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Effects of Population, Age, and Cultivation Methods on Ginsenoside Content of Wild American Ginseng (*Panax quinquefolium*)

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Genotype and environmental effects on ginsenoside content among eight wild populations of American ginseng (*Panax quinquefolium*) were investigated. Root concentrations of six ginsenosides were determined at the time of collection of plants from the wild (T0) and 2 years (T2) after transplanting roots from each of the eight populations to each of two different forest garden locations. Both location and population had significant effects on root and shoot growth. Overall, ginsenoside Rb1 was most abundant, followed by Rg1 and Re. Concentrations of Rg1 and Re were inversely related among and within populations. The relative ranking of populations differed depending upon the particular ginsenoside and sampling time. The relative importance of genotype and environment was not the same for all ginsenosides. Ginsenoside Re was influenced by population but not location, whereas Rb1, Rc, and Rb2 were influenced only by location (environment), while Rg1 and Rd were influenced by both. Ginsenoside levels were consistently lower, but growth was consistently higher at the more intensively managed garden location.

KEYWORDS: ***Panax quinquefolium*; ginseng; HPLC; ginsenoside; genotype; phytochemical variation**

INTRODUCTION

For at least 2000 years, Korean ginseng (*Panax ginseng* C. A. Meyer, Aralaceae family) has been valued as a medicinal herb in traditional Asian medicine. For nearly 300 years, American ginseng (*Panax quinquefolium*) has been harvested from wild populations across its range in eastern and central North America for export mainly to China (*1*). Since the eighteenth century, it has been cultivated horticulturally in North America as well (*2*).

The pharmacologically active constituents of *Panax* species are a group of triterpene saponins known as ginsenosides. *P. quinquefolium* is reported to contain 13 distinct ginsenosides (*3*). The six most abundant ginsenosides can be subdivided on the basis of the aglycone (dammarane) portion of the molecule, into 20(s)-protopanaxadiol (PD) ginsenosides (Rb1, Rb2, Rc, and Rd) and 20(s)-protopanaxatriol (PT) ginsenosides (Re and Rg1). Many biological and environmental factors affect ginsenosides quantitatively and qualitatively (qualitative effects pertain to relative contribution to total ginsenoside content). Variation among and within individual ginsenosides may be pharmacologically important because individual ginsenosides differ in their effects on human physiology (*4*). Given that cultivated American ginseng consists largely of undomesticated land races (*5*), wild populations may serve as reservoirs of genetic variation, which could prove valuable in breeding or clonal selection for genetic improvement. Moreover, substantial genetic variation in ginsenosides among wild populations might bear implications for conservation strategies for this increasingly threatened species.

Because the economic value of wild American ginseng is far greater than that of cultivated ginseng (>10-fold), it is often assumed that ginsenoside content must be higher in the former (*6*, *7*). Betz et al. (*8*) reported greater total ginsenoside content in the wild than in cultivated American ginseng, as did Foster (*9*), while Lui and Staba (*10*) reported minimal differences. Tanaka (*11*) found no significant difference in ginsenoside content between the wild and cultivated Asian ginseng, although Mizuno et al. (*12*) reported that ginsenosides Rg1, Re, and Rd were higher in the wild than in cultivated roots of Asian ginseng (*P. ginseng*), whereas the ginsenosides Rc, Rb2, and Rb1 were lower. In all of these comparisons of the ginsenoside content of the wild versus cultivated ginseng, one potentially confounding factor that was not accounted for is that of differences in age between the two types. An exception is a recent report by Assinewe et al. (*13*) that compared 4-year-old wild and cultivated roots and found no differences in the total ginsenoside content. Taking root age into consideration when comparing wild and cultivated populations is important, because there are several reports that ginsenosides increase with root age, and wild and cultivated ginseng is usually harvested at different ages. Cultivated roots typically are harvested at 3−4 years of age, whereas in most states, wild ginseng is typically harvested after 8 or more years (*14*, *15*). Studies based on relatively young (up

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to 7-year-old) cultivated ginseng indicate that root age is positively related to ginsenoside content in both Asian (*16*, *17*) and American ginseng (*18*, *19*), although Zito and Cheng (*20*) found no consistent difference in total or individual ginsenosides between 4- and 7-year-old American ginseng.

