Time of Day at Harvest Influences Carbohydrate Concentration in Crisphead Lettuce and Its Sensitivity to High CO₂ Levels after Harvest

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Abstract. Concentrations of starch and sugars were measured in the cap leaf (leaf no. 1) and in every fifth leaf (5, 10, 15, 20) in heads of crisphead lettuce (Lactuca sativa L.) harvested at 0700 HR (AM) or 1400 HR (PM) PDT. Starch content increased in the cap leaf from the AM to the PM harvest, but remained unchanged in other leaves. Sucrose concentration was <5 mg g⁻¹ dry weight in AM-harvested lettuce, but the cap leaf, leaf 20, and stem tissue contained 43, 24, and 61 mg g⁻¹ dry weight, respectively, in lettuce from the PM harvest. AM harvested lettuce contained 70% to 260% more glucose and 20% to 120% more fructose than PM-harvested lettuce. Glucose and fructose concentrations were greatest in leaf 10 (110 and 120 mg g⁻¹ dry weight, respectively) and decreased 20% to 50% in inner and outer leaves. Exposure of lettuce to 7.5% or 10% CO₂ for 12 days at 2.5°C followed by air for 3 days at 10°C caused more severe injury on AM- than on PM-harvested lettuce. Injury occurred primarily on leaves 7 through 17, with those between leaves 10 and 15 being most severely affected. High reducing sugar content at harvest did not appear to decrease the sensitivity of lettuce to high CO₂ during storage.

Crisphead lettuce stored in atmospheres containing elevated levels of CO₂ may develop brown stain (11, 18). The sensitivity of lettuce to this disorder varies with cultivar, growing region, and even among lots of a given cultivar from the same region (2, 19).

Time of day at harvest may be one factor that influences the sensitivity of lettuce to high CO₂. The tolerance of plants to the environmental stress of chilling has been reported to change diurnally (9, 12, 14) and carbohydrate content may influence the response of the plant to this stress (14). Leaves have been shown to fluctuate diurnally in their carbohydrate composition (4). Lipton suggested that photosynthetic activity of lettuce prior to harvest may increase the carbohydrate reserves and thus lower the sensitivity of the plant to CO₂ (10). Sugar content of the leaves at harvest has been correlated with the storage life of crisphead lettuce (20). However, little is known about how carbohydrate concentrations vary diurnally in nonphotosynthetic leaves, such as those found inside a head of lettuce, and how such variations affect the response of leaves to elevated levels of CO₂ in storage. Increasing the tolerance of lettuce to high CO₂ would prevent injury caused by accidental build-up of CO₂ in tight transport vehicles and would allow the use of high CO₂ levels of C O₂ in storage. Increasing the tolerance of plants to the environmental stress of chilling has been reported to change diurnally (14). Leaves have been shown to fluctuate diurnally in their carbohydrate composition (4). Lipton suggested that photosynthetic activity of lettuce prior to harvest may increase the carbohydrate reserves and thus lower the sensitivity of the plant to CO₂ (10). Sugar content of the leaves at harvest has been correlated with the storage life of crisphead lettuce (20). However, little is known about how carbohydrate concentrations vary diurnally in nonphotosynthetic leaves, such as those found inside a head of lettuce, and how such variations affect the response of leaves to elevated levels of CO₂ in storage. Increasing the tolerance of lettuce to high CO₂ would prevent injury caused by accidental build-up of CO₂ in tight transport vehicles and would allow the use of high CO₂ levels of C O₂ in storage. Increasing the tolerance of plants to the environmental stress of chilling has been reported to change diurnally (14). Leaves have been shown to fluctuate diurnally in their carbohydrate composition (4). Lipton suggested that photosynthetic activity of lettuce prior to harvest may increase the carbohydrate reserves and thus lower the sensitivity of the plant to CO₂ (10). Sugar content of the leaves at harvest has been correlated with the storage life of crisphead lettuce (20). However, little is known about how carbohydrate concentrations vary diurnally in nonphotosynthetic leaves, such as those found inside a head of lettuce, and how such variations affect the response of leaves to elevated levels of CO₂ in storage. Increasing the tolerance of lettuce to high CO₂ would prevent injury caused by accidental build-up of CO₂ in tight transport vehicles and would allow the use of high CO₂ levels of C O₂ in storage. Increasing the tolerance of plants to the environmental stress of chilling has been reported to change diurnally (2, 19).

