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Growth characteristics of American Ginseng (*Panax quinquefolius* L.) woods and field - cultivated at Northern Europe

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Abstract. In Latvia, Northern Europe, American ginseng was grown in three forest types with different dominant species, as well as in agricultural field conditions - cultivated under artificial shade with three different types of mulches. Field cultivation yielded higher yields, root length, and root weight than wood cultivation under dominant species *Corylus avellana, Betula pendula,* and *Picea abies*. Mulching had a positive impact on ginseng growth in the field. Mulching with straw and buckwheat hulls resulted in longer and heavier roots.
In American ginseng roots, the contents of six ginsenosides were determined: Rg1, Re, Rb1, Rc, Rb2, and Rd. Re was the most abundant ginsenoside, followed by Rb1 > Rd > Rg1 = Rb2 > Rd. The total content of ginsenosides in our study did not reach the 4 percent threshold set by US Pharmacopeia.
These findings show that *Panax quinquefolium* can be grown in Northern Europe at 57°N, but it takes more than four years to achieve adequate yields and ginsenoside content.

Key words: *Panax quinquefolium*, woods - cultivated, field - cultivated, ginsenosides, Northern Europe.

INTRODUCTION

Farmers in the Nordic - Baltic region have recently expressed an increased interest in cultivating novel medicinal and aromatic plant species, such as honeysuckle (Vinogradov et al., 2020), thyme, and lavender (Nekrošiene, 2009). Additional knowledge on their adaptation to various climates, production practices, and the best varieties is, nevertheless, required.

American ginseng (*Panax quinquefolius* L.) is a native flora of Northern American hardwood forests, where it has been harvested in the wild for the last three centuries (Burkhart et al., 2021) with increasing intensity, eventually reaching over - exploitation levels. Since the late 1800s, American ginseng has been cultivated to meet the increasing demand (Mcgraw et al., 2013; Liu et al., 2021). American ginseng can be grown in temperate climates with cold winters because their seeds require cold stratification to

break dormancy and germinate (Olson, 2014; Schmidt et al., 2019). The upper Midwest of the United States and Ontario, Canada are major cultivation regions (Liu et al., 2021), but there are reports of American ginseng cultivation in various Northern and Southern Hemisphere countries, such as New Zealand (Chen et al., 2020), Poland (Ligor et al., 2005), Denmark (Olson, 2014), France (Men-Olivier et al., 2006), and China (Nian He et al., 2008). American ginseng grows naturally in latitudes ranging from 30 to 50°N, and its northern limit in Canada is 53°N. Our experiments were conducted in Northern Europe under the Nemoral Climate zone at 57°N, but the proximity to the North Atlantic Ocean and the dominating west and south - west winds make winters warmer than indicated by latitude (Olson, 2014).

American ginseng can be grown in two different agroecosystems: in the forest or in an agricultural field with artificial shade. Ginseng cultivation in raised beds in agricultural fields (also known as 'field - cultivated ginseng') is the most common method of production in North America. This type of cultivation increases root yields and reduces harvesting time from 8 to 4 years (Lim et al., 2005; Roy et al., 2011; Liu et al., 2021). Mulching, soil density, bed shape, seedling density, light intensity, and other agrotechnological methods can be used to manage root size, shape, and yield (Lim et al., 2005; Roy et al., 2011).

There are two approaches for ginseng cultivation in the forest: so called 'woods cultivated' and 'wild-simulated.' The first approach is an intensively managed approach that includes raised beds, vegetation management, and soil tillage in a forest setting. The second method is to cultivate ginseng in forest soil with minimal intervention, such as site preparation before sowing seeds or planting plantlets in the forest (Burkhart et al., 2021).

