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ELEVATED TEMPERATURES INCREASE LEAF SENESCENCE AND ROOT SECONDARY METABOLITE CONCENTRATIONS IN THE UNDERSTORY HERB *PANAX QUINQUEFOLIUS* (ARALIACEAE)[1]

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The response of understory species to elevated temperatures is not well understood but is important because these plants are highly sensitive to their growth conditions. Three-year-old plants of *Panax quinquefolius*, an understory herb endemic to the eastern deciduous forests of North America, were grown in a greenhouse at 25/20°C (day/night) or 30/25°C for one growing season and analyzed each month. Plants grown at high temperatures had an early onset of leaf senescence and therefore accumulated less carbon. From May to July, *P. quinquefolius* grown at high temperatures had decreased photosynthesis (52%), stomatal conductance (60%), and root and total biomass (33% and 28%, respectively) compared to plants grown at low temperatures. As *P. quinquefolius* prepared to overwinter, plants grown at high temperatures had less root biomass (53%) than plants in low temperatures. The amount of storage-root ginsenosides was unaffected by temperature, and differences in storage root size may explain why plants grown at high temperatures had greater concentrations of storage root ginsenosides (49%) than plants grown at low temperatures. *Panax quinquefolius* is clearly sensitive to a 5°C increase in temperature, and therefore other understory species may be negatively impacted by future increases in global temperature.

Key words: Araliaceae; ginseng; ginsenosides; global warming; *Panax quinquefolius*; temperature response; understory herb.

Forest understory plant species are often highly sensitive to their environment, and increases in temperature are likely to profoundly affect their physiology and ecology. Mean surface air temperatures of the earth have increased 0.6°C during the last century, and global circulation models project a global warming of 1.4 to 5.8°C by 2100 (Houghton et al., 2001). Numerous studies have examined the effects of elevated temperatures on plants (Berry and Bjorkman, 1980; Morison and Lawlor, 1999; Rustad et al., 2001), including forest species. Few studies, however, have examined the effects of increased temperatures on understory herbaceous plants (Farnsworth et al., 1995).

Understory herbs are likely to be more affected by increases in temperature than the dominant woody species (Farnsworth et al., 1995). Elevated temperatures reduce stomatal conductance and, subsequently, reduce photosynthesis and growth of many plant species (Berry and Bjorkman, 1980). The photochemical efficiency of photosystem II also decreases at elevated temperatures, indicating increased stress (Gamon and Pearcy, 1989; Maxwell and Johnson, 2000). When plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed not allocated to growth is instead allocated to secondary metabolites (Mooney et al., 1991). Several studies have examined the effects of increased temperatures on secondary metabolite production of plants, but most of these studies have contradictory results. Some report that secondary metabolites increase in response to elevated temperatures (Litvak et al., 2002), while others report that they decrease (Snow et al., 2003).

Panax quinquefolius L. (Araliaceae), or American ginseng, is a slow-growing herb endemic to the eastern hardwood forests of North America (Charron and Gagnon, 1991; Anderson et al., 1993). Populations of this species are rare to uncommon and are decreasing in size as a result of a number of factors, including harvesting (Cruse-Sanders and Hamrick, 2004) and deer-browsing (McGraw and Furedi, 2005). *Panax quinquefolius* is an understory shade obligate with palmately compound leaves and a large storage root (Lewis and Zenger, 1982; Charron and Gagnon, 1991; Nantel et al., 1996; Schluter and Punja, 2000; McGraw, 2001). Leaves generally emerge in late April when the tree canopy is partially to fully developed (Carpenter and Cottam, 1981; Lewis and Zenger, 1982; Anderson et al., 1993), while senescence often occurs in October but may occur as early as August (Anderson et al., 1993).

Panax quinquefolius produces secondary metabolites that are steroidal saponins known as ginsenosides (Attele et al., 1999). These dammarene-type triterpenes are divided into two classes of compounds: those composed of two sugars, the 20(*S*)-protopanaxadiols (including Rb_1, Rb_2, Rc, and Rd); and those with three sugars, the 20(*S*)-protopanaxatriols (Re and Rg_1) (Fournier et al., 2003). Ginsenosides are found in all organs of the plant (Li et al., 1996) and act as antifungal (Nicol et al., 2002) and antimicrobial agents (Suits, 2003). Studies have reported that concentrations of ginsenosides vary depending on geography (Li et al., 1996; Mudge et al., 2001; Yuan et al., 2001), season (Kim et al., 1981; Li and Wardle, 2002), plant age (Li and Wardle, 2002), soil conditions (Konsler et al., 1990; Li et al., 1996; Li and Mazza, 1999), and light levels (Fournier et al., 2003). No studies have reported the

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effects of elevated temperatures on ginsenoside concentrations in *P. quinquefolius*.

