

## The Effect of Temperature, Photoperiod, and Light Quality on Gluconasturtiin Concentration in Watercress (*Nasturtium officinale* R. Br.)

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The effects of different growth regimes on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.) were investigated. Watercress plantlets at the 5th mature leaf stage (ca. 2 weeks old) were exposed to different day and night temperatures, to long (16 h) or short (8 h) days, to red (R) or far-red (FR) light given during the main long day photoperiod, and finally to R or FR light given at the end of the main photoperiod. Watercress plants grown under long days contained a 30–40% higher gluconasturtiin concentration and had a higher fresh weight than watercress plants grown under short days. Watercress plants grown under long days and temperatures of 15 or 10 °C had at least a 50% higher gluconasturtiin concentration, but a lower fresh weight, than that of plants grown at 20 or 25 °C. Watercress plants grown under metal halide light enriched with R light had approximately a 25–40% higher concentration of gluconasturtiin as compared to the FR-enriched plants. Likewise, a brief R light exposure at the end of the main photoperiod resulted in approximately a 25% or higher concentration of gluconasturtiin as compared to a FR end-of-day exposure. These data indicate that the concentration of gluconasturtiin in watercress can be significantly increased by growing plants at lower temperatures, under long days, and by exposure to R light.

**KEYWORDS:** Chemoprevention; glucosinolates; gluconasturtiin; HPLC; isothiocyanates; light quality; temperature; PEITC; phenethyl isothiocyanate; photoperiod; phytochrome; watercress; *Nasturtium officinale* R. Br.

### INTRODUCTION

Glucosinolates are a family of natural plant compounds predominately found in the Brassicaceae family. Their chemical structure varies, but they characteristically have a  $\beta$ -thioglucose moiety, a sulfonated oxime moiety, and a variable side chain. Glucosinolates in vegetable crops contribute to flavor and have potential as anticarcinogens (1). Hydrolysis of glucosinolates leads to products with biological activity that can have positive and negative nutritional effects. Hydrolysis products also affect plant–herbivore interactions.

Watercress (*Nasturtium officinale* R. Br.) is a perennial herb found in abundance near springs and open-running waterways in Europe and the Americas and is a rich source of 2-phenethyl glucosinolate (gluconasturtiin). The hydrolysis product of gluconasturtiin, 2-phenethyl isothiocyanate (PEITC), is believed to be cancer preventing by inhibition of phase I enzymes (mostly monooxygenases and cytochrome P450s, resulting in addition of a single atom of oxygen as a hydroxyl, ketone, or epoxide to a foreign compound) and by induction of phase II enzymes

(leading to the formation of any of several classes of conjugates), thus resulting in carcinogen excretion (2, 3).

**Environment and Glucosinolates—Photoperiod, Temperature, and Light Quality.** Palaniswamy et al. (4) studied the effect of light level, photoperiod, and temperature on PEITC content of 21 day old watercress plants. They found that watercress grown with 12 h of light produced more PEITC as compared to plants grown under an 8 h day length. A temperature of 25 °C resulted in higher amounts of PEITC as compared to 15 °C.

Later, Palaniswamy et al. (5) reported on the ontogenic variations of PEITC concentrations in watercress leaves. They found that the highest concentration of PEITC was in the top mature leaves between 40 and 60 days after transplanting. Watercress plants at these stages had reached the highest fresh and dry weights. However, one limitation of their study was that they only collected PEITC data on the top three mature leaves (5); they did not address the question of whether PEITC concentrations might vary if stems and older leaves are included in the analysis.

Antonious et al. (6) found that colored mulches affected the total glucosinolate content of turnip (*Brassica rapa* L.). Turnip

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plants exposed to blue mulch showed higher total glucosinolates in root tissue than the plants exposed to green or white mulch. In another study, Loughrin and Kasperbauer (7) found that mulch color and thus light quality altered the aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves.

Light quality also has an effect on watercress germination (8). For example, it was shown that at 15 °C, 3.5% of watercress seed germinated in the dark. When watercress seed was exposed to 10 min of red light (R), the germination rate increased to 41.5%, and when the R light was followed by 20 min of far-red light (FR), the germination rate decreased to 5.0%. The reduction in germination of watercress seed due to an exposure of FR light suggests photoreversibility of germination and indicates that watercress germination is under the control of phytochrome. In their study of seed germination, Biddington and Ling (8) did not go on to investigate the role of R or FR on glucosinolate concentration in the watercress plants produced.

The total glucosinolate concentration, among other secondary plant metabolites, seems to vary due to environmental fluctuations in photoperiod, light quality, and temperature. However, there is a paucity of data in the literature on how these environmental factors affect gluconasturtiin concentration in watercress. Watercress is grown commercially both in natural streams and also in hydroponic cultures in greenhouses where the growing environment can be controlled. To determine conditions for potential commercial production of glucosinolate-optimized watercress, we initiated controlled-environment studies on the effect of photoperiod, temperature, and light quality on gluconasturtiin concentration.

