Controlled-atmosphere Storage of Sweet Basil

Diana L. Lange¹ and Arthur C. Cameron

Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

Additional index words. carbon dioxide, oxygen, *Ocimum basilicum*, chilling injury

Abstract. The effect of controlled atmospheres (CA) on the development of injury symptoms and storage life of sweet basil (*Ocimum basilicum* L.) cuttings was assessed. Three-node basil stem cuttings were placed in micro-perforated low-density polyethylene packages and stored in the dark at 20 °C in a continuous stream of nitrogen containing the following percentages of O₂/CO₂: 21/0 (air), 21/5, 21/10, 21/15, 21/20, 21/25, 0.5/0, 0.5/5, 1/0, 1.5/0, 2/0, 1/5, 1.5/5, 1.5/7.5, and 1.5/10. Cuttings stored in an atmosphere of <1% O₂ developed dark, water-soaked lesions on young tissue after only 3 days. Fifteen percent or more CO₂ caused brown spotting on all tissue. Sweet basil stored in 1.5% O₂/0% CO₂ had an average shelf life of 45 days compared with 18 days for the air control. None of the CA combinations tested alleviated chilling injury symptoms induced by storage at 5 °C.

Sweet basil can be stored for an average of 12 d at 15 °C in air with acceptable visual quality (Lange and Cameron, 1994). However, marketing and storage of perishable commodities is more commonly conducted at 5 °C, which can cause chilling injury (CI) in basil. The shelf life of basil is only ≈7 d at 10 °C and 3 d at 5 °C (Lange and Cameron, 1994). Pre- and postharvest temperature conditioning at 10 °C before storage at 5 °C can reduce, but will not eliminate, CI (Lange and Cameron, 1997).

The use of atmospheres containing elevated levels of CO₂, reduced levels of O₂, or both, to retard senescence has been well documented for a variety of vegetables and herbs. Vegetables that benefit from controlled atmosphere (CA) storage include broccoli (*Brassica oleracea* var. *italica* Plenck) (Makhoul et al., 1989), Brussels sprouts (*B. oleracea* var. *gemmifera* DC.) (Lipton and Mackey, 1987), head lettuce (*Lactuca sativa* var. *capitata* L.) (Watada et al., 1964), romaine lettuce (*L. sativa* var. *longifolia* L.) (Aharoni and Ben-Yehoshua, 1973), and spinach (*Spinacia oleracea* L.) (Murata and Ueda, 1967). Moreover, CA storage has extended the storage life of herbs such as parsley (*Petroselinum crispum* L.) (Apeland, 1971; Hruschka and Wang, 1979; Nsengimana and Bangerth, 1981) and watercress (*Nasturtium officinale* R.Br.) (Hruschka

and Wang, 1979). Parsley stored at 0 °C in an atmosphere of 10% O₂ and 11% CO₂ remained dark green and salable for 4 weeks, while air-treated cuttings lasted only 2 weeks (Hruschka and Wang, 1979). Aharoni et al. (1990) reported that yellowing-susceptible herbs, such as watercress and chives (*Allium schoenoprasum* L.), were best stored at modified atmospheres of 5% to 8% O₂ plus 8% to 12% CO₂.

Injury from high CO₂, low O₂, or both has been reported for some horticultural crops. Aharoni and Ben-Yehoshua (1973) reported that 64% of romaine lettuce crowns were damaged (brown discoloration) by cold storage in 3% CO₂. Low O₂-injured Brussels sprouts held at 0.5% O₂ had reddish-tan discoloration and bitter flavor (Lipton and Mackey, 1987).

In some cases, low O₂ alleviated certain chilling injury symptoms such as internal breakdown of apples (*Malus ×domestica* Borkh.) at low temperatures (Kidd et al., 1927). Avocados [*Persea americana* (Mill.)] held 3 to 4 weeks at 7 °C in 2% O₂ plus 10% CO₂ showed only traces of CI, while those held in air were severely injured (Spalding and Reeder, 1975).

On the other hand, cucumbers (*Cucumis sativus* L.) stored at chilling temperatures in 3% to 75% CO₂ were more severely pitted than those held in air. The level of O₂ had no effect on chilling injury of cucumber, except at extreme levels (<1% or 100%), where it enhanced pitting (Eaks, 1956). Carbon dioxide at 5% to 20% increased the incidence of pitting and decay in bell peppers (*Capsicum annuum* var. *annuum* L.) compared with that of fruit stored in air (Cappellini et al., 1984).

