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Understory light and root ginsenosides in forest-grown *Panax quinquefolius*

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Abstract

The objective of this study was to determine the relationship between light levels in the understory of a broadleaf forest and the content of six ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd) in 1- and 2-year-old American ginseng (*Panax quinquefolius* L.) roots. Our results revealed that ginsenoside contents in 1- and 2 year-old roots collected in September were significantly related to direct and total light levels, and duration of sunflecks. At this time, the effect of light levels accounted for up to 48 and 62% of the variation in ginsenoside contents of 1- and 2-year-old American ginseng roots. Also, red (R) and far red (FR) light, and the R:FR ratio significantly affected Rd, Rc, and Rg_1 contents in 2-year-old roots, accounting for up to 40% of the variation in ginsenoside contents.

Keywords: *Panax quinquefolius* L.; Araliaceae; Hemispherical photography; Light intensity; Light quality; Sunflecks; Saponins; Ginsenosides

1. Introduction

The medicinal value of American ginseng (*Panax quinquefolius* L.) is attributed at least in part, to the presence of ginsenosides, a group of triterpene saponins (Briskin, 2000). Over 30 types of ginsenosides have been isolated and identified in ginseng (Chuang et al., 1995), with six major ginsenosides found in American ginseng: Rb_1 (**1**), Rb_2 (**2**), Rc (**3**), Rd (**4**), Re (**5**), and Rg_1 (**6**) (Court et al., 1996), which are dammarene-type triterpenes composed of either two sugars called 20(*S*)-protopanaxadiols (**1**–**4**) or three sugars named 20(*S*)-protopanaxatriols (**5**,**6**) as their aglycones (Fig. 1). Triterpene saponins are synthesised via the mevalonate pathway in the cytosol (Chappell, 2002) from the cyclization of 2,3-oxidosqualene to 20(*S*)-dammarenediol (Kushiro et al., 1997), the last known intermediate in the synthesis of individual ginsenosides (Wang and Zhong, 2002).

The effect of light on root ginsenoside contents of ginseng plants is absent from the literature, except for a few studies (Lee et al., 1987; Park and Lee, 1993; Zhang et al., 1994), which revealed that the content of ginsenosides in Asian ginseng (*Panax ginseng* C.A. Meyer) increases when plants are cultivated under various environmental stresses, including high light levels (30% of the solar radiation) (Lee et al., 1987). Park and Lee (1993) showed that the ginsenoside content in 6-year-old Asian ginseng roots consistently increases from 13.6, 16.3, 17.7, and 19.1 mg g^{-1} dry weight (DW) when plants are exposed to 5, 10, 20, and 30% of the solar radiation. However, Zhang et al. (1994) showed that roots of *Panax pseudoginseng* exposed to 50% of the solar radiation contain less ginsenosides than those exposed to 35% of the solar radiation. Exposed to 36% of the solar radiation and over, ginseng plants are subject to photobleaching, and if the light conditions

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persist, premature leaf death (Parmenter and Littlejohn, 2000). The effect of light levels transmitted through the forest canopy on root ginsenoside contents in forest-grown American ginseng remains poorly studied.

Growing in forest understories, American ginseng plants are exposed to seasonal and daily light level variations. As the forest canopy develops, understory plants are exposed to rapidly decreasing light intensities with associated changes in light quality. Once the deployment of the canopy is terminated, understory plants continue to be exposed to direct sunlight transmitted through gaps in the forest canopy (sunflecks). Hemispherical photography permits the estimation of light levels transmitted through the heterogeneous forest canopy overlying ginseng plants (Frazer et al., 1999). Our objective was to determine the relationship existing between understory light conditions and ginsenoside contents in American ginseng roots.

