

ABSTRACT

Title of Thesis: **THE ESTABLISHMENT AND PERSISTENCE OF AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L.) IN MARYLAND FORESTS**

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American ginseng (*Panax quinquefolius* L.) is a profitable non-timber forest product with the potential of improving the sustainability of Maryland forests. In order to determine factors affecting Maryland ginseng production, ginseng seeds and roots were planted in forests in Eastern, Central, and Western Maryland in plots amended with no treatment, lime, or gypsum. The response variables measured included soil nutrients and ginseng persistence and establishment. In general, soil lime treatments improved establishment at the Eastern but not the Central or Western sites. The gypsum soil treatments did not significantly affect populations. Establishment of American ginseng grown from seed ranked by site was Western>Central>Eastern. Conversely, root transplant establishment was best at the Eastern site. Across sites, soil pH, Ca, Mg, and K were positively correlated with establishment and persistence. Thus, American ginseng was grown throughout Maryland and ginseng production was enhanced by lime addition at the Eastern site.

THE ESTABLISHMENT AND PERSISTENCE OF AMERICAN GINSENG
(*PANAX QUINQUEFOLIUS* L.) IN MARYLAND FORESTS

By

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DEDICATION

I would like to dedicate this thesis to my family, who has always been supportive and encouraging; to the love of my life – without her help I could not have done this; to all of my academic mentors including my thesis committee: Dr. Frank Coale, Dr. Joseph Sullivan; to my undergraduate mentors Dr. Katharine Keller and Dr. Victor Wexler who inspired me to learn more; and finally to my thesis advisor, Dr. Marla McIntosh for challenging me to grow and work through difficulties.

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CHAPTER 1: LITERATURE REVIEW

Medicinal Value

The wild American ginseng (*Panax quinquefolius* L.) root is highly valued in Asia and is related to Asian ginseng (*Panax ginseng* C.A. Meyer), which has been used as a panacea in traditional Chinese medicine for over 4,000 years (Duke, 1989). American and Asian ginseng roots contain various ginsenosides, which are dammarane-type triterpene saponins considered to have pharmacological properties (Li et al., 1996). Individual ginsenosides are thought to stimulate the central nervous system, sedate the central nervous system, balance metabolic processes, decrease blood sugar, improve muscle tone, stimulate the endocrine system, and maintain proper hormone levels (Persons, 1994). Reviews of the medicinal properties and chemical constituency of American ginseng roots can be found in Court (2000) and Duke (1989). Although many medicinal properties have been attributed to American ginseng, the paucity of controlled double-blind studies of the ginsenosides' medicinal effects has hindered Western medicine's appraisal of the ginsengs (Arnason, 2001).

Both American and Asian ginseng are native to shady mesophytic hardwood forests (in North America and Asia, respectively). Over the last century, extensive deforestation in Asia has decimated wild populations of Asian ginseng, which has caused increased demand in Asia for imported American species. Subsequently, over-harvest of the native American ginseng from American forests has caused the species to be considered threatened by the Convention on the International Trade of Endangered Species (CITES). Thus the harvest and export of wild American ginseng plants is regulated by the United States Fish and Wildlife Service (USFWS).

American ginseng roots are highly valued in Asian markets. From 1990 to 2001, the price of dried wild ginseng roots fluctuated from \$495 to \$1000 per kg (Chamberlain and Predny, 2002). Seven states, mostly in the Appalachian region, account for 82% of wild ginseng exported from the United States from 1978 to 1998. The export and sales of wild ginseng roots, which is regulated by the US Fish and Wildlife Service, generated an average of \$71.7 million per year for rural communities in the United States between 1983 and 2000 (Chamberlain and Predny, 2002).

Sustainable Forestry

Wild-simulated production of American ginseng could contribute to more sustainable forests in Maryland. Sustainable forestry depends on exploiting the multiple benefits of forests (ecological, social, and economic) while maintaining a, “long-term, stable relationship between humans and the environment” (Kimmins, 1996). Assignment of forest benefits is largely dependent on the value systems of stakeholders involved and society at large. For example, utilization of the economic benefit of timber harvest would not represent sustainable forest management if social and ecological valuations of the forest, like recreational use and wildlife habitat, are sacrificed to an unacceptable degree. Likewise, if invasive species are introduced as a result of recreational opportunities (e.g. hunting) and biodiversity is of great value to stakeholders, then management plans should try to reduce introduction of invasive species while maintaining hunting opportunities. Seeking to balance ecological benefits like biodiversity and wildlife habitat with social benefits like recreational and spiritual opportunities and economic benefits like timber harvest and non-timber forest product harvest is the basis for sustainable forest management.

Balancing multiple forest benefits requires assessment of the multiple impacts and interactions of each forest practice and function. The harvest and sales of non-timber forest products (NTFP), like American ginseng, is a form of agroforestry that provides forest economic benefits and can contribute to sustainable forestry. However, in order to create a sustainable forest management plan, assessment of the impact of NTFP removal on other forest benefits is necessary. Alexander (2003) expressed concern that although NTFP management may lead to profitability, it can be at the cost of ecosystem simplification and loss of biodiversity. Certification and domestication of NTFP production in agroforestry systems should allow for profitable production while maintaining sustainability (Alexander, 2003). The USDA defines agroforestry as, “*intentional combinations of trees with crops and/or livestock that involve intensive management of the interactions between the components as an integrated agroecosystem.*” (USDA, 2002). Agroforestry practices include alley cropping, forest farming, riparian forest buffers, silvopasture, and windbreaks. Wild-simulated ginseng production is a USDA recommended forest farm crop (Dix et al., 1997), that could also be farmed in riparian forest buffers and windbreaks with sufficient canopy cover.

Habitat

American ginseng is native to the shady mesophytic North American hardwood forests ranging from Georgia to southern Québec, and from east of the Appalachian mountains to as far west to Minnesota (Beyfuss, 1999). It is thought that ginseng does not thrive in coniferous forest because acidic soil, dense shade, and pine litter inhibit ginseng growth (Persons, 1994). Ginseng populations are typically found on slopes near a source of water in temperate mixed hardwood forests (Davis, 1997). Near southern

Québec, at the northern edge of its range, ginseng populations are found in forests dominated by sugar maple-hickory (*Acer saccharum-Carya condiformis*) associations, butternut (*Juglans cinerea* L.), American basswood (*Tilia americana* L.), and American beech (*Fagus grandifolia* L.)] (Charron, 1991). In New York, native ginseng populations are most frequently associated with sugar maple and mixed hardwoods (American beech, black cherry (*Prunus serotina* L.), red maple (*Acer rubrum* L.), white ash (*Fraxinus americana*), red oak (*Quercus rubra* L.), and American basswood (Beyfuss, 2000). In North Carolina, ginseng is often found growing with black walnut (*Juglans nigra* L.), mixed oak (*Quercus* sp.), yellow poplar (*Liriodendron tulipifera* L.), and American basswood (Davis, 1997). In Kentucky, ginseng is most often found under maple, beech, dogwood (*Cornus* sp.), yellow poplar, oak, hickory, walnut, redbud (*Cercis* L. sp.), gum (*Nyssa* L. sp.), birch, and elm (*Ulmus* L. sp.) (Persons, 1994).

The understory plants most often associated with native ginseng populations include overstory species' transgressives (young trees), flowering dogwood (*Cornus florida* L.), and hop-hornbeam (*Ostrya virginiana* (Mill.) K. Koch) (Fountain, 1986). A list of herbaceous vegetation commonly found growing in association with populations of ginseng is shown in Table 1. The scores are from Beyfuss (2000). Scores range from 0 to 10 and the higher scores are associated with better indicator plants of suitable sites for growing ginseng. Beyfuss (2000) considered these plants to be indicators for proper site conditions for ginseng growth in terms of light, moisture, and soil fertility status. All of the species listed are ombrophytes (shade-loving) and grow in moist well-drained soil (Schmid, 2002). Jack-in-the-pulpit, rattlesnake fern, and maidenhair fern are listed by the USDA as having low drought tolerance, a high fertility requirement, and growing in a

soil pH range between 4.8 and 7.0 (USDA NRCS, 2002). The other listed species live in soils that have similar pH ranges of moderately acid to very acid (Schmid, 2002).

Table 1. Herbaceous species common near American ginseng populations.

Species	Score ¹	Other citations	Locations
<i>Arisaema triphyllum</i> , Jack-in-the-pulpit	5	Anderson, 1993; Hankins, 2000; Davis, 1997; Persons, 1998.	IL, VA, NC, New England
<i>Podophyllum peltatum</i> , mayapple	5	Anderson, 1993; Hankins, 2000; Davis, 1997; Persons, 1994.	IL, VA, NC, New England
<i>Botrychium virginianum</i> , rattlesnake fern	8	Fountain, 1983; Lewis, 1982; Anderson, 1993.	AR, NY, IL, New England
<i>Trillium</i> sp., trillium	5	Hankins, 2000; Davis, 1997; Persons, 1998.	VA, NC, New England
<i>Pteridophyta</i> , fern phylum	5	Hankins, 2000; Davis, 1997; Persons, 1998.	VA, NC, New England
<i>Polystichum acrostichoides</i> , Christmas fern	6	Fountain, 1983; Anderson, 1993;	AR, IL, New England
<i>Sanguinaria canadensis</i> , bloodroot	5	Fountain, 1983; Anderson, 1993; Beyfuss, 2002.	AR, IL, New England
<i>Adiantum pedatum</i> , maidenhair fern	8	Fountain, 1983	AR, New England
<i>Asarum</i> sp., ginger		Hankins, 2000; Davis, 1997; Persons, 1998.	VA, NC
<i>Hydrastis canadensis</i> , goldenseal		Hankins, 2000; Davis, 1997; Persons, 1998.	VA, NC
<i>Polygonatum biflorum</i> , goldenseal		Hankins, 2000; Davis, 1997; Persons, 1998.	VA, NC

¹ From Beyfuss (2000).

Botany of American ginseng

The species has umbellate inflorescences with greenish white flowers that lead to fruits which mature to red and contain one to three seeds. The seeds require 18 to 22 months of cold-warm-cold treatment (5°/20°/5° C) to break dormancy. The seed endocarp splits after the warm period and the second cool period may be necessary for the embryo to break endogenous dormancy (Stoltz and Snyder, 1985). At the top of the

root, the shoot is attached to a rhizome on which two buds are produced in alternate arrangement annually – the smaller one remains dormant and the larger bud generates the aerial shoot and a portion of the rhizome which becomes the internode. Since dormant buds remain on the rhizome and are separated by short internodes, nodes can be counted to determine age of ginseng roots. The species only reproduces sexually; the rhizome does not create asexual propagules. The root is a taproot, sometimes with lateral roots, and is the economically valuable part of the plant.

Soil Fertility Requirements

Published research on soil factors that affect the growth and distribution of American ginseng is limited in scope and the methods used for analyzing soil properties differ between studies and reports. Beyfuss (1998) collected and analyzed soil samples collected adjacent to native populations of ginseng in New York, Massachusetts, and New Hampshire forests in order to determine the soil pH properties associated with favorable ginseng growth. Soil pH levels of the samples in this study ranged from 3.8 to 7.0 with a mean of 4.9 (Beyfuss, 1998). Persons (1994) found that the mean pH of soil samples from 30 forest ginseng farms across the Northeast United States was 5.2. Other studies reported that soil pH from areas where native ginseng populations were found ranged from 5.0 to 6.5 in Québec (Charron, 1991), 4.4 to 7.3 in Illinois (Anderson, 1993), 4.6 to 7.4 in Kentucky (Roberts, 1980), 4.6 to 6.8 in Arkansas (Fountain, 1986), and 4.0 to 5.0 in North Carolina (Davis, 1997).

Various pot and field studies have demonstrated the importance of soil pH on American ginseng growth. Konsler and Shelton (1984) grew ginseng in pots containing soil at a pH of 4.4, 5.5, and 6.5 for four years. Mean root dry weight was greatest after

four years for plants growing in soil at pH 5.5. Calcitic limestone (CaCO_3), which increases soil pH and Ca concentrations, has been found to significantly increase the emergence and survival of American ginseng planted in very acid soils of red maple forests in Québec (Nadeau, 2003). Limestone applied at 6000 kg per hectare increased soil pH from 4.1 to 4.4 and Mehlich III-extractable Ca from 143 to 6380 mg/kg. For five years following the lime application, the treated plots had a significantly higher plant density and less mortality than the control plots. Limed plots also had significantly higher leaf area and shoot mass for young ginseng plants. Improved winter survival in limed plots was attributed to the physiological role of Ca in cold hardening and resistance to diseases (Nadeau, 2003).

In New England, soil collected near native ginseng populations had a wide range of ammonium acetate extractable soil Ca concentration (100-7000 lb/a) and a high average Ca concentration (3289 lb/a) (Beyfuss, 1998). In a nutrient solution experiment, Stoltz (1982) found that omitting Ca from the solution caused the first reported foliar deficiency symptoms for ginseng, which appeared as 0.5 to 1.0 cm of ginseng leaflet tips collapsing after 25 days. In addition, without Ca, P, or Mg in the nutrient solution, fresh roots had reduced weight, higher sugar levels, and lower starch levels than roots grown with these elements. This suggested that these nutrients play a significant role in carbohydrate allocation (Stoltz, 1982). In a field study of mulch effects on American ginseng growth, Konsler and Shelton (1990) found that root size after six years was positively correlated with increased soil pH, K, Ca, and Mg. Ginseng root weight after four years was positively correlated with increased soil pH, K, Ca, Mg, and Na in a seeded pot experiment (Konsler, 1990). Gypsum (calcium sulfate) application of 3000

kg/ha and 4000 kg/ha increased fresh and dry root weight and total ginsenoside content of three-year old ginseng plants grown in a greenhouse for 12 weeks (Lee, 2004).

Calcium, the third most abundant element in plants, is necessary for growth and plays a role in plant resistance to pathogens (Taiz and Zeiger, 1998). Calcium is a primary component of Ca-pectates in the middle lamella of cell walls which give plants rigidity. Callose, which forms a physical barrier to tissue penetration, was found to be dependent on Ca uptake in numerous plant species (Kauss, 1989). Although no published study has examined the role of soil Ca in ginseng resistance to pathogenesis, soil Ca has been found to increase host resistance to pathogens that commonly infect American ginseng. Although *Phytophthora cactorum* is commonly regarded as the most serious ginseng pathogen, pre-emergence damping-off and post-emergence seedling root rot caused by *Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp., have been known to kill 50% of seedlings in a wet season (Duke, 1989).

Kao and Ko (1986) found that soil Ca addition, via CaCO₃ or CaSO₄, significantly reduced damping off in cucumber seedlings caused by *Pythium splendens*. They proposed that Ca increased root growth and general growth rate, and significantly increased antagonistic microbial populations. Increased microbial populations then contributed to significant decreases in pathogenicity of *P. splendens*. Soil Ca addition also decreased the propagule density of *Fusarium solani* in a wheat-peas rotation in the top 15 cm of soil over three years (Allmaras 1987). In peas, Ca may have contributed to plant physiological defense against infection (i.e. Ca-pectates in root cortex), impaired saprophytic growth, and favored microbial antagonism (i.e. *Bacillus* spp. and *Streptomyces* spp. populations) to *Fusarium* incidence. *Bacillus cereus*, a possible

biological control agent for *Phytophthora cactorum*, was found to be dependent on Ca to generate ionic conditions which lysed *P. cactorum* oospores (Gilbert 1990). Adequate soil Ca not only contributes to plant health and survival, but may also augment plant resistance to pathogens through roles in signal transduction, defense activation, and biological control of the most common ginseng pathogens.

It has been hypothesized that the unique combination of acid soil and high Ca found in New England suppresses the most prominent pathogenic genera which threaten American ginseng (i.e., *Phytophthora* spp., *Pythium* spp., and *Fusarium* spp.) (Beyfuss, 1999; and Hankins, 2000). Northeastern forest soils are usually acidic and many are fairly low in Ca. In order to replicate a high Ca/low pH soil environment and improve ginseng survival and growth, Hankins (2001), suggested adding about 2400 kg gypsum ($\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$) per hectare to bring ammonium acetate extractable Ca levels to about 4000 lb/acre to forest soils with pH near 5.

Population Characteristics

There are few published studies of wild native American ginseng populations. Studies of plant populations have been conducted for populations containing approximately one hundred individuals in Québec (Charron, 1991), New York (Lewis, 1984; Lewis, 1988; Lewis 1982), Illinois (Anderson, 1993), Wisconsin (Carpenter, 1982), and Missouri (Lewis, 1988). These studies described plant populations by morphological class based on the number of prongs per plant rather than plant age. Classification by morphological class is easier and faster than classification by age, which requires examination of the underground rhizome to count the number of nodes. Although significant positive correlations were found between age and morphological class in

several studies (Lewis, 1984; Carpenter, 1982; and Lewis, 1983), each morphological class contained a wide range of age classes. All plants less than three years old had one-prong. Plants from three to six years old were also observed to have one prong. Two-pronged plants in these populations were observed to be from three to sixteen years old, averaging about five years old (Lewis, 1982; Anderson, 1993). Three-pronged plants ranged from four to seventeen years old averaging eight years old and all four-pronged plants were at least eleven years old (Anderson, 1993).

Ginseng seedling emergence in the wild in natural conditions appears to be quite low for any particular year. A few authors have estimated the size of seedbanks (viable seeds buried or partially buried in leaves and/or soil from previous years) from known fruit production in order to estimate ginseng seedling emergence percentages (Lewis and Zenger, 1982; Charron, 1991). For a wild ginseng population in Missouri, Lewis and Zenger (1982) estimated that only 0.55% of seeds derived from an estimated number of fruits, developed into seedlings twenty months later. For four wild populations in Québec, Charron (1991) estimated that 1% to 15% of seedlings emerged from fruits produced. Seeds that drop from plants are subjected to freezing, drying, and other obstacles that decrease seedling emergence. Ginseng seedling emergence was greatly increased by planting seeds in forests. In three Illinois forests, Anderson (1993) sowed ginseng seeds (from a local population) 1.25 cm deep and found 48% to 80% (average 66%) emergence after twenty months. In a forest near Sainte-Croix de Lotbinière, Canada, Nadeau et al. (2003) broadcast and incorporated pre-stratified seeds into the top 2 cm of soil and found 72% seedling emergence in control plots. Wide disparity between the low estimated emergence rates in Missouri and Québec and the much higher rates in

Illinois and Canada may be due to seeds being covered by soil and use of pre-stratified seed in the latter studies. Soil cover protected seeds from predation, freezing, and drying and likely contributed to greatly increased seedling emergence.

Several authors report high mortality rates for American ginseng seedlings (Nadeau et al, 2003; Charron, 1991; Lewis, 1988). In four populations studied over four years in Québec, 69% to 92% of seedlings died annually (Charron, 1991) and in Sainte-Croix de Lotbinière, 82% of seedlings died in one year (Nadeau et al, 2003). In Missouri, Lewis (1988) reported that 69% of seedlings died after an unusually dry late summer and early fall. However, Lewis (1982) reported no seedling mortality or 6% seedling mortality in separate years with temperatures and precipitation comparable to thirty-year averages. In contrast to high seedling mortality, three- and four-pronged plants were found to have less than 10% annual mortality (Charron, 1991). Although weather may play a significant role in ginseng seedling survival, examinations of small numbers of seedlings (less than fifty) for less than three years, without examination of climate may not be sufficient to draw conclusions about American ginseng seedling survival.

Studies of wild American ginseng populations reveal varying population demographics. Wild American ginseng population studies reviewed here consisted of plants ranging from one- to twenty-years old. In New York, Lewis (1984) reported that the largest age class was one-year old plants which represented 60% of the population. The two-year and three-year old age classes represented about 20% of the population and four-year through twelve-year old age classes represented 20% of the population (Lewis, 1984). In Illinois, Anderson (1993) estimated age classes based on morphological class and reported that the one-year old age class was about 6% of the population, the two-year

old age class was about 20%, three-year to six-year old age classes was about 52%, and seven-year to nine-year old age classes were about 8% of the population. In Missouri, Lewis (1982) reported that the one-year old age class varied from 2% to 15% of the population annually. The two-year to ten-year old age classes were normally distributed around the largest age class which was six-years old at the start of the observations and composed around 21% of the population for the three years of the study (Lewis, 1982). Charron (1991) reported population distributions for four Québec populations. The seedling class represented between zero and 44% of a population depending on the year and the specific population studied.

Correlations between flower production and morphological class; or between flower production and age class were found to be positive (Lewis, 1984; Carpenter, 1982; Lewis, 1983). However, the minimum age of flowering plants differed among populations, one-pronged plants in all studied populations were juvenile (non-flowering). One study concluded that 14% of two-year old (average 1.5 prongs) plants and 60% of three-year old (2 prongs) flowered (Lewis, 1984), whereas two studies concluded that no two- or three-year old plants in those populations flowered (Anderson, 1993; Carpenter and Cottam, 1982). The discrepancy between these studies may be due to natural variation between populations, environmental differences, and/or small sample sizes.

In general, larger and older ginseng plants (three-pronged and four-pronged) produce more flowers and fruit per plant and have longer seasonal persistence than smaller and younger ginseng plants (Lewis and Zenger, 1982; Anderson, 1993; Lewis, 1984; Carpenter, 1982). For plants older than six years old, 100% of a NY population (Lewis, 1984) and 70% of a WI population (Carpenter and Cottam, 1982) produced

flowers. Lewis (1982) found that three-pronged plants (eight-years old on average) produced between four and six fruits per plant and four-pronged plants (fourteen-years old) produced twelve fruits per plant. Anderson (1993) found that six- to eight-year old plants (averaging 2.5 to 2.9 prongs) averaged four to seven fruits per plant and that nine-year old to eleven-year old plants (averaging 3.1 to 3.6 prongs) averaged nine to thirteen fruits per plant. In wild populations in Wisconsin in late July, over 30% of one- and two-pronged plants had senesced whereas only 12% of three-pronged plants and no four-pronged plants had senesced. By mid-August, over 50% of one-pronged plants had senesced, 40% of two- and three-pronged plants were no longer above-ground, and all four-pronged plants were still above-ground.

Studies of population characteristics (e.g., seedling emergence and mortality, establishment, and seasonal persistence) have been used to ascertain and predict the rate of change in wild ginseng populations. The population growth rate, λ , measures change in a given population, and is usually dependent on plant size and morphological class (Carpenter, 1982; Lewis, 1983; and Charron, 1991). The population growth rate depends on probabilities of survival within and among morphological classes as well as the reproductive contribution of individuals within classes. When λ is less than 1, a population is expected to decrease if prevailing conditions continue. If λ is equal to 1 a population is expected to stay the same in number. Otherwise if λ is greater than 1 a population is likely to increase in number given the continuation of conditions noted prior to measurement. Wild ginseng populations appear to have a narrow range of population growth rates that average λ near 1. Constant population growth rates for these herbs have been attributed to stable forest habitats which provide a niche for these populations

(Charron, 1991). Ginseng populations were found to have λ near 1 (0.87 to 1.19 in Québec (Charron, 1991) and $\lambda = 1.2$ in a Wisconsin population (Sverdlove, 1981)), which indicated that the populations on average were not increasing or decreasing over time.

