

Partial-Root Harvest Of American Ginseng (*Panax quinquefolius* L.): A Non-Destructive Method For Harvesting Root Tissue For Ginsenoside Analysis

Ian Sabo
Department of Biology
The University of North Carolina Asheville
One University Heights Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Jonathan L. Horton

Abstract

American ginseng (*Panax quinquefolius* L.) is an economically important, but increasingly threatened, herbaceous perennial species native to eastern North America. The roots have long been prized in Asian medicine, and are beginning to be used more in the West for herbal supplements. Much research is focused on ginsenosides, the active medicinal compounds found in American ginseng. Given the conservation concerns regarding wild American ginseng, we began experimenting with a partial-root harvest method in 2014 for extracting tissue for ginsenoside analysis without killing individual plants or causing long-term declines in wild populations. Partial-root harvest samples were taken from 57 plants in four wild populations throughout western North Carolina. Of the 57 plants subjected to partial-root harvest in 2014, 51 (89%) reemerged in 2015, 45 (79%) reemerged in 2016, 22 (39%) reemerged in 2017, and 28 (49%) reemerged in 2018. These resprout rates were similar to paired unharvested plants (86%, $p = 0.568$; 82%, $p = 0.668$; 46%, $p = 0.400$; and 41%, $p = 0.390$, respectively). Partial-root harvested plants had significantly shorter stems ($p = 0.0289$) and smaller leaf area ($p = 0.0001$) than unharvested plants the 1st year after harvest. However, there were no significant differences in any plant metric between these two groups of plants by the 2nd year after harvest. Additional analyses also compared preharvest reproductive and morphological attributes for a larger set of plants ($n = 219$) in 20 populations subjected to partial-root harvest from 2014-2016 with the attributes for each subsequent year after harvest. Some trends that emerged in this preharvest - postharvest study were stem length being significantly lower in each postharvest year compared to the preharvest year, and the 2nd year after harvest being the only year where all plant metrics were significantly lower than the preharvest year. However, trends in this study were more variable than those from the paired comparison study. This variability could be due to several factors such as interannual variation in weather, the larger number of populations sampled as well as their difference in protection status, and the fact that some data for these analyses will not be collected until the 2019 and 2020 field seasons. Overall, the results of the more rigorous paired comparison study demonstrate that partial-root harvest could be an effective way for ginsenoside researchers to reduce their impact on wild American ginseng populations.

1. Introduction

American ginseng (*Panax quinquefolius* L.) is an economically important, perennial herb endemic to the deciduous forests of eastern North America with a range extending from northern Georgia north to southern Quebec and west to eastern Oklahoma and eastern Minnesota^{1,2,3}. The dried roots of the plant have been prized in Asian medicine since they were first exported to China in the early 18th century⁴. Products containing American ginseng are also on the rise in North America and Europe due to the growing popularity of eastern medicine in the West⁵. This use has led to an increased demand for the roots of this species, resulting in overharvesting, increasing rarity, and loss of genetic diversity in much of its native range^{6,7}. Additionally, climate change⁸ acid deposition³, increased herbivory due to increased white tailed deer densities⁹, as well as destruction of habitat due to logging, surface mining, and urban sprawl³ are all factors that potentially threaten existing populations.

In recent years, much research has been aimed at understanding the composition of the secondary metabolites, known as ginsenosides, produced by American ginseng. Ginsenosides belong to a class of molecules known as triterpenoid saponins, which are considered the main medicinal compounds found in the plant^{10,11,12}. It is theorized that these compounds are produced by the plant for both their antimicrobial¹³ and antifeedent¹⁴ properties. Recent pharmacological research suggests that specific ginsenosides may have different pharmacological effects including: antidiabetic^{15,16}, immune boosting¹⁷, cardioprotective¹⁸, cancer inhibiting^{19,20,21}, and neuroprotective²². Additionally, previous studies have demonstrated that chemotypes vary between organs (leaves, roots, and stems) in cultivated²³ and wild²⁴ plants, vary among roots of different plants within the same populations and among different populations^{5,25}, and also vary depending on plant age²⁶. This chemotypic variability is thought to be the result of both genetic²⁷ and environmental factors such as temperature²⁸, understory light²⁹, and soil moisture and nutrient availability³⁰. Furthermore, research also suggests that using different horticultural methods²⁶ and applying high heat treatments to roots³¹ can change the ginsenoside profile of the plants.

