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Postharvest Storage of Sweet Basil

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Diana L. Dostal

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POSTHARVEST STORAGE
OF SWEET BASIL

By

Diana L. Dostal

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ABSTRACT

POSTHARVEST STORAGE OF SWEET BASIL

By

Diana L. Dostal

The optimal storage temperature for packaged sweet basil [*Ocimum basilicum* (L.)] cuttings was 15C. Dark, moist lesions associated with chilling injury were observed at 0, 5, and 10C. Harvesting later in the day or evening extended the storage life of sweet basil stored at 5 to 25C.

Chilling injury was alleviated by using pre- and postharvest temperature conditioning at 10C. Daily 4-hour pulses of 10C for only 2 days prior to harvest delayed chilling injury for 3 days when stored at 5C. Postharvest conditioning at 10C for 1 day in the dark caused a 100% extension in the storage life of sweet basil at 5C.

Controlled atmosphere storage at 20C of packaged sweet basil cuttings in 1.5% O_2 and 0% CO_2 caused a 260% increase in storage life compared to packaged basil cuttings stored in air. Chilling injury of sweet basil was not alleviated by the use of CA storage at 5C.

To my parents and fiance;
and in memory
of my brother, Danny

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CHAPTER 1

POSTHARVEST SHELF LIFE OF SWEET BASIL AS INFLUENCED BY TEMPERATURE

Abstract. Freshly-harvested greenhouse-grown sweet basil [*Ocimum basilicum* (L).] placed into 800 cm^2 perforated packages lasted an average of 1, 3, 8, 12, 7, and 7 d at 0, 5, 10, 15, 20, and 25C, respectively. Mold growth and chilling injury were the primary factors limiting shelf life of sweet basil. Dark, moist lesions associated with chilling injury were observed at 0, 5, and 10C. In a second experiment, greenhouse-grown basil harvested at 1800 or 2200 HR (10 or 14 hr after the beginning of the light period) and held at 15C had an average shelf life of 17 or 15 d, respectively. In comparison, basil harvested at 0200 or 0600 HR (6 or 10 hr after the beginning of the dark period) had an average shelf life of 11 or 10 d, respectively. Shelf life was not extended at the 1800 or 2200 HR harvest times when greenhouse-grown basil was stored at 0 or 5C. However, in a subsequent study at 5C, greenhouse-grown basil acclimated to growth chamber conditions (constant 25C and 200 μmol s^{-1} m^{-2}) for 1 week prior to harvest and harvested at 1800 HR lasted significantly longer than basil harvested at 0600 HR. Three-day exposures to 7.5 or 10C did not cause chilling injury of sweet basil, although 5C and below for 3 d was harmful. High humidity storage of basil plants or cuttings in perforated packages at 5C resulted in 3 d of storage life, as compared to just 1 d of storage life for unpackaged plants.

Introduction

The fresh herb market is growing and has tremendous potential for further expansion considering modern cooking trends and the emphasis on fresh foods. Fresh herb production has expanded to meet this increased demand, but the quality of herbs at the retail market is often unacceptable. Growers must produce a high quality product and then prevent the loss of that quality during shipping, handling and marketing. A current problem is that growers, distributors, and retailers have limited information on the postharvest storage and handling practices for fresh herbs (Joyce et al., 1986; Morris, 1982). Postharvest storage studies such as the determination of the optimum storage temperatures or expected shelf lives for fresh herbs still need to be conducted.

Good temperature control is considered to be the most important factor in postharvest management (Hardenburg et al., 1986). In general, the storage life of a horticultural commodity increases as temperature decreases down to freezing. Therefore, a storage temperature near 0C is optimal for storage of chilling injury-resistant species. Hardenburg et al. (1986) recommend storage at 0C and 95 to 100% RH for parsley and watercress. Packaging is usually used to maintain such a high humidity.

One of the most highly consumed fresh herbs, sweet basil, is sensitive to chilling injury. Saltveit (1990) reported that basil is chilling-sensitive but no specific chilling temperature was given. Joyce et al. (1986) reported that storage at 5 to 7C is optimal for long-term basil storage. Chilling injury of basil appeared as darkened, moistened, pitted lesions on the youngest leaf tissue. In extreme cases, all the tissue turned black.

Both temperature and duration of exposure are involved in chilling injury. Chilling injury symptoms may appear in a short time if temperatures are considerably below the critical temperature, but a product may be able to withstand temperatures a few degrees below the critical temperature for a longer time. Therefore, additional research is necessary to determine the specific critical temperature and time combinations that will cause chilling injury of sweet basil.

Retailers often hold sweet basil at room temperature (20 to 25C) to avoid chilling injury symptoms. However, these high temperatures can potentially cause desiccation or enhance the growth of decay organisms which are detrimental to the quality of the produce. No shelf life data for sweet basil at room temperature (20 to 25C) has been reported.

Several studies have been directed toward treatments designed to alleviate the symptoms resulting from chilling injury at 5C (Lyons, 1973). King et al. (1982) found that chilling tomato seedlings at the end of the day, rather than at the beginning of the day, reduced chilling injury in tomato seedlings. Presently, the effect of harvest time during the day on chilling injury of leafy green tissue is not available in the literature.

Wilson (1976) reported that chilling injury on green bean plants can be prevented for 7 to 10 days simply by enclosing the plants in polyethylene bags. The symptoms of chilling injury on green bean plants are wilting and pitting of leaf tissue which are also symptoms of water loss. Most likely, the chilling injury symptoms on the bean plants were alleviated due to the increased relative humidity inside of the package.

In this research, the technique of packaging was used to control water loss from the cuttings. Packaging might also alleviate chilling injury symptoms on basil if the problem is related to water loss.

The objectives of this research were to characterize: 1) the shelf life response of basil cuttings stored at a wide range of temperatures, 2) the chilling injury response of basil stored at low temperatures for short exposures, and 3) the effect of chilling at different times within the day-night cycle.

Materials and Methods

Sweet basil [*Ocimum basilicum* (L).] seeds (Mountain Sterling Farms, Knoxville, Tenn.) were sown in 527 ml plastic azalea pots. A commercially available peat-based soilless mix was used in all experiments. (Baccto Professional Growers Mix, Michigan Peat, Houston, Tex.). Two seeds were placed in each of 3 holes, and the seedlings were thinned to 3 plants per pot after 2 weeks. This method of sowing and thinning produced more biomass at harvest than 1 or 2 plants per pot (data not shown).

Plants were grown in a computer-controlled greenhouse under 24/20C day/night temperatures. On sunny days, the greenhouse temperature sometimes increased to 30C. In all experiments, plants received at least 12 hours of light (either naturally or from high pressure-sodium lamps which supplied 175 to 225 μmol s^{-1} m^{-2}) from 10 September 1988 through the winter. The basil plants were fertilized daily with 150 ppm N using a 20-20-20 fertilizer. Basil stems were harvested after 6 weeks by cutting below the third node from the apical end. Each cutting had 6 to 8 leaves and weighed 3 to 4 grams. Unless otherwise stated, basil cuttings were harvested at 1400 HR on sunny days. The cuttings were held in unsealed plastic bags from harvesting through packaging (less than 30 min). Two cuttings (free from visual defects) were sealed in an 800 cm^2, 3 mil low-density polyethylene (LDF 550, Dow Chemical Co.) film package using a Magneta heat sealer-620 series.

Four $26^{1/2}$-gauge needle holes were punched through each package as recommended by Joyce et al. (1986). One ml-samples from perforated packages were injected into a Type 225 infrared CO_2 gas analyzer (Analytical Development Co. Limited, Hoddesdon, England) connected in series to an Ametek S-3A O_2 analyzer (Pittsburgh, Pa.). The N_2 flow rate passing through the analyzers was 150 ml/min. The average O_2 and CO_2 concentrations in the perforated packages were 19% and 0.30%, respectively (data not shown). These data were collected on 10 perforated packages after 1 week of storage at 20C. Harvesting took approximately 30 min and packaging, 60 min. Basil packages were held in the dark under black plastic for the duration of all experiments.

Analysis of variance was performed on most experiments using SAS statistical software (SAS Institute Inc., Cary, NC). Means of shelf life were compared using Duncan's multiple range test (α = 0.05).

Experiment 1. Four packages of basil were placed in each of the following controlled temperature chambers: 0, 5, 10, 15, 20, and 25C. In all chambers, the temperature was maintained at +/- 1C of the setpoint temperature. Basil was inspected daily at 1600 HR and visually rated for necrosis, surface molds, water-soaked lesions and leaf abscission. Shelf life was defined as the time at which the first sign of deterioration was noticed. The experiment was repeated 6 times between 12 July and 29 August 1988. The data were analyzed as a randomized complete block design with 6 blocks over time and 4 sample packages per treatment per block.

Experiment 2. Basil cuttings were harvested every 4 hr beginning at 0200 HR and ending at 2200 HR. After each harvest, the cuttings were handled and packaged as described above, and 4 packages were immediately placed at each of the following controlled temperatures: 0, 5, 10, 15, 20, and 25C. Observations, as described above, were made daily at 1600 HR. The experiment was conducted 3 times between 17 August and 3 October 1988. The data were analyzed as a randomized complete block design (blocked over time) in a factorial arrangement of 6 daily harvest times x 6 temperatures. There were 4 sample packages per treatment combination.

A related study was conducted to re-evaluate chilling injury of sweet basil cuttings harvested at 0600 and 1800 HR and stored at 5C. Plants were greenhouse-grown for 5 weeks as described above (1 January to Feb 8 1990), and then were moved to 2 growth chambers for 1 additional week prior to harvest. The growth chambers were both programmed for constant 25C, 75% RH. Fluorescent lamps supplied 12 hr-photoperiods from either 0800 to 2000 HR or from 2000 to 0800 HR at light intensities of 175 to 225 μmol s^{-1} m^{-2}. Daily fertilization with 150 ppm N using a 20-20-20 fertilizer occurred at 1000 HR. Basil plants were harvested at 1800 HR. By manipulating the photoperiods as described above, the beginning-of-day and end-of-day harvests were harvested simultaneously. The packaging procedure was conducted as described previously (except only 1 cutting was placed in each package), and the packages were evaluated daily at 1800 HR and visually rated based on the appearance of chilling injury in the form of darkened, water-soaked lesions. Cuttings with 2 to 5% or more damaged tissue were determined to be damaged by chilling injury. The experiment was repeated twice between 15 February and 1 March 1990 using 5 and 6 week-old basil.

The 2 runs of the experiment were combined and the data were analyzed as a randomized complete block design (blocked over age) in a one-way analysis of variance with 25 replications per harvest time treatment.

Experiment 3. Six week-old, greenhouse-grown plants (15 October to 10 November 1988) were harvested at 1600 HR and packaged as described in the last experiment. The packages were then stored in dark controlled temperature chambers programmed at temperatures of 0, 2.5, 5, 7.5, and 10C (with 75% RH) and durations of 0, 1, 2, and 3 days. Four packages per treatment combination were removed at 1600 HR after 1 through 3 days and were held in a 20C, 75% RH, dark controlled-temperature chamber. The packages were visually rated after 24 hr at 20C. The rating scale included 5 (no damage), 4 (up to 5% damage), 3 (6 to 10% damage), 2 (11 to 25% damage), and 1 (>25% damage). Basil cuttings with chilling injury were defined as those cuttings which had 1 to 5% of the tissue covered with darkened, water-soaked lesions (a rating scale 4 or less).

The experiment was conducted with 6 week-old plants between 7 November and 12 November 1988 and was analyzed as a completely randomized design in a two-way analysis of variance (temperature X duration of exposure).

In a subsequent study, storage at 5C was more closely evaluated over durations of 0 to 5 days. Basil plants were greenhouse-grown for 5 weeks (1 February to 7 March 1990) and growth chamber-grown (25C, 75% RH, 0800 to 2000 HR photoperiod) for the following week. (See greenhouse and growth chamber production methods above). Daily fertilization with 150 ppm N using a 20-20-20 fertilizer occurred for all 6 weeks. Sixty cuttings were harvested at 1800 HR and were packaged as usual. Fifty of the 60 packages plus 50 of 60 plants were placed into a dark growth chamber programmed at 5C. The other 10 packages and plants were controls. After 1, 2, 3, 4, and 5 days, 10 packages and plants were removed from 5C storage and were placed in a dark 25C and 75% RH growth chamber. Twenty-four hours later, the packages and plants were visually evaluated and the percentage of basil cuttings with chilling injury was determined as described in the first part of this experiment. This experiment was conducted only once from 7 March to 11 March 1990. The percentage of damaged basil was calculated at each duration and the data were plotted versus time.

Experiment 4. The effect of high humidity from packaging materials used in this research was determined by evaluation of chilling injury symptoms after a typical storage experiment. Basil that was grown for 5 weeks in the greenhouse and for 1 week in a growth chamber (see previous experiment methods and dates) was used in this experiment. Packaged cuttings, packaged plants, and unpackaged plants were prepared or selected for 5C storage at 1800 HR. The cuttings and plants were packaged as described above, with the exception that the plants were in 2150 cm^2 packages with 8 perforations. The unpackaged plants, packaged plants, and packaged cuttings were stored in 5C storage for 0, 1, 3, or 5 days. At each duration, 12 of each type of plant plus 12 cuttings were removed and placed in a dark 25C chamber. Visual evaluation and the determination of the percentage of basil cuttings with chilling injury occurred 24 hr later. (See evaluation procedure above). This experiment was conducted only once between 7 March and 13 March 1990. The % of basil cuttings with chilling injury was plotted versus time for each treatment.

Results

The primary types of chilling injury observed on sweet basil were darkened, water-soaked lesions on the youngest tissue and moldy, chlorotic damage on the oldest leaves. Chilling injury at 0, 5 and 10C appeared as necrotic lesions on the terminal growth, whereas at 20 and 25C, mold and chlorosis limited storage life. Basil stored at 10C usually received minor chilling injury symptoms followed by mold growth.

Experiment 1. The average shelf life of packaged basil held at 15C was 12.5 days, which was a significantly longer shelf life ($P = 0.0001$) than was found at the other temperatures (Table 1). Basil stored at 0C had the shortest average shelf life of 1.6 days. Non-linear regression analysis was performed on the raw data (using PlotIt statistical software, E. Lansing, Mich.) over the range of six temperatures (significant to the $P = 0.0001$ level)(Fig. 1). The function curve shows the rise to maximum shelf life at 15C with a decrease in longevity at lower and higher temperatures. At 5C, 75% of the basil packages were unacceptable after just 3 days due to the appearance of chilling injury symptoms, and the remainder of the packages were unacceptable after 6 days. Ninety-two percent of the basil packages stored at 15C were acceptable through 18 days of storage.

The shelf life of sweet basil stored at 15C was the most variable, as was represented by the largest standard deviation value of 5.4 days (Table 1). The average shelf life of basil stored at 0 and 5C was the least variable with standard deviation values of 0.6 and 1.2 days, respectively.

Experiment 2. The effect of storage temperature in this experiment was significant ($P = 0.0001$), with basil stored at 15C again having the longest shelf life (Fig. 2). The effect of harvest time during the day was significant ($P = 0.0001$), but was not consistent over temperatures ranging from 0 to 25C (interaction was significant at $P = 0.0001$).

