

High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var. *italica* L.)

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Abstract

High temperature causes unevenly-sized flower buds on broccoli inflorescences. This deformity limits production of broccoli to areas where summer temperatures rarely exceed 30 °C. The stage of development sensitive to heat was determined by exposing plants of 'Galaxy' broccoli at varying developmental stages to 35 °C day temperature for 1 week, and subsequently analysing the head structure. During the high temperature exposure, the development of certain flower buds was arrested. There was no corresponding cessation of bud initiation at the apex. No injury resulted if heat was applied before the reproductive induction, nor was there injury to differentiated flower buds. Meristems were affected only if heat was applied during inflorescence production or the floral initiation process. Shorter heat exposures produced little injury, and longer exposures were lethal. The plant's development at this sensitive period still appeared vegetative externally, but the youngest leaves had just begun to reorientate as a consequence of the reduced stem elongation rate. The meristem was less than 1 mm wide, and floral primordia were just forming, still subtended by leaf primordia. The injury was fully expressed by the time the head was first exposed (approximately 5–10 mm wide), though it became more apparent as the head matured. The buds that were delayed in development by the high temperature developed into normal flowers.

Key words: *Brassica oleracea*, broccoli, flowering, heat injury, developmental arrest.

Introduction

Floral development of broccoli (*Brassica oleracea* var. *italica*) is disrupted by temperatures over 30 °C, temper-

atures near optimal for vegetative growth. This lack of heat tolerance restricts broccoli production to maritime climates lacking extreme temperatures. The alteration in the inflorescence structure has been described primarily in terms of the marketability of the commercial product (Heather *et al.*, 1992).

Inflorescence development in broccoli involves proliferation of the meristem to make a highly ramified inflorescence. Developmental arrest occurs during floral bud development and internode elongation (Carr and Irish, 1997). In contrast, cauliflower has greater meristem proliferation and is arrested earlier, after the first florally-determined gene expression but before any bud development (Carr and Irish, 1997). Exposure to high temperature during a time window several weeks before harvest maturity disrupts broccoli flower development (Heather *et al.*, 1992), giving parts of the inflorescence the superficial resemblance to cauliflower. This paper describes the development of the broccoli inflorescence, the disruption of that development that is brought on by excessive temperatures and the stage of development that is affected by heat.

Materials and methods

Plant raising

Seeds of cv. Galaxy were sown into a peat-vermiculite growing medium in 'Speedling No. 100A' modular trays, containing 930 cells m⁻², each having a volume of 23 ml. Trays were placed in a glasshouse at day/night temperatures of 21/15 °C. Seedlings were fertilized 5 times weekly with liquid fertilizer containing 100 mg l⁻¹ N, 44 mg l⁻¹ P and 83 mg l⁻¹ K. After 30 d, the seedlings were vernalized for 10 d in a growth chamber at day/night temperatures of 13/11 °C with a 12 h photoperiod (Miller, 1988; Wurr *et al.*, 1995). Plants were then transplanted into 15 cm pots containing 1 l of Cornell Mix A (Boodley and Sheldrake, 1977) and 5 g slow-release fertilizer (Osmocote 14–14–14), and subsequently fertilized three times weekly at

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watering with liquid fertilizer as above. The small pots restricted the plant size, but bud development was otherwise normal. Growth continued under the glasshouse conditions described above.

Field-grown plants shown in Fig. 1 were started in trays as described above and transplanted in a research field at the Vegetable Research Farm of the New York State Agricultural Experiment Station on May 31, 1995. They experienced temperatures over 30 °C frequently during July and were photographed on July 27.

Heat treatment

When plants reached the appropriate stage for heat treatment, they were placed in a growth chamber (Conviron E15, Winnipeg, Manitoba, Canada) for 1 week at a day/night temperature of 35/22 °C with a 14 h photoperiod ($300 \mu\text{mol m}^{-2}\text{s}^{-1}$) and 75% humidity.

The first experiment tested susceptibility to injury at specified chronological ages. Groups of 12 plants were placed in the heat twice weekly beginning 65 d after seeding and continuing through 90 d after seeding. Control plants were kept in the glasshouse throughout. The mean leaf number was 11.6 ± 1.1 leaves (> 1 cm) at 65 d increasing to 19.2 ± 1.8 leaves at 90 d. The variation in development rate is typical for a hybrid broccoli (Wurr *et al.*, 1995).