In addition to age, environmental variation also severely limits conclusions regarding the relative importance of genetic and environmental effects that can be drawn from previously published comparisons among or between wild and cultivated populations. For example, in a recent study by Assinewe et al. (*13*), although root age was constant, the wild population sample consisted of a mixture of only 2−5 roots from each of 10 different wild populations from widely dispersed locations (Maine to Wisconsin), whereas the cultivated sample consisted of 12 roots from a single commercial garden. Similarly, no attempt was made to control for or describe environmental variation between wild and cultivated populations in studies completed by Betz et al. (*8*), Foster (*9*), Lui and Staba (*10*), Tanaka (*11*), and Mizuno et al. (*12*). Comparing ginsenoside content among cultivated populations from dissimilar environments, Li et al. (*21*) found that Re, Rb1, Rc, Rb2, Rd, and total ginsenoside contents were significantly different among populations, while Jackson et al. (*19*) reported that total ginsenoside content was significantly different between two Canadian ginseng farms in Ontario and British Columbia. The importance of environmental effects on ginsenoside content is apparent from controlled studies involving single populations. Li and Mazza (*22*) reported weak correlations between root ginsenoside levels and the level of various soil mineral nutrients, and Zito et al. (*20*) reported that wood mulches from different tree species significantly affected ginsenoside content in 7-year-old but not 4-year-old American ginseng. Ginsenoside Rg1 in American ginseng roots showed a negative correlation with soil phosphorus (*23*). Although ginseng is a shade-adapted species, ginsenoside levels were increased by increasing light levels up to 35% of full sun (*24*). It therefore appears that questions regarding the relative "potency" (ginsenoside content) of wild versus cultivated ginseng and the relative contribution of genotype and environment to interpopulation variation remain unresolved.

The objective of this research was to determine the relative contribution of genotype (population) and environmental (location) effects on ginsenoside levels in wild American ginseng populations collected from a geographically limited region. The study focused on wild populations from the Catskill Mountains region of New York State, where ginseng is reputed to be of exceptionally high quality (*26*) and sold at premium prices (*27*). The experimental approach entailed comparing the growth and ginsenoside content of multiple wild populations of American ginseng before and after transplanting into each of two different environments.

MATERIALS AND METHODS

Ginseng Collection and Transplanting. Ginseng plants were collected from eight wild populations located on privately owned forested lands in five contiguous counties in and adjacent to the Catskill Mountains region of New York State. The counties within the Catskill region from which plants were collected and the number of populations collected from each were Otsego (1), Schoharie (1), and Delaware (2). Populations were also collected from two adjacent counties, Broome (1) and Chenango (3), immediately to the west of the Catskills. Collections were made in october 2000, during the legal ginseng harvest season. The senescing aboveground shoot was discarded, while the entire remaining living plant, including the underground rhizome with its associated dormant bud, the attached storage root, and associated secondary lateral roots, was collected intact. Plant samples were stored in zip-lock plastic bags with a small quantity of forest soil. Plant samples from each of the eight wild ginseng populations were subdivided as follows: approximately 10 plants per population were destructively sampled, as described below, for initial (T0) estimation of dry weight and ginsenoside content. The remaining plants from each wild population were transplanted to each of the two forest gardens within 1 week of collection, and a subsample was harvested at the end of the second growing season (T2) for growth measurements and ginsenoside analysis. The number of independently extracted and analyzed roots (*n*) for each collection time and forest garden location is indicated below. Before separation into two subgroups (T0 and T2), the age of each plant was estimated by counting the annual bud scars along the rhizome (*28*). Age distributions among populations at T0 and the subsequent T2 harvest were approximately equivalent. The forest gardens, into which wild-collected ginseng plants were transplanted for this study, were typical of small-scale, "woods-grown" ginseng forest farming (agroforestry) production systems practiced under natural forest canopies in the eastern U.S. (*29*, *30*). This cultivation scheme contrasts sharply with that of the much more intensive field or artificial shade commercial ginseng production systems utilized primarily in Wisconsin, Ontario, and British Columbia (*15*). The two forest garden locations were selected to represent the two typical management systems for producing woods-grown ginseng (*29*), wild-simulated (WS) and woods-cultivated (WC), which differ in intensity of cultivation. The less intensive WS forest garden was located at Cornell University's Arnot Teaching and Research Forest, near Van Etten, New York (Chemung County). This site was located within a mature sugar bush managed primarily for maple syrup production, beneath a closed canopy consisting primarily of sugar maple (*Acer saccharum*). The site was on an approximately 25% north-facing slope. The soil type was a Mardin Channery silt loam. The native forest soil in two adjacent 3 × 6 m beds was rototilled lightly and fenced to exclude deer. No organic matter or other soil amendments were incorporated at the time of bed preparation or during the course of the experiment. During the 2-year experiment, this WS garden was hand-weeded but no pesticides, fungicides, or fertilizers were applied. The more intensively managed WC forest garden was located at a privately owned commercial ginseng forest farm near Oxford, New York. This hardwood forest site was predominantly red oak (*Quercus rubrum*) and sugar maple. The soil type was Mardin/Wellsboro. A raised bed, typical of the more intensive WC ginseng system, was prepared on gently sloping (<5%) ground by rototilling several times, with incorporation of 10.2 cm of dried, shredded hardwood leaves and 2.3 kg/9.3 m^2 of granular gypsum ($CaSO_4 \cdot 2H_2O$). Roots at both sites were planted so that the rhizome was approximately horizontal and the bud was on the downhill side, approximately 1 cm below the soil line. After planting, the beds were mulched to a depth of approximately 8 cm with dried leaves from the forest floor nearby. During the following two growing seasons, plants at the WC garden but not at the WS garden were treated several times with fungicides for Alternaria and Phytopthora.