Harvest Impacts

Given that some small wild ginseng populations have been found to be relatively stable (λ near 1), it is unlikely that populations are sustainable if many reproductive individuals were harvested. A key aspect of any self-sustaining ginseng population is the existence of mature plants producing viable seeds. For a population to maintain its size, mature plants must produce seeds of sufficient number to contribute to population continuation. Nantel et al. (1996) modeled harvest impact on four ginseng populations in Québec by creating population matrix models to assess population growth rate. They estimated changes in population characteristics and population growth rates under various harvest intensities (1%, 5%, 10%, 30%) and regimes (1 yr, 5 yr, 10 yr) for mature plants with at least two prongs. They predicted that the minimum viable population to survive without harvest for 100 years was 172 plants (1068 including seeds) and the extinction threshold was 91 plants (560 including seeds) (Nantel, 1996). If a given ginseng population had $\lambda = 1.045$, then maximum annual harvest of mature plants must be no more than 5% in order to maintain the population size for 100 years (Nantel, 1996).

In order to comply with the Convention on International Trade of Endangered Species (CITES), the United States Fish and Wildlife Service (USFWS) requires that states regulate the harvest of wild ginseng to prevent irreparable harm to ginseng populations. In Maryland, the Department of Agriculture requires that in order to harvest ginseng:

- plants must have three prongs, each with five leaflets
- if extant, seeds must be mature
- roots may only be collected from August 20th through December 1st
- all seeds from collected plants must be planted immediately in the vicinity of the collected plants (Maryland Department of Agriculture, 1999).

Since American ginseng has been generally found to be mature when it has three prongs, adherence to the law will likely decrease the chance of removing juvenile plants (having generally less than three prongs). In addition, provisions requiring harvesters to wait to harvest plants with seeds (fruit) until the seeds (fruit) are mature and by requiring that seeds be planted in the vicinity of collected plants, adherence to the law will contribute to seedbank preservation. However, loss of reproductive individuals (likely three prongs or more) will decrease the amount of fruit produced annually by a population. Anderson et al (1993) found that if all seven- to eleven-year old plants were removed from a non-harvested site in Illinois, then almost 70% of fruit typically produced by the population would be lost. By establishing harvest dates from late summer through fall, fruits from harvestable individuals have time to ripen and seeds have increased chance of germination and emergence after requisite planting. Some states do not require fruits from harvestable plants to be ripe before planting which is deleterious to population sustainability (Robbins, 2000).

Production Method

In 2000, 69% of exported American ginseng roots were grown under artificial shade in fields. Shade cultivation has been practiced since the 19th century in response to greatly diminished wild populations (Chamberlain and Predny, 2002). Shaded, field-cultivated American ginseng is grown from seeds which are previously stratified seeds and sown (78 to 157 kg/ha) from October to November in raised mulched beds under

wooden laths or polypropylene shade cloth. Plant density in cultivated beds is very high (43 to 258 plants/m²) (Konsler, 1982), disease incidence is often high, and it is estimated that nine fungicide applications are made per growing season (Proctor et al, 2003).

Artificial-shade grown ginseng roots grow more quickly than wild roots, and can be harvested after a minimum of four years compared to wild ginseng, which is not legally harvested until it has three prongs (at least six years old) in Maryland. Cultivated roots are smooth, white, and thick and tap-rooted. About 2000 to 4000 kg of dry roots is produced per hectare and may be sold for thirty to sixty dollars per kg (Chamberlain and Predny, 2002). A growing concern with ginseng produced in field conditions is the heavy use of pesticides – especially systemic fungicides, since buyers of medicinal end-products might be less likely to purchase the roots if they knew the plants contained systemic fungicides (Arnason, 2001).

About 31% of American ginseng exported from the United States is woods-cultivated, wild-simulated, or wild (Chamberlain et al, 2002). Woods-cultivated ginseng is similar to artificial-shade grown ginseng, except that trees are used for shade rather than cloth or laths. Seeds are sown in raised mulched beds with fertilizer and pesticide inputs similar to those of shaded field grown American ginseng. Beds are tilled and pH is often adjusted to between pH 5 and 6. Roots are harvested after six years and are fairly smooth, cream colored, and thick, with some branching. In 1999, woods-cultivated roots sold for eighty-eight dollars per kg (Hankins, 2000).

Wild-simulated ginseng production is the least scientifically documented method of commercially or privately growing ginseng. The goal is to produce roots which most closely resemble wild roots – a criterion with few prescriptive guidelines. In native

populations, plants have been found at low densities of 0.2 plants/m² (Lewis, 1983) and 0.7 plants/m² (Anderson, 1993). In order to replicate low plant densities, seeds can be sown in rows, hills, or broadcast at a fairly low rate (<11 kg/ha) and covered with soil (Davis, 1997). It is suggested that plants be left to the “vagaries of nature,” in order to replicate conditions in the wild (Hankins, 2000). The appearance associated with wild roots may result from protracted competition for water and nutrients. In Eastern medicine, there is a perception that ginseng roots gain curative power from a long life on the forest floor, which means roots become more valuable as they become older (Duke, 1989).

There is a paucity of information dealing with projected economic returns from a wild-simulated ginseng production system. Hankins (2000) itemized costs for seeds, soil amendments, labor (planting, harvest, and drying), and miscellaneous expenses for growing wild simulated ginseng. He estimated that over one hectare of sown seeds could yield 112 kg to 224 kg of dry roots after six to ten years. Thus a grower could gross between \$64,000 and \$128,000 per hectare (net income: \$46,000 to \$92,000) (Hankins, 2000). The density at harvest of Hankins’ theoretical crop would be between 2.5 plants/m² and 24.6 plants/m², given an average dried root weight of 0.91 g to 4.5 g (Beyfuss, 1998; Robbins, 2000.).

Overall Objectives

Although native American ginseng has not been found on the Eastern Shore of Maryland, it may have potential as a NTFP or contribute to sustainable forestry in this region. It may be possible to grow American ginseng in a non-native location or with species different from those in its native range (i.e. *Acer* sp., *Quercus* sp., *Juglans* sp.,

etc.) if environmental factors limiting ginseng establishment, growth, and survival can be identified. Production practices such as amending the soils' chemical properties could improve a site's suitability for growing and/or expanding its production range.

Production practices for specific site selection could enhance forests in Maryland by providing a NTFP within a diverse native forest. The most valuable ginseng roots are ones that have survived competition within a forest. The markets for American ginseng are aesthetic-driven; darker, more gnarled wild roots are much more valuable than whiter, smoother cultivated roots. Unlike most agronomic crops, a commercial or private wild-simulated ginseng grower attempting sustainable production would need to balance root biomass, aesthetic value of roots, population longevity, and resiliency of a population to harvest (Beyfuss, 1998; Hankins, 1997; Duke, 1990).

American ginseng is a threatened species which is native to Western and Central Maryland forests and is a profitable non-timber forest product. American ginseng's unique life history and factors which limit the species' establishment have not been previously characterized in Maryland forests. This thesis intends to characterize the unique life history of American ginseng in Maryland forests by examining the species' growth from seeds and transplanted roots in Western, Central, and Eastern Maryland forests. By examining the emergence and seasonal aboveground shoot populations in Eastern Maryland forests, this thesis seeks to characterize the species' growth in this non-native location and examine its potential as an NTFP there. Successful ginseng production in Eastern Maryland could potentially expand the species' germplasm and decrease harvest pressure on wild populations in Maryland. In addition, this thesis seeks to examine the effects of altering soil pH and soil Ca on the emergence, percent absent

and percent present aboveground, and establishment in those forests. By examining the roles soil pH and soil Ca play in ginseng's life history, this thesis sought to address factors that may affect the species' potential use as an alternative crop in native and non-native regions of Maryland.

CHAPTER 2: CALCIUM SEED EXPERIMENTS

Introduction

American ginseng, native to Maryland forests, is highly valued in its wild form and shows potential as a non-timber forest product for Maryland forest landowners. The primary objective of these experiments was to study the unique life history, or phenology, and establishment of American ginseng seeded in a wild-simulated production system, in three physiographic regions in Maryland. The secondary objective was to evaluate the effects of soil calcium amendments on soil fertility and to examine the relationships between soil fertility and phenology and establishment of these populations.

Wild-simulated ginseng production utilizes existing forest land, requires minimum inputs, and may be economically feasible over a range of forest sizes and types (e.g., a few hectares in tree windbreaks to several hundred hectares in primary or secondary forests). However, there are no published scientific studies of ginseng production in Maryland. Thus, this study will be the first to report on growing American ginseng in three physiographic regions of Maryland. In Maryland, ginseng is native to the Piedmont and the Ridge and Valley physiographic regions but has not been found or cultivated in the Coastal Plain region. This study compared the phenology and establishment of American ginseng grown from seed in a forest site in each of these three regions to investigate the potential of growing wild-simulated ginseng within and outside the native range.

Because population sustainability is of primary importance to wild simulated ginseng production and biodiversity, the phenology of American ginseng was studied to document population dynamics in Maryland. Three measures of phenology were used,

based on non-destructive observational study. The first measure, emergence, is an indication of seed viability and site suitability for germination and hypocotyl elongation.

The second measure of phenology is termed “absent aboveground” which measures the ginseng plants have died or senesced from plants which had emerged during a growing season. This measure has been used previously to examine American ginseng’s life history (Lewis and Zenger, 1982). The length of ginseng seedling leaf area duration is of interest because it is related to plant mortality and crop production. Early senescence will reduce the period of active photosynthesis and thus, carbohydrate production, root growth, and plant survival may be affected. Mortality will reduce the value of a crop per hectare.

The third measure of phenology is the percent of plants present aboveground at a given date based on the number of total seed sown. The percent of plants present at a given date during the growing season is a function of the number of plants emerged by a given date, the number of plants absent aboveground at that date, and the number of seeds sown. This measure, which can be calculated during each growing season, provides an indication of population stability and establishment. In addition, curves of percent of plants present aboveground illustrate the establishment and growth of American ginseng populations over time from an initial seed population.

Soil pH and Ca are considered important factors affecting American ginseng growth (Nadeau, 2003; Beyfuss, 1998; Hankins, 2000). Calcium is the third most abundant element in plants and is integral in providing cell rigidity, cell elongation and division, membrane permeability, and critical enzyme activation. Beyfuss (1998) suggested that 2443 kg/ha gypsum (calcium sulfate dihydrate) should be applied to soils

in order to duplicate high soil Ca and low soil pH found in soil samples taken near native ginseng populations in New York. However, these are based on data from New England and may be region specific. Thus, in order to determine the potential for improving ginseng production in Maryland using Ca soil amendments, soils at each experimental site were treated with differing rates of gypsum and lime. Gypsum was added at the rate suggested by Beyfuss (1998) and twice that rate, to increase soil Ca. Lime was added to increase soil Ca, as well as to increase soil pH.

The objectives of the experiments in chapter II were to study American ginseng using a wild-simulated production system in three forests in different physiographic regions of Maryland in order to:

- 1) Determine differences in phenology (emergence and absence aboveground) and establishment (seasonal presence aboveground) of American ginseng among physiographic regions in Maryland.
- 2) Determine and compare the effects of lime and gypsum on soil fertility and on American ginseng phenology and establishment among physiographic regions in Maryland.
- 3) Determine relationships between soil properties and American ginseng phenology and establishment.

Calcium Seed Experiment I (CS I): 2001 – 2003

Materials and Methods

Site Descriptions.

Experiments were established in the fall of 2001 at three forested locations in

Maryland. Two experimental locations, the Western Maryland Research and Education Center (West) and the Central Maryland Research and Education Center (Central) were within American ginseng's native range. The other location, the Wye Research and Education Center (East) was outside of ginseng's native range.

The West site is located near Keedysville, Maryland in Washington County at 39°29'N and 77°42'W with an elevation of 128.0 m (419.8') in the Ridge and Valley physiographic region. The Central site is located near Clarksville, MD in Howard County at 39°15'N and 76°56'W and an elevation of 112.8 m (370.0') in the Piedmont region. The East site is located near Wye Mills, Maryland in Queen Anne's County at 38°55'N and 76°08'W at an elevation of 6.1 m (20.0') in the Coastal Plain region. Temperatures and precipitation for 2001-2003 and the twenty year average for each location are given in Table 2 and Table 3, respectively.

The experimental plots at the West site were in a forest with dominant overstory of 30- to 40-year old mixed hickory (*Carya* sp.) and black walnut (*Juglans nigra* L.) and an understory of predominantly tree of heaven (*Ailanthus altissima* (P.Mill.) Swingle) and flowering dogwood (*Cornus florida* L.). The groundcover was mostly mayapple (*Podophyllum peltatum* L.), wild leek (*Allium tricoccum* Ait.), garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande), and multiflora rose (*Rosa multiflora* Thunb. ex. Murr.). The soil at the site was a rocky silt loam, a mesic Typic Hapludalf of the Hagerstown series.

At the Central site, the experimental plots were within a forest with a dominant overstory of 60- to 70- year old mixed oak (*Quercus* sp.) and hickory (*Carya* sp.) along with tulip poplar (*L. tulipifera* L.) and red maple (*Acer rubrum* L.). The understory was

mostly flowering dogwood (*C. florida* L.), slippery elm (*Ulmus rubra* Muhl.), and black gum (*Nyssa sylvatica* March.). The groundcover was a mixture of vines, (Japanese honeysuckle, *Lonicera japonicum* Thunb.; Virginia creeper, *Parthenocissus quinquefolia* (L.) Planch.; and poison ivy *Toxicodendron radicans* (L.) Kuntze.) shrubs, (spicebush, *Lindera benzoin* (L.) Blume, and Japanese barberry, *Berberis thunbergii* DC.), and herbaceous vegetation (mayapple, *P. poltatum* L.; Virginia strawberry, *Fragaria virginiana* Duchesne). The soils were silt loams, mesic Typic Hapludults of the Chester and Fairfax series. Slopes in the forest ranged from 0-7 percent but averaged less than 2 percent.

The East site was within a forest with a dominant overstory of 30- to 40- year old loblolly pine (*Pinus taeda* L.), tuliptree (*L. tulipifera*), and cherry (*Prunus* sp.). The understory consisted of red maple (*A. rubrum* L.) and American beech (*Fagus grandifolia* Ehrh.). The groundcover consisted of poison ivy (*T. radicans*). The soil was a sand loam, mesic Aquic Hapludults of the Woodstown series.

In the winter of 2001-2002, temperatures at the three sites were warmer than twenty-year averages and there was much less precipitation than average (Table 2 and Table 3). The extremely dry winter of 2001-2002 and the dry spring of 2002 were responsible for the drought of 2002. The winter of 2002-2003 was colder than average in Maryland. The late spring and early summer of 2003 were much wetter than average.

Table 2. Temperature means by month in 2001, 2002, 2003, and twenty-year averages for three forested experimental sites in Maryland.

Month	West				Central				East			
	2001	2002	2003	20-year	2001	2002	2003	20-year	2001	2002	2003	20-year
-----°C-----												
Jan.		2.7	-3.2	-0.8		2.8	n/a	-0.3		3.8	-2.0	-1.3
Feb.		2.7	-1.8	0.5		3.3	n/a	1.3		3.6	-0.8	0.7
Mar.		6.2	5.1	5.6		6.3	5.9	6.0		7.0	6.8	5.9
Apr.		12.3	10.7	12.0		13.3	10.8	11.3		13.5	11.3	11.2
May		15.7	15.1	17.3		16.1	14.6	16.8		16.9	14.9	16.3
Jun.	21.0	21.8	19.7	21.7	22.3	22.2	20.0	21.5	23.1	23.1	21.3	21.3
Jul.	23.6	24.0	22.9	23.8	22.1	23.9	23.7	23.9	22.2	25.5	24.8	23.7
Aug.	17.2	23.3	24.4	23.0	23.8	24.4	24.3	22.9	24.8	25.7	25.3	23.1
Sep.	n/a	19.2	19.4	19.3	17.7	20.5	19.4	19.3	18.7	20.9	20.8	19.5
Oct.	12.1	11.4		12.9	n/a	12.3		12.6	14.2	13.7		13.0
Nov.	8.2	5.4		6.8	9.2	6.0		7.1	10.4	1.3		7.4
Dec.	3.3	n/a		1.2	4.5	0.1		2.1	5.9	2.1		2.3

Table 3. Precipitation means by month in 2001, 2002, 2003, and twenty-year averaged for three forested experimental sites in Maryland.

Month	West				Central				East			
	2001	2002	2003	20-year	2001	2002	2003	20-year	2001	2002	2003	20-year
-----mm-----												
Jan.		43	64	71		44	n/a	87		57	44	76
Feb.		4	126	57		7	n/a	75		14	167	75
Mar.		91	77	86		84	67	105		100	108	99
Apr.		65	73	89		82	56	89		70	60	93
May		84	164	95		85	134	120		85	166	128
Jun.	54	81	176	100	195	42	186	98	153	79	156	117
Jul.	95	89	46	85	47	46	86	102	191	50	142	107
Aug.	47	91	120	92	104	98	94	99	155	43	120	106
Sep.	n/a	94	302	84	51	89	162	106	67	138	206	100
Oct.	16	118		80	19	164		89	17	165		96
Nov.	30	87		77	62	92		90	18	90		89
Dec.	34	n/a		73	28	83		89	38	118		94

Experimental Design and Layout.

In December 2001, the soil treatments (Table 4) were randomized and applied in three complete blocks, 5.1 m by 2.5 m, at each location. Each plot was seeded with four rows of pre-stratified American ginseng seed. Each row contained ten hills spaced

twenty-five cm apart with five seeds per hill planted at one to two cm depths. Leaves and debris were removed from the soil surface before planting seed.

Calcium Treatment Application.

At each location, there were five soil treatments (Table 4): a control (0 kg Ca/ha), a 1X and 2X lime rates, and 1X and 2X gypsum rates. The gypsum used was fertilizer grade that averaged 15.4% moisture and contained 21.8% Ca as (CaSO₄ · 2 H₂O). The gypsum was applied to plots at 2443 kg/ha, the rate recommended by Beyfuss (1998) for optimum ginseng growth and double this rate at 4886 kg/ha. The 1X and 2X gypsum treatments added 450 kg Ca/ha and 900 kg Ca/ha, respectively. The lime, also fertilizer grade, contained 37.9 % Ca as (CaCO₃) and averaged 1.2% moisture. The lime was applied at rates of 1823 kg/ha or 3346 kg/ha. The 1X and 2X lime treatments added 683 kg Ca/ha and 1366 kg Ca/ha respectively, and also increased soil pH. These lime rates are within the range suggested by van den Driessche (1984) to raise sandy loams of forest nurseries from 4.4 to 5.0 and 5.5, respectively. The Ca amendments were hand-applied to each plot in December 2001.

Table 4. Rate of total Ca added as either gypsum (CaSO₄ · 2 H₂O) or lime (CaCO₃) to comprise the five treatments in Calcium Seed Experiments.

Treatment	Total Ca added	Amendments	
		Gypsum	Lime
-----kg/ha-----			
C	0	0	0
G1	450	2443	0
G2	900	4886	0
L1	683	0	1823
L2	1366	0	3346

Soil Samples and Chemical Analyses.

Soil samples were collected in November 2001 before seeding and treatment

application and in late September of 2002 and 2003. Leaves, debris, and organic matter were removed from the soil surface before sampling the soil. Six soil cores were taken to a 7.5 cm depth within each block and composited to form one sample. Soils were dried at 50°C for 24 h and crushed to pass through a 20-mesh screen. A 2.5 mL scoop of soil was weighed, placed in a 70 mL plastic extraction cup, and 20 mL of Mehlich 3 extractant was placed in the cup (Mehlich, 1984). Cups were shaken on an Eberback shaker for 5 minutes and then poured through #1 paper filters into filter tubes. Filtrate was placed in autosampler tubes for analysis on the inductively coupled plasma spectroscope (ICP) for elemental Ca, Mg, K, P, and Fe. A twenty mL sample of soil was mixed with twenty mL of distilled water in a 70 mL plastic cup. After an hour, the 1:1 solution was stirred and pH was measured using an electrode.

Plant Measurements and Samples.

The dates of treatment applications and sampling procedures are shown in Table 5. During the first growing season, data were collected at each location biweekly from the first week of April 2002 until the second week of September 2002. Data recorded were plant counts and qualitative data involving insect damage, associated groundcover, browsing, and general plant vigor. Three measures of phenology were calculated: percent emerged, percent absent aboveground, and percent present aboveground of total seed sown.

Table 5. Sampling, sowing, and treatment application dates for the Calcium Seed Experiment I (2001 to 2003).

Month	Year		
	2001	2002	2003
	<u>Activity</u>		
January			
February			
March		Plant Counts [‡]	Plant Counts
April		Plant Counts	Plant Counts
May		Plant Counts	Plant Counts
June		Plant Counts	Plant Counts
July		Plant Counts	Plant Counts
August		Plant Counts	Plant Counts
September		Plant Counts	Plant Counts
October	Pre-Treatment Soil Sampling	Post-Treatment Soil Sampling I	Post-Treatment Soil Sampling II
November	Seeds Sown		
December	Treatments Applied		

[‡] – Plant counts were biweekly in 2002 and monthly in 2003.

Percent emerged ginseng plants in 2002 was calculated by dividing the number of plants counted emerged by the total number of seeds sown in 2001 on a per-plot basis. In some cases, seed could not be planted at the intended location of a hill due to physical obstacles. Thus, total seed sown per plot was similar but not constant. As the ginseng canopy developed over a period of a month, newly emerging plants were distinguished from plants that had been counted as emerged at the previous sampling date. Percent emerged plants in 2003 was calculated by dividing the number of plants counted as emerged in 2003 by the total number of plants counted as emerged in 2002. In both 2002 and 2003, percent total emerged was the cumulative emerged plants calculated for the last sampling date of the season.

The second measure of phenology, the percent absent aboveground was collected starting the second week of May 2002 and second week of April 2003. When the number of plants in a hill decreased, these were counted as absent aboveground. Percent absent

aboveground plants in 2002 and 2003 was calculated by dividing the number of plants counted as absent aboveground at a given date by the number of plants counted as emerged by that date for each respective year. Without using destructive sampling techniques, it was not possible to determine if American ginseng plants that were absent aboveground had died or had senesced by a given sampling date in 2002 or 2003.

The third measure of phenology, the percentage of plants present, was calculated by subtracting the number of plants counted as absent aboveground at a given date from the number counted as emerged by that date and dividing by the total number of seed sown in 2001.

During the second growing season, data were collected monthly on plant counts and qualitative plant health data. In addition, shoot data (categorized as three-leaflets, five-leaflets, and number of prongs) was also recorded. The sampling dates are shown in Table 5.

Statistical Analysis.