We conducted a greenhouse study to investigate the effects of a 5°C increase in temperature on the growth and physiology of *Panax quinquefolius*. We hypothesized that elevated temperatures would reduce photosynthesis and biomass production of *P. quinquefolius*, but that storage root ginsenoside concentrations would increase as a result of stress imposed from elevated temperatures. Understanding the effect of elevated temperatures on seasonal patterns of biomass acquisition and allocation of *P. quinquefolius* will help us understand how this important understory plant will respond to a changing climate.

MATERIALS AND METHODS

Plant material—Three-yr-old *Panax quinquefolius* (Araliaceae) roots were obtained from a woods-grown source (Shady Oaks Ginseng Co., Putnam County, West Virginia, USA). Initial fresh biomass of each root was measured to use as a covariate in the statistical analyses. In mid-March, roots were transplanted into 2-L pots containing a 1 : 3 mixture of sand and forest soil obtained from the West Virginia University Forest (Morgantown, West Virginia, USA) where *P. quinquefolius* grows naturally. Plants were grown at ambient temperatures before placement in the West Virginia University Life Science Building greenhouses (Morgantown, West Virginia, USA). Plants in the greenhouse were maintained under 65% shade cloth, and the light level was 216 $\mu mol \cdot m^{-2} \cdot s^{-1}$ averaged across the growing season. The volumetric water content, measured for five random plants from each temperature treatment every day with a time domain reflectometry (TDR) probe (HydroSense, Campbell Scientific, Inc., Logan, Utah, USA), was maintained at 20%, and no fertilizer was added to the soil.

Greenhouse treatments—*Panax quinquefolius* plants were randomly assigned to one of two temperature treatments ($N = 30$ per treatment) after they had emerged at the end of April. The temperature treatments represented the estimated mean summer temperature for understory conditions in a central hardwood forest around Morgantown, West Virginia (low temperature) and a 5°C increase reflective of the upper range of projected elevated temperatures as a consequence of global warming (high temperature). Air temperatures in the greenhouses were monitored with a LI-1400 datalogger (Li-Cor, Lincoln, Nebraska, USA). Mean daytime temperatures (from 0700 to 2100 hours) for the low and high temperature treatments were 26.8 ± 3.0°C and 31.2 ± 2.2°C, respectively. Mean nighttime temperatures (from 2100 to 0700 hours) for the low and high temperature treatments were 21.2 ± 1.7°C and 26.56 ± 1.3°C, respectively.

Photosynthesis, stomatal conductance, and chlorophyll fluorescence—Light-saturated net photosynthesis (A_{sat}) and stomatal conductance (g_s) were measured every 4 wk from 30 May until 25 August on four randomly selected *P. quinquefolius* plants from each temperature treatment using an open-flow gas exchange system (Li-Cor 6400). Gas exchange was measured with saturating light (PPFD = 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, predetermined with photosynthetic light response curves) on the largest terminal leaflet of each plant at ambient CO_2 (360 $\mu L \cdot L^{-1}$) and between 1000 and 1500 hours to minimize diurnal effects. Because their leaves began senescing in July, plants in the high temperature treatment were photosynthetically inactive by August and were not measured.

At the beginning of the July harvest, dark-adapted chlorophyll fluorescence was measured on all remaining plants ($N = 2$ in the high temperature treatment, $N = 16$ in the low temperature treatment) with a pulse-modulated fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Leaves were dark-adapted for 10 min, and fluorescence was measured under steady state conditions (F_s) following a saturating pulse of white light (F_m'). The photochemical efficiency of photosystem II (F_v/F_m) was then calculated as: $F_m' - (F_s/F_m')$ (Genty et al., 1989).

Biomass production—After gas exchange was measured, each plant was harvested and separated into 'shoots' (aerial stem, leaves, and inflorescences, if present) and 'roots' (rhizome, storage root, and fibrous roots). Leaf area was measured (Li-Cor LI 3100 Area Meter). No plants from the high temperature treatment were harvested in August because of the early onset of leaf senescence. Roots from these senesced plants were later collected in September, at the same time roots from the low temperature treatment were harvested, for measurements of the final overwintering biomass. Leaves were considered senesced when they were no longer mostly green and/or had detached from the belowground parts of the plant. All plant tissues were dried at 60°C for over 48 h and weighed to determine the biomass.

