5-1964

Effects of Pre- and Post- Harvest Applications of 6-Furfurylaminopurine and N6-Benzyladenine on Physio-Chemical Changes in Lettuce

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EFFECTS OF PRE- AND POST-HARVEST APPLICATIONS OF 6-FURFURYLAMINOPURINE AND N6-BENZYLADENINE ON PHYSIO-CHEMICAL CHANGES IN LETTUCE (*Lactuca sativa* L.)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Food Science and Technology

UTAH STATE UNIVERSITY
Logan, Utah

1964

ACKNOWLEDGEMENTS

The writer wishes to express his most sincere gratitude and thanks to Dr. D. K. Salunkhe, Associate Professor of Horticulture and Food Technology, for his help and valuable guidance throughout this study.

The writer also extends his sincere appreciation to the committee members, Dr. L. H. Pollard, Professor and Head of the Horticulture Department, and Dr. H. H. Wiebe, Professor of Botany and Plant Pathology, for their advice and review of this study.

Grateful acknowledgment is made to Dr. N. R. Bohidar, Assistant Professor of Applied Statistics for assistance in statistical analyses and interpretation of the data.

To the United Arab Republic Government which gave me the opportunity to continue my studies in the United States of America, I express my deep gratitude.

H. I. El-Mansy

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INTRODUCTION

The extent and nature of physio-chemical changes that take place in detached leaves after harvest and during storage have been reviewed and discussed by Osborne (1962) and Rogers (1955). These changes include loss of moisture (Wittwer et al., 1962), chlorophyll degredation (Person et al., 1957), Protein loss (Thimann and Manmahan, 1960), and result in subsequent appearance of the visual manifestations of senescence of plant tissues.

As lettuce, like most leafy vegetables, deteriorates rapidly and steadily after harvest. Loss of quality is inevitable and can only be minimized by rapid handling and with the best possible storage conditions (Pratt et al., 1954).

In recent years, abundant work has been done to delay senescence by the use of various chemicals. Among the investigated chemicals, kinetin (6-furfurylaminopurine) and its related compounds show some promise. Van Overbeek et al. (1941) reported a potent new growth-promoting factor (kinetin) in coconut milk. This chemical is active in causing many of the growth reactions of coconut milk at exceedingly small dosages. Subsequently several aminopurine compounds were synthesized. One of which is SD 4901 (Verdan), N6-benzyladenine, an experimental senescence inhibitor, was developed by Shell Development Company, Modesto, California in 1960. Many reports showed that this chemical is capable of delaying senescence of plant tissues on the basis of restoring protein molecules and respiration inhibition. On the other hand, others have shown stimulation of respiration and delaying of senescence.

Paucity of scientific literature on the stability of those chemicals on leafy vegetables gave impetus to a study of the comparative influence of pre- and post-harvest applications of 6-furfurylaminopurine and N6-benzyladenine as related to successive harvest times. Such studies may have considerable economic bearing upon storing and shipping leafy vegetables to distant markets.

This thesis presents effects of different concentrations (5, 10, and 20 ppm.) of pre- and post-harvest applications of 6-furfurylaminopurine and N6-benzyladenine as related to three successive harvest times (at one week intervals) on chlorophyll content, moisture content, total nitrogen, insoluble and soluble nitrogen, oxygen uptake, and CO_2 production during storage (at 40^{o} F. and 85 percent RH) of "Great Lakes" variety of lettuce.

REVIEW OF LITERATURE

The chemical changes that occur in leaves as they grow old have been well characterized for many species of plants. The endogenous factors which control and regulate these changes in plant cells remain, to a great extent, obscure, and the problem of why the cell eventually dies is yet unsolved.

Osborne (1962) reported there is a continuous decline in protein level in Xanthium leaf blades. The most rapid fall occurs during senescence and is associated with irreversible yellowing, loss of chlorophyll, and eventual death of the organ. He concluded that these symptoms occur especially with excised mature leaves, providing the petioles do not form roots. Rogers (1955) found with excised red kidney bean leaves that the decrease in protein content of the blade is not necessarily due to lack of carbohydrates, nitrogen or other nutrients, nor to an inability of the cells themselves to synthesize amino acids, but is due rather to failing ability of the cells to incorporate amino acids into proteins, providing absence of roots which may metabolize and supply the blade with certain factors necessary for the continuous synthesis of protein. Person et al. (1957) noted that both chlorophyll degradation and protein loss in detached wheat leaves are retarded if the blades are floated on solutions of benzimidazole at concentrations of 50 mg. per liter. Richmond and Lang (1957) showed similar effects could be obtained if excised leaves of

Xanthium are kept with their petioles dipped in a solution of kinetin at concentrations of 1.5 mg. per liter. Wang and Waygood et al. (1959) have shown that benzimidazole maintains the normal rate of chlorophyll biosynthesis and prevents the destruction of the green pigment in the light and in darkness in detached Khapli wheat leaves. It has also been reported by Person et al. (1957) that benzimidazole maintains the insoluble nitrogen content of detached leaves of "little club" wheat at a level higher than the water control.

Osborne and Hallaway (1960) reported that the retention of the green color in autumn leaves of Prunus serrulata is 10-20 days longer than the controls as a result of 2, 4, Dichlorophenoxyacetic acid (2, 4-D) application to the blades. This retention is localized in the treated areas and associated with an actively photosynthetic chlorophyll and a maintenance of the initial protein level. Thimann and Manmohan (1960) found that protein nitrogen rises rapidly on the expense of soluble nitrogen in kinetin treated internode sections of pea stems. They observed that the increase in protein nitrogen in all sections treated with kinetin from different places in the stem. They attributed that kinetin promotes the translocation of nitrogen to the growing points and this action was localized in the treated areas. Sigiura et al. (1962) reported that protein level was maintained in Nicotiana tabaccum when treated with kinetin at a concentration of 10^{-5} M. This effect was not observed in Nicotiana rustica except in the presence of light and sugars. They concluded that kinetin maintains protein levels by accelerating ribonucleic acid synthesis in which sugars are participating.

It has been reported (unpublished report of Shell Development Company, 1960) that after a crop is harvested, general degradation sets in. This results in the destruction of the soluble ribonucleic acid. Thus, protein synthesis slows down and as the mechanism of protein formation is disturbed, the pigments and other constituents disintegrate.

Salunkhe *et al*. (1962) explained:

> the primary step in the degradation of the soluble ribonucleic acid is thought to involve the loss of the end group adenine. A treatment with N6-benzyladenine therefore should provide the necessary adenine and restore the soluble ribonucleic acid molecule. Protein synthesis thus would be maintained and the treated produce would stay fresh for longer time.

Bessey (1960) found that lettuce treated with N6-benzyladenine was maintained in a fresh green condition considerably longer than the untreated heads. Zink (1961) reported that the same material acts as a senescence inhibitor and was effective in delaying the visual manifestations of senescence in lettuce, endives, Brussels sprouts, sprouting broccoli, mustard greens, radish tops, parsley, celery, green onions and asparagus.

Tuli *et al*. (1962) noted that post-harvest treatment of freshly harvested sweet cherries with N6-benzyladenine resulted in more attractive and greener fruit pedicels with a higher chlorophyll content and less loss of fresh weight of the fruit during storage for seven days at 70^{o} F. They also noted that there was no beneficial effects on the retention of chlorophyll in the strawberry fruit calyxs or caps as a result of such applications. Salunkhe *et al*. (1962) indicated that application of N6-benzyladenine at concentrations of 5, 10 and 20 p.p.m. resulted in no increase of the shelf-life of sweet cherries while increased the shelf-life of cauliflower, endives, parsley, snap beans, lettuce (head, leaf, and Romaine), radishes, bunching onions, and cabbage. Dedolph et al. (1961) explained such observed phenomena on the basis of respiration inhibition in asparagus spears. The same authors and also Gilbart (1962) found in addition to extending the shelf-life and maintaining the appearance of freshly harvested broccoli as a result of post-harvest dipping of N-6 benzylaminopurine at concentration of 10 p.p.m., there was also a reduction in weight loss, CO_2 evolution, and oxygen uptake under specified temperature conditions. On the other hand Smock et al. (1962), Wittwer et al. (1962), and Wittwer and Dedolph (1963) suggested that such inhibition of respiration related to the stage of maturity when application occurred. Moreover, Lipton and Ceponis (1962) observed a retardation of senescence and stimulation of oxygen consumption of kinin-treated lettuce tissues. Katsumi (1963) reported that both elongation and water uptake of etiolated pea stem sections were inhibited in the presence of Kinetin. He observed that such inhibition is accompanied by an inhibition of respiration. In order to find substances which can overcome the kinetin inhibition, DNA, RNA, cystein, methionine, arginine, uracil, ATP and calcium were tested. None of these substances were effective in reversing kinetin inhibition of respiration.

It may be rather confusing that kinetin, one of the growth substances, inhibits respiration while most growth substances, when applied to plant tissues, accelerate oxygen uptake. The acceleration of oxygen uptake by Indoleacetic acid (IAA) and 2, 4-D has been reported by many authors. Marre et al. (1960) showed that auxin treatments enhanced oxygen uptake as a consequence of the activation of some oxidative system. Their conclusion was that the high energy phosphate level increases, while the level of phosphate acceptor decreases during the first period following the auxin treatment of isolated pea internode segments, and consequently, there is a marked increase in respiration. Similar observations were reported by Osborne and Hallaway (1960) when respiratory measurements showed that there was continuous increase in oxygen-uptake as a result of 2, 4-D or 2, 4, 5-T applications on leaf disks of Prunus serrulata.

Zink (1961) found that N6-benzylaminopurine applications on lettuce, were essentially ineffective when applications were more than three to four days. The most satisfactory response was on the day of harvest. His findings suggest that the effect of this material disappears rapidly under field conditions. The same author attributed delaying senescence by maintaining cell vigor and delaying proteolysis. Richmond and Lang (1957) reported that kinetin can reduce or prevent the accelerated protein loss that is typical of detached leaves; at the same time, it delays the loss of chlorophyll and extends the life span of the leaf. They attributed that such treatment, thus, not a biocide buc acts through its effect on the physiology of the tissue.

MATERIALS AND METHODS

Lettuce transplants (Great Lakes variety) were grown on the Utah State Experimental Station farm at Farmington, Utah. After they reached a suitable size, 240 plants were transplanted into wide-mouth, one gallon jars containing a horticulture grade vermiculite as a soil medium for the plants. The plants were watered daily with equal amounts of half Hoagland No. I nutrient solution (Hoagland and Arnon, 1950) except once a week in which water only was employed in order to help leaching of accumulated salts. Table 1 presents the formula for the preparation of culture solution. Logan tap water was used for dilution in all instances. The nutrient solution was prepared daily in a 55 gallon polyethylene barrel.

Table 1. Formula for the preparation of culture solution for complete Hoagland's solution No. I. (Hoagland and Arnon, 1950)

Major nutrients	Concentration moles per liter	Micronutrients	Concentration ppm. of elements
$NH_4 H_2 PO_4$	0.001	$H_3 BO_3$	0.5
$K NO_3$	0.006	$MnCl_2.4 H_2O$	0.5
$Ca (NO_3)_2$	0.004	$ZnSO_4 .7 H_2O$	0.05
$Mg SO_4$	0.002	$Cu SO_4 .5 H_2O$	0.02
		$H_2MoO_4 . H_2O$	0.05
		Fe tartrate	1.37

As the plants approach maturity (after about three months from the time of transplanting), 147 plants were selected for three successive harvest times. Each harvest represented individual experiments and received the following treatments:

Experiment I: Effects of Pre-harvest Applications of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

Forty-nine plants of uniform size were selected and subdivided into seven treatments, each comprized of control, 6-furfurylaminopurine (at concentration of 5, 10, and 20 ppm.), N6-benzyladenine (at concentration of 5, 10, and 20 ppm.). Surfactant used was "Triton B 1956" (product of Rohm and Haas Company, Philadelphia, Pa.) at a concentration of 0.21 percent.

The plants were sprayed with these materials and their concentrations on July 11, 1963, July 21, 1963, and harvested on July 22, 1963. After harvest, lettuce heads were transferred immediately to storage rooms maintained at 40° F. and 85 percent R.H., and the following physio-chemical determinations (in duplicates) were made:

Respiration

A. CO_2 evolution during storage at 40° F. and 85 percent R.H., using the method of Claypool and Keefer (1942).

B. Oxygen uptake, in which ten leaf disks were floated on 2 ml. Hoagland No. I nutrient solution, using Warburg respirometer in a water bath at 98° F. (Stanley and Tracewell, 1955).

Moisture

Percent moisture content during storage was determined at five-day intervals according to the method described by Wittwer, et al., 1962.

Chlorophyll

Total chlorophyll content in leaves (greenness) during storage was measured at five day intervals using the method described by Osborne, 1963.

Nitrogen

A. Percent total nitrogen was determined during the storage at five-day intervals, using modified method of Kjeldahl nitrogen analysis outlined by Jackson (1958), and two-step mixed indicator was used for titration in an attempt to secure precise end point (Sher, 1955).

B. Water insoluble organic nitrogen was measured during the storage at five-day intervals, using A.O.A.C. method (1960).

C. Percent water soluble nitrogen was obtained by subtracting percent insoluble from percent total nitrogen.

Quality

Visual observations for quality were made at two to five-day intervals. A subjective score rating was used as follows: 9 = Excellent, 7 = Good, 5 = Fair, 3 = Unsalable, 1 = unusable. Table 2 describes the basis of such evaluations.

Table 2. Description of numerical quality ratings used to evaluate "Great Lakes" lettuce

Numerical rating[a]	Descriptive rating	Visual observations
9	Excellent	Field fresh, bright green appearance, and free from defects.
7	Good	Miner defects present but not objectionable. Green color slightly bleached and dull. Good retail sales appeal.
5	Fair	Outer leaves showing slight yellowing. Could be returned to acceptable condition with slight trimming.
3	Unsalable	Objectionable defects correctable by trimming. No more than slight decay.
1	Unusable	Slime and other defects serious; would not be eaten.

[a] Intermediate scores used when appropriate.

Experiment II: Effects of One Pre-harvest Treatment of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

To study effects of an application of 6-furfurylaminopurine and N6-benzyladenine, another set of 49 plants of uniform size was selected, subdivided into seven treatments as discussed under experiment I, and sprayed with the same materials and concentrations one day before harvest on July 29, 1963. Physio-chemical determinations were made as discussed under experiment I.