Analysis of variance (ANOVA) was conducted on the measured variables described previously. The analysis of variance was combined over sites with years considered to be a repeated measure. All factors except for blocks were considered fixed. Sources of variation and F-tests were according to McIntosh (1985). The linear model was:

$$M_{ijkl} = \mu + S_i + B(S)_j + T_k + TS_{ik} + TB(S)_{lkj} + Y_l + SY_{il} + BY_{jl} + TY_{kl} + TYS_{ikl} + e_{ijkl},$$

where M is the observation of the l th year Y and the k th calcium treatment T in the j th replication B within site S ; μ is the general mean, e is the variation due to random error or the residual, and TS , $TB(S)$, SY , BY , TY , and TYS are the interactions. The block within

site, $B(S)$, term was used to test site effects, the $TB(S)$ term was used to test treatment and treatment by site interaction effects, and the random error, e_{ijkl} was used to test remaining effects. Variances were considered to be homogeneous among sites except for post-season soil K in 2002 and 2003 and for percent present at any date in 2003. Analysis of variance by site was also conducted for dependent variables that exhibited significant site effects or interactions with site. These analyses used the following model:

$$M_{ijk} = \mu + B_i + T_j + TB_{ij} + Y_k + TY_{kj} + e_{ijk},$$

where M is the observation of the k th year Y and the j th calcium treatment T in the i th replication B ; μ is the general mean, e is the variation due to random error or the residual, and TB and TY are the interactions. The treatment by block interaction, TB , was used to test treatment effects and the random error was used to test all other effects. When analyses of variance for individual years and sites using the following model.

$$M_{ij} = \mu + B_i + T_j + e_{ij},$$

where M is the observation of the j th calcium treatment T in the i th replication B ; μ is the general mean, e is the variation due to random error or the residual. The random error was used to test all effects. PROC GLM in SAS (SAS Institute, 1990) was used to conduct ANOVA's. Analyses of variance tables include F-values for sources of variation not associated with error or block effects.

Results and Discussion

Soils

Pre-Treatment (2001).

Soil nutrient means and standard errors for each site prior to soil treatments are shown in Table 6. Soil pH, Ca, Mg, and K concentrations at the Central and West sites, the sites within the native range (West and Central) of American ginseng, were higher than at the non-native (East) site. Soil P concentrations were similar at all sites.

Table 6. Pre-treatment soil pH and Mehlich-3 extractable nutrient concentration means and standard errors at three experimental sites in Maryland in 2001.

Site	pH	Ca	Mg	K	P
West	5.6 (0.8) [‡]	1023 (600)	118 (63)	262 (52)	85 (26)
Central	5.8 (0.4)	1116 (174)	201 (38)	83 (16)	69 (23)
East	4.3 (0.1)	397 (27)	60 (8)	55 (7)	83 (16)

[‡] – Standard errors in parentheses.

Soil pHs at the native sites were in the typical range for Piedmont and Ridge and Valley forests (Brady and Weil, 2002). Soil Ca concentrations at the West site were 50% less than the average ammonium acetate extractable concentrations (2425 ± 269 mg/kg) reported for twenty-seven limestone-based hardwood forest soils in the Ridge and Valley region (Sutherland, 2003). The ammonium acetate extraction is known to extract roughly 1.1 times as much soil Ca as the Mehlich-3 method, which suggests that Ca concentrations are similar for the methods (Sparks, 1996). At the West site, Ca concentrations were low, relative to other limestone-based hardwood forest soils probably because previous clear-cutting and harvesting of trees significantly reduced soil Ca levels (Johnson et al, 1991). The soil pH at the East site was more acid than the other

experimental sites, which is common to Eastern coniferous forests (Barnes, 1998). The average soil Ca concentration at the East site was one third of the Ca concentration at the other sites.

Lower extractable soil Ca, Mg, and K at the East site may have been due to greater acidity, coarser soil texture, and different parent material. The pH range of soils for the three experimental sites was within the pH range of soils associated with native ginseng populations or successful forest ginseng production (Beyfuss, 1998; Davis, 1997; Konsler, 1990; and Persons, 1994).

Post-season (2002 and 2003).

The analyses of variance for post-season soil pH and extractable soil nutrients in 2002 and 2003 appear in Table 7. The associated treatment means are presented for pH and Ca in Table 7 and Mg, P, and Fe in Table 8. The main effect of site was tested for significance using the variation for the blocks within sites, which was highly significant ($p < 0.001$) for soil pH. Thus, the large block within site variation resulted in decreased sensitivity of the test and could result in a high Type II error rate. However, the treatment by year and treatment by site interaction was highly significant ($p < 0.01$) for soil pH indicating that the effect of site on soil pH was dependent on the treatment and year. The main effect of treatment was also highly significant ($p < 0.001$). Lime treatments increased soil pH at all three sites in 2002, but not in 2003 (Table 8). Gypsum did not significantly affect soil pH. In 2002, mean soil pH of L1 and L2 treatments were 0.5 to 1.2 units higher than control, respectively; but in 2003, mean soil pH of L1 and L2 were only 0.1 to 0.7 units higher than control. Typically, soil pH increases are greatest

within the first year after application (given adequate rainfall) and decrease in following years (Brady and Weil, 2002).

Table 7. Analysis of variance for post-season soil pH and Mehlich-3 extractable soil nutrients at three forested experimental sites in Maryland averaged over 2002 and 2003[‡].

Source of variation	pH	Ca	Mg	P	Fe
			<u>F-values</u>		
Site (S)	3.0	1.1	1.5	0.1	9.9*
Treatment (T)	37.9***	16.9***	9.5***	1.2	3.2*
T x S	2.0	1.3	2.5*	1.1	0.8
Year (Y)	1.6	64.2***	25.3***	12.6**	0.3
S x Y	1.7	2.6	0.2	1.2	1.8
T x Y	8.3***	7.2***	2.7	1.2	0.5
T x Y x S	4.0**	4.2**	1.2	1.8	0.8

†, *, **, *** indicates significance at $p < 0.10, 0.05, 0.01,$ and 0.001 respectively

‡ - Combined ANOVA for soil K not included because variances were not homogeneous across sites.

Table 8. Treatment means and LSD's for post-season soil pH and Mehlich-3 extractable soil Ca at three forested experimental sites in Maryland in 2002 and 2003.

Treatment	pH						Ca					
	West		Central		East		West		Central		East	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
	-----mg/kg-----											
C	5.4	5.6	5.7	5.8	4.2	4.5	1097	1156	979	832	362	277
G1	5.4	5.5	5.7	5.6	4.1	4.4	1443	939	1264	825	736	355
G2	5.3	5.5	5.5	5.5	4.3	4.4	1446	957	1330	840	884	306
L1	5.7	5.8	6.2	6.0	5.2	4.9	1273	1246	1372	1005	1071	818
L2	6.3	5.7	6.2	6.2	5.4	5.2	2637	1256	2042	1562	1142	893
LSD (0.05)	0.4	0.5	0.4	0.3	0.5	0.3	617	608	640	307	829	338
Mean ¹	5.6	5.6	5.9	5.8	4.6	4.7	1504	1111	1397	1013	839	530

¹ LSD (0.05) = 1.2 and 1.3 for comparing pH site means for 2002 and 2003, respectively.

LSD (0.05) = 1274 and 1092 for comparing Ca site means for 2002 and 2003, respectively

The significant effects for soil pH were also significant for soil Ca. Additionally, the main effect of the year was significant. The main effect of site was not significant for soil concentrations even though sites differed by almost 600 mg/kg. Like soil pH, this is probably due to decreased sensitivity when testing for site effects with large block within site variation. A large sampling error probably contributed to the type II error rate. Each of the six soil cores that formed the composite sample for each plot was only 2 cm in diameter, which may not have been an adequate sample size to achieve the desired precision for estimating plot means. This sampling variation may have been especially high in 2002, when drought conditions prevented the complete dissolution of surface applied amendments.

In 2002 at the West and Central sites, the L2 treatment significantly increased soil Ca over control by 1500 and 1000 mg/kg, respectively. At those sites, the L1 treatment was not significantly different from control. At the East site, although both lime treatments increased soil Ca by over 700 mg/kg, neither was significantly higher than control. Wide variation (200 to 2000 mg/kg) at the East site in 2002 may have been partly due to incomplete dissolution of lime. Undissolved lime in soil samples may have sporadically increased measured concentrations of soil Ca. In 2003, lime treatments at all sites had lower soil Ca than in 2002, which may be related to the lower pH in limed plots in 2003 compared to 2002. Thus, at the West site in 2003, there were no significant differences between any of the soil treatments and the control for soil pH or soil Ca. At the Central site, the L2 treatment had significantly higher soil Ca than control and was 0.4 pH units higher than control, whereas the L1 treatment had pH and Ca similar to

control. At the East site in 2003, both lime treatments had significantly higher soil Ca than control by 500 mg/kg and had 0.4 to 0.7 units higher soil pH than control.

In 2002 and 2003, no gypsum treatment at any site had significantly higher soil Ca than control. In 2002, gypsum treatments increased soil Ca by about 400 mg/kg at the West site, by about 300 mg/kg at the Central site, and by about 400 mg/kg at the East site. In 2003, soil Ca means for the gypsum treatments were lower than in 2002 and were not significantly different from the control. Since gypsum treatments did not significantly increase soil pH, there was not likely any change in pH-dependent cation exchange capacity (CEC) of these soils. Therefore, any Ca that was added in 2002 was probably leached by 2003.

Nadeau et al. (2003) found that ginseng plant density was significantly lower on forest soils with 360 to 520 mg/kg of Mehlich-3 extractable soil Ca than soils with 720 to 1970 mg/kg soil Ca. This suggested that less than about 600 mg/kg of Mehlich-3-extractable soil Ca may be sub-optimal for American ginseng production. Plots in this study with less than 600 mg/kg of Mehlich-3 extractable soil Ca occurred at the East site. The relationship between Ca levels and plant emergence, absence aboveground, and present aboveground in a later section of Chapter 2.

Soil Mg concentrations were significantly affected by soil treatment and a treatment by site interaction. At the West and Central sites, soil Mg significantly decreased with increasing gypsum rate; whereas at the East site, gypsum treatment means were not significantly different from the control (Table 9). The addition of gypsum to soil has been found to decrease soil Mg concentration when MgSO_4 is formed and leaches into the soil (Shainberg, 1988). Soil Mg was much lower at the East site than at

the other sites at the beginning of the experiments and the cation exchange capacity (CEC) in gypsum-applied plots was also probably low (due to low pH). Thus, soil Mg was held more tightly to soil colloids. Soil Mg was slightly lower in L1 but not the L2 plots compared to control at the West and Central sites. Adding Ca to soils with high Ca concentrations could have displaced Mg from soil colloids which would be subjected to leaching. There was a highly significant difference in soil Mg concentration between years. In general, soil Mg higher in 2002 than in 2003. Excess precipitation in 2003 may have caused some soil Mg to be leached. In gypsum-applied plots, as gypsum continued to react with soil, additional Mg was probably leached with added sulfate ions.

Table 9. Treatment means and LSD's for Mehlich-3 extractable soil nutrients at three forested experimental sites in Maryland in 2002 and 2003.

Site	Treatment	Mg		K		P		Fe	
		2002	2003	2002	2003	2002	2003	2002	2003
		mg/kg							
West	C	114	91	274	203	81	98	165	177
	G1	74	55	231	199	99	80	168	170
	G2	53	50	239	173	89	76	178	180
	L1	102	93	274	215	94	88	175	172
	L2	130	77	279	195	101	86	140	159
	LSD (0.05)	64	51	106	69	24	16	27	39
Central	C	211	172	277	245	51	43	160	158
	G1	145	107	238	203	87	69	160	167
	G2	91	77	202	186	94	100	155	164
	L1	127	151	230	225	102	60	172	152
	L2	179	134	236	197	105	88	140	142
	LSD (0.05)	61	24	63	53	67	52	41	27
East	C	57	38	68	56	89	82	245	231
	G1	45	29	65	52	92	92	250	258
	G2	61	31	62	49	90	76	258	223
	L1	68	61	68	53	85	88	221	221
	L2	62	48	71	55	102	87	236	218
	LSD (0.05)	36	27	13	13	30	32	41	35
Year means		101	81	188	154	91	81	188	186

Because error variances between sites were very heterogeneous for soil K, analysis combined over sites was not conducted. Soil K concentrations differed between years but were not significantly affected by treatments at any site (Table 10).

Table 10. Analyses of variance for Mehlich-3 extractable soil K at three forested experimental sites in Maryland in 2002 and 2003.

Source of variation	West	Central	East
		<u>F-value</u>	
Treatment (T)	0.5	2.2	0.9
Year (Y)	37.4***	15.0**	36.5***
T x Y	0.7	0.9	0.1

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

For soil P concentrations, the only significant effect was due to differences between years. The mean soil P concentration was lower in 2003 than in 2002.

There were significant differences in soil Fe concentration among sites. Fe concentration was significantly higher at the East site than the West and Central sites (Table 9), probably caused by the parent material at the East site having higher Fe content.

Plants

Emergence.

During the first season, plants emerged by April 2002 for all sites site (Figure 1). Plants began to emerge at all sites by the second and third weeks of April. The number of plants emerging increased rapidly until about the second week of May. Average total percent emerged plants was 44% of seeds planted in 2001 at the East site, 54% at the Central site, and 55% at the West site. These average total percent emerged plants of seeds planted were slightly lower than the average percent emerged (66%) reported by Anderson (1993) and less than the 72% average reported by Nadeau et al. (2003).

Dates of plant emergence were similar to those reported for twenty wooded sites in southern Wisconsin, where plants emerged between April and May (Carpenter and Cottam, 1982) and those observed at thirty-three sites in Illinois where plants emerged between April and May (Anderson, 1993). All first-season plants had one-pronged trifoliolate shoot morphology, as reported by other authors (Anderson, 1993; Lewis, 1983; Proctor and Bailey, 1987; Proctor et al, 2003).

In 2003, the second growing season, by the first week of April, 14%, 15%, and 23% of the total number of plants that had emerged in 2002 had reemerged at the East, West, and Central sites. There was a sharp increase in reemergence at all sites between the first and fourth weeks of April, after which reemergence reached a plateau (Figure 2). Onset of plant emergence was slightly later in 2002 than in 2003, which may have been due to less precipitation throughout the early season in 2002 or could be attributable to different physiology in second-year of growth. The West site had the highest percent reemerged plants (60%), followed by the Central site (47%), and the East site (30%).

The site x year interaction was the only significant source of variation for percent total emerged plants (Table 11). In 2002, the East site had significantly fewer percent emerged plants than the West or Central sites (Figure 1). There was no significant difference in total percent emerged between the West and Central sites. In 2003, the East and Central sites had fewer percent emerged plants than in 2002 (Figure 1 and Figure 2). Conversely, the West site had more percent emerged plants in 2003 than in 2002. In 2003, the East site again had the fewest percent emerged plants. However, in 2003 the Central site had significantly fewer plants emerged than the West site (Figure 1 and Figure 2).

Table 11. Analysis of variance for total percent emerged American ginseng plants combined over three forested experimental sites in Maryland and combined over 2002 and 2003.

Source of variation	F value
Site (S)	5.1
Treatment (T)	2.1
T x S	1.4
Year (Y)	2.8
S x Y	5.7**
T x Y	1.0
T x Y x S	1.0

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 , respectively

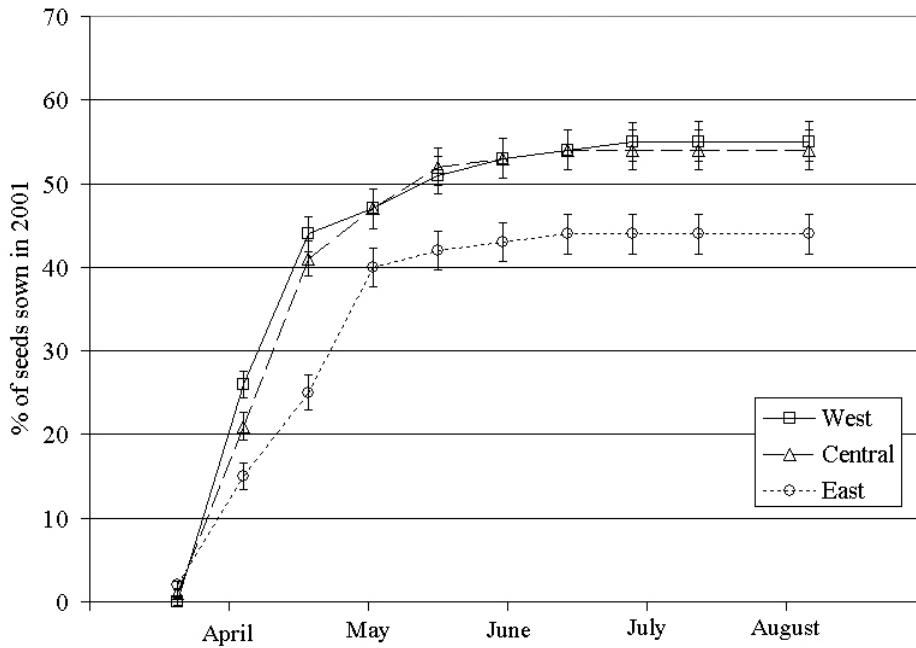


Figure 1. Percent emerged first-season American ginseng at three forested experimental sites in Maryland in 2002.

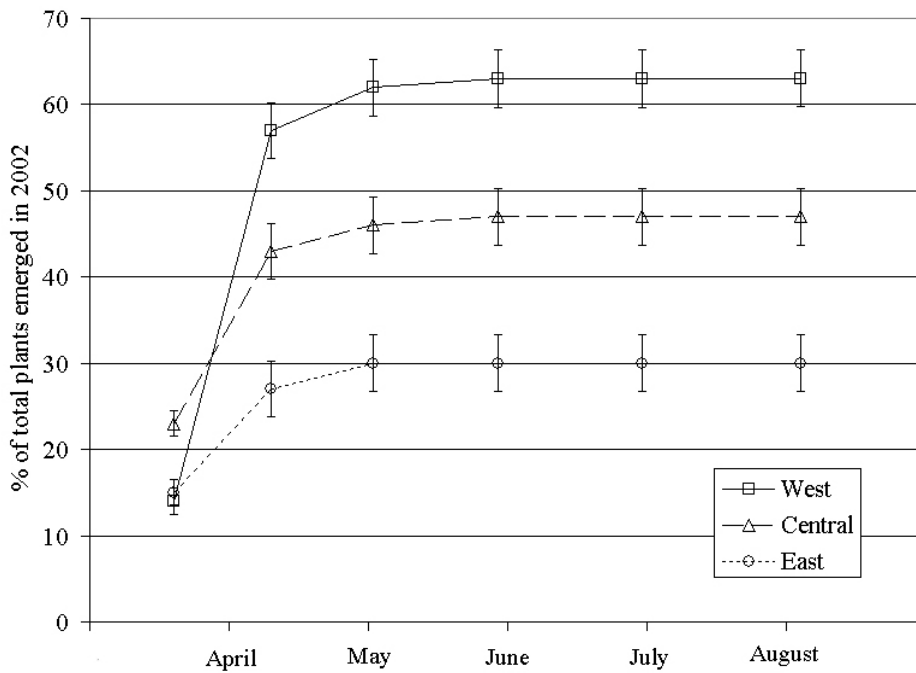


Figure 2. Percent emerged second-season American ginseng at three forested experimental sites in Maryland in 2003.

Analyses of variance on percent emerged were conducted by site to examine treatment effects by site more accurately. Although the treatment effects were not significant for the analysis combined over sites (Table 11), analyses of variance conducted by site found treatment effects to be significantly different for the East, but not the Central and West sites (Table 12).

Table 12. Analyses of variance for total percent emerged American ginseng plants at three forested experimental sites in Maryland in 2002 and 2003.

Source of variation	West	Central	East
		<u>F-value</u>	
Treatment (T)	0.2	0.3	14.2**
Year (Y)	2.1	3.4	28.9***
T x Y	1.1	0.1	4.9*

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

At the East site, total percent emerged was affected by treatment, year, and the interaction between treatment and year. The L2 treatment had higher total percent emerged plants than the control in 2002 and the L1 treatment had higher total percent emerged plants than the control in 2003 (Table 13). Lime treatments increased soil pH in 2002 and increased soil pH and Ca in 2003 (Table 8). Gypsum treatments, which added less Ca than lime treatments, did not affect soil pH or Ca. Nadeau et al. (2003) found that increasing soil pH on very acid forest soil increased survival and density of American ginseng in forest plots. They attributed increased survival in limed plots to increased nutrient availability and hypothesized that Ca provided for increased winter hardiness (Nadeau et al., 2003). In 2002, when total percent emerged was not different between lime and gypsum treatments at the East site, soil pH was higher with lime treatments than gypsum treatments and soil Ca was similar for both lime and gypsum treatments. In 2003, when total percent emerged was significantly higher for lime treatments than

gypsum treatments, soil pH and soil Ca were significantly higher, which suggests that one or both of these soil factors play a role in establishment of American ginseng at that site. Soil Ca, added with lime remained in the soil during the second season and may have contributed to the overall health of plants by providing greater structural rigidity and resistance to desiccation.

Table 13. Treatment means for total percent emerged American ginseng at three forested experimental sites in Maryland in 2002 and 2003.

Treatment	<u>Total Percent Emerged 2002</u>			<u>Total Percent Emerged 2003</u>		
	<u>West</u>	<u>Central</u>	<u>East</u>	<u>West</u>	<u>Central</u>	<u>East</u>
	% of total seeds sown in 2001			% of total plants emerged in 2002		
C	61	57	36	52	50	26
G1	62	53	44	61	46	15
G2	46	53	41	62	41	19
L1	57	53	40	66	45	53
L2	50	55	48	67	50	35
LSD (0.05)	24	16	9	30	25	15

Percent absent aboveground.

Without using destructive sampling techniques, it was not possible to determine if American ginseng plants that were no longer present aboveground had died or had senesced by a sampling date in 2002 or 2003. Emerged plants began to be absent aboveground at all three sites in early May 2002, less than a month after emergence, and percent absent plants increased until the end of the season (Figure 3). By June 7, almost 25% of all ginseng plants that had emerged by that date were absent. By August 8, almost 50% of plants were absent aboveground. By September 5, almost 100% were absent. A severe drought during the 2002 growing season led to observed stress in ginseng plants throughout the season and contributing to mortality and/or early senescence (see Table 3 for precipitation). During the summer, plants were often wilted. Less than 1% of ginseng plants died from diagnosed disease throughout the season.

The percent of plants absent aboveground was similar to American ginseng populations observed at twenty wooded sites in southern Wisconsin (Carpenter and Cottam, 1982). Carpenter and Cottam (1982) found that by July 22, approximately 35% of the first leaf stage ginseng plants (three to five-leaflets, corresponding to one- to four-year old plants) were absent aboveground and by August 11, approximately 55% were absent aboveground. During the second season of this experiment, plants began to be absent aboveground at all three sites in early May 2003, less than a month after reemergence, and increased steadily until the end of the season (Figure 4). Almost one quarter of all plants were absent aboveground by June 15 and almost half were absent by August 17.