## MATERIALS AND METHODS

**Effects of Photoperiod.** Watercress (*N. officinale* R. Br., Johnny's Selected Seeds, Albion, ME) was seeded in 12.5 cm square pots in moist soil-less media (SunGro Horticulture, SunShine SB-300 Universal, Bellevue, WA) containing sphagnum peat, bark, perlite, and vermiculite and grown in two growth chambers (model GCW-15, Environmental Growth Chambers, Chagrin Falls, OH). Emerging seedlings were grown at constant 20 °C and under long days [long-day photoperiod (LD), 16 h, photosynthetically active radiation (PAR) = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , R:FR = 2.1; the R:FR ratio was defined as the irradiance from 650 to 670 nm divided by the irradiance from 720 to 740 nm]. Plantlets were thinned to one plant per pot 1 week after seedling emergence. When plantlets reached the 5th mature leaf stage (approximately 14 days after seeding), 30 randomly chosen plants were exposed to LDs of 16 h in one growth chamber, and in a different growth chamber, 30 randomly chosen plants were exposed to short days (SD) of 8 h. The light source consisted of six Philips 400 W metal halide (MH) lamps delivering PAR = 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (the PAR is the total irradiance between 400 and 700 nm) and a R:FR = 2.1. The ambient temperature was constant at 20 °C day and night. Plants were watered daily and fertilized every 2 days with 100 ppm N by 15-5-15 Ca and Mg Peters Exel Water Soluble Fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH). All above ground tissue of sample plants was harvested, frozen in liquid nitrogen, and stored at -80 °C at the start of the photoperiod experiment and after 1 and 2 weeks of exposure to LD or SD.

**Effects of Temperature.** Watercress plants were grown at 20 °C under LD (16 h) to the 5th mature leaf stage as described above. When this stage was reached, the randomly chosen plantlets were moved to different growth chambers: constant at 25 °C, constant at 20 °C, constant at 15 °C, and constant at 10 °C. In addition, one growth chamber was set at a day temperature of 20 °C and a night temperature of 15 °C. Each temperature treatment contained 30 plants. All chambers had a 16 h photoperiod using metal halide lamps; PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and R:FR = 2.1. Watercress plants were watered and fertilized as described above. Individual plants were weighed (fresh weight, FW) and frozen after 1 or 2 weeks of growth at the indicated temperature.

**FR and R Light Enrichment.** Watercress plants were grown at 20 °C under LD (16 h) to the 5th mature leaf stage as described above. When this stage was reached, 90 randomly chosen plantlets were moved to different growth chambers under LD photoperiods: one with MH lamps enriched with FR light, one with MH lamps enriched with R light, and one chamber with MH lamps without additional light (control). The total PAR = 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The R light was provided by three R fluorescent lamps (Sylvania F48T12/2364/HO) filtered through an Encapsulite red tube guard (Lighting Plastics of Minnesota, St. Louis Park, MN). The plants grown under the R light regime were exposed to a R:FR = 3.5. The FR light was provided by six FR fluorescent lamps (Sylvania F48T12/232/HO) filtered through an Encapsulite FR tube guard (Lighting Plastics of Minnesota). The plants grown under the FR light regime were exposed to a R:FR = 1.5. All chambers were adjusted to equal PAR at the plant level by altering the distance between the lights and the plants. In the R growth chamber, the R lamps provided about 7.5% of the total PAR, and the contribution of the FR lamps to PAR was, as expected, negligible (about 0.1%). The R and FR lamps were switched off 1 min after the MH lamps because the MH lamps emitted a glimmer of light for about 30 s after being switched off. Plants were watered and fertilized as described under the photoperiod experiment. Individual watercress plants were harvested after 1 and 2 weeks of variation in light quality.

**End-of-Day R or FR Exposure.** Watercress plants were grown at 20 °C under LD (16 h) to the 5th mature leaf stage as described above. When this stage was reached, randomly chosen plantlets were moved to different growth chambers: (i) In one chamber, plants received no end-of-day light treatment after 16 h of MH light. (ii) In the second chamber, watercress plants were exposed to 16 h of MH, and at the end of the main photoperiod, they were exposed to 10 min of R light [provided by three R fluorescent lamps (Sylvania F48T12/2364/HO) filtered through an Encapsulite red tube guard (Lighting Plastics of Minnesota)]. (iii) In the third growth chamber, watercress plants were exposed to 16 h of MH, and at the end of the main photoperiod, they were exposed to 15 min of FR light [provided by six FR fluorescent lamps (Sylvania F48T12/232/HO) filtered through an Encapsulite FR tube guard (Lighting Plastics of Minnesota)]. PAR = 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in all three chambers. Plants were watered and fertilized as described under the photoperiod experiment. Individual watercress plants were harvested after 1 and 2 weeks of variation in light quality at the end of the day.

In the four experiments described above, all sampling was carried out between 13:00 h and 14:00 h. Preliminary experiments indicated that the gluconasturtiin concentration stabilizes after 13:00, after the lights had been on for 6 h (data not shown). Rosa (9) showed that in cabbage a similar trend in the leveling off of total glucosinolates occurred after 13:00 h (i.e., 6 h into the main photoperiod).