Basil stored at 15 and 20C and harvested at 1800 HR lasted approximately 6 d longer than basil harvested at 0200 or 0600 HR. The effect of harvest time during the day was similar for basil stored at 10 and 25C, but to a lesser extent than when stored at 15 or 20C. The effect of harvest time during the day on greenhouse-grown basil was found to be non-significant at 0 or 5C.

In a subsequent study using basil plants preharvest acclimated at constant 25C for 1 week, harvesting sweet basil at 1800 HR as opposed to 0600 HR significantly increased ($P = 0.0001$) the average shelf life of cuttings from 3 to 6 days. The difference in the percentage of basil damaged by chilling injury was not evident until after the third day of 5C storage (Fig. 3). On the sixth day, 100% of the basil harvested at 0600 HR was damaged compared to 50% damage on the basil cuttings harvested at 1800 HR. The 6 week-old basil was significantly more chilling-tolerant ($P = 0.0019$) than the 5 week-old basil (data not shown), but the same effect of harvest time during the day was observed in both runs, therefore the data were merged.

The coefficient of variability (cv), defined as the ratio of the population variability to the mean, was 54%. This high value for cv was a result of variable responses of sweet basil harvested at a similar time to 5C storage.

Experiment 3. No chilling injury was evident after basil cuttings were exposed to 7.5 and 10C for 3 days (Fig. 4). In this experiment, basil stored at 5C for 2 days or less were only slightly damaged (an average visual rating of 4.5), but 3 days of 5C resulted in basil that was 50% damaged by chilling injury (an average visual rating scale of 3). The storage of basil at 2.5C for one day resulted in 25% chilling injury damage, and increasing the duration, increased the chilling injury losses to 50% or higher. Basil stored at 0C was 100% damaged after only 1 day. In all cases, the basil cuttings were held at 20C for 1 day prior to evaluation of chilling injury symptoms.

Seventy percent of the basil cuttings stored at 5C for 3 days were damaged by chilling injury, whereas only 1 or 2 days of 5C storage resulted in 20% or less chilling injury losses (Fig. 5). These results were similar to the previous experiment in which 3 days of storage caused 50% chilling injury losses and less exposure was only slightly damaging (10% losses or less).

As the exposure temperature was decreased, the symptoms of chilling injury on sweet basil cuttings significantly increased ($P = 0.0001$)(Fig. 4). Similarly, increasing the duration of exposure at any chilling temperature significantly increased ($P = 0.0001$) the occurrence of chilling injury on basil.

Experiment 4. The use of packaging (with either plants or cuttings) reduced 70% or more of the chilling injury damage observed on unpackaged plants up through the third day of 5C storage (Fig. 6). After 1 day of 5C storage, all of the unpackaged plants were damaged. After 5 days of 5C exposure, at least 40% of the packaged cuttings and packaged plants showed chilling injury symptoms.

Discussion

Storage at 15C provided the longest shelf life for sweet basil in each of the first two experiments (Table 1, Fig. 2). This result contradicts those reported by Joyce et al. (1986) which stated that 5C was the best storage temperature for perforated polyethylene-packaged sweet basil stored for durations of one and two weeks. Joyce et al. (1986) also reported that 15C storage caused an unacceptable visual rating after only one week, whereas in this research, 15C was the best storage temperature with some acceptable packages remaining after 30 days. These contrasting results could be due to different sizes of perforations or different thicknesses of film in the packages. The average shelf life of sweet basil at 15C was 12.5 days with a corresponding standard deviation of 5.4 days. The shelf life of sweet basil cuttings stored at 15C ranged from 5 to 30 days. Storage at 15C could be the best solution for a highly perishable crop such as sweet basil if the shelf life was consistently 1 week or more.

Storage temperatures of 0 and 5C were found to be unsuitable for long-term storage of harvested sweet basil (Fig 1., Fig. 2). Storage of basil plants at 0C for as little as 0.5 hours, caused 25% chilling injury damage, whereas 1 hour was enough to cause >50% damage (data not shown). The symptoms were similar to chilling injury of basil reported by Joyce et al. (1986) and included discoloration, wilting, water-soaking, and decay as has been observed on other leafy tissues (Morris, 1982; Wang, 1982; King et al., 1982). Temperature and time-related studies revealed that the commercial refrigeration temperature (5C) for 3 days is enough to cause severe chilling injury damage of 50% or more (Fig. 5). This result was in agreement with similar results from Expt. 1 and 2 (Table 1, Fig. 1, Fig. 4).

Basil cuttings held at temperatures of 7.5 and 10C for durations of 7 days or more were damaged by chilling injury (Table 1, data not presented from Expt. 3). However, short-term storage (3 days or less) at 10C would be acceptable (Fig. 4).

Techniques for the alleviation of chilling injury are very important since basil, a minor crop, is likely to be stored with major crops at 5C or lower in the distribution or marketing channels. King et al. (1982), reported that potted tomato seedlings chilled at 2C beginning at 0700 HR were killed within 3 days, as opposed to seedlings chilled at 2200 HR, which were killed after 6 days. At temperatures of 15 and 20C, basil harvested at the 1800 and 2200 HR harvest times lasted the longest. In contrast, the initial results from 5C storage indicated that harvest time had no significant effect on reduction of chilling injury symptoms on basil cuttings. However, when part of this experiment was repeated in controlled-temperature chambers, the basil cuttings harvested at 1800 HR, as opposed to 0600 HR, lasted an average of 2 days longer at 5C. In the first 5C storage study, the day/night temperature of the greenhouse fluctuated which may have interfered with the results. Further research would be useful to try to clarify the harvest time effect on chilling injury of basil.

The advantage of harvesting at the end of the day, rather than in the morning, might be due to the cyclic depletion of endogenous carbohydrates in the early morning hours (King et al., 1982; King et al., 1988).

In all of these chilling injury studies, packaging was used to maintain high humidity in order to control water loss. In addition, packaging resulted in a delay of chilling injury symptoms (Fig. 6). Wilson (1976) reported that chilling injury symptoms on green bean plants were delayed as much as 7 to 10 days by using packaging techniques. Packaging of sweet basil in polymeric film will control water loss after harvest, as well as reduce chilling injury symptoms. This reduction may be due to the alleviation of symptoms related to water loss (pitting and desiccation). Postharvest storage of packaged commodities is also easy to facilitate.

The optimal temperature for long-term storage of packaged basil was found to be 15C. Temperatures from 20 to 25C actually resulted in longer shelf life than 0 and 5C so retailers should be encouraged to store basil at slightly warmer rather than slightly cooler temperatures. If these higher storage temperatures are used, harvesting later in the day or evening would be beneficial. Attention should be given to the length of storage if a chilling temperature such as 10C is chosen. Packaging, or other means of maintaining high humidity, should be integrated into the temperature management of chilling-sensitive crops.

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Table 1. The average shelf life of packaged sweet basil cuttings as influenced by temperature. The cuttings were harvested at 1600 HR and were stored in the dark. Each mean +/- s.d. was based on the observation of 24 packages.

Temperature (C)	Average Shelf Life (days)	S. D. (days)
0	1.6	0.6
5	3.2	1.2
10	8.3	2.7
15	12.5	5.4
20	7.3	3.0
25	6.8	2.4

Figure 1. The effect of temperature on the shelf life of packaged sweet basil cuttings harvested at 1600 HR and stored in the dark. Non-linear regression and confidence belt lines (significant at P = 0.0001) represent 24 data points per temperature.

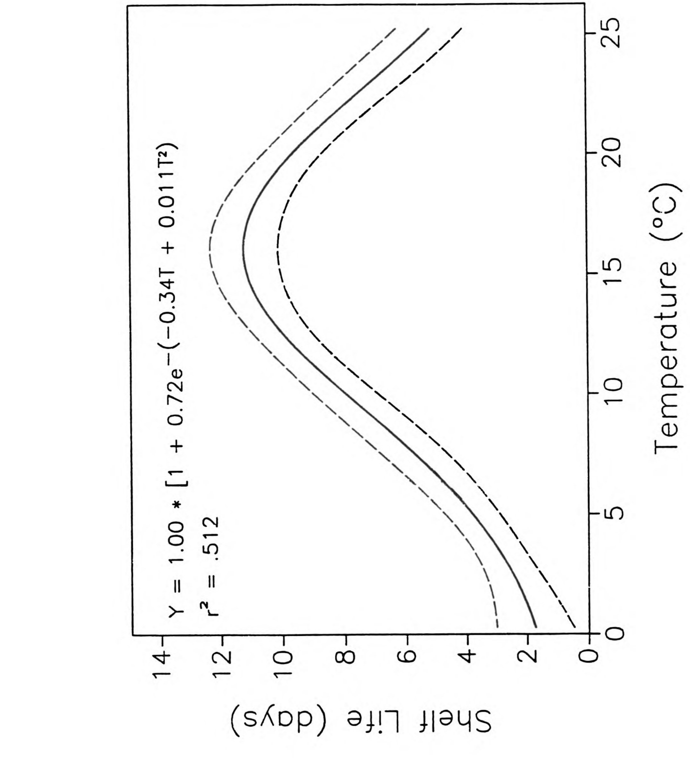

Y = 1.00 * [1 + 0.72e−(−0.34T + 0.011T²)
r² = .512
Shelf Life (days)
Temperature (°C)
0
2
4
6
8
10
12
14
0
5
10
15
20
25

Figure 2. The effect of daily harvest time on the average shelf life of packaged sweet basil cuttings stored at temperatures of 0 to 25C in the dark. Each bar represents the mean of 8 packaged cuttings. Means were separated using Duncan's multiple range test at $\alpha = 0.05$.

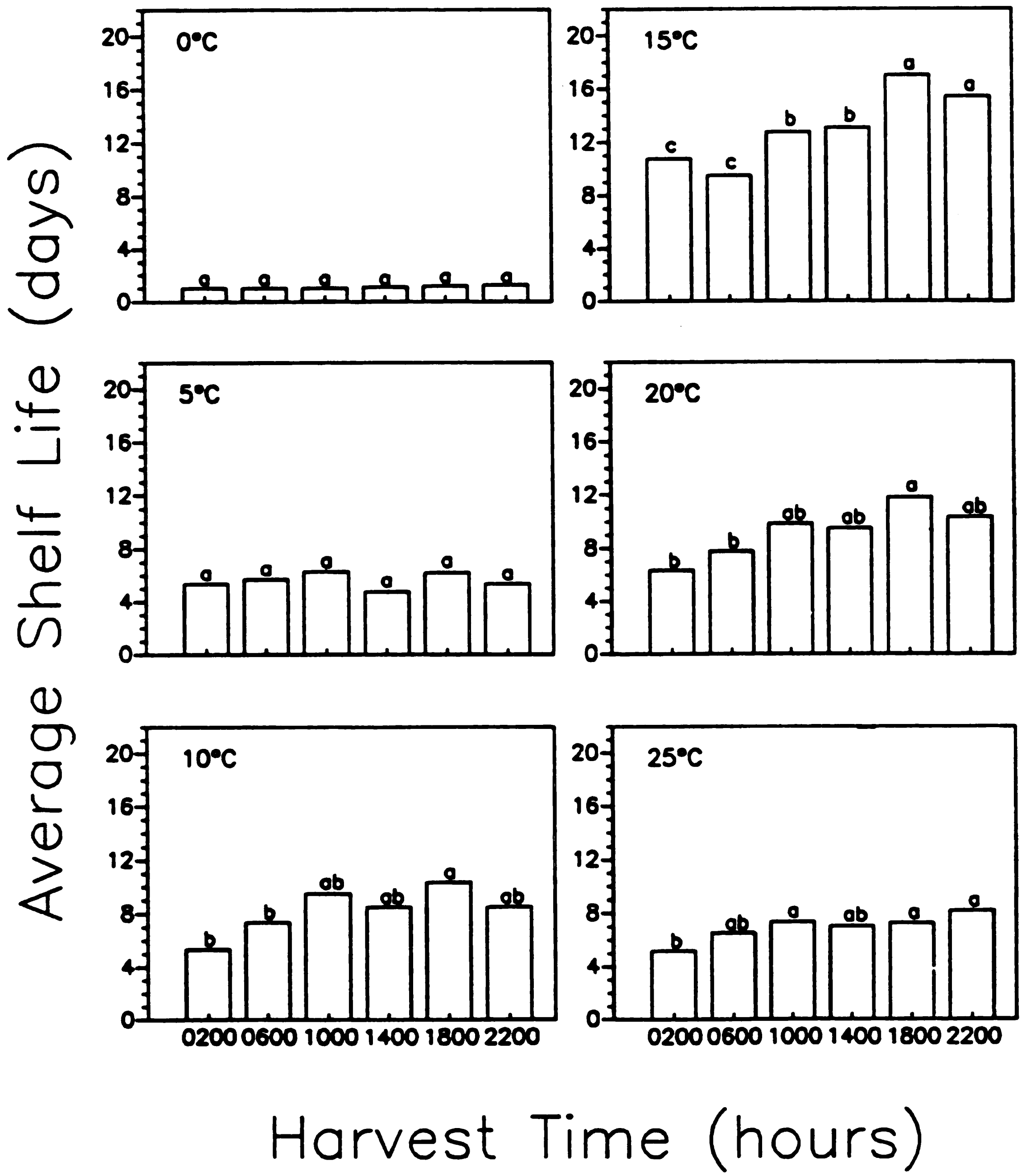

Average Shelf Life (days)
0°C
15°C
5°C
20°C
10°C
25°C
0200 0600 1000 1400 1800 2200
Harvest Time (hours)

Figure 3. The effect of 0600 and 1800 HR harvest times on the chilling injury response of packaged sweet basil cuttings stored at 5C in the dark. Each line represents 50 packaged cuttings from 2 consecutive runs. The average shelf life of basil harvested at 1800 HR was significantly longer than at 0600 HR. (Duncan's multiple range test at $\alpha = 0.05$.)