There were significant differences for percent absent aboveground between years and sites by year interactions for all sampling dates except August when only the site by year interaction was significant (Table 14). During 2002, the East site had the highest percent absent aboveground plants, followed by the West site, followed by the Central site (Figure 3). In 2003, the West site had the highest percent absent aboveground plants until July when sites were similar (Figure 4). The treatment effects were not significant for the analysis combined over sites, but there was a significant treatment x site interaction in May and July. Analysis of variance by site revealed that the only significant treatment effect was at the East site in July (Table 15), when in 2002, the G2 treatment had higher percent absent aboveground plants than control and the L1 had lower percent absent aboveground plants than control, and in 2003, when the G1 and L2 treatments had significantly lower percent absent aboveground plants (Table 16).

Table 14. Analyses of variance by month for percent absent aboveground American ginseng plants combined over three forested experimental sites in Maryland, combined over 2002 and 2003.

Source of variation	May 16	June 12	July 12	August 12
			<u>F-value</u>	
Site (S)	1.5	1.2	1.8	5.7
Treatment (T)	1.2	1.2	1.5	1.6
T x S	8.3**	1.7	2.5*	1.2
Year (Y)	27.0***	29.6***	31.7***	3.2
S x Y	8.3**	5.4*	5.6**	12.3***
T x Y	1.2	1.2	1.1	0.2
T x Y x S	0.4	0.3	0.1	0.2

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

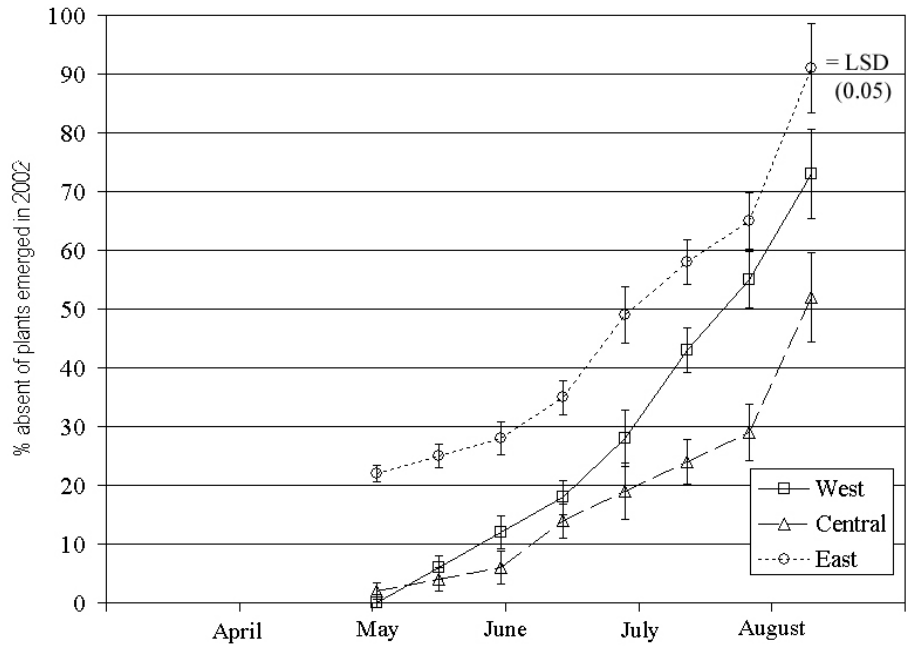


Figure 3. Percent absent aboveground first-season American ginseng at three forested experimental sites in Maryland in 2002.

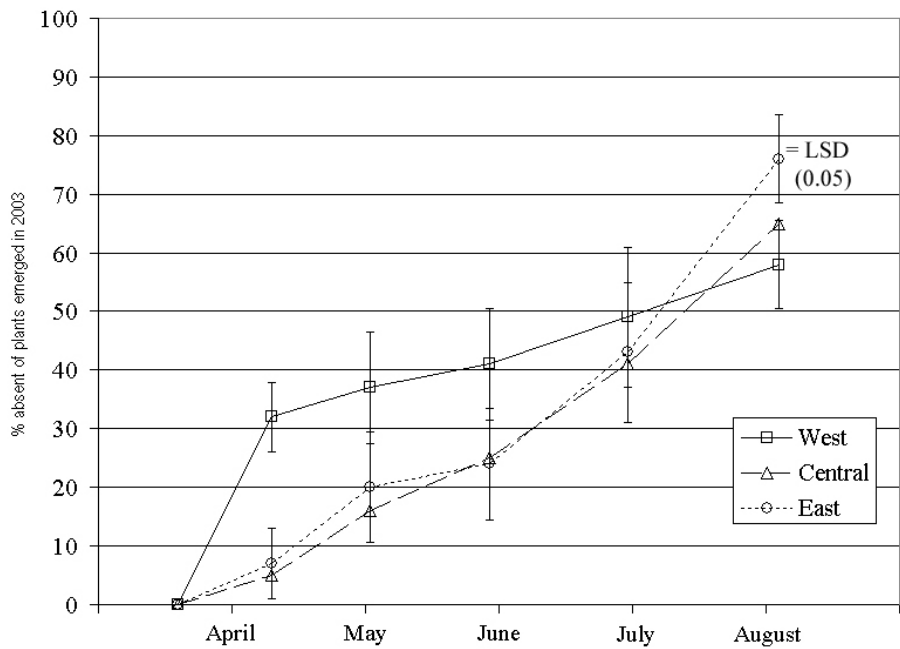


Figure 4. Percent absent aboveground second-season American ginseng plants at three forested experimental sites in Maryland in 2003.

Table 15. Analyses of variance by month for percent absent aboveground American ginseng plants at three forested sites in Maryland combined over 2002 and 2003.

Source of variation	May 15	June 13	July 12	August 8
	<u>F-value</u>			
	<u>West</u>			
Treatment (T)	1.2	0.2	0.0	0.7
Year (Y)	157.5***	215.6***	60.9***	70.6***
T x Y	8.0*	1.8	0.8	1.6
	<u>Central</u>			
Treatment (T)	1.9	1.3	0.8	0.8
Year (Y)	22.6**	32.3***	31.0***	47.8***
T x Y	0.7	1.0	0.6	1.2
	<u>East</u>			
Treatment (T)	2.0	2.0	7.0**	1.9
Year (Y)	2.5	2.5	2.2	12.2*
T x Y	0.7	0.6	1.0	0.1

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 16. Treatment means for percent absent aboveground American ginseng at three experimental sites in Maryland in July of 2002 and 2003.

Treatment	Percent Absent <u>Aboveground by 7/02</u>			Percent Absent <u>Aboveground by 7/03</u>		
	West	Central	East	West	Central	East
	-----%-----					
C	27	16	50	50	40	50
G1	28	23	46	47	53	32
G2	29	17	71	48	35	46
L1	25	18	30	54	45	42
L2	31	22	47	46	35	41
LSD (0.05)	16	18	9	24	26	9

Percent plants present aboveground.

The analyses of variance for plants present aboveground of total seeds sown in 2001 combined over sites are shown in Table 17. There were no significant effects on percent present aboveground except for site on August 2002. In 2002, the Central site had the highest percent plants present aboveground from mid-July through September whereas the West site had the highest percent plants present aboveground during this time period in 2003. In 2002, the percent plants present was as high as 48% at the West site, 50% at the Central site, and 32% at the East site (Figure 5). As plants were found to be absent aboveground, the percent plants present decreased to 12%, 5% and 2%, for the Central, West, and East sites, respectively. In 2003, percent present aboveground was at 32% in April at the West site and decreased to 18% by August (Figure 6). At the Central site, percent present aboveground was 23% in April and decreased to 10% by August. At the East site, percent present aboveground was 15% in May and decreased to 7% by August. Differences in percent plants present aboveground in each year could have been attributable to the weather differences between years, different physiology between seedlings and the second year of growth, or a combination of the two. Drought in 2002 may have contributed to mortality and therefore to less plants surviving and reemerging in 2003. It is also possible that American ginseng plants have a more stable growth rate during their second year of growth, which could explain the more constant curve in 2003.

Although the treatment effects were not significant for the analysis of variance of percent present aboveground combined over sites, analyses of variance conducted by site found significant treatment effects for the East, but not for the Central or West sites in 2002 and 2003 (

Table 18). At the East site in 2002 and 2003, lime treatment means for percent plants present aboveground were higher than the control mean, whereas gypsum

treatment means were not significantly different from the control means (Table 19). The treatment effects for percent plants present aboveground were similar to the treatment effects for emergence. Since percent plants present aboveground is dependent on emergence, it is expected that treatments would have similar effects on both dependent variables. Higher percent present plants in 2002 for limed plots suggested that soil pH and/or Ca affected percent present plants at the East site similar to the effect on emergence. It is possible that increasing soil pH increased nutrient availability and increased plant health which could have resulted in a greater percentage of plants remaining aboveground for a longer time in both years.

An examination of percent present aboveground over time (Figures 5 and 6) helped to portray the establishment of American ginseng populations during the first two growing seasons. The percent plants present aboveground was highest during the first growing season and was much lower during the second growing season. The lower percent present during the second season may have been due to drought-induced mortality of first-season plants or dormancy of second year plants. Although reports of seedling mortality have conflicted, it is possible that high seedling mortality could be indicative of a Type III growth curve with regard to population dynamics of the American ginseng (Molles, 1999). Type III growth curves are indicative of populations with high mortality rates for the youngest individuals and low mortality rates for older individuals.

Table 17. Analyses of variance for percent American ginseng plants present aboveground of total seed sown combined over three forested experimental sites in Maryland in 2002.

Source of variation	April 18	May 15	June 13	July 12	August 8
	<u>F-value</u>				
Site (S)	1.3	1.7	1.7	2.6	6.9*
Treatment (T)	0.7	1.8	1.1	1.1	1.0
T x S	1.4	1.3	0.8	0.8	0.9

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 18. Significance of treatment effects on percent American ginseng plants present aboveground at three forested experimental sites in Maryland in 2002 and 2003.

Year	Site	April 4	April 18	May 15	June 13	July 12	August 8
		<u>F-values</u>					
2002	West	n/a	0.6	1.3	0.4	0.4	0.4
2002	Central	0.8	0.5	0.1	0.1	0.3	0.3
2002	East	2.6	6.4*	5.1*	6.8*	5.1*	3.7
2003	West	1.3	0.2	0.2	0.2	0.2	0.4
2003	Central	0.1	0.4	0.5	0.4	0.5	1.0
2003	East	8.3**	9.8**	14.2**	15.7**	14.7**	1.6

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 19. Treatment means and LSDs for percent American ginseng plants present aboveground at three forested experimental sites in Maryland in May of 2002 and 2003.

Treatment	<u>May 15, 2002</u>			<u>May 16, 2003</u>		
	West	Central	East	West	Central	East
	-----%-----					
C	53	48	27	20	24	11
G1	55	45	32	26	18	12
G2	37	46	21	25	20	13
L1	51	47	41	22	19	22
L2	39	47	37	20	25	18
LSD (0.05)	23	16	11	19	15	6

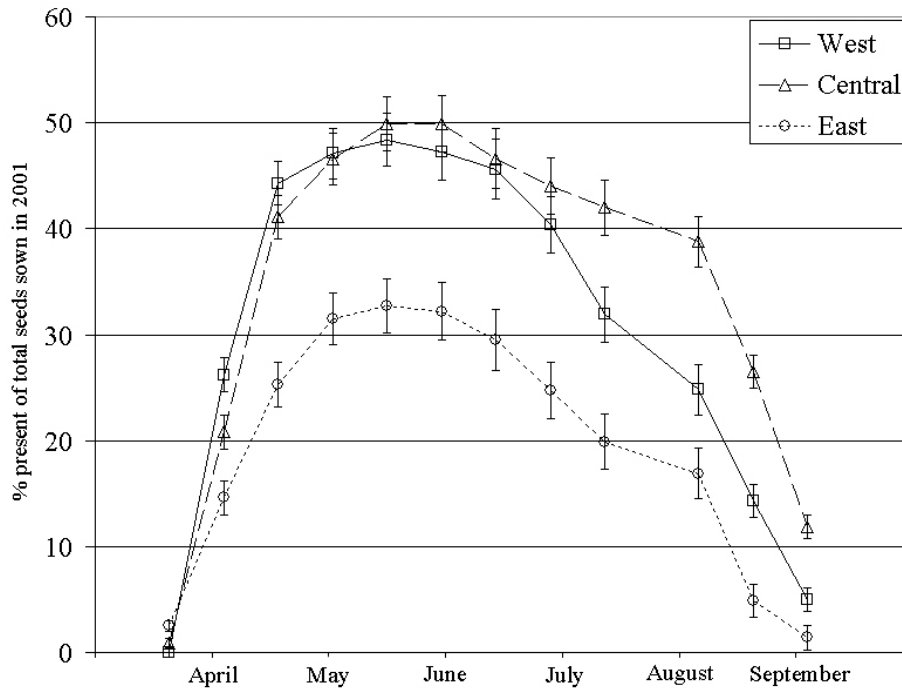


Figure 5. Percent American ginseng plants present aboveground of total seeds sown at three forested experimental sites in Maryland in 2002.

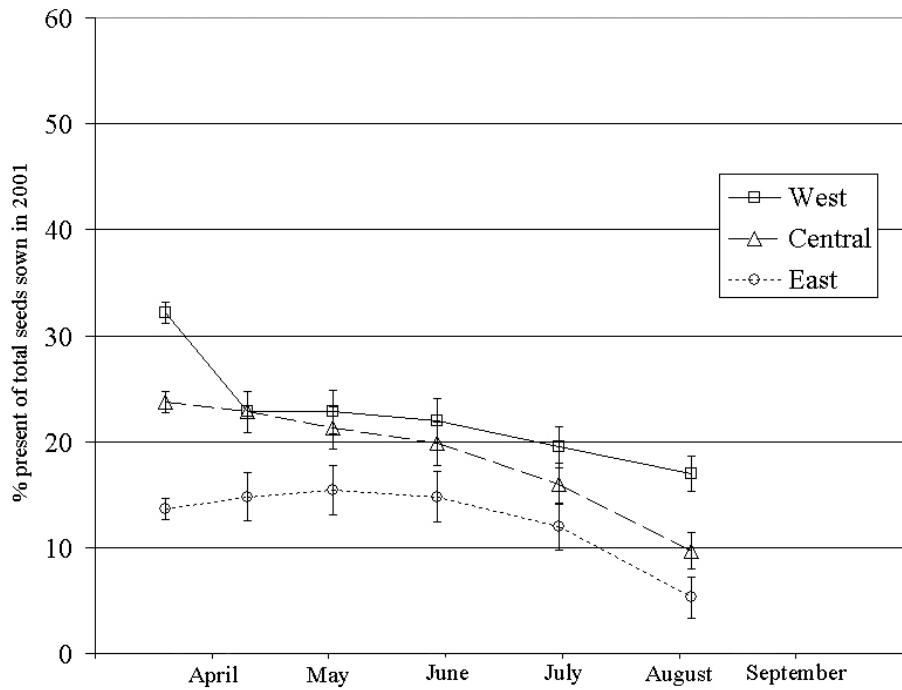


Figure 6. Percent American ginseng plants present aboveground of total seeds sown at three forested experimental sites in Maryland in 2003.

Second-season shoot morphology.

During the second season, shoot morphology differed among individual plants and were classified into three morphological classes: trifoliolate (three-leaflets), quinquefoliate (five-leaflets), and two-prong. The East site had the largest three-leaflet class, followed by the West site, and the Central site (Table 20). The Central site had the largest five-leaflet class followed by the West site, and the East site. The West site had the largest two-prong class followed by the East site and Central site.

At the West site, the three-leaflet morphological class was the largest and averaged 42% of the population and five-leaflet and two-prong each averaged 29% of the population. At the Central site, the five-leaflet class was the largest and averaged 40% of the population, the three-leaflet class averaged 36%, and the two-prong class averaged 24%. The largest morphological class at the East site was the three-leaflet class, which averaged 53% of the population. The two-prong class averaged 26% of the population and the five-leaflet class averaged 21% of the population.

Table 20. Treatment and site means for percent morphological class size of second-season American ginseng grown at three forested experimental sites in Maryland in 2003.

Treatment	<u>West</u>			<u>Central</u>			<u>East</u>		
	3- leaflet	5- leaflet	2- prong	3- leaflet	5- leaflet	2- prong	3- leaflet	5- leaflet	2- prong
	-----%-----								
C	40	26	34	28	41	30	68	12	19
G1	49	25	26	33	43	24	72	22	6
G2	40	29	31	38	36	26	58	13	28
L1	36	31	32	41	37	22	46	22	32
L2	45	33	22	42	41	17	42	29	28
LSD (0.05)	13	11	17	21	15	20	30	14	27
Mean ¹	42	29	29	36	40	24	53	21	26

¹ LSD (0.05) = 29, 10, and 27 for comparing site means for 3-leaflet, 5-leaflet, and 2-prong classes, respectively.

Soil-Plant Relationships

Soil Fertility Correlations.

Significant correlations between soil fertility factors and percent total emerged, percent plants absent aboveground in August, and percent present aboveground in August appear in Table 21. Across sites, there was a significant positive correlation between total percent emerged and soil K and a significant negative correlation with soil Fe. Across sites, there were significant negative correlations between percent absent aboveground in August and soil pH, Ca, Mg, and K and conversely a significant negative correlation between percent plants absent aboveground and soil Fe. Across sites, there were significant positive correlations between percent present in August and soil Ca, Mg, and K and conversely there was a significant negative correlation between percent present in August and soil Fe. The significance of any correlation is dependent on sample size and ranges of the dependent variables. Thus, correlations may not be apparent within sites if the range of one or both of the dependent variables is narrow.

Also, the sample size (n=90) for correlations across sites is three times the sample size at each site (n=30). Scatter plots are presented to display relationships between the variables that are not evident based on linear correlation coefficients. The scatter plots displayed in Figures 7, 8, and 9 show plot means of total percent emerged, percent absent and percent present aboveground in August and soil fertility values. Because of the confounding of various soil fertility values and site, the correlation of soil fertility values and phenology measures are confounded. Scatterplots, as well as correlations by site (Table 21) may clarify relationships between soil fertility and phenology measures.

Figure 7 displays the plot means for percent total emerged plants and soil fertility levels. Tendencies observed in the scatter plots (Figure 7): A. soil pH less than 4.8 was common at the East site and those plots had low total emergence. B. Soil Ca concentrations less than 1000 mg/kg Ca were associated with percent emergence greater than 50% at the Central site but less than 50% at the East site. C. Soil Mg displayed a relationship similar to soil Ca for percent emergence. D. The K concentrations at the East site were tightly clustered around 50 mg/kg for the East site and the percent emergence was below 70% for all plots. The K concentrations were higher and more variable at the West and Central sites and the plots with the highest percent emerged had K concentrations near 150 mg/kg K. E. Soil P was particularly variable at the West site, but was the soil nutrient that consistently had no significant effect on the phenology of American ginseng. F. Soil Fe concentrations were highly negatively correlated to soil pH (Table 21) and the scatter plots of soil Fe showed the reverse patterns of the soil pH scatter plots.

Table 21. Correlation coefficients of soil fertility values and percent absent, percent present, and total percent emerged by August at three forested experimental sites in Maryland averaged over 2002 and 2003.

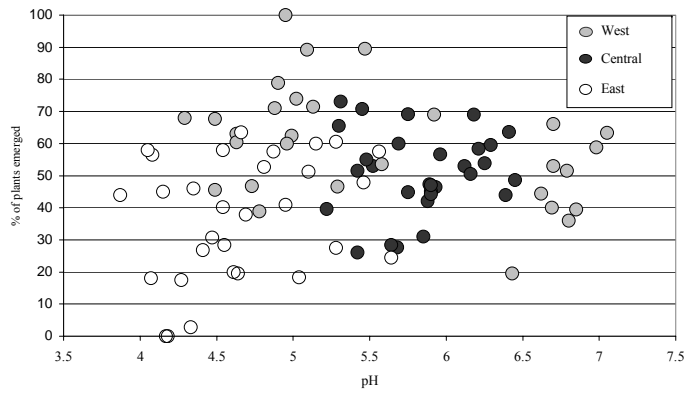
	% Total emerged	% Absent in August	% Present in August
Across sites			
pH	ns	-0.45***	ns
Ca	ns	-0.24*	0.21*
Mg	ns	-0.42***	0.28**
K	0.23*	-0.46***	0.29**
P	ns	ns	ns
Fe	-0.35***	0.54***	-0.29**
West			
pH	-0.40*	ns	ns
Ca	-0.47*	ns	-0.44*
Mg	-0.40*	ns	ns
K	-0.46*	ns	-0.46*
P	ns	ns	ns
Fe	ns	ns	ns
Central			
pH	ns	ns	ns
Ca	ns	-0.41*	-0.44**
Mg	0.42*	-0.42*	ns
K	ns	ns	-0.46*
P	ns	ns	ns
Fe	ns	ns	ns
East			
pH	ns	ns	ns
Ca	ns	ns	ns
Mg	ns	ns	ns
K	ns	0.47**	-0.45*
P	0.48**	ns	ns
Fe	-0.44*	0.50**	0.50**

Figure 8 displays the plots means for percent absent aboveground in August and soil fertility levels. Trends in Figure 8: A. Soil pH less than 4.8 was common at the East site and plots had high percent absent. B. Soil Ca concentrations ranged widely at the West site and concentrations less than 1000 mg/kg Ca were associated with typical percent absent greater than 70%. C. Soil Mg concentrations at the East site were

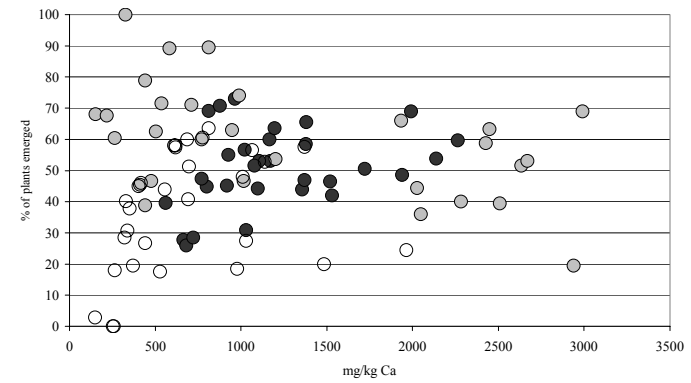
generally clustered around 75 mg/kg and were associated with higher percent absent. D. The K concentrations at the East site were tightly clustered around 50 mg/kg for the East site and total percent emerged was above 70% for most plots. F. Soil Fe concentrations were the inverse of soil pH plots.

Figure 9 displays the plots means for percent absent aboveground in August and soil fertility levels. Trends are (Figure 9): B. The East site had Ca values below 1000 mg/kg and was associated with lower percent present aboveground. C. Mg at the East site was clustered below 100 mg/kg and was associated with lower percent present aboveground than higher values. D. A tight cluster of low K values at the East site was associated with low percent present aboveground. F. The East site had Fe values generally above 200 mg/kg, which were associated with lower percent present aboveground than plots with less Fe.

