Supplemental Light before Harvest Increases Phenethyl Isothiocyanate in Watercress under 8-hour Photoperiod

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Watercress (*Nasturtium officinale* R.Br.) plants were grown in growth chambers at 15 °C or 25 °C and either 8- or 12-h photoperiod (PP). The photosynthetic photon flux (PPF) was 265 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in all chambers, but beginning 1 week before harvest, half of the plants in each chamber were subjected to a higher PPF (435 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). At harvest, watercress leaves and stems were analyzed for phenethyl isothiocyanate (PEITC) concentration. Without supplemental PPF, watercress grown at 25 °C and 12-h PP produced higher PEITC concentration in leaves and stems than plants grown at 15 °C and 12-h PP, or plants grown at 8-h PP and either temperature. With one week of supplemental PPF before harvest, plants grown at 15 or 25 °C and the 8-h PP produced PEITC concentrations as high as plants exposed to 12-h PP and similar temperatures. However, a week of supplemental PPF did not alter PEITC concentrations in plants grown at the 12-h PP, regardless of temperature. At 25 °C, plants grown under the low PPF and the 12-h PP produced 62% greater dry mass than plants exposed to a week of high PPF and the 8-h PP, but did not differ in PEITC content. Thus, the effect of one week of high PPF on PEITC concentration depended on photoperiod.

Cultural conditions, including the quality and quantity of light, daylength, temperature, and nutritional balance, can dramatically influence phytochemical yield (Letchamo and Gosselin, 1994). Phenethyl isothiocyanate (PEITC) is a promising phytochemical that inhibits several types of cancer (in rats and mice) caused by tobacco-specific carcinogens (Stoner et al., 1995). PEITC is undergoing clinical trials to help develop human dietary recommendations.

Gluconasturtin, the glucosinolate precursor that yields PEITC on hydrolysis, is abundant in watercress (*Nasturtium officinale* R.Br. Brassicaceae), at 12.6 mmol/100 g⁻¹ fresh sample (Daxenbichler et al., 1991). Recently, we found that increasing sulfur levels in a hydroponic solution increased the PEITC concentration in watercress leaves (Palaniswamy et al., 1995). Isothiocyanates in radish (*Raphanus sativus* L.) were reported to be influenced by daylength (Neil and Bible, 1973). Ju et al. (1980) reported seasonal variation in

thiocyanate ion concentration in rutabaga (*Brassica napobrassica* Mill.) and turnip (*Brassica rapa* L.) cultivars. In these studies, the environmental conditions were not carefully controlled or monitored. However, Hamner and Parks (1944) quantitatively related the ascorbic acid concentration of turnip leaves at harvest to the light intensity received 1 week before harvest. The objective of our study was to establish whether four combinations of temperature and photoperiod, or increased light intensity before harvest, would alter the PEITC concentration in watercress leaves and stems.

Materials and Methods

Watercress seedlings (21 d old) were transplanted into 500-cm³ square pots containing the commercial medium Metro 510 (O.M. Scotts, Marysville, Ohio) and 40 pots were transferred into each of four growth chambers (EGC model G-10, Chagrin Falls, Ohio). Nitrogen was applied to plants at 25 mg·L⁻¹ for the first week and then 50 mg·L⁻¹ until harvest using a 20N-4.36P-16.6K water-soluble fertilizer in the irrigation water.

Each chamber was programmed to maintain 15 or 25 °C and a photoperiod of 8 or 12 h. The average photosynthetic photon flux (PPF) was maintained at 265 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in all the chambers until 1 week before harvest. At this time, half of the plants in each chamber were exposed to PPF of 435 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by moving them closer to the light source. The remaining plants continued to receive 265 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ until harvest. The PPF in the growing area was highest along the center row of the chamber and lowest along the opposite side wall of the chamber. The gradient for 265 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ranged from 273 along the center row to 257 along the opposite side wall. The gradient for 435 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ranged from 448 along the center row to 422 along the opposite side wall. To ensure that all plants received similar PPF during the study, the plants in each treatment were rotated every 3 d across the light gradient in each treatment. The PPF levels reported in this study were obtained by averaging the light measurements taken at various locations of the growing area every 5 d throughout the study period.

Treatments were arranged in a split-plot design with the four combinations of photoperiod (PP) and temperature as main plots and the PPF during the 1 week before harvest as subplots. The experiment was conducted three times. Treatments were randomly assigned to chambers, resulting in a different chamber being used for each treatment each time the study was repeated. During one run of this experiment, the growth chamber with the combination of 25 °C and 8-h PP failed 1 week before harvest. Therefore, data were analyzed as an experiment with unequal number of replications using SAS General Linear Models procedure (SAS Inst., 1985).

