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Effects of Interactions Among Age, Cultivation Method (Location) and Population on Ginsenoside Content of Wild *Panax Quinquefolium* L. One Year after Transplanting from Wild

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ABSTRACT : To evaluate the effects of cultivar, environment, age and cultivation times on ginsenoside content among 8 wild populations of American ginseng (*Panax quinquefolium*), the concentrations of 6 ginsenosides in root were determined at the time of collection (T0) of plants from the wild and 1 year after (T1) transplanting the roots 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. The second most abundant ginsenoside were Re and Rg1, however the contents of them were not significantly different from each other. Concentrations of Rg1 and Re were inversely related. Ginsenoside Re was influenced by population and location. Ginsenoside Rg1, Rb1, Rc, Rb2 and Rd were influenced by population, location and age. Ginsenoside levels were consistently lower but growth was consistently higher at the more intensively managed garden location.

Key words **:** *Panax quinquefolium*, age, cultivation, population, ginsenoside

INTRODUCTION

For nearly 300 years, American ginseng (*Panax quinquefolium*) has been harvested from wild populations in eastern and central North America for export mainly to China (Schorger, 1969). Since the eighteenth century, it has been cultivated horticulturally in North America as well (McGraw, 2001).

The pharmacologically active constituents of the Panax species are a group of triterpene saponins known as ginsenosides. *P. quinquefolium* is reported to contain 13 distinct ginsenosides (Cho & Yuk, 1998). The six most abundant ginsenosides are Rg1, Re, Rb1, Rc, Rb2 and Rd. Many biological and environmental factors affect ginsenosides quantitatively and qualitatively. Variation of ginsenosides may be pharmacologically important since individual ginsenosides differ in their effects on human physiology (Ki *et al.*, 1998). Because the cultivated American ginseng consists largely of undomesticated land races (Schluter & Punja, 2002), wild populations may serve as reservoirs of genetic variation for genetic improvement.

Since 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. Betz *et al.* (1984) and Foster (1996) reported greater total ginsenoside content in wild ginseng than in cultivated American ginseng while Lui and Staba (1980) reported minimal differences between them. Tanaka (1987) found no significant difference in ginsenoside content between wild and cultivated Asian ginseng, although Mizuno *et al.* (1994) reported that ginsenosides Rg1, Re, and Rd were higher in wild roots than in cultivated roots of Asian ginseng (*P. ginseng*), whereas the ginsenosides Rc, Rb2, and Rb1 were lower. In all these comparisons of the ginsenoside content of wild vs. cultivated ginseng, one potentially confounding factor that was not accounted for is that of differences in age between wild and cultivated ginseng. Cultivated roots typically are harvested at 3 to 4 years of age, whereas in most states wild ginseng is typically harvested after 8 or more years (Hankins, 2000; Proctor & Bailey, 1987). Studies based on relatively young (up to 7-year-old), cultivated ginseng indicate that root age is positively related to ginsenoside content in both Asian (Kim *et al.*, 1987; Soldati & Tanaka, 1984) and American ginseng (Court *et al.*, 1996b; Jackson *et al.*, 2003), although Zito & Cheng (1986) 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 about 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.* (2003), although root age was constant, the wild population sample consisted of a mixture of only 2 to 5 roots from each of 10 different wild populations. Similarly, no attempt was

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made to control for or describe environmental variation between wild and cultivated populations in studies completed by Betz *et al.*, (1984), Foster (1996), Lui and Staba (1980), Tanaka (1987), and Munzino (1994). Comparing ginsenoside content among cultivated populations from dissimilar environments, Li (1996) found that Re, Rb1, Rc, Rb2, Rd, and the total ginsenoside contents were significantly different among populations while Jackson (2003) 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 (1999) reported weak correlations between root ginsenoside levels and the level of various soil mineral nutrients, and Zito *et al.* (1986) reported that wood mulches from different tree species significantly affected ginsenoside content in 7-year-old but not in 4-year-old American ginseng. Ginsenoside Rg1 in American ginseng roots showed a negative correlation with soil phosphorus (Konsler *et al.*, 1990). Although ginseng is a shade-adapted species, ginsenoside levels were increased by increasing the light levels up to 35% of full sun (Fournier *et al.*, 2003). It therefore appears that questions regarding the relative "potency" (ginsenoside content) of wild vs. cultivated ginseng, and the relative contribution of genotype and environment to inter population variation, remain unresolved.

