¹⁴C Carbon-labeled Photosynthate Partitioning in *Ilex crenata* 'Rotundifolia' at Supraoptimal Root-zone Temperatures

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Abstract. *Ilex crenata* Thunb. 'Rotundifolia' split-root plants were grown for 3 weeks with root zones at 30/30, 30/34, 30/38, 30/42, 34/34, 38/38, and 42/42C. The 38C root-zone treatment was the upper threshold for several growth and physiological characteristics. A portion of the root system grown at or near the optimum temperature could compensate, in terms of shoot growth, for part of the root system exposed to supraoptimal root-zone temperatures up to 38C. Higher root-zone temperatures did not affect short-term photosynthetic rates or root : shoot ratios, but altered photosynthate partitioning to various stem and root sinks. Although no differences were found for total ¹⁴C partitioned to the roots, partitioning of ¹⁴C into soluble and insoluble fractions and the magnitude of root respiration and exudation were influenced by treatment. Heating half of a root system at 38C increased the amount of ¹⁴C respired from the heated side and increased the total CO₂ respired from the nonheated (30C) half. Exposure of both root halves to 42C resulted in membrane damage that increased the loss of ¹⁴C-labeled photosynthates through leakage into the medium.

Supraoptimal root-zone temperatures reduce the growth of container-grown plants. Temperatures as high as 58C have been recorded in media of container-grown plants in Florida (Martin and Ingram, 1988). Ingram et al. (1988) reported reductions in growth and changes in root distribution of *Ilex crenata* 'Helleri' when containers were exposed to direct radiation (average maximum of 50C in the western quadrant) compared to plants in shielded containers (average maximum of 42C in the western quadrant). Laboratory experiments have shown that roots of 'Helleri' holly were injured by a 30-min exposure to 51C (Ingram, 1986).

Foster (1986), working with *Ilex crenata* 'Rotundifolia', found more ¹⁴C-labeled photosynthate in leaf tissue and less in the root fraction when the root zone was maintained for 7 days at 28C compared to 40C. He hypothesized that 1) the decrease of labeled ¹⁴C in the roots with increasing temperature was due to a physical blockage, thereby decreasing the amount of partitioned label or 2) the decrease in the root was due to an increase in transport of labeled assimilates to the roots, with subsequent loss of label via root respiration at increased root-zone temperatures.

Environmental conditions that restrict plant growth alter the distribution of C between plant and soil fractions and C loss through respiration (Whipps, 1984). Roots depend on C assimilates imported from the shoot. Loss of C compounds from roots is influenced by several environmental factors, including temperature (Lambers, 1987). Import rate is determined by the use of assimilates in the sink organ. Wardlaw (1968) suggested that differences in development between shoots and roots may be due to competition for available C at high temperatures. The objectives of this study were to determine if 1) a portion of the root system of *Ilex crenata* 'Rotundifolia' grown at or near the optimum temperature could compensate, in terms of growth, for

a portion of the root system exposed to temperatures above optimum, 2) heating only a portion of the root system would alter the pattern of photosynthate partitioning to various plant parts, and 3) high root-zone temperatures caused increased loss of current photoassimilates through root respiration.