In all of the above experiments, data were obtained on FW of the plants and gluconasturtiin concentration at the end of the growth period. Usually, eight randomly chosen plants were individually weighed, extracted, and analyzed per experimental treatment. In a few experiments, five individual plants were randomly chosen from each treatment group for dry weight (DW) determination. Each experiment was replicated at least three times over time with substantially the same results, and data from a representative experiment are presented. All experimental treatments were arranged in a completely randomized design. Data are expressed as means  $\pm$  standard error (SE). Analysis of variance was calculated, and means were compared using the least significant difference (LSD) test at  $P < 0.05$ . All data were analyzed with the Statistix (Analytical Software, St. Paul, MN) program.

**Light Measurements.** PAR was measured with an Apogee Quantum Meter, model QMSW-SS (Apogee Instruments, Inc., Logan, UT), and spectral quality, which allowed calculation of R:FR ratios, was measured with an Apogee model SPEC-UV/PAR Spectroradiometer (Apogee Instruments, Inc.). The PAR was measured twice weekly, and height adjustments were made to keep the irradiance level as specified across the growth chamber. The watercress plants under each light treatment were rotated once weekly from the center to the edge of the growth chamber to avoid irradiance level discrepancies (e.g., slightly higher PAR in the center of the growth chamber as compared to the edge).

**Glucosinolate Extraction.** Frozen watercress samples were ground with a mortar and pestle that had been prechilled with liquid nitrogen. The ground plant material was transferred into rapidly boiling deionized distilled water, and boiling was continued for 10 min. Boiling water extraction was used to avoid handling large amounts of hot methanol, the extraction solvent often employed [see, for example, Prestera et al. (10)]. Preliminary experiments indicated comparable recoveries (data not shown). The plant extract was filtered through Whatman #1 (9 cm) filter paper, and the filtered extract was brought up to a final volume of 20 mL g<sup>-1</sup> FW of tissue. The aqueous extract was mixed thoroughly, and aliquots of 15 mL were transferred to glass vials for ammonium sulfate precipitation. Ammonium sulfate was added at 2.8 g per 5 mL extract, the samples were left overnight at 4 °C to precipitate extracted protein, and then, the precipitate was removed by centrifugation at 10000g for 30 min. Ammonium sulfate precipitation was found to reduce protein content and allowed for longer high-performance liquid chromatography (HPLC) column life. Finally, the aqueous extract was filtered using a 0.2 μm syringe-fitted filter unit (Nalge Co., Rochester, NY). The filtrate was stored at -20 °C prior to analysis by HPLC.

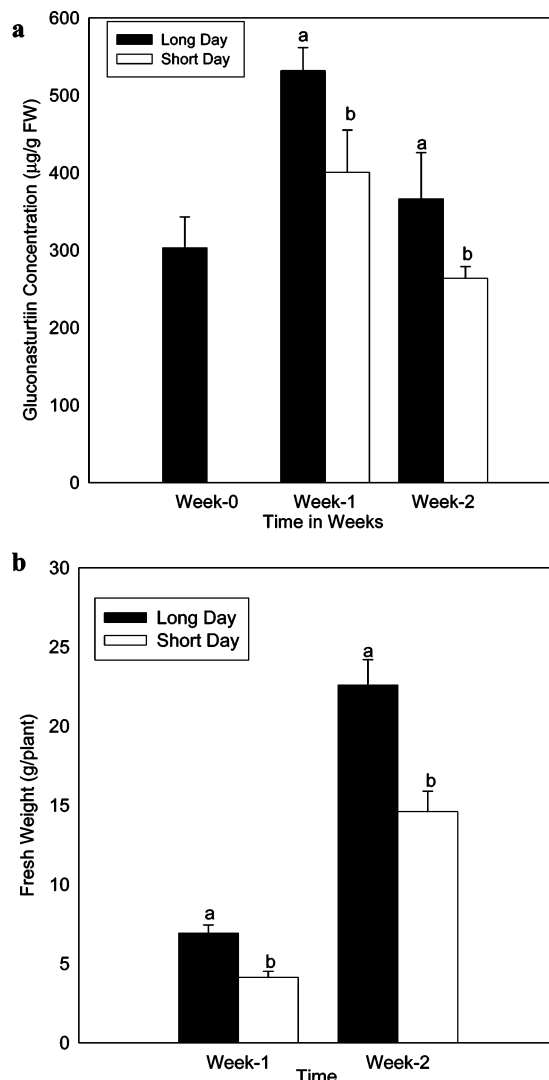
When a gluconasturtiin standard was added to the boiling water extraction at a level approximately equal to the endogenous level, approximately 80% of the added gluconasturtiin was recovered through the purification procedures, as determined by HPLC analysis. For both added and endogenous gluconasturtiin (nonspiked samples), about 90% of the total extractable compound was recovered in a single extraction step, and a second extraction yielded 10% or less additional compound. A third extraction yielded no additional gluconasturtiin (L. Wong, personal communication).