Sample Preparation and Ginsenoside Extraction. Within several days of collecting plants in plastic bags, samples were rinsed with tap water to remove soil, blotted dry, and then air-dried with a forced air food dehydrator (FD 50/30, American Harvest, Inc., Chaska, MN) at 35 °C for 3 days. This method of drying by gentle heating, as opposed to freeze-drying or forced air-drying at a higher temperature, was used because it more closely approximates the drying method used by ginseng collectors/growers. After drying, the rhizome and fibrous secondary roots were removed and the remaining storage root was prepared for analysis by weighing and grinding to a fine powder with a tissue grinder (AG-2005, Angel Electronic, Inc., Seoul, South Korea). Powdered samples were stored at room temperature in airtight, sealed glass scintillation vials.

The procedure for ginsenoside extraction and analysis was modified from Court et al. (*31*). A 100-mg powdered sample of each root was extracted in 10 mL of 100% HPLC-grade methanol (Fisher Chemicals, Fairlawn, NJ) in a plastic centrifuge tube and placed in a sonicator bath for 15 min at room temperature. The sample tube was centrifuged at 4500 rpm for 1 min, and the supernatant was collected. The pellet was re-extracted two additional times with 10 mL of solvent each time, and the supernatants were combined. The supernatant was reduced to

dryness under vacuum with a rotary evaporator (Buchi 011, Buchi Analytical, Inc., New Castle, DE) at 38 °C, and the residue was redissolved in 2 mL of 100% methanol. This was dried under a stream of N_2 at 38 °C and finally redissolved in 500 μL of 73% acetonitrile diluted with HPLC-grade water. A 15-μL sample was injected for HPLC analysis.

The HPLC was a Waters model 2690 Separations Module with a PDA detector (Waters 996 Photodiode Array) to determine absorption at 203 nm. Empower Pro software (Build 1154) was used for gradient programming and integration of absorption peaks. An HPLC column (Chromapack Standard Columns, LiChrosorb RP18, 5 μm, 250 × 3 mm) was used with a guard column (Chromsep Guard R), and a gradient of two solvents, (A) phosphate buffer (10.3 mM KH_2PO_4 at pH 5.8) and (B) acetonitrile: 0−20 min, 84−82% A and 16−18% B; 20−60 min, 82−60% A and 18−40% B, at a flow rate of 1.15 mL/min. M-cresol was used as an internal standard. Ginsenoside standards included Rg1, Re, Rb1, Rc, Rb2, and Rd (Indofine Chemical Company, Hillsborough, NJ). Qualitative identification of ginsenoside peaks was determined by cochromatography (equivalent retention time) with chemically pure standards, and quantification was based on the integration of the peak area compared with a standard curve (*32*). Results are reported as percent ginsenoside on a dry weight basis.

Experimental Design and Statistical Analysis. The experimental unit of analysis was the entire storage root of a single ginsenoside plant. The number of replicate root samples (*n*) for each population is indicated in parentheses for the T0, T2 WS, and T2 WC locations, respectively, as follows: P1 (11, 13, 10), P2 (10, 10, 10), P3 (7, 9, 10), P4 (10, 10, 10), P5 (7, 6, 10), P6 (5, 3, 10), P7 (10, 10, 10), and P8 (6, 10, 10). Initial ginsenoside content (T0) and plant growth and ginsenoside content at the end of the second growing season (T2) were treated as separate experiments for purposes of statistical analysis. Separate analyses were performed using as the dependent variable dry weight, shoot height, each of the six individual ginsenosides including Rg1, Re, Rb1, Rc, Rb2, and Rd, and total ginsenoside (sum of all six). The analyses for the T0 and T2 experiments included the independent variables population (P), with eight levels, and age (A) as a continuous variable treated as a covariate. The analyses for the T2 experiment also included the variable forest garden location (L) at two levels. For both experiments, a general linear model was fitted using the GLM procedure in SAS (SAS Institute, Inc., Cary, NC). The dependent variables root dry weight and shoot height were evaluated for the T2 experiment using the GLM procedure in SAS. In the case of the discrete variables P and L, Duncan's multiple comparison test was used for mean separation when the main effects or interactions were significant at the 5% level. When the continuous variable, A, was statistically significant, the least-squares (LS) mean for ages 4 and 10 years old (±1 standard deviation from the mean age of 7 years old) was used for graphic presentations in the figures that follow.

RESULTS AND DISCUSSION

Growth and Development. Both forest garden location (L) and population (P) had statistically significant effects on shoot height ($p \leq 0.01$) and on root dry weight ($p \leq 0.01$) (**Figure 1**). Both shoot height and root dry weight were clearly affected by population, with population 7 exhibiting the greatest root dry weight at either location and the greatest shoot height at the WC location. Across populations, shoot height and root dry weight were greater at the more intensively managed WC location. When these results were taken together, they suggested that the WC garden location, as expected, was more conducive to growth than the WS garden location.