Most, if not all, of this previous phytochemical analyses of American ginseng appears to have resulted in the destruction of the entire plant root. Given the conservation concerns regarding American ginseng, we began experimenting with a non-destructive, partial-root harvest method in 2014 in an attempt to extract tissue for ginsenoside analysis without killing or causing long-term declines in wild populations. We believed that carefully harvesting small amounts of tissue (~ 300 mg fresh tissue) and replanting the remaining root would allow plants to continue to grow and would not have negative effects on their growth or survival. If successful, this partial-root harvest method would provide a usable, non-destructive method to extract root phytochemicals without killing plants.

2. Methods

2.1. Paired Comparison Study

113 mature (three or four prong) *P. quinquefolius* plants (Fig. 1A) from four protected wild populations in western North Carolina were monitored. Of these plants, partial-roots were harvested from 57 in 2014, while the other 56 were of comparable size, but were left as unharvested controls. Soil was carefully removed around roots to expose them and approximately 300 mg of root tissue was harvested. If present, roots branching from the main root were harvested (Fig. 1B). If no side root was present, then part of the side of the main root was harvested with care to not damage the vascular cylinder in the center of the root. Roots were then replanted into the same hole from which they came. The populations were tagged and mapped following the techniques used by Searels et al.²⁵. Reproductive and morphological data (reproductive status, number of berries, number of leaves, total number of leaflets, stem height (cm), peduncle length (cm), largest leaf rachis (cm), largest leaflet length (cm), and largest leaflet width (cm)) was collected during mid growing season in 2014 before harvest and each subsequent year harvest (2015-2018). Leaflet length (LL) and leaflet width (LW) were used in an allometric equation (1) developed by Mooney and McGraw³² calculate leaf area (LA) of the largest leaf.

$$LA = 11.4597 + 4.5774LL - 4.5091LW + 0.5786LL \times LW \quad (1)$$

T-tests were used to compare the number of berries, number of leaves, number of leaflets, leaf area, and stem length between harvested plants and unharvested plants before harvest and each postharvest year (2015-2018). Survivorship was compared between the harvested plants and the unharvested plants each postharvest year (2015-2018) using a chi-squared test.

2.2. Preharvest - Postharvest Study

In addition to the 57 plants subjected to partial-root harvest in 2014, we harvested tissue from an additional 74 plants from 10 populations in 2015 and 88 plants from eight populations (six new populations and two that were sampled in 2016). The additional samples in 2015 and 2016 did not have paired unharvested plants for comparison. The lack of paired unharvested plants was the result of small population sizes or the resampling of previously sampled populations that did not have enough plants of similar size suitable to be paired plants with those that were harvested. The same

procedure to map plant locations was used and the same morphological and reproductive metrics were collected as those collected in the paired comparison study. All plants that had root tissue harvested from 2014 – 2016 (n = 219 total) were resurveyed either annually or biannually. Paired t-tests were used to compare the number of berries, leaves, and leaflets, leaf area, and stem length from the harvest year (year 0) with subsequent years after harvest (years 1 – 4).