**Glucosinolate Analysis.** Gluconasturtiin and other glucosinolates were separated and quantified by reverse phase HPLC on a Waters system (Dual model 6000A pumps and a model 680 controller, Waters Associates, Milford, MA) equipped with a Waters model 712 WISP autosampler, a UV detector (model 783A, Applied Biosystems, Foster City, CA) set at 235 nm, and a Hewlett-Packard (Palo Alto, CA) model 3396 integrator. We utilized an Ultra Aqueous C18 (150 mm × 4.6 mm, 5 μm) column (#9178565, Restek Corp., Bellefonte, PA; see application note 59335, <http://www.restekcorp.com/restek/images/external/59335.pdf>). The HPLC protocol was modified from Lewke et al. (11): The flow rate was 1.5 mL min<sup>-1</sup> using a gradient of 0.1 M ammonium acetate (solvent A) and 30% methanol in 0.1 M ammonium acetate (solvent B). The elution gradient was as follows: 0–4 min, 100% A; 4–14 min, linear gradient to 70% B; 14–17 min, linear gradient to 100% B; hold at 100% B for 5 min; 22–23.5 min, linear gradient to 100% A. A 15.5 min post-run reequilibration with 100% A followed each separation sequence. Gluconasturtiin was identified by coelution with a purified gluconasturtiin standard (LKT Laboratories, St. Paul, MN); that is, the same peak (with a retention time of 13–14 min) increased in size when the tissue extract was “spiked” with the authentic compound. Quantification was obtained by comparison of peak area from the sample to that obtained from a concentration series of gluconasturtiin standards.

## RESULTS

**Effects of Photoperiod.** Watercress plants that were grown under SD conditions showed a significantly lower gluconasturtiin concentration as compared to the plants grown under LD conditions at both weeks sampled (Figure 1a), and the gluconasturtiin concentration decreased from week 1 to week 2 under both LD and SD treatments. After 1 week, the plants grown under LD had approximately 33% more gluconasturtiin on a FW basis than those grown under SD (532 ± 30 vs 401 ± 48 μg/g FW). After 2 weeks, the LD plants had 39% more gluconasturtiin than the SD plants (366 ± 60 vs 264 ± 15 μg/g FW). The SD photoperiod treatment also resulted in a significantly lower FW as compared to the LD treatment (Figure 1b). As expected, FW continued to increase for both photoperiod treatments after 2 weeks of exposure.

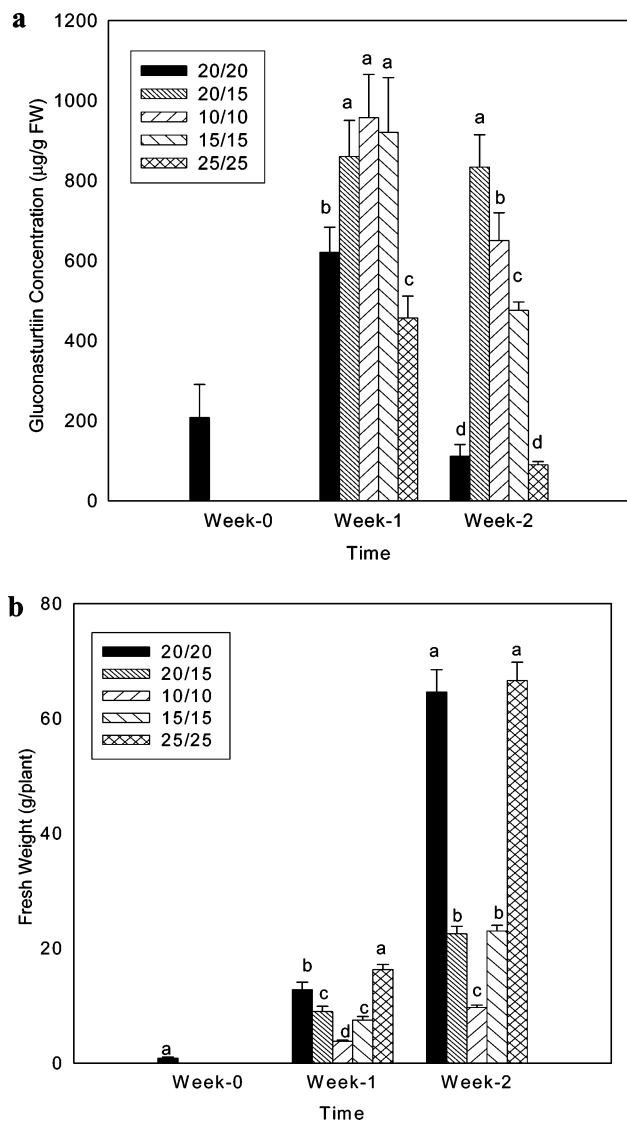
**Effects of Temperature.** The gluconasturtiin concentration was higher in samples of watercress plants grown at constant



**Figure 1.** (a) Effect of a LD (16 h) or SD (8 h) photoperiod on gluconasturtiin concentration ( $\mu\text{g/g FW}$ ) of watercress plants. Plantlets were exposed to different day lengths after they reached the 5th mature leaf stage. PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps with the temperature constant at 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05. (b) The effect of a LD (16 h) or SD (8 h) photoperiod on FW (g) of watercress plants. Plantlets were exposed to different day lengths after they reached the 5th mature leaf stage. PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps with the temperature constant at 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05.

