Effect of N^6-Benzylaminopurine on Respiration & Storage Behavior of Broccoli (Brassica oleracea var. italica) [1, 2]

R. R. Dedolph, S. H. Wittwer, V. Tuli, & D. Gilbart

Department of Horticulture, Michigan State University, East Lansing, Michigan

N^6-Benzylaminopurine [3] applied as a preharvest spray and a post-harvest dip has been found effective in prolonging storage life and preventing chlorophyll breakdown in green leafy vegetables (1, 5, 7). We have suggested that these observed phenomena and many of the diverse effects of this and other kinins are explicable on the basis of respiration inhibition (3). The experiments reported here relate respiration inhibition, losses in fresh weight, and temperature of storage to subjective evaluations of fresh appearance or market quality in broccoli following post-harvest treatment with N^6-benzylaminopurine.

Materials & Methods

► Weight Changes & Subjective Evaluations. Heads of broccoli (cultivar *Spartan Early*), transferred immediately to the laboratory after harvest, were selected for uniformity of size, maturity, and high market quality. Forty samples of two heads averaging about 250 g each were withdrawn from this lot and weighed. Half of the samples were selected at random, dipped into an aqueous solution containing 0.10 % Tween 20 and 10 ppm of N^6-benzylaminopurine. The remaining samples were dipped in an aqueous solution containing a wetting agent, 0.10 % Tween 20 (Atlas Powder Co., Wilmington, Del.), as a control. Both the N^6-benzylaminopurine treated heads and the control heads were then separated at random into four lots of five samples each. Each sample was wrapped in waxed paper. They were then stored in a common container at 4, 10, 15, or 21 C temperatures.

After 2 days of storage, all samples were visually examined by five judges, none of whom was familiar with the treatments or coding system used. The judges were asked to classify each of the two-head samples as excellent, good, fair, poor, or unacceptable according to appearance. Fresh weights were also recorded at the times that freshness and appearance were appraised. The samples stored at 21 C were discarded after 2 days. Those stored at 15 C were discarded after 6 days. After 8 days of storage the samples at 4 and 10 C were again weighed and judged, and those stored at 10 C discarded. The samples stored at 4 C were judged and weighed again after 10 and 20 days.

Each judge's quality appraisal, for each of the five samples of a given treatment, was considered to be independent. Percentage differences in ranking between treated and control samples at a given temperature and period of storage were compared by a Chi Square test of independence. The degrees of freedom for these tests were determined by the number of classes in which samples were placed by the judges. Treatment was purported to have an effect on the quality retention as measured subjectively only when there were significant deviations among the number of samples placed in the various classes in the treated and control groups. Final fresh weight of a sample was converted to percentage of initial weight before dipping, and summarized by analysis of variance or regression methods where appropriate. Weights of the same sample at successive holding periods were considered independent observations.

► Respiration Rates & Respiration Quotients. Twenty-four terminal heads of broccoli, each weighing approximately 250 g, were selected and weighed. Treatment methods and concentrations were the same as in the previous experiment. Twelve heads were treated with N^6-benzylaminopurine, and an equal number used as controls. Single heads were placed in 24 respirometers held at 21 C. Carbon dioxide evolution of each head was measured colorometrically (2) at 4 hour intervals for 92 hours, whereupon they were reweighed and discarded.

Fitted regression lines presented describing carbon dioxide evolution in treated and control broccoli were obtained. The lines presented are adequate data descriptions which are significant at the 1 % level. Equations of the lines were further utilized in determining the minimum, inflection, and maximum points in the curves, and in determining the calculated values for percentage inhibition of carbon dioxide evolution.

Bud clusters of broccoli (0.2–0.5 g) removed from the main head were treated either with N^6-benzylaminopurine or as controls as in the previously described tests. The bud clusters were then held for

[1] Received Jan. 5, 1962.

[2] Journal Article No. 2905 of the Michigan Agricultural Experiment Station.

[3] N^6-benzyladenine. The N^6-benzylaminopurine used in these tests was supplied by the Shell Development Co., Modesto, Cal.

24 hours at 21 C. Carbon dioxide evolution and oxygen uptake were then determined on the individual bud clusters by the direct method in Warburg manometers. Linear regressions constrained through the origin were fitted to these data, with each line representing the data from four manometers.

Results & Discussion

► Weight Changes & Appearance According to Temperature & Length of Holding Period. The numbers of samples placed in each of the quality classes by the judges were expressed as percentages of the total treated or control lots (table I). After 2 days at 21 C, the judges clearly preferred the N^6-benzylaminopurine treated samples. These samples stored at 15 C were also appraised as more acceptable after both 2 and 6 days of storage. Samples stored at 10 C were not significantly different after 2 days, but after 6 and 8 days of storage the treated samples were judged as superior. Significant differences favoring treatment were not detected at 4 C until 10 days after treatment.