Table 1
Coefficient of determination (r^2) for linear regression analysis between understory light levels and ginsenoside contents (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd) in 1- and 2-year-old American ginseng roots (September) cultivated in a broadleaf forest

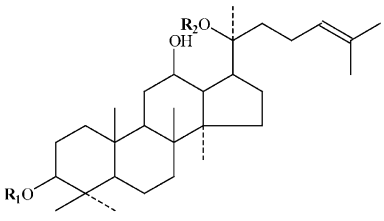
	Rg_1 (**6**)	Re (**5**)	Rb_1 (**1**)	Rc (**3**)	Rb_2 (**2**)	Rd (**4**)
One-year-old plants						
Daily light						
Direct	0.37*[a]	0.09[ns]	0.31*	0.04[ns]	0.25[ns]	0.45**
Diffuse	0.04[ns]	0.13[ns]	0.10[ns]	0.01[ns]	0.14[ns]	0.32*
Total	0.24[ns]	0.12[ns]	0.25[ns]	0.03[ns]	0.24[ns]	0.46**
Sunfleck	0.36*	0.09[ns]	0.32*	0.04[ns]	0.27*	0.48**
Two-year-old plants						
Cumulative light						
Direct	0.07[ns]	0.22[ns]	0.45*	0.62**	0.41*	0.51**
Diffuse	0.26[ns]	0.04[ns]	0.03[ns]	0.05[ns]	0.00[ns]	0.02[ns]
Total	0.17[ns]	0.23[ns]	0.43*	0.58**	0.32*	0.46*
Sunfleck	0.08[ns]	0.19[ns]	0.36*	0.54**	0.39*	0.48*

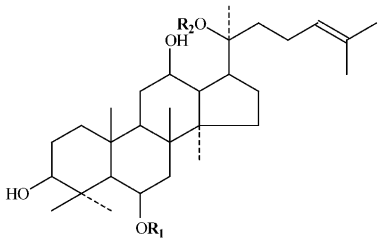
[a] Each regression was performed using the data collected in 15 experimental units (9 and 5 samples per unit for 1- and 2-year-old plants, respectively). The slope is positive.
[NS] *, **: non-significant and significant at $P \leqslant 0.05$ and $P \leqslant 0.01$.

2. Results and discussion

In September 1999, **5** and **3** contents in 1-year-old roots were not significantly linked to light levels, while daily direct light and duration of sunflecks were significantly positively linearly related to **6**, **1**, **4** (Table 1), and total root ginsenoside contents (Fig. 2). For example, 31% of the variation in total ginsenoside content of one-year-old American ginseng roots was due to total light levels that ranged from 119 μmol m^{-2} s^{-1} to 334 μmol m^{-2} s^{-1} at the end of the growing season (Fig. 2). Except for **4**, daily diffuse and total light levels had no effect on individual ginsenoside contents in 1-year-old American ginseng roots (Table 1).

Except for the slightly significant relationship between daily total light and **5** content ($r^2 = 0.26$; $P \leqslant 0.05$), daily light levels were not significantly related to individual or total ginsenoside contents in roots of 2-year-old American ginseng plants ($P \leqslant 0.05$) (data not shown). In contrast, cumulative direct and total light levels, and cumulative duration of sunflecks were positively related to **1**–**4** (Table 1), and total ginsenoside contents in 2-year-old roots (Fig. 3). Notably, 44% of the variation in total ginsenoside content of 2-year-old American ginseng roots was due to cumulative total light levels at the end of the growing season (Fig. 3). However,





1 Rb_1 : R_1=D-Glc(β1-2)D-Glc-
R_2=D-Glc(β1-6)D-Glc-

2 Rb_2 : R_1=D-Glc(β1-2)D-Glc-
R_2=L-Ara(α1-6)D-Glc-

3 Rc : R_1= D-Glc(β1-2)D-Glc-
R_2= L-Araf(α1-6)D-Glc-

4 Rd : R_1= D-Glc(β1-2)D-Glc-
R_2=D-Glc-

5 Re : R_1=L-Rha(α1-2)D-Glc-
R_2=D-Glc-

6 Rg_1 : R_1=D-Glc-
R_2=D-Glc-

Fig. 1. Chemical formulae of six dammarene-type triterpenes called ginsenosides, Rg_1, Re, Rb_1, Rc, Rb_2, and Rd, identified in American ginseng roots. Glc: glucopyranosyl; Ara: arabinopyranosyl; Araf: arabinofuranosyl; Rha: rhamnopyranosyl.