Root ginsenosides—Ginsenoside concentrations from extracts of storage roots were measured from each harvested plant using reverse-phase high performance liquid chromatography (RP-HPLC). After drying, the rhizome and fibrous roots were removed, and the storage roots were finely ground and stored in a drying oven at 60°C until extractions were performed. Ultrasonic extraction and analytical separation procedures followed Court et al. (1996), with the following modifications. Ground storage root samples (50 mg) were extracted in 100% methanol, and 30 μL were injected into the RP-HPLC. Samples ran for 74 min on a Waters 2690 Separations Module (Waters, Milford, Massachusetts, USA) consisting of a Waters 996 Photodiode Array Detector adjusted to 203 nm and a C_{18} reversed-phase column and guard column (Chrompack Standard Columns, Varian Inc., Palo Alto, California, USA). The gradient employed the eluents (A) water, (B) acetonitrile, and (C) phosphate buffer solution at a flow-rate of 1.15 mL/min with the following profile: 0–15 min, 0% A, 19% B, 81% C; 15–24.5 min, 0% A, 21% B, 79% C; 24.5–29 min, 0% A, 26.3% B, 73.7% C; 29–43 min, 0% A, 27% B, 73% C; 43–47 min, 0% A, 34% B, 66% C; 47–54 min, 0% A, 36% B, 64% C; 54–55 min, 0% A, 43% B, 57% C; 55–64 min, 15% A, 85% B, 0% C; and 64–74 min, 0% A, 19% B, 81% C. Ginsenosides were quantified using standard curves of known concentrations of individual ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd from INDOFINE Chemical Co., Hillsborough, New Jersey, USA), and the concentrations of all ginsenosides were calculated as a percentage of the dried storage root tissue (mg ginsenoside per mg of dried storage root × 100%). The concentrations of ginsenosides Rb_1, Rb_2, Rc, and Rd were added to determine the total concentration of 20(S)-protopanaxadiols (PD), and the concentrations of Re and Rg_1 were combined to give the total concentration of 20(*S*)-protopanaxatriols (PT). Total ginsenoside concentration was determined as the sum of all of the individual ginsenosides. The ginsenoside content, or the absolute amount of ginsenosides, in each storage root was calculated as the milligrams of ginsenosides multiplied by the dried storage root biomass (mg).

Statistical analysis—A two-way analysis of variance (ANOVA) was used to determine whether growth temperature or harvest date significantly affected net photosynthetic rates, stomatal conductance, and storage root ginsenoside concentrations during the growing season (May, June, and July). Ginsenoside concentrations in the overwintering storage root measured in September and chlorophyll fluorescence measured in July were analyzed using a one-way ANOVA with temperature as the main effect. Differences in individual ginsenosides between the pretreatment period (mean of March and April harvests) and the mean over the growing season and between the pretreatment period and mean final concentrations in September were analyzed using orthogonal contrasts following a one-way ANOVA with month as the main effect.

Biomass production was analyzed using a two-way analysis of covariance (ANCOVA) with temperature and harvest date as the main factors and the initial dry root biomass as a covariate to remove any effects of differences in initial root size on biomass measurements. Biomass data from May, June, and July were used; data from August were not used because shoots senesced early for plants in the high temperature treatment. A one-way ANCOVA with initial root biomass as the covariate was used to determine whether the final overwintering root biomass measured in September was significantly affected by temperature. All statistical analyses were performed using SAS JMP 3.2.5 (Cary, North Carolina, USA).

RESULTS

Photosynthesis, stomatal conductance, and chlorophyll fluorescence—Light-saturated net photosynthetic rates (A_{sat}) of *P. quinquefolius* grown in both temperature treatments

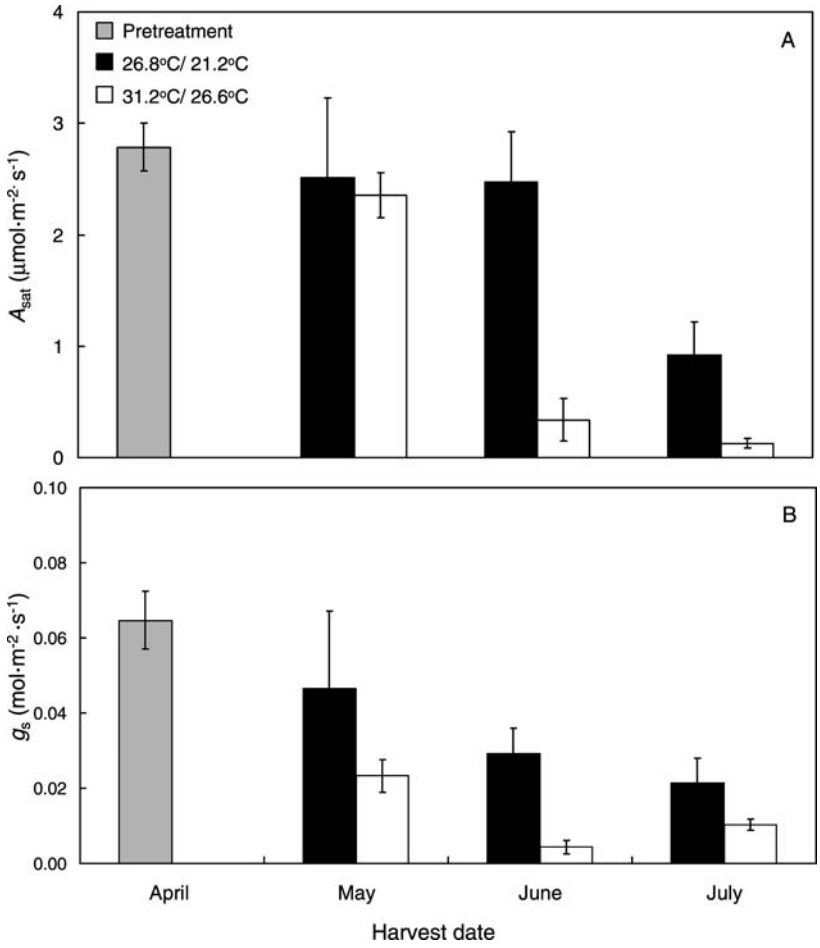

Fig. 1. (A) Light-saturated net photosynthetic rates, A_{sat}, and (B) stomatal conductance, g_s, of *Panax quinquefolius* before temperature treatments ("pretreatment"), or after one growing season at low or high temperatures. Data points are the mean (±SE) for 2–4 plants.