The subjective evaluations presented in table I show that N^6-benzylaminopurine was effective in slowing the degeneration of marketable appearance of broccoli at all temperatures employed. At high temperatures visible differences in acceptability, as a benefit of treatment, appeared earlier than at lower temperatures. There was, however, a time sequence following treatment in which the benefits of N^6-benzylaminopurine became equally apparent at all temperatures. It occurred after 2 days at 21 C for the one temperature extreme, compared to 20 days at the other extreme of 4 C.

Differences in fresh weight loss related to period of storage were discernible at all temperatures where this parameter could be measured (table II). Weight losses from the N^6-benzylaminopurine treated samples were significantly less than for the controls in the samples held at 4 C. At this temperature losses in weight were a function of time. After 20 days they were about twice as great in the controls as in the treated samples. At temperatures above 4 C, however, N^6-benzylaminopurine had no effect. These results suggest a different physiological response to N^6-benzylaminopurine when the living explant is subsequently held at low, as compared to high, temperatures.

An increase in weight over the initial weight which persisted at least through the first 2 days of storage occurred with samples held at 15 C or lower. As no free water was apparent on the surface of the samples after this storage interval, it appears that the solution initially left on the broccoli heads after dipping may have been absorbed directly by the developing buds that constitute the broccoli heads, as well as by the cut surface at the base of the stalk.

► Metabolic Activity & Respiration Quotients. The average rate of carbon dioxide evolution from 4 to 92 hours after treatment shows respiration inhibition attributable to treatment of broccoli with N^6-benzyl-

Table I

Appearance Ratings of N^6-Benzylaminopurine Treated & Control Broccoli as Affected by Length of Storage & Temperature

Length of storage Days	Temperature C		% Samples in each class					Odds*
			Excellent	Good	Fair	Poor	Unacceptable	
2	21	Treated	15	65	20	0	0	99:1
		Control	0	0	0	60	40	
2	15	Treated	75	25	0	0	0	19:1
		Control	25	35	25	15	0	
6		Treated	0	0	5	70	25	99:1
		Control	0	0	0	35	65	
2	10	Treated	65	35	...	...	...	...
		Control	35	65	...	...	...	
6		Treated	10	55	35	...	...	99:1
		Control	...	...	...	80	20	
8		Treated	...	...	50	50	...	99:1
		Control	...	...	...	25	75	
2	4	Treated	80	20	...	...	...	...
		Control	75	25	...	...	...	
6		Treated	50	50	...	...	...	...
		Control	55	45	...	...	...	
8		Treated	50	45	5	...	...	...
		Control	30	65	5	...	...	
10		Treated	45	40	15	...	...	19:1
		Control	10	40	50	...	...	
20		Treated	10	20	30	10	30	99:1
		Control	...	...	5	35	60	

* Odds that treated and control samples have dissimilar distributions among the five grade classes. Blank spaces indicate that the odds were less than 19:1.

Table II
Fresh Weight Changes in Broccoli as Affected by Treatment With N^6-Benzylaminopurine, & Temperature & Duration of Storage

Length of storage Days	Storage temperature in Centigrade							
	4		10		15		21	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
	Mean % original weight							
2	103.2	101.4	102.7	103.1	102.9	101.9	94.4	93.3
6	100.7	99.5	99.6	99.3	99.3	97.8	...	...
8	99.2	97.5	97.4	97.6	...	...	...	...
10	98.1	96.2	...	...	...	...	...	...
20	94.2	90.3	...	...	...	...	...	...

Weight changes differed significantly for duration of storage at 4, 10, and 15 C; for treatment at 4 C (Odds 99 : 1).

aminopurine (fig 1). The calculated degree of inhibition obtained by determining the area between the fitted lines for treated and control samples before intersection showed that the average respiration of the N^6-benzylaminopurine treated samples was 20.8 % less than that of the control. The observed value for inhibition during a comparable period was 20.0 %. If one compares the relative respiration rates over the entire 92 hours in which carbon dioxide evolution was measured, the calculated inhibition resulting from the N^6-benzylaminopurine treatment amounted to 18.6 % and for the observed 18.4 %. It is noteworthy that, though the general pattern of these curves differs from those presented for asparagus (3), the magnitude of inhibition of respiration is of the same order (16 % for asparagus vs. 20 % for broccoli), suggesting that like systems are affected in both of these diverse species. The fact that these curves cross after relatively long periods of high respiration in the control can tentatively be explained on the basis of degeneration of metabolic systems in the explant.