Ginsenosides are unique components that contribute to the pharmacological activity of ginseng roots. Rb1, Rb2, Rc, Rd, Re, and Rg1 are the six major ginsenosides found in American ginseng (Fournier et al., 2003). The European Pharmacopoeia (01/2016:1523) does not list American ginseng. The dried root of *P. quinquefolius* is listed in the US Pharmacopeia with quality criteria of at least 4% total ginsenosides calculated on a dried basis. Climate, seasonal changes (Hao et al., 2020), light levels (Fournier et al., 2003), soil fertility and calcium content in soil (Konsler et al., 2019), mulching (Roy et al., 2011), plant population, and plant age all influence ginsenoside content (Lim et al., 2005).

Given that there are no research reports on American ginseng cultivation in Latvia, the goal of this study was to determine American ginseng cultivation potential in Latvia.

The study's main objectives were to assess root length, fresh root weight, and ginsenoside content in four-year-old roots of *Panax quinquefolius* grown in Latvia in various woods - cultivated and field - cultivation settings. Three different forest types were investigated, as well as three types of artificially shaded agricultural fields marked by different, locally available mulches.

MATERIALS AND METHODS

Seed propagation and planting

Seedlings were grown from stratified seeds purchased from a commercial supplier (Harding's wild mountain herbs). In March 2018, the seeds were sown in pots and grown in a nursery with an average moisture content of 56%, an average temperature of 16 °C,

sodium lamps with light intensities ranging from 30,00 to 4,000 Lux, and a photoperiod of 16 hours.

In May 2018, plantlets were planted in three forest locations: first with dominant species *Coryllus avellana* (57°12'N 25°06'E), second with dominant species *Betula pendula* (57°14'N 25°12'E) and third with dominant species *Picea abies* (57°14'N 25°12'E). The species composition differed across the forest pilot sites (Table 1).

Table 1. Plant species in forest pilot sites

Forest layer	Dominant canopy species in woods		
	Corylus avellana	*Betula pendula*	*Picea abies*
Tree layer	*Pinus sylestris*, *Quercus robur*, *Tilia cordata*	*Betula pendula*	*Betula pendula*, *Picea abies*
Shrub layer	*Corylus avellana*, *Tilia cordata*, *Rubus idaeus*, *Acer plantonoides*, *Quercus robur*, *Padus avium*	*Amelanchier spicata*, *Rubus idaeus,* *Lonicera xylosterum,* *Picea abies*	*Amelanchier spicata*, *Lonicera xylosteum*, *Picea abies*, *Rubus idaeus*
Herb layer	*Galeobdolon luteum*, *Luzula pilosa*, *Solidago virgaurea*, *Lupinus polyphyllus*, *Fragaria vesca*, *Geum rivale*	*Aegopodium podagraria*, *Fragaria vesca*, *Dryopteris filix - mass*, *Veronica chamaedrys*, *Mycelis muralis*, *Lupinus polyphyllus*, *Equisetum sylvaticum*,	*Luzula pilosa,* *Lupinus polyphyllus*, *Solidago virgaurea*, *Fragaria vesca*, *Geum rivale*

Plantlets were planted in a shadowed system on raised soil beds in our organically certified agricultural experimental field (57°19'N 25°19'E) in June 2018. Shading was provided by wooden board roofing, with boards 10 cm wide and 2 cm apart, reducing solar radiation to 3–8 percent of full sunlight on sunny days and 6–30 percent on cloudy days. Each treatment bed measured 17 m long, 0.90 m wide, and 0.15 m tall. In both the - wood and field trials, the distance between plants was 10×15 cm. Mulch was used to cover the planting beds after they were finished. Mulching with buckwheat hulls, birch leaves, and reed (*Phragmites australis*) straws was tested.

All of the testing sites had sod-podzolic sandy loam soil. Table 2 summarises soil agrochemical properties. Because of the low pH level, granulated lime was applied at a rate of 400 $kgha^{-1}$ in all testing locations prior to sowing. Mechanical removal and traps (on the field) were used to control slugs, and traps were used to control rodents (in the forest). If necessary, hand weeding was performed.

