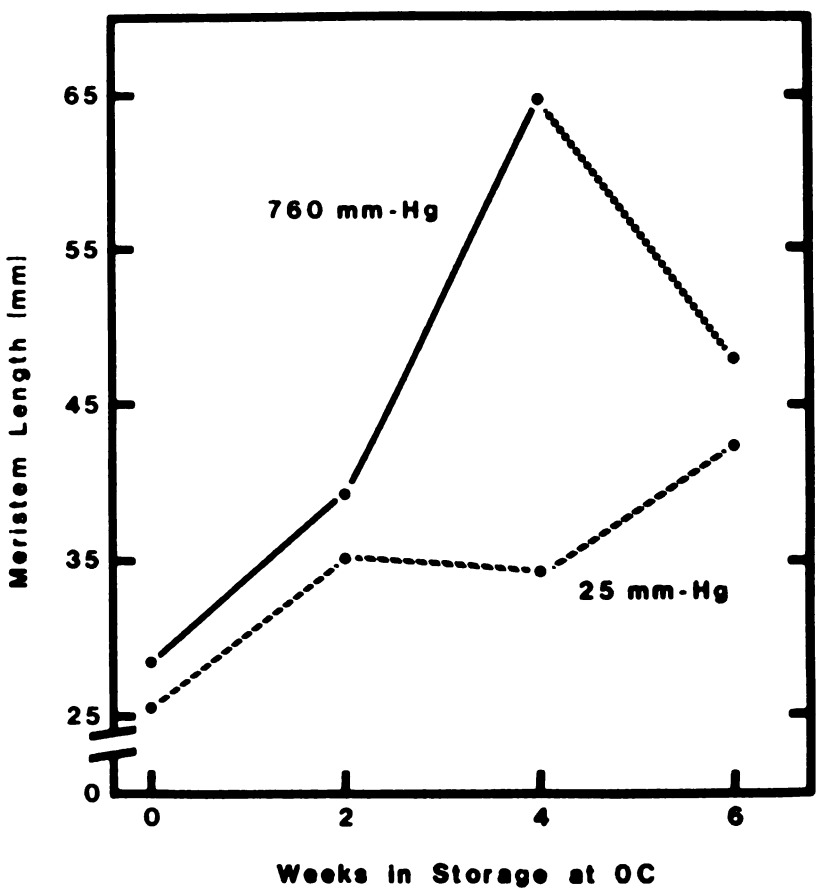

Figure 5. Effect of storage pressure and duration at 0°C on meristematic development of marketable celery plants.

Table 5. Effect of Storage Pressure and Duration at 0°C on Percentage Survival for Clou and Self-Blanche Cauliflower After Outplanting.

Variety	Pressure (mm-Hg)	Duration (weeks)	% Survival
Self-Blanche	control	0	79.4ab[z]
"	25	2	93.3ab
"	150	2	98.3a
"	760	2	96.7ab
"	25	4	30.0a
"	150	4	93.3ab
"	760	4	93.3ab
Clou	control	0	96.7[y]
"	25	2	93.3
"	150	2	91.7
"	760	2	95.0
"	25	4	91.7
"	150	4	95.0
"	760	4	85.0

[z]Mean separation by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

[y]Absence of letters indicates nonsignificance.

conditions than was Self-Blanche. This is consistent with the acclaimed heat tolerance difference of these varieties. All other treatments showed fair to good recovery with the exception of the Self-Blanche plants held at 25 mm-Hg for 4 weeks which gave only a 30% recovery. There is no apparent explanation for this high mortality. It appears to be a variety characteristic. Further testing is necessary to determine the underlying physiological basis. In general, the rate of survival decreased as the pressure decreased indicating a stress created by the low pressure.

Like celery, the purpose of the cauliflower experiment was to alter a response to cold treatments. Table 6 lists the percentage of buttoned heads per hectare for both varieties. For Self-Blanche, though not significantly different, the number of buttoned heads tended to decrease as the pressure was decreased. This difference was significant for Clou. At ambient pressure, over 42% of the heads buttoned while at 25 mm-Hg, none of the plants buttoned even after 4 weeks of storage at $0^{o}C$. A pressure of 150 mm-Hg gave an intermediate amount of buttoning indicating that the effects of the low pressure treatment are additive. The data is consistent with the hypotheses put forth previously for celery. The attenuation of vernalization by low oxygen tensions still appears to be the most feasible. The intermediate oxygen level yielded an intermediate number of buttoned heads. In addition, the results appear to be in agreement with Skapski's (57) observation that buttoning is, in part, variety dependent. The reason the summer variety was more susceptible to buttoning than the fall variety may be that summer varieties do not require as low a temperature or as long a duration of cold to trigger the vernalizing mechanism.