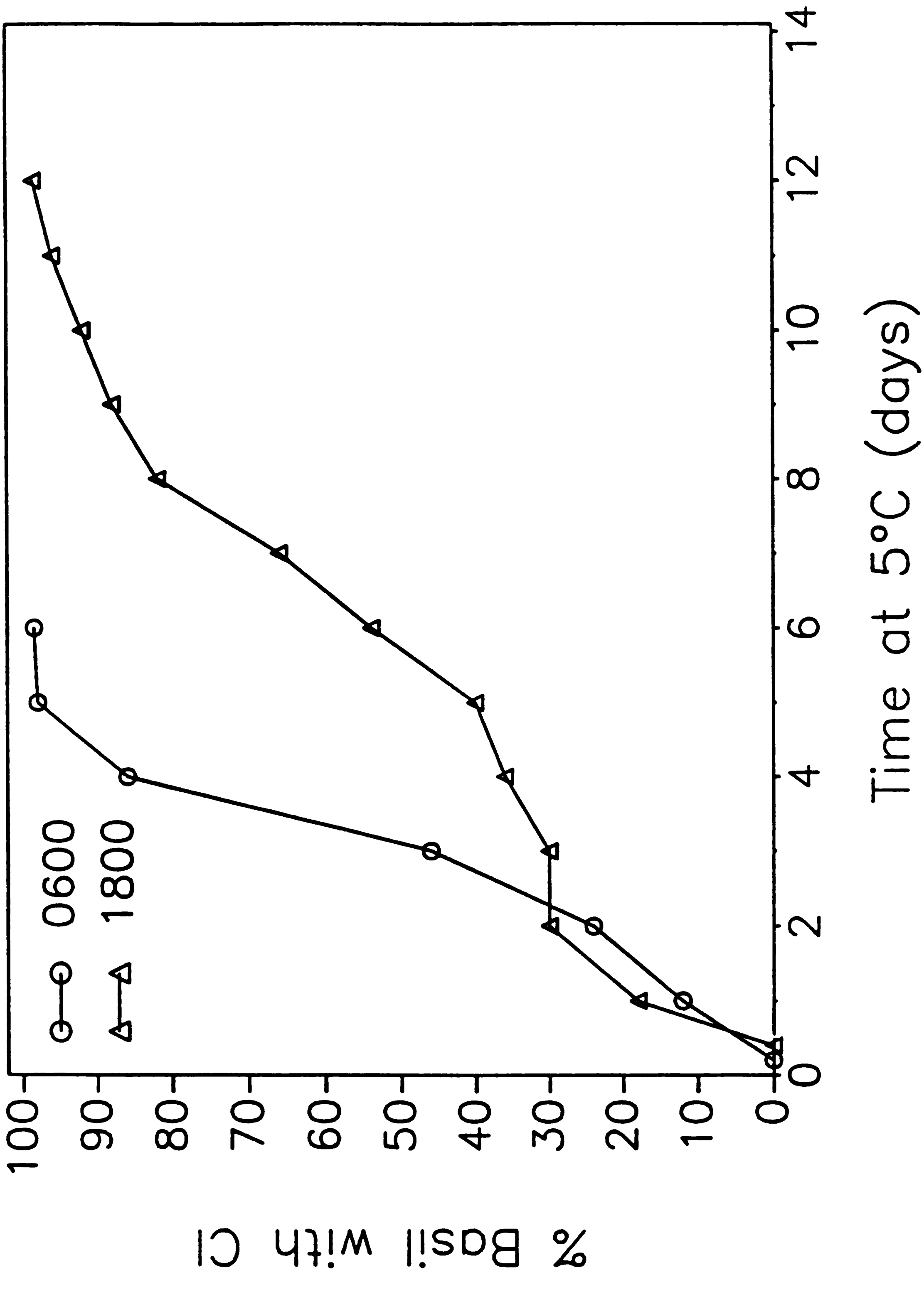
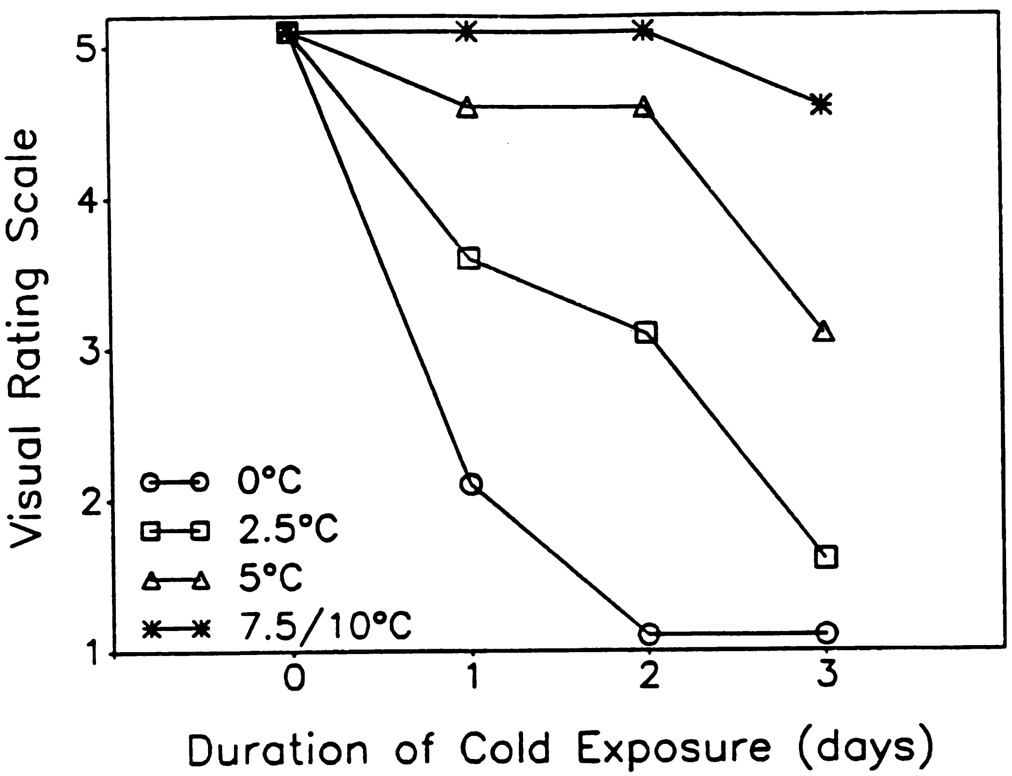
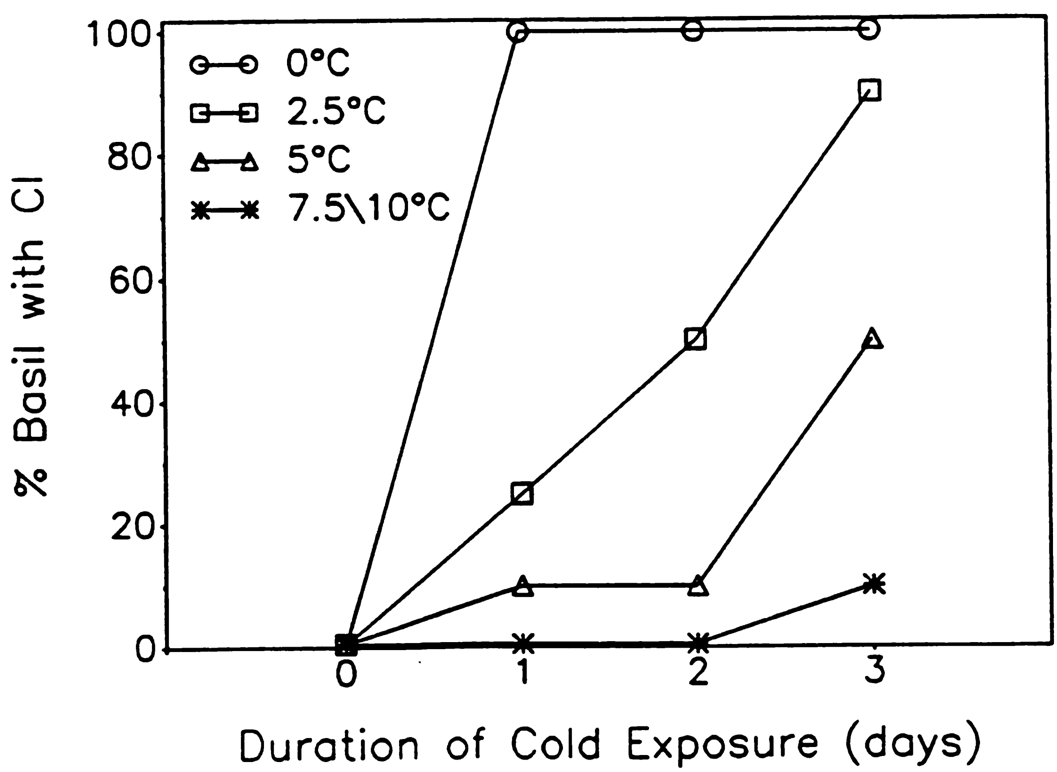

0600
1800
Time at 5°C (days)
% Basil with CI
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60
70
80
90
100

Figure 4. The effect of low temperatures for short durations on the chilling injury response of packaged sweet basil cuttings stored in the dark. A visual rating scale was derived with 5 = 0-2% damage, 4 = 2-5%, 3 = 5-10%, 2 = 10-25%, and 1 = >25% (top). The % of basil with chilling injury was determined by counting the number of cuttings with an injury rating of 4 or less (bottom). One point represents the mean of 4 packaged cuttings.



5
4
3
2
1
Visual Rating Scale
0°C
2.5°C
5°C
7.5/10°C
0
1
2
3
Duration of Cold Exposure (days)



100
80
60
40
20
0
% Basil with CI
0°C
2.5°C
5°C
7.5\10°C
0
1
2
3
Duration of Cold Exposure (days)

Figure 5. The effect of dark storage at 5C for short durations on the chilling injury response of packaged sweet basil cuttings. Each bar represents 10 packaged cuttings.

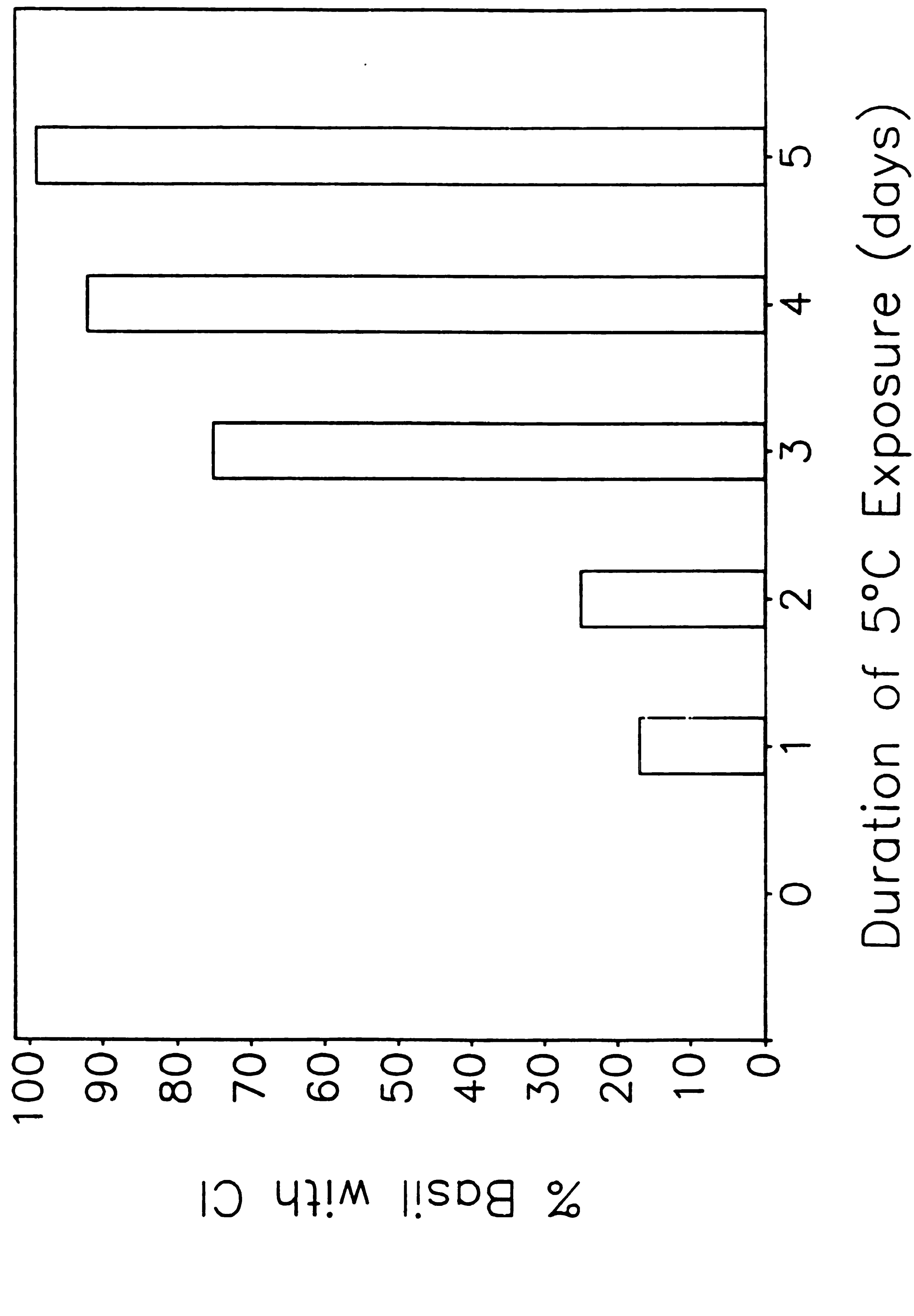
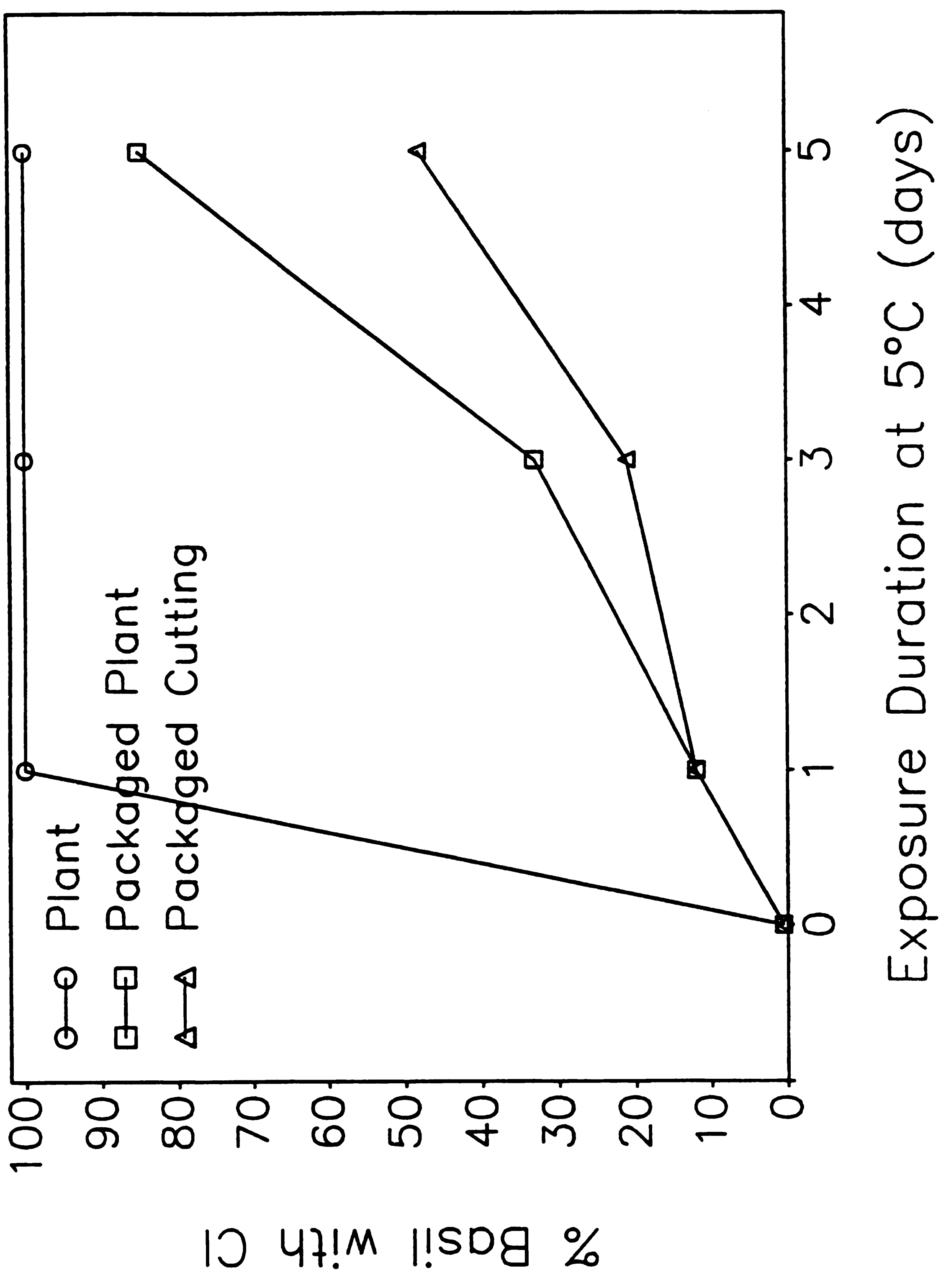

% Basil with CI
Duration of 5°C Exposure (days)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5

Figure 6. The effect of packaging on the chilling injury response of sweet basil plants and cuttings. Data were based on the observation of 24 plants or cuttings merged from 2 consecutive runs.