Experiment III: Effects of Post-harvest Dipping of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

For a post-harvest treatment of 6-furfurylaminopurine and N6-benzyladenine, 49 plants of uniform size were selected, received a post-harvest dip immediately after harvest, of the same materials and concentrations of experiments I and II.

Physio-chemical analyses were made as discussed in detail under experiment I.

The reasons for conducting these three experiments were to evaluate the effects of 6-furfurylaminopurine and N6-benzyladenine in relation to various stages of maturity (three successive harvest times at one week intervals); effectiveness of two applications vs. one application; or a post-harvest dipping is as well effective.

Statistical analyses were made (Snedecor, 1961) and data are presented in the Appendix.

RESULTS

Physio-chemical changes in relation to pre- and post-harvest applications of 6-furfurylaminopurine and N6-benzyladenine are presented in the following three experiments.

Experiment I: Effects of Pre-harvest Applications of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

Respiration

CO_2 evolution. An inhibitory effects of CO_2 evolution during storage was directly related to the concentrations of 6-furfurylaminopurine and N6-benzyladenine applications, Figures 1 and 2 and Tables 3 and 4. With the exception, however, of 10 p.p.m N6-benzyladenine treatment, where the inhibitory effects were more than when lettuce was treated with 20 p.p.m. of N6-benzyladenine in the early stages (first eight days) of storage.

O_2 uptake. Oxygen consumption by lettuce was stimulated under the action of both 6-furfurylaminopurine and N6-benzyladenine. It is apparent from Figures 3 and 4 that higher the concentrations of the chemicals the less stimulation of oxygen uptake under the conditions of this experiment.

Moisture

The moisture content in lettuce treated with several concentrations of 6-furfurylaminopurine was higher than that treated with N6-benzyladenine, Tables 3 and 4 in the text and Table 11 in Appendix. The untreated control lettuce heads had lower moisture content.

Total chlorophyll

Figures 5 and 6 show that the loss of total chlorophyll during the refrigeration storage of lettuce was much higher in the untreated controls as compared with lettuce treated with different concentrations of both 6-furfurylaminopurine and N6-benzyladenine.

Nitrogen

Total nitrogen. Table 13 indicates that lettuce treated with 20 p.p.m 6-furfurylaminopurine had higher total nitrogen content, followed by 10 p.p.m 6-furfurylaminopurine, 10 p.p.m N6-benzyladenine, 5 p.p.m 6-furfurylaminopurine, 5, 20 p.p.m N6-benzyladenine and the untreated controls, respectively.

Water insoluble organic nitrogen. It is apparent from Tables 3 and 4 that percent water insoluble organic nitrogen was higher in both 6-furfurylaminopurine and N6-benzyladenine treatments of head lettuce than the untreated controls. The magnitude of increasing order in organic nitrogen is similar to that of total nitrogen. No significant change in the percent insoluble nitrogen during the storage duration was evidenced when lettuce was treated with 20 p.p.m and 10 p.p.m of 6-furfurylaminopurine and 10 p.p.m N6-benzyladenine. On the other hand the untreated control lettuce showed a significant progressive degredation of insoluble nitrogen content during storage, Table 14.

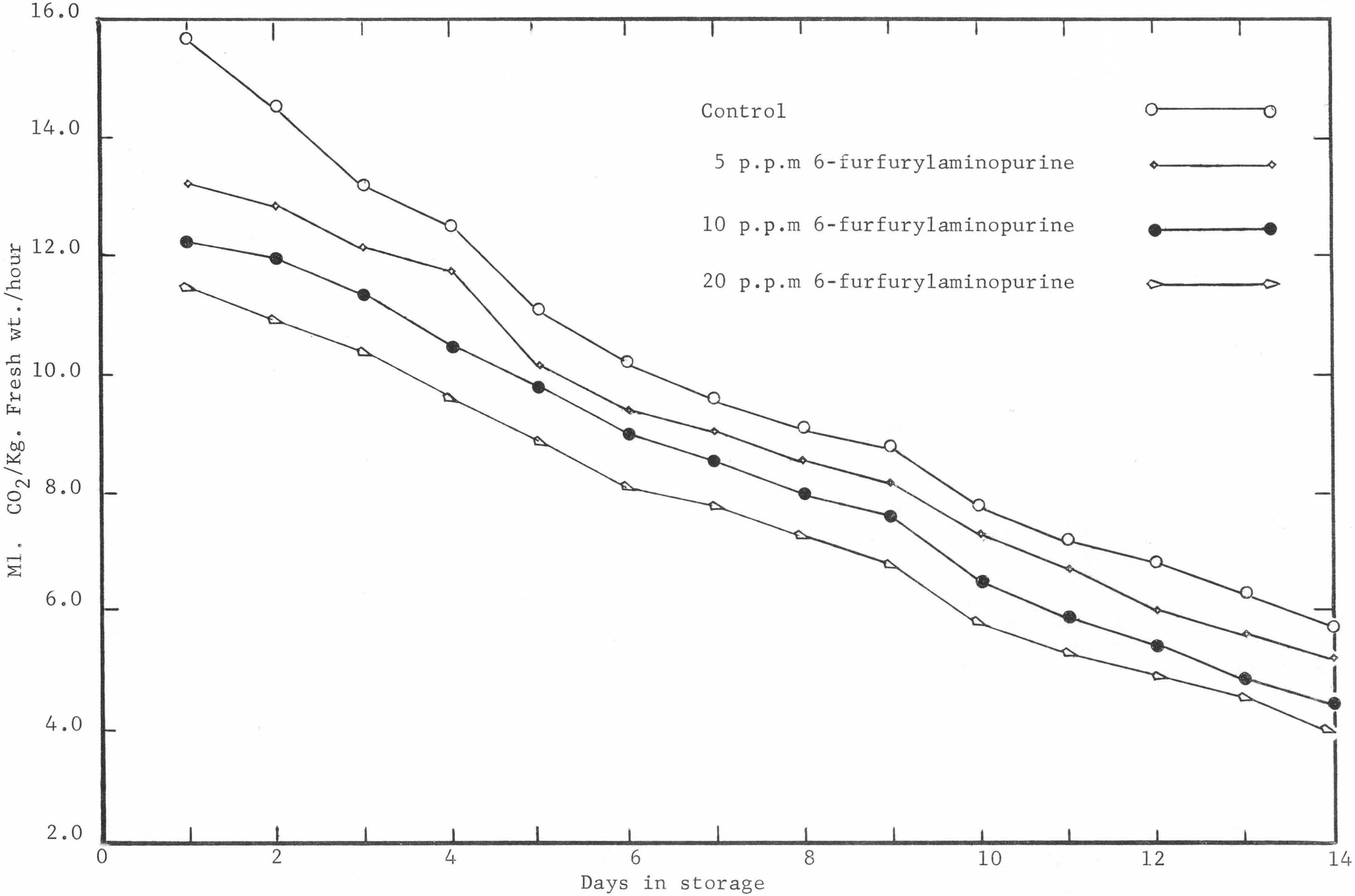

Figure 1. Respiration levels of head lettuce in relation to pre-harvest applications of 6-furfurylaminopurine during storage at 40° F and 85 percent R.H. (First harvest: July 22, 1963)

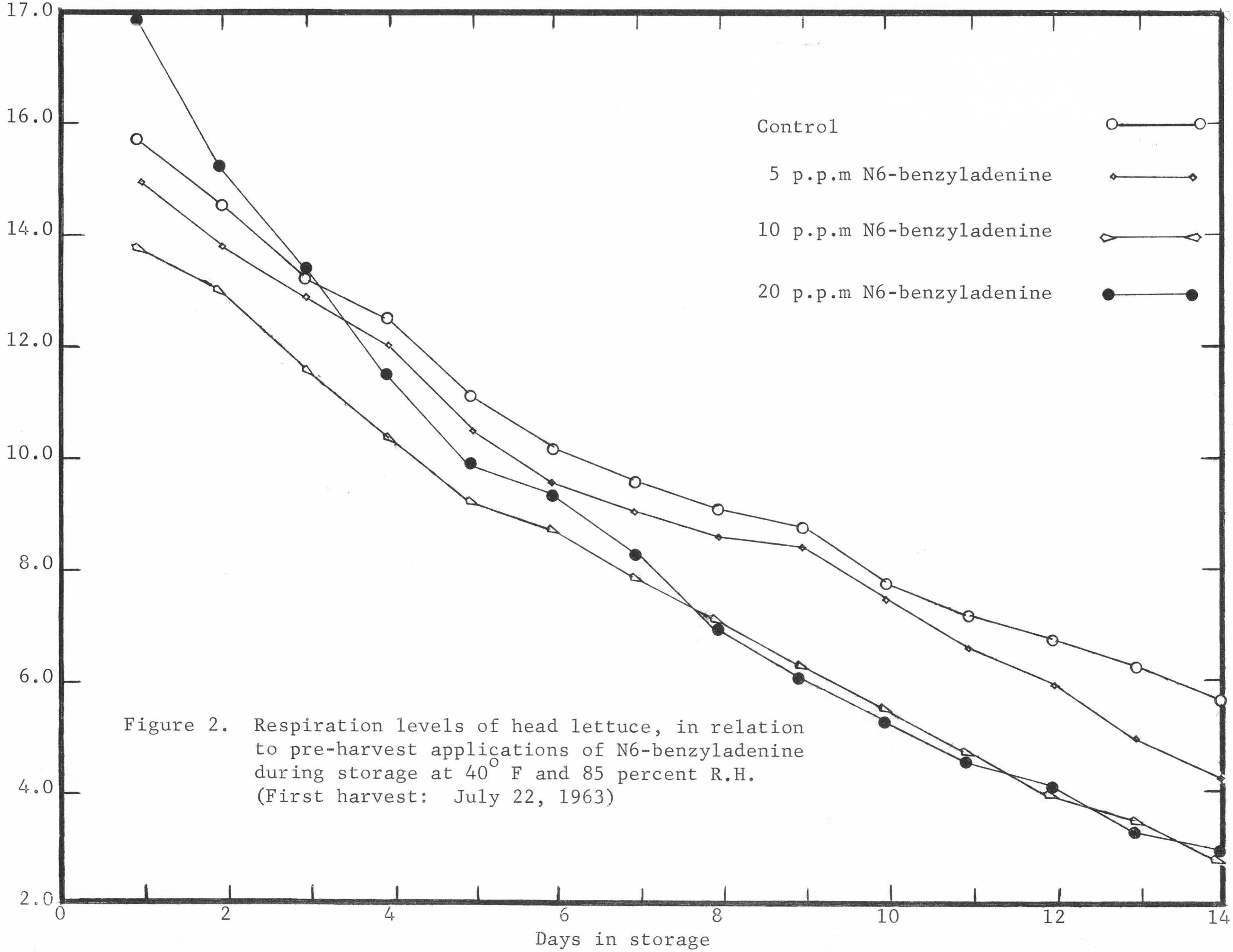

Figure 2. Respiration levels of head lettuce, in relation to pre-harvest applications of N6-benzyladenine during storage at 40° F and 85 percent R.H. (First harvest: July 22, 1963)

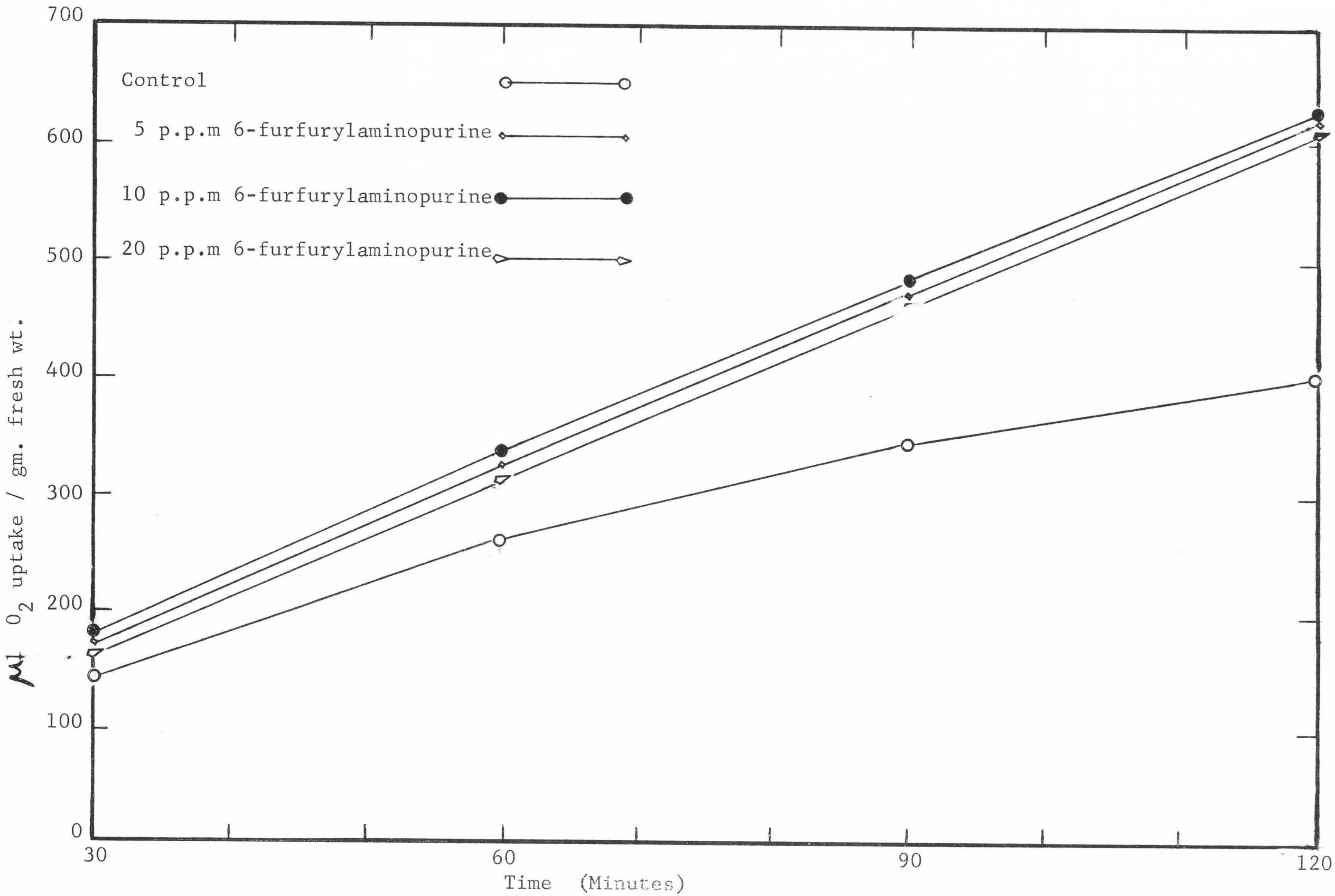

Figure 3. Oxygen uptake of lettuce leaf segments at 98° F, in relation to pre-harvest applications of 6-furfurylaminopurine (First harvest: July 22, 1963)