Materials and Methods

Stem-tip cuttings of *Ilex crenata* 'Rotundifolia' were taken during the first week of Sept. 1987 and rooted in an 80 perlite :20 sphagnum peat mixture (v/v) under intermittent mist for 12 weeks. Rooted cuttings were potted into paired 500-ml square plastic pots containing Metro-Mix 300 (W.R. Grace and Co., Cambridge, Mass.) with half of the roots in each container. After 12 weeks, the split-root plants were bare-rooted and transplanted into paired 1200-ml clear plastic bags containing 1100 cm³ washed quartz sand. Each bag was then placed into polyvinyl chloride cylinders (8 x 24 cm) that provided light exclusion and physical support. After 6 weeks of growth, the plants were moved from the greenhouse to a growth room. Experiments were initiated 1 week later and were conducted in a 3.0 x 7.6-m walk-in growth room. Irradiance was supplied by 18 1000-W phosphor-coated metal-arc HID lamps (GTE Sylvania Corp., Manchester, N.H.). A photosynthetic photon flux of 1000 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ was measured at canopy height by use of a quantum radiometer (LI-COR, Lincoln, Neb.). The photoperiod was 13 hr (0500 to 1800 HR), with the dark period interrupted for 3 hr (2200 to 0100 HR) by incandescent light. Air temperature was maintained at 28 $\pm$ 1C during the light period and 21 $\pm$ 1C during the dark period. Relative humidity was held at $\approx$ 40% during the light period and 90% during the dark period. Plants were watered as needed and fertilized twice weekly (200 ml) with a solution of 300 mg N/liter of soluble 20N-8.8P-16.6K (Peters 20-20-20, W.R. Grace and Co.).

Root-zone temperature combinations for the split-root systems (left/right) were 30/30, 30/34, 30/38, 30/42, 34/34, 38/38, and 42/42C. Treatments were imposed for 21 days in a randomized complete-block design replicated six times over 8 weeks. Root zones were maintained at $\pm$ 1C using an electronically controlled root heating system described by Foster (1986). Shoot length and branch number were recorded on days 1 and 21. On

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day 21, each plant was moved to an assimilation chamber for labeling studies.

Assimilation chamber. A split-root assimilation chamber consisting of three separate plexiglass compartments was designed for $^{14}\text{CO}_2$ -labeling the foliar portion of a plant, while allowing the collection of respired ^{14}C from the separate split-root halves maintained at treatment temperatures. The foliar compartment measured $14 \times 15 \times 28.5$ cm (L $\times$ W $\times$ H). Each split-root half compartment measured $14 \times 14 \times 28$ cm (L $\times$ W $\times$ H). Each of the three compartments had an additional volume measuring $9 \times 6 \times 19$ cm (L $\times$ W $\times$ H) to accommodate a circulating fan. Each box fan (Dayton Electric Manufacturing Co., Chicago), $7.9 \times 3.8 \times 7.9$ cm (L $\times$ W $\times$ H), circulated air in the chamber at a rate of $46 \text{ m}^3 \cdot \text{hr}^{-1}$.

$^{14}\text{CO}_2$ -labeling. Each plant was transported to the laboratory and sealed inside the assimilation chamber between 0730 and 0800 HR. Root-zone temperatures were electronically controlled as described by Foster (1986). A high-pressure sodium lamp (Lumalux 400W, GTE Sylvania Corp.) was used to provide a photosynthetic photon flux of 700 to $800 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ at canopy level. Shoot and root-zone temperatures were monitored using a digital thermocouple, thermometer (Cole Palmer Instrument Co., Chicago). Shoots were between $30 \pm 1^\circ\text{C}$ at the beginning of the labeling period and $32.5 \pm 1^\circ\text{C}$ 6 hr later. Relative humidity was monitored using an Airguide hygrometer (Chicago) and was maintained at $64\% \pm 3\%$.

The shoot chamber and air flow system were flushed with N_2 gas to zero the infrared gas analysis system (Anarad Model AR-600R, Anarad, Santa Barbara, Calif.). Each plant was then sealed in the chamber and flushed with N_2 for 2 min (CO_2 levels of $=150$ to $200 \mu\text{l} \cdot \text{liter}^{-1}$) before filling the shoot system with $311 \mu\text{l CO}_2/\text{liter}$. The system was then pulsed for 60 sec with $15.3 \mu\text{Ci}$ ($1 \text{ Ci} = 37 \text{ GBq}$) $^{14}\text{CO}_2/\text{liter}$. Total CO_2 uptake was monitored for 30 min using a closed system before another 60-sec pulse of $^{14}\text{CO}_2$ was added. After the 60-min pulse with $^{14}\text{CO}_2$, the shoot chamber was flushed with N_2 followed by a 5-hr chase period using ambient air ($380 \pm 20 \mu\text{l CO}_2/\text{liter}$). A circulating pump (Neptune Dyna-Pump, Universal Electric Co., Owosso, Mich.) and Dwyer flow meters (Dwyer Instruments, Michigan City, Ind.) were used to control the flow rate through the shoot compartment system at $1.5 \text{ liters} \cdot \text{min}^{-1}$.