Plant
Packaged Plant
Packaged Cutting
% Basil with CI
Exposure Duration at 5°C (days)
0 10 20 30 40 50 60 70 80 90 100
0 1 2 3 4 5

CHAPTER 2

PRE- AND POST HARVEST TEMPERATURE CONDITIONING OF SWEET BASIL

Abstract. Postharvest shelf life of fresh sweet basil [*Ocimum basilicum* (L.)] at 5C was only 3 to 4 d due to chilling injury. Plants which were hardened at 10C for 4 hr daily (2 hr at the end of the light period and 2 hr at the beginning of the dark period) for 2 d prior to harvest had 3 d of extended postharvest shelf life. Increasing the duration of preharvest conditioning beyond 2 d did not improve the shelf life. Plants were chill-hardened for 1 wk at different periods during the day. Four, 5, and 6 week-old basil plants were harvested in each of three consecutive runs. With the 4- and 5 week-old basil, chill-hardening at the beginning of the dark extended average shelf life by 1 and 1.5 d, respectively. Shelf life was either decreased or not affected by the other periods of preharvest chilling. Postharvest chill-hardening of packaged sweet basil for 1 d at 10C before transfer to 5C increased shelf life by 5 d. Great potential exists for postharvest chill-hardening of packaged sweet basil since this method is effective and convenient.

Introduction

One of the most highly consumed fresh herbs, sweet basil, is sensitive to chilling injury. Joyce et al. (1986) reported that 5 to 7C is optimal for long-term basil storage, whereas Duke (1978) reported that 7C is the lower limit of growing temperatures for field-grown basil. Chilling injury symptoms of basil cuttings (darkened lesions and water-soaking) have been observed at temperatures as high as 10C (after 7 days or more storage). The optimum long-term storage temperature was found to be 15C (Chapter 1).

Commercially, fresh herbs are marketed either as potted plants or as cuttings. Field- or greenhouse-grown plants and cuttings are typically harvested and shipped in mixed loads to retail markets. The majority of horticultural commodities are stored at temperatures near 5C. Mixed load shipments of herbs and leafy vegetables at near 5C cause problems due to the occurrence of chilling injury damage of chilling-sensitive crops, especially basil.

Since chilling is often unavoidable, treatments to either increase the tolerance of the tissue before chilling or to reduce the development of injury symptoms after chilling would be highly desirable. In addition to storage at the optimum temperature, there are several temperature manipulation techniques that have been used to decrease chilling injury for several other crops (Harding et al., 1957; Lyons, 1979; McColloch, 1962; Wheaton and Morris, 1967). Conditioning, defined as the holding of sensitive tissue slightly above the critical chilling temperature for a period of time, has been shown to increase tolerance of tissue to chilling (Wheaton and Morris, 1967). Several studies have been directed toward postharvest treatments designed to alleviate the symptoms resulting from chilling injury. Most of the conditioning research has been conducted on harvested fruits and vegetables. Harding et al. (1957) reported that exposure of grapefruit to 7 days at 10 or 15C prevented or significantly reduced CI during storage at 0 or 1C. In similar findings, McColloch (1962) found that sweet peppers exposed to 10C for 5 or 10 days had reduced chilling injury symptoms at 0C. There are no known reports of a temperature conditioning treatment for vegetative cuttings.

Hetherington et al. (1983) found that corn seedlings grown on a 16C-day and 6C-night temperature regime were more tolerant to chilling at 0C than plants grown on a 20C-day and 15C-night regime. Research has not been reported in which preharvest intermittent exposures were applied at a non-chilling temperature to herbaceous cuttings.

Postharvest intermittent warming has been reported to reduce chilling injury of bell peppers (Wang and Baker, 1979), cucumbers (Cabrera and Saltveit, 1990; Wang and Baker, 1979; Hirose, 1985) and okra (Ilker and Morris, 1975). Many crops are responsive to short pulses of warm, non-chilling temperatures during storage, so perhaps short pulses of low (non-chilling) temperatures during production would help to "harden" the plant prior to storage.

King et al. (1982) found that harvesting at the end of the day reduced chilling injury in tomato seedlings. Previous research indicated that packaged basil cuttings harvested at 1800 HR as compared to cuttings harvested at 0600 HR could have longer shelf life due to delayed chilling injury symptoms (see Chapter 1). If diurnal variation is a factor to consider with chilling-tolerance, than this variation might also be a factor to consider during selection of a daily conditioning period.

The objectives of this research were to compare the chilling injury response of sweet basil conditioned : 1) preharvest with daily 4 hour-exposures at 10C, 2) preharvest at 10C and at several different periods within the day, and 3) postharvest at 10C for 1 day.

Materials and Methods

Production methods. Sweet basil [*Ocimum basilicum* (L.)] seeds from Mountain Sterling Farms (Knoxville, Tenn.) were sown into 208-cell plug trays containing Fisons Sunshine Mix #3 (a commercially-available special fine peat-based soilless mix from Fisons Western Corp., Vancouver, BC Canada) in consecutive experiments from 10 October 1989 through 5 February 1990 at Michigan State University, E. Lansing, MI. The basil seeds were germinated under an intermittent mist system at 24/20C day/night temperatures (in a computer-controlled greenhouse) with natural lighting only. The night temperature of the greenhouse sometimes dropped down to 17C on nights when the outside temperature was less than -5C. After 2 weeks, 3 seedlings were transplanted into 527 ml azalea pots containing Baccto Professional Growers Mix (a commercially available peat-based soilless mix from Michigan Peat Co., Houston, Tex.) and were grown under natural lighting for 1 week. During the next 3 to 5 weeks, the plants received at least 12 hours of light (either naturally or from high pressure-sodium lamps at 150-200 μmol s^{-1} m^{-2}) and daily fertilization with 150 ppm N using a 20-20-20 fertilizer.

Packaging and storage methods. The cuttings were harvested below the third node from the apical end. Each cutting had 6 to 8 leaves and weighed 3 to 4 grams. The cuttings were held in unsealed plastic bags from harvesting until packaging. A package consisted of one cutting sealed in an 800 cm^2, 3 mil low-density polyethylene (#550) film package using a Magneta heat sealer-620 series. Four $26^{1/2}$-gauge needle holes were punched through each package as recommended by Joyce et al. (1986). Harvesting took approximately 30 min, and packaging, 45 min.

Basil packages were held in the dark at 5C under black plastic for the duration of all experiments. In all experiments, basil was inspected daily for necrosis and was considered to have chilling injury if 1 to 5% of the leaf surfaces were observed to have chilling injury symptoms. End of shelf life was determined when the first visual symptoms of chilling injury were observed. Analysis of variance was performed on all experiments. Means of shelf life were compared using Duncan's multiple range test ($\alpha = 0.05$).

Experiment 1. Seventy, 6-week-old, greenhouse-grown basil plants were placed into a Micro-Pro 2000 microprocessor-based growth chamber. The chamber was programmed at constant 25C. Fluorescent lamps supplied 12 hour-photoperiods (0800 to 2000 HR) at light intensities of 175 to 225 μmol s^{-1} m^{-2}. Daily fertilization as described above occurred between 0800 and 1000 HR. After 1 week, 35 basil plants were moved to a chamber set for 20 hours at 25C, and for 4 hours (1800 to 2200 HR) at 10C. Five cuttings were harvested at 1730 HR from each chamber (see harvesting methods) after 2, 4, 6, 8, 10, 12, and 14 days. The experiment was conducted from 5 January to 26 January 1990.

The experiment was analyzed as a completely randomized design in a two-way analysis of variance. The two factors (and corresponding levels) were 7 durations and 2 temperature treatments with 5 replications per treatment combination.

Experiment 2. Four-, 5-, and 6-week-old basil plants were used in each of three consecutive runs. Each run of the experiment began with 1 week of growth chamber-conditioning as in Expt. 1. After the first week, 20 plants were moved to 4 growth chambers programmed at 10C for 4 hours at 0400 to 0800 HR, 0800 to 1200 HR, 1600 to 2000 HR, or 2000 to 2400 HR. Plants were maintained as described for Expt. 1. After one week, the plants were harvested at 1500 HR and 100 packages were placed in 5C storage by 1630 HR. Each of the runs was performed similarly between 5 February and 2 March 1990.

The experiment was analyzed as a randomized complete block design blocked over age of the basil plants at harvest. The only variable was period of temperature conditioning during the day; 20 replications were used per treatment.

Experiment 3. Five-week-old, greenhouse-grown basil plants were harvested and packaged as previously described. The packaged basil cuttings were either chill-hardened in the dark at 10C for 1 day prior to 5C dark storage or were stored immediately after harvest in 5C dark storage. The experiment was performed from 15 December until 31 December 1989 in two consecutive runs. Data from both runs were combined and analyzed as a completely randomized design in a one-way analysis of variance. There were 10 total replications for each temperature treatment.

Results

Experiment 1. Five week-old basil plants conditioned for 2 to 14 days at 10C (4 hours per day), were delayed in chilling injury development when compared to untreated plant tissue (Fig. 1). The average shelf life of conditioned basil was 6.5 days, approximately 3 days longer than the shelf life of the control cuttings at 3.7 days (significant to P = 0.0001). All of the untreated cuttings had chilling injury damage by 7 days as opposed to 13 days for the conditioned basil cuttings. An additional, significant benefit was not observed with an increase in duration of conditioning beyond 2 days.

The coefficient of variability (cv), defined as the ratio of population variability to the mean, was 48%. Although the average shelf life was significantly increased, the range of shelf life data (over all durations) was 1 to 12 days for the conditioning treatment and 1 to 7 days for the control.

Experiment 2. Basil tissue conditioned during the beginning of the dark (BOD) period and stored at 5C had an average shelf life of 8.2 days, approximately 1 day longer than the control cuttings (not conditioned)(Fig. 2). At other time periods during the day, conditioning caused a small but significant decrease in average shelf life (P = 0.0001) compared to the controls (not conditioned).

The experiment was blocked by age of the basil plants used for harvesting. The oldest tissue was consistently the most tolerant over all periods of conditioning. Six week-old basil cuttings (used in the third run) had an average shelf life of 7.5 days as opposed to 6.9 and 6.4 days for 4 and 5 week-old basil, respectively.

The cv as defined above was 29% which was less than in Expt. 1 (48%). The average shelf life of combined 4, 5, and 6 week-old (Expt. 2) control cuttings (7.5 d) was higher than the average shelf life of 5 week-old controls in Expt. 1 (3.7 days).

Experiment 3. Postharvest conditioning of 5 week-old basil plants for 1 day at 10C delayed the subsequent onset of chilling injury symptoms at 5C (Fig. 3). Twenty percent of the cuttings stored for 1 d at 10C were undamaged by the 17th day of storage. Untreated cuttings were all damaged after 9 days. One day of 10C storage significantly extended ($P = 0.0064$) the average shelf life of basil by 193% (10.4 days) when compared to the untreated controls (5.4 days). The experiment was repeated twice with statistically similar results so the data were combined.

The cv was 46% which was very similar to the cv of 48% from Expt. 1. The average shelf life of the untreated controls was 5.4 days.

Discussion

Preharvest conditioning of basil at 10C for as little as 2 days (4 hours daily) was effective in the delay of chilling injury by an average of 3 days (Fig. 1). Preharvest conditioning at 7.5C for more than 2 days caused increased susceptibility to chilling injury symptoms (data not shown). Similarly, Kader et al. (1974) found that vegetable plants exposed to chilling temperatures in the field or during handling had increased susceptibility to additional injury when exposed to chilling storage temperatures. Thus choosing the proper temperature and duration for conditioning requires careful consideration.

The best time to condition was at the BOD (2000 to 2400 HR) period. All other periods of conditioning negatively affected the onset of chilling injury and the related shelf life (Fig. 2). These results "somewhat" correlate with results of King et al. (1982) and results presented in Chapter 1 that showed that it is least damaging to apply cool temperatures to seedlings or cuttings at the end of the day rather than in the early morning hours.

Postharvest conditioning of basil at 10C for only 1 day delayed the onset of chilling injury symptoms by 5 days. Harding et al. (1957) and McColloch (1962) found that 7 to 9 days of 10C exposure was effective in the reduction of chilling injury symptoms on grapefruit and bell peppers, respectively. The technique of postharvest conditioning of basil looks promising due to the effectiveness of conditioning at 10C for only 1 day. In addition, this technique is relatively simple and practical. A drawback would be that a separate controlled temperature facility would be necessary for temporary storage at 10C.

The chilling injury response of the sweet basil population was highly variable as was expressed with the high coefficients of variability (29 to 48%) in the three experiments. As was reported in Chapter 1, high plant-to-plant variability was common in all experiments. Special efforts were made to standardize the selection of cuttings and the production methods, but the variation may be due to genetic differences in the seed lot or other factors, such as the stage of development of individual leaves.

Wang (1990) reported that the effectiveness of conditioning is affected by maturity of the commodity. In Expt. 2, the basil was blocked by age which was significant ($P = 0.0001$). Terminal cuttings from 6 week-old plants were more tolerant than cuttings from 4 and 5 week-old plants. The age of cuttings was difficult to standardize within a given experiment or block within an experiment due to non-uniform germination rates within the seed crop.

The most tenable proposal for the primary event of chilling injury is that there is a temperature-induced change in molecular ordering of membrane lipids (Lyons, 1979). This proposal supports the observation that membrane permeability is dramatically increased in chilling-sensitive plants after chilling (Wilson, 1976). Lyons (1973) speculated that a phase change in the membrane lipids might serve as a primary event that causes a series of secondary events that lead to visual symptoms of chilling injury.