decreased as the season progressed ($F_{2,20} = 14.98$, $P = 0.0003$; Fig. 1A), but temperature affected the timing of this seasonal decrease ($F_{2,20} = 4.76$, $P = 0.0251$). In the low temperature treatment, the seasonal decrease in photosynthesis of *P. quinquefolius* occurred between June and July, whereas in the high temperature treatment the seasonal reduction occurred between May and June. Averaged across all measurement dates, the A_{sat} was 52% less in the high temperature treatment than in the low temperature treatment ($F_{1,20} = 14.25$, $P = 0.0018$). Stomatal conductance (g_s) also declined as the growing season progressed ($F_{2,20} = 4.68$, $P = 0.0264$) and as a result of elevated growth temperature ($F_{1,20} = 13.43$, $P = 0.0023$; Fig. 1B).

In July the photochemical efficiency of photosystem II (F_v/F_m) averaged 0.50 (±0.09) in the low temperature treatment but was reduced by 66% in the high temperature treatment to 0.17 (±0.03) ($F_{1,17} = 13.25$, $P = 0.0022$). By August, shoots of *P. quinquefolius* grown at high temperatures were completely senesced.

Biomass production—Total biomass production of *P. quinquefolius* increased across the growing season in both temperature treatments ($F_{2,22} = 8.23$, $P = 0.0035$), but the observed increases were 28% less for plants grown at high temperatures ($F_{1,22} = 5.83$, $P = 0.0281$). Changes in total biomass production were reflected by changes in root biomass but not shoot biomass. Shoot biomass was not affected by harvest date ($F_{2,22} = 0.56$, $P = 0.5825$) or temperature ($F_{1,22} = 0.01$, $P = 0.9106$; Fig. 2A). Conversely, root biomass of *P. quinquefolius* increased across the growing season ($F_{2,22} = 15.14$, $P = 0.0002$), but 33% less root mass was produced by plants in the high temperature treatment compared to those in low temperatures ($F_{1,22} = 11.80$, $P = 0.0034$; Fig. 2B). Root to shoot ratios (data not shown) increased throughout the growing season regardless of temperature treatment ($F_{2,22} = 11.45$, $P = 0.0008$). This increase was less for plants grown at high temperatures than for plants grown at low temperatures (2.49 ± 0.16 compared to 3.12 ± 0.17 for all plants across the growing season; $F_{1,22} = 8.94$, $P = 0.0087$). Biomass production of all plant tissues depended upon the initial root biomass measured at the beginning of the experiment (Total: $F_{1,22} = 35.27$, $P < 0.0001$; Root: $F_{1,22} = 34.93$, $P < 0.0001$; Shoot: $F_{1,22} = 21.92$, $P = 0.0003$).

The biomass of roots harvested in September, after plant senescence, was affected by temperature treatments such that roots grown at high temperatures weighed 53% less than roots grown at low temperatures ($F_{1,13} = 18.41$, $P = 0.0013$; Fig. 2B).