Soil Composition Analysis. Table 1 shows that the WC sites exhibited 3-fold greater soil calcium and 7-fold greater manganese than the WS site but soil organic matter was only approximately half as great at the WC site. Soil calcium in particular is thought to play an important role in ginseng growth and development, to the extent that application of gypsum ($CaSO_4 \cdot 2H_2O$) is often recommended for WC ginseng production (*33*).

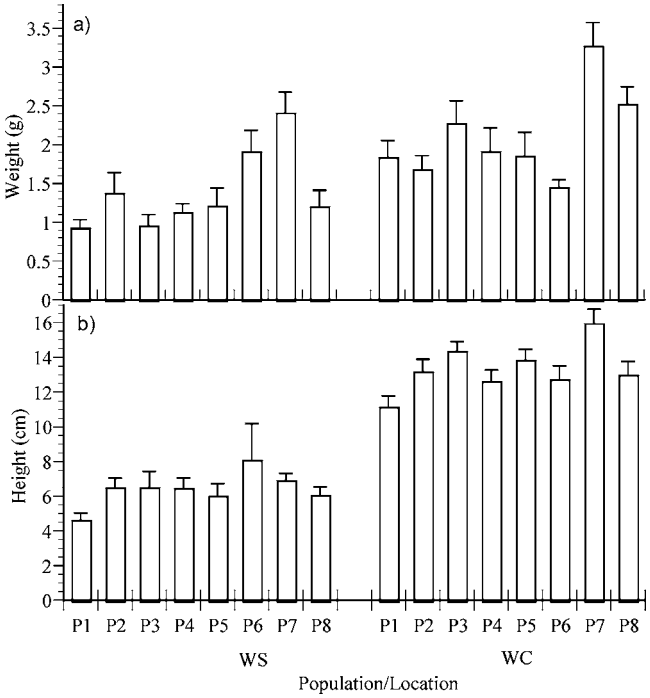

Figure 1. Effect of wild ginseng populations (P1–P8) and forest garden location to which populations were transplanted (WS, wild-simulated; WC, woods-cultivated) on (a) root dry weight and (b) shoot height at the end of the second growing season (T2) after transplanting. Growth responses are the mean ± SEM.

Table 1. Soil Nutrient Analysis for Wild-Simulated (WS) and Wood-Cultivated (WC) Forest Garden Locations[a]

	forest garden location	
nutrient	WS	WC
pH	4.5	4.3
P	8	10
K	353	510
Mg	387	460
Ca	2472	8262
Mn	184	1295
Zn	16	16
organic matter (%)	11.9	5.4

[a] Content of P, K, Mg, Ca, Mn, and Zn as kg/hc.

Ginsenoside Content. Overall, averaged across all populations, forest garden locations, and sampling times, the relative abundance of the six ginsenosides was Rb1 > Rg1 > Re > Rc > Rb2 = Rd (**Figure 2**). In several previous studies involving American ginseng (roots), the relative abundance of the three most abundant ginsenosides was always Rb1 > Re > Rg1, with Rg1 considerably lower than Re, ranging from an Re/Rg1 ratio of 1.5:13.7 (*13*, *18*, *19*, *21*, *22*, *34*, *35*). In this study, on the other hand, Rg1 was significantly higher (averaged across all populations, locations, and harvest times) than Re (Re/Rg1 ratio < 1.0) and nearly as high as Rb1. There was, however, considerable variation observed between the relative levels of Rg1 and Re, among populations and even among replicate roots within a single population sample. Moreover, an inverse relationship was observed to occur frequently between the levels of these two ginsenosides. **Figure 3** shows the root-to-root variation within a single representative sample of 11 roots (P1 at T0) in the levels of the three most abundant ginsenosides, Rb1, Rg1, and Re. A total of 4 of the 11 replicate roots in this sample had no measurable Re, and in each such case, Rg1 levels

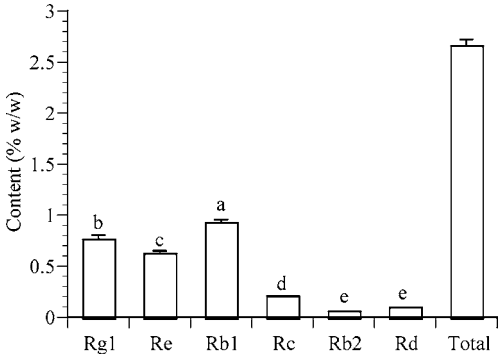

Figure 2. Relative abundance of six major ginsenosides and total ginsenoside averaged across all populations, forest garden locations, and collection times. Ginsenoside content is the mean ± SE of 319 individual root samples. Means accompanied by the same letter are not significantly different using Duncan's multiple comparison tests ($p < 0.05$).