Beneficial effects of temperature preconditioning have been attributed to various physiological changes. Increases in the degree of unsaturation of fatty acids in response to hardening or chilling temperatures have been demonstrated in several plants, including cotton seedlings (St. John and Christiansen, 1976) and alfalfa leaves (Kuiper, 1970). These increases in fatty acid unsaturation during low-temperature conditioning have been proposed to be a result of altered fatty acid desaturase activity and not of preferential biosynthesis of individual phospholipids (Harris and James, 1969).

Some of the criticisms of this hypothesis are that the degree of fatty acid unsaturation in bulk lipids rarely correlates with the level of chilling sensitivity and that the sterols and membrane-associated proteins have been ignored (Guye, 1988). King et al. (1988) hypothesized that increased sucrose in the plant cells might be able to stabilize the cell membranes. This hypothesis should be further researched as another explanation for the efficacy of temperature preconditioning.

Basil cuttings that were conditioned during the beginning-of-dark period (BOD) lasted significantly longer than the cuttings conditioned during all other periods. Within a 24-hour day, all of the conditioned basil received the same daily average temperature. Therefore, for example, conditioning at the beginning or the end of the dark period seemingly should make no difference. King et al. (1988) reported that the carbohydrate reserves are highest in tomato seedlings during the end-of-light (EOL)(1600 to 2000 HR) and beginning-of-dark (BOD)(2000 to 2400 HR) periods. However, the EOL treatment showed no positive effect on the conditioning of basil. As in the case of maize, light could induce chilling injury during production (Miedema, 1982). Possibly, conditioning at 10C in the presence of light pre-induced chilling injury on the basil cuttings.

Chilling temperatures (10C and below for 7 days or less) will most likely be encountered during the shipping, handling, and retail display of sweet basil. Basil is a minor crop, therefore it will be marketed with commodities which have optimum storage temperatures that are damaging to chilling-sensitive crops. Both pre- and postharvest temperature manipulation were shown to alleviate chilling injury. In earlier work (Chapter 1), harvest time was shown to be an important consideration (for storage temperatures of 5 to 25C); the best results were with basil harvested at the EOL period.

The postharvest conditioning technique probably holds the most promise due its effectiveness after just 1 day (Fig. 3) and also due to its convenience and practicality. King et al. (1988) reported that one night of storage at 10C increased the chilling-tolerance of tomato seedlings. In this research, 4 hours of conditioning at 10C during the BOD period (for at least 2 days) was effective in the reduction of chilling injury. Possibly, one day of this conditioning treatment would be enough. A possible solution to large losses due to chilling injury might be to harvest in the late afternoon or early evening, followed by overnight postharvest storage of the chilling-sensitive crops at 10C before refrigerated shipment. This procedure would utilize the beneficial techniques reported in these studies.

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Figure 1. The effect of preharvest intermittent cooling at 10C on the % of packaged sweet basil cuttings damaged when stored at 5C in the dark. Plants were conditioned at 10C daily from 1800 to 2200 HR. Each point represents 35 packaged cuttings merged over 2 to 14 d of conditioning. The effect of conditioning was significant at P = 0.0001 as determined by analysis of variance.

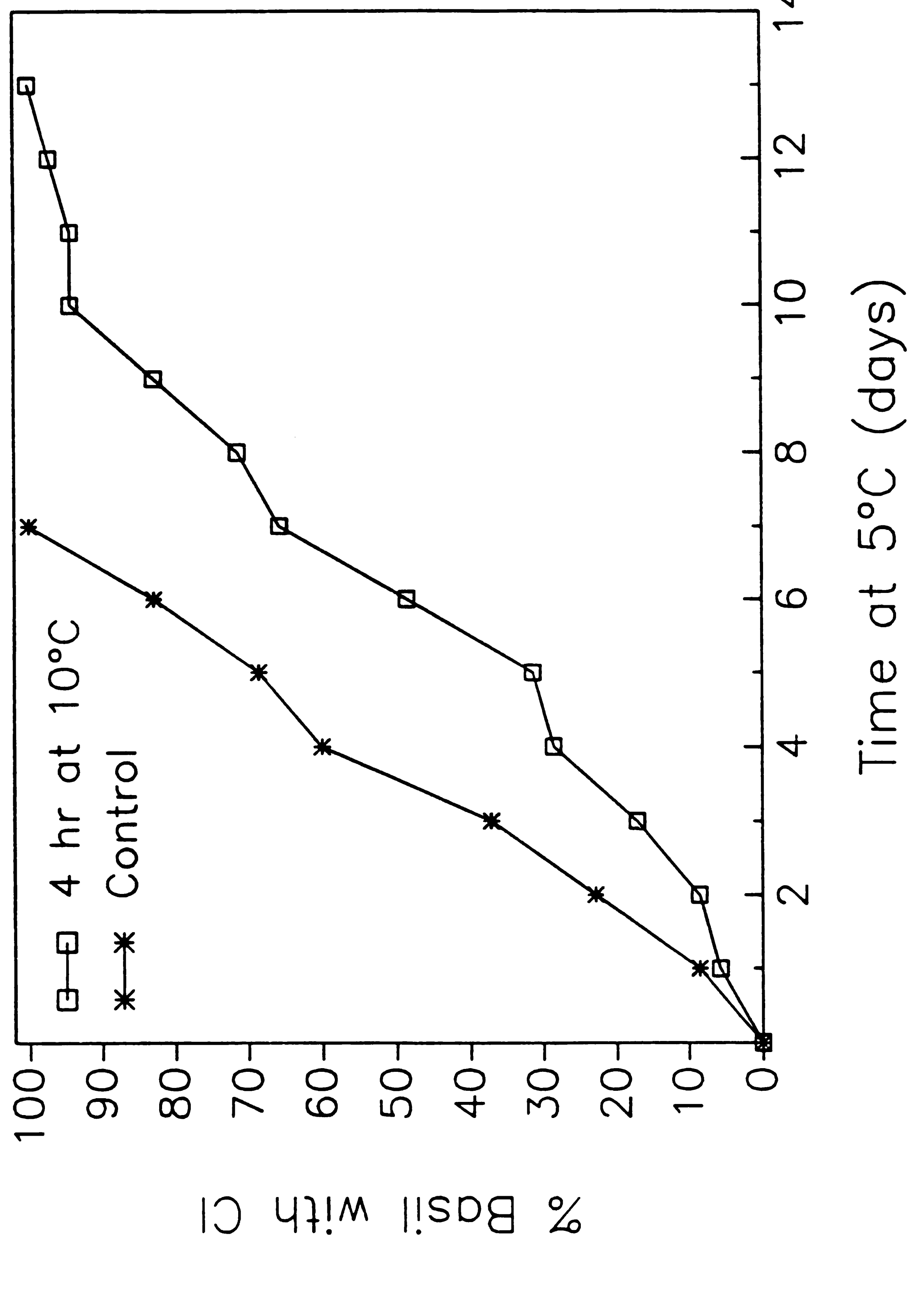

4 hr at 10°C
Control
% Basil with CI
Time at 5°C (days)
0
10
20
30
40
50
60
70
80
90
100
2
4
6
8
10
12
14

Figure 2. The effect of preharvest intermittent cooling at 10C for 4 hours daily at 4 different periods within the day on the chilling injury response of packaged sweet basil cuttings. Each bar represents 60 packaged cuttings from 3 combined runs. Darkened bars signify periods of darkness. The effect of period of conditioning was significant at $P = 0.0001$. Treatment means were separated by Duncan's multiple range test at a significance level of $\alpha = 0.05$.

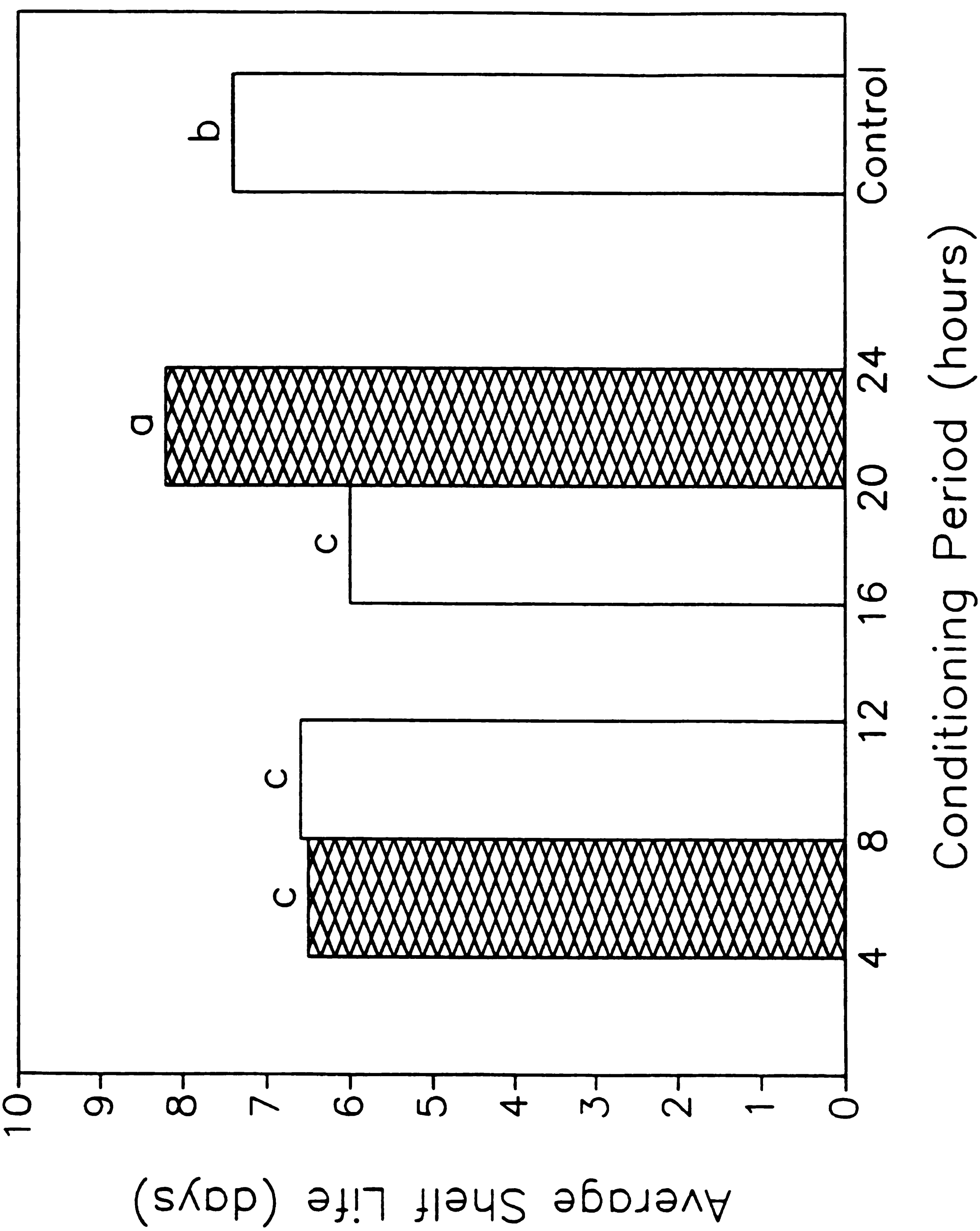
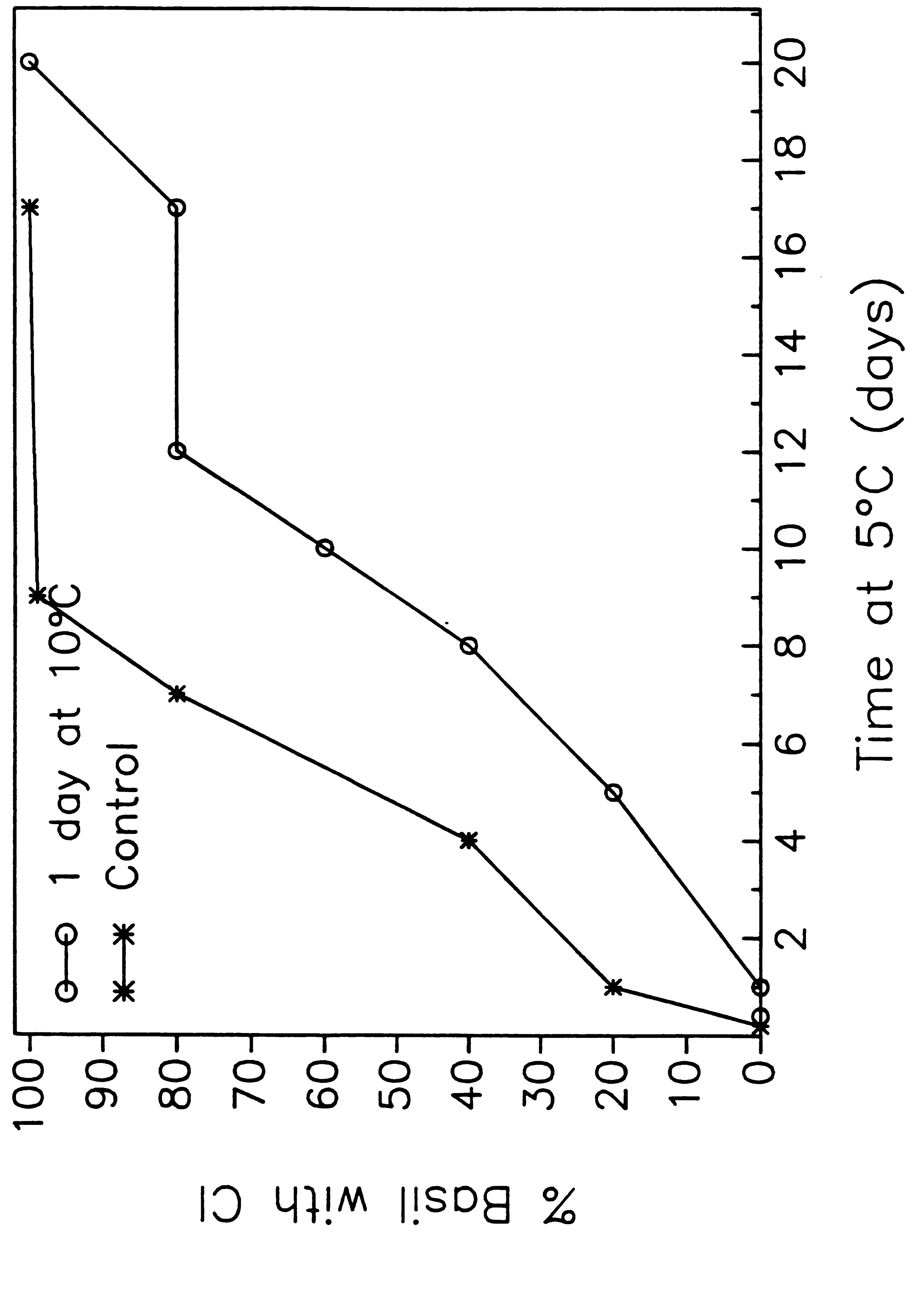

Average Shelf Life (days)
0
1
2
3
4
5
6
7
8
9
10
Conditioning Period (hours)
4
8
12
16
20
24
Control
a
b
c
c
c
c

Figure 3. The effect of postharvest conditioning for 1 day at 10C (in the dark) on the % of cuttings damaged by chilling injury in storage at 5C in the dark. Ten observations were combined from 2 runs. The effect of postharvest conditioning was significant at $P = 0.0001$. The treated cuttings were in storage longer than the controls due to the extra day at 10C.