cumulative diffuse light levels did not affect ginsenoside contents in American ginseng roots (Table 1, Fig. 3). While our results showed that there is a linear relationship between light levels and ginsenoside contents, exposing ginseng plants to more 36% of the solar radiation would not result in higher root ginsenoside contents since Zhang et al. (1994) showed that plants exposed to 35 and 50% of the solar radiation have decreasing ginsenoside contents. The canopy opening in our experimental site, estimated using hemispherical photography, allowed less than 35% of the solar radiation to penetrate to the understory even during emergence of ginseng plants in the spring (data not shown).

Our study further revealed that American ginseng plants exposed to longer sunflecks contained higher root ginsenoside contents than those exposed to shorter sunflecks (Table 1, Figs. 2 and 3). For example, 35% of the variation in total ginsenoside content of 1-year-old American ginseng roots was due to sunfleck durations that ranged from 0.7 to 3.7 h d^{-1} at the end of the growing season (Fig. 2). To our knowledge, there are no reports that sunflecks stimulate the accumulation of ginsenosides in American ginseng or other plants. However, the highest daily sunfleck durations (up to 3.7 h d^{-1}) and light levels (up to 18% of the solar radiation) measured in 1999 were shown to induce mild photooxidation in forest-grown 2-year-old American ginseng plants (Fournier, 2001), which could have stimulated plant defence mechanisms such as the induction of jasmonate-response genes by jasmonate (Creelman and Mullet, 1995). Methyl jasmonate has been shown the greatly increase ginsenoside contents in Asian ginseng and *Panax notoginseng* cell cultures (Lu et al., 2001; Wang and Zhong, 2002). However, daily sunfleck durations (from 0.4 to 2.0 h d^{-1}) and light levels (from 5% to 7% of the solar radiation) (data not shown) measured in September 2000 were not sufficiently elevated to induce photooxidative damage, which could explain why daily sunflecks and light levels did not significantly affect ginsenoside contents in 2-year-old American ginseng roots in September 2000.

Light quality also influenced root ginsenoside contents in 2-year-old American ginseng plants where those exposed to higher red (R) and far red (FR) light levels, and higher R:FR ratios had elevated contents of **6**, **3**, and **4** in their roots (Fig. 4). However, blue (B) light did

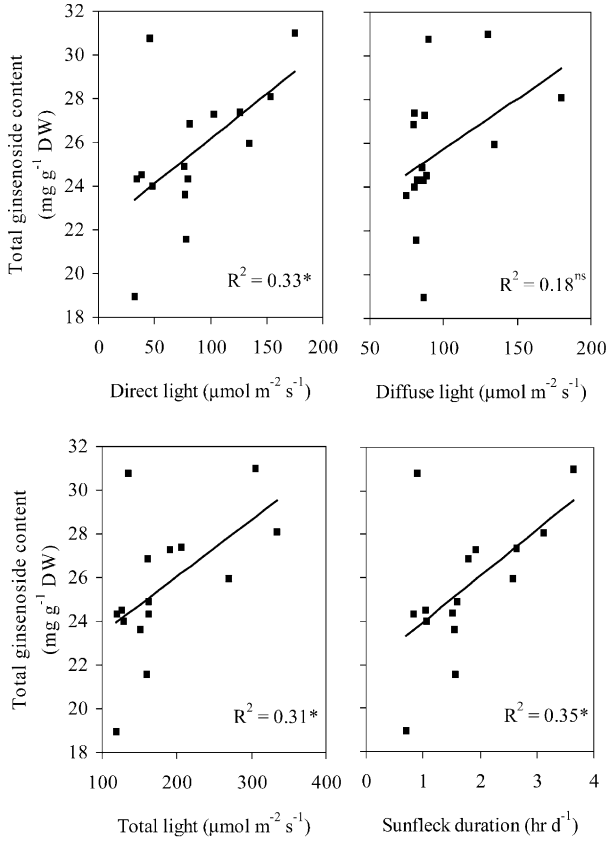


Fig. 2. Coefficient of determination (r^2) for linear regression analysis between daily understory light levels and total ginsenoside content in 1-year-old American ginseng roots collected in September 1999 in a broadleaf forest. Each regression was performed using data collected in 15 experimental units (nine samples per unit). NS, *: non-significant and significant at $P \leqslant 0.05$.