The objective of this research was to determine the interactions 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 (Clemans, 2004), and sold at premium prices (Harris, 1999). The experimental approach entailed comparing the growth and ginsenoside content of multiple wild populations of American ginseng after transplanting it to two different environments.

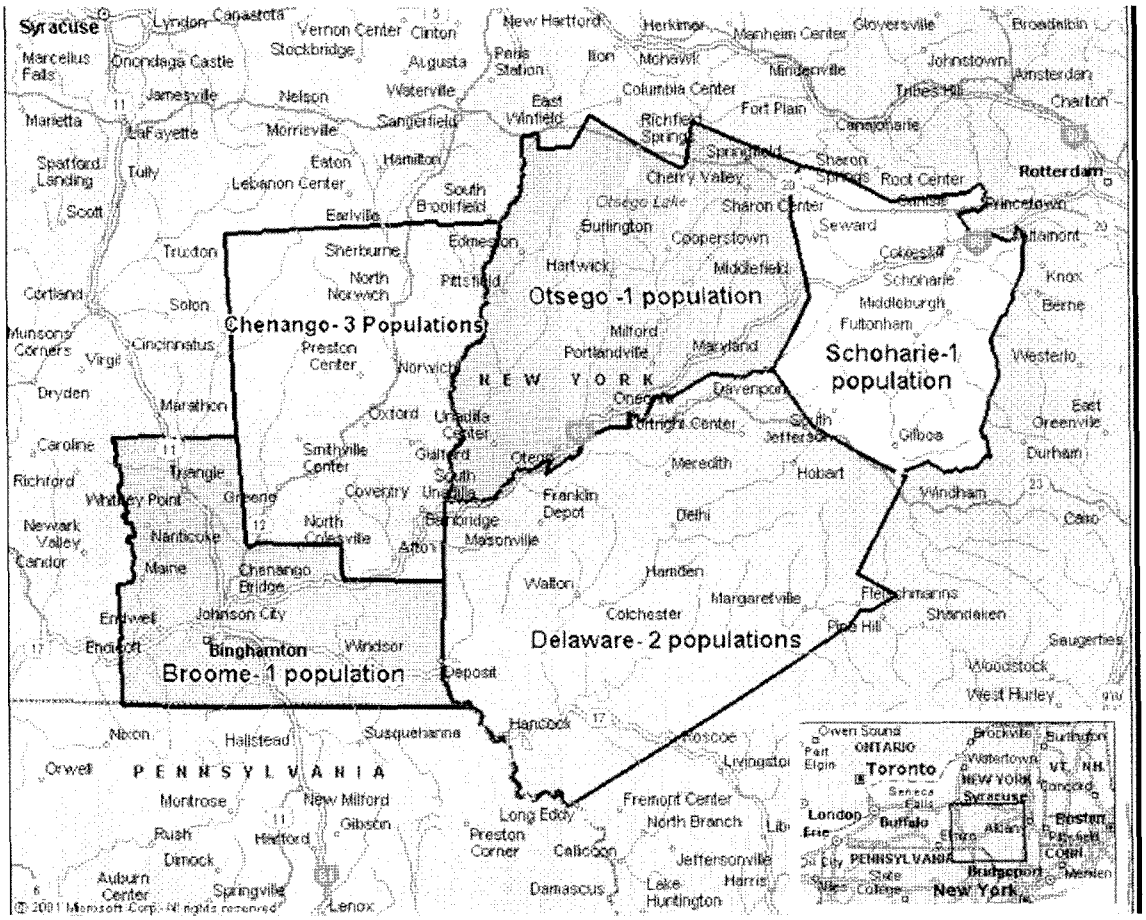

Fig. 1. A map showing the 5 counties (Chenango, Otsego, Schoharie, Delaware, and Broome) in New York State where plants of Panax quinquefolium were collected from wild populations. Otsego, Schoharie, and Delaware counties are in the Catskill Mountain region. Inset shows the location of these counties within NY State.

