Relationship between Carbohydrate Level and Floral Initiation in Broccoli[1]

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Abstract. Floral induction of broccoli (*Brassica oleracea italica*) 'Waltham 29' at low temp (5°C) was associated with increasing levels of starch and sugar in the shoot tip. This same relationship was found in experiments concerning plant age, duration of cold treatment, leaf removal before and during cold treatment, and treatment with succinic acid 2,2-dimethylhydrazide (SADH) or gibberellic acid (GA_3).

Greenhouse experiments with plants grown continuously at 16-21°C or 21-27°C indicated that high carbohydrate levels are not necessarily associated with flowering. Flowering was delayed at the higher temp with little difference in carbohydrate level.

There are a number of reports in the literature suggesting a relationship between developmental changes in plants and carbohydrate level (1, 4, 8, 9, 10). In cold-requiring plants low temp is reported to induce flowering and simultaneously increase the level of carbohydrates (9, 11). Temperatures resulting in reduced carbohydrate levels in cauliflower also resulted in reductions in the number of plants which flowered (9). Evidence, however, is lacking for a causal relationship between carbohydrate level and flowering.

With broccoli low temp enhanced flowering and a higher growing temp of 24-27°C blocked flowering (3). However, flowering occurs without cold treatment if plants are grown continuously at temp below 24-27°. Thus, broccoli was used to study further the relationship between carbohydrates and flowering.

Materials and Methods

Broccoli, *Brassica oleracea* var. *italica,* cv. Waltham 29 was used in these experiments. The plants were first grown in a growth chamber at 24° day and 27°C night under 1800 ft-c of light supplied for 16 hr daily (incandescent 20% and cool white fluorescent 80%). Flowering was induced by a 3-week exposure to 5° during which light was supplied for 16 hr daily at an intensity of 350 ft-c (cool white fluorescent). The second method of plant culture was to grow the plants in a greenhouse (February-April) at 16-21° and 21-27°. Succinic acid 2,2-dimethylhydrazide (SADH) and gibberellic acid (GA_3) were applied as foliar sprays at concn of 2500 and 1000 ppm, respectively.

One-half cm portions of shoot tip with all but small primordial leaves removed were used for carbohydrate determinations. Each sample consisted of 3 apices which were stored in a frozen state until extraction with 95% ethanol in a soxhlet apparatus for 10 hr. The ethanol was quantitatively transferred to volumetric flasks and made to volume. Following filtration an aliquot of the ethanol was evaporated to dryness, redissolved in water and used for sugar determinations.

Ethanol insoluble residue remaining in the extraction thimble was dried, weighed, and transferred to 50-ml centrifuge tubes. Starch was solubilized twice with 52% perchloric acid according to the method of McCready et al. (7). Alcohol soluble sugars and solubilized starch were analyzed with anthrone (7). Glucose was used as a standard and results expressed as mg glucose/g fresh wt. The alcohol soluble fraction is referred to as sugars and that fraction which resulted from perchloric acid digestion is referred to as starch.

Results

Broccoli plants were induced to flower by transferring them from a 24-27°C growth chamber to a cold room (5°) 5 weeks after seeding. Samples were taken for carbohydrate analysis at weekly intervals. Exposure of 5-week old plants to 1 week of 5° resulted in an increased level of sugar when compared to shoot tips of plants grown continuously at 24-27° (Fig. 1). This difference remained constant for the following 2 weeks with increased levels at both temp. At 5° the starch content increased with time while at 24-27° the starch level remained unchanged. Floral initiation was not observed in plants grown at 24-27°, but 87% of the plants exposed to 5° for 3 weeks flowered.

Since carbohydrates may accumulate during cold treatment independent of floral induction, an attempt was made to block carbohydrate synthesis during cold treatment. To accomplish this, all but small unexpanded leaves were removed from 5-week-old plants prior to cold treatment or following 2 weeks at 5°C. Table 1 shows that the removal of leaf surface resulted in lower carbohydrate levels and also a corresponding decrease in the percentage of plants that subsequently flowered.