10 and 15 °C than at constant 20 °C and constant 25 °C at both weeks (Figure 2a). After 1 week at either 10 or 15 °C, the plants contained about 50% more gluconasturtiin than the plants grown at a constant 20 °C (957 ± 108  $\mu\text{g/g FW}$  at 10 °C or 921 ± 136  $\mu\text{g/g FW}$  at 15 °C vs 621 ± 63  $\mu\text{g/g FW}$  at 20 °C). Watercress grown under 20/15 °C day–night also had a higher gluconasturtiin concentration than plants grown at a constant 20 °C, about 38% greater after 1 week (860 ± 90  $\mu\text{g/g FW}$  at 20/15 °C day–night vs 621 ± 63  $\mu\text{g/g FW}$  at a constant 20 °C). Plants grown at 25 °C had the lowest gluconasturtiin concentration of all temperature treatments, about 25% less than those grown at 20 °C after 1 week. As would be expected, FW was significantly lower in the plants grown at the lower temperatures as compared to continuous 20 or 25 °C (Figure 2b). The plants grown at 25 °C had long fibrous stems and relatively little leaf area as compared to the leafier and more

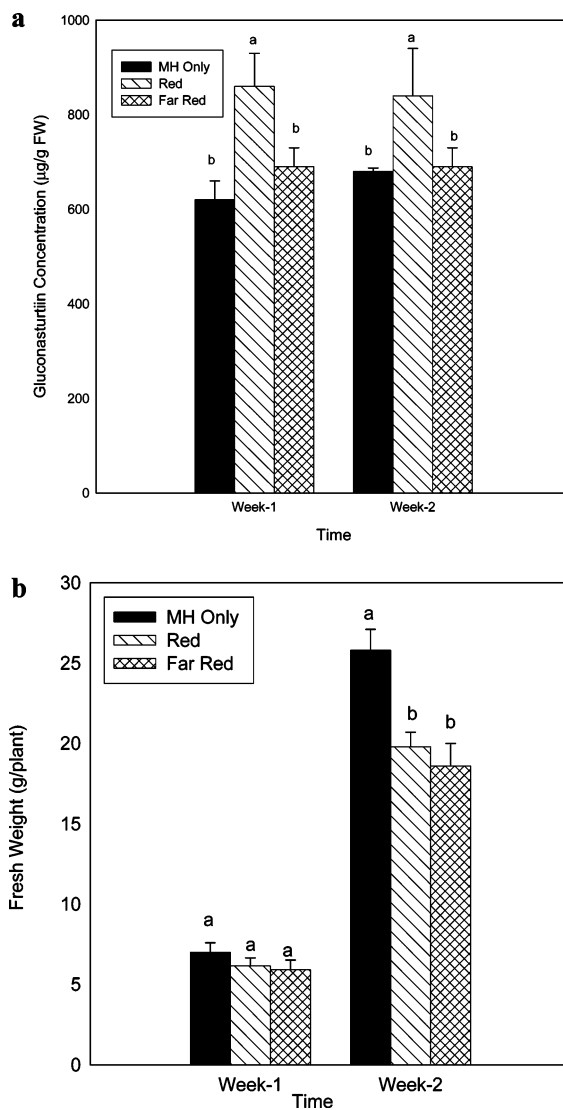




**Figure 2.** (a) Effect of various temperatures on gluconasturtiin concentration ( $\mu\text{g/g FW}$ ) of watercress. Plantlets were grown at 20 °C under LD (16 h) and then exposed to different temperatures (day/night) after they reached the 5th mature leaf stage. PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05. (b) The effect of various temperatures on FW of watercress. Plantlets were grown at 20 °C under LD (16 h) and then exposed to different temperatures (day/night) after they reached the 5th mature leaf stage. PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05.

compact plants grown at 15 °C (data not shown). In other experiments comparing growth at different temperatures, gluconasturtiin concentration was always lower at continuous 20 or 25 °C as compared to 10 or 15 °C, and cooler night temperatures always produced higher gluconasturtiin concentrations. However, the effect was not always as dramatic as that shown in the week 2 data in **Figure 2**.