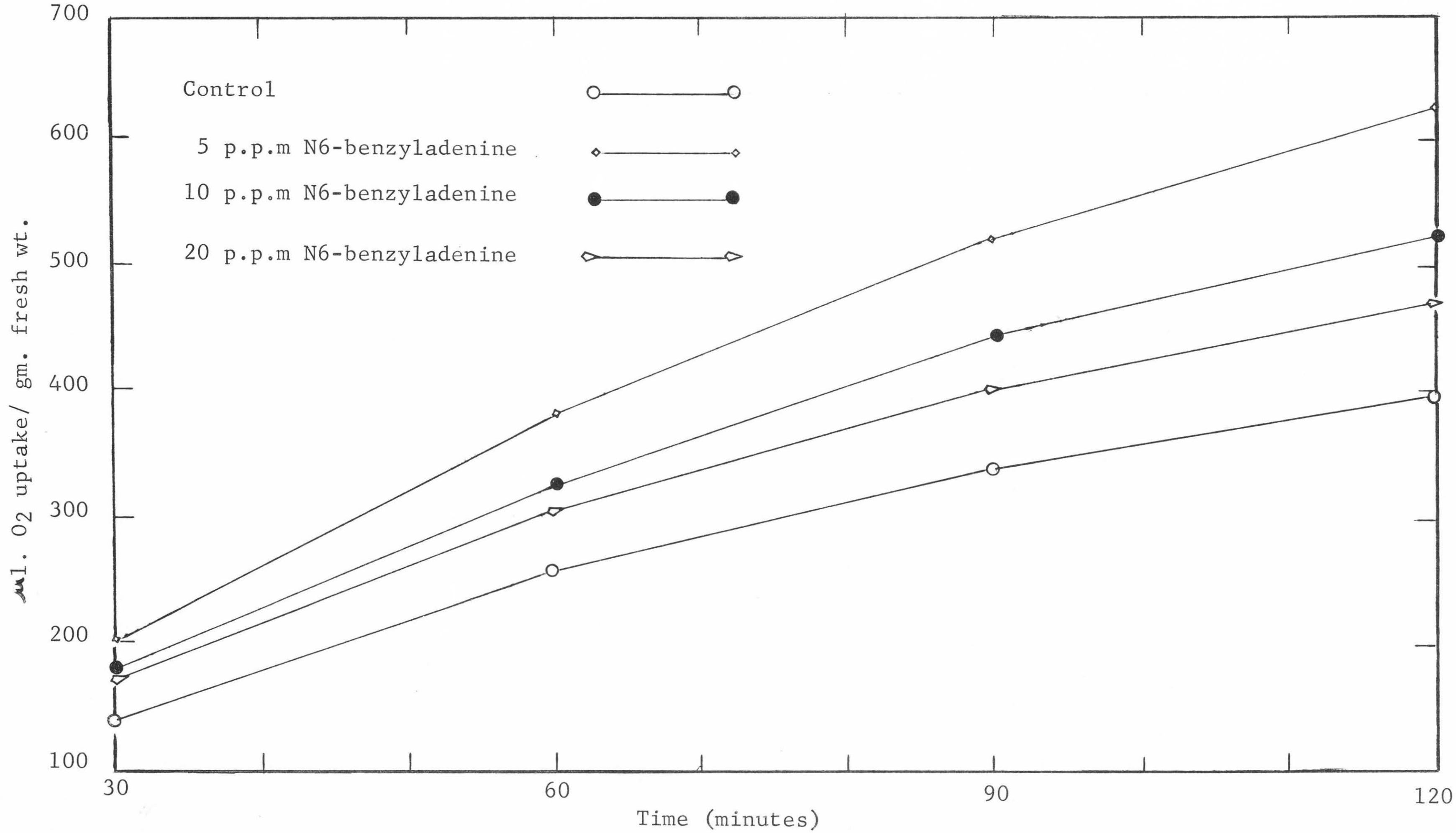

Figure 4. Oxygen uptake of lettuce leaf segments at 98° F, in relation to pre-harvest applications of N6-benzyladenine (First harvest: July 22, 1963)

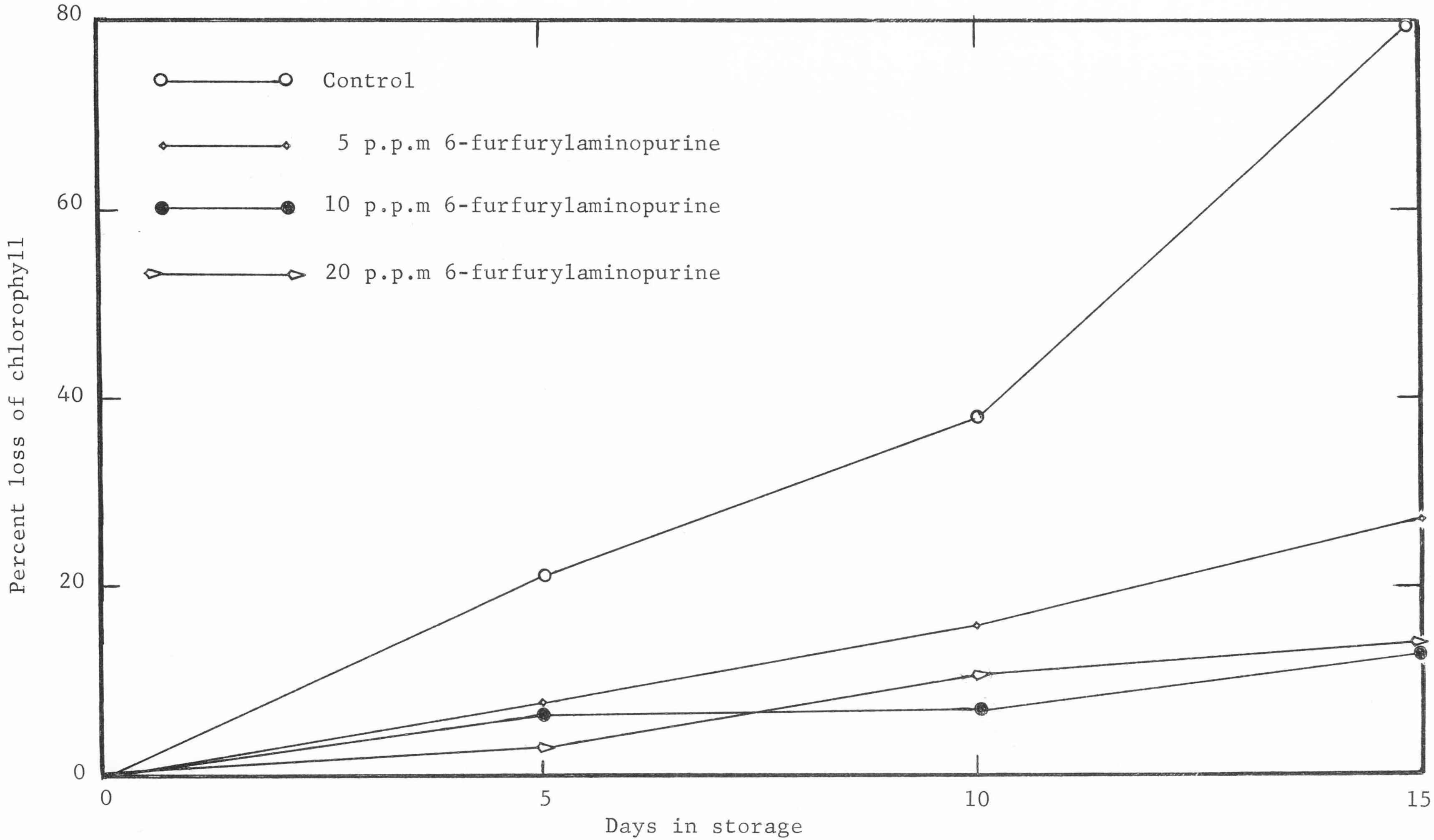

Figure 5. Percent loss of total chlorophyll in lettuce leaves treated with pre-harvest applications of 6-furfurylaminopurine (First harvest: July 22, 1963)

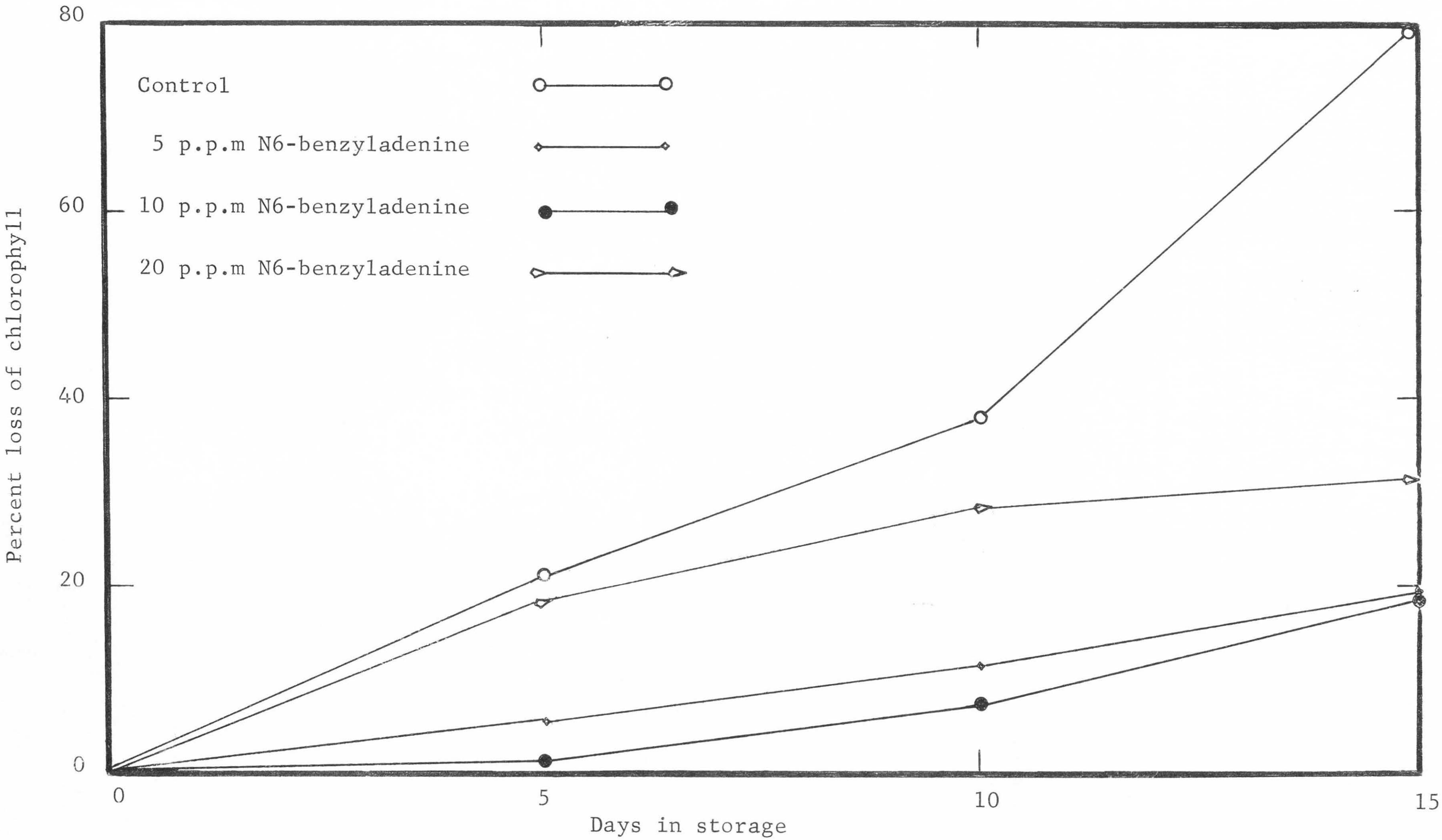

Figure 6. Percent loss of total chlorophyll in lettuce leaves treated with pre-harvest applications of N6-benzyladenine (First harvest: July 22, 1963)

Table 3. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40° F. in relation to pre-harvest applications of 6-furfurylaminopurine (First harvest: July 22, 1963)

Treatment	Moisture (percent)	Total[a] chlorophyll (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control									
At harvest	92.7	40.0	2.95	1.90	1.05	138.5	401	7	--
After 5 days storage	92.0	48.5	2.92	1.70	1.22			5	
After 10 days storage	91.0	55.0	2.64	1.50	1.14			5	
After 15 days storage	89.2	72.0	2.55	1.20	1.35			1	
5 p.p.m 6-furfurylaminopurine									
At harvest	95.5	41.0	3.01	2.08	0.93	126.5	616	9	10
After 5 days storage	95.0	44.0	2.98	2.06	0.92			9	
After 10 days storage	94.6	47.5	2.84	2.00	0.84			7	
After 15 days storage	94.0	52.0	2.83	1.80	1.03			7	
10 p.p.m 6-furfurylaminopurine									
At harvest	95.0	37.0	3.21	2.60	0.61	116.2	624	9	10
After 5 days storage	95.0	39.5	3.15	2.60	0.55			9	
After 10 days storage	94.8	39.5	3.08	2.55	0.53			9	
After 15 days storage	94.5	41.5	3.02	2.40	0.62			7	
20 p.p.m 6-furfurylaminopurine									
At harvest	95.0	33.0	3.47	2.94	0.53	105.7	606	9	10
After 5 days storage	95.0	34.0	3.33	2.92	0.41			9	
After 10 days storage	94.9	36.5	3.27	2.93	0.34			9	
After 15 days storage	94.7	37.5	3.15	2.90	0.25			9	