Juvenile plants were transferred from the 24-27°C growth chamber 3, 4, and 5 weeks after germination and placed at 5° for 3 weeks. Since juvenile plants cannot be induced to flower, carbohydrates should increase less during cold treatment if the level of carbohydrate is related to floral induction. Shoot tips were taken for carbohydrate analysis immediately following cold treatment. The following results were obtained: as plant age at the beginning of cold induction increased from 3 to 4 to 5 weeks, the percentage of plants flowering increased from 37 to 75 to 85%, respectively. This agrees with earlier findings where broccoli was shown to have a juvenile period during which flowering cannot be induced (3). Plants grown continuously at 24-27° remained vegetative.

Low temp treatment resulted in an increased level of sugar at all ages with very little difference between juvenile and adult plants (Fig. 2). The level of starch following 3 weeks at 5°C increased sharply from 3- to 4-week old plants and coincides with a large increase in the number of plants flowering and the passing of juvenility.

Results from this first series of experiments show that compared to high temp, low temp enhances floral induction in broccoli and increases the carbohydrate level of shoot tips. However, the experimental methods used do not reveal a cause and effect relationship between them. Therefore, a second approach was attempted.

Plants were started in a growth chamber at 24-27°C and transferred into 16-21° or 21-27° greenhouses 32 days after

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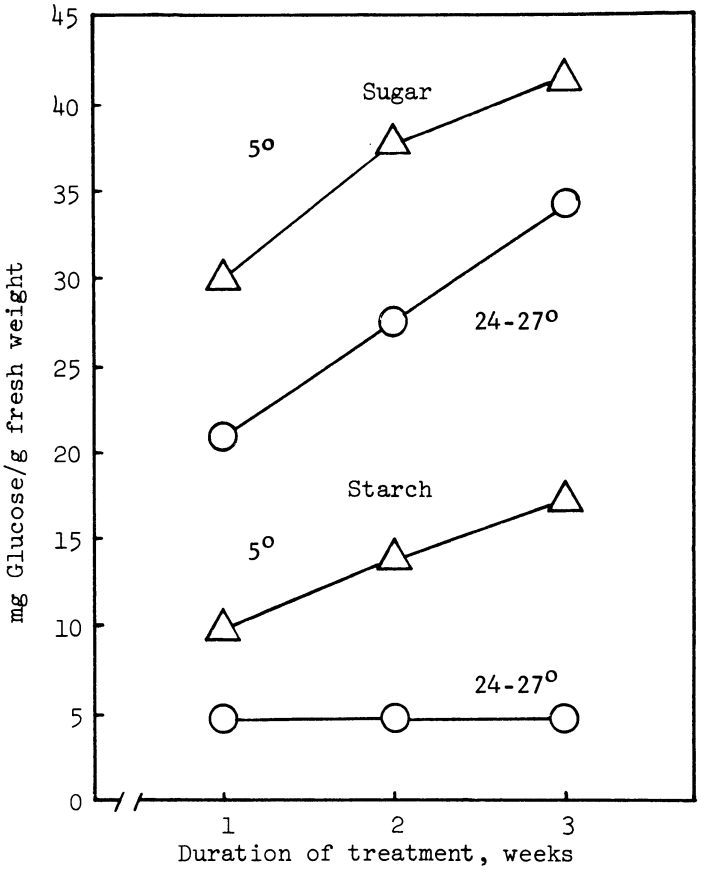

Fig. 1. The effects of temp on sugar and starch content of the shoot tips of 5-week old 'Waltham 29' broccoli plants. Each value is a mean of 3 replications.

seeding. Growth regulators, SADH and GA_3, were applied as sprays 40 and 47 days after germination. Samples for carbohydrate analysis were taken at the time of the last spray and at 10-day intervals thereafter.