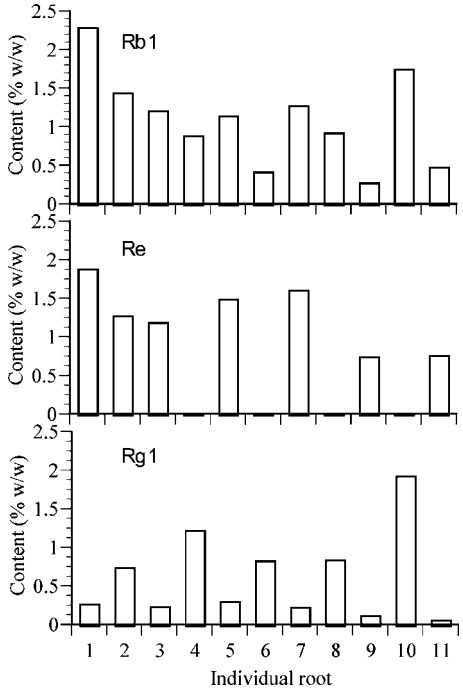

Figure 3. Concentration (%) of three ginsenosides (Rb1, Re, and Rg1) in each of 11 individual ginseng roots sampled from population 1, at the time of collection from the wild (T0).

were relatively high (>0.5% dry weight). On the other hand, in the remaining 7 roots with measurable Re content, Rg1 levels were low (<0.5% dry weight), with the exception of replicate number 2. The same pattern is evident when comparing the levels of Re and Rg1 for all roots sampled across all populations. **Figure 4** suggests a complex relationship between Re and Rg1, at lower levels of Rg1, but clearly, at T0, nearly all roots with Rg1 > 0.5% dry weight had little if any Re (**Figure 4a**) and, similarly, at T2, nearly all roots with Rg1 > 1.6% had essentially no Re (**Figure 4b**). Overall, Rg1 was found in relatively greater abundance compared with Re (**Figure 2**). A total of 35% of all individual roots sampled in this study contained no measurable Re (e.g., **Figure 3**). The percentage of roots without detectable Re differed significantly among populations across both sampling times (χ^2, $p \leq 0.001$), as follows: population 5 (70% of individual roots had no Re), population 4 (61%), population 2 (57%), population 1 (26%), population 7 (18%), population 3 (17%), population 6 (14%), and population 8 (13%). The inverse relationship between Rg1 and Re levels is apparent not only

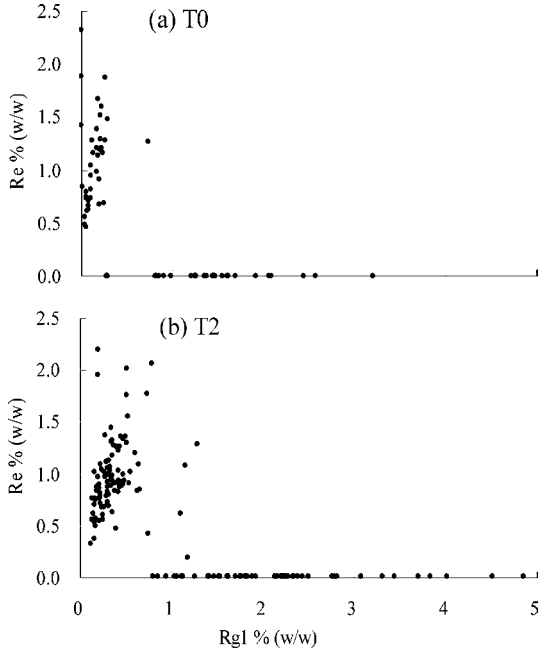

Figure 4. Relationship between the concentration of ginsenosides Rg1 and Re for all root samples of *Panax quinquefolium* across all populations at (a) the time of collection from the wild (T0, $n = 66$ root samples) and (b) the end of the second growing season after transplanting to two forest garden locations (T2, $n = 151$ root samples).

among individual roots within a population sample (e.g., **Figure 3**) but also among populations based on the level of each of these ginsenosides averaged across all roots in each of the eight populations. This can be seen both at T0 (Rg1, **Figure 5a**, versus Re, **Figure 5c**) and also for T2 (Rg1, **Figure 6a**, versus Re, **Figure 8**). The inverse relationship between Rg1 and Re is most extreme in the case of population 2 at T0 (**Figure 5**), which had the highest level of Rg1 for any population at that sampling time, in contrast to nearly 0 detectable Re. Both of these ginsenosides are structurally related to PT based on the presence of the triol aglycon subunit. The other 4 ginsenosides analyzed, including Rb1, Rb2, Rc, and Rd, are PD ginsenosides. The nature of the interaction among ginsenosides, suggested by **Figure 4**, is worthy of further investigation.