MATERIALS AND METHODS

Ginseng Collection and Transplanting.

Ginseng plants were collected from 8 wild populations located in five contiguous counties in and adjacent to the Catskill 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 (Fig. 1). Collections were made in October 2000, during the legal ginseng harvest season. The senescing above ground shoot was discarded while the entire root was collected intact.

Plant samples of the 8 wild ginseng populations were subdivided as harvesting year. Approximately 10 plants per population were destructively sampled, as described below, for initial (T0) estimation of fresh weight and ginsenoside content. The remaining plants from each wild population were transplanted to each of the two forest gardens within one week of collection, and a subsample was harvested at the end of the first growing season (T1) for growth measurements and ginsenoside analysis. Before the separation into two subgroups (T0, T1), the age of each plant was estimated by counting the annual bud scars along the rhizome (Anderson *et al.*, 1993). Some of the upper parts of the storage root was transplanted to pots in a green house to maintain the populations and the remaining lower parts were used for ginsenoside analysis. Age distributions among the populations at T0 and the subsequent T1 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. (Beyfuss, 1999; Hill & Buck, 2000). 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 (Proctor & Bailey, 1987).

The two forest garden locations were selected to represent the two typical management systems for producing woods-grown ginseng (Beyfuss, 1999)-wild-simulated (WS) and woods-cultivated (WC)-which differ in intensity of cultivation. The less intensive wild simulated (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 beneath a closed canopy consisting primarily of sugar maple (Acer saccharum). The soil type was a Mardin Channery silt loam. The native forest soil in two adjacent 3×6 meter beds was rototilled lightly. No organic matter or other soil amendments were incorporated at the time of bed preparation or during the course of the experiment. During the one-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 by rototilling several times, with incorporation of 10.16 ㎝ (4 inches) of dried, shredded hardwood leaves and 2.3 ㎏/9.3 m^2 of granular gypsum ($CaSO_4 \cdot 2H_20$). After planting, the beds were mulched to a depth of approximately 8 ㎝ 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.

The procedure for ginsenoside extraction and analysis was modified from Court, *et al.*(1996a). This modified procedure is well described in Lim *et al.* (2005).

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 parenthesis for the T0, the T1 WS and the T1 WC locations, respectively, as follows : P1 (11, 13), P (10, 10), P3 (7, 9), P4 (10, 10), P5 (7, 6), P6 (5, 3), P7 (10, 10), P8 (6, 10). The initial ginsenoside content (T0) and plant growth and the ginsenoside content at the end of the first growing season (T1) were treated as separate experiments for purposes of statistical analysis. Separate analyses were performed using as the dependent variable fresh weight and shoot height for each of the 6 individual ginsenosides including Rg1, Re, Rb1, Rc, Rb2, and Rd and total ginsenoside (the sum of all 6, Total).

The analyses for the T0 and the T1 experiments included the independent variables population (P) with 8 levels, and age (A) as a continuous variable treated as a covariate, but only the analyses for the T1 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 fresh weight and shoot height were evaluated for the T1 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 main effects were sig-

nificant at the 5% level. When the continuous variable A was statistically significant, the least squares mean (LS mean) for ages 4 and 10 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 fresh weight ($p \leq 0$ .01) (Fig. 2). Both shoot height and root fresh weight were clearly affected by population. The WC garden location was more conducive to growth than the WS garden location. Population 7 exhibited the greatest root fresh weight at either location and the greatest shoot height at the WC location. Across populations, shoot height and root fresh weight were greater at the more intensively managed WC location.