Air was pumped through each of the root compartments during the 6 hr of treatment at a rate of $1.0 \text{ liter} \cdot \text{min}^{-1}$ after being bubbled through 400 ml of 0.2 N NaOH to reduce the CO_2 concentration to $=10 \mu\text{l CO}_2/\text{liter}$. The background level of CO_2 accounted for $<5\%$ of the total CO_2 trapped. Air exiting each root compartment was bubbled through 200 ml of 0.2 N NaOH to trap respired CO_2 .

Sampling and analysis. $^{14}\text{CO}_2$ -labeled plants were separated into leaves, stem, and root halves before being weighed. Samples were frozen in liquid N_2 , boiled in 80% (v/v) ethanol for 2 hr, and then ground in a Virtis homogenizer (Virtis Co., Gardiner, N.Y.). Leaf area was estimated using a prediction equation based on leaf fresh weight [$R^2 = 0.99$, leaf area = 5.20 (leaf fresh weight) + 39.37]. Ethanol-soluble material was separated from ethanol-insoluble material by filtering the ground extracts through filter paper and thoroughly washing with ethanol. Samples of ethanol-soluble material ($50 \mu\text{l}$) were added to 10 ml of scintillant (ScintiVerse II, Fisher Scientific, Orlando, Fla.) and 3 ml of distilled H_2O . Ethanol-insoluble material was oven-dried for 5 days at 63°C before adding a 0.02-g sample to the scintillant. Label in the potting medium was extracted by boiling 10-cm^3 samples in ethanol.

Excess BaCl_2 was added to the NaOH traps to precipitate the CO_2 as BaCO_3 . The precipitate was allowed to settle overnight, collected in centrifuge tubes, washed with deionized water, centrifuged for 5 min at 2200 rpm, and dried at 63°C for 7 days before analysis. The dried BaCO_3 was then ground into a fine powder, 0.05-g samples were added to 10 ml of scintillant with 3 ml of distilled H_2O , and analyzed via liquid scintillation spectrophotometry to quantify respired $^{14}\text{CO}_2$. Total respired CO_2 (plant plus microbial) was calculated from the total BaCO_3 collected as precipitate.

Radioactivity was determined via liquid scintillation spectrophotometry using a LKB 1214 liquid scintillation counter after a quench correction (LKB Wallac, Turku, Finland). Labeled C in the leaves, stems, root halves, growth medium, and C respired from the roots was expressed as a percentage of the total labeled C recovered from the plant. The data were analyzed as a general linear model (Statistical Analysis System, Raleigh, N.C.), with orthogonal contrasts designed to address research objectives.

Results and Discussion

Growth analysis. Growth values for the above-ground portions of *Ilex crenata* 'Rotundifolia' decreased as temperature increased when both root halves were heated in relation to the control ($30/30^\circ\text{C}$) (Table 1). Leaf area ($R^2 = 0.94$), leaf fresh weight ($R^2 = 0.95$), and shoot elongation ($R^2 = 0.96$) decreased quadratically with increasing root-zone temperatures from 30 to 42°C when both root halves were at the same temperature. Growth and morphological responses known to be influenced by root-zone temperatures include plant height; leaf expansion, shape, and color; root extension; branching; root and shoot dry weight; flowering; and fruiting (Cooper, 1973). Growth generally increases up to an optimum temperature and then decreases at higher temperatures.