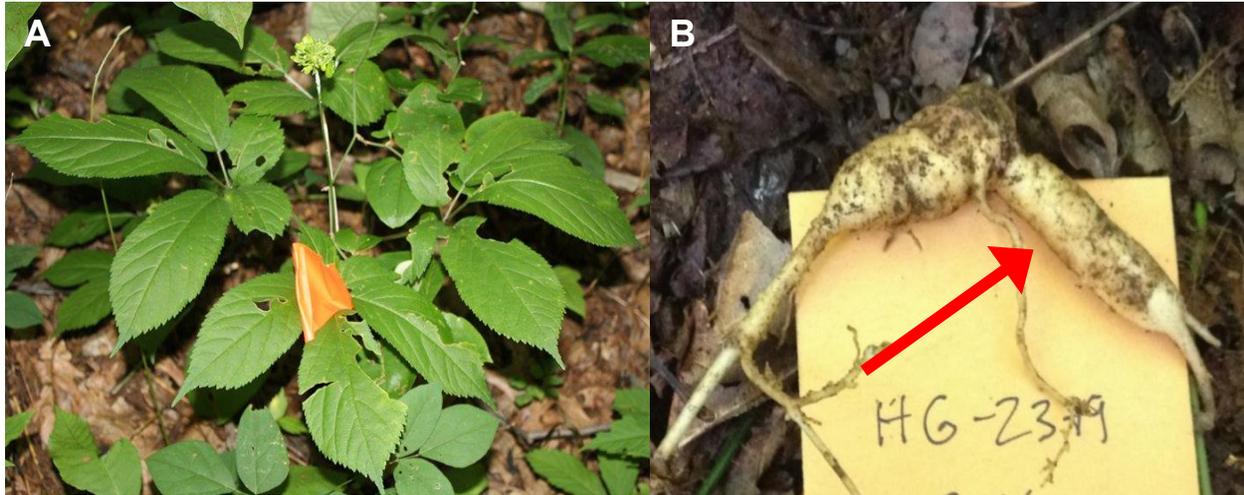


Figure 1. A. Four leaved (four prong) *Panax quinquefolius* plant with inflorescence. B. Root showing a side root that was partially harvested Photos by J. Horton.

3. Results

3.1 Paired Comparison Study

Prior to partial-root harvest in 2014, there were no significant differences in morphology between partial-root harvested and unharvested plants (Table 1; Fig. 3), confirming that our paired control plants were good matches with our harvested plants. There was no significant difference in reemergence between harvested and unharvested plants in the four years after harvest (Table 2). The number of berries, number of leaves, and number of leaflets per plant did not differ significantly between harvested and unharvested plants the first year after harvest (2015) (Table 1; Fig. 4A). Both stem length and leaf area decreased between 2014 and 2015 for harvested plants, but not for unharvested plants, and these differences were significant (Table 1; Fig. 4A). By the second year, there were no significant differences between harvested and unharvested plants for any attribute. This pattern continued throughout the study (until 2018) (Table 1; Figs 4B-4D).

Table 1. Statistical results from t-tests comparing morphological parameters between partial-root harvested and unharvested plants.

Parameter	Pre-harvest (n = 113)		1 st year after (n = 99)		2 nd year after (n = 91)		3 rd year after (n = 48)		4 th year after (n = 51)	
	t	p	t	p	t	p	t	p	t	p
# of Berries	-1.33	0.1874	0.60	0.5510	0.81	0.4214	0.77	0.4464	0.83	0.4133
# of Leaves	0.03	0.9791	-1.42	0.1584	1.04	0.3007	-1.77	0.0828	-0.41	0.6824
# of Leaflets	-0.63	0.5329	-1.65	0.1025	1.41	0.1608	-1.34	0.1873	-1.26	0.2170
Stem Length	-0.59	0.5567	2.22	0.0289	0.56	0.5788	0.58	0.5690	0.21	0.8375
Leaf Area	-0.46	.6440	3.95	0.0001	1.19	0.2391	0.32	0.7537	-0.75	.4583

Table 2. Statistical results from chi-squared test comparing survivorship between partial-root harvested and unharvested plants.

	2015 - 1 st year after (n = 99)	2016 - 2 nd year after (n = 91)	2017 - 3 rd year after (n = 48)	2018 - 4 th year after (n = 51)
χ^2	0.368	0.184	0.709	0.739
p	0.5442	0.668	0.400	0.390