1 day at 10°C
Control
Time at 5°C (days)
% Basil with CI
0 10 20 30 40 50 60 70 80 90 100
2 4 6 8 10 12 14 16 18 20

CHAPTER 3

CONTROLLED ATMOSPHERE STORAGE OF SWEET BASIL

Abstract. The effect of controlled atmospheres on the storage life of sweet basil cuttings [*Ocimum basilicum* (L.)] was assessed. The cuttings were placed in perforated packages and were stored within a number of different O_2 and CO_2 gas mixtures. Storage at 1% or less O_2 was limited by the appearance of dark, water-soaked lesions on the youngest tissue. Ten percent or more CO_2 caused brown spotting on all tissue. The optimum gas mixture for CA storage of sweet basil was 1.5% O_2/0% CO_2 at which the average shelf life was extended 260% from 17.5 d (air control) to 45.3 d. Storage of sweet basil cuttings at 5C in 1.5 O_2/5% CO_2 did not alleviate chilling injury symptoms.

Introduction

Sweet basil stores well for an average of 12.5 days at 15C in air with acceptable visual quality (Chapter 1). However, this temperature is not presently used during marketing and storage of perishable commodities. Temperatures of 10C or below have been shown to cause chilling injury and a reduction in average shelf life from 12.5 days at 15C to approximately 7 days at 10C and 3 days at 5C (Chapter 1). Temperature conditioning at 10C before storage at 5C resulted in some alleviation of chilling injury, but did not eliminate the problem (Chapter 2).

Controlled-atmosphere (CA) storage is a technique for maintaining the quality of produce in an atmosphere that differs from air in respect to the proportion of O_2, CO_2, or N_2. Research into the use of atmospheres containing elevated levels of CO_2 and/or reduced levels of O_2 to retard senescence has been well documented for a variety of vegetables and herbs. Examples of vegetables that benefit from CA storage are broccoli (Makhlouf et al., 1989), Brussels sprouts, (Lipton and Mackey, 1987), head lettuce (Watada et al., 1964), romaine lettuce (Aharoni and Ben-Yehoshua, 1973) and spinach (Murata and Ueda, 1967). Moreover, CA storage has extended the storage life of herbs such as parsley (Apeland, 1971; Hruschka and Wang, 1979; Nsengimana and Bangerth, 1981) and watercress (Hruschka and Wang, 1979). Parsley stored at 0C in an atmosphere of 10% O_2 and 11% CO_2 remained dark green and salable for an additional 2 weeks longer than control cuttings that only lasted 2 weeks (Hruschka and Wang, 1979). Aharoni et al. (1990) reported that yellowing-susceptible herbs, such as watercress, chives, chervil and sorrel, were best stored at modified atmospheres of 5 to 8% O_2 and 8 to 12% CO_2.

High CO_2 injury or low O_2 injury are reported on some horticultural crops. Aharoni and Ben-Yehoshua (1973) reported that 64% of the heads of 'Great Lakes' lettuce were damaged due to cold storage in 3% CO_2. Low O_2 injury (reddish-tan discoloration and bitter flavor) was observed on Brussels sprouts held at 0.5% O_2 (Lipton and Mackey, 1987).

In some cases, CA storage has been reported to alleviate chilling injury symptoms on commodities such as apples (Kidd et al., 1927) and avocados (Spalding and Reeder, 1975). Kidd et al. (1927) was the first to test the influence of CA storage on chilling injury. Low O_2 levels in storage at 0C inhibited the formation of internal breakdown (a symptom of chilling injury in some cultivars of apples), while high CO_2 levels tended to increase the incidence and severity of internal breakdown. Spalding and Reeder (1975) reported that avocados held 3 to 4 weeks at 7C in 2% O_2 and 10% CO_2 showed only traces of chilling injury, while those held in air were severely injured.

On the other hand, cucumbers stored at chilling temperatures in 3 to 75% CO_2 were more severely pitted than cucumbers held in air. However, the level of O_2 had no effect except at extreme levels (less than 1 or 100%), at which it enhanced pitting (Eaks, 1956). Carbon dioxide at 5 to 20%

increased the incidence of pitting and decay in bell peppers when compared with fruit stored in air (Cappellini et al., 1984).

The objectives of this research were to determine : 1) the injurious levels of O_2 and CO_2 at which sweet basil cuttings cannot be safely stored at 20C, 2) whether or not storage in various O_2/CO_2 combinations can extend the storage life of basil, and 3) the effect of an optimized O_2/CO_2 combination on the prevention of chilling injury damage on sweet basil stored at 5C.

Materials and Methods

Sweet basil [*Ocimum basilicum* (L.)] seeds from Mountain Sterling Farms (Knoxville, Tenn.) were sown into 527 ml plastic pots filled with Baccto Professional Growers Mix (a commercially available peat-based soilless mix from Michigan Peat Co., Houston, Tex.). The seedlings were thinned as described in Chapter 1. The basil plants were grown at 24/20 day/night temperatures in a computer-controlled greenhouse from 3 Jan to 7 March 1989 at Michigan State University, E. Lansing, Mich. The night temperature in the greenhouse sometimes dropped down to 17C on nights when the outside temperature was less than -5C. Plants were fertilized daily with 150 ppm N using a 20-20-20 fertilizer from transplant to harvest. Natural daylengths were extended to 12 hr photoperiods (0800 to 2000 HR) with high pressure sodium lamps that supplied 150 to 200 μmol s^{-1} m^{-2}.

Three-node, 6 week-old basil cuttings were harvested at 1600 HR and were packaged individually in 800 cm^2, 3 mil low-density polyethylene (#550) (LDPE) film packages that were sealed with a Magneta heat sealer-620 series. Each package had eight $26^{1/2}$ perforations so that the internal CO_2/O_2 concentrations of the package were similar to the storage chamber concentrations. Eight packages were placed into an 120 cm X 30 cm chamber made out of double layer, 3 mil low-density polyethylene. The chamber was heat sealed and inlet and outlet ports were made on either end of the chamber. The ports were constructed out of 10 cm-long, 0.64 cm-wide plastic tubing that was inserted through the double layer of film and sealed with silicone caulking. Twenty cm of latex tubing was connected to the outside of the exit port for sampling. The inlet port was attached to 20 cm of amber tubing that was connected to the gas-mixing system. The polyethylene chambers were sampled twice daily for gas analysis. One ml-samples from both the chambers and interior packages were injected into a Type 225 infrared CO_2 gas analyzer (Analytical Development Co. Limited, Hoddesdon, England) connected in series to an Ametek S-3A O_2 analyzer (Pittsburgh, Pen). The N_2 flow rate passing through the analyzers was 150 ml/min.

Desired concentrations of CO_2 and O_2 were achieved by the mixture of air and CO_2 into the N_2 carrier gas that was flowing at 2 liters per hour. Nitrogen gas (for the gas mixtures) and air (for the control) were humidified by passage through 5 consecutive sealed jars filled with deionized water in order to increase the RH of the flushing gas to 90% or more (Shirazi, 1989). The CO_2 concentrations used in this experiment ranged from 0 to 25% and the O_2 used ranged from 0.5 to 21%. The following O_2/CO_2 % were used: 0.5/5, 1/5, 1.5/5, 1.5/7.5, and 1.5/10. The concentrations of gases within the individual packages were always +/- 5% of the desired concentrations entering the chambers. Four to 6 treatments were CA-stored at one time. An air (21% O_2, 0% CO_2) control was used in each sub-experiment for comparison.

Controlled atmosphere storage of sweet basil cuttings was maintained in a dark 20C storage chamber from 5 February to 25 March 1989. Basil stored under all treatments, including air, was observed to be wilted after storage durations of 2 weeks or more.

The storage life of sweet basil cuttings was determined visually. A cutting was considered damaged or unsalable when the first symptom of high CO_2 injury, low O_2 injury, or mold was observed.

Results

Basil cuttings stored in atmospheres of 0.5 or 1% O_2 showed symptoms of low O_2 injury which appeared as dark, water-soaked lesions on only the youngest tissue (similar to chilling injury symptoms observed in Chapters 1 and 2). This low O_2 injury reduced the average shelf life of sweet basil stored in 0.5% O_2 from 19.3 days for the air-stored basil to 2.8 days for the 0.5% O_2 treatment (Table 1). However, the average shelf life of basil stored at 1% O_2 was only reduced by 3 days (compared to the air control). In one study, storage in 1.5% O_2 did not cause low O_2 injury and also resulted in a 27 day-extension of the average shelf life (45 days in 1.5% O_2, 18 days in air). The addition of 7.5 or 10% CO_2 to 1.5% O_2 reduced the average shelf life by approximately 10 days due to the appearance of high CO_2 injury in the form of brown spots on all ages of leaf tissue. Basil cuttings stored in atmospheres of 10, 15, 20, and 25% CO_2 (in air) were damaged by symptoms typical of high CO_2 injury. The average shelf life of basil was only reduced at CO_2 concentrations of 15 to 25% (Table 3). Storage of basil in 5% CO_2 in air extended the average shelf life by 9 days over the basil stored in air (data not presented) and this treatment never caused high CO_2 injury.

The best O_2/CO_2% for CA storage of sweet basil was either 1.5/0 or 2/0 (Table 2) which caused an extended average shelf life of 45.3 and 44.0 days (respectively), as compared to an average shelf life of 17 days for the basil held in air. The addition of 5% CO_2 to 1.5% O_2 reduced the average shelf life of sweet basil by 40% (compared to basil stored in 1.5% O_2), but the use of the 1.5/5 treatment did extend the average shelf life by 40% when compared to the air control.

Storage of sweet basil cuttings in a 1.5% O_2 and 5% CO_2 atmosphere at 5C had no effect on the prevention of chilling injury of sweet basil. The CA-stored basil was 30% more damaged than the air-stored basil on days 6 through 8 of 5C storage.

The type of decay was not determined but the decay appeared as mainly gray, fuzzy molds on the surface of the leaves only.

Discussion

The optimum gas composition for CA storage of sweet basil at 20C was 1.5% to 2% O_2 with no added CO_2 (Table 2). The addition of 5% CO_2 actually shortened the average shelf life of sweet basil cuttings by 40% when compared to storage at 1.5% O_2 alone. This result contrasts with the results of Lipton and Mackey (1987) in which 10% CO_2 further improved storage of Brussel sprouts as compared to storage with 1 to 2% O_2.

Basil was damaged by CO_2 concentrations of 10 to 25%, although treatment with 10% CO_2 did not reduce the average shelf life as compared to the control. Controlled atmosphere storage with 10 to 40% CO_2 was recommended for spinach (Murata and Ueda, 1967); parsley and watercress are best stored at approximately 10% CO_2 (Hruschka and Wang, 1979). Not all of the herbs will benefit from high concentrations of CO_2 as was shown with sweet basil cuttings (Table 3).

The positive effects of low O_2 and high CO_2 were hard to separate during visual analysis, since both treatments caused color retention and delay of decay. The high quality of CA-stored basil at 20C was maintained best by storage in 1.5% O_2 (260% increase in the average shelf life as compared to the air control), but storage in 1.5% O_2/5% CO_2, or 5% CO_2 in air at 20C also extended the average shelf life of sweet basil by 40% over the air control. Lipton and Mackey (1987) reported that the use of low O_2 resulted in color retention but did not delay the development of decay at 5 to 7.5C. High CO_2 only reduced decay development with no effect on color retention. Makhlouf et al. (1989) reported that high CO_2 did reduce chlorophyll loss in broccoli buds at 25C, but low O_2 was more effective than high CO_2.

Controlled atmosphere storage (1.5% O_2, 5% CO_2) was ineffective in the alleviation of chilling injury on sweet basil stored at 5C (Fig. 1). However, this same mixture of gases caused a delay in senescence at 20C (Table 3). Other commodities have been shown to respond negatively to CA storage at chilling temperatures. Carbon dioxide at 5 to 20% increased chilling injury of bell peppers at 5C when compared to peppers stored in air at 5C (Cappellini et al., 1984). Similarly, cucumbers had increased pitting when stored at 3 to 75% CO_2, although levels of O_2 between 1 and 100% were not damaging (Eaks, 1956).

Kidd et al. (1927) reported that low O_2 levels inhibited the incidence of internal breakdown in apples, but high CO_2 increased the incidence and severity of internal breakdown. Therefore, 5C storage of sweet basil in 1.5% O_2 and atmospheric CO_2 should be tested to discover if O_2 alone can alleviate chilling injury of sweet basil.

Ethylene has long been known to enhance leaf senescence (Burg, 1968) and to reduce the market quality of fruits, vegetables, florist crops, and ornamental foliage (Kader, 1985). In many plant tissues, CO_2 was found to antagonize the senescence-enhancing effects of ethylene (Burg and Burg, 1967 and Aharoni et al., 1979). Makhlouf et al. (1989)

reported that low O_2 delayed senescence by reducing respiration and ethylene biosynthesis in broccoli buds. High CO_2 delayed chlorophyll loss during senescence by reducing respiration and ethylene action.

In all experiments at 20C, the basil stored in air lasted an average of 14 days or longer which was almost double the shelf life of basil used in Chapters 1 and 2. This might be due to extended storage life caused by decreased RH in the packages. The RH of the packages was not measured in this study but the relatively high flow rate in the chambers could have caused a drop in RH compared to packages which were not stored in CA.

Further studies need to be carried out to clarify the effect of CA storage treatments on the eating quality of sweet basil but a highly beneficial effect on the visual quality (especially color retention) was evident and no noticeable loss in aroma was noted.

The use of short term CA at ambient temperature (Wills et al, 1979) could be of value during transportation and marketing of minor crops, such as fresh herbs, where it is not always feasible to maintain produce at low temperatures. Sweet basil might be successfully stored in air at 20C after application of a short CA treatment prior to storage. Storage at 5C should be avoided due to the high incidence of chilling injury of sweet basil at 5C. Produce could be given the short CA treatment just after harvest by the grower or packinghouse and then marketed without the need for any further special facilities to give adequate retention of quality. Extension of storage life by 2 weeks should be acceptable for such highly-perishable crops as herbs.

Even if CA storage was considered to be too expensive to be feasible for minor crops like basil, at least the information gathered in these studies could be used to design an optimized package for sweet basil cuttings. This package would be designed so that the respiration of the basil cuttings would consume enough O_2 to drive the O_2 concentration down to near 1.5%. Carbon dioxide should be scrubbed out of the packages so that the concentration never exceeds 5% CO_2. This technique would be simple and inexpensive.

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Table 1. The effect of low O_2 and low O_2/high CO_2 on the average shelf life of packaged sweet basil cuttings stored at 20C in the dark. Each mean was based on the observation of 8 packaged cuttings.

