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Carbon Dioxide-induced Injury of Romaine Lettuce Stored in Controlled Atmospheres

Werner J. Lipton

Horticultural Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 2021 South Peach Avenue, Fresno, CA 93727

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Abstract. **Romaine lettuce (*Lactuca sativa* L.) was stored for 2 weeks at 0°, 2.5°, or 5°C with O_2/CO_2 (%/%) levels of 21/0 (air), 20/5, 20/7.5, 20/10, 20/15, 20/20, 20/30, 0.25/0, or 0.5/0. Controlled atmosphere combinations of 20/15, 20/20, and 20/30 resulted in brown, sunken patches on the green lamina at all temperatures. The 20/10 treatment induced this injury only at 0° and 2.5°, whereas 20/7.5 induced it only at 0°. No injury developed in 20/5 or 21/0. Brown stain, a typical symptom of CO_2 injury on midribs of crisphead lettuce, was absent in 20/30, 20/20, 20/5, and 21/0, but developed in the other atmospheres, mainly at 0°. Low levels of O_2 (0.5% or 2%) combined with 7.5% CO_2 did not consistently enhance sensitivity of romaine to CO_2 injury. Salability was retained at least as well in romaine stored at 0° or 2.5° in air as in any of the controlled atmospheres.**

Romaine lettuce (*Lactuca sativa*) that was stored in closed film bags or liners in which CO_2 levels reached 2% to 7% and in which O_2 levels ranged from 2% to about 14% reportedly was less yellow and required less trimming than that held in open bags (1). The same authors noted that the lettuce was damaged only in samples in which CO_2 reached 15% and O_2 decreased to <1%. Thus, the market life of romaine apparently can be extended by use of certain modified atmospheres. However, these results are of limited usefulness, because the research did not clarify whether the benefit was derived from high CO_2, low O_2, or from their combination. Also, no information was developed on the range of concentrations of the gases that would be beneficial or the levels that would induce damage. Finally, the influence of temperature on the response of romaine to modified atmospheres remained unexplored. Since the response of crisphead lettuce to storage with elevated CO_2 is strongly influenced by O_2 concentration (11) and temperature (2), it seemed advisable to study the response of romaine to these variables. Without such knowledge, the optimal storage or transit conditions for romaine would remain uncertain. The research reported here, therefore, delineated the response of romaine lettuce to high levels of CO_2, low levels of O_2, and their combination at several temperatures. Also, specific injuries induced by high levels of CO_2 are described.

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Romaine lettuce 'Parris Island' was harvested, packed, and vacuum-cooled commercially in coastal valleys of central California from Oct. 1985 through May 1986 and hauled to Fresno under refrigeration. Experiments were set up within about 30 hr of harvest. The heads were rinsed before storage in some of the tests because of adhering soil. A thin slice of the stem was cut off before storage to permit evaluation of the discoloration of the cut surface under the various atmospheres. Leaves with severe physical damage were removed, but those with small lesions of downy mildew, which affected many heads, were not removed.

A wide range of CO_2 concentrations was evaluated in two unreplicated, preliminary tests to see which concentrations would injure the lettuce and which would have no beneficial effects, and to permit description of symptoms of injury. Very low O_2 levels were included to determine whether they alone would injure the romaine. The first preliminary test included O_2/CO_2 (%/%) combinations of 21/0 (air control), 20/10, 20/20, 20/30, 0.25/0, and 0.5/0; the second of these tests included 20/5, 20/10, 20/15, the low O_2 levels, and the air controls. The low O_2 levels and 20/10 were repeated to ascertain that the origin of the romaine did not influence symptom occurrence or expression. Once the limits of sensitivity were established, the following O_2/CO_2 combinations were evaluated in three tests: 21/0, 0.5/0, 0.5/7.5, 2/7.5, and 20/7.5. The tests spanned the period from Oct. 1985 through May 1986. Precision of the gas mixtures was ±0.05% for 0.25% and 0.50% O_2, ±0.2% for 2% O_2 and ±0.5% for the CO_2 concentrations and for 20% O_2. General storage procedures and methods of mixing and analyzing the gases have been described (6–8). Types of injuries induced by high CO_2 and/or low O_2 atmospheres were described. The severity of the injuries and of decay and the general appearance of all heads were evaluated subjectively by use of rating scales in which ratings for quality decrease (from 9 to 1) as those for defects increase (from 1 to 9) (4). Color of the cut surface (butt color) was recorded but not quantified. Heads rated 5 or higher for overall quality, which considered all aspects of appearance, were considered salable.