**FR and R Light Enrichment.** Watercress grown under MH enriched with R light had a higher gluconasturtiin concentration as compared to plants grown under MH alone (control) or MH enriched with FR light at both weeks (**Figure 3a**). After 1 week, the R-enriched plants had about 39% more gluconasturtiin than the MH-grown plants ( $860 \pm 70$  vs  $620 \pm 40$   $\mu\text{g/g FW}$ ), and after 2 weeks, the difference was 24% ( $840 \pm 100$  vs  $680 \pm 7$   $\mu\text{g/g FW}$ ). The gluconasturtiin concentration in the control and



**Figure 3.** (a) Effect of R or FR light enrichment during the main photoperiod on gluconasturtiin concentration ( $\mu\text{g/g FW}$ ) of watercress. Plantlets were exposed to R- or FR-enriched light environments after they reached the 5th mature leaf stage. PAR = 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps with a photoperiod 16 h and a temperature of 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05. (b) The effect of R or FR light enrichment during the main photoperiod on FW (g) of watercress. Plantlets were exposed to R- or FR-enriched light environments after they reached the 5th mature leaf stage. PAR = 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by metal halide lamps with a photoperiod 16 h and a temperature of 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05.

FR-enriched plants did not significantly differ. The FW among the three different light treatments was not different at week one (**Figure 3b**). However, at week two, the control plants had a significantly higher FW as compared to the plants grown under the FR- or R-enriched light regimes (**Figure 3b**).

Because our goal in these experiments was to determine environmental conditions that would enrich gluconasturtiin concentration in watercress and because watercress is usually eaten fresh in salads, we determined gluconasturtiin concentration on a FW basis and did not routinely calculate it on a DW basis. However, we did determine the gluconasturtiin concentration on a DW basis for the experiment shown in **Figure 3**. These results are shown in **Table 1**. As is apparent, the same trends hold when the data are expressed on a DW or FW basis.

**Table 1.** R or FR Enrichment and Gluconasturtiin Concentration in Watercress<sup>a</sup>

light treatment	week 1		week 2	
	gluconasturtiin ( $\mu\text{g/g}$ FW)	gluconasturtiin ( $\mu\text{g/g}$ DW)	gluconasturtiin ( $\mu\text{g/g}$ FW)	gluconasturtiin ( $\mu\text{g/g}$ DW)
	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE
metal halide	620 $\pm$ 40	7713 $\pm$ 566	680 $\pm$ 7	8248 $\pm$ 255
R-enriched	860 $\pm$ 70	10744 $\pm$ 833	840 $\pm$ 100	10510 $\pm$ 1200
FR-enriched	690 $\pm$ 40	8609 $\pm$ 498	690 $\pm$ 40	8701 $\pm$ 581

light treatment	week 1		week 2	
	FW (g/plant)	DW (g/plant)	FW (g/plant)	DW (g/plant)
	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE
metal halide	7.00 $\pm$ 0.6	0.56 $\pm$ 0.14	25.8 $\pm$ 1.3	2.10 $\pm$ 0.54
R-enriched	6.16 $\pm$ 0.5	0.49 $\pm$ 0.12	19.8 $\pm$ 0.9	1.73 $\pm$ 0.43
FR-enriched	5.92 $\pm$ 0.6	0.47 $\pm$ 0.12	18.6 $\pm$ 1.4	1.49 $\pm$ 0.37

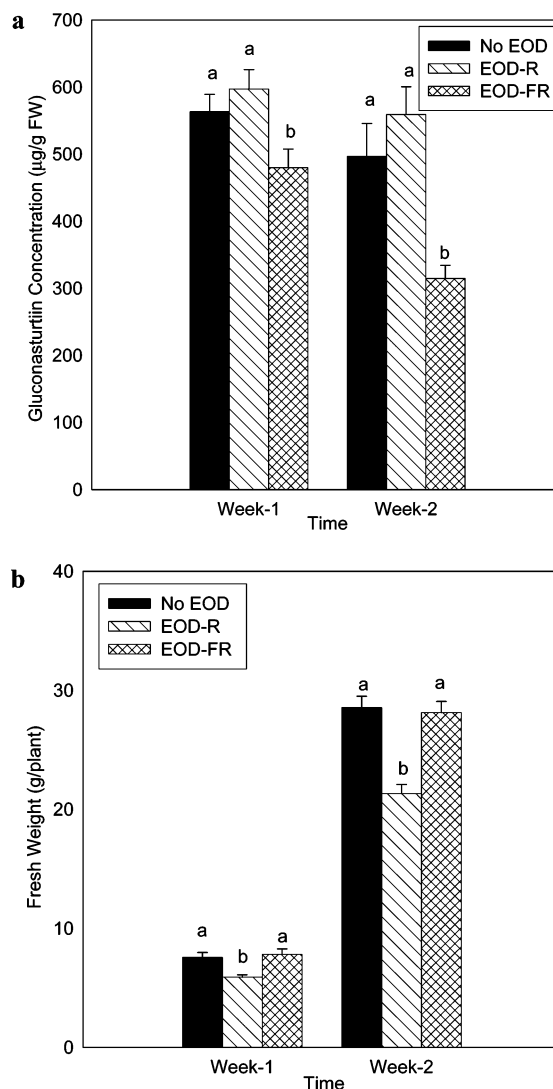
<sup>a</sup> Watercress plants were exposed to MH under a 16 h photoperiod with or without supplemental R or FR light. FW values, from **Figure 3**, are shown here for comparison;  $n = 8$  for each treatment and time point.