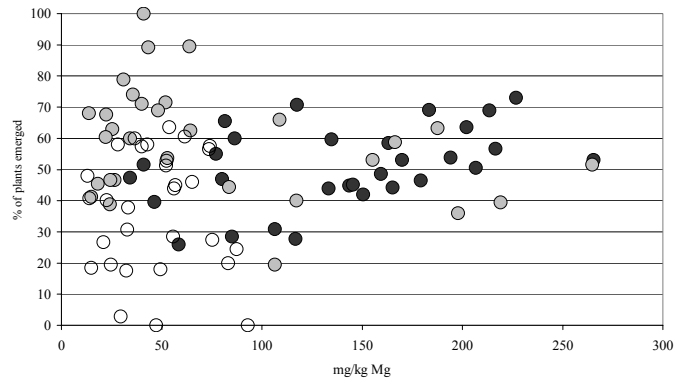
Figure 7. Scatter plots of soil fertility values by percent total emerged ginseng plants at three experimental sites in Maryland in 2002 and 2003.



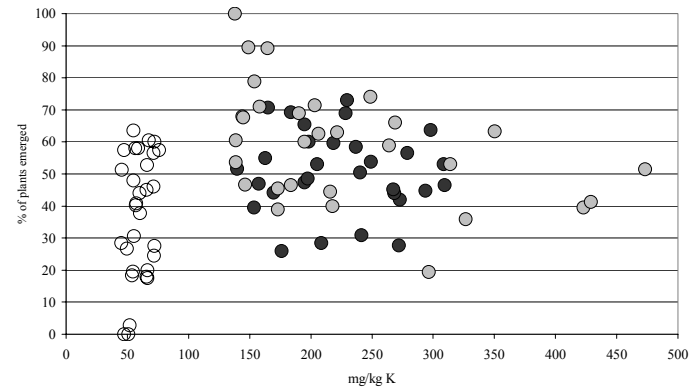
A.



B.

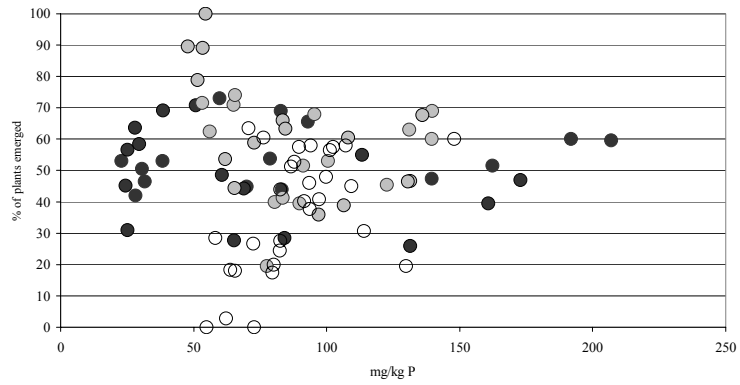


C.

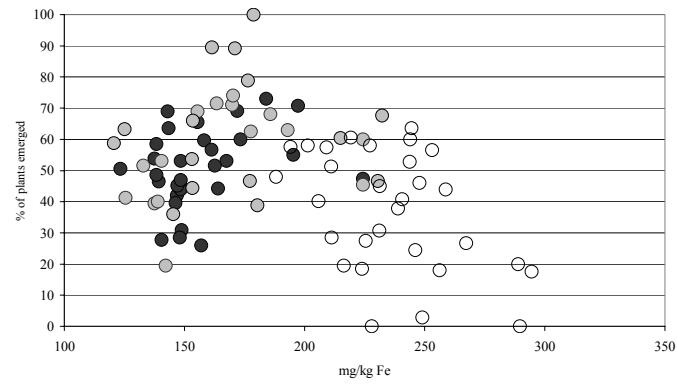


D.

Figure 7 (continued). Scatter plots of soil fertility values by percent total emerged ginseng plants at three experimental sites in Maryland in 2002 and 2003.

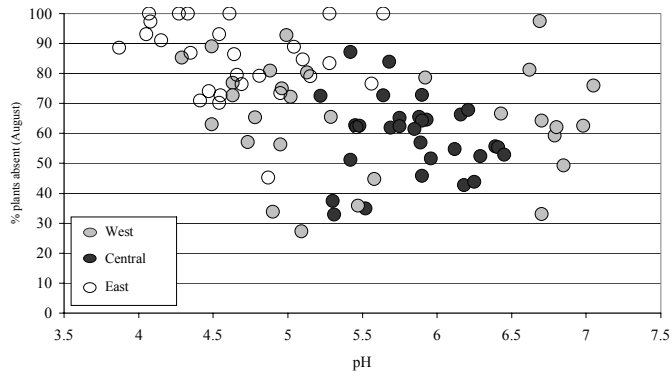


E.

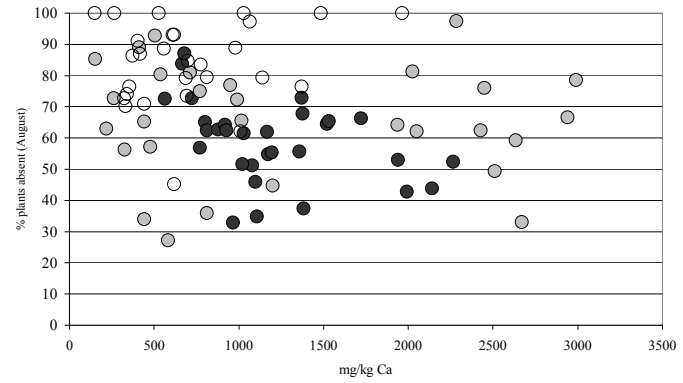


F.

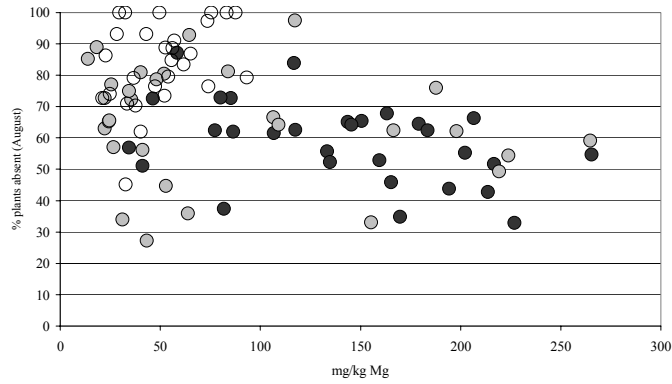
Figure 8. Scatter plots of soil fertility values by percent ginseng plants absent aboveground by August at three forested experimental sites in Maryland in 2002 and 2003.



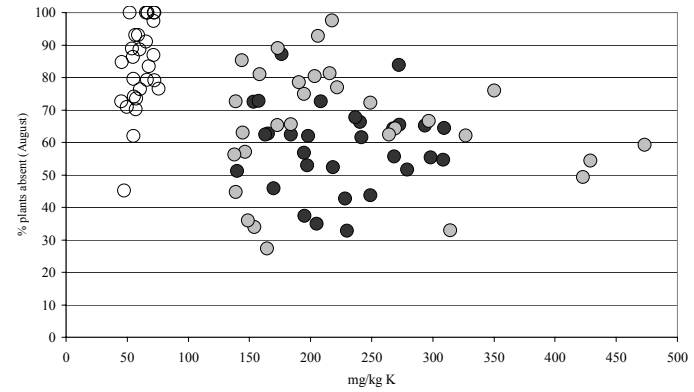
A.



B.

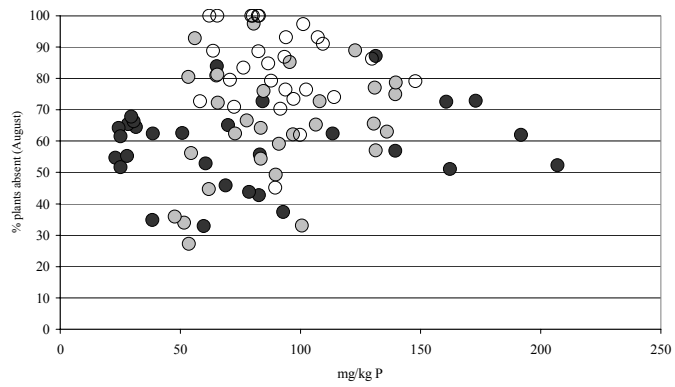


C.

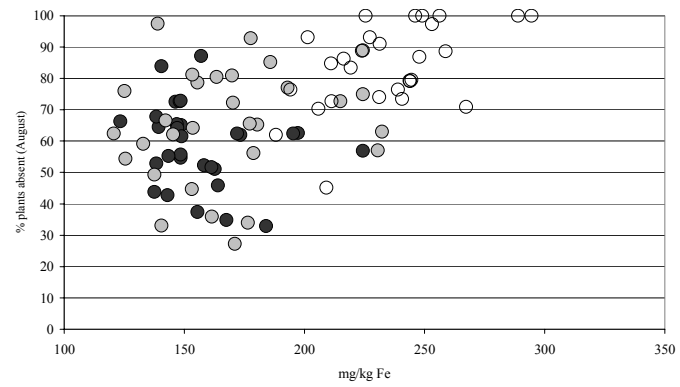


D.

Figure 8 (continued). Scatter plots of soil fertility values by percent American ginseng plants absent aboveground by August at three forested experimental sites in Maryland in 2002 and 2003.

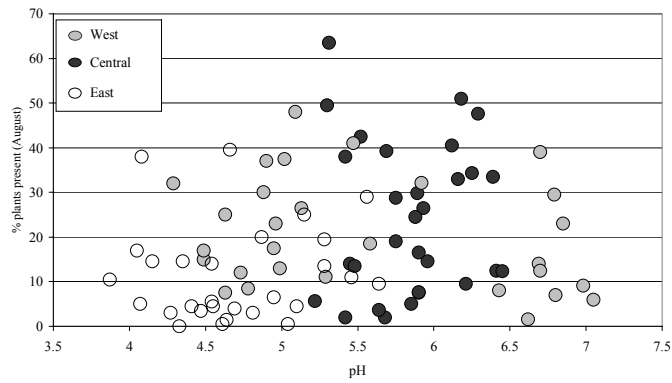


E.

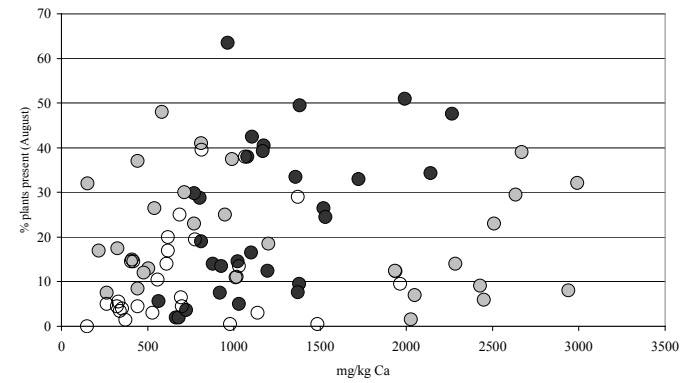


F.

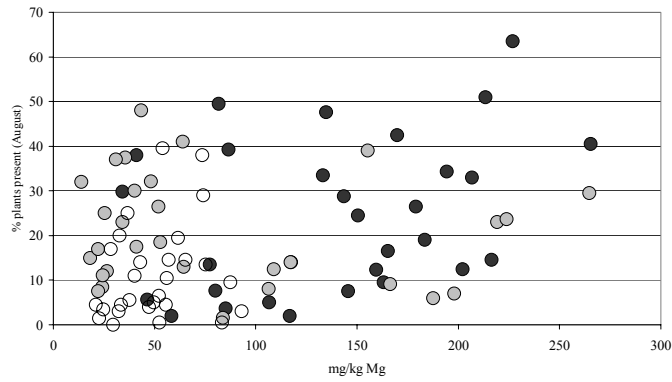
Figure 9. Scatter plots of soil fertility values by percent ginseng plants present aboveground in August at three forested sites in Maryland in 2002 and 2003.



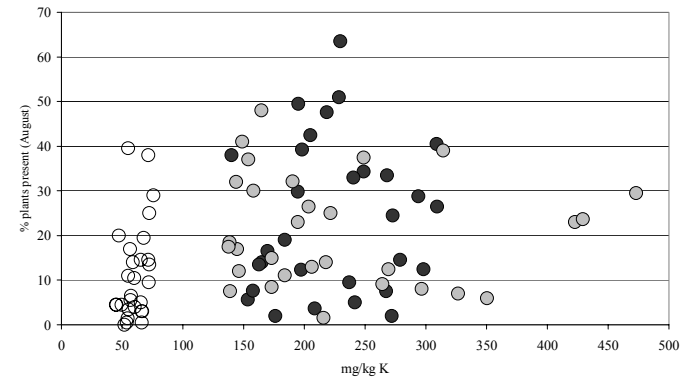
A.



B.

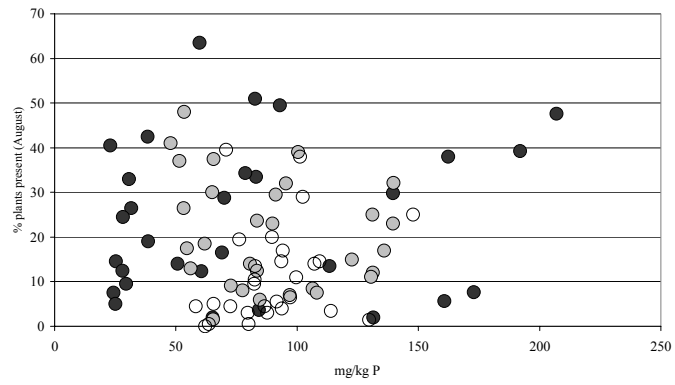


C.

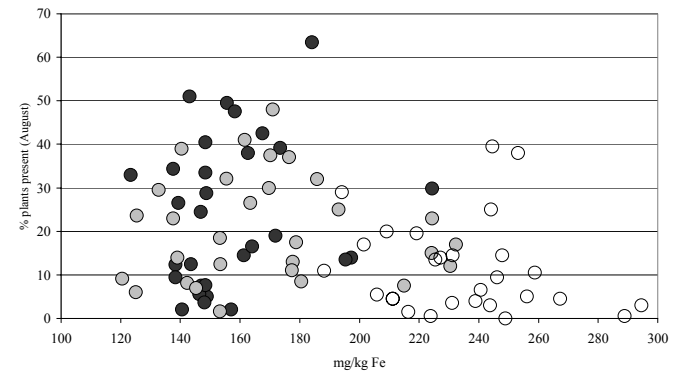


D.

Figure 9 (continued). Scatter plots of soil fertility values by percent American ginseng plants present aboveground in August at three forested sites in Maryland in 2002 and 2003.



E.



F.

Across sites, there were significant positive correlations between soil pH and Mehlich-3 extractable Ca, Mg, and K and significant negative correlations between soil Fe and soil pH, Ca, Mg, and K (Table 22). These correlations were not surprising given that Ca, Mg, and K are increasingly available with increasing pH. Correlations at the West site were nearly identical to correlations across sites. This may be the case because the West site had the widest range of soil fertility values (Figure 7, Figure 8, and Figure 9). Correlations at the Central site did not match correlations across sites as closely as the West site did. This may be due to a narrower range of soil fertility values at that site. Correlations at the East site matched across site correlations the least and may have been due to the narrowest range of most soil fertility values.

Table 22. Correlation coefficients of soil fertility values at three forested experimental sites in Maryland averaged over 2002 and 2003.

	Ca	Mg	K	P	Fe
Across sites					
pH	0.83***	0.75***	0.80***	ns	-0.81***
Ca		0.63***	0.64***	ns	-0.59***
Mg			0.76***	-0.39***	-0.62***
K				ns	-0.80***
P					ns
West					
pH	0.92***	0.88***	0.77***	ns	-0.85***
Ca		0.78***	0.75***	ns	-0.78***
Mg			0.93***	ns	-0.77***
K				ns	-0.67***
P					0.49***
Central					
pH	0.67***	0.51**	0.51**	ns	-0.44*
Ca		0.40*	ns	ns	-0.42*
Mg			0.64***	-0.68***	ns
K				-0.60***	-0.45*
P					ns
East					
pH	0.64***	ns	ns	ns	-0.45*
Ca		0.76***	0.52**	ns	ns
Mg			0.65***	ns	ns
K				0.36*	ns

Calcium Seed Experiment II / Mulch Seed Experiment I (CS II/MS I): 2002 – 2003

Materials and Methods

Site Descriptions.

In the fall of 2002, the CS II/MS I supplementing the CS I experiments were established in different plots at the same three sites. These new experiments were conducted to provide additional data on the establishment of American ginseng and also to determine whether wheat straw mulch would improve American ginseng growth by reducing plant competition.

The CS II/MS I experiment at the West site was conducted within a forest with different species composition and soil parent material than the CS I experiment at the West site. The forest was dominated by 30 and 40 years old mixed oak (*Quercus* sp.) and tuliptree (*Liriodendron tulipifera* L.). The understory was primarily tree of heaven (*Ailanthus altissima* (P.Mill.) Swingle). The groundcover was garlic mustard (*Alliaria petiolata* (Bieb.) Cavara & Grande), and multiflora rose (*Rosa multiflora* Thunb. ex. Murr.). The soil was an extremely rocky silt loam, a mesic Typic Hapludalf of the Hagerstown series.

The CS II/MS I experiment at the Central site was conducted within the same forested area as the CS I experiment. The CS II/MS I experiment at the East site was conducted within a forest with 30 to 40 year old loblolly pine (*Pinus taeda* L.) and white oak (*Quercus alba* L.). The understory was American beech (*Fagus grandifolia* Ehrh.). The groundcover was poison ivy (*Toxicodendron radicans* (L.) Kuntze). The soil was a sandy loam, a mesic Aquic Hapludult of the Woodstown series.

Experimental Design and Layout.

In December 2002, the CS II/MS I treatments were randomized in three complete split-blocks, 5.1 m by 4.3 m, at each location. Each plot was seeded with four rows of pre-stratified American ginseng seed. Each row contained fourteen hills spaced twenty-five cm apart with five seeds per hill planted at one to two cm depths. Leaves and debris were removed from the soil surface before planting seed. Half of the block (twenty rows of seven hills) was randomly assigned to either no mulch or wheat straw mulch, which was applied by hand to create a 7 cm layer of mulch. Thirty cm sections of ten cm diameter PVC pipe were placed over wooden plant markers to keep mulch five cm away from the center of each hill. After mulch was applied, leaves were returned to the no-mulch part of the split-block. The same Ca treatments were applied in the CS II/M I experiments as the CS I experiments (Table 4).

Soil sampling, chemical analyses, and plant measurements were identical to procedures used in CS I experiments.

Statistical Analysis.

The analyses of variance were similar for the CS II/M I and the CS I experiments except that that mulch was included as a factor. The full model was:

$$M_{ijkl} = \mu + S_i + B(S)_j + T_k + TS_{ik} + TB(S)_{lkj} + L_l + SL_{il} + BL_{jl} + TL_{kl} + TLS_{ikl} + e_{ijkl},$$

where M is the observation of the l th mulch L and the k th calcium treatment T in the j th replication B within site S ; μ is the general mean, e is the variation due to random error or the residual, and TS , $TB(S)$, SL , BL , TL , and TLS are the interactions. The block within site, $B(S)$, term was used to test site effects, the $TB(S)$ term was used to test treatment and treatment by site interaction effects, and the random error, e_{ijkl} was used to test remaining

effects. In the analysis, site, treatment, and mulch were considered fixed and blocks were considered random. Variances were not homogeneous among sites for post-season soil K in 2003. For that dependent variable and when analyses of variance by site combined over years were of interest, analysis of variance was done by means of the following model:

$$M_{ijk} = \mu + B_i + T_j + TB_{ij} + L_k + TL_{kj} + e_{ijk},$$

where M is the observation of the k th mulch L and the j th calcium treatment T in the i th replication B ; μ is the general mean, e is the variation due to random error or the residual, and TB and TL are the interactions. The treatment by block interaction, TB , was used to test treatment effects and the random error was used to test all other effects. When analyses of variance by mulch were of interest, analysis of variance was done by means of the following model:

$$M_{ij} = \mu + B_i + T_j + e_{ij},$$

where M is the observation of the j th calcium treatment T in the i th replication B ; μ is the general mean, e is the variation due to random error or the residual. The random error was used to test all effects. PROC GLM in SAS (SAS Institute, 1990) was used to conduct ANOVA's.

Results and Discussion

Soils

Pre-treatment (2002).

Soil pH and Mehlich-3 extractable nutrient means and standard errors are shown in Table 23. Soil pH ranged from neutral at the West site (6.5), to moderately acid at the

Central site (5.6), to strongly acid at the East site (4.1). The West field site for this experiment (CS II) had a greater percentage of limestone outcrops and different dominant tree species (*Quercus alba*, *Acer rubrum*) than the CS I experimental site and was more typical of nearby areas containing wild ginseng. Thus, the soil pH and Ca means were higher at the West site for the CS II plots than in the CS I plots. At the Central site, the field plots for the CS II experiment were adjacent to the field plots for the CS I experiment and their soil pH, Ca, Mg, and P means were similar to those reported for CS I experiments. At all sites, soil K was higher in 2002 than reported for 2001, which could be the result of a drought in 2002 that contributed to less K uptake during the growing season and more available K by fall sampling dates. The East site experimental plots for the CS II experiment were located in a different section of the same forest as the CS I experimental plots. Mean soil pH was slightly lower and soil Ca and P were much lower for the CS II experiment than for CS I experiment. The lower soil pH found in CS II experimental plots probably caused less Ca and P to be available.

Table 23. Soil pH and Mehlich-3 extractable nutrient means and standard errors prior to treatment application at three experimental sites in Maryland in 2002.

Site	pH	Ca	Mg	K	P	Fe	
		-----mg/kg-----					
West	6.5 (0.05) [‡]	2409 (86)	193 (5)	320 (5)	31 (2)	129 (2)	
Central	5.6 (0.03)	1154 (52)	158 (8)	229 (7)	43 (4)	141 (2)	
East	4.1 (0.03)	80 (17)	28 (3)	46 (2)	23 (2)	307 (12)	

[‡] – Standard errors in parentheses.

Post-season (2003).

The analyses of variance for soils collected after the first growing season were applied are shown in Table 24. There were significant differences among sites for soil

pH and all of the measured soil nutrients except P (Table 24). Across sites, mulch treatments did not affect any of the measured soil parameters whereas the gypsum and lime treatments had significant effects on pH and Mg (Table 24).