[a] Percent transmittance at the absorbtion maximum of chlorophyll a (665 mμ)

[b] Percent nitrogen expressed on dry weight basis

[c] Results expressed on fresh weight basis

[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

Table 4. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40° F. in relation to pre-harvest applications of N6-benzyladenine (First harvest: July 22, 1963)

Treatment	Moisture (percent)	Total[a] chlorophyll (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control						138.5	401		--
At harvest	92.7	40.0	2.95	1.90	1.05			7	
After 5 days storage	92.0	48.5	2.95	1.70	1.22			5	
After 10 days storage	91.0	55.0	2.64	1.50	1.14			5	
After 15 days storage	89.2	72.0	2.55	1.20	1.35			1	
5 p.p.m N6-benzyladenine						129.0	628		5
At harvest	94.0	43.0	2.88	2.02	0.86			7	
After 5 days storage	93.4	45.5	2.82	2.02	0.80			7	
After 10 days storage	92.9	48.0	2.80	1.88	0.92			7	
After 15 days storage	92.7	51.0	2.77	1.80	0.97			6	
10 p.p.m N6-benzyladenine						107.9	524		10
At harvest	93.5	39.0	2.96	2.13	0.83			9	
After 5 days storage	93.1	39.5	2.92	2.04	0.88			9	
After 10 days storage	92.5	42.0	2.85	1.98	0.87			9	
After 15 days storage	92.4	46.5	2.83	1.89	0.94			7	
20 p.p.m N6-benzyladenine						118.1	476		5
At harvest	92.0	44.0	3.02	1.98	1.04			7	
After 5 days storage	91.2	52.0	2.90	1.76	1.14			7	
After 10 days storage	90.0	56.5	2.81	1.55	1.26			7	
After 15 days storage	89.8	58.0	2.77	1.42	1.35			4	

[a] Percent transmittance at the absorbtion maximum of chlorophyll a (665 mμ)
[b] Percent nitrogen expressed on dry weight basis
[c] Results expressed on fresh weight basis
[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

Water soluble nitrogen. It can be seen from Tables 3, 4, and 15 that the percent water soluble nitrogen was higher in the untreated control and with 20 p.p.m N6-benzyladenine treatments of head lettuce, followed by 5 p.p.m and 10 p.p.m N6-benzyladenine, 5 p.p.m, 10 p.p.m and 20 p.p.m 6-furfurylaminopurine treatments respectively.

Quality

Tables 3 and 4 show that 20 p.p.m 6-furfurylaminopurine treatment has the best quality rating after harvest and during the entire storage period. Treatments of 10 p.p.m 6-furfurylaminopurine and 10 p.p.m N6-benzyladenine have rather similar quality score, followed by 5 p.p.m 6-furfurylaminopurine, 5, 20 p.p.m N6-benzyladenine, and the untreated controls of head lettuce was poor in quality and unacceptibly shrivelled and turned yellow.

Experiment II: Effects of One Pre-harvest Treatment of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

Respiration

CO_2 evolution. With the exception of the relative decrease in CO_2 evolution during storage in this experiment, the inhibitory effects in relation to 6-furfurylaminopurine and N6-benzyladenine treatments were similar to that of experiment I, Figures 7 and 8 and Tables 5 and 6.

O_2 uptake. Respiration treands as measured by oxygen consumption followed the same pattern as under experiment I, Figures 9 and 10, except with 20 p.p.m N6-benzyladenine treatment where an inhibitory effects were observed (after 90 minutes) in oxygen consumption.

Moisture

Similar results in moisture content were obtained, Tables 5, 6 and 11 as under experiment I.

Total chlorophyll

Except with 20 p.p.m N6-benzyladenine treatment, Figure 12, where loss of chlorophyll was even more than the untreated controls (after 5 days) in the early stages of storage, similar results were obtained in all other treatments as under experiment I, Figures 11, and 12.

Nitrogen

Total nitrogen content was the highest in this experiment, Table 13. Similar results of insoluble nitrogen, and soluble nitrogen were obtained as noted under experiment I.

Quality

Tables 5 and 6 show that 10, and 20 p.p.m 6-furfurylaminopurine treatments to head lettuce had the best quality rating after harvest and during the entire storage period. Treatments of 10 p.p.m N6-benzyladenine had a rather high quality score, followed by 5 p.p.m 6 furfurylaminopurine; 5 p.p.m and 20 p.p.m N6-benzyladenine; and the untreated control lettuce had the lowest quality score.

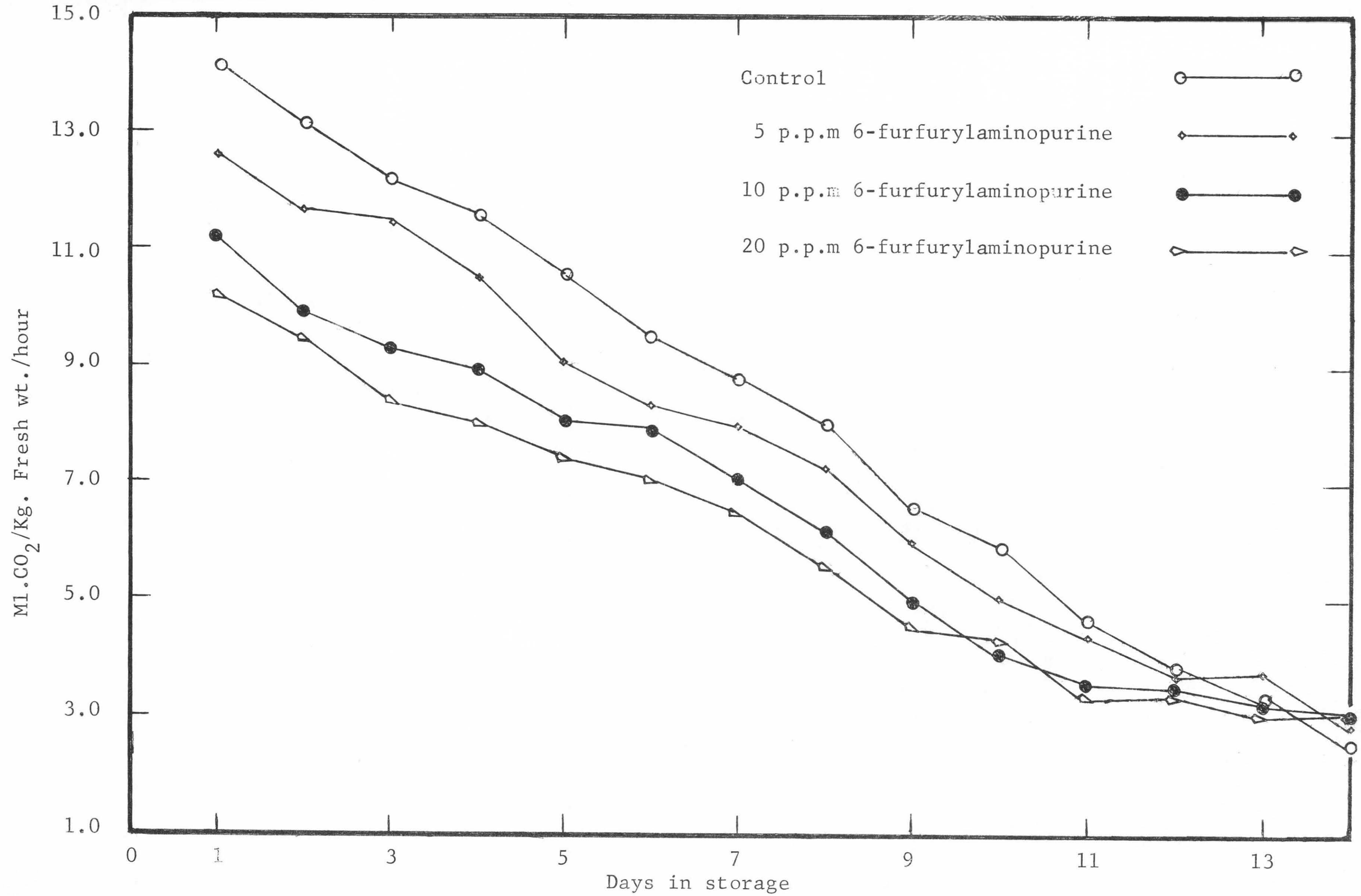

Figure 7. Respiration levels of head lettuce, in relation to pre-harvest applications of 6-furfurylaminopurine during storage at 40° F and 85 percent R.H. (Second harvest: July 29, 1963)

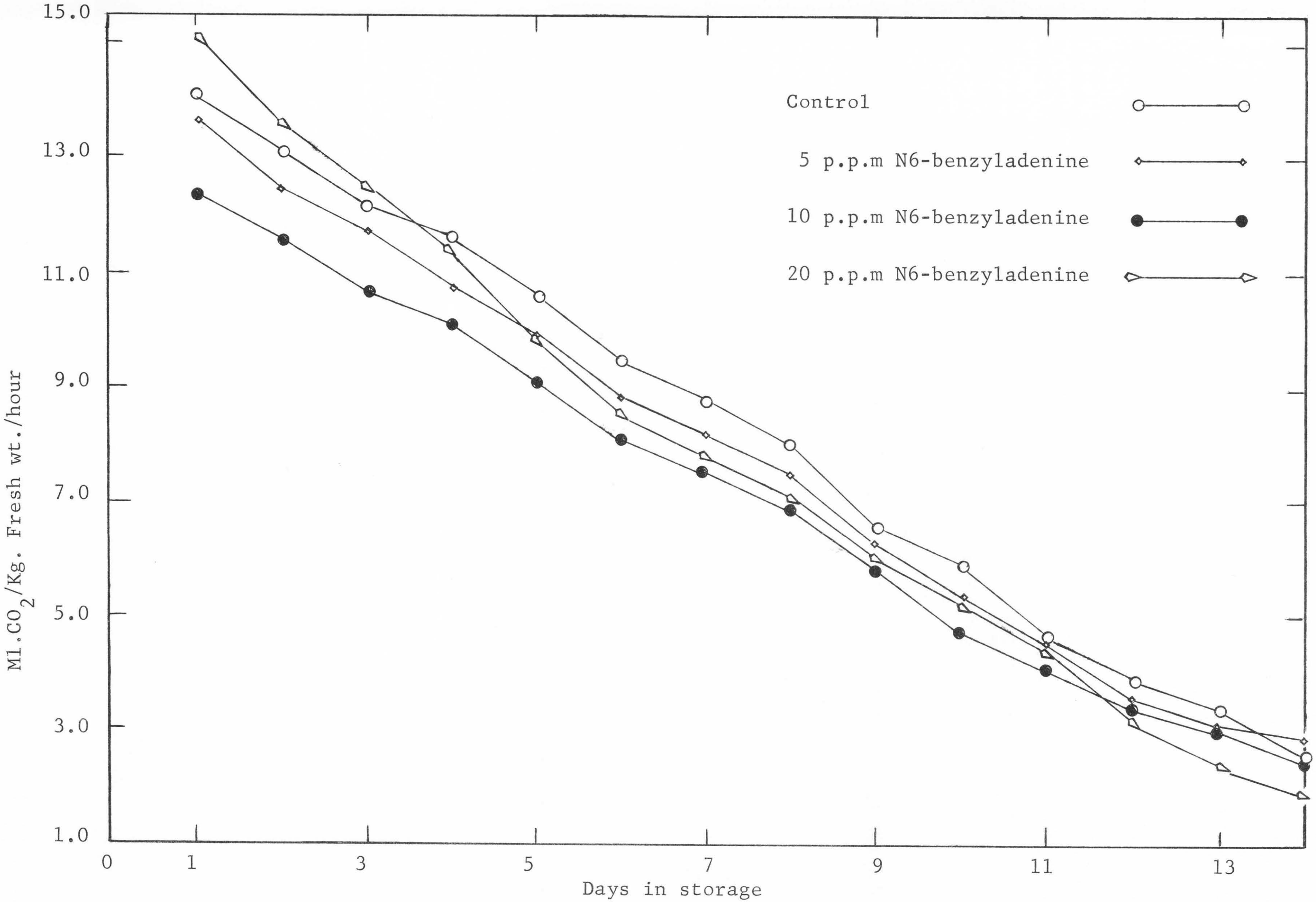

Figure 8. Respiration levels of head lettuce in relation to pre-harvest applications of N6-benzyladenine during storage at 40° F and 85 percent R.H. (Second harvest: July 22, 1963)