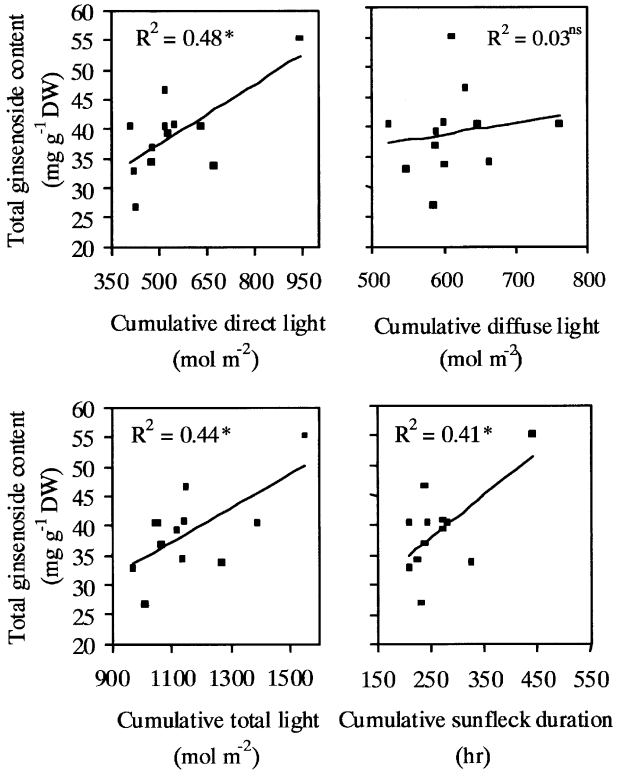


Fig. 3. Coefficient of determination (r^2) for linear regression analysis between cumulative understory light levels and total ginsenoside content in 2-year-old American ginseng roots collected in September 2000 in a broadleaf forest. Each regression was performed using data collected in 15 experimental units (five samples per unit). NS, *: non-significant and significant at $P \leqslant 0.05$.