The second experiment tested susceptibility to injury at specified growth stages: the first external sign of reproductive development (straightened stage in Fig. 2), a few days later (bowed stage) and the last stage before the head was visible without dissection (crown stage). Groups of 20 plants were assigned to each treatment and were moved into the heat when they reached the assigned growth stages. Twenty control plants were kept in the glasshouse throughout. A greater number of plants was used per treatment because greater statistical power was needed to avoid Type II errors with fewer treatments. The stage of development was determined by measuring the width the shoot tip at the thickest point of the petioles of two oldest appressed leaves, with the widths being 6, 7 and 9 mm for the three stages. The plants had 12 ± 0 , 13.0 ± 0.2 and 13.1 ± 0.3 leaves (> 1 cm) at the three stages, respectively, for the data in Figs 6 and 7. Leaf number was not a consistent predictor: in other experiments the bowed stage was reached between 11 and 14 leaves.

The third experiment tested how injury differed with different durations of treatment. The durations of the heat treatments were 0, 1, 2, 4, 7, and 10 d. Eight plants were randomly assigned to each heat treatment, then placed in the growth chamber for the indicated time beginning at the bowed stage.

Scoring injury

The unevenness of the bud sizes was quantified using a 7-point scale (Heather *et al.*, 1992), scoring when the oldest buds were 2–3 mm long. A score of 1 was a perfect head, 3 had small buds showing at the centre of each whorl, 5 had about 50% of the area of each whorl in small yellow buds, 7 had few or no normal buds at the time when the head should have been mature. Bud sizes were measured on the terminal whorl and on the central whorl of the first branch about 4 d after scoring, to allow the buds to enlarge until they were more easily measurable. The sepal length was measured for each bud starting with the oldest bud and working inward along the phyllotactic spiral. For the duration of injury experiment, bud sizes were measured on the oldest outward-facing whorl on the third branch of each plant. These were both consistent and representative of the quality of the whole head.

Scanning electron microscopy

Broccoli apices were excised and fixed with 3% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 6.5 for 1.5–2 h. They were rinsed in phosphate buffer, distilled water, then dehydrated in an acetone series and finally dried in a critical point dryer (CPD020, Balzers AG, Balzers, Switzerland). The specimens were coated with 100 Å Au/Pd on a sputter coater (SCD040, Balzers AG, Balzers, Switzerland) and viewed on a Hitachi S-530 microscope at an acceleration voltage of 20 kV.

Results

Heat injury in broccoli is characterized by an uneven appearance of the head, caused by exposed flower buds having a much greater range of sizes than in uninjured heads (Fig. 1). The exposed lower buds on normal heads of Galaxy broccoli were fairly uniform in size, with sepals about 2 mm long at harvest maturity (Fig. 1a, b). Moderately heat injured heads had normal sized buds on the outside of each whorl, but many smaller ones exposed in the centre (Fig. 1c, d). Severely injured heads had predominantly small buds, but with a few normal buds (Fig. 1e, f).