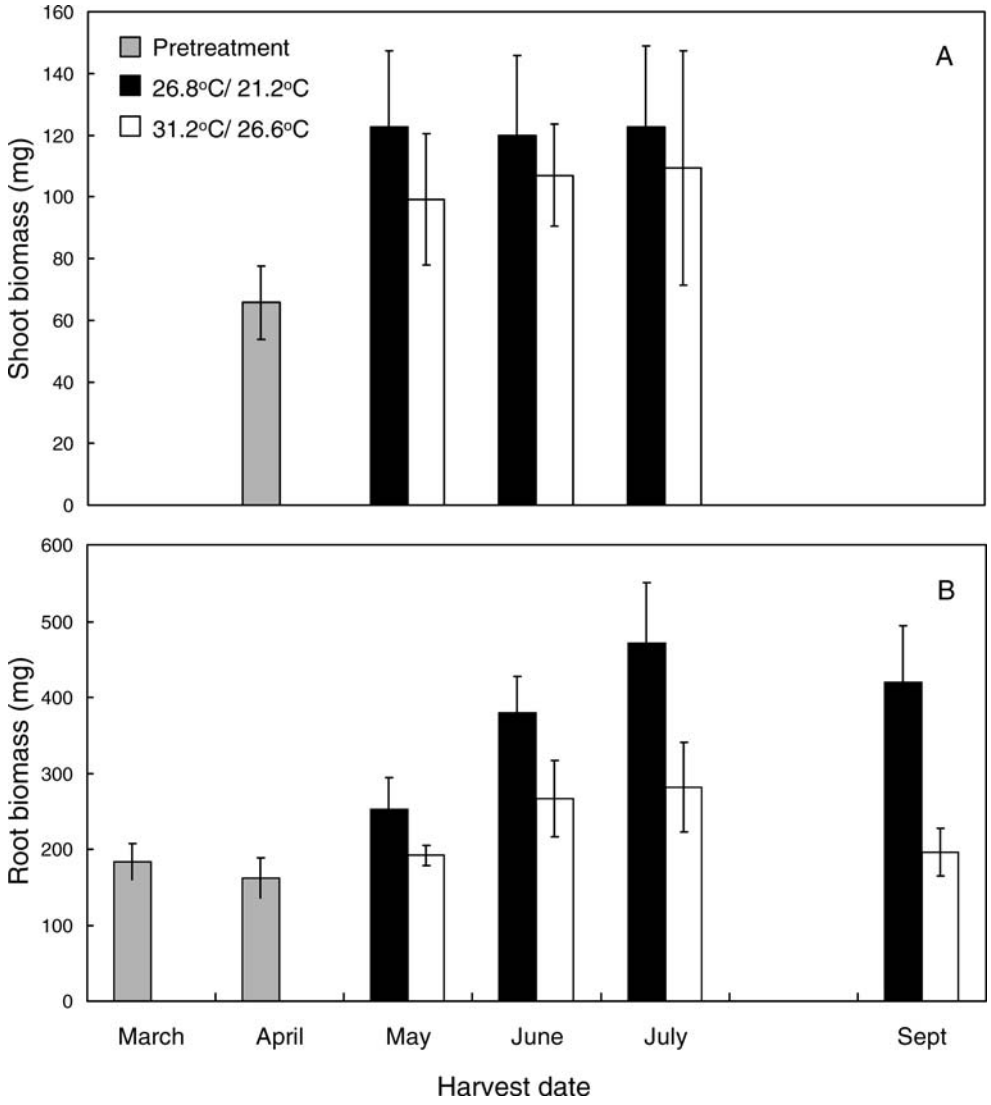

Fig. 2. (A) Shoot biomass and (B) root biomass of *Panax quinquefolius* before temperature treatments ("pretreatment"), or after one growing season at low or high temperatures. March and April values of the mean of 7–10 plants (±SE); May-July values are the mean of 3–4 plants (±SE); and September root biomass is the overwintering biomass measured after aboveground senescence, and each value is the mean of 5–9 roots (±SE).

Root ginsenosides—The concentrations of all individual ginsenosides from plants grown at low temperatures decreased from the pretreatment period (mean of March and April) to the growing season (mean of May, June, and July), except for ginsenosides Rd and Rg_1 (R_{b1}: $F_{1,38} = 9.38$, $P = 0.0057$; R_{b2}: $F_{1,38} = 7.52$, $P = 0.0119$; R_c: $F_{1,38} = 5.48$, $P = 0.0288$; R_e: $F_{1,38} = 10.20$, $P = 0.0042$; Figs. 3 and 4). However, the concentration of most individual ginsenosides from plants grown at high temperatures remained constant during this time, and only ginsenosides Rb_1 and Re decreased (R_{b1}: $F_{1,38} = 5.65$, $P = 0.0262$; R_e: $F_{1,38} = 4.84$, $P = 0.0381$).

Regardless of temperature treatment, Rb_1 and Re did not change throughout the growing season, but the concentrations of the other ginsenosides decreased over time (R_{b2}: $F_{2,20} = 6.47$, $P = 0.0241$; R_c: $F_{2,20} = 4.80$, $P = 0.0241$; R_d: $F_{2,20} = 12.45$, $P = 0.0007$; R_{g1}: $F_{2,20} = 12.96$, $P = 0.0005$). In both high and low temperature treatments, the 20(*S*)-protopanaxadiols (PD), 20(*S*)-protopanaxatriols (PT), and total storage root ginsenosides also decreased from May to July (PD: $F_{2,20} = 6.50$, $P = 0.0092$; PT: $F_{2,20} = 4.08$, $P = 0.0386$; Total: $F_{2,20} = 5.83$, $P = 0.0134$; Table 1). During this time, all individual ginsenosides were insensitive to temperature, except Rb_2 which was 30% higher in storage roots grown at high temperatures compared to those in low temperatures ($F_{1,20} = 6.29$, $P = 0.0241$).