Table 24. Post-season analyses of variance for soil pH and Mehlich-3 extractable soil nutrients combined over three forested experimental sites in Maryland in 2003.[†]

Site	pH	Mg	P	Fe
<u>F-value</u>				
Site (S)	66.1***	19.4**	0.9	15.9**
Mulch (M)	1.8	1.0	0.1	1.5
S x M	0.5	0.5	1.1	0.4
Treatment (T)	14.6***	36.9***	0.8	1.4
M x T	1.5	0.8	0.7	0.1
S x T	0.5	5.9***	1.8†	1.2
S x M x T	0.5	0.3	0.7	0.4

† - Between sites, soil Ca and K means were very heterogeneous and a combined analysis was not conducted.

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Post-season differences among sites for soil pH were similar to pre-treatment differences (Table 23 and Table 25). Although lime treatments significantly increased soil pH at the West site by 0.2 or 0.3 units over control, the increases may not have had biological significance (Table 26). The limestone-based soils at this site may have buffered changes in pH. For the more acid Central site soil, lime treatments significantly increased soil pH by 0.4 to 0.5 units, which may have been biologically and chemically significant enough to alter nutrient availability. At the East site, lime treatments only raised soil by 0.1 or 0.2 units, which was statistically significant for the latter increase, but probably not biologically significant. The extreme acidity and coarse soil at this site, in combination with high precipitation, probably caused lime to move down the horizon

as it reacted, without significantly altering the soil pH of the top 7.5 cm of soil. Soil pH means following gypsum treatments was similar to control means at all three sites.

Table 25. Analyses of variance for Mehlich-3 extractable soil Ca and K at three forested experimental sites in Maryland in 2003.

Source of variation	Ca			K		
	West	Central	East	West	Central	East
	<u>F-value</u>					
Mulch	0.1	0.5	0.3	889.3**	101.3**	4.0
Treatment	3.2*	3.3*	4.0*	5.5**	6.1**	3.7*
M x T	0.3	0.5	1.9	0.1	1.5	1.9

†, *, **, *** indicates significance at $p < 0.10$, 0.05 , 0.01 , and 0.001 respectively

Because of different parent material, the soil Ca concentrations differed substantially among sites, variances were not homogeneous, and analysis of variance was conducted by site. The mean soil Ca concentrations at the sites ranged from 2170 mg/kg at the West site to 960 mg/kg at the Central site, to only 170 mg/kg at the East site. Both lime treatments at all sites, except for the L1 treatment at the West site increased soil Ca (Table 26). Neither gypsum treatment increased soil Ca except for the G2 treatment at the East site. Although the largest increases in soil Ca occurred at the Central site, the largest proportional increases occurred at the East site. Larger proportional increases were due to lower initial soil Ca at the East site, while low total increases (70 to 140 mg/kg) may have been due to leaching and because increases in pH and pH-dependent CEC may have limited adsorption of added Ca.

Table 26. Treatment means for soil pH and Mehlich-3 extractable soil Ca at three experimental sites in Maryland in 2003 combined over mulch treatments.

Treatment	pH			Ca		
	West	Central	East	West	Central	East
	-----mg/kg-----					
C	6.7	5.6	4.1	1990	620	80
G1	6.7	5.7	4.0	2000	900	150
G2	6.5	5.6	4.0	1970	910	210
L1	6.9	6.0	4.2	2360	1120	180
L2	7.0	6.1	4.3	2540	1230	220
LSD (0.05)	0.2	0.3	0.2	440	390	90
Mean ¹	6.8	5.8	4.1	2170	960	170

¹LSD (0.05) = 0.6 for comparing soil pH site means and 540 for comparing Ca site means.

Soil Mg concentrations showed significant differences among sites similar to the soil Ca differences (Table 29). Soil Mg concentration ranged from 123 mg/kg at the West site, to 82 mg/kg at the Central site, to 19 mg/kg at the East site. Less than 60 mg/kg of Mg is considered low and may cause deficiency in vegetable crops (Schonbeck, 2004). The soil treatments significantly affected soil Mg concentrations and also interacted with sites (Table 24). At the West and Central sites, gypsum additions to the soil decreased the Mehlich-3 extractable Mg (Table 27). Leaching of soil Mg with sulfate added by gypsum probably caused this decrease. At the East site, extractable soil Mg concentrations were not significantly different between gypsum treatments and the control, which may have been due to very low initial soil Mg.

Table 27. Treatment means and LSD's for Mehlich-3 extractable Mg, P, and Fe concentrations at three forested experimental sites in Maryland in 2003.

Site	Treatment	Mg	P	Fe
		-----mg/kg-----		
West	C	143	38	143
	G1	106	42	148
	G2	82	40	147
	L1	136	40	142
	L2	148	44	133
	LSD (0.05)	19	6	9
	Mean	123	41	143
Central	C	100	57	164
	G1	58	58	164
	G2	37	58	163
	L1	109	59	159
	L2	106	52	152
	LSD (0.05)	24	6	14
	Mean	82	57	160
East	C	21	34	263
	G1	11	31	272
	G2	15	38	289
	L1	23	30	263
	L2	25	34	282
	LSD (0.05)	9	8	33
	Mean	19	33	274
	Site LSD (0.05)	10	4	12

Soil K concentrations differed substantially among sites, variances were not homogeneous, and analysis was conducted by site (Table 25). Soil K was the only nutrient that was significantly affected by mulch. The effect of mulch on soil K was significant at the West and Central sites, but not at the East site (Table 25). Mulch increased the extractable K by 100 mg/kg and 60 mg/kg at the West and Central sites, respectively (Table 28). At the East site, K concentrations were low and more variable than the other sites and the difference of 20 mg/kg between mulched and non-mulched treatments was not statistically significant. It is possible that the wheat straw mulch,

which is known to have 1 to 2% potassium, added K to soils. Added soil K from mulch may have been leached at the East site because low soil pH-dependent CEC may have prevented adsorption of K. Also, suppression of the herbaceous layer, which is known to have high foliar K, may have contributed to more available K by the end of the growing season (Gilliam and Roberts, 2003). The effect of mulch at the West and Central sites may have been greater than at the East site due to a denser herbaceous layer. There were also significant treatment effects on soil K at all three sites. Gypsum treatments had lower soil K than control at the West and East sites. At the Central site, the gypsum treatments were not significantly different from the control for soil K.

Table 28. Treatment means and LSD's for Mehlich-3 extractable soil K concentration with and without mulch at three forested experimental sites in Maryland in 2003.

Treatment	<u>West</u>		<u>Central</u>		<u>East</u>	
	No Mulch	Mulch	No Mulch	Mulch	No Mulch	Mulch
	-----mg/kg-----					
C	208	303	168	201	52	77
G1	199	291	152	227	42	54
G2	171	255	140	180	46	61
L1	197	297	173	265	45	81
L2	216	308	169	223	48	62
LSD (0.05) ¹	43		46		19	
Mean ²	198	291	161	220	47	67

¹ LSD for comparing treatment means within either No Mulch or Mulch columns.

² LSD (0.05) = 13 (West), 26 (Central), and 45(East) for comparing mulch means within a site.

For soil P, there were no significant differences due to any of the sources of variation. There was a significant site effect on soil Fe, which was similar at the West and Central sites and significantly higher at the East site.

Plants

Percent plants emerged.

Graphs of the percent plant emergence are shown for the growing season in Figures 10 and 11. The tick marks for each month correspond to the middle of the month. At the first sampling date of March 2003, plants had not emerged. By the first week of April 2003, plants had emerged from 10% of seeds sown in 2002 at the West site, 9% at the Central site, and 8% at the East site. Total percent emerged was 51% at the West site, 37% at the Central site, and 26% at the East site. The only significant source of variation for total percent emerged plants was mulch by site interaction (Table 29). The treatment means and LSD's for total percent emergence are shown in Table 31. Total percent emerged at the West site was significantly greater with the mulch treatment. Conversely, total percent emerged at the Central site was higher for the no-mulch treatment. At the East site mulch treatments were not different (Figure 10 and Figure 11). Although the percent total emerged at the East site was less than the other sites, the means were not significantly different due to high block within location variability which decreased the sensitivity of the test for site effects. There was no significant treatment effect across sites or by sites (Table 30).

Table 29. Analysis of variance for total percent emerged first-season American ginseng combined over three forested experimental sites in Maryland in 2003.

SOV	Total Percent Emerged
	<u>F-value</u>
Site (S)	1.5
Mulch (M)	1.3
M x S	12.1**
Treatment (T)	0.9
T x S	2.0
T x M	1.0
T x M x S	0.2

*, **, *** indicates significance at $p < 0.05$, 0.01, and 0.001 respectively

Table 30. Analyses of variance by site for total percent emerged American ginseng plants at three forested experimental sites in Maryland in 2003.

Source of variation	West	Central	East
		<u>F-value</u>	
Treatment (T)	0.5	2.1	1.7
Mulch (M)	17.0**	9.5*	2.9
M x T	1.1	0.8	0.4

*, **, *** indicates significance at $p < 0.05$, 0.01, and 0.001 respectively

Table 31. Mulch and soil treatment means and LSD's for total percent emerged American ginseng plants by mulch treatment at three forested experimental sites in Maryland in 2003.

Treatment	<u>West</u>		<u>Central</u>		<u>East</u>	
	No Mulch	Mulch	No Mulch	Mulch	No Mulch	Mulch
	-----%-----					
C	48	48	48	31	28	19
G1	42	52	46	30	22	18
G2	49	58	45	41	23	24
L1	50	62	34	26	33	32
L2	44	55	38	35	34	27
LSD (0.05) ¹	11		16		13	
Mean ²	46	55	42	33	28	24

¹ LSD for comparing treatment means within either No Mulch or Mulch columns.

² LSD (0.05) = 5% (West), 7% (Central), and 6%(East) for comparing mulch means within a site.

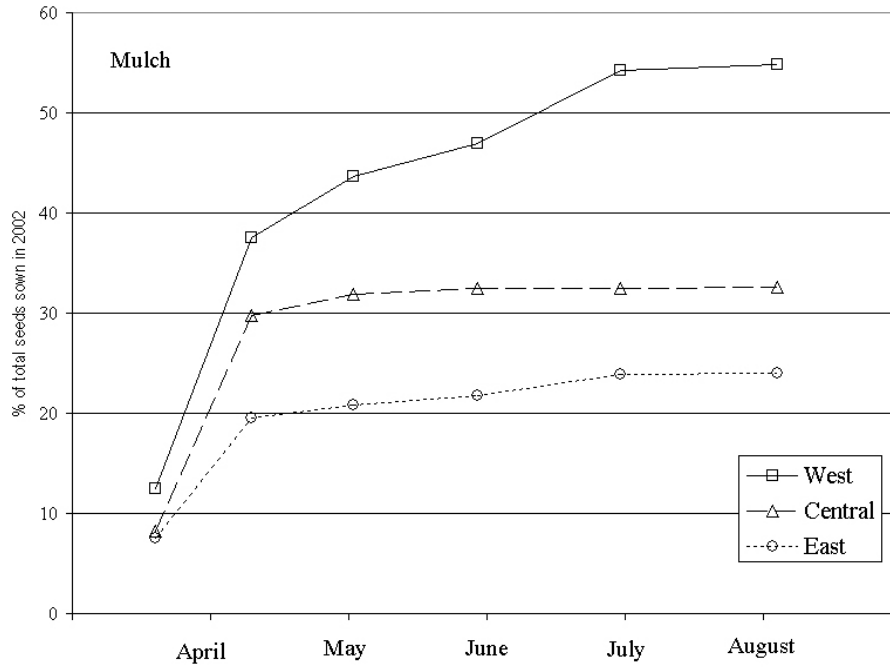


Figure 10. Percent emerged American ginseng plants in mulched plots at three forested experimental sites in Maryland in 2003.

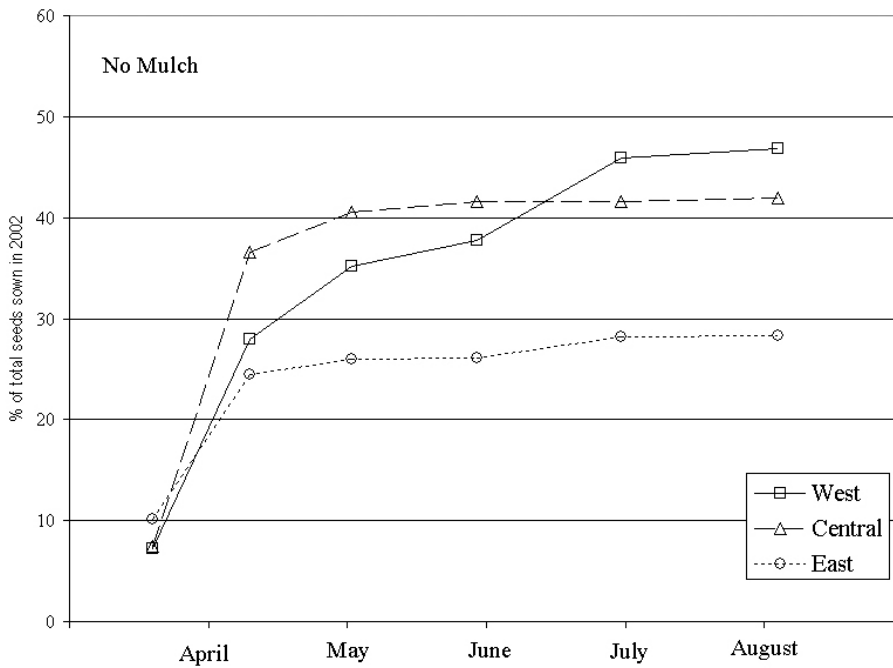


Figure 11. Percent emerged American ginseng plants in non-mulched plots at three forested experimental sites in Maryland in 2003.

Percent plants absent aboveground.

Plants were first observed to be absent aboveground at all three sites in early May, less than a month after emergence, and percent absent aboveground increased until the end of the growing season (Figure 12 and Figure 13). In June and July, there were significant effects on percent absent aboveground due to site, treatment, mulch x site, and treatment x site interactions (Table 32). In August, the effects of site and treatment x site were no longer significant. The mulch x site interaction occurred because the no mulch treatment at the Central site had significantly higher percent absent aboveground than the no-mulch treatment whereas there was no difference between mulch treatments at the West and East sites.

Analysis of variance for percent absent aboveground was conducted for the July sampling date because this date represents maximum early senescence and mortality (Table 33). Treatment effects were significant at the East site but not the West or Central sites (Table 33). At the East site, the L2 treatment for mulch and no-mulch treatments had significantly lower percent absent aboveground plants by July than the control or gypsum treatments (Table 34). It is possible that increased pH in limed plots increased the availability of plant nutrients which increased the overall health of plants.

Table 32. Analysis of variance of percent absent aboveground first-season American ginseng at three forested experimental sites in Maryland in 2003.

SOV	6/12	7/11	8/15
		<u>F-value</u>	
Site (S)	6.0*	8.8*	4.1
Mulch (M)	5.4	4.3	1.8
M x S	7.5*	6.1*	6.1*
Treatment (T)	6.3***	5.4**	4.4**
T x S	2.7*	2.2*	1.2
T x M	0.5	0.4	0.9
T x M x S	0.7	1.0	0.3

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 33. Analysis of variance for percent absent aboveground first-season American ginseng plants at three forested experimental sites in Maryland during the week of July 11, 2003.

Source of variation	West	Central	East
		<u>F-value</u>	
Treatment (T)	1.7	2.5	6.6*
Mulch (M)	5.3	59.5***	5.3
M x T	0.7	0.2	1.6

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 34. Treatment means of percent absent aboveground first-season American ginseng plants at three forested experimental sites in Maryland during the week of July 11, 2003.

Treatment	<u>West</u>		<u>Central</u>		<u>East</u>	
	No Mulch	Mulch	No Mulch	Mulch	No Mulch	Mulch
	-----%-----					
C	46	30	60	88	77	91
G1	38	30	57	86	81	92
G2	45	42	52	75	94	85
L1	26	25	52	77	71	80
L2	42	27	50	80	57	73
LSD (0.05) ¹	14		10		12	
Mean ²	39	31	54	81	76	84

¹ LSD for comparing treatment means within No Mulch or Mulch columns

² LSD (0.05) = 9% (West), 8% (Central), and 8%(East) for comparing mulch means within a site.

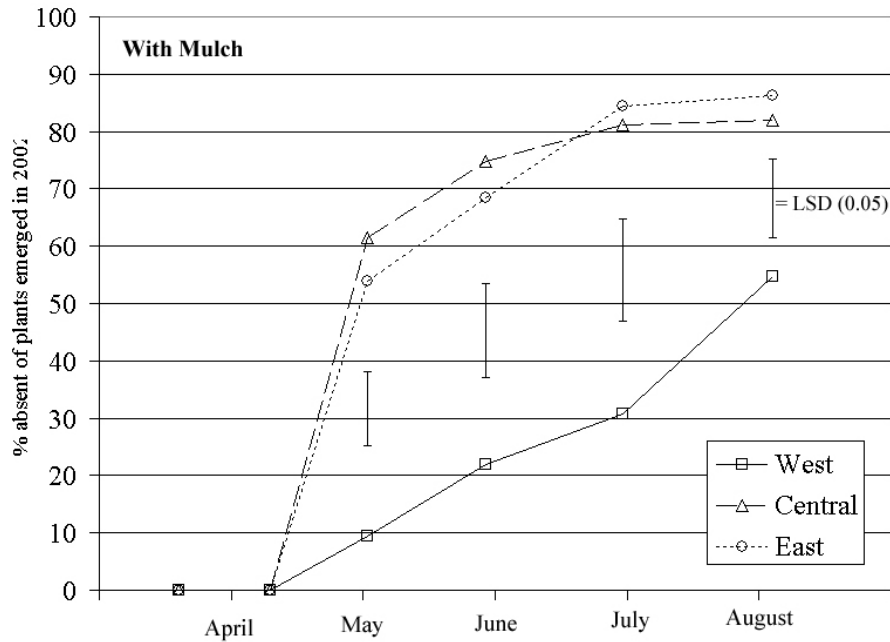


Figure 12. Percent absent aboveground American ginseng plants in mulched plots at three forested experimental sites in Maryland in 2003.

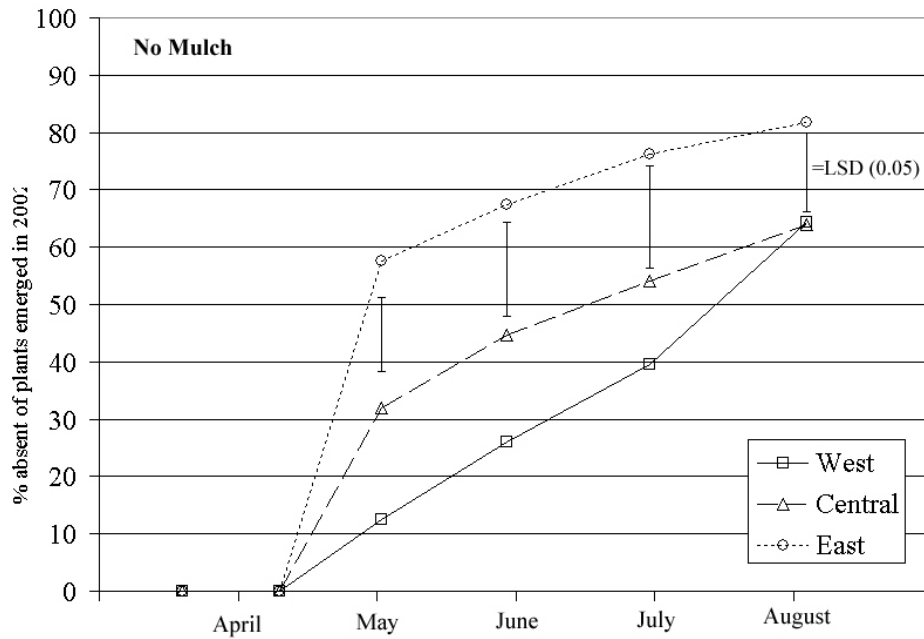


Figure 13. Percent absent aboveground American ginseng plants in non-mulched plots at three forested experimental sites in Maryland in 2003.

Percent plants present aboveground.

In May, the percentage plants present of total seeds sown increased to a maximum of 40% at the West site, 36% at the Central site, and 23% at the East site (Figure 14 and Figure 15). By August, as plants became absent aboveground, the percent plants present decreased to 31%, 13% and 8%, for the Central, West, and East sites, respectively.

There was a significant site x mulch interaction throughout the growing season (Table 35). At the West site, the mulch treatment had higher percent plants present aboveground than the no-mulch treatment, whereas at the Central site, the no-mulch treatment had higher percent plants present aboveground. At the East site, except for the July sampling date, there were no significant differences between the mulch treatments. Thus, the effect of mulch on percent plant present was highly site-specific.

The percent plants present aboveground were not significantly different among sites until August when the West site had significantly higher percent plants present aboveground than the Central and East sites. There were no significant effects of treatment on percent plants present aboveground across or within sites for any sampling date (Table 36).

Figures 5 and 15 can be used to compare percent present aboveground over time for the establishment year of the American ginseng populations. These figures depict the results of seeding ginseng in very dry, mild winter (2001 to 2002) and in a wetter, colder winter (2002 to 2003) at three forested sites. In general, the percent present aboveground for seedlings was higher at all sites in 2002 than 2003. The relative rankings of the East and West sites were similar both years. However, the Central site had similar percent plants present aboveground in 2002 but less percent plants aboveground in 2003 as

compared to the West site. The difference could be due to the wide differences in weather for each year and/or site conditions for each year.

Table 35. Analyses of variance across sites for percent American ginseng plants present aboveground in August at three forested experimental sites in Maryland in 2003.

Source of variation	April 4	April 18	May 15	June 13	July 12	August 8
	<u>F-values</u>					
Site (S)	0.1	0.8	1.2	3.0	5.5	8.3*
Mulch (M)	1.3	0.3	1.2	7.8**	5.8*	5.5*
S x M	5.2*	10.8***	11.5***	25.5***	23.8***	17.6***
Treatment (T)	1.4	0.8	0.7	1.3	0.9	0.7
M x T	1.2	1.3	1.2	1.3	0.4	0.3
S x T	0.8	1.5	1.2	1.1	1.1	1.0
S x M x T	0.4	0.3	0.2	1.2	0.6	0.6

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

Table 36. Analyses of variance by site for percent American ginseng plants present aboveground in August at three forested experimental sites in Maryland in 2003.