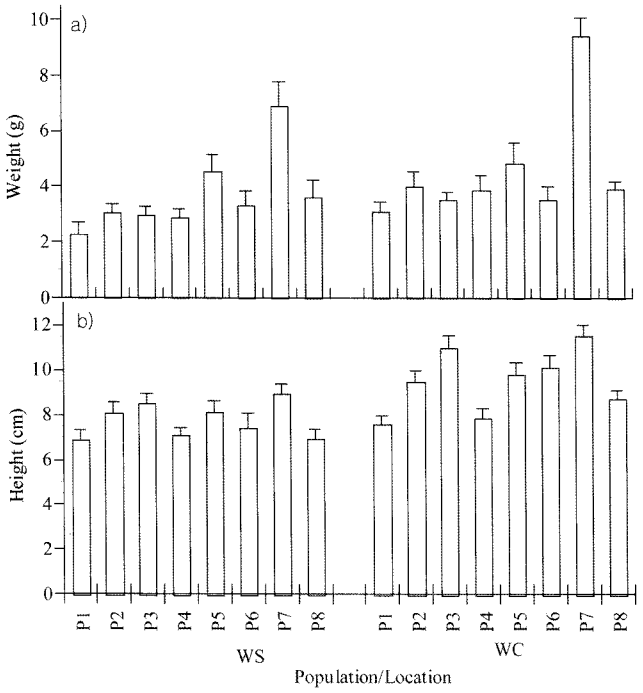

Fig. 2. Effect of wild ginseng populations (P1-P8) and forest garden location to which populations were transplanted (WS, wild-simulated; WC, woods-cultivated) on (a) root fresh weight, (b) shoot height at the end of the second growing season (T1) after transplanting. Growth responses are the Mean ± SEM.

Soil Ca and Organic Matter Analysis.

Ca content in the WC site (8262 kg/hc) was higher than in the WS site (2472 kg/hc). The application of gypsum ($CaSO_4 \cdot 2H_2O$) is often recommended for woods-cultivated ginseng production (Beyfuss, 1999; Hankins, 2004). Beyfuss (1999) reported that application of gypsum increased the survival rate of ginseng. Soil organic matters in WS and WC site were 5.4 % and 11.9%, respectively..

Ginsenoside Content.

The relative abundance of the 6 ginsenosides was Rb1 > Re = Rg1 > Rc > Rd = Rb2 (Table 1) across all populations, forest garden locations, and sampling times. In several previous studies involving American ginseng (roots), Rg1 was considerably lower than Re. The range of Re/Rg1 ratio was from of 1.5 to 13.7 (Assinewe *et al.*, 2003; Court *et al.*, 1996b; Jackson *et al.*, 2003; Ko *et al.*, 1995; Li & Mazza, 1999; Li *et al.*, 1996; Li & Wardle, 2002). In this study, the Rg1 content was almost same with Re (Re/Rg1 ratio = 0.92) across all populations, locations, and harvest times. An inverse relation-

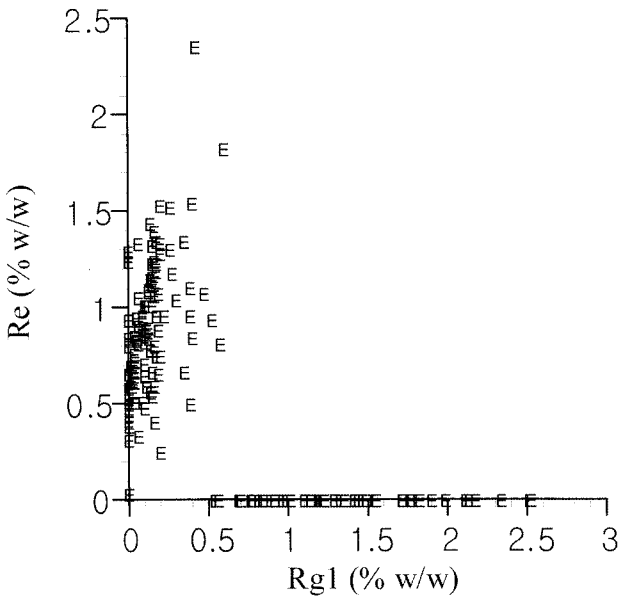

Fig. 3. Relationship between concentration of ginsenosides Rg1 and Re for all root samples of Panax quinquefolium) across all populations at the end of the first growing season after transplanting to two forest garden locations (T1, n = 154 root samples).