The respiration rates of both the N^6-benzylaminopurine treated and the control samples were adequately described by equations of the second order. The minimum, inflection, and maximum points of the curve depicting carbon dioxide evolution in the control samples occur earlier than did the corresponding points in the curve describing the respiration of treated samples. These critical points in the respiration pattern are reasonable indicators of a delay in physiological degeneration as a consequence of treatment with N^6-benzylaminopurine. The progressive and uniform movement of these points indicates that the factor responsible for the compound's activity in respiration inhibition has a consistent effect once incorporated in the metabolic cycle. The consistency and duration of this effect is indicative of an irreversible incorporation of N^6-benzylaminopurine into one or more metabolic systems. The failure to inhibit respiration completely suggests that once this compound is incorporated in the system, only the magnitude of functional activity of the system is decreased.

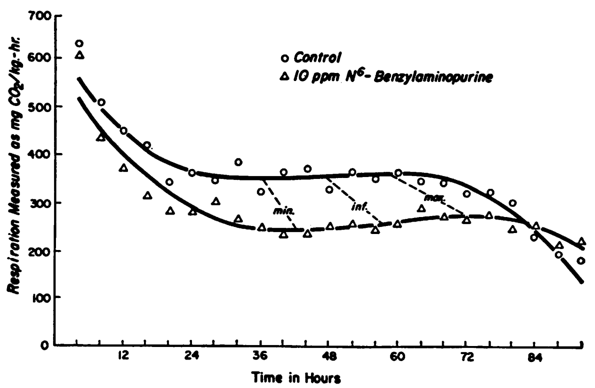

Fig. 1. Respiration rates of broccoli held at 21 C as modified by N^6-benzylaminopurine.

The data for carbon dioxide evolution and oxygen uptake 24 to 26.5 hours after treatment (fig 2) clearly show that the N^6-benzylaminopurine treated samples have lower rates of oxygen uptake, and lower rates of carbon dioxide evolution than corresponding control samples. The respiration quotient of the N^6-benzylaminopurine treated samples and the control samples were similar. These data indicate that like substrates were probably metabolized at the time of the above measurements, and that the differences in the rates of carbon dioxide evolution were not from a shift toward anaerobic respiration. They further suggest that N^6-benzylaminopurine acts as an inhibitor of catabolism without materially altering metabolic pathways.

The Warburg measurements at 30 C indicated a carbon dioxide evolution rate of ca. 550 mg CO_2/kg/hour for the N^6-benzylaminopurine treated, and ca. 740 mg CO_2/kg/hour for the control at 30 C. The corresponding rates in the large sample respirometers at 21.1 C, at comparable times after treatment, were ca. 275 mg CO_2/kg/hour for the treated, and ca. 370 mg CO_2/kg/hour for the control. It is unlikely that within these temperature ranges N^6-benzylaminopurine has an appreciable effect on the respiration temperature coefficient of broccoli.

► Miscellaneous Studies. The Spartan Early variety of broccoli was treated with post-harvest aqueous dips of N^6-benzylaminopurine and other chemicals

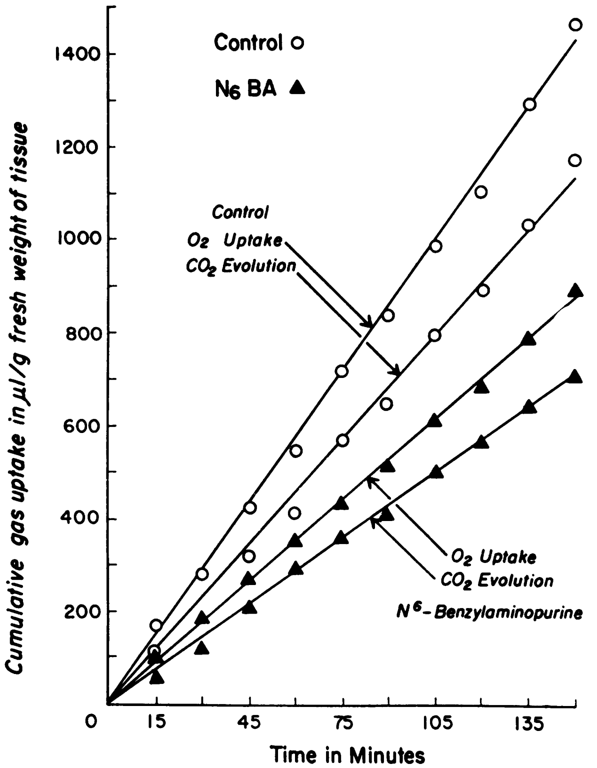

Fig. 2. Oxygen uptake and carbon dioxide evolution in broccoli buds as modified by N^6-benzylaminopurine.

plus 0.10 % Tween 20, and then placed at 21 C. Adequate replication was used for valid estimates of response. Chlorophyll and proto-chlorophyll contents were determined by methods described by Withrow, et al. (6).