The objectives of this research were to determine 1) injurious levels of O₂ and CO₂ for sweet basil cuttings stored at 20 °C, 2) whether storage in various O₂/CO₂ combinations can extend the storage life of basil, and 3) the effect of an optimized O₂/CO₂ combination on the prevention of CI damage on sweet basil stored at 5 °C.

Sweet basil seeds were sown in 527-mL plastic pots, filled with a commercial peat/perlite/vermiculite mix. The basil plants were grown at 24 °C day/20 °C night in a computer-controlled greenhouse from 3 Jan. to 7 Mar. Plants were fertilized daily with 150 ppm N using 20N–8.7P–16.6K from the first true-leaf stage to harvest. Natural day lengths were extended to 12-h photoperiods (0800 to 2000 HR) with high-pressure sodium lamps that supplied 150 to 200 mmol·m⁻²·s⁻¹.

Three-node, 4- or 6-week-old basil cuttings were harvested at 1600 HR and packaged individually in 800 cm², 3 mil (76 μm), low-density polyethylene film (LDF 550; Dow Chemical U.S.A., Midland, Mich.) packages sealed with a Magneta 620 heat sealer (Packaging Aids Corp., San Francisco). Each package had eight perforations of ≈0.4 mm diameter. Eight packages were placed into an 120 × 30-cm chamber made of double-layer, 3-mil (76 μm), low-density polyethylene film. The chamber was heat-sealed and inlet and outlet ports were made on either end. The ports were constructed of 10-cm-long, 0.64-cm-wide plastic tubing that was inserted through the double layer of film and sealed with silicone caulking. Perforations ensured that the concentrations of gases within the individual packages were always within 5% of the desired concentrations entering the chambers (data not shown). The internal atmospheres were measured by inserting a needle through silicone caulking on the chamber and on the individual package. In this manner, the injection hole was resealed by using silicone caulking.

Desired concentrations of CO₂ and O₂ were achieved by mixing air and CO₂ with N₂ carrier gas that was flowing at 12 L·h⁻¹. Nitrogen gas (for the gas mixtures) and air (for the control) were humidified by passage through five sealed 4-L jars filled with deionized water to increase the relative humidity (RH) of the flushing gas to >90% (Shirazi and Cameron, 1993).

Experiment 1. Four-week-old basil cuttings were stored at 20 °C and exposed to O₂ levels that ranged from 0.5% to 21% and CO₂ concentrations that ranged from 0% to 10%. The following O₂/CO₂ percentages were used: 21/0, 0.5/0, 1/0, 1.5/0, 1/5, 1.5/7.5, and 1.5/10. Several of the CA combinations were tested again on 6-week-old cuttings from the same greenhouse-grown basil crop in order to determine the optimum storage conditions (see Expt. #2).

Experiment 2. Six-week-old basil cuttings were stored at 20 °C and exposed to the following O₂/CO₂ percentages: 21/0, 1.5/0, 2/0, and 1.5/5. The O₂/CO₂ atmosphere of 1.5/0 was tested again to confirm its efficacy as an optimized CA atmosphere. In this second experiment, 2% O₂ was tested to determine if a slightly higher O₂ concentration would further extend the shelf life of basil beyond storage in 1.5% O₂. In addition, the CA combination of 1.5% O₂ and 5% CO₂ was tested to determine if the addition of CO₂ would further extend shelf life beyond that in 1.5% O₂ alone.

Experiment 3. Six-week-old basil cuttings

Received for publication 25 Mar. 1997. Accepted for publication 24 Oct. 1997. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by Michigan State Univ. and does not imply its approval over other products or vendors that may also be suitable. 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.

¹Current address: Postharvest Physiologist, Landec Corp., 3603 Haven Ave., Menlo Park, CA 94025-1010.

were stored at 20 °C and exposed to elevated CO₂ concentrations of 5%, 10%, 15%, 20%, and 25% in combination with 21% O₂ to evaluate the potential of high CO₂ concentrations alone.

Experiment 4. Six-week-old basil cuttings were stored at 5 °C and exposed to 1.5% O₂ and 5% CO₂ to determine if CA could prevent or reduce the incidence of CI.