Table 1. Relative abundance of 6 major ginsenosides and total ginsenoside averaged across all populations and forest garden locations on year after transplanting (T1). Ginsenoside content is the mean +/– SE of 154 individual root samples. Means accompanied by the same letter are not significantly different using Duncan's multiple comparison tests ($P < 0.05$)

Rg1	Re	Rb1	Rc	Rb2	Rd	Total	
T1	0.549b	0.594b	0.748a	0.168c	0.051d	0.057d	2.166

Table 2. Significance levels for effects of population (P), age (A), and forest garden location (L) and interactions on concentration of six individual and total ginsenosides at the time of collection from the wild (T0) and one years after transplanting (T1) to each location

T0	Ginsenoside						
	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
P	**	**		*	**	**	
A			**	*	**		*
PxA			*				

T1	Ginsenoside						
	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
L	**		**	**	**	**	**
P	**	**	**	*	**	**	**
A							
L*P	**	**	*	**	**	**	*
A*L							
A*P	**		**	*	**	*	*
A*L*P	**		*	*	**		

* 0.01= P-value=0.05, ** : P-value≤0.01

ship was observed between the levels of these 2 ginsenosides (Fig. 3). Fig. 3 suggests a complex relationship between Re and Rg1. At lower levels of Rg1, all roots at T1 with Rg1 content over 0.6% (w/w) had little Re. Thirty four percent of all individual roots sampled in this study contained no measurable Re at T1. We found similar inverse relationship of Rb1 and Re at T0 (Lim *et al.*, 2005). Both of these ginsenosides are structurally related 20-S-Protopanaxatriols (PT), based on the presence of the triol aglycon subunit. The other 4 ginsenosides analyzed, including Rb1, Rb2, Rc, and Rd, are 20-S-Protopanaxadiol (PD) ginsenosides.

At the time of collection from the wild (T0), the effects of root age and population differed considerably, depending on ginsenoside. Root age had significant effects on Rb1, Rc, Rb2 and Total, and a significant PxA interaction for Rb1, but there was no significant effect of age on the ginsenosides Rg1, Re, and Rd (Table 2). The detailed individual ginsenoside content at T0 was already been published (Lim *et al.*, 2005;Mudge *et al.*, 2004). Table 2 shows the statistical significance of the factors P, L, and A, and all possible interactions for each individual ginsenoside at T1. At T1, it was possible to test the effect of environment, since ginseng plants from each of the 8 populations harvested from the wild had been transplanted to the WS and WC forest garden locations. Except Re, all ginsenosides including Total had significant location effects and interactions between age and population. All ginsenosides with Total had significant population effects and interactions between location and population. Rg1, Rb1, Rc and Rb2 had complex three-way interactions among age, location and population. However, all ginsenosides with Toal had no significant age effects. Because of three way interactions among age, location and population, T1 ANOVA table was subdivided to WS and WC ANOVA table (Table 3). At the T1 WC location, there were no age related effects, however at the T1 WS location, except Re, all ginsenosides with Total had interactions between age and population. At both T1 locations, there were no main effects of age. The main effect of age did not look apparent because it was confounded by population and location interactions related with age. These results showed that Rg1, Rb1, Rc and Rb2 in some populations significantly increase or these ginsenosides in the other populations significantly decrease with age at T1 WS location (Fig. 4). However, there were no age related effects at more intensively managed T1 WC location (Table 3).

Table 3. Significance levels for effects of population (P) andage (A) interactions on concentration of six individual and total ginsenosides at one year after transplanting (T1)to wood simulated (WS) and wood cultivated (WC) locations

WS	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
P	**	**	*	*	**	**	
A							
A*P	**		**	**	**	*	*

WC	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
P	**	**	**	**	**	**	**
A							
A*P							

For the T1 location and population main effect, 7 years was used for the LS mean because the average age of all roots was 7 and the standard deviation was 4. Four and ten year were used for graphic presentations. Seven populations in Rg1 (Fig. 3a), six populations in Rb1 (Fig. 3b), five populations in Rc (Fig. 3c), five populations in Rb2 (Fig. 3d), five populations in Rd (Fig. 5a) and six populations in total (Fig. 5b) had higher content in the WS location than in the WC location. These ginsenosides were significantly influenced by location.