Table 6. Percentage of Cauliflower Plants Per Hectare Which Buttoned as Influenced by Storage Pressure and Duration at 0°C.

Variety	Pressure (mm-Hg)	Duration (weeks)	% Buttoned
Self-Blanche	control	0	0.0[y]
"	25	2	0.0
"	150	2	0.0
"	760	2	0.0
"	25	4	0.0
"	150	4	1.9
"	760	4	3.6
Clou	control	0	0.0a[z]
"	25	2	0.0a
"	150	2	12.8ab
"	760	2	18.9b
"	25	4	0.0a
"	150	4	37.8c
"	760	4	42.6c

[z]Mean separation by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

[y]Absence of letters indicates nonsignificance.

The foregoing results argue against traditional beliefs concerning the cause of buttoning. As reviewed earlier, Carew and Thompson (14), Robbins et al. (50) and others agree that no single factor contributes to buttoning but rather it is a combination of factors including nitrogen supply, temperature, transplant age and transplant size. The plants used in this experiment were of a normal size and age and they were planted in soil with an adequate supply of nitrogen. Growing conditions were favorable until well after the time the plants began buttoning. Accordingly, there are two possible conclusions; first, low temperature alone may be enough to induce buttoning in cauliflower. Alternatively, young plants with 4-8 leaves are capable of being vernalized. This is consistent with Wiebe (71) and at odds with Sadik (51) and Aamlid (1) who claim that a cauliflower plant requires from 16-20 leaves before it is capable of being vernalized. However, the age at which juvenility ends may be variety dependent. The plants used in this experiment were young, but old enough to be vernalized according to Wiebe's parameters.

Yield studies were confounded by the early harvest. Because plantings were made at different times, no statements can be made concerning differences caused by the duration of storage. The original intent was to use curd weight as a measure of yield. Since a majority of the marketable plants did not develop curds by harvest time, the fresh weight of the above ground portion of the plant was substituted. The results are presented in Table 7. The decreased yields for Self-Blanche at low pressure are a direct result of the poor survival rate. The increased yields at low pressure for Clou are a result of fewer buttoned heads.

Table 7. The Effect of Storage Pressure on the Fresh Weight of Cauliflower Plants Per Hectare.

Variety	Pressure (mm-Hg)	Kg/Ha
Self-Blanche	25	27513a[z]
"	150	29395a
"	760	30071a
Clou	25	27258a
"	150	26341a
"	760	23561b

[z]Mean separation by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

When an average weight per plant was computed, plants of the Clou variety stored at 25 mm-Hg were significantly larger than those stored at 760 mm-Hg (See Table 8). There were no differences between 25 and

Table 8. The Effect of Storage Pressure on the Average Plant Weight of Clou Cauliflower Plants.

Variety	Pressure (mm-Hg)	Grams/Plant
Clou	25	1060a[z]
"	150	1063a
"	760	939b

[z]Mean separation by Duncan's multiple range test. Means followed by the same letter are not significantly different at the 0.05 level.

150 mm-Hg. Differences for Self-Blanche were nonsignificant although there was a tendency towards smaller plants as the pressure was decreased. The larger average plant size for Clou may be due to a higher retention of chlorophyll and carbohydrate reserves during the storage period. Tolle (68), Wu et al. (74) and Bangerth (6) have all demonstrated that low pressure storage inhibits chlorophyll degradation in tomatoes. Dilley and Price (24) have also demonstrated that chlorophyll is retained in pepper and tomato transplants at low pressure. With greater chlorophyll retention, the photosynthetic capacity of the low pressure stored plants would be greater for the first few days after transplanting which may be vital to reestablish plant growth.

SUMMARY AND CONCLUSIONS

Celery plants were held at 0°C for two weeks at 25 mm-Hg with no seedstalk formation occurring after transplanting while those stored at ambient pressure showed a tendency to bolt. At durations longer than two weeks, some bolting occurred but considerabley less than that observed in plants stored at ambient pressure. The 6 week storage treatment at 0°C and 760 mm-Hg had 91% bolters while those stored under the same conditions but at 25 mm-Hg had only 25% bolters. Cauliflower showed a similar response with the 25 mm-Hg pressure preventing buttoning even after 4 weeks of storage at 0°C. Those stored 4 weeks at ambient pressure showed approximately a 40% buttoning rate for the Clou variety. Yield differences in celery were related to both pressure and duration with stalk size decreasing with duration but increasing at lower pressures.

Prevention of floral initiation for two weeks should be enough time to move plants overland from California to Michigan or other production sites. A cost analysis will be necessary to determine the cost/benefit ratio. Lougheed (43) has estimated that hypobaric storage has a 36% higher cost than conventional controlled atmosphere storage for apples. Hypobaric storage and transportation facilities are expensive and the costs may not justify the benefits for seedling transplants, but this remains to be determined.

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