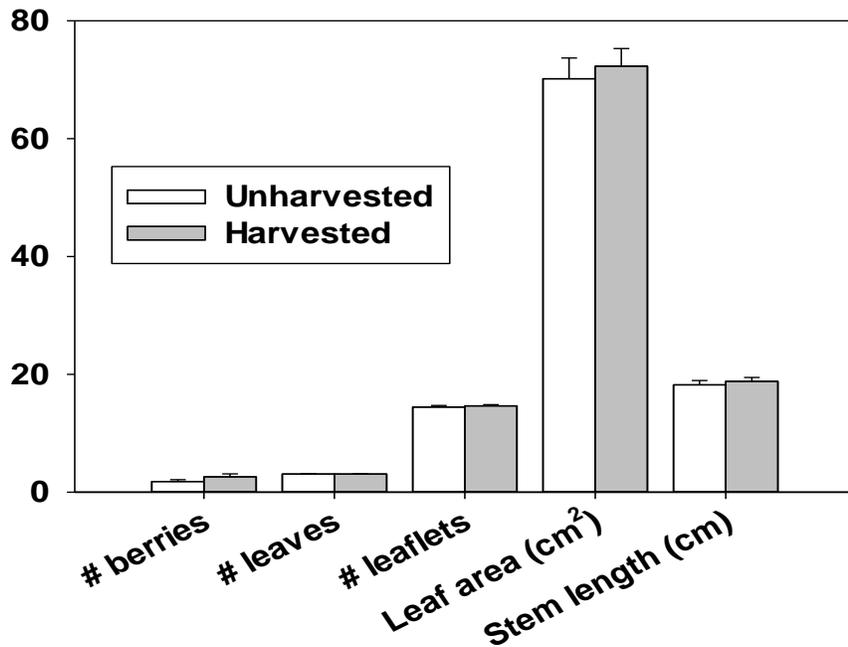


Figure 3. Mean (± 1 se) pre-harvest morphological measurements did not vary significantly ($p > 0.05$ for all) between the partial-root harvested and unharvested plants.