Materials and Methods

Crisphead lettuce (‘Salinas’) grown in the Salinas Valley of California was harvested at 0700 HR (AM) or 1400 HR (PM) PDT in four tests conducted between June and October. The cap leaf (leaf no. 1) and leaves 5, 10, 15, and 20 from five heads (heads average 24 ± 2 leaves > 25 mm long) at each harvest were separated from the head, dissected into blade and midrib portions, and frozen on dry ice immediately after harvest. The stem from the base of the cap leaf to the apex was also frozen. Frozen samples were lyophilized, ground, and held at room temperature in a desiccator for 3 weeks or less. Samples weighing 100 mg were extracted in 5 ml of 12 chloroform : 5 methanol : 3 water (by volume) for 30 min and the soluble extract was saved for sugar analysis. The residue was dried at 50°C, suspended in 10 ml of 100 mM acetate buffer (pH 4.8), and boiled for 10 min. Starch then was digested by adding 20 mg of amyloglucosidase (Sigma) to each sample and incubating at 55°C for 90 min (3). The supernatant was analyzed for glucose using glucose oxidase, peroxidase, and o-dianisidine (Sigma, Tech. Bul. 510). Corn starch was used as a standard.

Soluble sugars in the extracts were separated from the pigments and lipids by adding 3 ml of distilled water per 5 ml of extract. Samples were mixed, centrifuged (1100 × g, 5 min) and the water-alcohol phase was used for sugar analysis (6). Glucose, fructose, and sucrose were analyzed using high-performance liquid chromatography (HPLC) (5). Samples (50 μl) were injected into a 4.6 mm (i.d.) x 250 mm (length) column packed with 10 μm Lichrosorb NH₂. The mobile phase was HPLC-grade 80 acetonitrile : 20 water (v/v) at a flow rate of 1.5 ml·min⁻¹. Sugars were detected using refractive index and identified by comparison with chromatograms of known sugars.

For evaluation of sensitivity to CO₂, lettuce was harvested immediately after the AM and PM samples had been collected. It then was vacuum-cooled and transported to Fresno, Calif., where it was held overnight at 2.5°C. The following day, the lettuce was placed into 118-liter stainless steel chambers (18 heads/chamber) and held at 2.5°C for 12 days. Atmospheres within the chambers were maintained at 21% O₂ combined with either 0%, 7.5%, or 10% CO₂ by a continuous flow of humidified gas (600 ml·min⁻¹). After cold storage, nine heads from each treatment were rated for brown stain (8). The remaining lettuce was rated after being held for 3 days at 10°C in air. During this last examination, each leaf of three heads chosen randomly from the AM harvest held in 10% CO₂ was evaluated for brown stain to determine the location of this disorder within the head.

Received for publication 23 Feb. 1987. We thank Bruce Church, Inc. and Bud Antle, Inc., both of Salinas, Calif. for their cooperation in providing the lettuce used in these tests. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.
Each of the four tests was treated as a block and data were analyzed using a randomized complete block design. Mean separation was determined using Fisher's LSD at the 5% level.

**Results**

Starch, a major storage carbohydrate in most plants, was at low concentrations in most lettuce tissues (Fig. 1A). However, the blade of the cap leaf, a photosynthetically active tissue, contained 5 mg starch/g dry weight in PM-harvested lettuce.

The blade of the cap leaf also contained nearly 45 mg sucrose/g dry weight in the PM harvest, but only ≈7 mg·g⁻¹ dry weight in the AM harvest (Fig. 1B). The blade and midrib of leaf 20 and the stem of PM-harvested lettuce also contained significant amounts of sucrose. Only trace amounts of sucrose were detected in leaves 5, 10, and 15 of PM-harvested lettuce and in all leaves of AM-harvested lettuce.

Glucose and fructose were the major sugars found in the lettuce midribs and blades and comprised >70% of the sugars in all leaves (Fig. 1). AM-harvested lettuce contained 70% to 260% more glucose (Fig. 1C) and 20% to 120% more fructose (Fig. 1D) than PM-harvested lettuce. Concentrations of these sugars in midrib and blade tissues were similar except for leaf 20, where the midrib of AM-harvested samples contained more than the blade. Stem tissues from AM-harvested lettuce also contained more glucose and fructose than PM-harvested lettuce. Leaves at position 10 contained about 20% to 50% more glucose and fructose than the cap leaf. The highest concentrations, however, were found in the midribs of leaf 20 in AM-harvested lettuce.