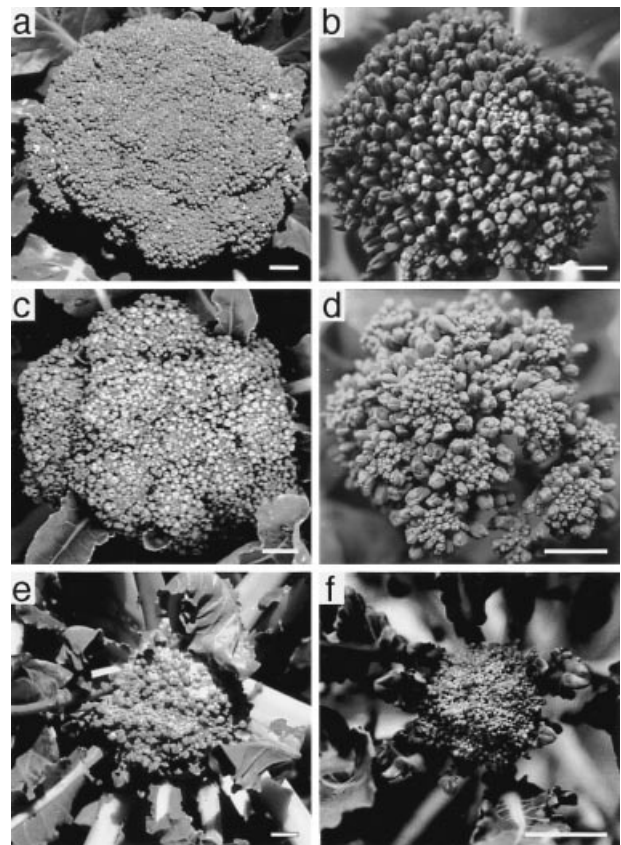


Fig. 1. Appearance of heat injury to broccoli inflorescences. (a, c, e) Field-grown plants; (b, d, f) greenhouse-grown plants. (a, b) Normal appearance with uniform flower bud size (injury rating=1); (c, d) moderate symptoms, outer buds in whorl larger (injury rating=4); severe symptoms, only a few large buds (injury rating=7). Scale bar = 1 cm.

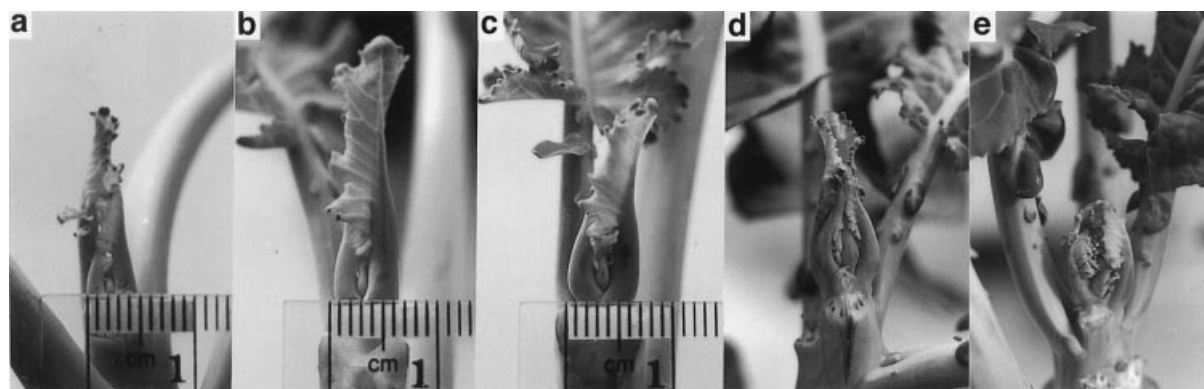


Fig. 2. External appearance of shoot tip during reproductive transition. The growth stages are (a) vegetative, (b) straightened, (c) bowed, (d) crown, (e) headed.

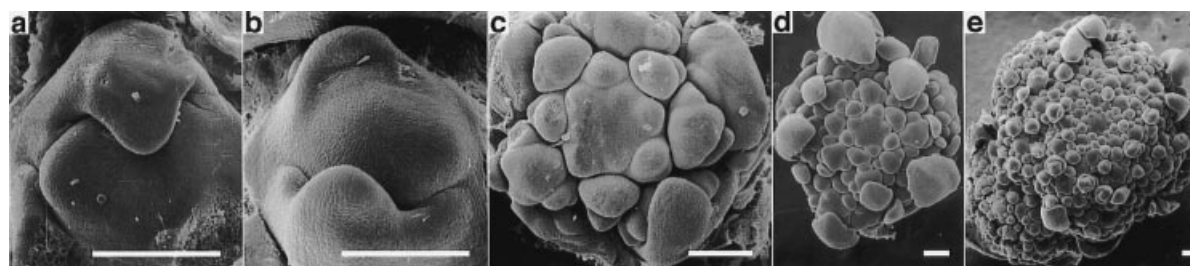


Fig. 3. Microscopic appearance of meristem during the reproductive transition. The shoots were dissected when the external appearance corresponded to the pictures in Fig. 2: (a) vegetative, (b) straightened, (c) bowed, (d) crown, (e) headed. Scale bars = 0.2 mm.

In order to apply heat stress at specific stages of reproductive development, changes in the external appearance of the shoot were identified. The shoot tip was a constant size from when leaf 6 reached 1 cm until the reproductive transition, which occurred at leaf 11 to 15. The width of the shoot tip was determined by measuring across the petioles of the two largest leaves that were still appressed, as shown in Fig. 2a–c. The tip was 4–5 mm across during the vegetative stage (Fig. 2a). The first change in the appearance of the shoot tip was an increase in this measurement to 6 mm, and a slight straightening of the youngest unfurled leaves in the ‘straightened’ stage (Fig. 2b). Three to four days later, the apex was 7 mm across, and the petioles curved slightly inward at the ‘bowed’ stage (Fig. 2c). After 3–4 additional days, the apex was 9 mm across and highly curved, suggesting the existence of a small head inside at the ‘crown’ stage (Fig. 2d). An additional 4 d thereafter, the head became visible at the ‘headed’ stage (Fig. 2e).