Gas samples were removed twice daily from polyethylene chambers. One-milliliter samples from both the chambers and interior packages were injected into an infrared gas (CO₂) analyzer (ADC 225-MK3; Analytical Development Co., Hertfordshire, England) connected in series to an O₂ analyzer (Ametek S-3A/II with a calcia-zirconia electrochemical detection cell; Ametek, P&A Instrument Div., Pittsburgh).

Consecutive CA storage experiments were maintained in a dark, microprocessor-based storage chamber (20 °C, 75% RH) from 5 Feb. to 25 Mar. 1989. Basil stored under all treatments, including air, lost water ($\approx 1\%$ fresh mass) after storage durations of 2 weeks or more, probably because of the high flow rate (12 L·h⁻¹) through the polyethylene chambers.

The storage life of sweet basil cuttings was determined visually. A cutting was considered damaged or unsalable when the first symptom of high-CO₂ injury, low-O₂ injury, or mold was observed. The gas mixtures and controls were compared by using the LD₍₅₀₎ (the time in days at which 50% of the basil cuttings in a treatment were unsalable because of 2% to 5% surface area necrosis caused by injury or decay).

Results and Discussion

Experiments 1 and 2. Four-week-old basil cuttings stored in O₂/CO₂ atmospheres of 0.5/0, 1/0, or 1/5 at 20 °C in the dark showed symptoms of low-O₂ injury (Table 1), which appeared as dark, water-soaked lesions on only the youngest tissue, and were similar to CI symptoms observed by Lange and Cameron (1994). In all cases, shelf life was either limited by the development of surface molds and/or chlorosis of the older tissue, or by the development of O₂- and/or CO₂-injured tissue. Low-O₂ injury was observed within 3 d in the 0.5/0 treatment (Table 1). The average shelf life in 1/0 was 16 d vs. 19 d for the air-stored basil. Addition of 5% CO₂ to 1% O₂ appeared to accelerate the appearance of low-O₂ injury in 4-week-old cuttings, further reducing the average shelf life to 12 d. Storage in 1.5/0 did not cause low-O₂ injury and resulted in a 4- or 28-d extension of the average shelf life for 4- (Table 1) or 6-week-old cuttings (Table 2), respectively. The addition of 7.5% or 10% CO₂ to 1.5% O₂ had no positive effect on the average shelf life of 4-week-old cuttings, because of the incidence of high-CO₂ injury, which appeared as interveinal brown spotting on leaf tissue of all ages, and appeared at the same time as mold was observed in the air control (Table 1).

The best O₂/CO₂ treatments for CA storage of 6-week-old basil were either 1.5/0 or 2/0,

which resulted in average shelf lives of 45 and 44 d, respectively, as compared to 17.5 d for 6-week-old basil held in air (Table 2). The 1.5/5 treatment extended the average shelf life by about 77% (to 31 d) compared with the air control (17.5 d). However, the addition of 5% CO₂ to 1.5% O₂ reduced the average shelf life by about 32% compared to storage in 1.5/0. Lipton and Mackey (1987) found that the addition of 10% CO₂ further improved shelf life of Brussels sprouts compared with storage in 1% to 2% O₂ alone. Similarly, Makhoul et al. (1989) reported that high CO₂ atmospheres reduced chlorophyll loss in broccoli buds at 25 °C, but when used alone, low O₂ was more effective than high CO₂.

In this study, we found that elevated CO₂ levels were not beneficial and often reduced shelf life. This reduction was probably due to basil's sensitivity to CO₂ injury. We also found that low O₂ used alone was the most effective treatment in extending shelf life of sweet basil.

The disparity of results in Tables 1 and 2 probably is due to differences in tissue age. The data in Table 1 were generated using 4-week-old basil, and those in Table 2 from 6-week-old basil. We have found that more mature basil cuttings are less susceptible to low-O₂ injury (data not shown). This may be due to a more developed cuticle, which would better protect the leaf tissue from low-O₂ injury. The difference in shelf life of air control and 1.5% O₂-treated cuttings in these consecutive experiments may also be due to preharvest effects, such as varying amounts of disease pressure and/or RH in the greenhouse, or because 4-week-old basil cuttings are inherently more perishable than 6-week-old cuttings.