As expected, floral initiation occurred earlier at 16-21°C

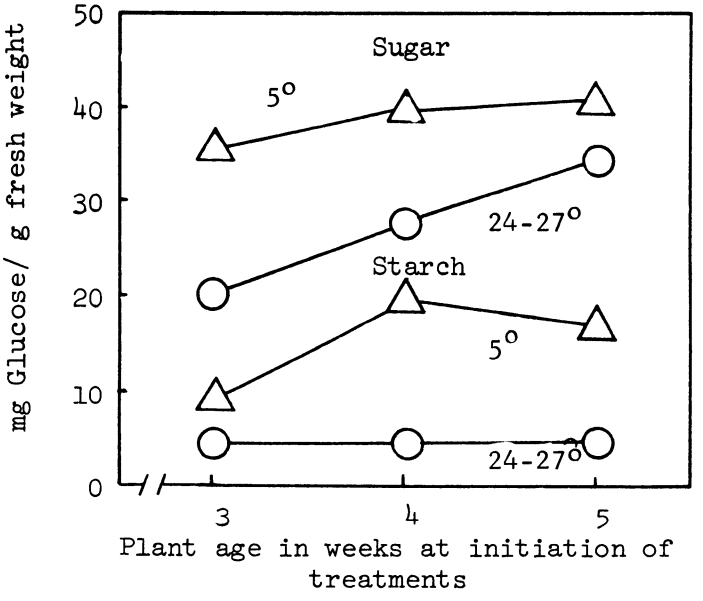

Fig. 2. The effects of plant age and temp on the starch and sugar content of shoot tips of 'Waltham 29' broccoli. Each value is a mean of 3 replications.

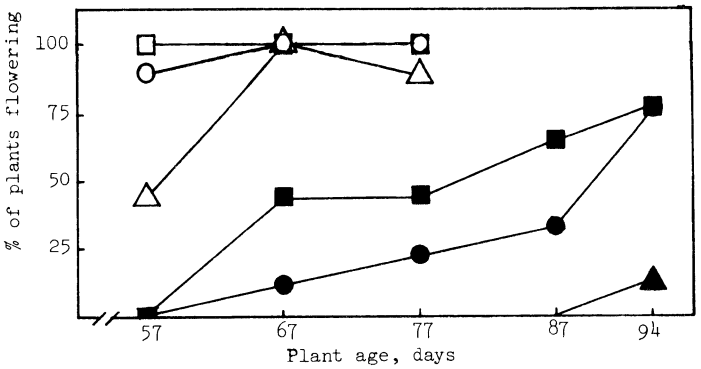

Fig. 3. The effects of SADH, GA_3, and growing temp on floral initiation of 'Waltham 29' broccoli. Treatments: ○ 16-21°C; △, 16-21° + SADH; □, 16-21° + GA_3; ●, 21-27°; ▲, 21-27° + SADH; ■, 21-27° + GA_3. Percentages are calculated on the basis of 9 plants per treatment.

(Fig. 3). SADH delayed floral initiation at both temp (2) while GA_3 enhanced flowering at 21-27°.

Since floral induction was so rapid at 16-21°C, samples of the apical growing point could not be attained past the third harvest date (Fig. 4). Temperature did not affect the level of sugar, but flowering did occur much earlier at 16-21°. SADH reduced the level of sugar and delayed flowering at both temp. Flowering did occur, however, at somewhat lower carbohydrate levels in these plants. GA_3 did not affect the level of sugar, but appeared to slightly increase starch.

Discussion

A relationship between carbohydrate accumulation and floral induction in cold requiring plants was suggested by Sadik and Ozbun (9). In their studies maximum levels of carbohydrates

Table 1. The effects of leaf removal on sugar, starch, and flowering of 'Waltham 29' broccoli plants grown for 3 weeks at 5°C. Values for sugar and starch are means of 3 replications.

Treatments	Mg glucose/g fwt		% of plants flowering
	Sugar	Starch	
Leaves removed at initiation of 5°	13.3[z]	10.6[z]	0.0
Leaves removed after 2nd week of 5°	15.1	16.4	28.5
No. leaves removed	41.5	17.5	87.5

[z]Mean of 2 replications.

were attained after 1 week at 5°C, while 2 weeks of cold were required to induce flowering in all plants. Sadik and Ozbun (9) suggested that the carbohydrate build-up during the first week of cold treatment might be a preparatory step leading to synthesis of a substance responsible for flowering. The data presented in this study for broccoli are in general agreement with their findings. Namely, treatments which promoted carbohydrate accumulation such as low temp also promoted flowering, whereas treatments tending to prevent carbohydrate accumulation such as SADH treatment, high temp, use of juvenile plants, or leaf pruning reduced flowering. Defoliation has previously been reported to decrease flowering in cabbage (1), carrots and beets (6). Cabbage plants can be induced to flower even when defoliated once they have attained a certain stem size, suggesting an adequate carbohydrate reserve (5).