The development of the meristem at each of these stages was then identified. Heads dissected at the vegetative stage had only leaf primordia (Fig. 3a); at the straightened stage, cauline leaf primordia with a flat spot at the incipient axillary meristem (Fig. 3b); paraclades developing from the axillary meristems (Fig. 3c); and flower buds developing on the paraclades (Fig. 3e).

Differentiation of the reproductive shoots had two

phases. In the first phase, axillary meristems developed, producing reiterating paraclade meristems (Fig. 3c, d). In the second phase, all the meristems produced flower buds (Fig. 3e).

When plants were exposed to high temperature during the vegetative period, no injury was observed (Fig. 4). The unevenness can be expressed in terms of flower-bud size. Heat during each of the early reproductive stages resulted in an uneven head. The decrease in bud size

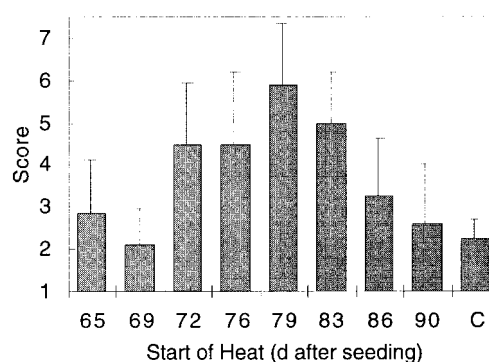


Fig. 4. Injury to broccoli heads when plants were exposed to 35 °C for 1 week beginning at different times after planting. Individual plants were at somewhat different growth stages on an individual day, but the crown stage occurred at approximately day 84. The 7 point rating scale of increasing injury is described in the text and illustrated in Fig. 1. The error bars indicate the standard error.

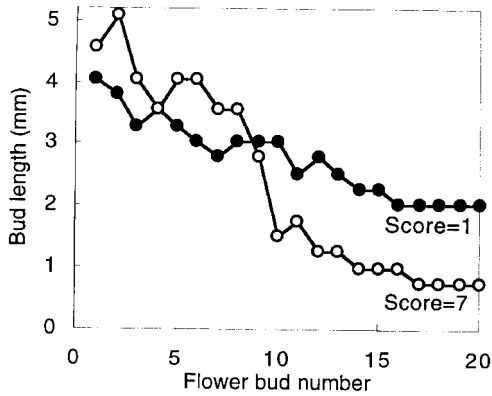


Fig. 5. The size of flower buds on the terminal floral whorl of broccoli inflorescences relative to their position on the apex and the subjective score of heat injury. The buds were numbered from the outside and increased in order along the phyllotactic spiral. The bud size was measured as the length of the sepals when the oldest buds were about 4 mm long. The lines are for a whorl from a representative uninjured head (Injury rating=1), and from a severely injured head (Injury rating=7).

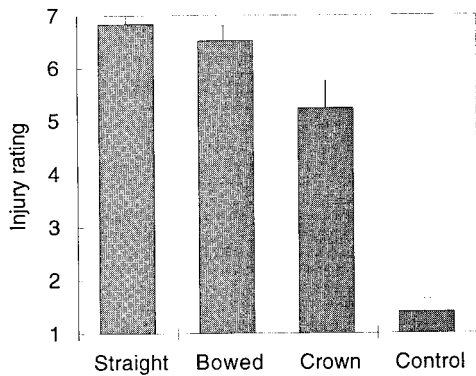


Fig. 6. The extent of injury resulting in broccoli inflorescences when plants were exposed to 35°C for 1 week beginning at different stages of reproductive transition. The error bars indicate the standard error.

along the phyllotactic spiral was monotonic in uninjured inflorescences, but showed a discontinuity in injured ones (Fig. 5). The early reproductive stages were similarly sensitive to stress (Fig. 6), with a larger number of uninjured buds on the terminal whorl the later the stress was applied (Fig. 7). When the stress was 4 d or less, the discontinuity was too small to observe, with 7 d of treatment it was distinctive, and with 10 d the plants lost leaves, had severely reduced head size and 3 of 8 did not recover from the heat treatment (Fig. 8). The size distributions of 7 and 10 d treatments were different from the control at >95% using the Kolomogroff-Smirnoff test. The mean injury ratings (1 to 7 scale) were: 2.0 (control), 2.1 (1 d), 1.4 (2 d), 2.5 (4 d), 5.3 (7 d), and 6.8 (10 d). Thus, only the 7 d treatment produced substantial injury without mortality.