Experiment 3. Six-week-old basil cuttings stored in atmospheres of 21/10, 21/15, 21/20, or 21/25 showed symptoms typical of high-CO₂ injury. However, the average shelf life was reduced only by CO₂ concentrations of 15% to 25% (Fig. 1). In a separate experiment, storage of 6-week-old basil in 21/5 extended the average shelf life by 9 d over storage in air (data not presented) and never caused high-CO₂ injury. Controlled atmosphere storage

using 10% to 40% CO₂ was recommended for spinach (Murata and Ueda, 1967), whereas parsley and watercress are best stored at $\approx 10\%$ CO₂ (Hruschka and Wang, 1979). However, not all herb types will benefit from $>10\%$ atmospheric CO₂, as was shown with basil cuttings (Fig. 1).

Experiment 4. Storage of basil cuttings in a 1.5/5 atmosphere at 5 °C did not prevent CI (Fig. 2). Instead, the CA treatment increased CI symptoms relative to air storage. The incidence of CI in CA was about 25% greater than that in air on days 6 through 8 of 5 °C storage.

The positive effects of low O₂ and high CO₂ concentrations were difficult to separate by visual analysis, since both treatments resulted in better color retention and delayed the onset of decay. The quality of CA-stored basil at 20 °C was maintained best by storage in 1.5/0 [about a 160% increase in the average shelf life of 6-week-old cuttings compared with that of the air control (Table 2)], but storage in either 1.5/5 (Table 2) or 21/5 (data not presented) also extended the average shelf life by about 77% over the air control. Lipton and Mackey (1987) reported that exposure of Brussels sprouts to low O₂ prolonged the retention of green color but did not delay the development of decay at 5 to 7.5 °C. Alone, high CO₂ exposure reduced decay development with no effect on color retention. Controlled atmosphere storage (1.5/5) did not alleviate CI in basil stored at 5 °C (Fig. 2), but caused a delay in senescence at 20 °C (Table 2). Other commodities have responded negatively to CA storage at chilling temperatures. Carbon dioxide levels of 5% to 20% increased CI of bell peppers compared with that of peppers stored in air (Cappellini et al., 1984). Similarly, cucumbers had increased pitting when stored in 3% to 75% CO₂ at chilling temperatures, although levels of O₂ between 1% and 100% were not damaging (Eaks, 1956). In this study, the potential of low O₂ exposure alone to alleviate CI was not evaluated.

In CA experiments at 20 °C, the basil stored in air lasted an average of 17 d or longer, which was double the shelf life of basil in

Table 1. The effect of low O₂ and low O₂/high CO₂ on the average shelf life of 4-week-old packaged sweet basil cuttings (n = 8) stored at 20 °C in the dark (Expt. 1).

Treatment (%)		Avg shelf life (days) $\pm$ SD	Limiting factor
O ₂	CO ₂		
21.0	0.03	19.3 $\pm$ 2.1	Mold
0.5	0.00	2.8 $\pm$ 0.2	Low-O ₂ injury
1.0	0.00	16.3 $\pm$ 1.4	Low-O ₂ injury
1.5	0.00	23.0 $\pm$ 1.2	Mold
1.0	5.00	11.8 $\pm$ 0.9	Low-O ₂ injury
1.5	7.50	21.0 $\pm$ 0.6	High-CO ₂ injury
1.5	10.00	20.0 $\pm$ 0.3	High-CO ₂ injury

Table 2. The effect of O₂ and CO₂ concentrations on the average shelf life of 6-week-old packaged sweet basil cuttings (n = 8) stored at 20 °C in the dark (Expt. 2).

Treatment (%)		Avg shelf life (days) $\pm$ SD	Limiting factor
O ₂	CO ₂		
21.0	0.03	17.5 $\pm$ 1.8	Mold
1.5	0.00	45.3 $\pm$ 3.7	Mold
2.0	0.00	44.0 $\pm$ 3.1	Mold
1.5	5.00	31.0 $\pm$ 2.2	Mold

Fig. 1. Effect of high CO_2 in combination with 21% O_2 on the average shelf life of packaged 6-week-old sweet basil cuttings ($n = 8$) stored at 20 °C in the dark. Vertical bars represent standard deviation above and below the mean (Expt. 3).