Source of variation	April 4	April 18	May 15	June 13	July 12	August 8
<u>F-value</u>						
<u>West</u>						
Treatment (T)	0.5	0.8	0.6	0.8	0.7	0.7
Mulch (M)	10.8*	9.3*	8.0*	13.6**	10.5*	6.7*
T x M	0.8	0.8	1.0	2.6	0.4	0.2
<u>Central</u>						
Treatment (T)	1.3	3.4	2.2	0.6	0.4	1.0
Mulch (M)	0.2	5.2	9.3*	44.3***	89.1***	71.8***
T x M	0.8	0.7	0.7	1.0	1.2	1.0
<u>East</u>						
Treatment (T)	1.2	0.9	1.1	1.6	1.9	1.4
Mulch (M)	2.4	6.3*	4.9	3.6	0.3	3.6
T x M	0.8	0.6	0.5	1.5	1.2	1.2

*, **, *** indicates significance at $p < 0.05$, 0.01 , and 0.001 respectively

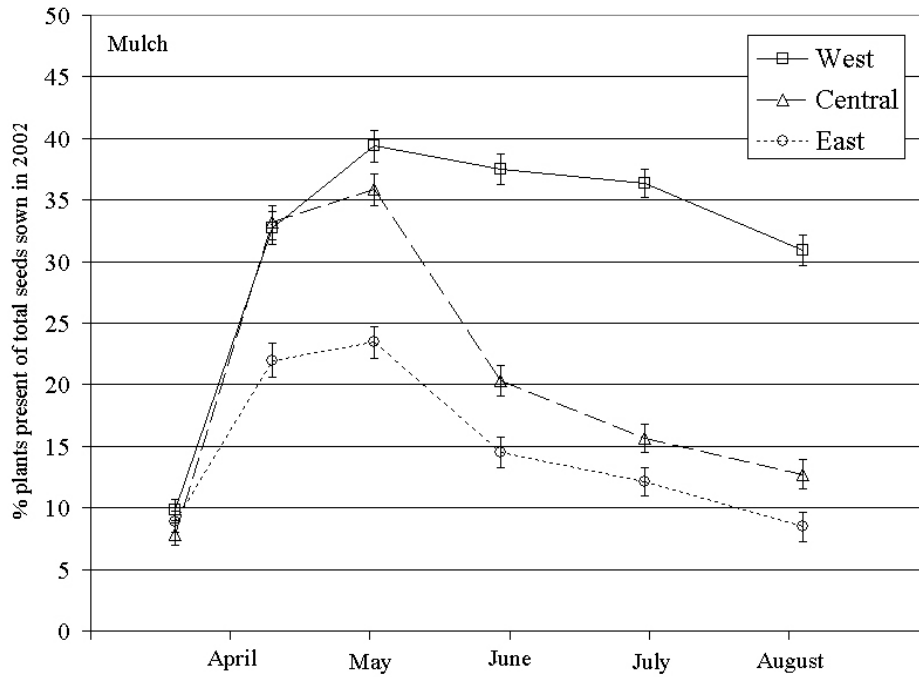


Figure 14. Percent of ginseng plants present aboveground in mulched plots at three forested experimental sites in Maryland in 2003.

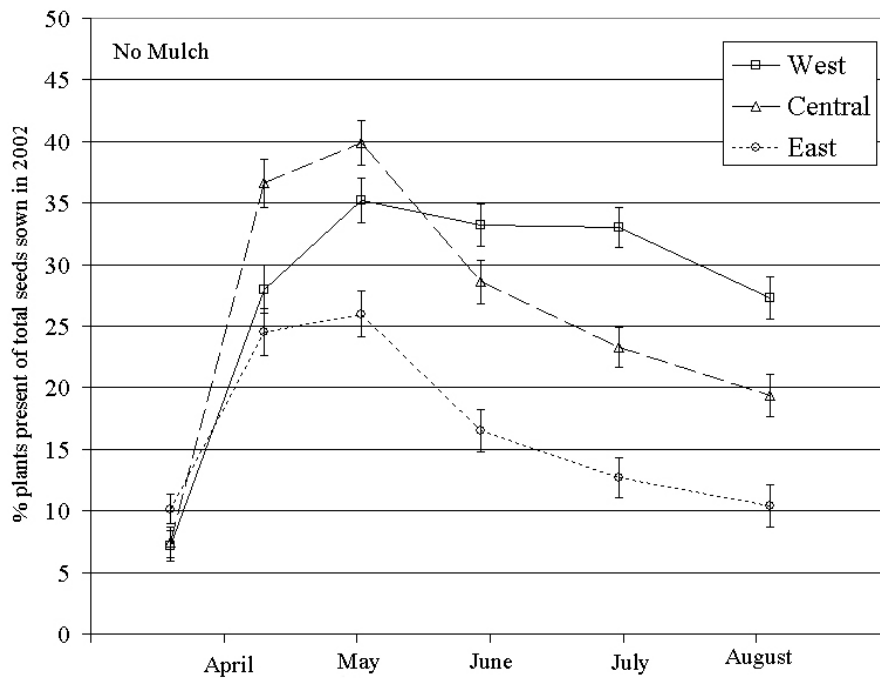


Figure 15. Percent of ginseng plants present aboveground in non-mulched plots at three forested experimental sites in Maryland in 2003.

Soil-Plant Interaction

Soil fertility correlations.

Across sites, there were significant positive correlations between soil pH, Ca, Mg, K and percent total emerged plants (Table 37). Across sites, there were significant negative correlations between soil pH, Ca, Mg, K and percent absent plants by August. Across sites, there were significant positive correlations between soil pH, Ca, Mg, K, and percent plants present aboveground. The scatter plots displayed in Figures 16, 17, and 18 show plot means of total percent emerged, percent absent and percent present aboveground in August and soil fertility values. Because of the confounding of various soil fertility values and site, the correlation of soil fertility values and phenology measures are confounded. Scatterplots, as well as correlations by site (Table 37) may clarify relationships between soil fertility and phenology measures.

Trends in Figure 16: A. Sites values are mostly separated – the West site had the highest pH values (about 7) and average percent emerged plants (about 55%), the Central site had lower pH (5 to 6) and percent emerged (about 35%), and the East site had the lowest pH (about 4) and emergence (about 20%). B. Soil Ca values were largely separated by site, and displayed a relationship similar to soil pH for percent emergence. All plots at the East site had less than 500 mg/kg Ca and averaged low emergence. The Central site had Ca concentrations ranging from 500 to 1500 mg/kg and averaged about 35% emergence. The West site averaged 1500 to 3000 mg/kg Ca and averaged about 55% emergence. C. Sites had wider variation and greater overlap in soil Mg concentration than soil pH or Ca and an upward trend was less evident. D. Soil K displayed a relationship similar to soil Mg for percent emergence. E. Soil P was

somewhat variable and no trend was evident. F. Soil Fe values were separated by site, as for pH and Ca. The highest emergence occurred at the West site which was clustered around 10 mg/kg Fe, followed by the Central site clustered around 150 mg/kg, followed by the more variable East site which had the highest Fe and the lowest emergence.

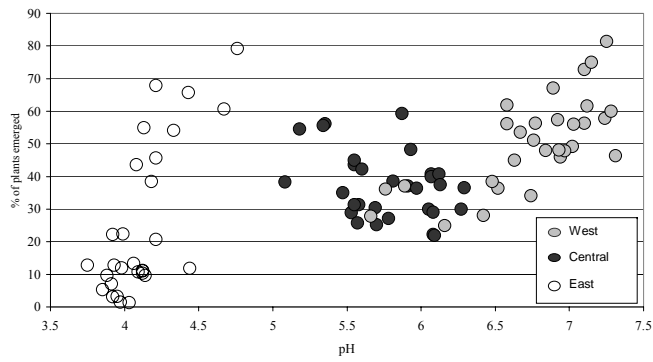
Several trends that are apparent in Figure 17: A. Soil pHs by site were grouped similar to 7A. However, percent plants absent aboveground decreased with increasing pH. B. There was more overlap in soil Ca values than in soil pH and higher Ca concentrations occurred with lower percent absent aboveground plants. C. Soil Mg displayed a relationship similar to soil Ca for percent absent aboveground plants. D. Soil K also displayed a relationship similar to soil Ca for percent absent aboveground plants. E. Soil P was variable and no trend was obvious. F. Soil Fe concentration was similar.

Trends in Figure 18 were similar to trends observed in Figure 16.

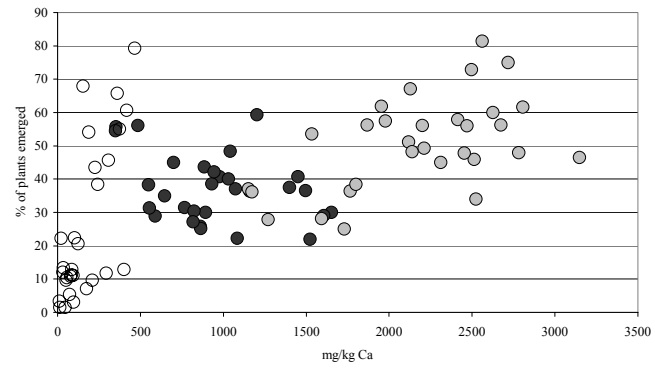
Table 37. Correlation coefficients for soil fertility factors and percent absent by August, percent of total seeds sown, and percent total emerged ginseng plants at three experimental sites in Maryland in 2003.

	% Total emerged	% Absent in August	% Present in August
Across sites			
pH	0.61***	-0.63***	0.64***
Ca	0.58***	-0.62***	0.65***
Mg	0.49***	-0.52***	0.52***
K	0.53***	-0.51***	0.49***
P	ns	ns	ns
Fe	-0.68***	0.62***	-0.59***
West			
pH	0.71***	-0.57**	0.67***
Ca	0.54**	-0.41*	0.49**
Mg	0.40*	ns	0.42*
K	0.50**	-0.51**	0.55**
P	-0.50**	ns	-0.57**
Fe	-0.51**	0.37*	-0.60***
Central			
pH	ns	ns	ns
Ca	ns	ns	ns
Mg	-0.40*	ns	ns
K	-0.41*	0.64***	ns
P	ns	ns	ns
Fe	ns	ns	ns
East			
pH	0.73***	-0.72***	0.78***
Ca	0.71***	-0.73***	0.71***
Mg	0.87***	-0.83***	0.91***
K	0.64***	-0.46*	0.56**
P	ns	ns	ns
Fe	-0.84***	0.70***	-0.84***

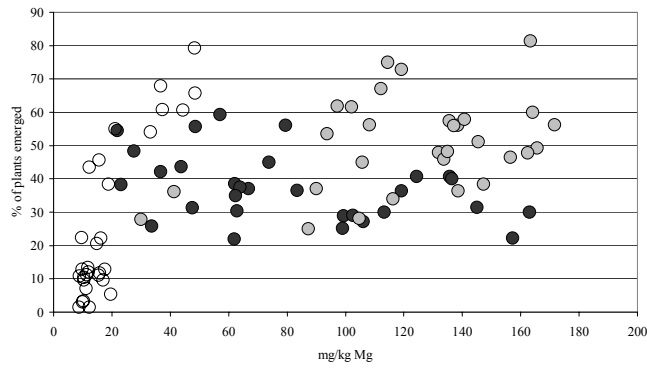
Figure 16. Scatter plots of soil nutrient concentrations by percent total emerged ginseng plants at three experimental sites in Maryland in 2003.



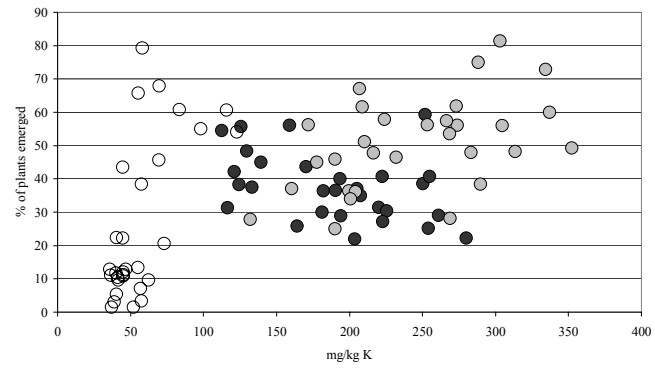
A.



B.

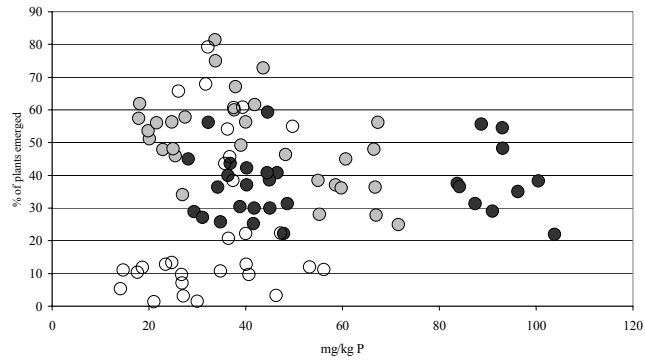


C.

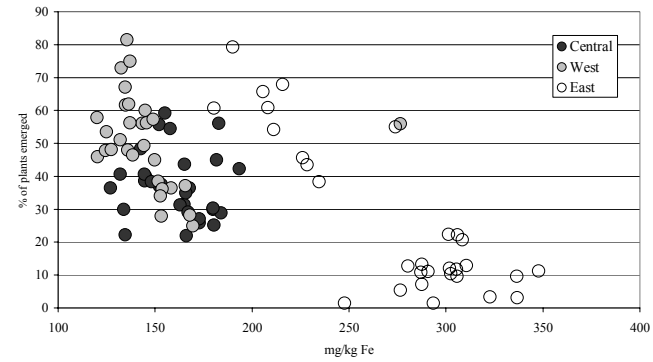


D.

Figure 16 (continued). Scatter plots of soil nutrient concentrations by percent total emerged ginseng plants at three experimental sites in Maryland in 2003.

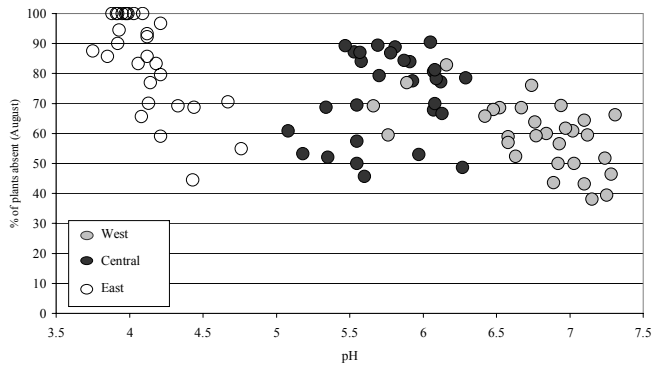


E.

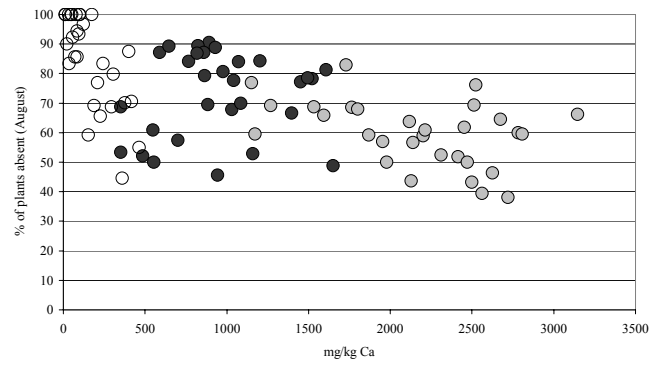


F.

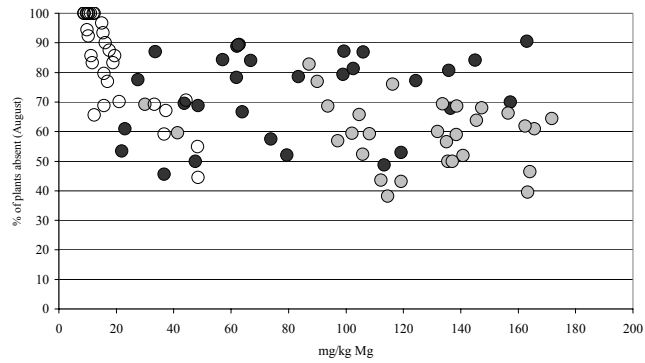
Figure 17. Scatter plots of soil nutrient concentrations by percent American ginseng plants absent aboveground by August at three experimental sites in Maryland in 2003.



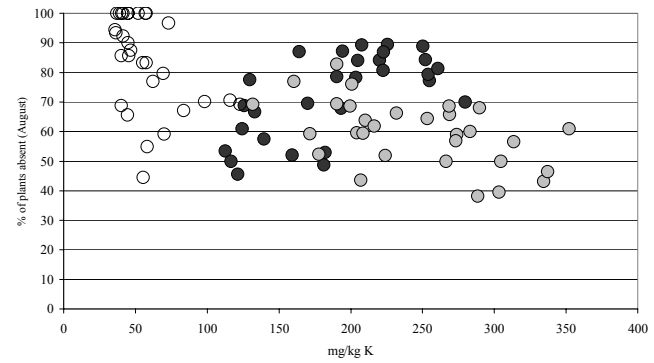
A.



B.

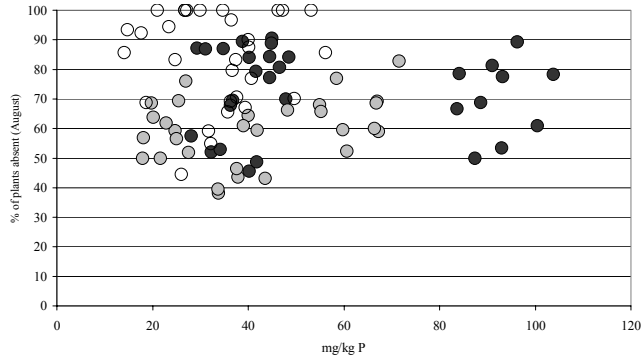


C.

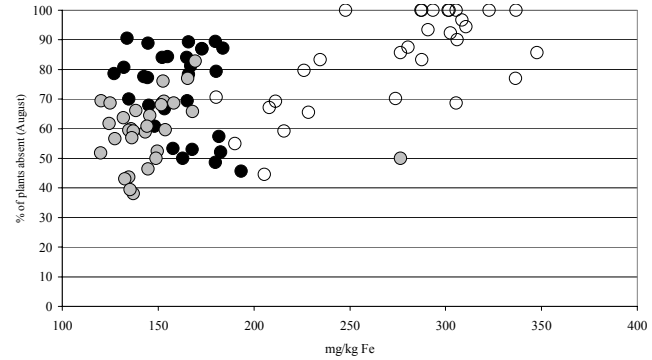


D.

Figure 17 (continued). Scatter plots of nutrient concentrations values by percent American ginseng plants absent aboveground by August at three experimental sites in Maryland in 2003.

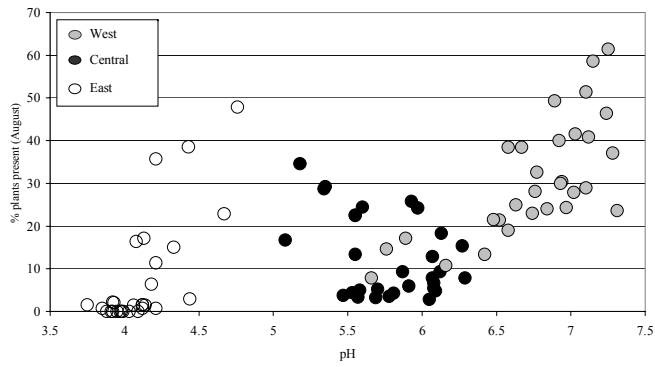


E.

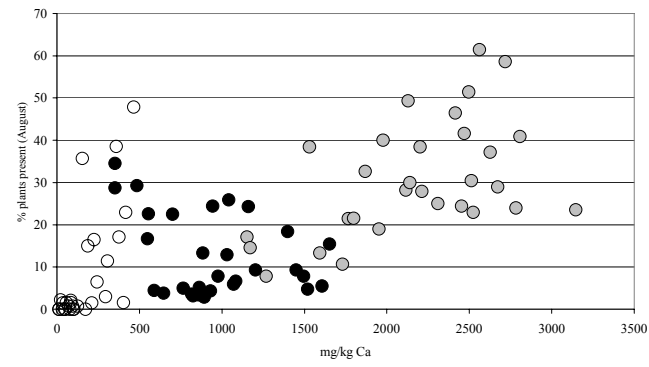


F.

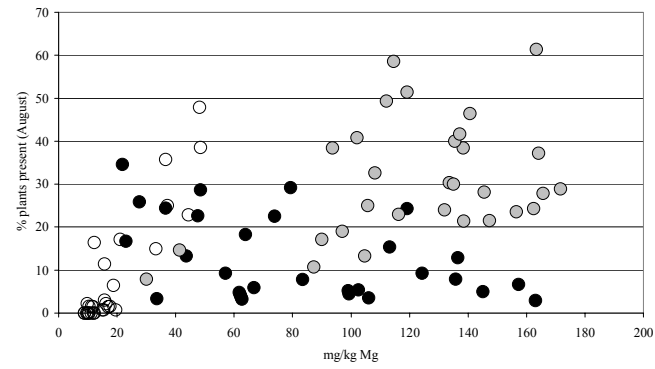
Figure 18. Scatter plots of soil nutrient concentrations by percent American ginseng plants present aboveground at three experimental sites in Maryland in 2003.



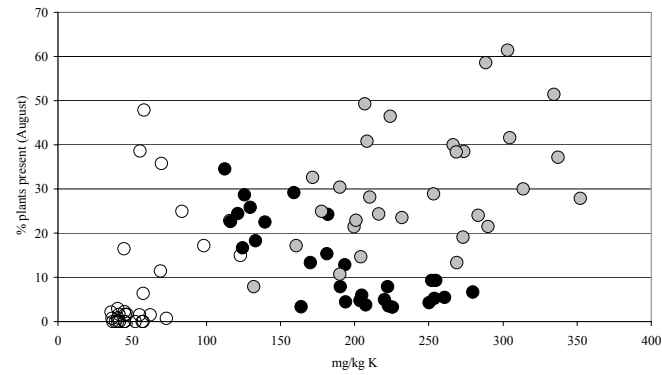
A.



B.

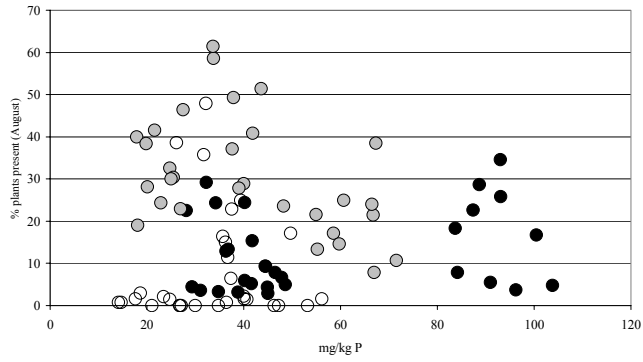


C.

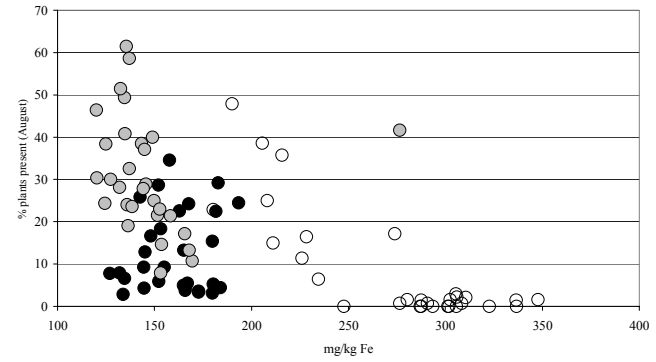


D.