The effect of temperature became prominent at the final measurement in September when the concentration of total storage root ginsenosides was 49% greater in storage roots grown at high temperatures than in storage roots grown at low temperatures ($F_{1,7} = 7.00$, $P = 0.0383$). The concentrations of ginsenoside Re and the total protopanaxatriols were also higher (57% and 51%, respectively) in plants grown at high temperatures (R_e: $F_{1,7} = 50.42$, $P = 0.0004$; PT: $F_{1,7} = 25.83$, $P = 0.0023$). At this final measurement, the concentrations of most storage root ginsenosides were similar to concentrations observed before treatment. Only the concentration of ginseno-

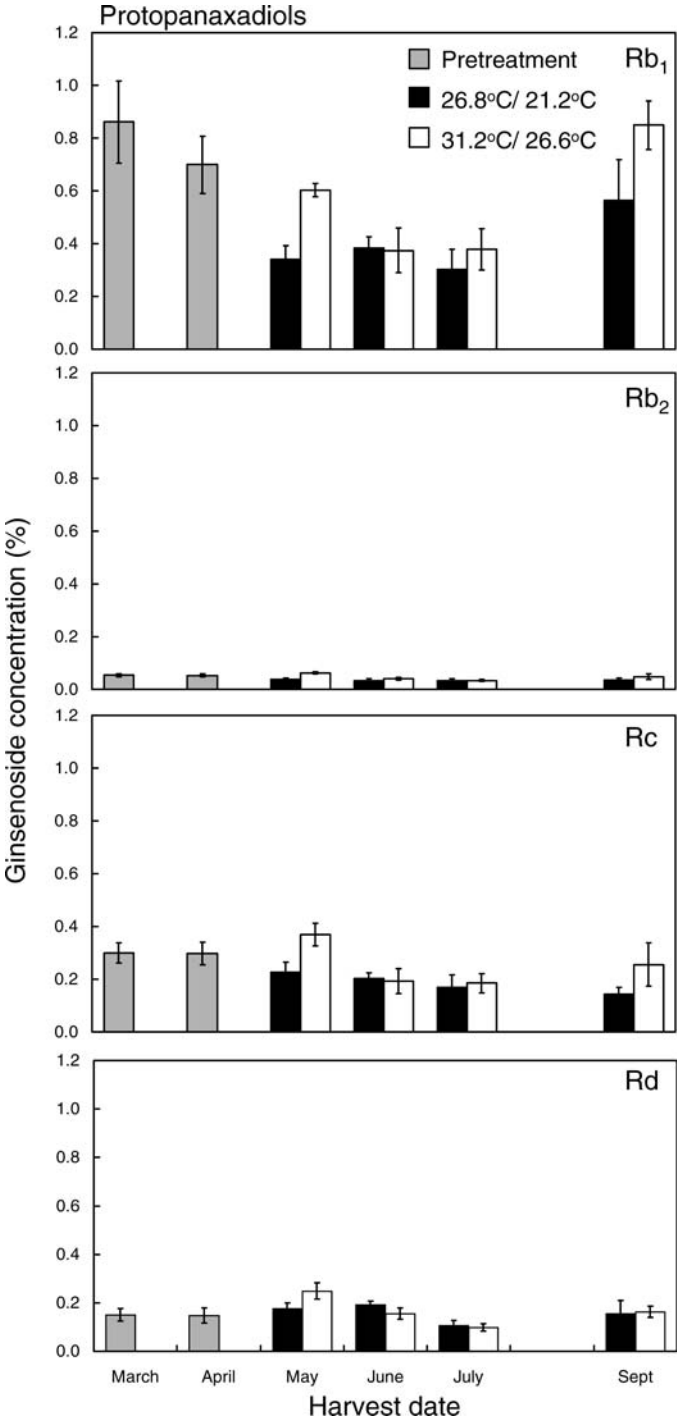

Fig. 3. Concentrations of protopanaxadiol ginsenosides (Rb_1, Rb_2, Rc, and Rd) from storage roots of *Panax quinquefolius* before temperature treatments ("pretreatment"), or after one growing season at low or high temperatures.

side Rc from plants grown at low temperatures was not restored and instead decreased between the pretreatment period and September ($F_{1,24} = 6.34$, $P = 0.0212$). The absolute amount of ginsenosides in each storage root, on the other hand, was not affected by temperature treatment at the final measurement ($P \geq 0.05$ for all individual ginsenosides; Table 2).