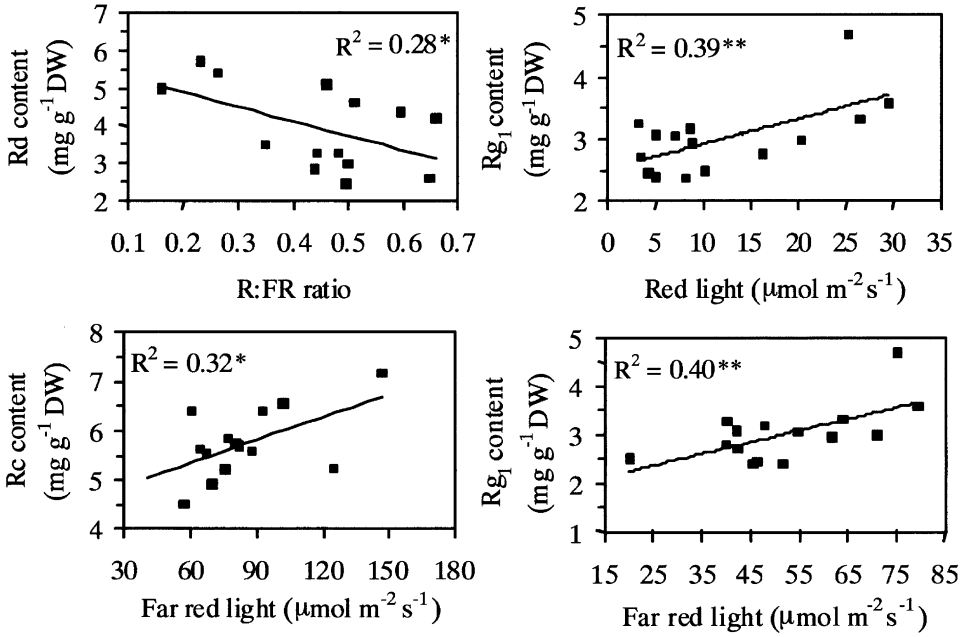


Fig. 4. Coefficient of determination (r^2) for linear regression analysis between Rg_1, Rc, and Rd contents in 2-year-old American ginseng roots, and understory red (R) and far red (FR) light levels, and R:FR ratio measured on 28 May 2000 (Rc) and 4 July 2000 (Rg_1 and Rd) in a broadleaf forest. Each regression was performed using data collected in 15 experimental units (five samples per unit). *, **: Significant at $P \leqslant 0.05$ and $P \leqslant 0.01$.

not influence the accumulation of ginsenosides in 2-year-old roots of American ginseng plants ($P \leqslant 0.05$) (data not shown). Since R and FR light significantly influenced ginsenoside contents (Fig. 4), it suggests that phytochrome, a photoreceptor that is influenced by light (Mohr and Schopfer, 1995) could affect enzyme activity and gene expression of intermediates in the pathway involved in ginsenoside synthesis. However, the regulatory effect of light intensity and quality on the process of ginsenoside synthesis remains elusive and the process of triterpene biosynthesis remains poorly studied, even considering the interest in these compounds as natural medicines (Haralampidis et al., 2001; Osbourn, 2003).

3. Conclusions

Our study showed, for the first time, the role of light intensity, quality, and duration of sunflecks on ginsenoside contents in American ginseng roots grown in a broadleaf forest. Precisely, results of this study showed that direct and total light levels, and duration of sunflecks were positively linearly related to ginsenoside contents in American ginseng roots at the end of the growing season. Moreover, R and FR light, and R:FR ratio significantly affected the content of several ginsenosides in American ginseng roots. A conservative pruning of the trees forming the forest canopy could allow ginseng growers to optimise understory light levels, maximising the accumulation of ginsenosides in American ginseng roots. However, beyond 36% of the solar radiation, ginseng is exposed to high light levels that not only cause reduced ginsenoside accumulations (Zhang et al., 1994), but also photoinhibition and premature leaf death (Parmenter and Littlejohn, 2000). Growing American ginseng in a broadleaf forest is profitable since roots contain higher ginsenoside contents than plants cultivated under artificial shade (Court et al., 1996) and even wild-grown plants (Betz et al., 1984).

4. Experimental

4.1. Experimental design

In October 1998, stratified American ginseng (*Panax quinquefolius* L.) seeds (Panax Q Farm Ltd, Vernon, B.C., Canada) were planted in 15 experimental units each measuring 6 m^2 in a broadleaf forest at Île d'Orléans, Québec, Canada (Lat. 46.57 North; Long. 70.56 West). The location of each experimental unit was allotted randomly on a total forest surface area of 1.2 ha. Due to the heterogeneous nature of the forest canopy, there was no repetition of light levels, which were estimated at fixed points (one point per experimental unit) in each experimental unit. Prior to sowing seeds at a density of 30 kg ha^{-1}, the soil was churned to a depth of 15 cm and lime was incorporated into the soil mixture (6 t ha^{-1}) to elevate soil pH to an average of 5.2 ± 0.3. The average soil mineral concentrations (ppm $\pm$ standard deviation) during the years 1999 and 2000 were as follows: 15 ± 8 P, 86 ± 22 K, 3085 ± 1062

Ca, 50±10 Mg, 1568±245 Al, 258±34 Fe, 2.0±0.8 Cu, 10.3±6.9 Mn, 20.24±7.48 Zn, 0.17±0.04 B, 12±1% organic matter and electrical conductivity of 114±28 μS dm^{-1}. Linear regressions were performed using SAS, version 6.12 (SAS Institute Inc., NC, USA) to elucidate the relationship existing between light levels transmitted through the forest canopy and ginsenoside contents in American ginseng roots.