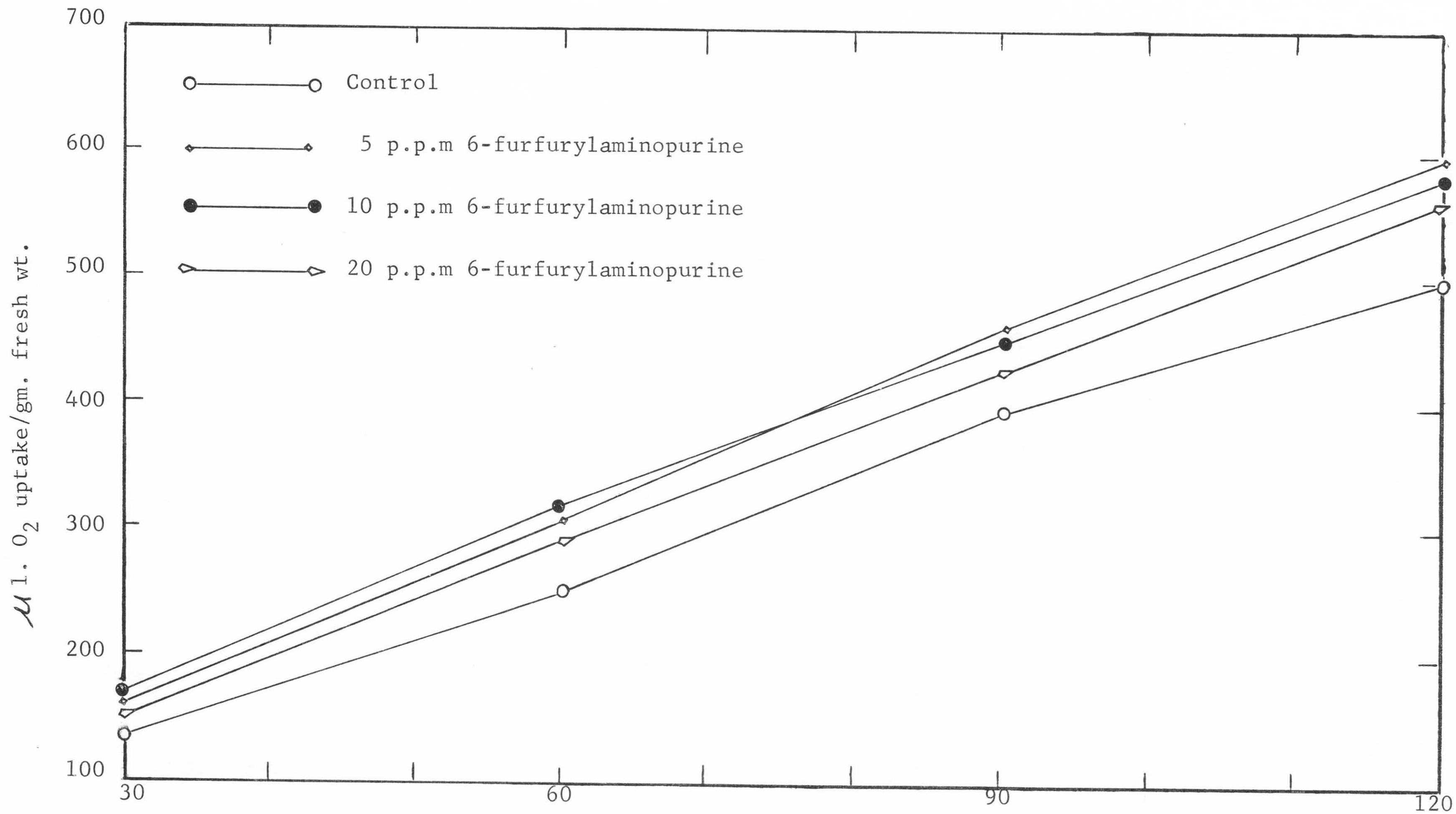

Figure 9. Oxygen uptake of lettuce leaf segments at 98° F, in relation to pre-harvest applications of 6-furfurylaminopurine (Second harvest: July 29, 1963)

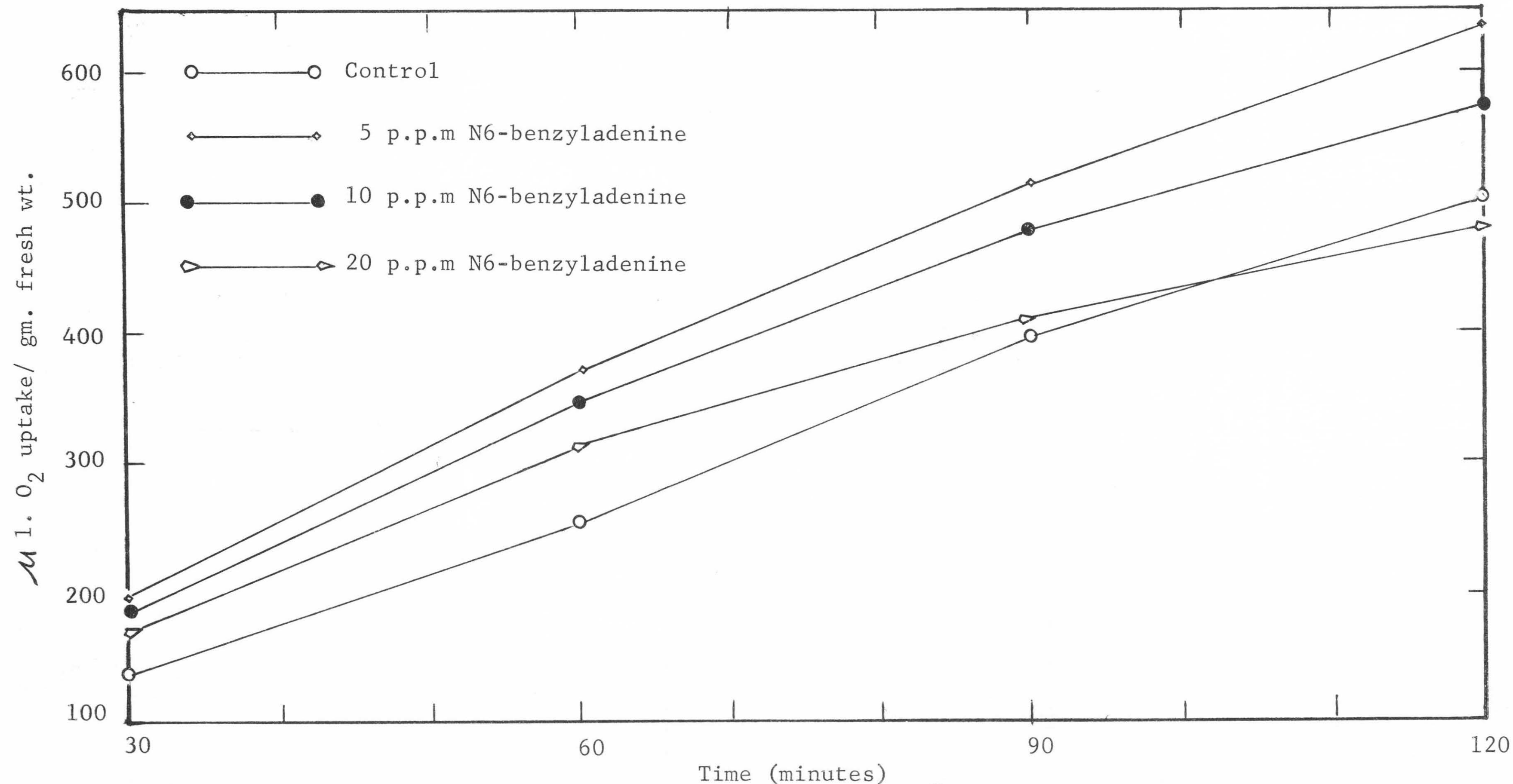

Figure 10. Oxygen uptake of lettuce leaf segments at 98° F, in relation to pre-harvest applications of N6-benzyladenine (Second harvest: July 29, 1963)

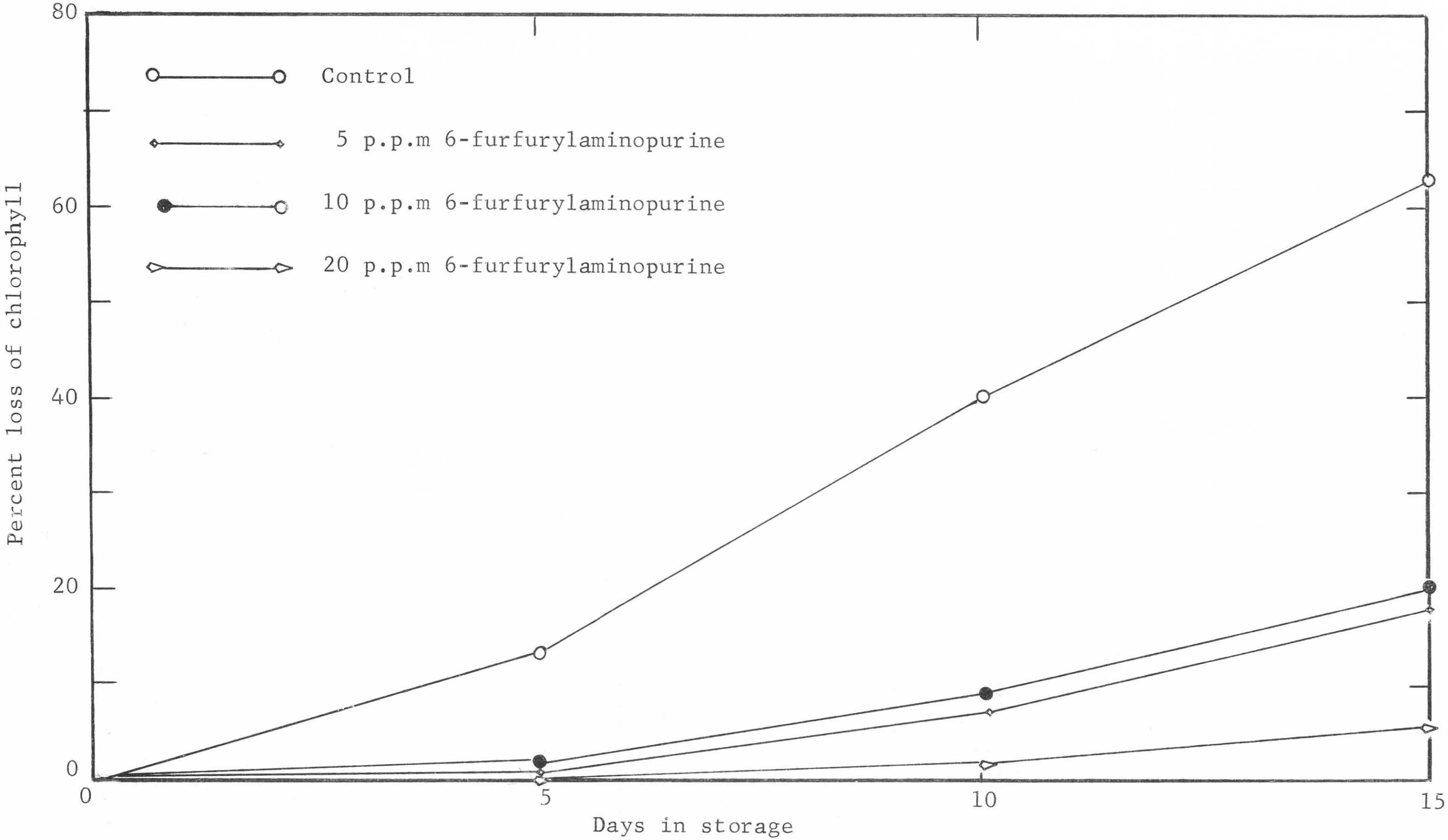

Figure 11. Percent loss of total chlorophyll in lettuce leaves treated with pre-harvest applications of 6-furfurylaminopurine (Second harvest: July 29, 1963)

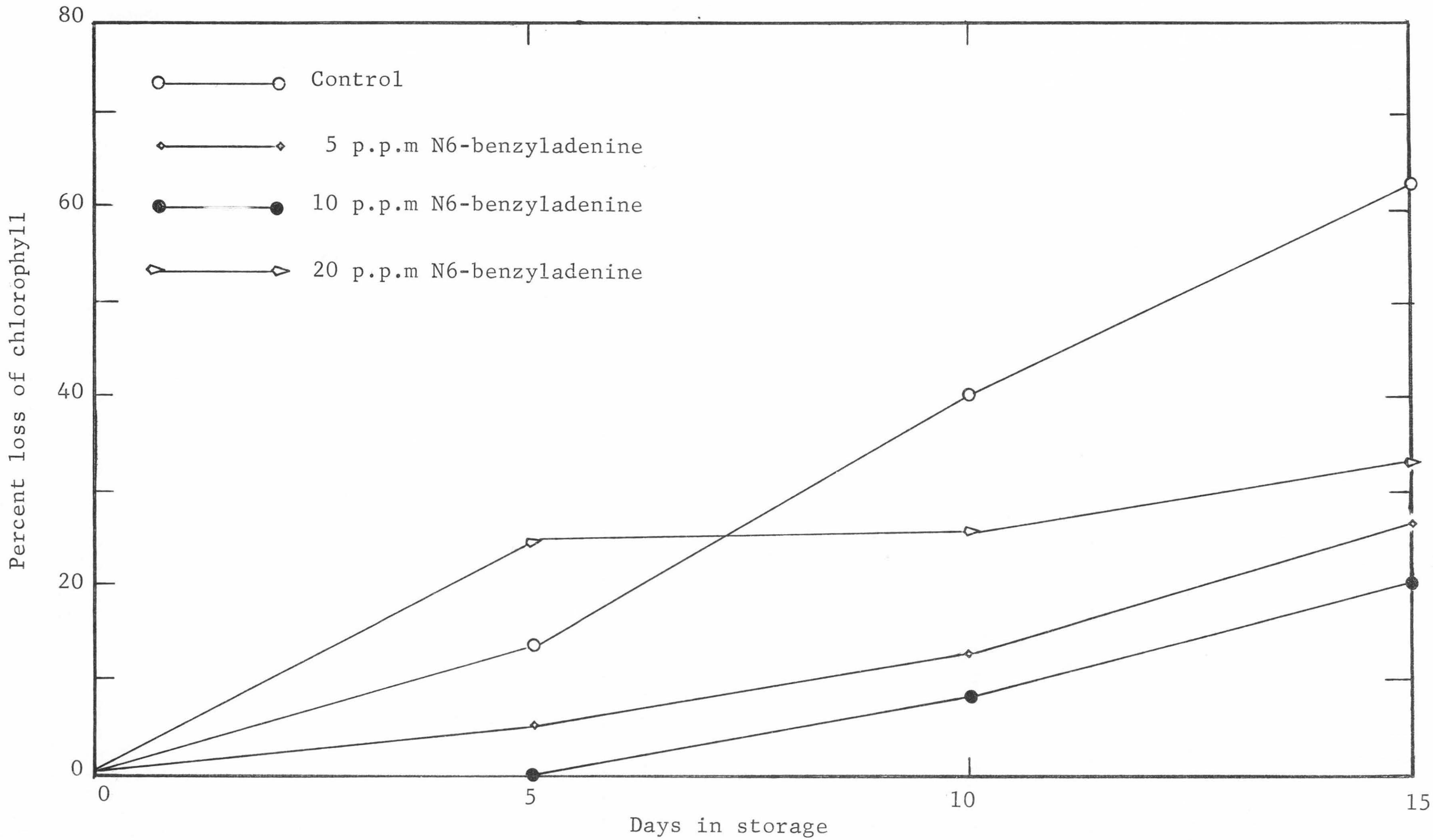

Figure 12. Percent loss of total chlorophyll in lettuce leaves treated with pre-harvest applications of N6-benzyladenine (Second harvest: July 29, 1963)

Table 5. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40° F. in relation to pre-harvest applications of 6-furfurylaminopurine (Second harvest: July 29, 1963)