Leaf area, shoot elongation, and leaf and shoot fresh weights decreased compared to the control when one-half of the root system was exposed to 42°C for 3 weeks (Table 1). Stem fresh weight decreased at $30/42^\circ\text{C}$ and $38/38^\circ\text{C}$ compared to $30/30^\circ\text{C}$ and $30/38^\circ\text{C}$, respectively. For leaf area and leaf and stem fresh weights, these results show that exposing one-half of the root system of 'Rotundifolia' holly to 38°C was less detrimental than exposing the whole root system to 38°C . However, holding one-half of the root system at 30°C could not prevent a reduction in top growth resulting from exposure of the other root half to 42°C . Shoot elongation decreased quadratically ($R^2 = 0.99$) as root-zone temperature increased when one-half of the root system was heated.

The only significant difference detected for total root fresh weight was between the $30/38^\circ\text{C}$ and $38/38^\circ\text{C}$ treatments (Table 1). The $30/38^\circ\text{C}$ treatment produced 1.8 times more root fresh weight. The ratio of left root fresh weight to right root fresh weight was not significantly influenced by treatment (data not shown). Harrison (1989) found no difference in the dry weight gain of *Ilex crenata* 'Rotundifolia' when grown with root zones at 28 , 34 , and 40°C for 6 weeks. Root-zone temperature up to 40°C for 6 hr daily did not affect shoot or root dry weight for ixora or citrus, but 40°C had decreased the root : shoot ratio after 90 days (Ingram et al., 1986a). Heating the right root half above 30°C did not influence the fresh weight of the left root half in our tests.

The partitioning of photoassimilates between shoots and roots and differences in root : shoot ratios are often influenced by root-zone temperatures (Cooper, 1973; Johnson and Ingram,

Table 1. Effects of root-zone temperature treatment of split-root systems on selected growth characteristics of *Ilex crenata* 'Rotundifolia' after 21 days.

Treatment (left/right)	Leaf area (cm ²)	Shoot elongation ^z (cm)	Fresh wt (g)				
			Leaf	Shoot	Total	Root	
						Left	Right
30/30	302	37	7.5	5.8	16.4	8.8	7.6
30/34	257	35	6.4	5.2	13.3	9.1	4.2
30/38	324	28	8.1	5.7	17.2	10.7	6.5
30/42	225	14	5.6	4.6	13.5	8.1	5.4
30/30	302	37	7.5	5.8	16.4	8.8	7.6
34/34	219	25	5.4	4.2	10.3	4.6	5.7
38/38	215	11	5.3	4.1	9.8	5.6	4.2
42/42	208	13	5.2	4.4	11.1	4.9	6.2
Contrasts							
30/30 vs. 30/34	NS	NS	NS	NS	NS	NS	*
30/30 vs. 30/38	NS	NS	NS	NS	NS	NS	NS
30/30 vs. 30/42	*	*	*	*	NS	NS	NS
30/34 vs. 34/34	NS	NS	NS	NS	NS	**	NS
30/38 vs. 38/38	NS	**	**	**	**	**	NS
30/42 vs. 42/42	NS	NS	NS	NS	NS	*	NS

^zMean initial shoot length was 52 cm.

NS, *, **Nonsignificant or significant at P = 0.05 or 0.01, respectively.

1984). Although differences were found in this study for individual growth criteria, no differences were detected among treatments for root : shoot ratios (data not shown). Total plant biomass ranged from 19.2 to 31.0 g. Therefore, while temperature influenced the overall size of the plants, the root : shoot ratios remained the same. In contrast, Ingram et al. (1988), using various container-spacing strategies to study the influence of temperature on *Ilex crenata* 'Helleri' growth through a growing season, found decreased root growth and subsequently decreased root : shoot ratios associated with elevated root-zone temperatures. Lack of root : shoot ratio differences in this study were probably due to the short growth period (3 weeks).

Distribution of ¹⁴C-photosynthates. Photosynthetic rates were measured during the first 30 min of the labeling period. Although photosynthetic rates ranged from 2.3 (30/30C) to 3.6 μ mol CO₂/sec per square meter (38/38 C), no differences were detected in response to root-zone temperature, probably due to a large degree of variability within treatments. Foster (1986) found that shoot C exchange rates decreased in 'Rotundifolia' holly when grown with root-zones at 36 and 40C for 1 week. Lack of differences after 3 weeks at supraoptimal root-zone temperatures may have been due to changes in leaf protein and pigment contents (Ruter, 1989). Other researchers have also reported decreases in photosynthetic rates in response to long-term exposure of woody plants to supraoptimal root-zone temperatures (Gur et al., 1972; Johnson and Ingram, 1984). Elevated demands by sink tissues for assimilates can increase photosynthetic supply by raising rates of CO₂ fixation (Gifford and Evans, 1981).