The considerable magnitude of the intrapopulation variation shown in **Figure 3** for a typical population is consistent with the report by Smith et al. (*25*) that roots within a 1 m^2 plot exhibited severalfold variation in total ginsenoside. Similarly, Assinewe et al. (*13*) reported a severalfold variation in ginsenoside content among individual roots from wild populations. Although the considerable intrapopulation variation shown in **Figure 3** and observed repeatedly throughout this study suggests that considerable genetic gain could be achieved through clonal selection of individual plants, after *in vitro* ginseng cloning strategies become more reliable, it also suggests that relatively large sample sizes are necessary for statistically meaningful comparisons among populations or other experimental treatments. Many previously published reports on the effects of various experimental treatments on ginsenoside levels have utilized samples consisting of only a few roots per treatment (*13, 21, 36*) or population sample (*13, 19*) and may consequently have under-estimated the statistical significance of differences among treatments or populations. Given the level of intrapopulation variation observed in these experiments, we used a sample size ≥10, except when limited by the population size

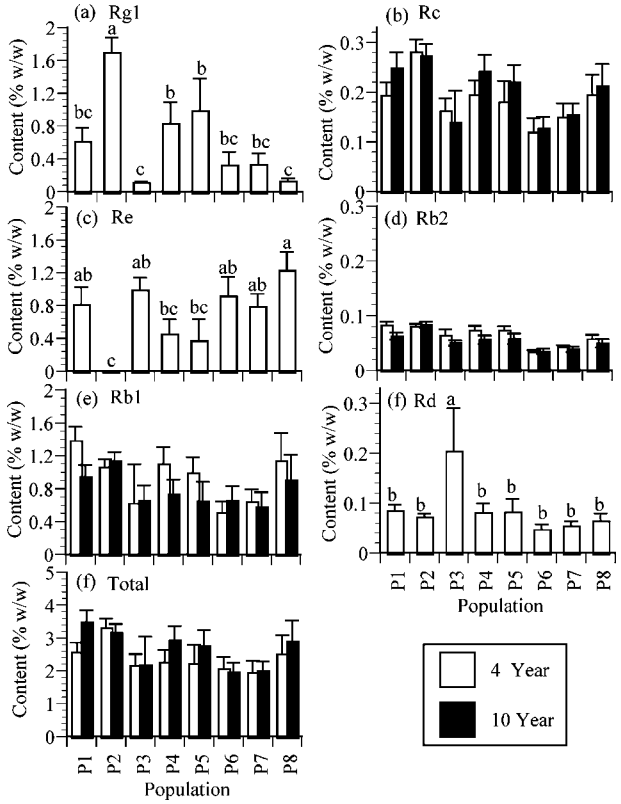

Figure 5. Concentration (mean ± SEM) of six ginsenosides and total for eight populations (P1–P8) at the time of collection from the wild (T0). Means accompanied by the same letters are not significantly different in Duncan's multiple comparison test ($p < 0.05$). For Rc, Rb2, Rb1, and the total, the estimated LS means for ages 4, 7, and 10 years are shown because ANOVA indicated a significant effect of age or population × age interaction (**Table 2**).

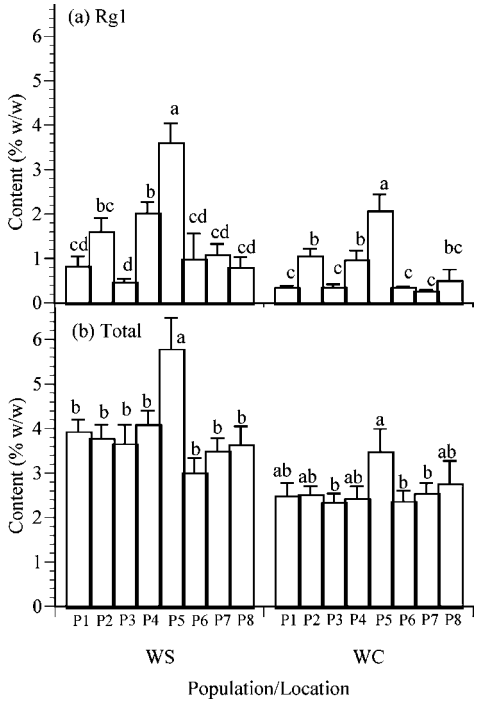

Figure 6. Concentration (mean ± SEM) of ginsenoside Rg1 (a) and total ginsenoside (b) for eight populations (P1–P8) of *Panax quinquefolium* at the end of the second growing season (T2) at the wild-simulated (WS) and woods-cultivated (WC) forest garden locations. Means accompanied by the same letter within the location are not significantly different in Duncan's multiple comparison test ($p < 0.05$).

Table 2. Significance Levels for Effects of Population (P), Age (A), and Forest Garden Location (L) and Interactions on the Concentration of Six Individual and Total Ginsenosides at the Time of Collection from the Wild (T0) and 2 Years after Transplanting (T2) to Each Location

	ginsenoside						
T0	Rg1	Re	Rb1	Rc	Rb2	Rd	total
P	*a*	*a*		*b*	*a*	*a*	
A			*a*	*b*	*a*		*b*
P×A			*b*				

	ginsenoside						
T2	Rg1	Re	Rb1	Rc	Rb2	Rd	total
P	*a*	*a*				*a*	*a*
L	*a*		*a*	*a*	*a*	*a*	*a*
A						*b*	
L×P						*b*	
A×P							
A×L							
A×L×P							

[a] p value ≤ 0.01. [b] p value ≤ 0.05.

at the time of collection from the wild (caption of **Figure 5**) or by plant death before harvest from the forest gardens.