Treatment	Moisture (percent)	Total[a] chlorophyll (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control									
At harvest	91.5	47.0	3.37	1.99	1.38	115.0	501	7	--
After 5 days storage	90.5	53.5	3.06	1.74	1.32			5	
After 10 days storage	89.3	64.0	2.96	1.48	1.48			5	
After 15 days storage	87.2	76.5	2.68	1.28	1.40			1	
5 p.p.m 6-furfurylaminopurine									
At harvest	93.0	46.0	3.36	2.44	0.92	105.1	599	9	5
After 5 days storage	92.4	46.5	3.14	2.42	0.72			9	
After 10 days storage	92.0	49.5	3.08	2.42	0.66			7	
After 15 days storage	91.3	54.0	3.00	2.34	0.66			6	
10 p.p.m 6-furfurylaminopurine									
At harvest	92.0	43.0	3.37	2.68	0.69	91.3	582	9	10
After 5 days storage	91.6	44.0	3.16	2.60	0.56			9	
After 10 days storage	91.4	47.0	3.07	2.60	0.47			9	
After 15 days storage	91.1	47.5	3.02	2.54	0.48			9	
20 p.p.m 6-furfurylaminopurine									
At harvest	92.5	45.0	3.39	2.78	0.61	84.5	566	9	12
After 5 days storage	92.5	45.0	3.14	2.78	0.36			9	
After 10 days storage	92.1	46.0	3.07	2.76	0.31			9	
After 15 days storage	92.1	47.5	3.04	2.74	0.30			9	

[a]Percent transmittance at the absorbtion maximum of chlorophyll a (665 mμ)
[b]Percent nitrogen expressed on dry weight basis
[c]Results expressed on fresh weight basis
[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

Table 6. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40° F. in relation to pre-harvest applications of N6-benzyladenine (Second harvest: July 29, 1963)

Treatment	Moisture (percent)	Total chlorophyll[a] (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control									
At harvest	91.5	47.0	3.37	1.99	1.38			7	
After 5 days storage	90.5	53.5	3.06	1.74	1.32	115.0	501	5	--
After 10 days storage	89.3	64.0	2.96	1.48	1.48			5	
After 15 days storage	87.2	76.5	2.68	1.28	1.40			1	
5 p.p.m N6-benzyladenine									
At harvest	92.0	46.5	3.35	2.21	1.14			7	
After 5 days storage	91.1	49.0	3.11	2.01	1.10	108.5	635	7	5
After 10 days storage	90.6	52.5	3.04	1.72	1.32			7	
After 15 days storage	89.7	59.0	3.02	1.56	1.46			6	
10 p.p.m N6-benzyladenine									
At harvest	92.0	47.0	3.41	2.70	0.71			9	
After 5 days storage	91.4	47.0	3.17	2.52	0.65	99.7	567	9	10
After 10 days storage	90.8	51.0	3.08	2.48	0.60			9	
After 15 days storage	90.6	56.5	3.04	2.42	0.62			8	
20 p.p.m N6-benzyladenine									
At harvest	92.0	46.0	3.32	2.41	0.91			7	
After 5 days storage	91.0	57.5	2.98	1.99	0.99	108.9	480	7	--
After 10 days storage	89.4	58.0	2.87	1.74	1.13			5	
After 15 days storage	88.1	61.5	2.77	1.39	1.38			3	

[a]Percent transmittance at the absorbtion maximum of chlorophyll a (665 mμ)
[b]Percent nitrogen expressed on dry weight basis
[c]Results expressed on fresh weight basis
[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

Experiment III: Effects of Post-harvest Dipping of 6-furfurylaminopurine and N6-benzyladenine on Physio-chemical Changes in Lettuce

Respiration

CO_2 evolution. There was a significant progressive decrease in CO_2 evolution under this experiment, Table 9. The inhibitory effects of 6-furfurylaminopurine and N6-benzyladenine on CO_2 evolution during storage were similar to experiments I and II, Figures 13 and 14.

O_2 uptake. Figures 15 and 16 showed the stimulation of oxygen consumption in both 6-furfurylaminopurine and N6-benzyladenine treatments. The stimulative effects of N6-benzyladenine treatments were more with various concentrations while it was less with 6-furfurylaminopurine and different concentrations have almost the same stimulative effects on oxygen consumption.

Moisture

The moisture contents of both 6-furfurylaminopurine and N6-benzyladenine treatments, were significantly higher, Tables 7, 8 and 11, than the untreated controls.

Total chlorophyll

It is apparent from Figures 17 and 18 that the loss of total chlorophyll during the refrigeration storage of lettuce was similar to experiments I and II. The effectiveness of 6-furfurylaminopurine treatments (where no loss was observed after 5 days in storage, Figure 17) was more than with N6-benzyladenine treatments, Figure 18.

Nitrogen

The lowest amount of total nitrogen content was observed under this experiment, Table 13. Insoluble and soluble nitrogen were the same as under experiments I and II, Tables 7 and 8.

Quality

Tables 7 and 8 show that better quality score is directly related to concentrations of 6-furfurylaminopurine treatments. On the other hand, 10 p.p.m N6-benzyladenine treatment has better quality than with 5 p.p.m and 20 p.p.m N6-benzyladenine treatment. The untreated controls had the lowest quality scores.

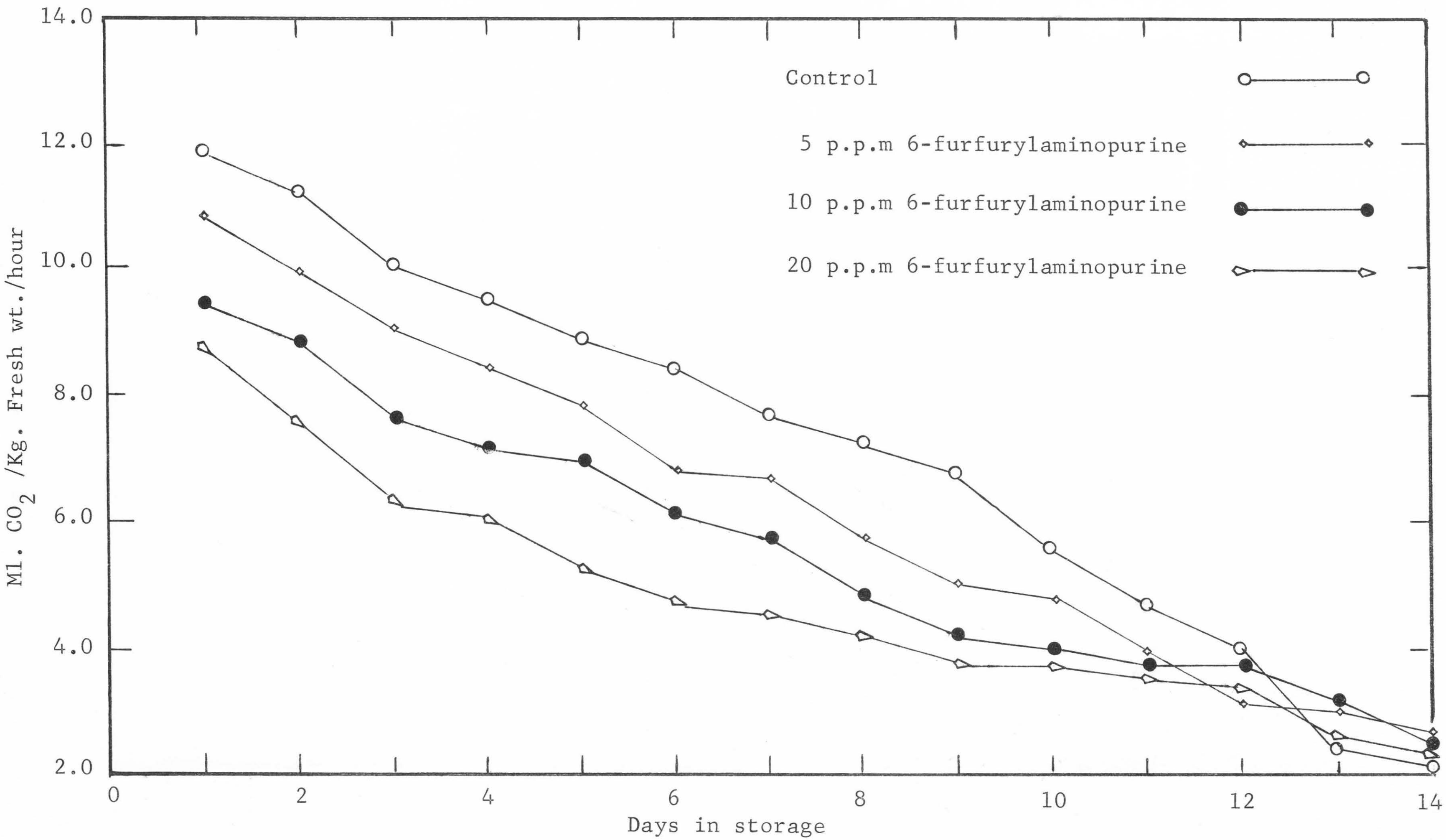

Figure 13. Respiration levels of head lettuce, in relation to post-harvest applications of 6-furfurylaminopurine during storage at 40° F and 85 percent R.H. (Third harvest: August 4, 1963)

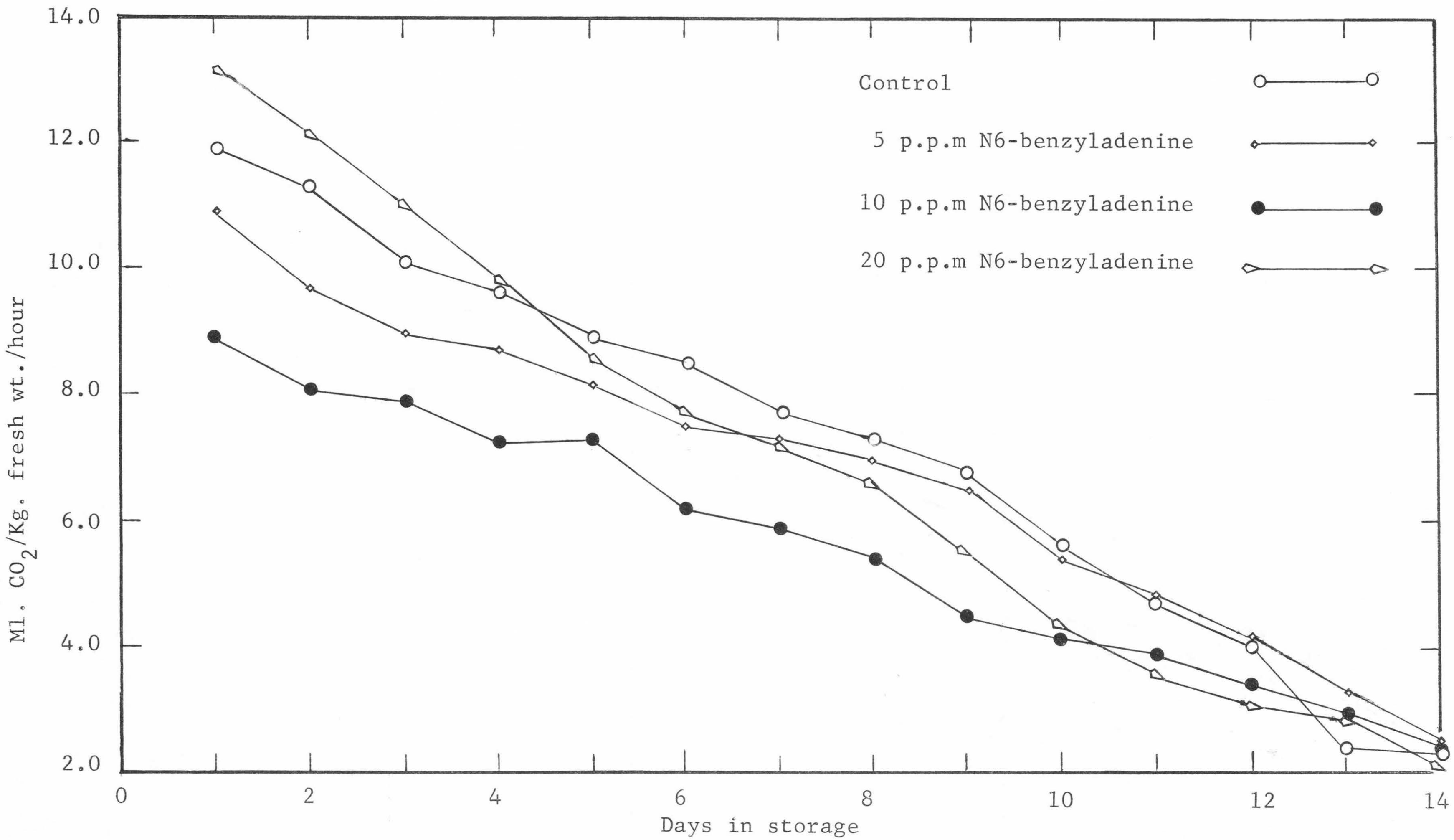

Figure 14. Respiration levels of head lettuce in relation to post-harvest applications of N6-benzyladenine during storage at 40° F and 85 percent R.H. (Third harvest: August 4, 1963)