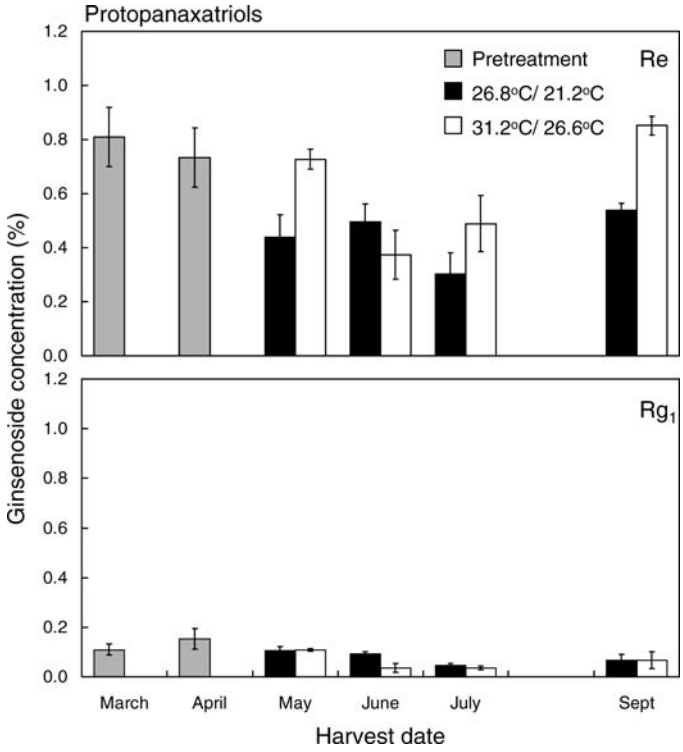

Fig. 4. Concentrations of protopanaxatriol ginsenosides (Re and Rg_1) from storage roots of *Panax quinquefolius* before temperature treatments ("pretreatment"), or after one growing season at low or high temperatures.

DISCUSSION

Panax quinquefolius was highly sensitive to a 5°C increase in growth temperature. Elevated temperatures reduced net photosynthetic rates and stomatal conductance and caused premature leaf senescence. At the end of the growing season, plants grown at the high temperature treatment had significantly less belowground biomass but higher concentrations of total storage root ginsenosides than plants in the low temperature treatment. Therefore, increased temperatures may have detrimental consequences for the growth of understory species, such as *P. quinquefolius*.

Physiological response to elevated temperatures and heat stress in P. quinquefolius—Temperatures strongly influence photosynthetic processes, ontogenetic development, and the carbon balance of plants, and the influence can be positive or negative (Morison and Lawlor, 1999). Moderate increases in temperature may increase biomass production of overstory forest species (Kirschbaum, 2000). Understory forest species, however, have been found to be more sensitive to elevated temperatures than shrub or tree species (Farnsworth et al., 1995), and shade-tolerant species are particularly susceptible to temperature changes (Bassow et al., 1994). Therefore, even small increases in growth temperature may induce heat stress for shaded understory herbs. The decreased stomatal conductance and photosynthesis of *P. quinquefolius* observed in this experiment may be indicative of reduced chloroplast and membrane integrity (Berry and Bjorkman, 1980) and increased cell and tissue death (Liu and Huang, 2000) associated with heat stress. *Panax quinquefolius* may be especially sensitive to

TABLE 1. Ginsenoside concentrations (total, protopanaxadiol [Rb_1, Rb_2, Rc, and Rd], and protopanaxatriol [Re and Rg_1]) in storage roots of *Panax quinquefolius* grown at low (26.8°C day/21.2°C night) or high (31.2°C day/26.6°C night) temperatures. March ($N = 10$) and April ($N = 7$) values are pretreatment ginsenoside concentrations; May to July ($N = 3$–4 per temperature treatment) values are the ginsenoside concentrations during the growing season; and September ($N = 4$ per temperature treatment) values are the overwintering ginsenoside concentrations after shoots had senesced. Each value is the mean (±SE).

Concentration (%)	Growth temperature	Pretreatment		Growing season			Overwintering
		March	April	May	June	July	September
Total ginsenosides	Low	2.23 (0.32)	2.08 (0.30)	1.32 (0.19)	1.40 (0.09)	0.96 (0.18)	1.50 (0.25)
	High			2.12 (0.12)	1.17 (0.23)	1.22 (0.19)	2.24 (0.13)
Protopanaxadiols	Low	1.36 (0.22)	1.20 (0.17)	0.78 (0.09)	0.81 (0.02)	0.61 (0.12)	0.90 (0.23)
	High			1.28 (0.09)	0.76 (0.14)	0.70 (0.09)	1.32 (0.16)
Protopanaxatriols	Low	0.92 (0.12)	0.89 (0.14)	0.54 (0.10)	0.59 (0.07)	0.35 (0.07)	0.61 (0.03)
	High			0.84 (0.03)	0.41 (0.09)	0.53 (0.11)	0.92 (0.05)

increased growth temperatures. Lee et al. (1980) found that the photosynthetic rates of *P. ginseng* C. Meyer (Asian ginseng) leaves were reduced by 25% when measured at 30°C rather than 20°C. Our study, using a more moderate increase in temperature, found that light saturated photosynthetic rates were 52% lower for *P. quinquefolius* growing at 30°C rather than 25°C.

The low values of photochemical efficiency of photosystem II that we observed in July indicated that *P. quinquefolius* leaves in both temperature treatments were senescing and that the senescence process had progressed further in the shoots grown in the high temperature treatment (Liu and Huang, 2000). Photosynthetic capacity of a senescing leaf gradually declines until the leaf dies (Gepstein, 1988). Leaf senescence is controlled by a combination of environmental factors, such as photoperiod and temperature, as well as endogenous factors including age, reproductive maturity, and plant hormones (Munne-Bosch and Alegre, 2004). Temperature strongly influences metabolic activity and plant ontology, and high temperatures can induce premature leaf senescence (Morison and Lawlor, 1999). The decreased leaf longevity in *P. quinquefolius* grown at elevated temperatures reduced the time available for photosynthesis over the growing season and probably caused the reduction in biomass accumulation.