The injury was observable even when the apex was only 2 mm across, with unheated apices having a uniform

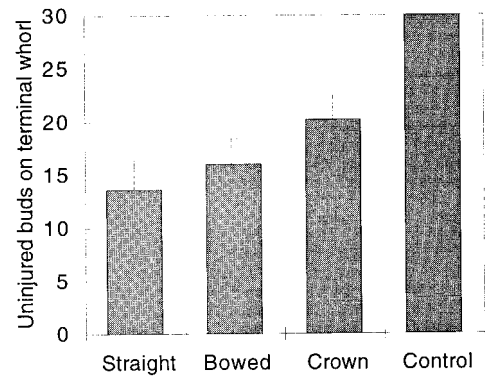


Fig. 7. The number of uninjured buds on the terminal whorl of broccoli inflorescences when plants were exposed to 35°C for 1 week beginning at different stages of reproductive transition. Only the first 30 buds were large enough to judge accurately. A maximum of 30 buds were measured. The error bars indicate the standard error. For comparison, the injured inflorescence in Fig. 5 was scored as having 8 uninjured buds on the terminal whorl.

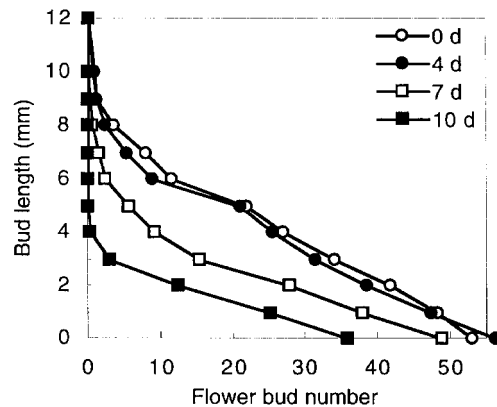


Fig. 8. Bud size distributions caused by different durations of exposure to 35/22°C day/night temperature. The curves are the means of 8 plants, except 10 d in which only five plants survived. The 1 and 2 d treatments are omitted for clarity. The bud size was measured as the length of the sepals when the oldest buds were about 4 mm long.

appearance (Fig. 9a), but a few large buds showing on the heat-treated apices (Fig. 9b). This bud size discontinuity was similar when the head was 20 mm wide. The flower buds that were delayed in development by the high temperature were observed at anthesis to have normal structure and had anther sacs containing pollen.

Discussion

High temperature causes uneven flower head formation in broccoli. Plants that were heat-treated at specific stages of development showed characteristic injury. The external development was consistently correlated with the meristem development. While plants in the vegetative stage were not affected by heat treatment (Heather *et al.*, 1992; Fig. 4), those at the earliest discernible reproductive phase were strongly affected. The plants treated at the beginning

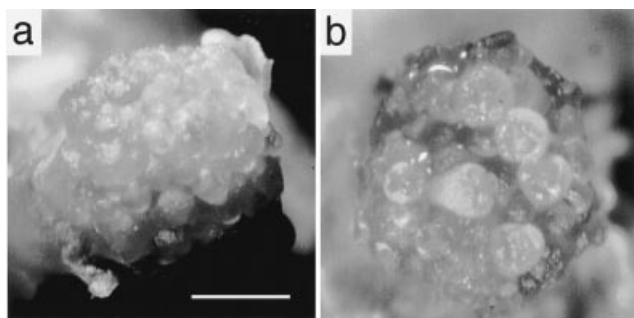


Fig. 9. Appearance of broccoli inflorescence with the abrupt change in flower bud size associated with high-temperature injury. (a) Normal immature inflorescence, (b) injured immature inflorescence. Scale bar = 1 mm.

of reproductive development had few buds escape injury, with the number increasing the later the treatment was initiated. The sensitive stage of development passed by the time the head is 5–10 mm, which was the estimate made by Heather *et al.* (1992) based on field-grown material. Nevertheless, the time from the sensitive stage until the buds were mature, 3 weeks, was similar.