Treatment	Avg. Shelf Life (days)	Type of Damage
Air Control	19.3	Mold
0.5% O_2	2.8	Low O_2 injury
1% O_2	16.3	Low O_2 injury
1.5% O_2	23.0	Mold
1% O_2, 5% CO_2	11.8	Low O_2 injury
1.5% O_2, 7.5% CO_2	21.0	High CO_2 injury
1.5% O_2, 10% CO_2	20.0	High CO_2 injury

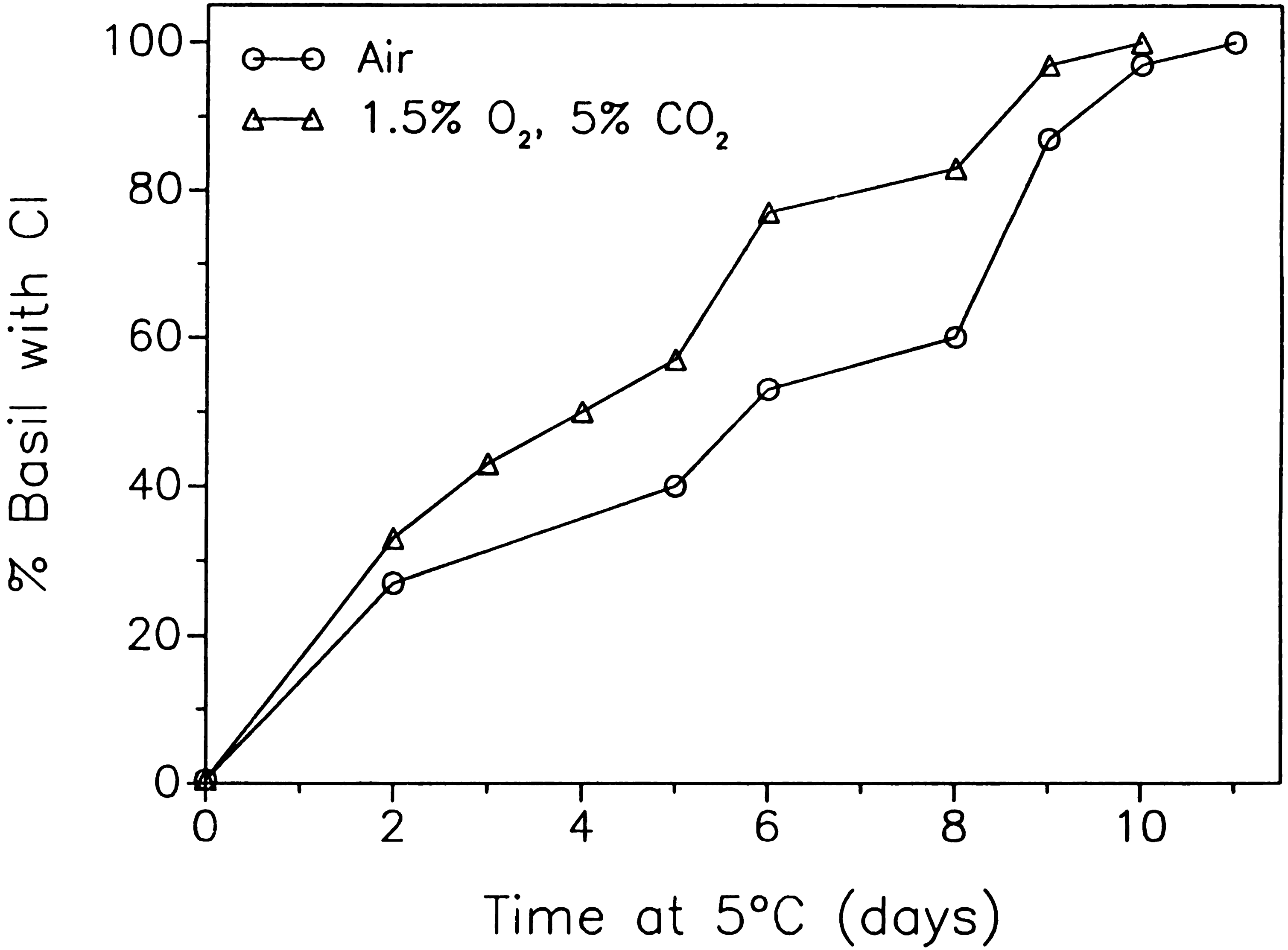
Table 2. The effect of optimized O_2 and CO_2 concentrations on the average shelf life of packaged sweet basil cuttings stored at 20C in the dark. Each mean was based on the observation of 8 packaged cuttings.

Treatment	Avg. Shelf Life (days)	Type of Damage
Air Control	17.5	Mold
1.5% O_2	45.3	Mold
2% O_2	44.0	Mold
1.5% O_2, 5% CO_2	31.0	Mold

Table 3. The effect of high CO_2 on the average shelf life of packaged sweet basil cuttings stored at 20C in the dark. Each mean was based on the observation of 8 packaged cuttings.

Treatment	Avg. Shelf Life (days)	Type of Damage
Air Control	18.8	Mold
10% CO_2 in Air	17.5	High CO_2 injury
15% CO_2 in Air	11.5	High CO_2 injury
20% CO_2 in Air	3.0	High CO_2 injury
25% CO_2 in Air	3.0	High CO_2 injury

Figure 1. The effect of controlled atmosphere storage in 1.5% O_2 and 5% CO_2 on the chilling injury response of packaged sweet basil cuttings stored at 5C in the dark. Each point represents 8 packaged cuttings.


% Basil with CI
Time at 5°C (days)
Air
1.5% O_2, 5% CO_2
0
20
40
60
80
100
0
2
4
6
8
10

SUMMARY AND RECOMMENDATIONS

The postharvest shelf life of packaged sweet basil at various temperatures, daily harvest times, and storage atmospheres was found in this research. In addition, the effect of preharvest and postharvest conditioning on the subsequent shelf life of sweet basil was demonstrated.

In Chapter 1, sweet basil lasted the longest when stored at 15C and harvested in the late afternoon or early evening. Chilling injury damaged basil cuttings at temperatures of 10C or below.

The chilling injury on sweet basil (stored at 5C) was reduced with the use of either pre- or postharvest conditioning. Preharvest conditioning at 10C (4 hours daily) for at least 2 days delayed chilling injury for 3 days. This technique was only beneficial if applied between 1800 and 2400 HR or later. More importantly, postharvest conditioning caused an 100% extension in average shelf life of the basil cuttings (from 5 to 10 days).

Controlled atmosphere storage of basil at 20C was most effective when 1.5% O_2 was applied. Carbon dioxide concentrations of 10% or more caused high CO_2 damage on the basil leaves. Storage of basil in 1.5% O_2 and 5% CO_2 did not alleviate chilling injury symptoms at 5C.

Most likely, the basil will be stored at 5C during transit and marketing. The results presented suggest that postharvest temperature conditioning should be used to reduce chilling injury symptoms. Basil cuttings harvested later in the day or early in the evening should be packaged immediately, and stored overnight at 10C, prior to 5C shipment. The shelf life of sweet basil might be further improved if 1.5% O_2 is found to reduce chilling injury at 5C.

Low O_2 storage at 20C extended the shelf life of sweet basil. Controlled atmosphere storage is not feasible for storage of minor crops like herbs. However, CA storage can be used as a technique to discover the best O_2 and CO_2 concentrations for storage of basil. Subsequently, a package can be designed that will maintain the desirable modified O_2 and CO_2 atmospheres around the respiring basil. Modified atmosphere packaging (MAP) is much more cost effective than CA storage due to the need for special facilities and equipment for CA storage.

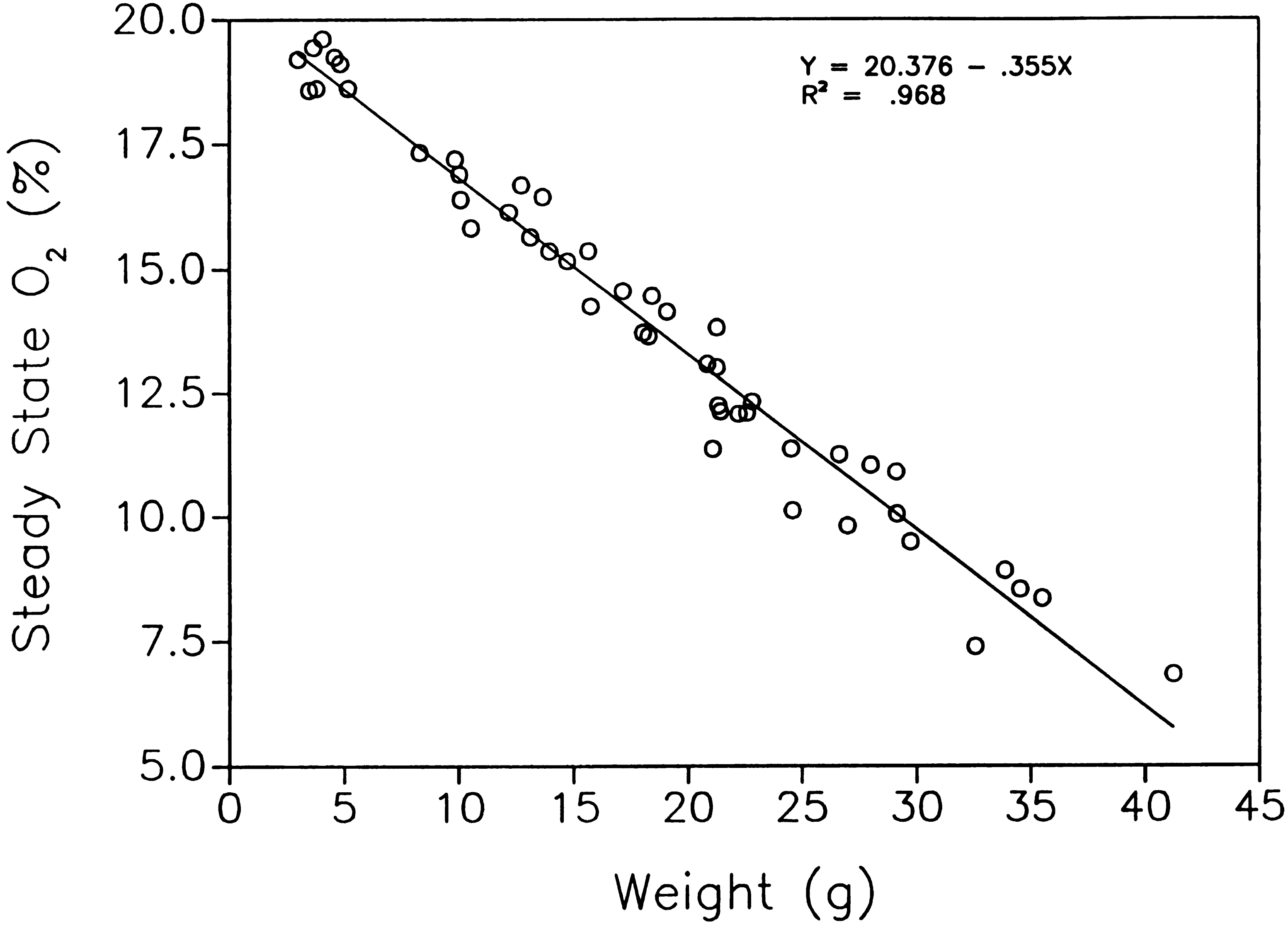
Another alternative would be to stick cuttings in water picks (as used with cut roses) at 20C. The average shelf life of basil (held in this manner) was 41 days. This technique supplies the necessary water to the cut stem, without increasing the humidity around the leaves. Therefore, mold growth is not a problem; the cutting lasts as long as the stem is able to take up water.

One technique currently being used is to market basil as small plants growing in an artificial medium like rockwool. The consumer can harvest basil as he needs it. Another advantage is that basil can be grown in the same container in which it is sold.

APPENDIX A

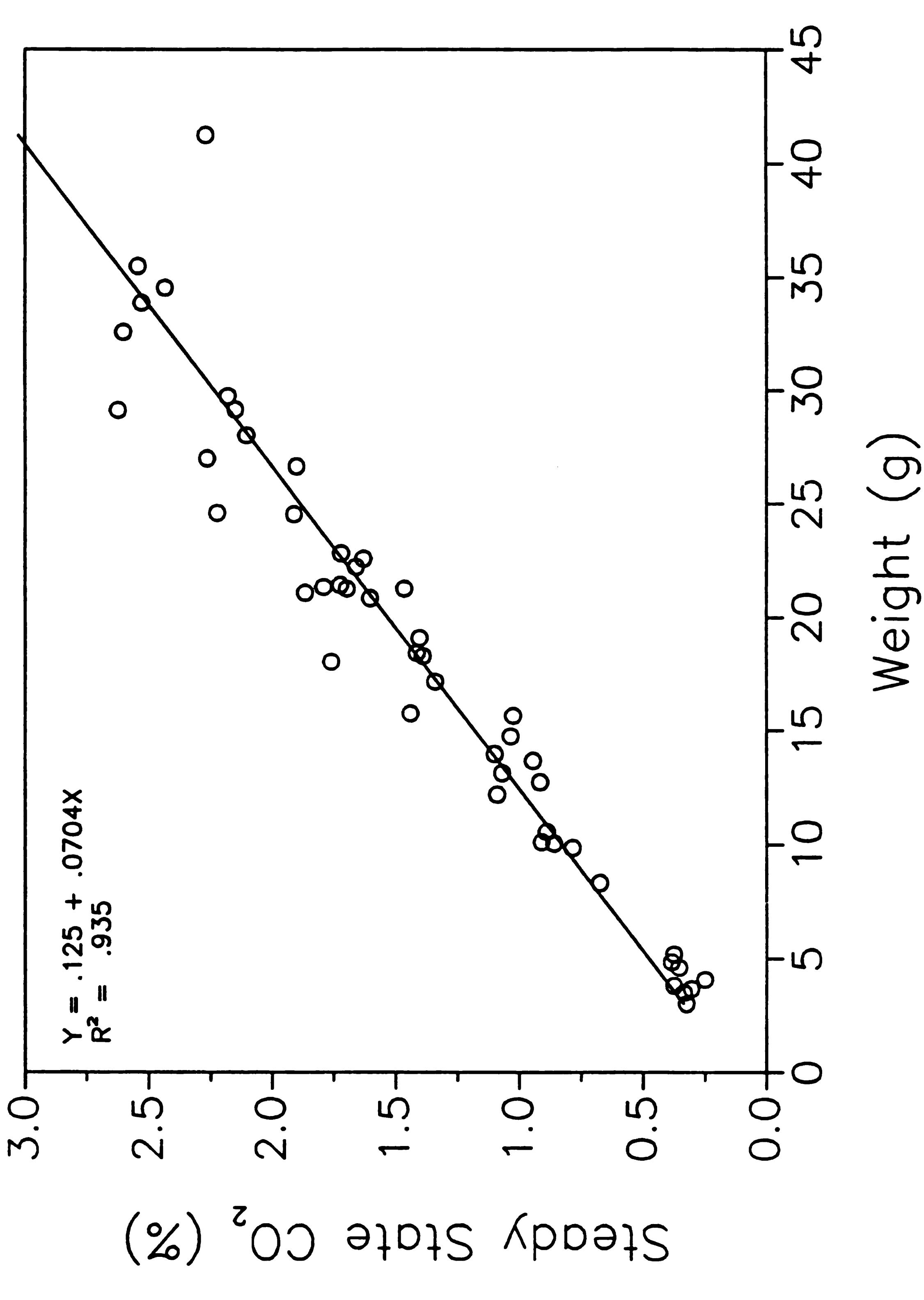
MODIFIED ATMOSPHERE PACKAGING AND STEADY-STATE RESPIRATION OF SWEET BASIL

The effect of sweet basil weight on the O_2 concentration present in the package at equilibrium. The basil cuttings were packaged into 3 mil, 800 cm^2 low-density polyethylene film at harvest. The packages were held at 20C in the dark. Gas analysis occurred once daily for 3 to 5 days after harvest or until the CO_2 and O_2 concentrations had equilibrated. The average concentrations per package were plotted. Data were based on the average of 8 packages. Non-linear regression was performed on the raw data at $\alpha = 0.05$.