In the preliminary and replicated tests, samples consisted of four heads, each taken from a different box. Two samples (eight heads) comprised a treatment, with half the heads being examined after 14 or 15 days of storage at 0°, 2.5°, or 5°C (±0.5°) in the atmospheres noted and the other half after an additional 2 days of holding in a humidified air stream (aeration) at 10°. The samples weighed between 1.4 and 2.0 kg. Weights obtained at each examination, rather than initial weights, were used to calculate rates of ethylene production because of substantial free moisture on the heads before they were stored. Each test encompassed five or six atmospheres, three temperatures and the two examinations noted previously. Because the samples were in the same or in serially connected containers, the data from the two examinations were analyzed separately.

CO_2-induced injury on the lamina typically involved browning and sinking of patches of various sizes. In the preliminary

Table 1. Incidence of CO_2-induced injury on the midribs of romaine lettuce stored 2 weeks at low temperature and following 2 days of aeration at 10°C.

Storage temperature (°C)	Percent injured heads (±SD)[z]		
	O_2/CO_2 concn (%/%)		
	0.5/7.5	2/7.5	20/7.5
	Low temperature		
0	8 (±8)	17 (±14)	50 (±50)
2.5	0	17 (±29)	5
5	0	0	0
	Two days aeration at 10°C		
0	75 (±43)	67 (±38)	75 (±0)
2.5	8 (±14)	33 (±38)	17 (±14)
5	0	8 (±14)	0

[z] Injury principally consisted of brown stain and occasionally of russet-like spots. No similar symptoms developed in any samples stored in air only. All means are based on 12 heads, four each from three tests.

Table 2. Percent salable romaine lettuce stored 2 weeks at low temperature and following 2 days of aeration at 10°C.

Storage temperature (°C)	Percent salable heads (±SD) O_2/CO_2 concn (%/%)				
	21/0[z]	0.5/0	0.5/7.5	2/7.5	20/7.5
	Low temperature				
0	100	100	100	100	100
2.5	100	100	100	92 (±14)	100
5	92 (±14)	83 (±29)	100	92 (±14)	100
	Two days aeration at 10°C				
0	100	92 (±14)	83 (±29)	92 (±14)	100
2.5	100	100	100	100	100
5	8 (±14)	75 (±25)	92 (±14)	83 (±28)	75 (±25)

[z] Air. All means are based on 12 heads, four each from three tests.

tests, this injury was evident in all heads stored in 20/30, 20/20, or 20/15, regardless of temperature. At 0°C, however, these atmospheres also induced such lesions in bands along the leaf margin. Temperature strongly affected the results for romaine stored in 20/10—virtually all heads were injured at 0° or 2.5°, but only one at 5°. Among samples held with 7.5% CO_2, injury was confined to heads stored at 0° (incidence 0% to 17%); heads stored with 5% CO_2 remained uninjured. When injury developed, it tended to intensify during aeration at 10°.

A relatively evenly distributed tan bronzing of the midrib, but no brown stain (BS), developed in romaine stored 2 weeks in 20/30 or 20/20 in preliminary tests.

BS, with its discrete, halo-like lesions, is the typical symptom of CO_2-induced injury on the midrib of crisphead lettuce, regardless of storage temperature (11). BS was evident in romaine lettuce held with 20/15 or 20/10, but only at 0°C in preliminary tests. In the replicated tests in which 0.5/7.5, 2/7.5, and 20/7.5 were tested, BS developed in all three combinations at 0°, but only in 2/7.5 at 2.5°, and in none of the atmospheres at 5° (Table 1 upper). The incidence of BS increased during the 2 days of aeration at 10°, but most dramatically in samples previously held at 0° (compare Table 1 upper and lower). However, the mild BS (trace or slight) in many of these heads would not reduce salability of affected heads.

Small brown spots developed in a few heads stored at 0°C in 20/7.5 and 20/15 and sporadically during aeration following storage with 7.5% to 20% CO_2 at all temperatures. These spots, which resembled russet spotting on crisphead lettuce (9), also were noted by Stewart and Uota (10) on crisphead lettuce stored in 3/10.

Carbon dioxide injured heart leaves of romaine only in 20/30 and 20/20, as a bronzing of midribs at 0° and 2.5°C, but only as a necrosis of leaflet margins at 5°. The latter closely resembled the relevant injury in crisphead lettuce (9).

Low O_2 (0.25/0 or 0.5/0) induced no specific disorders, but led to soft rot development in the stem in a few samples held at 5°C.

From 0% to 67% of heads showed some decay after cold storage and from 16% to 100% after aeration at 10°C. However, there was no pattern of decay attributable to atmosphere composition, except for the influence of low O_2 on stem-end decay previously noted. The effect of temperature also was not clear-cut, although the incidence of decay usually was higher at higher temperatures for any given atmosphere. The relatively high incidence of decay and lack of regularity in its distribution among treatments is attributed to the presence of small lesions of downy mildew in many freshly harvested heads.