**End-of-Day R or FR Exposure.** Watercress plants exposed to end-of-day R light showed a higher gluconasturtiin concentration at weeks one and two as compared to plants exposed to FR at the end of the day. After one week, the EOD R treatment resulted in about 24% more gluconasturtiin than the EOD FR treatment ( $597 \pm 29$  vs  $480 \pm 28 \mu\text{g/g}$  FW). After 2 weeks, the difference was about 78% ( $559 \pm 41$  vs  $315 \pm 20 \mu\text{g/g}$  FW). (**Figure 4a**). The R end-of-day light treatment also possibly resulted in plants with a higher gluconasturtiin concentration than the plants without end-of-day light treatments, but this increase was not significantly different. The plants not exposed to end-of-day light treatments also showed higher gluconasturtiin concentrations as compared to the plants exposed to end-of-day FR. Thus, FR end-of-day light exposure reduced the gluconasturtiin concentration. The end-of-day R-exposed plants had significantly lower FW at weeks one and two as compared to the end-of-day FR treatment or the control. The watercress FW did not differ between the control and the FR end-of-day light treatments (**Figure 4b**).

## DISCUSSION

Long days (16 h) resulted in plants with higher gluconasturtiin concentrations and FWs as compared to short days (8 h) (**Figure 1**). Gluconasturtiin and FW seem to be affected by the total photosynthetic radiation when grown under MH without R or FR added at constant 20 °C. The lower FW in the SD-exposed plants was likely due to reduced photosynthesis (8 h less light as compared to the LD-grown plants). A typical marketable weight for watercress is between 30 and 50 g; thus, watercress plants grown under long days would reach this marketable weight earlier than short day-grown plants.

Temperatures of constant 10 and 15 °C resulted in a higher gluconasturtiin concentration as compared to plants grown at constant 20 or 25 °C (**Figure 2a**). However, the FW was significantly lower at the lower temperatures as compared to constant 20 or 25 °C grown plants (**Figure 2b**). Plants grown at 20 °C during the main photoperiod and 15 °C during the night had a higher gluconasturtiin concentration and intermediate FW. Plants grown under the 20/15 °C growth regime will reach the marketable FW of 30–50 g slightly later than the plants grown under constant 20 or 25 °C but will have a higher gluconasturtiin concentration. The constant 10 °C temperature



**Figure 4.** (a) Effect of R or FR end-of-day treatments on gluconasturtiin concentration ( $\mu\text{g/g}$  FW) of watercress. At the end of a 16 h day of MH, plantlets were exposed to R (5 min) or FR (10 min) light exposures or darkness after they reached the 5th mature leaf stage. PAR =  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  with a photoperiod of 16 h and a temperature of 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05. (b) The effect of R or FR end-of-day treatments on FW (g) of watercress. At the end of a 16 h day of MH, plantlets were exposed to R (5 min) or FR (10 min) light exposures or darkness after they reached the 5th mature leaf stage. PAR =  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  with a photoperiod of 16 h and a temperature of 20 °C. Different letters indicate a significant difference of means;  $n = 8$ ; LSD = 0.05.

regime, albeit resulting in a higher gluconasturtiin concentration, also results in plants that have a FW increase rate that may be too low to be practical. Although we do not know the mechanistic basis for these effects of lower temperature, it would be logical for these defense compounds to be produced in cool-running streams and other wet habitats typical for natural populations of watercress (12).

The spectral quality of the light source affects gluconasturtiin concentration in watercress (**Figure 3a**). Plants grown under metal halide lamps enriched with R light had a higher gluconasturtiin concentration when compared to the control and FR-enriched plants. However, the FW for the control plants was higher at week two as compared to the R- and FR-enriched plants. The higher FW in the control plants is not easily

explained by the quantity of light active in photosynthesis since the PAR for all three light regimes was 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Watercress exposed to end-of-day R light treatments had a higher gluconasturtiin concentration as compared to plants given FR end-of-day treatments (Figure 4a). These data on an increase in gluconasturtiin concentration support the notion that R light in general has a positive effect on gluconasturtiin concentration as compared to FR light. The control plants that were exposed to MH alone showed a gluconasturtiin concentration that was not statistically different from the end-of-day R light treatment. The ratio of R:FR in MH is between 2.1 and 2.5, indicating that R light is predominant in the light spectrum of the metal halide lamps. This probably accounts for the lack of a significant effect from the end-of-day R light treatment.

As indicated above, seed germination of watercress has been shown to be under control of the plant photoreceptor phytochrome (8). We are not aware of any report of phytochrome control of glucosinolate biosynthesis; however, because many other secondary plant products are under phytochrome control, e.g. (13), we carried out the experiments shown in Figures 3 and 4 to test the hypothesis that glucosinolate concentrations might be regulated by phytochrome. Our results are consistent with that hypothesis. Enrichment with R (Figure 3a) pushes the phytochrome photostationary photoequilibrium toward the active form of the pigment, while FR enrichment does not have that effect. An end-of-day FR treatment lowered the gluconasturtiin concentration (Figure 4a), whereas the EOD R treatment did not. EOD treatments are often indicative of phytochrome control (14) and are relevant to natural growth conditions since the proportion of R and FR in the natural environment changes at sunset (14). While these results are consistent with the view that gluconasturtiin concentration is under phytochrome control in watercress, they do not prove that hypothesis. Typically, promotion of a response by R and reversal of the effect of R by FR are required to demonstrate phytochrome control. Because our aim was to determine growth conditions that could be adapted to watercress production systems, we did not carry out additional light pulse experiments.