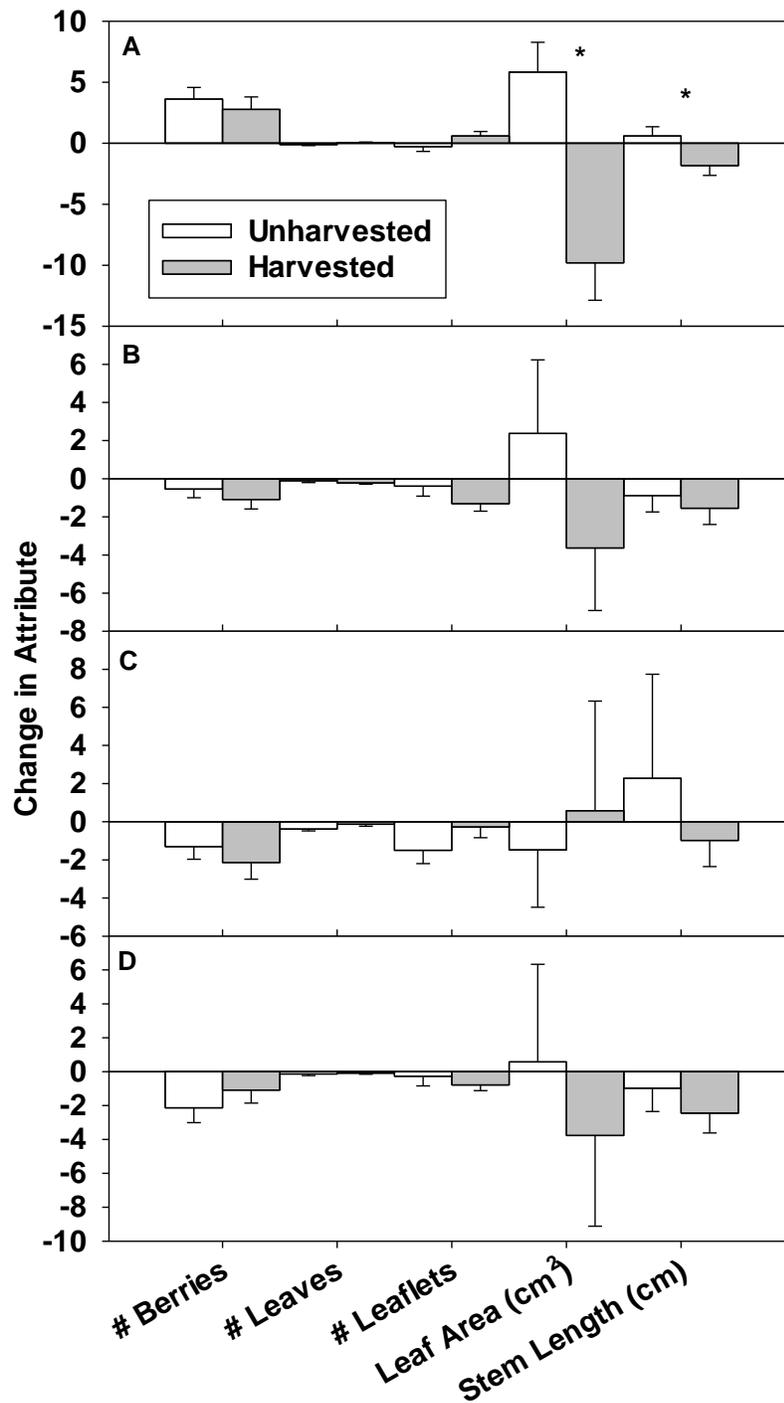


Figure 4. Mean (\pm 1 se) morphological attributes between 2014 – preharvest and (A) 2015 – one year postharvest, (B) 2016 – two years postharvest, (C) 2017 – three years postharvest, and (D) 2018 – four years postharvest. Significant differences between partial-root harvested and unharvested plants are noted with asterisk.

3.2. Preharvest – Postharvest Study

For all plants subjected to partial root harvest, neither number of leaves nor number of leaflets were significantly different the first year after harvest from preharvest values. Both leaf area and stem length were significantly smaller the first year after harvest, however, the number of berries was larger (Table 3; Fig. 5). All plant attributes were significantly smaller than preharvest values in the second year after harvest (Table 3; Fig. 5). Moreover, the mean number of leaves, number of leaflets, and stem length all exhibited their lowest values in the 2nd year after harvest. Means for all attributes increased between the 2nd and 3rd years after harvest (Fig 5). However, number of leaflets and stem length were significantly lower than preharvest values, while other parameters were not significantly different (Table 2; Fig. 5). By the 4th year after harvest, the only parameters that were significantly lower than preharvest year values were number of berries and stem length (Table 3; Fig. 5).

Table 3. Statistical results from paired t-tests comparing morphological parameters between pre-harvest (Y0) means for all plants subjected to partial-root harvest 2014-2016 and means for each subsequent year after harvest (Y1-Y4)

Parameter	1 st year after harvest		2 nd year after harvest		3 rd year after harvest		4 th year after harvest	
	t	p	t	p	t	p	t	p
# of Berries	-3.35	0.0010	2.64	0.0098	1.17	0.2485	2.51	0.0186
# of Leaves	0.33	0.7400	6.39	<0.0001	2.01	0.0509	0.57	0.5732
# of Leaflets	0.99	0.3257	6.24	<0.0001	2.09	0.0427	-0.11	0.9159
Stem Length	6.46	<0.0001	22.05	<0.0001	2.92	0.0058	2.40	0.0237
Leaf Area	8.80	<0.0001	2.48	0.0153	1.72	0.0933	-0.33	0.7472