4.2. Hemispherical photography and spectroradiometry measurements

Understory light conditions were evaluated using hemispheric photography (Fournier, 2001; Frazer et al., 1999) and spectroradiometry. Hemispheric photographs were taken on overcast days with uniform cloud cover using a Nikon CoolPix 800 digital camera equipped with a Fisheye lens Converter FC-E8 (Nikon Corp., Tokyo, Japan) assembled on a Manfrotto levelled tripod (Manfrotto Nord, Feltre, Italy) 1 m above ground level. Photographs were taken skyward once in September 1999 and at regular intervals (total of 15 times) during the 2000 growing season. From May to June 2000, photographs were recorded twice weekly in each experimental unit and at 3-week intervals thereafter. The images were analysed with an imaging software named Gap Light Analyser, version 2.0 (University of Victoria, BC, Canada), which estimated the amount of transmitted direct, diffuse, and total (direct + diffuse) radiation incident on a surface when there is blockage of light from the overlying forest canopy, and sunfleck durations (Frazer et al., 1999). Diffuse radiation is defined as atmospheric light energy dispersed by atmospheric particles and dust, while direct light is non-dispersed atmospheric light. Sunflecks are naturally found gaps in the forest canopy that permit the penetration of direct sunlight to the understory (Frazer et al., 1999). Cumulative light levels were determined by cumulating daily light levels (mol m^{-2} d^{-1}) and daily sunfleck durations (h d^{-1}) estimated from May to July 2000 (for July analyses), and from May to September 2000 (for September analyses). Cumulative light levels were not determined in 1999 since transmitted light levels were recorded once at the end of the growing season.

The spectral quality (300–1100 nm) of the understory light was characterised on 4 May, 28 May, and 4 July 2000 between 10:00 and 14:00 using a spectroradiometer (Licor 1800, Lincoln, N.E., U.S.A.) in each experimental unit. The light sensor was placed at a fixed location in the experimental unit to measure blue (B), red (R), and far red (FR) light levels, and red:far red (R:FR) ratio. Statistical analyses were performed using light quality measurements recorded during each sampling period with the ginsenoside content of 2-year-old American ginseng roots in July 2000.

4.3. Root ginsenoside analyses

In the 15 experimental units, nine (1-year-old) and five (2-year-old) American ginseng roots, collected in September 1999, July 2000, and September 2000, were randomly selected and pooled to measure root ginsenoside contents (per DW). One ginsenoside analysis was performed for each pooled sample (one analysis per experimental unit), for a total of 45 analyses. All root samples were dried in an oven at 45 °C for 72 h prior to ginsenoside analyses. After dried root samples were crushed, the root powder was mixed with 50% ethanol, boiled, and centrifuged at 3000 rpm for 10 min. The supernatant was then filtered and 80 μl of acetophenone in 500 ml of 50% ethanol was added to the supernatant to which 25 ml of 50% ethanol was incorporated. Lastly, 20 μl of the final solution was injected into HPLC apparatus (4.6×250 mm) for ginsenoside analyses using linear gradient A [10 mM KH_2PO_4–CH_3CN (80–20)] and gradient B [H_2O–CH_3CN (15–85)] with a flow rate of 1 ml min^{-1}. The linear gradient profile was as follows: 0–15 min, 98–96% A, 2–4% B; 15–25 min, 96–85% A, 4–15% B; 25–40 min, 85–75% A, 15–25% B; 40–50 min, 75–0% A, 25–100% B; 50–62 min, 100% B. Acetophenone was used as an internal standard solution (Chuang et al., 1995) and as a response factor, which enabled us to calculated the quantity of ginsenosides in root samples. The content of each ginsenoside was determined by comparing the area of the peaks of the ginsenosides in the root samples to standards (Extrasynthese, Genay, France).

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