The population main effects were substantively confounded by age and location effects at T1. Table 2 shows all ginsenosides with Total had population main effects and L*P interaction. Table 3 shows significant population main effects in both T1 WS and T1 WC. For example, population 5 in Rg1 (Fig. 4a) and Rb1 (Fig. 4b), and population 8 in Rc (Fig. 4c) had rela-

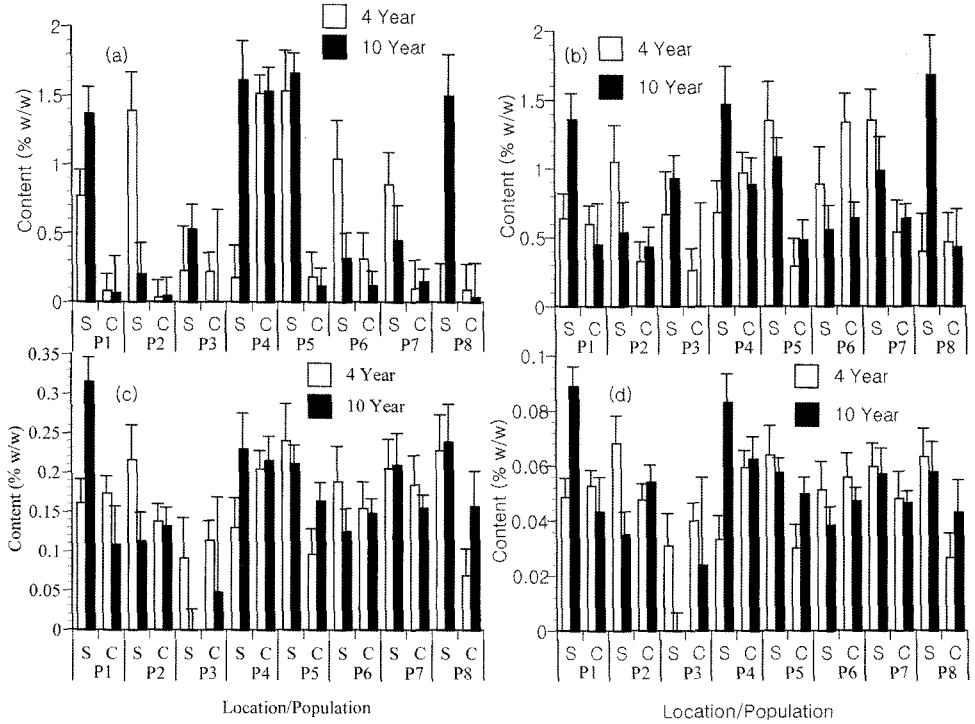

Fig. 4. Concentration (Mean ± SEM) of ginsenoside (a) Rg1, (b) Rb1, (c) Rc and (d) Rb2for 8 populations (P1-P8) of Panax quinquefolium at the end of growing season (T1) at the wild-simulated (S) and woods-cultivated (C) forest garden locations. LS means for ages 4 and 10 years old are given for each treatment combination because there was a statistically significant effect of age.