The relationship between floral induction and carbohydrate accumulation seemed to disappear when the plants were grown at 16-21° and 21-27°C in a greenhouse. In these experiments flowering was greatly delayed by the warmer temp but there was little difference in either sugar or starch levels While these data suggest that the carbohydrate level has little effect on

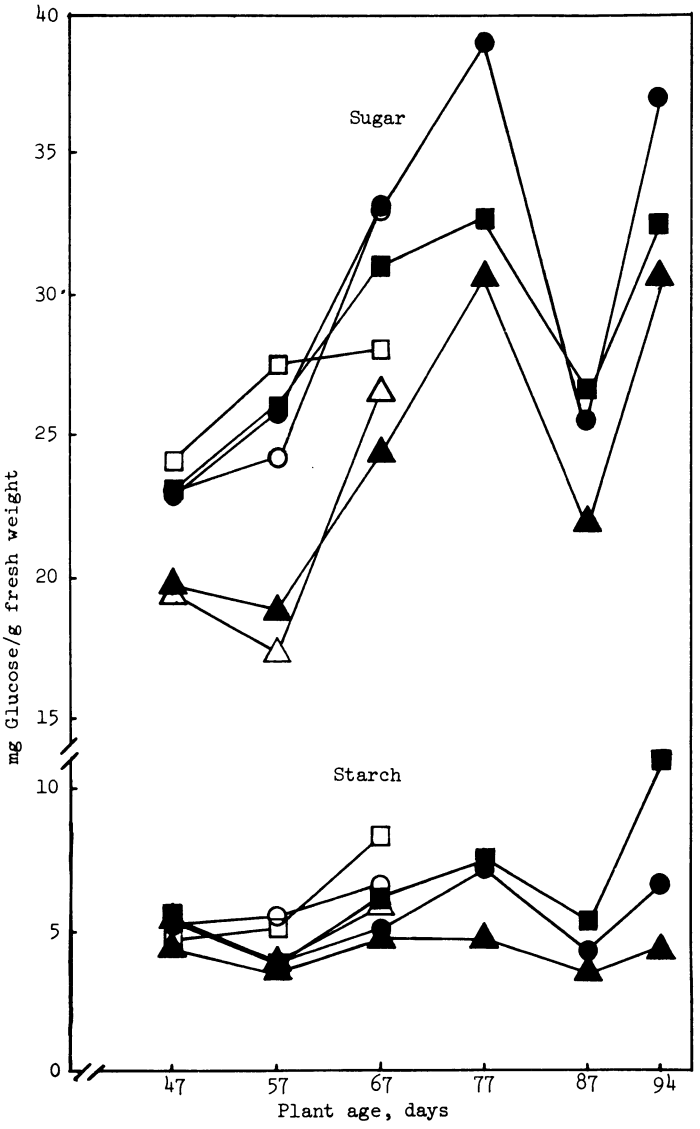

Fig. 4. Changes in starch and sugar content of shoot tips of 'Waltham 29' broccoli as affected by SADH, GA_3, and growing temp. Treatments: ○, 16-21°C; △, 16-21° + SADH; □, 16-21° + GA_3; ●, 21-27°; ▲, 21-27° + SADH; ■, 21-27° + GA_3. Each value is a mean of 3 replications.

flowering response, it should be remembered in interpreting the data that plants grown continuously at a relatively warm temp flowered at an older physiological age than in previous experiments. Under these conditions, flowering was possibly not limited by carbohydrate level, but rather by a subsequent reaction.

The data show that under some conditions the level of carbohydrate found in the shoot tip of broccoli is correlated with floral induction as previously reported for cauliflower (9). However, if plants are grown continuously at a warm temp, a high level of carbohydrate does not insure flowering. This suggests that the association between carbohydrate accumulation and floral induction is coincidental and carbohydrate accumulation is not directly responsible for floral induction as suggested by Trione (11).

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