For total ¹⁴C recovered in the leaf or root fractions (Table 2), no significant differences were detected among treatment comparisons, but differences were detected for total recovered ¹⁴C in the stem fraction. Percent ¹⁴C in the stem for the 30/34, 30/38, and 30/42C treatments decreased compared to the 30/30C control. No differences were detected for the percentage of labeled photosynthate remaining in the leaves; this result may indicate that the roots depended on a current supply of assimilates available from the stem at most temperature/split-root treatment combinations.

Table 2. Percent of recovered ¹⁴C partitioned to leaf, stem, and total root (both root halves) tissue of *Ilex crenata* 'Rotundifolia' as influenced by root-zone temperatures. Plants were pulsed with ¹⁴CO₂ for 1 hr, followed by a 5-hr chase with ambient air. The percent ¹⁴C partitioned to the stem is also expressed as the percentage occurring in the soluble and insoluble fraction. Each value is the mean of six replicate plants.

Treatment (left/right)	Leaf	Stem	Stem (soluble)	Stem (insoluble)	Root
Percent					
30/30	71.0	16.1	77.4	22.6	12.9
30/34	71.6	12.3	73.6	26.4	16.1
30/38	70.5	11.9	71.5	28.5	17.6
30/42	74.9	10.3	80.9	19.1	14.8
30/30	71.0	16.1	77.4	22.6	12.9
34/34	77.2	11.2	71.1	28.9	11.6
38/38	68.9	8.8	79.3	20.7	22.3
42/42	71.6	11.2	81.8	18.2	17.2
Contrasts					
30/30 vs. 30/34	NS	*	NS	NS	NS
30/30 vs. 30/38	NS	**	NS	NS	NS
30/30 vs. 30/42	NS	**	NS	NS	NS
30/34 vs. 34/34	NS	NS	NS	NS	NS
30/38 vs. 38/38	NS	*	*	*	NS
30/42 vs. 42/42	NS	NS	NS	NS	NS

NS, *, **Nonsignificant or significant at P = 0.05 or 0.01, respectively.

A greater portion of the ¹⁴C in the stem was in the insoluble fraction for the 30/38C treatment (28.5%) than for the 38/38C (20.7%) treatment (Table 2). This effect was also evident when data were expressed on a fresh-weight basis. A greater demand for readily available assimilates in the rapidly respiring roots was indicated compared to the stem, since there was a corresponding increase in the soluble stem fraction at 38/38C.

Differences were detected in the percentage of ¹⁴C recovered from the insoluble fractions in the roots and in the potting medium (Table 3), although no differences were detected among treatments for the percent recovered ¹⁴C that was transported to the roots (Table 2). The percent recovered ¹⁴C in the insoluble root fraction was reduced when one-half of the root system was

exposed to 34, 38, or 42C for 6 hr daily. No additional reduction in the percent recovered ^{14}C in the insoluble fraction was noted when both root halves were at elevated temperatures. There was 3.2 times more ^{14}C recovered in the medium fraction when both root halves were grown at 42C than when plants were grown at 30/30 and 30/42C.

Temperature influenced the allocation of ^{14}C -labeled photosynthates to the various fractions in each root segment (Table 4). The percentage of recovered ^{14}C in the soluble fraction of the left root half exposed to 30C was increased by a factor of 1.7 relative to 42C and the percentage recovered from the soluble fraction of the root half exposed to 42C was decreased. About twice as much total percent ^{14}C was recovered from the

30C left root half of the 30/42C treatment as from the 42/42C treatment. Roots at 30/34, 30/38, and 30/42C had about one-third the amount of ^{14}C in the insoluble fraction of the right root half held at the higher temperature than in the control (Table 4). At 42/42C, the percent recovered ^{14}C in the insoluble and soluble fractions of the right root half was 3- and 2-fold higher, respectively, than found in the 42C half of the 30/42C treatment, possibly indicating preferential partitioning to the nonheated 30C half.