This pattern of injury is consistent with heat preventing the initiation of bud elongation, but affecting neither bud production nor continued elongation. This model predicts several elements of the pattern of injury: those buds that are enlarging when the stress is begun continue to do so, creating a large size difference between affected buds and those only a few places lower in the phyllotactic spiral. The continued production of buds results in the many small buds at the centre of each paraclade being exposed. In a normal head, these would be so few as to be covered by the more mature buds, giving a highly uniform appearance.

In a normally-developing inflorescence, the flower buds on an individual head increased in size from the bud primordia at the apical meristem outward to the oldest bud. At harvest maturity the oldest flower buds are 2 mm long in this variety; buds of similar size cover the small young buds at the centre of the whorl. In heat-injured heads there were a variable number of older buds of age-appropriate size, and a larger number of small buds, with an abrupt discontinuity in bud size. In the centre of each whorl, small buds were exposed before they turned green. The exposed buds indicate that there is continued bud differentiation during the heat exposure rather than a cessation of all development. The number of normal-sized buds was related to the degree of differentiation of the reproductive apex at the time of heat exposure. The week-long heat stress was too long to permit the exact stage of bud arrest to be specified, but shorter stresses did not result in a distinguishable discontinuity. Such a determination will require determination of changes in the expression of relevant genes.

The alterations in bud development can be interpreted

in terms of the flowering genes that have been identified in broccoli and cauliflower. Since injury only occurred in plants that had already been induced to produce reproductive meristems, the sensitivity is not in any of the genes associated with floral induction and vernalization. At the other end of the developmental sequence, the injury does not affect flower structure, so the sensitivity is not in the well-studied *Brassica* floral organ identity genes. The injury occurred during the initiation of bud enlargement, which is part of the floral initiation process (Ma, 1994; Haughn *et al.*, 1995). In cauliflower, *BoAPI* expression peaks at the transition from reiterating meristems to floral primordia (Anthony *et al.*, 1996). Developmentally later genes (*BoiAP3*, *BoiCAL*) are expressed only in broccoli, not in cauliflower (Carr and Irish, 1997). Weakened or delayed expression of these genes by high temperature is likely to cause the structural alterations observed here. The localization of *BoiAP3* to the floral meristem rather than the inflorescence meristem (Carr and Irish, 1997) fits the site of arrest especially well. Expression of *BoAPI* is indeed reduced at high temperatures in broccoli (Anthony *et al.*, 1996), related genes expressed only in broccoli may be similarly sensitive.

An alternative candidate is the *tfl* gene, which suppresses flower development (Shannon and Meeks-Wagner, 1993) by affecting the timing of phase switching from inflorescence to flower (Schultz and Haughn, 1993). High temperature makes its phenotype stronger (Alvarez *et al.*, 1992). In broccoli, high temperature injury occurred when this gene would be turning off, and a delay should produce a phenotype similar to that observed. The injury caused by heat is consistent with an effect on the expression level on one or more of the suite of genes controlling the floral initiation process.

Broccoli can have many developmental defects when grown in warm seasons. The defect described here is the one requiring the least heat. In warmer conditions, lack of vernalization, leafy heads and flower death can occur. Timing of the heat stress also affects the type of injury. Non-vernalization occurs from exposure before floral evocation (Fontes *et al.*, 1967). Leafy heads are the result of elongation of the cauline leaves, which then emerge through the flowering head. Normally these leaf primordia at the base of each paraclade are suppressed (Hempel and Feldman, 1994). The reduced growth of flower buds could prevent suppression of the subtending leaf primordium, making leafy heads a secondary effect of inhibition of bud development. Flower death must occur after flower buds have differentiated.

High temperature injury to head development in broccoli was identified as a disruption of the floral initiation process. The disruption appears to be a delay in bud development on the inflorescence meristem, but not an inhibition of bud initiation. The sensitive stage can be identified microscopically by observing inflorescence

primordia rather than leaf primordia at the apex. It can also be identified without dissection by following the broadening of the shoot tip. The plant is in the sensitive stage when the horizontal distance across the petioles of the two oldest appressed leaves increases over what it was during vegetative growth. This result provides the basis for a technique that plant breeders can use to screen for heat tolerance.

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