Elevated CO_2 levels, especially those ≥7.5%, effectively retarded and even prevented darkening of the cut stem surface regardless of O_2 level. The effect was stronger at 0° and 2.5°C (where the surface remained almost white) than at 5° (where it ranged from light pink to red). Butt color ranged from white to red in romaine held in 0.5/0 regardless of temperature, while that of the controls ranged from light pink to reddish-brown. The surface darkened noticeably during the 2 days of aeration at 10° in all treatments, but no differences were discernible among treatments.

Overall quality of the romaine, expressed as the percentage of heads considered salable, was influenced principally by the injuries induced by high CO_2 levels, degree of yellowing of the leaves, and presence of decay. Almost all heads were salable after cold storage (Table 2 upper). Salability tended to decrease during aeration, except for lettuce that had been stored at 2.5°C, where CO_2 injury, yellowing, and decay were minimal. Salability declined most seriously in the controls from 5° (Table 2 lower), where yellowing and decay were prominent. Overall, salability was retained at least as well in romaine stored at 0° or 2.5° in 21/0 as under any other conditions.

Ethylene production averaged 0.2 $\mu l \cdot kg^{-1} \cdot hr^{-1}$ or less during storage of romaine at low temperature. Increases during aeration at 10°C were not attributable to either atmosphere composition or temperature, with one exception. In that test, in which romaine was held in 20/30, ethylene production during the first day of aeration was two to four times that of the controls, with the highest level (1.3 $\mu l \cdot kg^{-1} \cdot hr^{-1}$) following storage at 0°. Ethylene production was 0.3, 0.3, and 0.5 $\mu l \cdot kg^{-1} \cdot hr^{-1}$ for the control samples that had been held in air at 0°, 2.5°, or 5°, respectively. High levels of ethylene production in samples from 20/30 likely were a response to the injurious effect of the atmosphere.

The foregoing data established that romaine lettuce can be injured by CO_2 levels ≥7.5%, but that the degree of injury is not materially influenced by O_2 levels in the range of 0.5% to 20%. These results are in contrast to those with crisphead lettuce, in which low O_2 levels aggravated CO_2 injury (10, 11). However, the results for romaine resemble those for crisphead lettuce (2, 3) in that the CO_2-induced injuries were more serious at 0°C than at slightly higher temperatures. This influence of temperature on development of CO_2 injury was particularly prominent after subsequent holding of the samples for 2 days at 10° in air.

Overall, none of the controlled atmospheres had maintained salability better than storage in air when the romaine was examined immediately after storage at 0°, 2.5°, or 5°C. However, when cold storage and controlled atmospheres was followed by 2 days of aeration at 10°, earlier exposure to low O_2 (0.5%) or high CO_2 (7.5%) was beneficial, but only for lots from 5°. Thus, commercial use of O_2 reduction or CO_2 enrichment would be potentially useful only if romaine cannot be maintained between 0° and 2.5°.

Romaine lettuce had a lower incidence of BS than that reported for crisphead lettuce (3, 10, 11), which contains a much higher proportion of white tissue. Although romaine was free of BS after being held 2 weeks at 2.5°C with 7.5%, 10%, or 15% CO_2, BS reportedly affected 21% and 52% of crisphead lettuce stored only 1 week in 20/5 and 20/10, respectively, at nearly identical temperatures (10). The results after aeration were similar, i.e., the incidence in romaine was 50% to 25% that reported for crisphead lettuce (10). The susceptibility to CO_2 injury of the green lamina for the two types of lettuce cannot be compared because data for crisphead lettuce are lacking, undoubtedly because the high susceptibility of the latter to BS did not warrant experiments at substantially elevated CO_2 concentrations.

The foregoing results and their comparison with those for crisphead lettuce strengthen the evidence that green and nongreen tissue differ in their sensitivity to high levels of CO_2 during cold storage, as was suggested earlier (5). The basis for this difference remains to be explored.