The percent total ^{14}C (insoluble + soluble) in roots held at 30C was 1.6-, 2.4-, and 4.3-fold higher than in roots on the right side that were held at 34, 38, or 42C, respectively (Table 4). For the 30/30, 30/34, and 30/38C treatments, the percent recovered ^{14}C for each root half was =1:1. At 30/42C, this ratio was =3: 1. There was a difference in partitioning of total ^{14}C to each root half when only one side of the root system was grown at 42C.

The only difference in amounts of respiratory $^{14}\text{CO}_2$ released from roots was for the warmed half of the 30/38C treatment relative to both root halves at 30C (Table 5). At 30/38C, 84% of the total ^{14}C respired from both root halves came from the root half exposed to 38C, even though the fresh weight of roots in the half at 30C was 1.8 times higher than that in the half at 38C. Total CO_2 respired (Table 5) from the left root half was 1.8 times higher for 30/38C than for 30/30C, although this difference was not evident when expressed on a weight basis. More current nonlabeled photosynthates or previously accumulated photosynthates may have been respired from the nonheated portion of the root system in relation to the warmer right root half, although no increase occurred in ^{14}C respired from the nonheated root half. Kouchi et al. (1986) used a compartmental analysis model to study the partitioning of photoassimilated C in soybean plants and found the soluble pool in roots acted differently from soluble pools in other organs. This result suggested that the soluble fraction of the root can temporarily store C as a reserve respiratory pool. Such partitioning may occur in the nonheated root halves and could function to compensate for increases in currently respired ^{14}C and decreased soluble and insoluble C pools that occur in the root halves at the higher temperatures.

Table 3. Effect of root-zone temperature (21 days) on the allocation of insoluble and soluble below-ground ^{14}C -labeled assimilates between root tissue, respired $^{14}\text{CO}_2$, and the potting medium after a 1-hr pulse followed by a 5-hr chase with ambient air. The data presented are the total for both root halves and each value is the mean of six replicate plants.

Treatment (left/right)	Root tissue		Respired	Medium
	Insoluble	Soluble		
	Percent			
30/30	14.6	60.2	19.0	6.2
30/34	10.0	52.0	30.8	7.2
30/38	8.0	50.0	37.3	4.7
30/42	9.9	68.0	15.8	6.3
30/30	14.6	60.2	19.0	6.2
34/34	9.4	60.6	21.5	8.6
38/38	9.0	48.4	32.5	10.1
42/42	11.6	58.1	10.0	20.3
Contrasts				
30/30 vs. 30/34	*	NS	NS	NS
30/30 vs. 30/38	**	NS	NS	NS
30/30 vs. 30/42	**	NS	NS	NS
30/34 vs. 34/34	NS	NS	NS	NS
30/38 vs. 38/38	NS	NS	NS	NS
30/42 vs. 42/42	NS	NS	NS	*

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Table 4. Percent of total below-ground ^{14}C -labeled assimilates recovered from half-root systems of split-root *Ilex crenata* 'Rotundifolia' plants as affected by root-zone temperature. Plants were treated for 21 days at the respective root-zone temperatures. Plants were labeled with $^{14}\text{CO}_2$ for 1 hr followed by a 5-hr chase with ambient air. Each value is the mean of six replications:

Treatment (left/right)	Left root				Right root			
	Insoluble	Soluble	Respired	Medium	Insoluble	Soluble	Respired	Medium
	Percent							
30/30	8.2	32.3	10.1	3.2	6.5	27.9	8.9	2.9
30/34	7.8	30.4	10.8	3.9	2.2	21.6	20.0	3.3
30/38	5.7	35.1	6.0	2.4	2.3	14.9	31.3	2.3
30/42	7.8	55.4	8.0	3.3	2.1	12.7	7.7	3.0
30/30	8.2	32.3	10.1	3.2	6.5	27.9	8.9	2.9
34/34	4.4	26.5	15.8	4.3	5.0	34.1	5.7	4.2
38/38	6.2	31.4	10.7	5.2	2.8	16.9	21.9	4.9
42/42	5.6	26.5	4.8	10.4	6.0	31.6	5.2	9.9
Contrasts								
30/30 vs. 30/34	NS	NS	NS	NS	**	NS	NS	NS
30/30 vs. 30/38	NS	NS	NS	NS	**	*	**	NS
30/30 vs. 30/42	NS	**	NS	NS	**	**	NS	NS
30/34 vs. 34/34	NS	NS	NS	NS	NS	*	NS	NS
30/38 vs. 38/38	NS	NS	NS	NS	NS	NS	NS	NS
30/42 vs. 42/42	NS	**	NS	**	**	**	NS	**

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Table 5. Effect of root-zone temperature treatment on total CO₂ respired from each half-root system of split-root *Ilex crenata* 'Rotundifolia' plants.

Treatment (left/right)	CO ₂ (mg·hr ⁻¹)		CO ₂ (mg·hr ⁻¹ ·g ⁻¹ fresh wt)	
	Left	Right	Left	Right
30/30	18.5	21.5	2.33	2.83
30/34	28.5	25.0	3.67	6.83
30/38	32.8	21.3	4.17	3.33
30/42	23.8	22.3	4.33	6.00
30/30	18.5	21.5	2.33	2.83
34/34	22.7	18.0	6.17	3.16
38/38	24.5	24.5	5.17	7.50
42/42	28.0	24.5	6.00	4.67
Contrasts				
30/30 vs. 30/34	NS	NS	NS	*
30/30 vs. 30/38	*	NS	NS	NS
30/30 vs. 30/42	NS	NS	NS	NS
30/34 vs. 34/34	NS	NS	NS	*
30/38 vs. 38/38	NS	NS	NS	*
30/42 vs. 42/42	NS	NS	NS	NS

NS, *, ** Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Increases in total CO₂ respired per gram fresh weight (Table 5) were detected between 30/30C vs. 30/34C and 30/38C vs. 38/38C for the warmer right root half. No differences were detected for total CO₂ respired from the right root half for any of the treatment comparisons. Increases in current ¹⁴C respired could indicate a corresponding rise in the demand for readily available respiratory substrates, which, in turn, is likely to correspond to decreased insoluble and soluble photosynthates in the right root segments. Recently fixed C has been found to dominate the C released by plant roots as respired CO₂ (Meharg and Killham, 1988). Since no differences were detected for total CO₂ respired from the higher-temperature right root halves, this implied that long-term increases in root-zone temperature from 30 to 42C were not reflected in rates of respiratory CO₂ loss at the end of the 3 weeks. The short-term response of respiration rates in actively growing tissues about doubles with each 10C increase in temperature up to 45C. Shishido et al. (1987) found =75% of the imported labeled C to be lost from cucumber roots during 24 hr as temperature increased from 15 to 30C. Although in our tests, total CO₂ respired from the left (30C) root was higher at 30/38C than at 30/30C, there was no difference in the amount of currently labeled ¹⁴C respired from this portion of the root. More labeled ¹⁴C was respired from the 38C right root half of the 30/38C treatment, but the total respired CO₂ did not differ between halves. Therefore, the portion of the root system at 38C may have been a greater sink for current photosynthate than the root half at 30C. This result indicates that the unheated root half at 30C respired more previously fixed nonlabeled C.