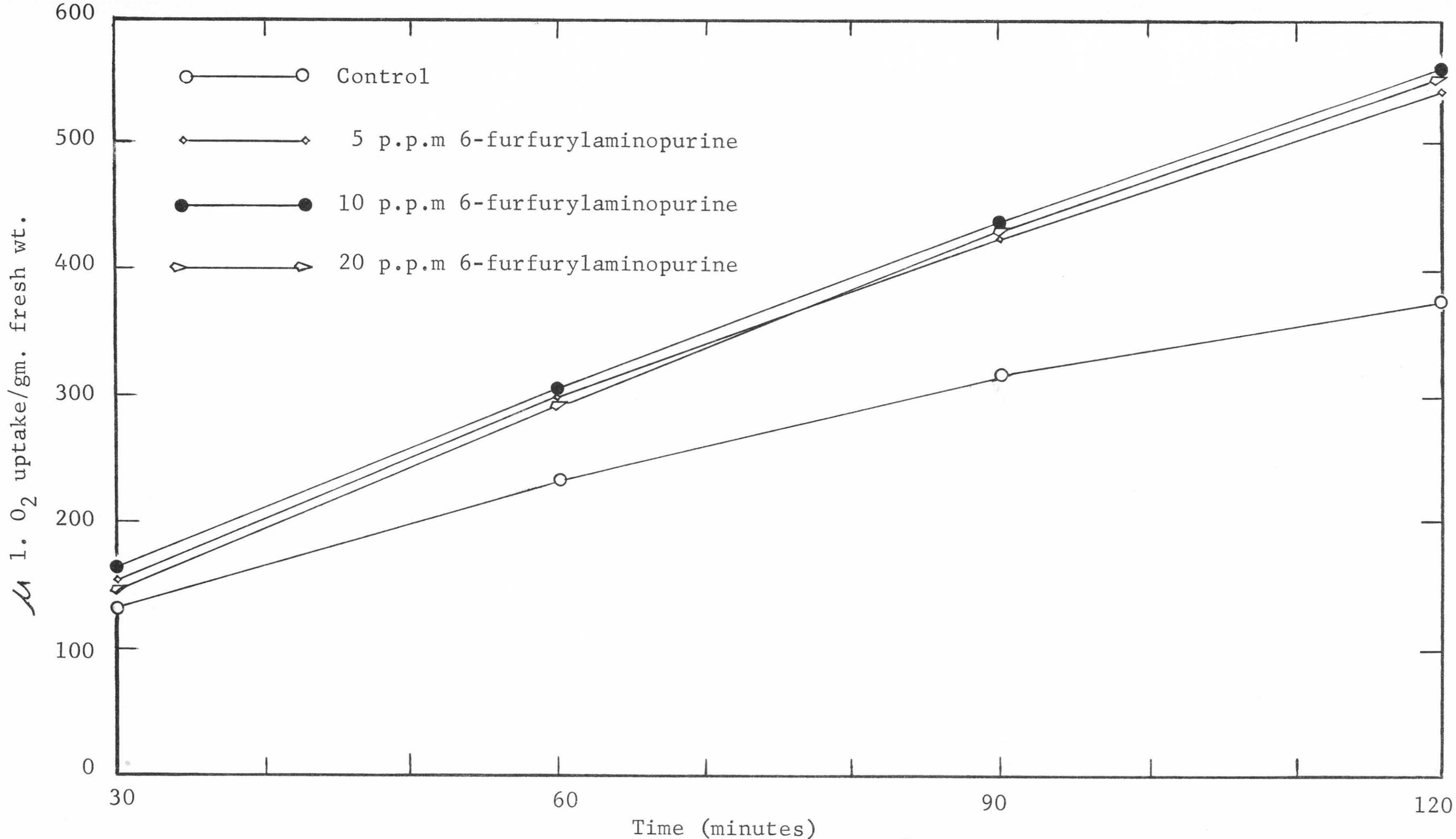

Figure 15. Oxygen uptake of lettuce leaf segments at 98° F, in relation to post-harvest applications of 6-furfurylaminopurine (Third harvest: August 4, 1963)

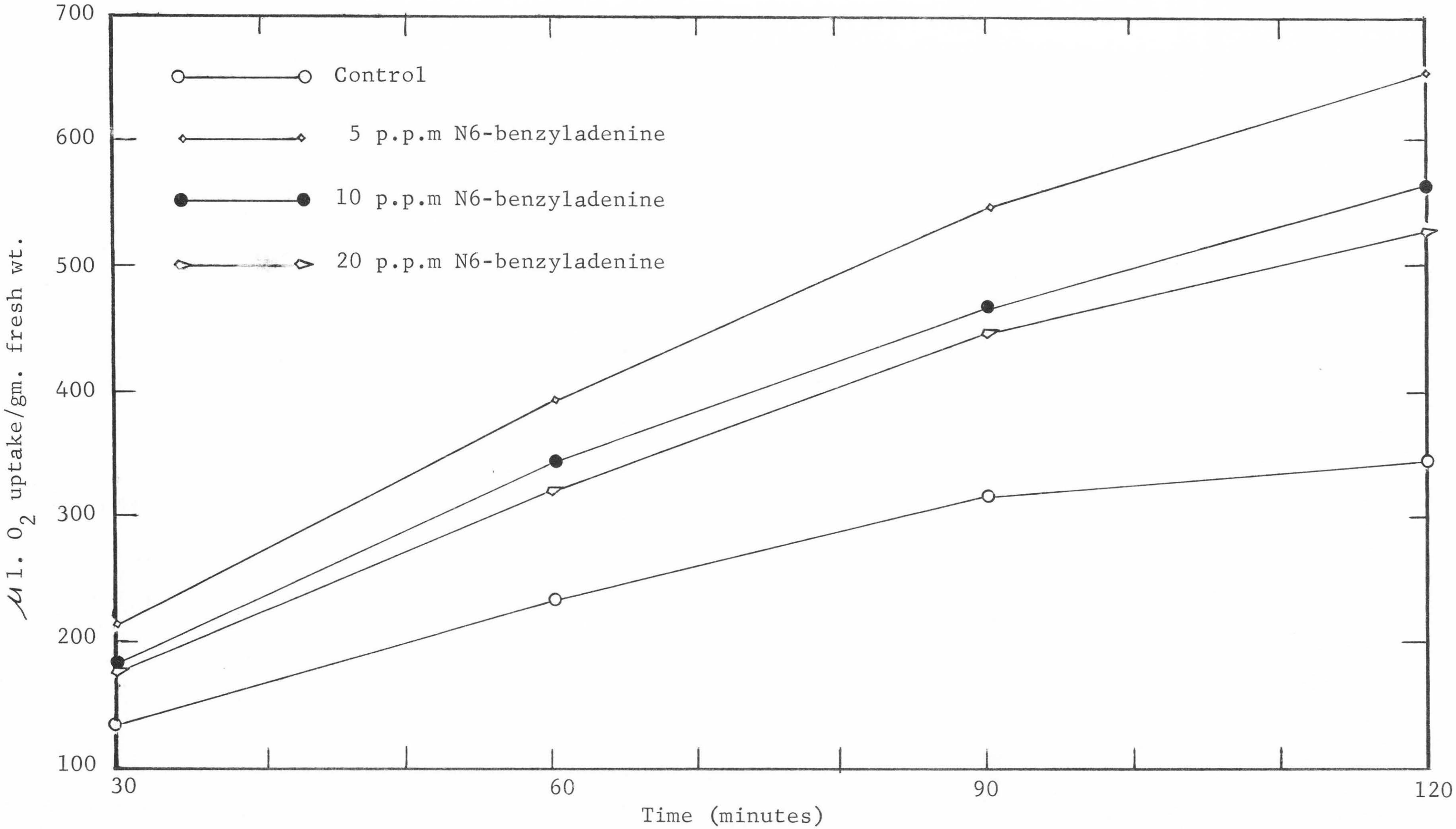

Figure 16. Oxygen uptake of lettuce leaf segments at 98° F, in relation to post-harvest applications of N6-benzyladenine (Third harvest: August 4, 1963)

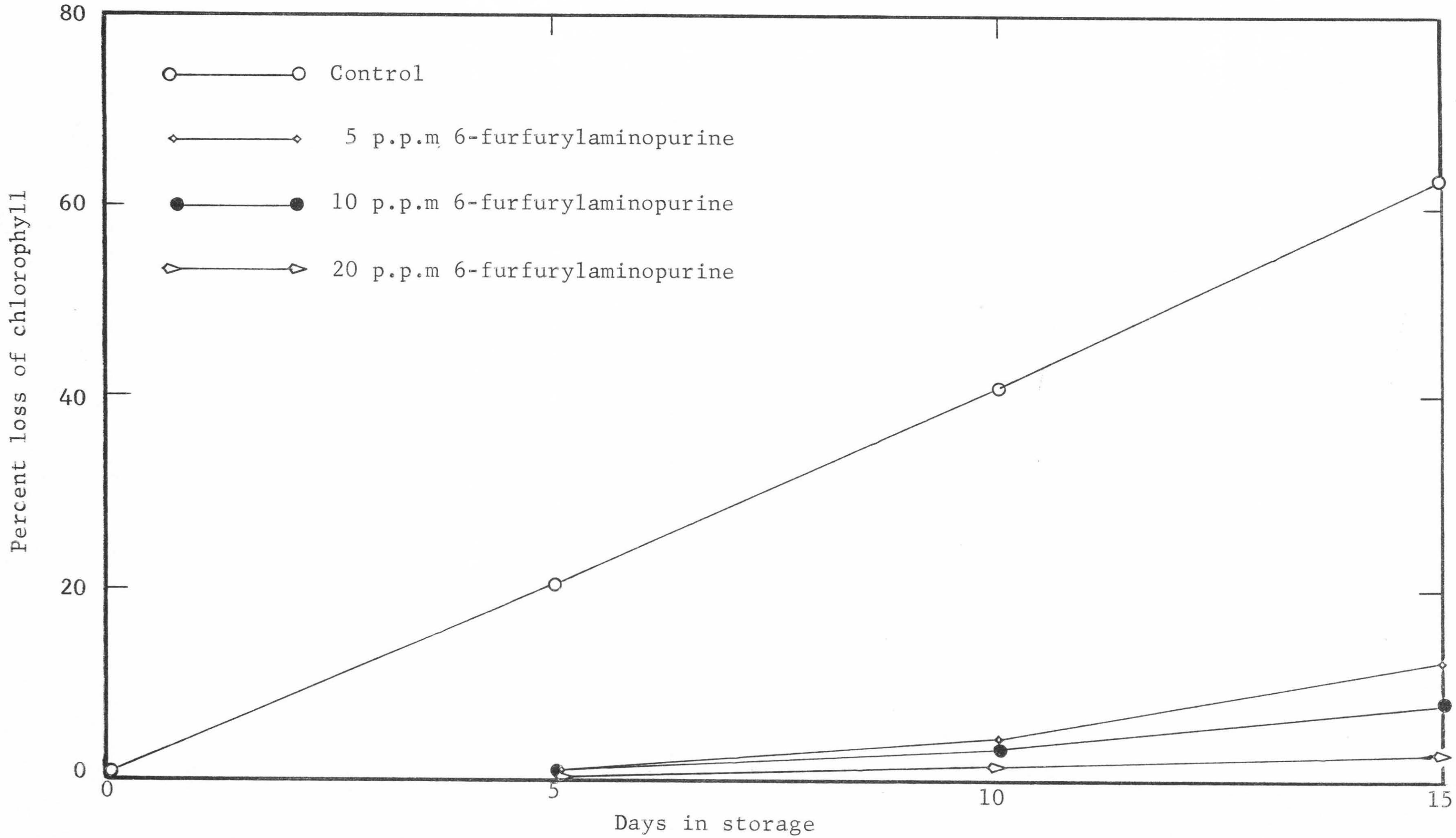

Figure 17. Percent loss of total chlorophyll in lettuce leaves treated with post-harvest applications of 6-furfurylaminopurine (Third harvest: August 4, 1963)

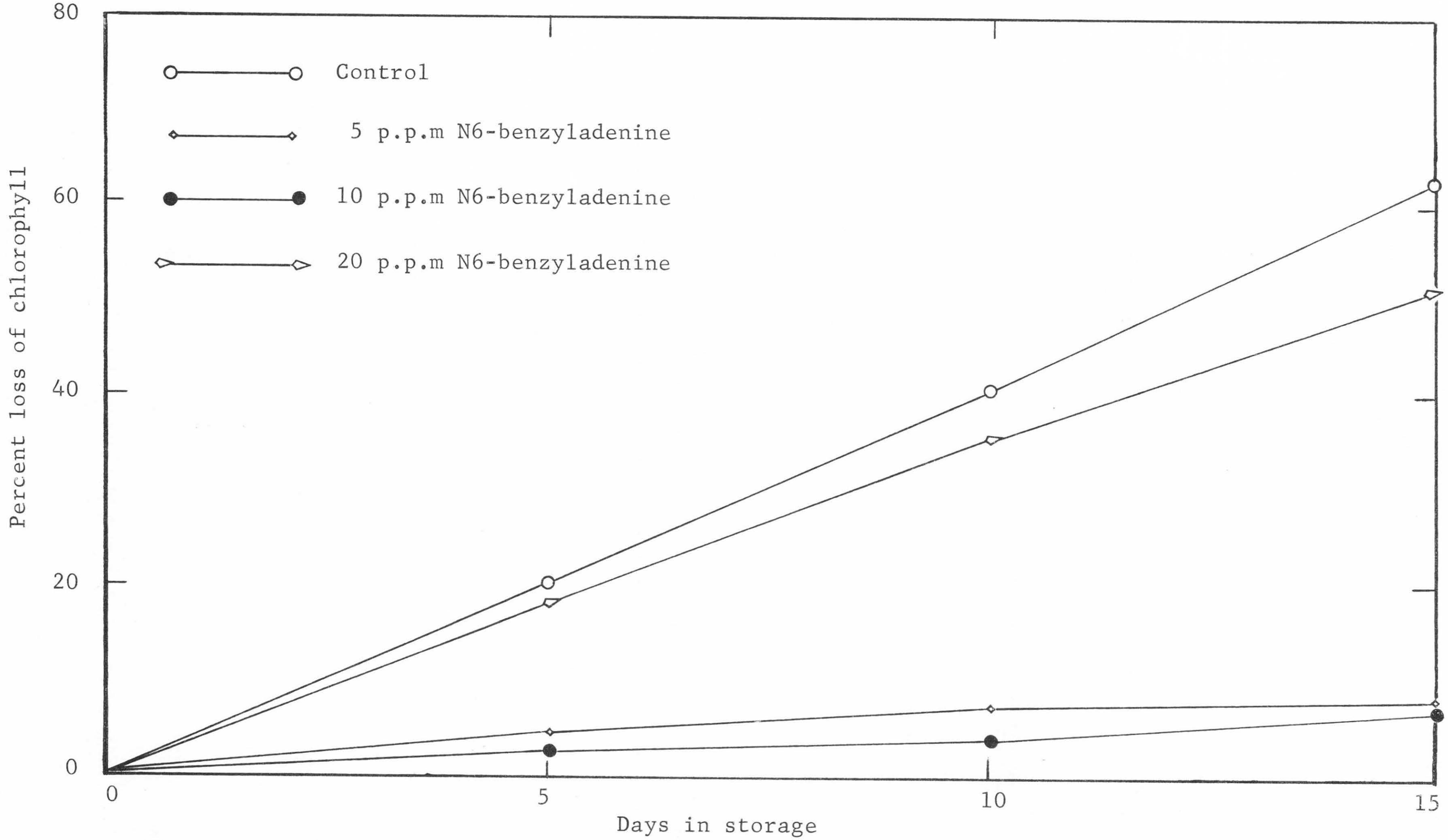

Figure 18. Percent loss of total chlorophyll in lettuce leaves treated with post-harvest applications of N6-benzyladenine (Third harvest: August 4, 1963)