The average of plant age for T0 and T2 samples was 6.9- and 7.5-years-old, respectively. At the time of collection from the wild (T0), the effects of root age and population differed considerably, depending upon ginsenoside. Root age had significant effects on Rc and Rb2 and a significant P×A interaction for Rb1, but there was no significant effect of age on the ginsenosides Rg1, Re, and Rd (**Table 2**). In **Figure 5**, LS-estimated means for ages 4 and 10 years (±1 standard deviation from the mean of 7 years old) are shown only for those ginsenosides for which A or P×A were significant (Rb1, Rc, Rb2, and the total). Duncan's mean separation test was performed only for the observed treatment means shown for Rg1, Re, and Rd, for which there was no significant effect of age or P×A. For Rg1, the difference between the highest and lowest populations was nearly 10-fold. Population 2 was significantly higher than other populations (**Figure 5a**), whereas this population had no detectable Re, as described above. In the case of the third ginsenoside for which there was a significant P effect, Rd, population 3 was substantially higher than in any other case (**Figure 5f**). **Figure 5e** illustrates the significant P×A interaction for the ginsenoside Rb1, in that for some populations (P1, P4, P5, and P8) the ginsenoside level increased with increasing LS age, whereas for other populations (P2 and P6) Rb1 decreased with increasing LS age. With respect to the effect of population on Rb1, populations 3, 6, and 7 tend to show lower effects than the others. The ginsenosides Rc (**Figure 5b**) and Rb2 (**Figure 5d**) exhibit significant main effects of both population and age. These ginsenosides tended to increase with increasing age, and populations 6 and 7 had particularly low levels of these ginsenosides in comparison with other populations.

At T2, it was possible to assess not only the effects of age and population on ginsenoside content but also broadly to test the effect of environment, because ginseng plants from each of the eight populations harvested from the wild had been transplanted to the WS and WC forest garden locations. This created conditions that differed in many environmental variables, as is inevitable for any two widely geographically separate growing sites. **Table 2** shows the statistical significance at T2

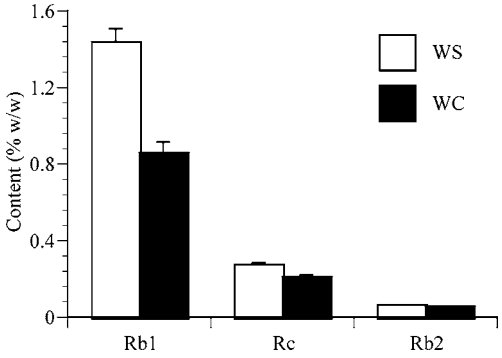

Figure 7. Concentration (mean ± SEM) of ginsenoside Rb1, Rc, and Rb2 for all eight populations (combined) at the end of the second growing season (T2) for the wild-simulated (WS) and woods-cultivated (WC) forest garden locations. Means accompanied by the same letter are not significantly different in Duncan's multiple comparison test.

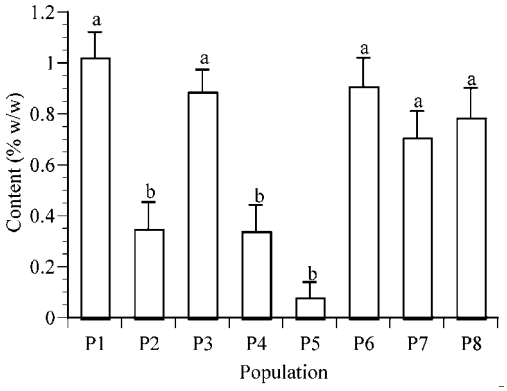

Figure 8. Concentration (mean ± SEM) of ginsenoside Re for eight populations (P1–P8) at the end of the second growing season (T2). The number of roots analyzed for each population was the same as indicated in **Figure 1**. Means accompanied by the same letter are not significantly different in Duncan's multiple comparison test.

of the factors P, L, and A and all possible interactions for each individual ginsenoside. In addition to significant main effects of the population and/or location on Rg1, Re, Rb1, Rc, and Rb2, there was a significant P×L interaction for Rd. The total ginsenoside content, calculated as the sum of six individual ginsenosides, was significantly affected by population and location (**Table 2**). It is notable that, for these five ginsenosides at T2, unlike T0 (wild collection sites), age did not influence total ginsenoside levels either as a main effect or in interaction with the population or location. **Figure 6b** shows the effect of population and location on the total ginsenoside level. For all populations, total ginsenoside was higher at the WS forest garden location than at the WC location. At both locations, total ginsenoside was significantly higher in P5 than in other populations ($p \leq 0.05$, on the basis of Dunnett's pairwise comparison of P5 versus each of the other populations).