Fig. 2. Effect of controlled atmosphere (CA) storage in 1.5% O_2 + 5% CO_2 (○) or air (21% O_2) (■) on the chilling injury response of 6-week-old sweet basil cuttings ($n = 8$) stored at 5 °C in the dark for up to 11 d. The effect of CA storage was not significant at $P \leq 0.05$, $cv = 61\%$ (Expt. 4).

earlier research (Lange and Cameron, 1994). This increase might be due to decreased RH in the packages. The RH of the packages was not measured in this study, but the high flow rate of humidified air in the chambers could have caused a slight drop in RH. In addition, constant ventilation of the chambers may have prevented ethylene accumulation that may have occurred in earlier studies (Lange and Cameron, 1994). Visual shelf life determination was used in this study and flavor quality was ig-

nored. The actual shelf life would probably be shorter because of loss of flavor and aroma.

Sweet basil would probably benefit from CA storage at 20 °C, especially if harvested after 6 weeks and exposed to 1.5% O_2 . A more practical approach may be to cool basil quickly to ≈ 15 °C to remove the field heat (Lange and Cameron, 1994). The next step would be for a grower/packinghouse to provide a short CA treatment of 1.5% O_2 (0% CO_2), followed by marketing. This handling should provide a

consistent extension of storage life of 2 or more weeks, which would be acceptable for highly perishable crops like herbs. Storage at 5 °C should be avoided because of the high incidence of CI.

A more economically feasible approach may be to utilize advances in modified atmosphere packaging (MAP). This may be achieved by using package flushes with optimal O_2/CO_2 atmospheres, or possibly by integrating O_2 scavengers and/or ethylene absorbers into the package.

Literature Cited

- Aharoni, N. and S. Ben-Yehoshua. 1973. Delaying deterioration of romaine lettuce by vacuum cooling and modified atmosphere produced in polyethylene packages. *J. Amer. Soc. Hort. Sci.* 98:464-468.
- Aharoni, N., A. Reuveni, and O. Dvir. 1990. Modified atmospheres in film packages delay senescence and decay of fresh herbs. *Acta Hort.* 258:255-262.
- Apeland, J. 1971. Factors affecting respiration and color during storage of parsley. *Acta Hort.* 20:43-52.
- Cappellini, M.C., P.A. Lachance, and D.E. Hudson. 1984. Effect of temperature and carbon dioxide atmospheres on the market quality of green bell peppers. *J. Food Qual.* 7:17-22.
- Eaks, I.L. 1956. Effect of modified atmospheres on cucumbers at chilling and non-chilling temperatures. *Proc. Amer. Soc. Hort. Sci.* 67:473-475.
- Hruschka, H.W. and C.Y. Wang. 1979. Storage and shelf life of packaged watercress, parsley and mint. *USDA Mkt. Res. Rpt.* 1102.
- Kidd, F.D., C. West, and M.N. West. 1927. Gas storage of fruit. *Dept. Sci. Indus. Res., London. Spec. Rpt.* 30.
- Lange, D.L. and A.C. Cameron. 1994. Postharvest shelf life of sweet basil (*Ocimum basilicum*). *HortScience* 29:102-103.
- Lange, D.L. and A.C. Cameron. 1997. Pre- and postharvest temperature conditioning of sweet basil. *HortScience* 32:114-116.
- Lipton, W.J. and B.E. Mackey. 1987. Physiological and quality responses of Brussels sprouts to storage in controlled atmospheres. *J. Amer. Soc. Hort. Sci.* 112:491-496.
- Makhlof, J., C. Willemot, J. Arul, F. Castaigne, and J.P. Emond. 1989. Regulation of ethylene biosynthesis in broccoli flower buds in controlled atmospheres. *J. Amer. Soc. Hort. Sci.* 114:955-958.
- Murata, T. and Y. Ueda. 1967. Studies on the CA-storage of spinach. *Jpn. Soc. Hort. Sci.* 36:449-454.
- Nsengimana, J. and F. Bangerth. 1981. Influence of different components of storage atmosphere on vitamin C content of parsley and corn salad. *Gartenbauwissenschaft* 46:84-87.
- Shirazi, A. and A.C. Cameron. 1993. Measuring transpiration rates of tomato and other detached fruit. *HortScience* 28:1035-1038.
- Spalding, D.H. and W.F. Reeder. 1975. Low-oxygen high-carbon dioxide controlled atmosphere storage for control of anthracnose and chilling injury of avocados. *Phytopathology* 65:458-461.
- Watada, A.E., L.L. Morris, and L. Rappaport. 1964. Modified atmosphere effects on lettuce, p. 83-85. In: *Proc. Fruit and Vegetable Perishables Handling Conf.*, Davis, Calif.