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Contribution of Endogenous Nitrogen Toward Continuing Growth in a Cranberry Vine

T. Stieber[1] and L.A. Peterson[2]
Department of Horticulture, University of Wisconsin–Madison, Madison, WI 53706

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Abstract. **Rooted cranberry cuttings (*Vaccinium macrocarpon* Ait. 'McFarlin') were placed in nutrient solution with NH_4^+ as the N source. The plants were grown for 20 weeks with complete nutrient solution for the first 8 weeks and a nutrient solution minus N for the remaining 12 weeks. The objective was to determine the contribution of endogenous N of a perennial toward continuing growth of cranberry vines. Growth and N composition were measured at 2-week intervals. Shoots increased in size from 5.3 g of dry matter per pot at 8 weeks to 35.8 g at 20 weeks. Although N deficiency symptoms were present, the cranberry vines were actively growing at the termination of the experiment. Shoot N concentration at this point was 5.5 mg·g^{-1} (0.55%).**

Nitrogen absorbed by plant roots generally is considered to constitute the major supply for plant growth. However, for a crop such as cranberry, N stored in its perennial structure is also a potential supply. Redistribution of N within a perennial structure has been shown by Kang et al. (6) and Weinbaum et al. (10) to be a major N source for early spring growth in apple and prune trees, respectively. The objective of this study was to examine the extent of reuse of the internal N content of cranberry vines in relation to its contribution to continuing vine growth.

The N concentration in cranberry has not been a reliable indicator of N status of the plant (4, 5, 9), as it has with other crop plants. Eaton (3) proposed that 10.0 mg·g^{-1} N (1.0% N) represented a luxury N level under field conditions. Torio (9), using sand culture, reported N deficiency at 7.8 mg·g^{-1} N (0.78%) and excessive growth at 9.8 mg·g^{-1} N (0.98%). Somogyi (8), using substrates with varying organic matter compositions, reported deficiency symptoms when N concentrations fell below 8.0 mg·g^{-1} (0.80%). Greidanus et al. (5), using solution culture, showed that 'Stevens' receiving NO_3^- resulted in poor growth despite a N content of 13.4 mg·g^{-1} (1.34%). Plants receiving NH_4^+-N produced vigorous growth with a tissue concentration of 9.1 mg·g^{-1} N (0.91%).

The cultivar 'McFarlin' was chosen for the experiment because previous unpublished data indicated it was vigorous in solution culture. Cuttings 6 cm in length from dormant vines were stripped of leaves and rooted in deionized water. About 25 days were required for good root development. Ten rooted cuttings were transplanted to a 3.3-liter pot of modified Hoagland's solution with 280 mg of NH_4^+-N as the N source. A total of 30 pots were established and arranged in a randomized complete block. The cuttings were suspended in polyurethane foam plugs and inserted in a styrofoam pot cover. The cultures were aerated, and solution volumes were maintained with deionized water additions. The pH of the solutions was monitored and adjusted to 5.0 ± 0.5 when necessary. Fluorescent lighting was used to insure a 15-hr daylength. Greenhouse temperatures were maintained at 21° ± 2.8°C. The amount of N in each pot was measured at the beginning and at 2-week intervals for 8 weeks by the Bremner and Keeney procedure (1).

Nitrogen in the nutrient solutions was nearly depleted for a majority of the pots after 8 weeks. The plants were very healthy at that time. At 8 weeks, the solutions in the remaining pots were changed to a modified Hoagland's minus the N component. An additional nutrient supplement minus N was made to the remaining pots at 14 weeks to ensure an adequate supply of all other nutrients.

At 2-week intervals from the initial starting date, plants from three pots were harvested and maintained as replicates. Plants from each pot were divided into shoot and root tissue with the latter being washed with deionized water prior to drying. The tissue samples were dried at 70°C, weighed, and ground in a Wiley mill to pass a 40-mesh sieve. Total N in the tissue was determined by the Kjeldahl procedure of Peterson and Chesters (7).

Yield and N concentration of shoots and roots of cranberry plants harvested at 2-week intervals are presented in Fig. 1. The shoots increased in size from 5.3 g/pot at 8 weeks to 35.8 g/pot at 20 weeks without further absorption of N by the roots. Root weight increased from 0.6 to 3.7 g/pot during this time. The change in yield and N concentration of shoots and roots was significantly different (Duncan's multiple range test) with each 2-week interval with the exception of shoot and root yields for weeks 2 and 4. Even though N deficiency symptoms were present during the latter part of the experiment, plant growth was continuing. Basal leaves were senescing while the meristems continued to produce new leaves. The plants harvested at 20 weeks had numerous shoots up to 1 m in length.

The N concentrations in the shoot tissue peaked at 6 weeks and the root tissue at 8 weeks with concentrations of 39.5 mg·g^{-1} N (3.95%) and 41.0 mg·g^{-1} N (4.10%), respectively. Tissue N concentrations then declined until the termination of the trial at 20 weeks. At that point, the shoot N concentration was 5.5 mg·g^{-1} N (0.55%) and the root concentration was 8.8 mg·g^{-1} N (0.81%).

By monitoring the N content in the nutrient solutions and plant tissue, a N balance sheet was established to account for the N used in the experiment. Recovery of N was calculated on a per pot basis as follows: N

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[1]Research Assistant.
[2]Professor.