Table 7. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40° F. in relation to post-harvest applications of 6-furfurylaminopurine (Third harvest: August 5, 1963)

Treatment	Moisture (percent)	Total chlorophyll[a] (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control									
At harvest	91.0	51.0	1.68	0.78	0.90	101.1	372	9	--
After 5 days storage	89.4	61.5	0.98	0.41	0.57			7	
After 10 days storage	88.1	72.0	0.66	0.32	0.34			5	
After 15 days storage	85.9	80.0	0.42	0.18	0.24			2	
5 p.p.m 6-furfurylaminopurine									
At harvest	91.0	51.5	1.67	0.83	0.84	88.8	542	9	10
After 5 days storage	90.0	51.5	1.42	0.79	0.63			9	
After 10 days storage	89.6	54.0	1.30	0.72	0.58			9	
After 15 days storage	89.0	58.0	1.00	0.57	0.43			7	
10 p.p.m 6-furfurylaminopurine									
At harvest	91.0	50.0	1.69	0.87	0.82	79.0	554	9	10
After 5 days storage	90.4	50.0	1.57	0.84	0.73			9	
After 10 days storage	90.3	52.0	1.52	0.84	0.68			9	
After 15 days storage	90.0	54.0	1.48	0.79	0.69			8	
20 p.p.m 6-furfurylaminopurine									
At harvest	91.0	52.0	1.70	0.82	0.88	67.6	551	9	12
After 5 days storage	90.7	52.0	1.61	0.92	0.69			9	
After 10 days storage	90.3	53.0	1.59	0.90	0.69			9	
After 15 days storage	90.2	53.5	1.47	0.87	0.70			9	

[a]Percent transmittance at the absorbtion maximum of chlorophyll a (665 $m\mu$)
[b]Percent nitrogen expressed on dry weight basis
[c]Results expressed on fresh weight basis
[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

Table 8. Physio-chemical changes in "Great Lakes" Lettuce, during storage at 40^{o} F. in relation to post-harvest applications of N6-benzyladenine (Third harvest: August 5, 1963)

Treatment	Moisture (percent)	Total[a] chlorophyll (percent)	Nitrogen[b] Total (percent)	Nitrogen[b] Insoluble (percent)	Nitrogen[b] Soluble (percent)	Total CO_2[c] production during storage (14 days) ml CO_2/kg/hr	Total O_2[c] consumption (2 hr period) ul O_2/gm	Quality rating[d]	Increase in shelf life over the controls (days)
Control									
At harvest	91.0	51.0	1.68	0.78	0.90	101.1	372	9	--
After 5 days storage	89.4	61.5	0.98	0.41	0.57			7	
After 10 days storage	88.1	72.0	0.66	0.32	0.34			5	
After 15 days storage	85.9	80.0	0.42	0.18	0.24			2	
5 p.p.m N6-benzyladenine									
At harvest	91.0	54.0	1.66	0.80	0.86	94.9	652	9	10
After 5 days storage	89.8	56.5	1.37	0.61	0.76			7	
After 10 days storage	89.1	58.0	1.22	0.58	0.64			7	
After 15 days storage	88.3	58.5	0.97	0.32	0.65			7	
10 p.p.m N6-benzyladenine									
At harvest	91.0	57.0	1.68	0.84	0.84	78.3	562	9	12
After 5 days storage	90.2	59.0	1.62	0.81	0.81			9	
After 10 days storage	89.6	59.5	1.49	0.77	0.72			9	
After 15 days storage	89.4	61.0	1.44	0.63	0.81			7	
20 p.p.m N6-benzyladenine									
At harvest	91.0	51.5	1.71	0.82	0.89	97.4	527	9	--
After 5 days storage	89.3	59.5	1.02	0.43	0.59			7	
After 10 days storage	88.0	70.5	0.92	0.40	0.52			5	
After 15 days storage	86.4	78.0	0.86	0.24	0.62			4	

[a] Percent transmittance at the absorbtion maximum of chlorophyll a (665 mμ)
[b] Percent nitrogen expressed on dry weight basis
[c] Results expressed on fresh weight basis
[d] 9: Excellent 7: Good 5: Fair 3: Unsalable 1: Unusable

DISCUSSION

A study was conducted to evaluate the effects of pre- and post-harvest applications of 6-furfurylaminopurine and N6-benzyladenine on the refrigerated-life of head lettuce, variety "Great Lakes."

The study was divided into the following three experiments:

Experiment I. Two times pre-harvest applications of both chemicals (First harvest)

Experiment II. One time pre-harvest application of both chemicals (Second harvest)

Experiment III. Post-harvest application of both chemicals (Third harvest)

The results obtained are discussed below.

Each of the three experiments showed an inhibition in CO_2 evolution during storage when either 6-furfurylaminopurine or N6-benzyladenine were applied at concentrations of 5 p.p.m, 10 p.p.m and 20 p.p.m. There was significant decrease in CO_2 evolution (at five percent level) among the three successive harvests. Early harvest has the highest amount of CO_2 evolution, followed by second and third harvests respectively. The inhibitory effects of such materials were significantly related to the concentrations applied, with the exception, however, with 20 p.p.m N6-benzyladenine treatments where the inhibitory effects were less than with 10 p.p.m N6-benzyladenine treatments. The inhibition of CO_2 evolution as a result of applications of such chemicals were reported by many other workers (Dedolph _et al_., 1962). On the other hand no significant differences were observed among the three harvests in oxygen uptake while there were stimulation of oxygen consumption adversely and significantly (at one percent level) related to the concentrations of both N6-benzyladenine and 6-furfurylaminopurine. These findings of higher rates of oxygen consumption agree with the findings of Osborne (1959), Lipton and Ceponis (1962), Smock _et al_, (1962), and may be explained together with lower rates of CO_2 evolution on the basis of CO_2 fixation into other metabolities.

The moisture content during storage of lettuce treated with both 6-furfurylaminopurine and N6-benzyladenine were significantly higher (at one percent level) than the untreated controls, and higher values were obtained at the first harvest followed by the second and third harvest respectively. There were significant differences (at one percent level) in moisture contents among various treatments, directly related to the concentrations of 6-furfurylaminopurine which were higher than with various N6-benzyladenine treatments.

There was significant (at five percent level) progressive degredation in total chlorophyll content among the three harvests. In all instances, the loss of total chlorophyll during storage was much higher in the untreated controls as compared with lettuce treated with both 6-furfurylaminopurine and N6-benzyladenine.

The total percentage of nitrogen contents was more than the untreated lettuce controls when both chemicals were applied. Similar results were obtained with water insoluble organic nitrogen. The stabilization of percent water insoluble organic nitrogen during storage as a result of 6-furfurylaminopurine and N6-benzyladenine applications were evident where no significant change was observed. The increase in percentage soluble nitrogen at the expense of insoluble nitrogen in the untreated lettuce controls rather than the treated plants may indicate that both 6-furfurylaminopurine and N6-benzyladenine stabilize water insoluble organic nitrogen which may be associated with higher percentage of total chlorophyll content.

These findings are in agreement with many other workers (Person et al., 1951; Richmond and Lang, 1957; Wang and Waywood, 1959; Thimann and Manmohan, 1961; and Sugiura et al., 1962).

Both 6-furfurylaminopurine and N6-benzyladenine are effective in delaying the visual manifestations of senescence of lettuce plants, whether pre- or post-harvest applications were made, and can be explained, together with higher rates of chlorophyll content, percentage total and insoluble nitrogen, moisture content and maintenance of fresh appearance and quality rating, on the basis of maintaining cell vigor and delaying proteolysis (Zink, 1961), and consequently delaying senescence and extending the shelf-life of the fresh produce.

In general, most promising results were obtained when post-harvest treatments were made as shown in Experiment III. With 6-furfurylaminopurine, 20 p.p.m seems to be the most effective, since it prolongs the shelf-life of lettuce by 12 days more than the untreated control lettuce.

SUMMARY AND CONCLUSIONS

Lettuce (variety: Great Lakes) was uniformly treated in the field and harvested three times at weekly intervals. Lettuce for the first harvest was sprayed two times (with a weekly interval prior to harvest) in the field with 6-furfurylaminopurine and N6-benzyladenine at concentrations of 0, 5, 10, and 20 p.p.m. Lettuce for the second harvest was treated in the same manner, except that both chemicals were applied only one day before harvest. Lettuce for the third harvest received a post-harvest dip of the same materials and concentrations. The lettuce heads were then stored at 40^{o} F and 85 percent R.H. and were analyzed for CO_2 evolution, O_2 uptake, moisture, total chlorophyll, water soluble and insoluble organic nitrogen, and quality ratings at five day intervals. In all instances inhibition of CO_2 evolution during storage was directly related to the concentrations of both 6-furfurylaminopurine and N6-benzyladenine. On the other hand the stimulation of oxygen consumption under the action of both chemicals was conversely related to their concentrations. Higher values of moisture content, total chlorophyll, total and insoluble nitrogen were observed during storage when both materials were applied as pre- or post-harvest treatments.

These findings suggest the effectiveness of both chemicals on delaying the visual manifestations of senescence as noticed by higher quality ratings.

Most promising results were obtained when post-harvest treatments were made. 6-furfurylaminopurine was more effective than N6-benzyladenine, and both maintain better quality ratings than the untreated control lettuce.

In order to maintain high quality of refrigerated head lettuce, and to keep the fresh produce for longer time, it is advisable to use such materials since it may have considerable economic bearing upon storing and shipping leafy vegetables to distant markets.

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APPENDIX

Table 9. Analysis of variance and interaction between harvests and chemical treatments on CO_2 evolution during storage of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. values
Harvest (H)	2	0.14	0.07	17.02[a]
Error (A)	3	0.01	0.00	
Treatment (T)	6	0.08	0.01	20.62[b]
T X H	12	0.00	0.00	1.27[n.s.]
Error (B)	18	0.01	0.00	

[a] Significance at 5 percent level of probability of Type I[*] error
[b] Significance at 1 percent level of probability of Type I error
[n.s.] Non significance

[*] Type I error signifies rejecting hypothesis when it is true.

Table 10. Analysis of variance and interaction between harvests and chemical treatments on O_2 consumption of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. values
Harvest (H)	2	0.00	0.00	6.53[n.s.]
Error (A)	3	0.00	0.00	
Treatment (T)	6	0.12	0.02	99.35[a]
T X H	12	0.02	0.00	9.19[a]
Error (B)	18	0.00	0.00	

[a] Significance at 1 percent level of probability of Type I error
[n.s.] Non significant

Table 11. Analysis of variance and interaction between harvests, chemical treatments, and storage on moisture content of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. values
Harvest (H)	2	402.88	201.44	52.50[a]
Error (A)	3	11.50	3.83	
Treatment (T)	6	201.76	33.62	76.19[a]
T x H	12	50.93	4.24	9.61[a]
Error (B)	18	7.94	0.44	
Storage (S)	3	95.26	31.75 (.001)	186.25[a]
H x S	6	2.24	0.37	2.29[n.s.]
T x S	18	34.70	1.92	12.30[a]
H x T x S	36	2.13	0.05	0.35[n.s.]
Error (C)	63	10.74	0.17	

[a] Significance at 1 percent level of probability of Type I error
[n.s.] Non significant

Table 12. Analysis of variance and interaction between harvests, chemical treatments, and storage on total chlorophyll of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. values
Harvest (H)	2	1240.00	620.00	26.76[a]
Error (A)	3	69.49	23.16	
Treatment (T)	6	1314.33	219.05	19.81[b]
T x H	12	271.48	22.62	2.14[n.s.]
Error (B)	18	199.03	11.05	
Storage (S)	3	1008.45	336.15	31.55[b]
H x S	6	44.17	7.36	0.66[n.s.]
T x S	18	798.91	44.38	4.13[b]
H x T x S	36	404.66	11.24	1.12[n.s.]
Error (C)	63	693.19	11.00	

[a] Significance at 5 percent level of probability of Type I error
[b] Significance at 1 percent level of probability of Type I error
[n.s.] Non significant

Table 13. Analysis of variance and interaction between harvests, chemical treatments, and storage on total nitrogen of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. Values
Harvest (H)	2	440.35	220.17	907.33[a]
Error (A)	3	0.72	0.24	
Treatment (T)	6	17.86	2.97	511.01[a]
T x H	12	10.97	0.91	136.22[a]
Error (B)	18	0.13	0.00	
Storage (S)	3	17.83	5.94	1110.89[a]
H x S	6	5.56	0.92	157.57[a]
T x S	18	5.47	0.30	61.66[a]
H x T x S	36	5.50	0.15	35.99[a]
Error (C)	63	0.37	0.00	

[a] Significance at 1 percent level of probability of Type I error

Table 14. Analysis of variance and interaction between harvests, chemical treatments, and storage on water insoluble organic nitrogen of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. Values
Harvest (H)	2	543.26	271.63	823.23[a]
Error (A)	3	0.98	0.32	
Treatment (T)	6	95.94	15.84	666.31[a]
T x H	12	7.74	0.64	27.13[a]
Error (B)	18	0.42	0.02	
Storage (S)	3	19.43	6.47	590.82[a]
H x S	6	1.15	0.19	17.58[a]
T x S	18	12.18	0.67	62.74[a]
H x T x S	36	2.51	0.06	6.46[a]
Error (C)	63	0.69	0.01	

[a] Significance at 1 percent level of probability of Type I error
n.s. Non significant

Table 15. Analysis of variance and interaction between harvests, chemical treatments, and storage on water soluble nitrogen of "Great Lakes" lettuce

Source of variance	Degrees of freedom	Sum of squares	Mean square	F. values
Harvest (H)	2	10.32	5.16	35.87[a]
Error (A)	3	0.43	0.14	
Treatment (T)	6	57.40	9.56	98.80[a]
T x H	12	60.35	5.02	51.93[a]
Error (B)	18	1.74	0.09	
Storage (S)	3	5.47	1.82	18.11[a]
H x S	6	6.42	1.07	11.05[a]
T x S	18	6.75	0.37	3.52[a]
H x T x S	36	10.74	0.29	2.90[a]
Error (C)	63	6.71	0.10	

[a] Significance at 1 percent level of probability of Type I error