Our results on the effect of photoperiod and temperature on gluconasturtiin concentration differ from those of Palaniswamy et al. (4) in several important ways. First, they found that a 12 h photoperiod resulted in more PEITC than a photoperiod of 8 h when the plantlets were grown at 265  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . However, when watercress was exposed to 435  $\mu\text{mol m}^{-2} \text{s}^{-1}$  1 week before harvest, there was no difference in PEITC concentration in plants grown under an 8 or 12 h photoperiod. Palaniswamy et al. (4) reasonably attributed the increase in PEITC under an 8 h photoperiod to the PAR increase. However, in our experiments, watercress at the 5th leaf stage (approximately 2 weeks old) was exposed to 1 or 2 weeks of 8 or 16 h photoperiod under a PAR equivalent to their maximum (PAR = 400–450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The LD-exposed plants had a higher gluconasturtiin concentration than the SD-grown plants at both sampling times. This implies that the day length effect was photomorphogenic rather than photosynthetic. A SD experiment in which the dark period was interrupted by a night break would determine if the increase in gluconasturtiin was due to photoperiod or PAR.

Second, Palaniswamy et al. (4) found that a growth temperature of 25 °C increased the PEITC concentration in watercress. In contrast, our data showed that lower night temperatures (15 and 10 °C) increased gluconasturtiin concentration as compared to continuous 20 or 25 °C. The discrepancy in the data between

two studies may lie in the fact that watercress in our study was harvested at 21 and 28 days after seedling emergence. In the study by Palaniswamy et al. (4), the plants were much older, since their experiments were initiated at 21 days after planting and harvested 33 days after treatment initiation.

Third, Palaniswamy et al. (4) measured the concentration of PEITC in the plant tissue, not the naturally occurring glucosinolate parent compound, gluconasturtiin, whereas we measured gluconasturtiin concentration. Because most of the material in the plant is not degraded, unless the glucosinolate was intentionally completely hydrolyzed to the isothiocyanate, it is not clear how to interpret PEITC data without parallel data on gluconasturtiin.

PEITC, the hydrolysis product of gluconasturtiin, is formed upon release of endogenous myrosinases during tissue disruption, as occurs when the plant is eaten. In the plant, myrosinase is physically segregated from gluconasturtiin, and cooking the vegetable leads to inactivation of the myrosinase. However, in humans, glucosinolates are converted to isothiocyanates after ingestion of cooked watercress (15). Although there is no generally accepted evidence for the presence of significant myrosinase activity in mammalian cells, there is good evidence for glucosinolate conversion by intestinal microflora (15, 16). The plant myrosinase probably plays an important role, though, since gnotobiotic rats fed plant myrosinase along with glucosinolates had high levels of isothiocyanates and isothiocyanate metabolites (17).

PEITC has been shown to have chemoprotective properties in laboratory animal studies (2, 3, 18). For instance, rats that had 489 ppm PEITC in their diet showed a dramatic decrease in lung tumorigenesis (19). Thus, increasing gluconasturtiin in watercress by optimizing environmental growth conditions may potentially lead to a “value-added” property for watercress producers. Of course, any such product must be evaluated by taste panels to determine if increased concentrations of gluconasturtiin, or other secondary products, reduce consumer acceptability.

Although we are not aware of any breeding experiments aimed toward increasing glucosinolate concentration in watercress, such experiments have been carried out in broccoli (20, 21). A 10-fold increase in the concentration of 4-methylsulfinylbutyl glucosinolate (the sulforaphane precursor) resulted in a 80–100-fold increase of the ability of the broccoli tissue to induce quinone reductase, a standard assay of phase II induction potential. Thus, an increase in glucosinolate concentration, as least in broccoli, can result in vegetables with increased chemopreventive potential.

In this research, we studied the effect of photoperiod, light quality, and temperature on gluconasturtiin concentration and FW increases in watercress. We found that gluconasturtiin concentration responds to variable environmental parameters. For example, long days (16 h), night temperatures below 20 °C, and supplementary R light resulted in increased gluconasturtiin concentrations in watercress plants. Watercress is increasingly grown in environmentally managed areas to control the quality of the product. Our data suggest that growers can change photoperiod, temperature, and light quality of the light source to optimize the quality and health benefits from their watercress product.

#### ABBREVIATIONS USED

DW, dry weight; FR, far-red light; FW, fresh weight; LD, long-day photoperiod; MH, metal halide light; PAR, photosyn-

thetically active radiation; PEITC, 2-phenethyl isothiocyanate; R, red light; SD, short-day photoperiod.

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