Brown stain injury increased with increasing CO₂ concentration and during subsequent holding of the lettuce in air for 3 days (Fig. 2). AM-harvested lettuce was injured more readily by CO₂ than PM-harvested lettuce, but the difference was evident at both examinations for 10% CO₂ but only after aeration for 7.5%.

Brown stain occurred primarily on leaves 7 through 17, with those between leaves 10 and 15 being most severely affected (Fig. 3). Leaves 1 to 5 and 19 to 22 did not express and injury in the reported tests. A pinkish-bronzing of the meristem and youngest leaves (<25 mm in length) also was observed in all lettuce stored in high CO₂, but not in air controls.

**Discussion**

Starch accumulates during the day in photosynthetically active leaves and is depleted during the night (4). The cap leaf was the only leaf analyzed that received enough light to produce a net gain of starch through photosynthesis and, thus, demonstrates this pattern of starch accumulation during the day and depletion at night.
Sucrose, which is the translocatable carbohydrate in most plants, also increased in the cap leaf from the AM to the PM harvest. The presence of sucrose in the afternoon in the stem and young leaves indicates that assimilates are being actively translocated in the afternoon from the photosynthetically active wrapper and cap leaves to the actively growing leaves inside the head. In the early morning, however, low levels of sucrose would indicate that assimilate translocation into the head is minimal at this time.

Sucrose is converted to glucose and fructose after it reaches the head leaves. Lettuce harvested near sunrise contained more glucose and fructose than PM-harvested lettuce. Thus, these higher levels of reducing sugars are not a result of the accumulation of current photosynthates. Translocation of assimilates from photosynthetically active leaves is known to continue through the night (4) and may partially account for the greater concentration of sugars in the morning than in the afternoon. In addition, low temperatures during the night would reduce the rate of sugar metabolism and thus reduce the respiratory loss of these reducing sugars that may occur at warmer daytime temperatures. Low temperatures have been reported to alter sugar metabolism, causing an accumulation of sugar in a wide variety of plants (1).

The higher concentrations of reducing sugars found in leaf 10 than in the cap leaf are in agreement with results of Ito (7). Fouse and Lipton (5), however, reported the highest levels of sugars in outer head midribs (leaves 1 and 2). The latter calculated their results on a fresh-weight rather than a dry-weight basis, which may explain this difference.

PM-harvested lettuce tolerated high CO₂ in the storage atmosphere better than AM-harvested lettuce. High levels of sugars in leaf tissues seem to provide some resistance in injury from stresses such as freezing (15), chilling (14), and high salt (13). However, high reducing sugar concentrations in lettuce did not appear to increase tolerance of the tissue to CO₂. In fact, leaf tissues having the highest levels of glucose and fructose, those of leaf 10 and the morning harvest, also had the most brown stain.

Sucrose has been reported in certain cases to be more effective than monosaccharides in increasing the tolerance of plant tissues to various stresses (14, 16). In our study, higher concentrations of sucrose were found in the relatively tolerant PM-harvested lettuce than in the AM-harvested lots. However, these same leaves (leaves 1 and 20) in the morning-harvested lettuce also were not injured. If sucrose increases the tolerance of lettuce leaves to CO₂, it would need to be translocated into the sensitive leaves after harvest or have its effect indirectly.

Factors other than assimilate levels associated with the time of day of harvest may also influence the sensitivity of lettuce to brown stain. Exposure of lettuce leaves to light after harvest was reported to decrease the incidence of brown stain (17). Light that lettuce receives prior to harvest in the afternoon may decrease the sensitivity of lettuce to CO₂, but the mechanism by which light is exerting this influence is unknown.

The water potential of the tissue at the time of harvest is another diurnal change that may be altering the response of lettuce tissue to CO₂. Turgidity tends to be greatest just before dawn and decreases through midday. This difference in water potential due to harvest time would be amplified further by water lost during vacuum cooling.

Further work is needed before we are able to understand how these pre- and postharvest environmental factors influence the physiological response of lettuce to elevated levels of CO₂ during storage.

Literature Cited