Both kinetin (6-furfurylaminopurine) and N^6-benzylaminopurine increased the chlorophyll retention of broccoli heads stored for 48 hours after treatment. The increases in retention of chlorophyll were linear in the 0 to 50 ppm range for both compounds as measured at 10 ppm increments. N^6-Benzylaminopurine was somewhat more efficacious than kinetin on a ppm basis. This was particularly apparent at the higher concentrations. Chlorophyll contents of the entire head samples, after 48 hours storage, ranged from 40 μg/g fresh weight for 0 ppm to 95 μg/g fresh weight at 50 ppm for the N^6-benzylaminopurine-treated samples, and 80 μg/g fresh weight for the samples treated with 50 ppm of kinetin. Protochlorophyll contents at this time ranged from about 8 μg/g fresh weight for the controls to about 15 μg/g fresh weight in the 50 ppm-treated samples for either kinetin or N^6-benzylaminopurine.

The retention of chlorophyll in N^6-benzylaminopurine-treated samples was not altered when 10 or 100 ppm of naphthaleneacetic acid, or indole-3-acetic acid, or 20 or 200 ppm of gibberellin A_3 was also applied. Benzimidazole at concentrations ranging from 0 to 100 ppm did not increase chlorophyll retention. Neither diffuse light nor darkness for 48 hours had any discernible effect on chlorophyll retention of control or treated broccoli.

These diverse observations suggest a possible use of broccoli bud clusters as bioassay system for kinins. A method using chlorophyll retention as a bioassay for kinins has already been reported by Osborne and McCalla (4).

Summary

N^6-benzylaminopurine applied as a post-harvest dip at 10 ppm to freshly harvested broccoli maintained the appearance, extended the duration of visual market acceptability, and retained the green color. There was also a reduction in weight loss, carbon dioxide evolution, and oxygen uptake under specified temperature conditions. Subjective evaluations during storage at 4, 10, 15, and 21 C showed that impartial judges favored the appearance of the N^6-benzylaminopurine treated broccoli. Weight losses of broccoli were linear with time at all temperatures. Only at 4 C were they significantly less as a result of treatment with N^6-benzylaminopurine. Significant reductions occurred in carbon dioxide evolution and oxygen uptake with treated broccoli at 21 and 30 C. Comparisons of carbon dioxide evolution over a period of 92 hours for N^6-benzylaminopurine treated and control samples revealed that the critical points in the respiratory pattern indexing the state of degradation were consistently and progressively shifted to later periods as a result of treatment. The respiration quotients of treated and control samples were not significantly different twenty-four hours after treatment.

Observations on the effects of chemical treatment on chlorophyll retention in broccoli showed that benzimidazole was inactive. The effects of N^6-benzylaminopurine were more pronounced than those for kinetin, independent of light, and not affected by simultaneous treatments with α-naphthaleneacetic acid, indole-3-acetic acid or gibberellin.

Literature Cited

1. Bessey, P. M. 1960. Effects of a new senescence inhibitor on lettuce storage. Ariz. Univ. Agr. Exp. Sta. Rept. No. 189: 5.
2. Claypool, L. L. & R. M. Keefer. 1942. A colorometric method for CO_2 determination in respiration studies. Proc. Am. Soc. Hort. Sci. 40: 177.
3. Dedolph, R. R., S. H. Wittwer, & V. Tuli. 1961. Senescence inhibition & respiration. Science 134: 1075.
4. Osborne, D. J. & D. R. McCalla. 1961. Rapid bioassay for kinetin & kinins using senescing leaf tissue. Plant Physiol. 36: 219.
5. Shell Development Co. 1961. Modesto, Cal. Commercial brochures.
6. Withrow, R. B., W. H. Klein, L. Price, & V. Elstad. 1953. Influence of visible & near infra red radiant energy on organ development & pigment syntheses in bean & corn. Plant Physiol. 28: 1.
7. Zink, F. W. 1961. N^6-benzyladenine, a senescence inhibitor for green vegetables. Agr. & Food Chem. 9: 304.