Root ginsenoside concentrations and content—Despite the negative effect of elevated temperatures on root biomass at the end of the growing season, the concentration of total storage root ginsenosides was 49% higher when plants were grown at higher rather than lower temperatures. The concentration of most ginsenosides from plants grown at low temperatures decreased from the pretreatment period through the growing season, while ginsenosides from plants grown at high temperatures remained relatively constant. As the plants prepared to overwinter, the concentrations of most ginsenosides were restored to pretreatment levels. Li and Wardle (2002) also studied the seasonal fluctuations in ginsenosides but reported that concentrations were highest in June and decreased through September for 3- and 4-yr-old *P. quinquefolius* plants.

Differences in the final overwintering concentrations of storage root ginsenosides can be attributed to dilution by the size of the storage root. The absolute amounts of ginsenosides in storage roots from plants grown in both temperature treatments were not different; therefore, as storage roots from plants grown at low temperatures increased in biomass, the concentrations of ginsenosides decreased. Likewise, greater concentrations of ginsenosides in *P. quinquefolius* grown at high temperatures were associated with smaller increases in storage root size.

The concentrations of secondary compounds in plant tissues often increase in response to stress (Mooney et al., 1991). The carbon-nutrient balance hypothesis (Bryant et al., 1983) and the growth differentiation balance hypothesis (Herms and Mattson, 1992) suggest that when plants are stressed, a trade-off occurs between allocating carbon to biomass production or to the formation of defensive secondary compounds. These hypotheses predict that carbon is allocated to biomass production in nonstressed plants but to formation of secondary defense and away from biomass in stressed plants. Hamilton et al. (2001) recently criticized these hypotheses, and studies of how secondary metabolites respond to climate change conditions have been inconsistent. For example, studies show that monoterpene levels in Douglas fir (*Pseudotsuga menziesii*) can either decline with a 3.5°C increase in temperature (Snow et al., 2003), increase with a 4°C increase (Litvak et al., 2002), or are unaffected by the combination of elevated CO_2 (550 μmol/mol) and a 4°C increase in temperature (Constable et al., 1999). A review by Morison and Lawlor (1999) shows that the interactive effect of elevated CO_2 and increased temperature

TABLE 2. Absolute amount of individual and total ginsenosides in storage roots of *Panax quinquefolius* grown at low (26.8°C day/21.2°C night) or high (31.2°C day/26.6°C night) temperatures in September. Each value is the mean (±SE) for $N = 4$.

Growth temperature	Ginsenoside (mg/root)						
	Rb_1	Rb_2	Rc	Rd	Re	Rg_1	Total
Low	3.34 (1.45)	0.18 (0.05)	0.67 (0.16)	0.84 (0.34)	2.99 (1.19)	0.35 (0.13)	8.36 (3.18)
High	2.15 (0.44)	0.12 (0.03)	0.64 (0.22)	0.43 (0.12)	2.11 (0.22)	0.17 (0.08)	5.61 (0.84)

may be negligible for both secondary metabolites and biomass production. In our experiment, a 5°C increase in temperature clearly induced stress in *P. quinquefolius* but did not stimulate production of storage root ginsenosides.

Implications of increased temperatures on P. quinquefolius—Increased temperatures across the globe have been linked to shifts in species' distributions (Walther et al., 2002). *Panax quinquefolius* naturally grows from southern Georgia to Ontario and Quebec in Canada (McGraw et al., 2003). In this study, *P. quinquefolius* was highly sensitive to a 5°C increase in growth temperature. Temperature, therefore, may be a key environmental factor controlling the present range limits of *P. quinquefolius*, preventing it from extending further south. This study suggests that future increases in temperature associated with global warming may shift the natural distribution of *P. quinquefolius* northward.

Conclusions—Carbon accumulation in *P. quinquefolius* was strongly affected by a 5°C increase in growth temperature. Leaf senescence was accelerated, and photosynthesis and stomatal conductance were reduced as the season progressed and the temperature increased. In September, the overwintering biomass of *P. quinquefolius* roots grown at higher temperatures was half that of plants grown at lower temperatures, but the absolute amount of ginsenosides per root was unaffected by temperature. Therefore, the concentration of total storage root ginsenosides was affected by the size of the root, and plants grown at higher temperatures had greater concentrations of ginsenosides than plants grown at low temperatures. We conclude that a 5°C increase in temperature is highly detrimental to the productivity of *P. quinquefolius* and that increased temperatures associated with climate change may induce heat stress, reducing the productivity and potentially leading to changes in current distribution of similar sensitive understory herbs.

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