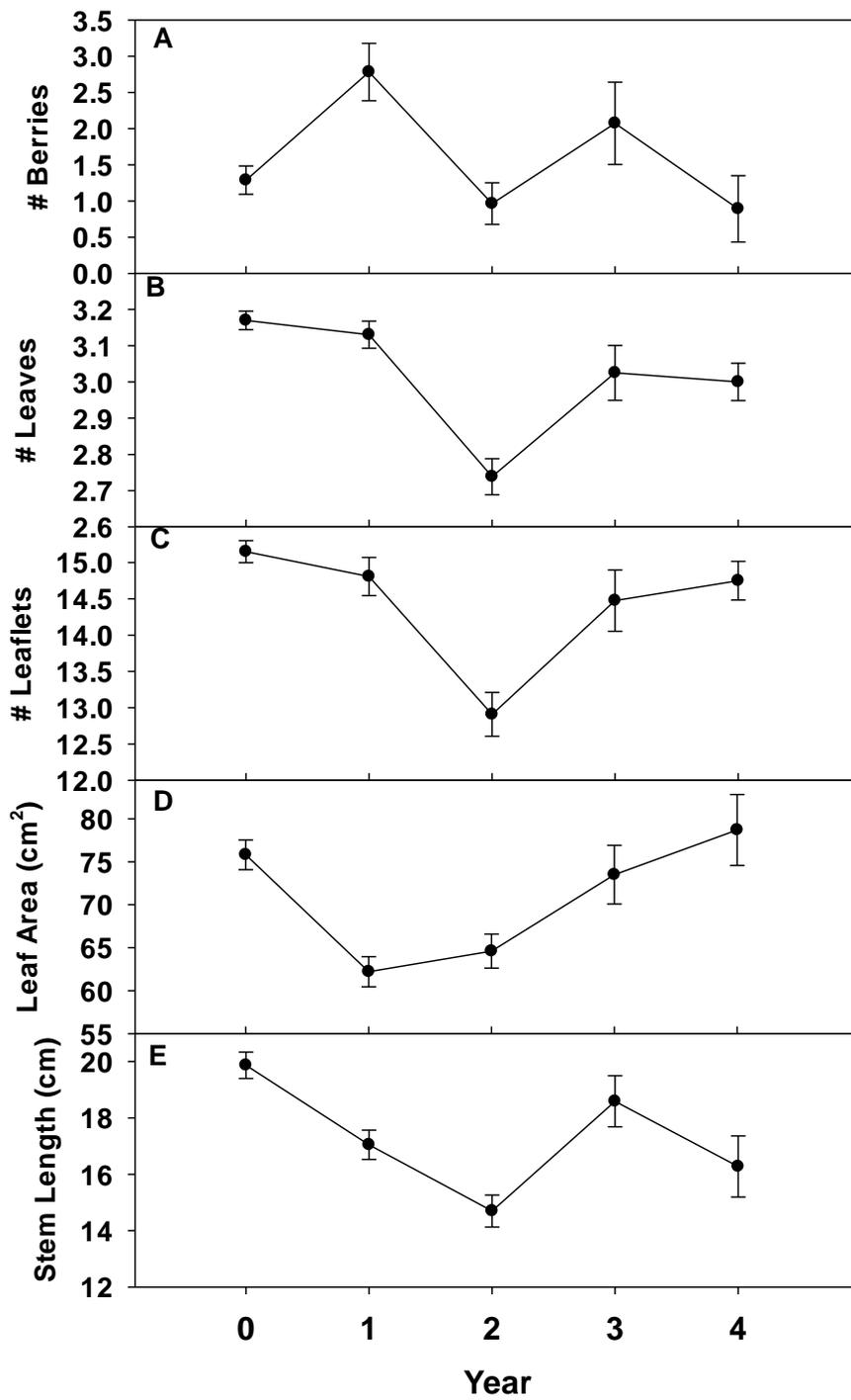


Figure 5. Mean (\pm 1se) morphological attributes from partial-root harvest plants in the harvest year (0) and each subsequent year after harvest (1 – 4).

4. Discussion

Partial-root harvest had no significant effect on survivorship in the four years following harvest (Table 2). However, there were significant decreases in stem length and leaf area in harvested plants compared to unharvested controls in the 1st year after harvest (Table 1; Fig. 4), but these were not present by the 2nd year after harvest. This decrease in stem length and leaf area in harvested plants could be a response to the simulated root herbivory from the partial-root harvest. Root and rhizome herbivory have been seen to cause size class reversion and dormancy in subsequent years in some American ginseng populations³³. It should also be noted that the considerably smaller number of individuals in the 3rd (n = 48) and 4th year after harvest (n = 51; Table 1) was due largely to one population being heavily poached in late summer 2016.