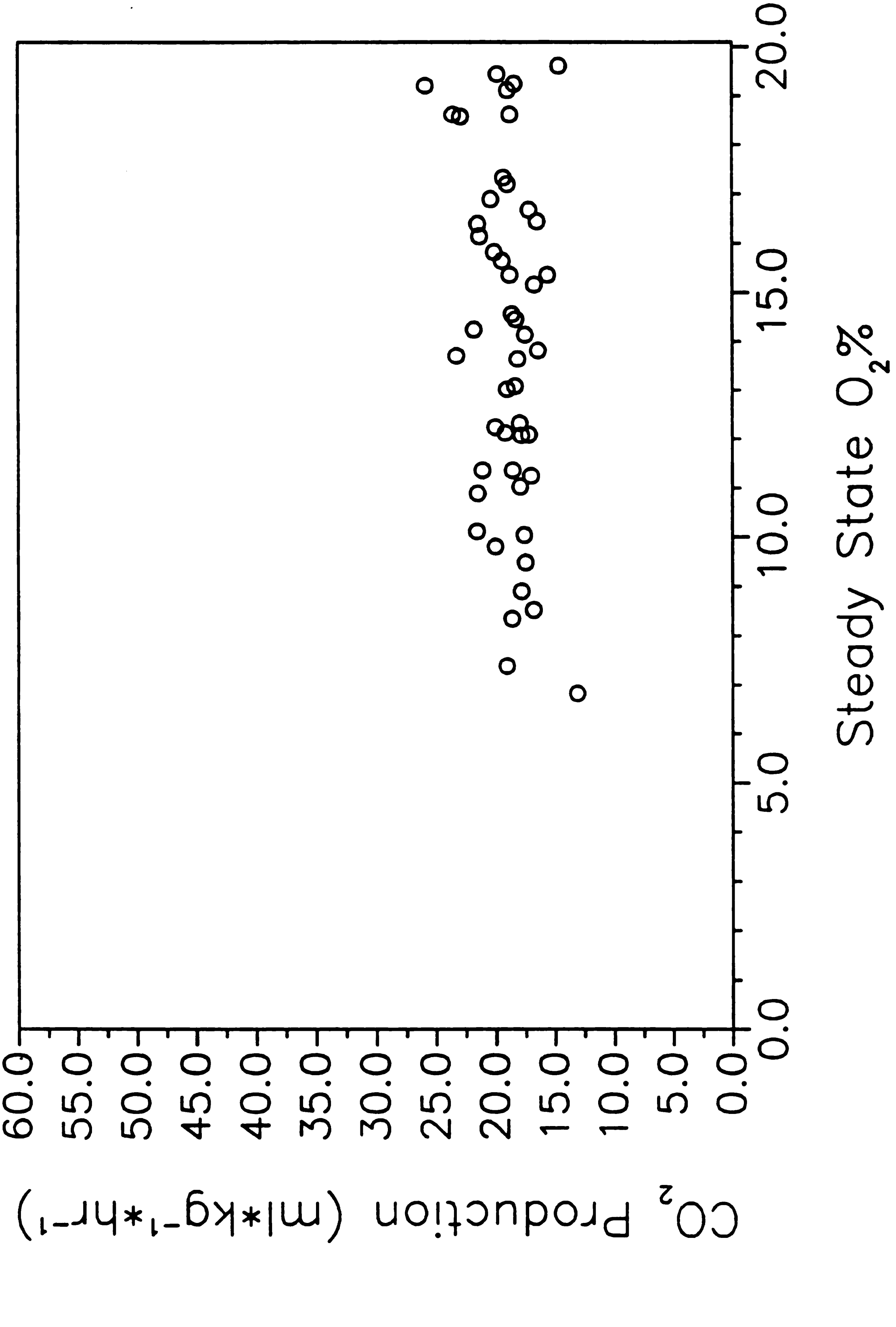


Y = 20.376 − .355X
R² = .968
Steady State O₂ (%)
Weight (g)
20.0
17.5
15.0
12.5
10.0
7.5
5.0
0
5
10
15
20
25
30
35
40
45

The effect of sweet basil weight on the CO_2 concentration present in the package at equilibrium. The basil cuttings were packaged into 3 mil, 800 cm^2 low-density polyethylene film at harvest. The packages were held at 20C in the dark. Gas analysis occurred once daily for 3 to 5 days after harvest or until the CO_2 and O_2 concentrations had equilibrated. The average concentrations per package were plotted. Data were based on the average of 8 packages. Non-linear regression was performed on the raw data at $\alpha = 0.05$.

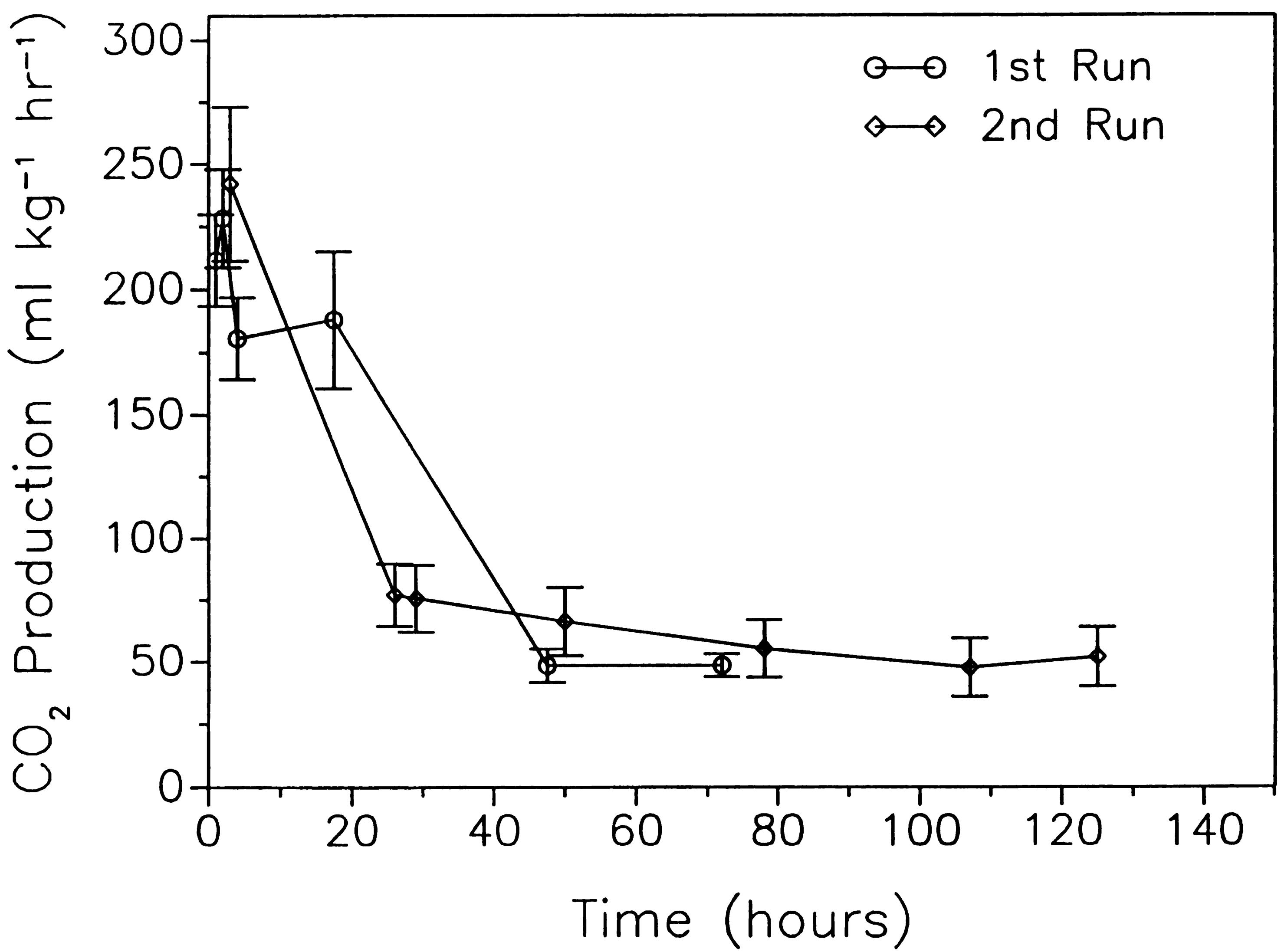

Y = .125 + .0704X
R² = .935
Steady State CO2 (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
45
Weight (g)

The effect of O_2 concentration at equilibrium on the CO_2 production of sweet basil cuttings. The basil cuttings were packaged into 3 mil, 800 cm^2 low-density polyethylene film at harvest. The packages were held at 20C in the dark. Gas analysis occurred once daily for 3 to 5 days after harvest or until the CO_2 and O_2 concentrations had equilibrated. The average concentrations per package were plotted. Data were based on the average of 8 packages.



CO_2 Production (ml*kg⁻¹*hr⁻¹)
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.0
5.0
10.0
15.0
20.0
Steady State O_2%

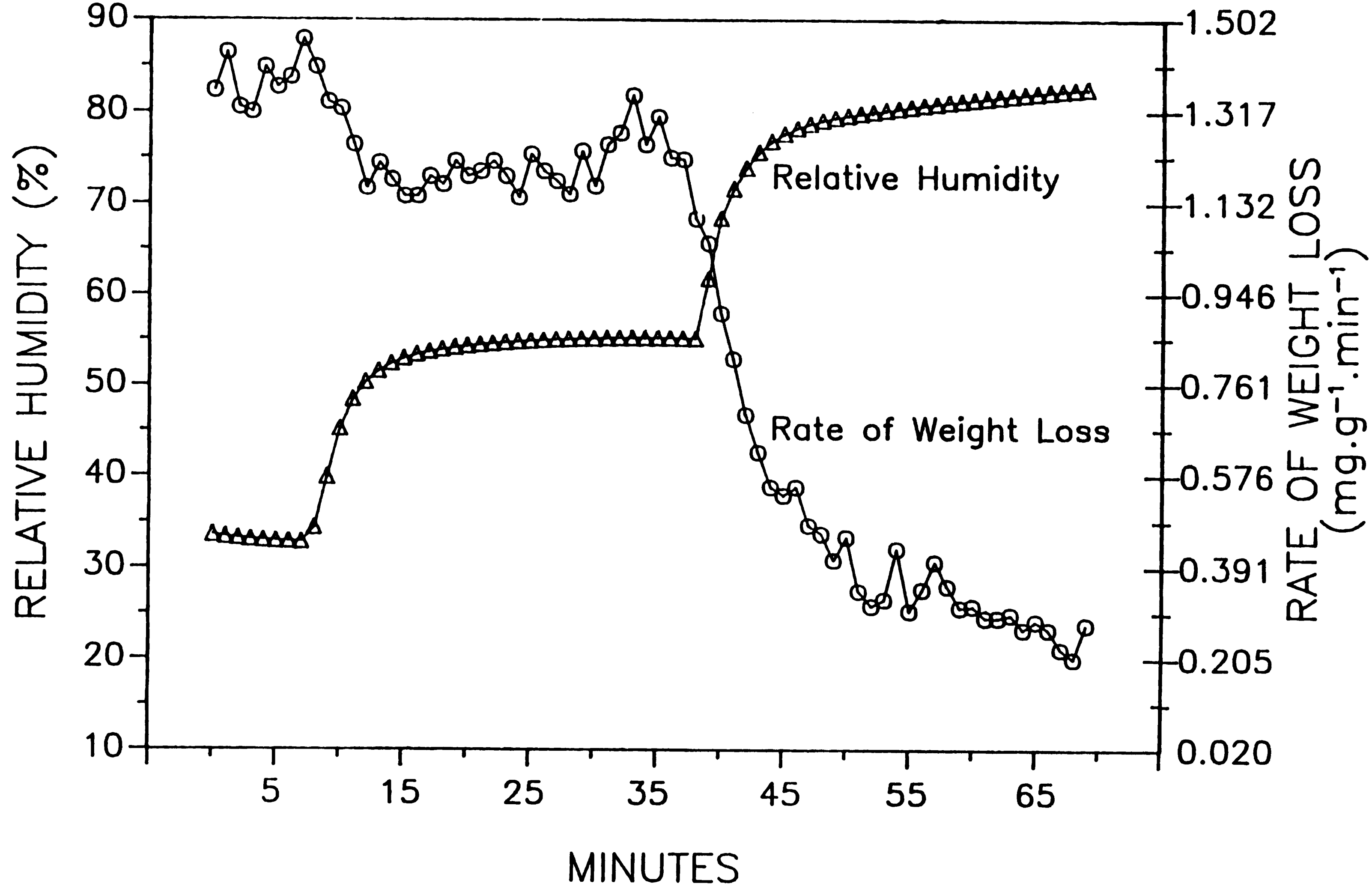
The respiration rate of sweet basil as determined in a flow-through system. The cuttings were harvested and immediately placed into containers connected to a continuous stream of air. The containers were held in at 20C in the dark. Means +/- s.d. were based on the average of 6 samples of basil per run.


1st Run
2nd Run
CO_2 Production (ml kg^{-1} hr^{-1})
0
50
100
150
200
250
300
0
20
40
60
80
100
120
140
Time (hours)

APPENDIX B

THE TRANSPIRATION RATE OF SWEET BASIL

The effect of 30, 50, and 80% RH on the transpiration rate of a sweet basil cutting at 23C. An ADC humidity generator supplied the desired relative humidities. The cutting weighed 0.732 grams. The weight of basil was recorded every minute using an analytical balance that was hooked up to a computer.



RELATIVE HUMIDITY (%)
90
80
70
60
50
40
30
20
10
5
15
25
35
45
55
65
MINUTES
Relative Humidity
Rate of Weight Loss
RATE OF WEIGHT LOSS
(mg.g⁻¹.min⁻¹)
1.502
1.317
1.132
0.946
0.761
0.576
0.391
0.205
0.020