Increased root-zone temperatures have been shown to increase the maintenance portion of root respiration in sunflower (Szaniawski and Kielkiewicz, 1982). Supraoptimal temperatures are known to increase maintenance respiration while causing decreases in growth due to depletion of the available carbohydrate pool (Gent and Enoch, 1983). Increased maintenance respiration with a corresponding decrease in growth respiration, due to greater maintenance costs for the root zone at 38C, could account for decreased root fresh weights (Table 1) and shifts in ¹⁴C partitioned to soluble fractions (Table 4). Lambers (1987) has suggested that 15% to 30% of all photosynthates are respired by roots, with an estimated 10% of daily photosynthesis going toward maintenance costs of the roots. The warmer

right root half of the 30/38C treatment respired 5.5% of the total ¹⁴C recovered from the plant after 6 hr of labeling (Tables 2–4). Respired ¹⁴C from both root halves of the 38/38C treatment accounted for 7.3% of the total recovered ¹⁴C. These respiration values are in line with those projected by Lambers (1987) if respiration values are considered over 24 hr.

Compared to the 30/42C treatment, a significant increase was detected in the root ¹⁴C media fractions (percent of total) for the left and right root halves at 42/42C. Loss of cellular components can increase with increasing temperatures due to loss of membrane integrity (Levitt, 1980). Critically high temperatures leading to 50% electrolyte leakage from root cell membranes of various woody plants decreased linearly as exposure time increased exponentially (Ingram, 1985, 1986; Ingram et al., 1986b). The predicted critical temperature for hex *crenata* 'Rotundifolia' after a 30-min exposure was 48 ± 1.5 C (Ruter, 1989). Foster (1986), using nonacclimated 'Rotundifolia' holly liners, found root-zone temperatures of 45C for 6 hr daily to be lethal within 48 hr. It appears that a 3-week exposure of both root halves to 42C caused enough membrane damage to increase the loss of labeled photosynthates into the growth medium. Preferential partitioning of photosynthates to the nonheated left root half at 30/42C may account for the lack of ¹⁴C found in the medium fraction from the 42C right root half for this treatment.

Photosynthates transported to the roots may be used by a variety of C sinks that include exudation, growth, and respiratory processes. The loss of C by exudation is generally considered to be $\leq 5\%$ of the total partitioned photosynthate, although this may vary under adverse conditions (Lambers, 1987). About 30% of the total ¹⁴C partitioned to the roots was lost through root leakage and respired CO₂ at 42/42C, which could have been fixed by soil microorganisms. During the 6 hr of treatment, 3.5% of the total recovered ¹⁴C was from the medium fraction for the 42/42C treatment (Tables 2 and 3). The total ¹⁴C found in or respired from both root halves at 42/42C was over three times greater than the ¹⁴C found in the medium at 30/42C.

In conclusion, 38C appeared to be the upper threshold for root-zone temperature tolerance, as indicated by a number of physiological and growth values in 'Rotundifolia' holly. Half of the root system exposed to 30C can partially compensate in terms of shoot growth for detrimental effects to the root half at 34 or 38C. When both root halves were grown at 38C or just half of the root system was exposed to 42C, shoot growth decreased. Compensation is important because the temperature profile within a container will vary several degrees depending on the orientation of the wall to incoming solar radiation (Martin and Ingram, 1988). Although total plant biomass varied between treatments, higher than optimal root-zone temperatures did not appear to alter photosynthetic rates or root : shoot ratios. Therefore, while high root-zone temperatures affected overall plant growth, increasing root-zone temperatures did not affect C partitioning so as to alter photosynthesis.

Foster (1986) proposed that decreases in the amount of ¹⁴C found in the root system of 'Rotundifolia' holly when exposed to root-zone temperatures from 28 to 42C could be due to a physical 'blockage' of the vascular system or an increase in partitioning to the roots, with subsequent loss of ¹⁴C due to increased respiration rates. A physical blockage of the vascular system did not appear likely since no difference in the percent total recovered ¹⁴C partitioned to the roots was found. However, the increased loss of respired ¹⁴C at 38C could account for the decrease in the labeled root fraction noted by Foster (1986). When both root halves were exposed to 42C, the primary loss

of current ^{14}C -labeled photosynthate most likely was through leakage due to loss of membrane integrity.

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