Figure 18 (continued). Scatter plots of nutrient concentrations by percent American ginseng plants present aboveground at three experimental sites in Maryland in 2003.



E.



F.

Across sites, there were significant positive correlations between soil pH, Ca, Mg, and K (Table 38). Since Ca, Mg, and K have increasing availability with increasing pH, this is not surprising. There was also a significant negative correlation between soil Fe and soil pH, Ca, Mg, and K. Soil Fe has decreasing availability with increasing pH. At the West site, correlations were very similar to across site correlations, which may have been due to the wide range of soil fertility values there (Figure 16, Figure 17, and Figure 18). Correlations at the Central site were similar to across site correlations except for soil Fe, which may have been due to the narrow range of Fe concentrations at that site. Correlations at the East site were very similar to across site correlations.

Table 38. Correlation coefficients of soil nutrient concentrations at three forested experimental sites in Maryland in 2003.

	Ca	Mg	K	P	Fe
Across sites					
pH	0.94***	0.89***	0.90***	ns	-0.89***
Ca		0.84***	0.82***	ns	-0.77***
Mg			0.87***	ns	-0.78***
K				ns	-0.82***
P					-0.26*
West					
pH	0.87***	0.78***	0.55**	-0.56**	-0.80***
Ca		0.67***	0.37*	ns	-0.73***
Mg			0.54**	ns	-0.64***
K				ns	-0.40*
P					0.69***
Central					
pH	0.89***	0.56***	0.49**	ns	-0.40*
Ca		ns	0.43*	ns	ns
Mg			0.64***	-0.43*	ns
K				ns	ns
P					ns
East					
pH	0.63***	0.77***	0.52**	ns	-0.64***
Ca		0.69***	0.47*	ns	-0.61***
Mg			0.59***	ns	-0.80***
K				ns	-0.58***
P					ns

Conclusions

This experiment includes the first known study of wild-simulated American ginseng grown from seed in Maryland forests. Randomized complete block experiments were conducted to study ginseng's unique life history and phenology over two growing seasons in three forested sites in Maryland. Two sites were within the native range of ginseng and one site was outside of ginseng's native range. Although ginseng was successfully grown from seed for two years in forests in three physiographic regions in Maryland, fewer plants emerged and the percent plants present aboveground by August of total seed sown were less in the Coastal Plain region than the Piedmont or Ridge and Valley sites. Lime application increased total percent emerged plants and percent present at the Coastal Plain site while gypsum did not affect total percent emerged or percent present. At the Piedmont and Ridge and Valley sites, neither lime nor gypsum affected plant counts or phenology. Population establishment in the first season of growth as indicated by curves of percent present aboveground over time may provide evidence that American ginseng develops from seed following a Type III population growth curve. However, more research is needed to further elucidate population dynamics in wild-simulated ginseng production.

Lime application raised soil pH and Ca at all three sites and gypsum decreased Mg and K at the Piedmont and Ridge and Valley sites. For these forests in Maryland, soil pH less than 4.8, Ca less than 500 mg/kg, Mg less than 25 mg/kg, K less than 100 mg/kg, or Fe greater than 250 mg/kg was associated with fewer percent emerged plants, higher percent absent aboveground plants in August, and fewer percent present aboveground plants in August, suggesting that these nutrients play a role in the growth

and development of American ginseng in these forests. Soil nutrient concentrations and parent materials varied within the experimental forests and influenced the response of the soils to the soil amendments. Thus, pH and fertility recommendations to successfully grow American ginseng should be based on soil test results and soil type. Further research is necessary to determine whether the ginseng populations could survive for seven years, which is the earliest that wild-simulated ginseng is usually harvested. This additional research is needed to predict the potential market value of ginseng per hectare in Maryland. In addition, a long-term study of the effects of soil nutrients on growth of American ginseng is needed to better define the role soil nutrients play in the life of the plant.

CHAPTER 3: CALCIUM ROOTLET EXPERIMENTS

Introduction

American ginseng can be grown in a wild-simulated production system from seeds and/or transplanted rootlets, however, rootlets cost at least ten times more than seeds (Davis, 1997). The advantage of transplanting rootlets is the decreased time to harvest of mature roots (Duke, 1988) and the greater probability of emergence and survival (Persons, 1992).

Several studies have shown that older American ginseng plants have lower annual mortality rates and greater fruit production than younger plants (Lewis, 1984; Carpenter, 1982; Lewis, 1983; Charron, 1991; and Carpenter, 1982). Charron (1991) examined four American ginseng populations in Québec for two to three years and reported that annual mortality rates for plants larger than seedlings was less than 10%. Lewis (1982) observed a ginseng population in Missouri for three years and reported that non-seedling mortality rates were between 0 and 4%. Several studies found significant positive correlations between age class and flower production in wild populations of American ginseng (Lewis, 1984; Carpenter, 1982; and Lewis, 1983). Lewis (1984) reported that 14% of two-year old plants in a NY population flowered, 60% of three-year old plants flowered, 87% of four-year old plants flowered, and 75% of five-year old plants flowered. In other studies, (Anderson, 1993; Carpenter and Cottam, 1982) plants less than four-years old did not flower. In the study conducted by Carpenter and Cottam (1982), the mean fruit per plant was 0.3 for four-year old plants and increased to two fruits per plant for five-year old plants.

Two of the measures of phenology used in chapter II were measured for rootlet

experiments: emergence and absent aboveground. Since populations of plants grown from rootlets were small ($n < 25$), percent present was not calculated. However, examination of plants emerging for a second growing season may give some indication of early establishment of ginseng populations from transplanted rootlets.

The objectives of the experiments reported here were to study American ginseng grown in three forests in the three physiographic regions of Maryland in order to:

- 1) Examine phenology (emergence, absence aboveground) and establishment of different age rootlets in each region.
- 2) Determine the effects of lime and gypsum on phenology of two- and four-year old American ginseng rootlets.

Calcium Rootlet Experiment (CR I): 2002 – 2003

Materials and Methods

Site Descriptions and Experimental Design.

The CR I experiments were planted in the fall of 2001 at the West, Central, and East sites in the same forested areas as the CS I experiments. Two-year-old and four-year-old roots were planted at each site as separate randomized complete block experiments. Each experiment had twenty-five plants consisting of five replications of five treatments, which were the same soil treatments at the CS experiments.

Experiments were 150 cm wide by 150 cm long containing five rootlets equally spaced 25 cm apart. Leaves and debris were removed from the soil surface and rootlets were planted 6-7 cm deep, and covered with soil. After planting, soil treatments were applied manually and leaves were returned to the soil surface.

Field Measurements and Sampling.

Data were collected during the first year of growth (2002) at each location biweekly from the first week of April until the first week of September. Data collected were plant counts and qualitative data involving insect damage, associated groundcover, browsing, and general plant vigor. During their second year of growth (2003), data were collected for these plants at each location the first and third weeks of April, the first week of May, and monthly thereafter until the third week of September. A table of experimental activities and dates is shown in Table 39.

Table 39. Sampling, planting, and treatment application dates for the Calcium Rootlet Experiment I (2001 - 2003).

Month	Activity		
	2001	Year 2002	2003
January			
February			
March		Plant Counts [‡]	Plant Counts
April		Plant Counts	Plant Counts
May		Plant Counts	Plant Counts
June		Plant Counts	Plant Counts
July		Plant Counts	Plant Counts
August		Plant Counts	Plant Counts
September		Plant Counts	Plant Counts
October			
November	Rootlets Planted		
December	Treatments Applied		

[‡] - Plant counts in were biweekly in 2002 and monthly in 2003.

The percent emerged ginseng plants from rootlets planted in 2002 was calculated by dividing the number of plants counted as emerged by the total number of rootlets planted in 2001. In 2003, the percent emerged plants was calculated by dividing the number of plants counted as emerged by the total number of plants emerged in 2002. Since populations of planted rootlets were small, the average number of days until plants were observed absent aboveground was calculated, rather than the percent absent aboveground

over time. Without using destructive sampling techniques, it was not possible to determine if ginseng plants that were absent aboveground had died or senesced by a given sampling date in 2002 or 2003. For each emerged plant, the number of prongs, date of fruit set, and fruit maturation were recorded. In 2003, plots were sampled monthly and days to absence aboveground and days to fruitset could not be accurately calculated and are not reported here.

Statistical Analysis.

In 2002, emergence was recorded as plant counts and percent emergence was calculated as the number of plants emerged divided by the number of rootlets planted. The percent of emerged plants or percent of morphological class were compared using chi-square statistics from PROC FREQ (SAS Institute, 1990). Analyses of variance for days to absence and days to fruitset used the following model:

$$M_{ijk} = \mu + S_i + B_j(S_i) + T_k + TS_{ik} + e_{ijk},$$

where M is the observation of the i th site S , j th block B , and k th calcium treatment T ; μ is the general mean, e is the variation due to random error or the residual, and TS is the interaction. The random error was used to test all effects.

Results and Discussion

Two-year old roots.

Emergence.

Percent of American ginseng plants that emerged in 2002 and reemerged in 2003 from transplanted two- and four-year-old rootlets at the West, Central, and East sites are shown for each treatment in Table 40. The reemerged in 2003 three- and five-year old plants. Plants were observed to emerge from two-year-old rootlets from late April to late

May 2002. In 2002, the overall mean percent total plants emerged from two-year-old rootlets was 45%. Mean percent plants emerged from planted rootlets was 40% at the West site, 28% at the Central site, and 68% at the East site. Percent emerged rootlets were significantly different between sites ($\chi^2 = 8.5$, $P < 0.01$). Based on approximate 95% confidence intervals, the East site had significantly higher emergence from planted two-year old roots in 2002 than the West or Central sites, which were similar. The mean percent emergences of each soil treatment appear in Table 40 for descriptive purposes. However, due to small sample sizes, chi-square statistics could not be used to test for differences among treatments.

During 2003, only two of the two-year old rootlets reemerged of the thirty-four which had emerged in 2002. One root was at the West site and one was at the Central site. Low reemergence in 2003 indicated that there was a high rate of mortality and/or seasonal dormancy for two-year old plants that had emerged 2002. It was not possible to determine the whether plants died or were seasonally dormant. Severe drought in 2002 could have caused mortality or induced dormancy.

Table 40. Percentages of American ginseng plants emerged from two- and four-year-old roots in 2002 and from three- and five-year old roots in 2003 at three forested experimental sites in Maryland.

Root Age	<u>West</u>				<u>Central</u>				<u>East</u>			
	<u>2002</u>		<u>2003</u>		<u>2002</u>		<u>2003</u>		<u>2002</u>		<u>2003</u>	
<u>Treatment</u>	2-year	4-year	3-year	5-year	2-year	4-year	3-year	5-year	2-year	4-year	3-year	5-year
C	0	60	0	40	60	80	0	60	80	100	0	80
G1	20	60	33	40	40	80	25	40	40	100	0	80
G2	60	60	0	40	40	80	0	20	80	100	0	80
L1	60	100	0	20	40	80	0	0	80	80	0	80
L2	0	60	0	80	20	80	0	20	60	100	0	80
Mean ± 95% CI	28 ± 18	68 ± 18	14 ± 26	65 ± 23	40 ± 19	80 ± 16	10 ± 19	35 ± 21	68 ± 18	96 ± 8	0	88 ± 13

Shoot morphology.

In 2002, emerged plants from two-year old roots had one or two-prongs (Table 41). In 2002, across sites, 26% of plants grown from two-year old roots had one-prong and 74% had two-prongs. In 2003, all of the plants from two-year old roots had two-prongs.

Fruitset.

None of the plants that emerged from two-year old roots in 2002 or three-year old plants in 2003 set fruit. However, there were only two three-year old roots that had reemerged.

Aboveground absence.

For 2002, the days to absence for the two-year-old roots and the associated ANOVA appear in Table 42. In 2002, plants began to be absent aboveground at all three sites between the first and third weeks of August. There was a significant site effect on days aboveground and the East site was found to have significantly fewer days aboveground than the West and Central sites, which were similar to each other (Table 42). Significantly fewer observed days aboveground for plants grown from two-year old roots at the East site may have been due to increased drought stress at that site. Finer-textured soils, like that found at the West and Central sites probably had better water retention capacity than coarser soils at the East site, which could be critical to plant survival in drought years like 2002. It is possible that other factors, like soil pH or mineral nutrition at the East site affected days aboveground for plants, but data was insufficient data to determine those effects. In 2003, the days to absence for the two plants that reemerged were approximately 125 for both the Central and West sites. No

plants grown from two-year old rootlets reemerged at the East site.

Table 41. Percentages of plants in morphological classes and setting fruit for American ginseng plants grown from two- and four-year-old roots at three forested experimental sites in Maryland in 2002 and 2003.

Root Age	<u>West</u>				<u>Central</u>				<u>East</u>			
	<u>2002</u>		<u>2003</u>		<u>2002</u>		<u>2003</u>		<u>2002</u>		<u>2003</u>	
	2-year	4-year	3-year	5-year	2-year	4-year	3-year	5-year	2-year	4-year	3-year	5-year
Prong class	-----%-----											
1-prong	29	6	0	0	40	0	0	18	18	0	n/a	0
2-prong	71	71	100	43	60	50	100	82	82	71	n/a	28.6
3-prong	0	24	0	57	0	50	0	0	0	29	n/a	61.9
4-prong	0	0	0	0	0	0	0	0	0	0	n/a	9.5
Fruitset ± 95% CI	0	71 ± 22	0	64 ± 28	0	25 ± 18	0	0	0	50 ± 20	n/a	62 ± 21

Table 42. Site means and ANOVA for days to absence for American ginseng plants emerged from two-year old rootlets at three experimental sites in Maryland in 2002.

Site	Days to absence
West	102
Central	94
East	73
Standard Error	6
Source of variation	F-value
Site (S)	2.4*
Treatment (T)	0.3
S x T	1.6

*, **, *** indicates significance at the p< 0.05, 0.01, and 0.001 level, respectively.

Four-Year Old Roots.

Emergence.

Plants were observed to emerge from four-year old roots transplanted in 2001 from late April to late May 2002 (Table 40). Across sites in 2002, mean percent total plants emerged from four-year old roots was 81% compared to only 45% for two-year old roots (Table 40). In 2002, mean percent plants emerged from four-year old planted roots was 68% at the West site, 90% at the Central site, and 86% at the East site. Total percent emerged roots was significantly different between sites ($\chi^2 = 6.5$, $P < 0.05$). Based on approximate 95% confidence intervals, the East site had significantly higher total percent emerged from planted four-year old roots measured in 2002 than the West site, which was within the same confidence interval as the Central site.

In 2003, five-year old plants emerged at the beginning of April. Across sites, plants reemerged from 59% of plants that had emerged in 2002. At the West site, two roots that had not emerged in 2002 emerged in 2003 and at the East site, one root which had not emerged in 2002 emerged in 2003. Thus, the phenomenon of seasonal dormancy was observed for planted American ginseng roots. The percent emerged roots was significantly different between sites ($\chi^2 = 16.7$, $P < 0.01$). Based on approximate 95% confidence intervals, the Central site had significantly lower percent reemerged plants (five-year old) in 2003 than the West or East sites, which were similar.

During the second season of growth (2003), there was a much higher percent (59%) of reemerged plants from originally transplanted four-year old roots than from originally transplanted two-year old roots (6%). Transplanted four-year old rootlets, which were much larger than transplanted two-year old rootlets, may have been better

able to survive the drought in 2002 because of greater carbohydrate storage and/or drought tolerance. Thus, transplanting four-year old rootlets may allow for greater establishment of ginseng populations by having a greater probability of survival. These results are similar to previous reports of lower mortality rates for older plants (Lewis, 1984; Carpenter, 1982; Lewis, 1983; Charron, 1991; and Carpenter, 1982).

Shoot morphology.

In 2002, emerged plants from four-year old roots had one-, two-, or three-prongs (Table 43). Only one of sixty-one plants had one-prong (2%). Across locations, 64% of plants had two-prongs and 34% had three-prongs. During the second season (2003), five-year old plants had two-, three-, or four-prongs. Across locations, the two-prong class was 28% of the total, the three-prong class was 67% of the total, and the four-prong class was 5%. The approximate 95% confidence interval of the difference between the two-prong and three-prong proportions at the West site was $33\% \pm 28$, indicating that the three-prong class was significantly larger. The approximate 95% confidence interval of the difference between the two-prong and four-prong proportions at the East site was $19\% \pm 23$, indicating that the proportions in these classes were not different.

Table 43. Percentages by soil treatment of plants emerged and in morphological classes of American ginseng plants grown from four-year-old roots in 2002 and from five-year old roots in 2003 at three forested experimental sites in Maryland.

Morphological class		C	G1	G2	L1	L2	Total
		-----%					
2002	Emerged	67	25	42	54	50	48
	1-prong	0	0	8	0	0	2
	2-prong	67	75	67	54	58	64
	3-prong	33	25	25	46	42	34
2003	Emerged	60	40	47	47	67	52
	1-prong	0	0	0	0	0	0
	2-prong	22	38	43	0	40	31
	3-prong	67	63	57	100	50	64
	4-prong	11	0	0	0	10	5

Fruitset.

Four-year plants set fruit from early July to mid-August 2002. Twenty-nine of the sixty-one plants (48%) produced fruit. At the West site, 71% of plants produced fruit after an average of 106 days (Table 41 and Table 44). At the Central site, 25% of plants produced fruit after an average of 72 days. At the East site, 50% of plants produced fruit after an average of 113 days. Days to fruitset were similar for the West and East sites and significantly shorter for the Central site (Table 44). Percentage of plants setting fruit were significantly different between sites ($\chi^2 = 7.8$, $P < 0.05$). Based on approximate 95% confidence intervals, the Central site had significantly lower percent plants setting fruit than the West site, which was in the same confidence interval as the East site. Low fruit set at the Central site may have been due to an absence of pollinators [i.e. *Dialictus* (Halictid sweat bees)], which may increase pollination and fruit production.

In 2003, five-year old plants set fruit in August. Twenty of the thirty-two plants (63%) produced fruit. At the West site, 64% of plants produced fruit. At the Central site,

no plants produced fruit. At the East site, 62% of plants produced fruit.

Aboveground Absence.

In 2002, plants that emerged from transplanted four-year old roots began to be absent aboveground at all three sites between the first and third weeks of May. The average number of days until plants became absent occurred in August at all three sites (Table 44). There was no difference in days to absence between sites. Although days to fruitset and days to absence were similar, the sample size for each was different, since plants that became absent before maturity did not set fruit.

Table 44. Site means and ANOVA for days to fruitset and days to absence of American ginseng plants grown from four-year old roots at three forested experimental sites in Maryland in 2002.

<u>Site</u>	<u>Days to fruitset</u>	<u>Days to absence</u>
West	106	108
Central	72	112
East	113	106
Standard Error	2	4
<u>Source of variation</u>	<u>F-value</u>	
Site (S)	61.2***	0.2
Treatment (T)	0.1	1.0
S x T	0.1	0.8

*, **, *** indicates significance at the $p < 0.05$, 0.01 , and 0.001 level, respectively.

Conclusions

These experiments studied the emergence, absent aboveground, and establishment of American ginseng from transplanted two- and four-year old roots in three physiographic regions in Maryland that were grown under different calcium soil amendment regimes. The study monitored ginseng growth from planted roots over two growing seasons at two sites within the native range of ginseng and one site outside ginseng's native range. Although ginseng was grown successfully from transplanted two- and four-year-old roots in forests in three physiographic regions of Maryland, a higher percentage of transplanted roots emerged at the Coastal Plain (East) site than the Piedmont (Central) or Ridge and Valley (West) sites. While the percent emergence of plants from transplanted roots was highest at the East site, the percent emergence of plants from seeds at the East site was the lowest. The reason for the differences between seeds and roots is not apparent. Due to small sample size, the effects of lime and gypsum on growth parameters were not statistically analyzed. Transplanted four-year old roots were much more likely to survive than two-year old roots and probably provide for better establishment than transplanted two-year old roots. Because transplanted four-year old roots produced fruit, they were considered mature and harvestable. However, more research is needed to predict the potential market value of ginseng per hectare in Maryland. In addition, future experiments should have greater sample sizes in order to better address the roles environment, soil type, and soil nutrients play in growth of American ginseng from transplanted roots.

CHAPTER 4: SUMMARY AND CONCLUSIONS

Growing wild-simulated American ginseng (*Panax quinquefolius* L.) as a non-timber forest product may be a profitable way to improve the sustainability of Maryland forests as well as a way to reduce the threat of the species' extinction in the wild. Experiments were conducted in Eastern, Central, and Western Maryland forests in order to determine factors affecting Maryland ginseng production. The experiments investigated the phenology and establishment of wild simulated American ginseng grown from seeds and transplanted roots. Based on the previous reports that indicate that adding calcium to the soil enhances American ginseng growth and survival, the experiments also included lime and gypsum treatments. The response variables measured included soil nutrient status, plant emergence, phenology, and morphology for the first two years after planting. The objectives of these studies were to learn about and compare the growth potential of wild-simulated American ginseng in the three physiographic regions of Maryland.

Ginseng populations grown from seed in two native forests (Piedmont and Ridge and Valley region) had higher percent emerged plants and higher plants present aboveground in August than in a non-native (Coastal Plain region) forest. Lime application increased the percent emerged and percent present plants at the Coastal Plain forest whereas gypsum did not affect growth measures. Based on curves of percent present aboveground over time, it appeared seeded populations may develop following a Type III growth curve, which is characterized by high mortality in young plants and lower mortality as plants become older.

Lime application on seeded experiments raised soil pH and Ca at all three sites and gypsum decreased Mg and K at the Piedmont and Ridge and Valley sites. For these forests in Maryland, soil pH less than 4.8, Ca less than 500 mg/kg, Mg less than 25 mg/kg, K less than 100 mg/kg, or Fe greater than 250 mg/kg was associated with fewer percent emerged plants, higher percent absent aboveground plants in August, and higher percent present aboveground plants in August, suggesting that these nutrients play a role in the growth and development of seeded American ginseng populations in these forests.

Ginseng populations may also be established by transplanting roots. Although transplanted two- and four-year old roots grew in all the three Maryland forests, transplanted four-year old roots survived better than transplanted two-year old ginseng roots. The greatest establishment from transplanted four-year old roots occurred at the Coastal Plain forest, which is contrary to the results of seeding ginseng in that forest. In future studies, sample sizes of experiments with transplanted ginseng roots will need to be larger than twenty-five in order to examine the effects of soil amendments on growth.

More research is needed to address whether wild simulated ginseng grown from seed or transplanted roots will survive to maturity. In addition, for mature populations grown from seed and transplanted roots, yield and crop quality will need to be determined. Further study is needed to address the role that soil fertility plays in the growth of seeded ginseng populations after the second growing season until maturity and harvest. Finally, more research is needed to examine the interrelationships of environment and soil fertility with the growth and establishment of wild simulated American ginseng populations.

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