Considering the six ginsenosides individually, the major ginsenoside Rb1 and minor ginsenosides Rc and Rb2 were significantly influenced by location but not by population (**Table 2** and **Figure 7**). Across all populations, Rb1 was significantly higher at the less intensively managed WS location than at the WC location. Rc and Rb2 followed the same pattern as Rb1, but the magnitude of the relative difference between locations was less for these minor ginsenosides. In the case of the two major ginsenosides, Re (**Figure 8**) and Rg1 (**Figure 6a**), there were significant differences among populations and a difference between locations was also significant for Rg1. As was the case

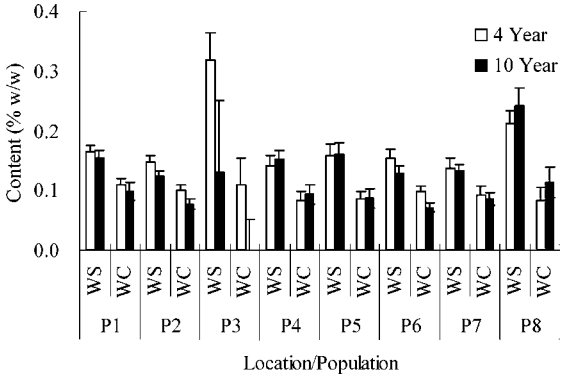

Figure 9. Concentration (mean ± SEM) of ginsenoside Rd for eight populations (P1–P8) of *Panax quinquefolium* at the end of the second growing season (T2) at the wild-simulated (WS) and woods-cultivated (WC) forest garden locations. LS means for ages 4 and 10 years old are given for each treatment combination because there was a statistically significant effect of age.

for total ginsenoside, Rg1 was higher in roots grown at WS than at WC (**Figure 6a**).

At T2, Rd exhibited a more complex response to the three experimental variables than was the case for the other ginsenosides. There were significant effects of P, L, and A and a significant L×P interaction (**Table 2**). Because Rd was the only ginsenoside significantly affected by age at T2 and it was also significantly affected by P, L, and L×P, **Figure 9** shows LS means for 4 and 10 years of age for each location and each population. Rd tended to decrease with increasing age, especially in the case of populations 1, 2, 3, 6, and 7. Rd levels weregreater in roots grown at the less intensively managed WS location than at the WC location, as was also the case with the ginsenosides Rg1, Rc, Rb1, and total ginsenoside.

Given that an important goal of this study was to determine the relative contribution of genotype and environment to ginsenoside variation, one of the most important conclusions from this study is that the relative importance of genotype and environment is not the same for all ginsenosides; each must be considered independently. After the effects of interactions or main effects associated with plant age (A) are taken into consideration, our results suggest that the eight populations exhibit primarily genotype-associated variation for the ginsenoside Re with an approximately 10-fold variation among populations. The ginsenosides Rb1, Rc, and Rb2 exhibit little variation among populations but considerable variation between the two locations of forest gardens, consistent with being primarily under environmental control. The ginsenosides Rg1 and Rd exhibit genotype×environment interaction (P×L) with approximately 5- and 2-fold variation, respectively, among populations and a consistent, nearly 2-fold, variation between the two environments in the case of Rg1 but with less variation in the case of Rd. Unlike this study, previous reports of differences in ginsenoside content among wild (*13*) and cultivated populations (*21*, *22*) from different locations cannot distinguish between the contribution of genetic and environmental factors because the different populations (genotypes) being compared were not all grown at environmentally uniform sites.

Because the two forest garden locations involved in this study represent the two different commonly recommended agroforestry forest farming systems (WS and WC) for ginseng production in the northeastern U.S., it is tempting to suggest that the differences in intensity of cultivation between the two (WC > WS) might account for the differences in growth (WC > WS) and ginsenoside production (WS > WC, except for Re).

Although this may be useful as a working hypothesis for future research, no firm conclusions can be drawn because the two forest garden locations involved in this study differed in many respects, like almost any two farms, and because the two different production systems (WS and WC) were not replicated in this experiment. Controlled, unconfounded experiments, preferably in a greenhouse or growth chamber, are needed to determine the relative contribution of specific environmental factors. Nonetheless, the severalfold difference in soil Ca and Mn and the 2-fold difference in organic matter between the two sites (**Table 1**) suggest that one or more of these factors might contribute to the observed differences. Light, rainfall, and temperature were not recorded at the two sites, but even an exhaustive and thorough site characterization would still not allow for unambiguous determination of the specific factors associated with the overall environment-related responses seen in this study. An overall assessment of the published literature reveals a surprisingly poor understanding of the role of environmental factors (light, temperature, moisture, nutrition, and cultural practices) on ginsenoside levels. Biomedical research has shown potential benefits of specific ginsenosides on cancer (*37*) and diabetes (*38*). Identification of specific populations (genotypes) and cultural (environmental) conditions that enhance production of these and other ginsenosides could impact commerce in this medicinal herb and the future role that it may play in public health.

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