Comparisons of preharvest to postharvest plant attributes for plants subjected to partial-root harvest are more variable than the results from the paired comparison study. However, there were some trends that did emerge. First, there were significantly lower means for all plant attributes the 2nd year after harvest compared to the preharvest year (Table 3; Fig 5). Second, stem length was significantly lower each year after harvest when compared to preharvest values. Both of these trends may be due to interannual variation in weather which has been linked to changes in morphology and reproductive parameters of American ginseng^{8,34}. Interannual variation in weather may further explain other discrepancies in the data such as the number of berries being significantly higher in the 1st year after harvest than in the preharvest year. For instance, berry production in American ginseng has been shown to be negatively affected by higher temperatures³⁵. Inter-annual changes in light availability could have also impacted morphological parameters, previous research has suggested length of longest sunfleck has a positive linear relationship with the growth of American ginseng³⁶.

Several potentially confounding factors were present in the preharvest – postharvest study. First, plants were harvested in different years, so some plant attributes may be responding to interannual variation in weather in addition to the partial-root harvest. Second, because of time constraints and the difficulty in accessing some sites, some populations were only surveyed every other year. Third, because of small population sizes, we were unable to utilize paired, unharvested plants for comparison. Additionally, the preharvest-postharvest had a larger number of populations, which varied in their protection status. Plants from 20 populations were used in the preharvest – postharvest study and several of these are on unprotected, public land, whereas only four protected populations were used in the paired plant study. Although it should be noted that there was evidence of illegal harvest resulting in loss of study plants in many of the populations regardless of protection status. In a 2004 study, Cruise-Sanders and Hamrick⁶ found age class structure of American ginseng to be significantly smaller for unprotected populations compared to protected populations. Similarly, Mooney and McGraw³² found that populations of American ginseng with high harvest pressure had 30% less leaf area, decreased sympodium length, and reduced berry production compared to populations with low harvest pressure. Additionally, in the preharvest – postharvest study, sample size was reduced in years 3 and 4 after harvest because these did not include plants harvest in 2015 and 2016. These populations will continue to be monitored and the addition of these data may better elucidate long term effects of partial-root harvest on plant morphometrics.

One topic that neither of these studies addressed was the impact of partial-root harvest on the plants' susceptibility to root pathogens. American ginseng is susceptible to many root pathogens including: *Cylindrocarpon destructans*, *Fusarium* spp., and *Phytophthora cactorum*^{37,38,39}. One study found that cultivated American ginseng plants intentionally wounded and inoculated with *C. destructans* were significantly more likely to suffer from root rot than unwounded plants³⁸. However, cultivated American ginseng plants are often more susceptible to disease than wild populations⁴⁰, possibly because of the much higher density of plants in cultivated settings or root damage caused during cultivation activities. Darmano et al.³⁷ found that indigenous soils harboring wild American ginseng plants contained strains of *P. cactorum* that were much less likely to be pathogenic than the strains found in gardened American ginseng soils. To better evaluate the potential negative effects of this partial root harvest method on long-term plant health and survivorship, further work on the potential susceptibility to postharvest pathogen infection should be investigated.

5. Conclusion

The two studies presented here showed somewhat different results. The high variability in the preharvest – postharvest study likely resulted from the different years in which plants were harvested and the effects of interannual variation in weather on the plant attributes we observed. The results from the paired study are more reliable because both

harvested and unharvested plants experienced the same environmental influences on growth. From this study, we can conclude that non-destructively harvesting root tissue causes reductions in plant size (stem length and leaf area), but not survivorship when compared with unharvested controls. These effects appeared only in the first year after harvest and were not seen in the subsequent three years of observation suggesting that partial-root harvest has little long term effect on growth or survivorship over time.

Because the partial-root harvest method had no significant effect on survivorship in either year and had only marginal and short-lived effects on morphology, it could prove to be an effective, non-destructive method for tissue collection for ginsenoside determination for research applications. One hindrance to studying the phytochemical makeup of wild American ginseng populations was the need to destructively harvest root tissue. This method will allow researchers to sample plants from wild populations without resulting in long-term declines in these populations from research harvesting.

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