Whole-plant fresh mass, dry mass (DM), leaf area, and the number of side shoots arising from the main stem were determined 33 days after treatment initiation for two plants in each treatment replication. The terminal five nodes of shoots from the remaining 18 plants were frozen (-59 °C) until analysis for PEITC. Plant leaf and stem tissues were analyzed in duplicate for PEITC concentration using gas chromatography (Gil and MacLeod, 1980), and expressed as mg·g⁻¹ DM.

Results and Discussion

Watercress leaves had higher concentrations of PEITC at the higher PPF and temperatures. Leaves of plants grown under the 8-h PP and then subjected to the 435 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 1 week before harvest produced $\approx 60\%$ higher

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Table 1. Effect of interactions of temperature (T) and photoperiod (PP) and increasing photosynthetic photon flux (PPF) 1 week before harvest on phenethyl isothiocyanate (PEITC) concentration in watercress leaves.

PPF 1 week before harvest ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	PEITC (mg·g ⁻¹ leaf dry mass) ^a			
	Photoperiod (h) and temperature (°C) combinations			
	8-h		12-h	
15	15	25	15	25
265	3.0	4.2	4.1	6.6
435	4.8	6.6	4.0	6.7

^aTo compare any two treatment combinations, LSD_{0.05} = 1.8

Table 2. Effect of photoperiod and temperature on the PEITC concentration in watercress stems, and shoot dry mass, leaf area, and number of side shoots of watercress plants.

Photoperiod (h)	Temp. (C°)	Characteristic ^a			
		PEITC in stems (mg·g ⁻¹ dry mass)	Shoot dry mass (g)	Leaf area (cm ²)	Side shoots (no.)
8	15	2.3 b	0.6 d	154 d	4.7 b
	25	2.7 b	1.1 c	488 b	8.2 b
12	15	2.9 b	1.5 b	292 c	7.8 b
	25	3.7 a	3.3 a	1054 a	17.0 a

^aMean separation within columns by LSD ($P \leq 0.05$).

concentration of PEITC than those that were grown continuously at $265 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ until harvest (Table 1). This trend was similar at both temperatures. However, PEITC concentration in the leaves of plants grown at 12-h PP was not affected by an increase in PPF before harvest at either temperature. Thus, the response of PEITC concentration to increased PPF 1 week before harvest depended on photoperiod.

At 25 °C, PEITC concentration in leaves of plants grown under 12-h PP and continuous $265 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ throughout did not differ from that of plants grown under 8-h PP (25 °C) and subjected to increased PPF for 1 week before harvest. However, DM at 25 °C and 12-h PP with continuous $265 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ throughout was about 3.2 times higher than DM for plants grown under 8-h PP and subjected to increased PPF for 1 week before harvest (3.39 and 1.06 g, respectively).

Plants grown under 25 °C and 12-h PP had a higher concentration of PEITC in stems, higher shoot DM, larger leaf area, and more side shoots than plants grown under 15 °C and 8-h PP (Table 2). These plant growth characteristics were not influenced by increased PPF during the last 7 d before harvest (data not shown).

On a DM basis, the PEITC concentration in watercress leaves was $\approx 72\%$ higher than the PEITC concentration in stems. Higher PEITC concentration in the leaves, compared to stems, indicates that photosynthetically active plant

parts, such as the leaves, are the probable sites of most of the synthesis and/or storage of the parent glucosinolate (gluconasturtin), as earlier reported for radish by Chong and Bible (1974).

The relationship between PEITC concentration in leaves and total DM varied with each particular environment. At 15 °C, the plants grown at 12-h PP produced a similar PEITC concentration in leaves, but a 145% greater total DM (leaves and stems), than the plants grown at 8-h PP. At 25 °C and 12-h PP, watercress leaves produced a 23% higher PEITC concentration and the plants had 194% greater total DM compared to those grown at 25 °C and 8-h PP.

Our results clearly show that PEITC concentration is influenced by higher PPF before harvest, but only for plants grown under a short (8-h) PP. Apparently, the effect of higher PPF before harvest was able to substitute for the longer PP (12-h) in stimulating PEITC production. The results also demonstrate that production of DM and PEITC were higher at 25 °C than at 15 °C.

Environmental factors such as temperature, PP, and a short-term increase in PPF before harvest apparently can affect the PEITC concentration in watercress leaves. However, the response of increased PEITC concentration is not necessarily associated with similar changes in plant DM. Since the main plot treatments in this study were combinations of only two temperatures and PPs, further experi-

mentation is necessary to develop a more complete picture of the individual effects of these key environmental factors on PEITC yield.

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