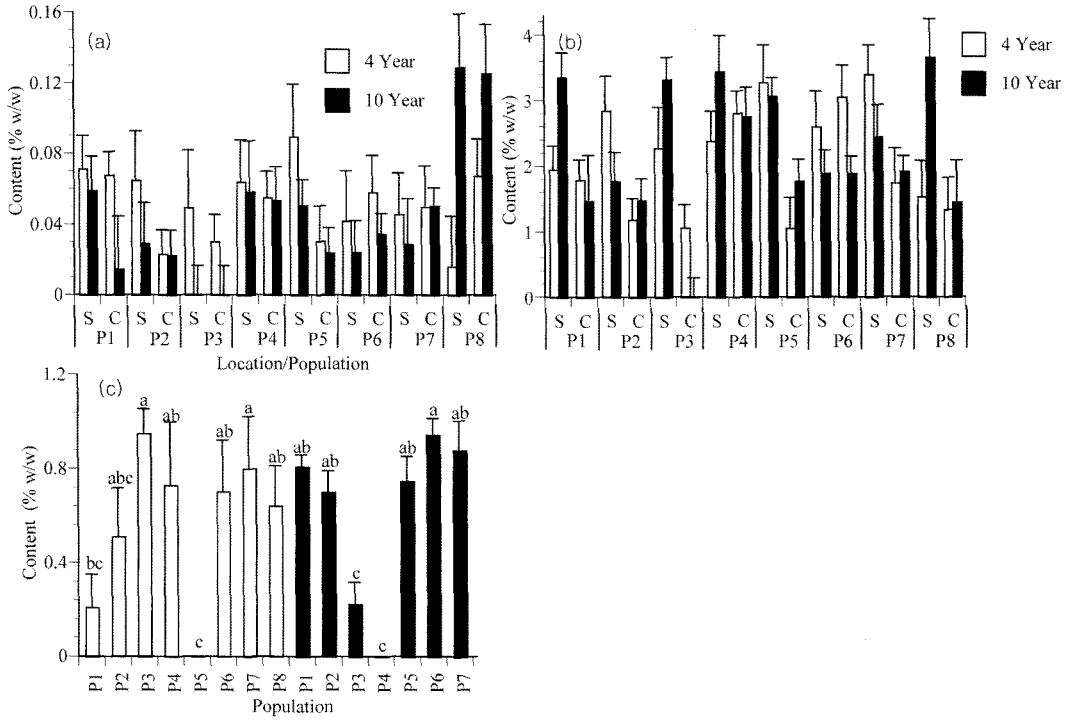

Fig. 5. Concentration (Mean ± SEM) of ginsenoside (a) Rd, (b) Total and (c) Re for 8 populations (P1-P8) of Panax quinquefolium at the end of growing season (T1) at the wild-simulated (S, white) and woods-cultivated (C, black) forest garden locations. LS means for ages 4 and 10 years old are given for each treatment combination because there was a statistically significant effect of age for (a) Rd and (b) Total. Means accompanied by the same letter within location are not significantly different in Duncan's multiple comparison test ($P < 0.05$).

tively higher content in WS location compared with other populations, however they had lower content in the WC location.

The Individual ginsenoside Re had population main effects and L*P interaction. Population 3 had the highest Re with population 3 in the WS location, however population 3 had the lowest Re with population 4 and 8 (Fig. 5c).

If we take account of the fact that an important goal of this study was to determine the relative contribution of genotype, age and environment to ginsenoside variation, one of the most important conclusions from this study is that age and environments have a considerable role for ginsenoside production. Genotype and age effects except Re were affected by environments. Although main effects of population were statistically significant, the populations effects were not clear. The reason of this might be that growing of one year was not enough to eliminate the environmental effects of the collection site.

Traditionally, north eastern Asians have believed that the older wild Asian ginseng was more valuable than younger cultivated ginseng. However, our result of American ginseng shows that wild American ginseng lost age effects in intensively cultivated location after transplanting (Table 3). Age effects were different by population and cultivation methods. To verify Asian belief, scientific research is needed although Asian wild ginseng is rare and extremely expensive compared with American wild ginseng.

Unlike this study, previous reports of differences in ginsenoside content among wild (Assinewe *et al.*, 2003) and cultivated populations (Li & Mazza, 1999; Li *et al.*, 1996) from different locations cannot distinguish the contribution of genetic and environmental factors because the different populations (genotypes) compared were not all grown at environmentally uniform sites. Since the two forest garden locations represent the two different commonly recommended agroforestry forest farming systems (WS, WC) for ginseng production in the northeastern U.S.A., 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 related with age (WS > WC; except for Re). Although this may be useful as a working hypothesis for future research, no firm conclusions can be drawn since the two forest garden locations differed in many respects and since the two different production systems (WS, WC) were not replicated in this experiment. Controlled experiments are needed to determine the relative contribution of specific environmental factors. Nonetheless, the several-fold difference in soil Ca and the 2-fold difference in organic matter between the two sites, suggest that one or more of these factors might have contributed to the observed differences. 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) and age on ginsenoside levels. The 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 it may play in public health.

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