Table 2. Soil agrochemical characteristics in woods and cultivated field trial sites

Properties	Dominant canopy species in the woods			Mulch types on the field		
	Corylus avellana	*Betula pendula*	*Picea abies*	Buckwheat hull	Birch leaves	Straw
pH KCl	5.6	4.3	4.3	5.4	5.4	5.4
Organic matter content, %	5.2	2.5	2.5	2.5	2.5	2.5
Ca mg kg^{-1}	1138	202	202	667	667	667
P_2O_5 mg kg^{-1}	15	67	67	131	131	131
K_2O mg kg^{-1}	100	68	68	194	194	194

Plant sampling and yield assessment

In October 2021, ten four-year-old plants were randomly selected from each experimental variant. The senescing aboveground shoot was removed, and the remaining underground part was washed and dried with paper towels, root length measured, and roots weighed. They were then pooled to determine the root ginsenoside content. Prior to ginsenoside analysis, samples were dried for 20 hours at a temperature of 45 °C. Each sample was analysed in three replications.

Chemical analysis

Ginsenosides Rg1, Re, Rb1 and Rf were purchased from Sigma-Aldrich. HPLC-grade acetonitrile and methanol were supplied by Fisher Scientific (Loughborough, UK). Deionized water was obtained through a Mill-Q system, purchased from Millipore, phosphoric acid was of analytical purity.

Dried roots were pulverized by a laboratory mill. Ultrasound-assisted extraction was carried out in an ultrasonic device with a thermostat. Samples (0.5 g) were extracted with 40 mL of 50% methanol at 70.0 °C for 60 min. Samples were cooled, centrifugated for 10 min at 4,000 rpm, and filtrated through a membrane filter with a nominal pore size 0.45 μm to remove the insoluble materials. The standard solution corresponding to 0.3 mg mL^{-1} of ginsenoside Rg1, Re, Rb1 and Rf was prepared by dissolving 3 mg of reference standards in 10 mL of methanol. The solution was sonicated for 5 min and filtered through a membrane filter with a nominal pore size 0.45 μm.

Chromatographic analyses were performed on a HPLC system Agilent 1290 Infinity II series (Agilent Technologies, Germany). LC separations were achieved based on the European pharmacopeia (01/20216:1523) by using an Agilent Eclipse XDB-18 3.5 μm, 4.6×150 mm (Zorbax) column (35 °C) with water as a mobile phase A adjusted to pH 2 with phosphoric acid and acetonitrile as mobile phase B, using the following gradient program: 0–8 min (20% B); 8–40 min (20–40% B); 40–45 min (40–60% B); 45–47 min (60–95% B); 47–52 min (95% B); 52–55 min (95–20% B); 55–75 min (95–20% B) with the flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL. Chromatograms were obtained on an Agilent WVD detector (Agilent Technologies, Germany) at a wavelength of 203 nm. The experimental data were handled using MassHunter Qualitative analyses 10.0 software (Agilent Technologies). For peak identification, retention times (tR) for standard solution and analysed samples were compared. The quantitative determination of sum of ginsenosides Rb1 and Rg1 in the samples expressed as percentage of dried drug was provided by inserting the related chromatographic peak areas in a mathematical equation, based on the European Pharmacopoeia.

Data analysis

T–tests were used to determine significance ($P \leq 0.05$) of mulching type under the shadowing system in field cultivation and the dominant tree species in woods - cultivation on root length and root weight. Two - way ANOVA with an ad - hoc *Tuckey test* was used to determine significance of growing conditions on composition of ginsenosides.

RESULTS AND DISCUSSION

Fresh root weight and length were significantly greater in 4-year-old field - cultivated plantlets than in 4-year-old woods-cultivated plantlets (Fig. 1). Fresh root weights among treatments ranged between 4.1 and 10.7 g in the field cultivation and 0.1 and 1.6 g in the woods cultivation, which is much less than the average fresh root weight of a 4 - year old ginseng taken in September (26.4 g) reported by Li and Wardle in Canada (Li & Wardle, 2002). Other authors have also reported that wood - cultivated ginseng has lower root weight than field - cultivated ginseng (Lim et al., 2005).

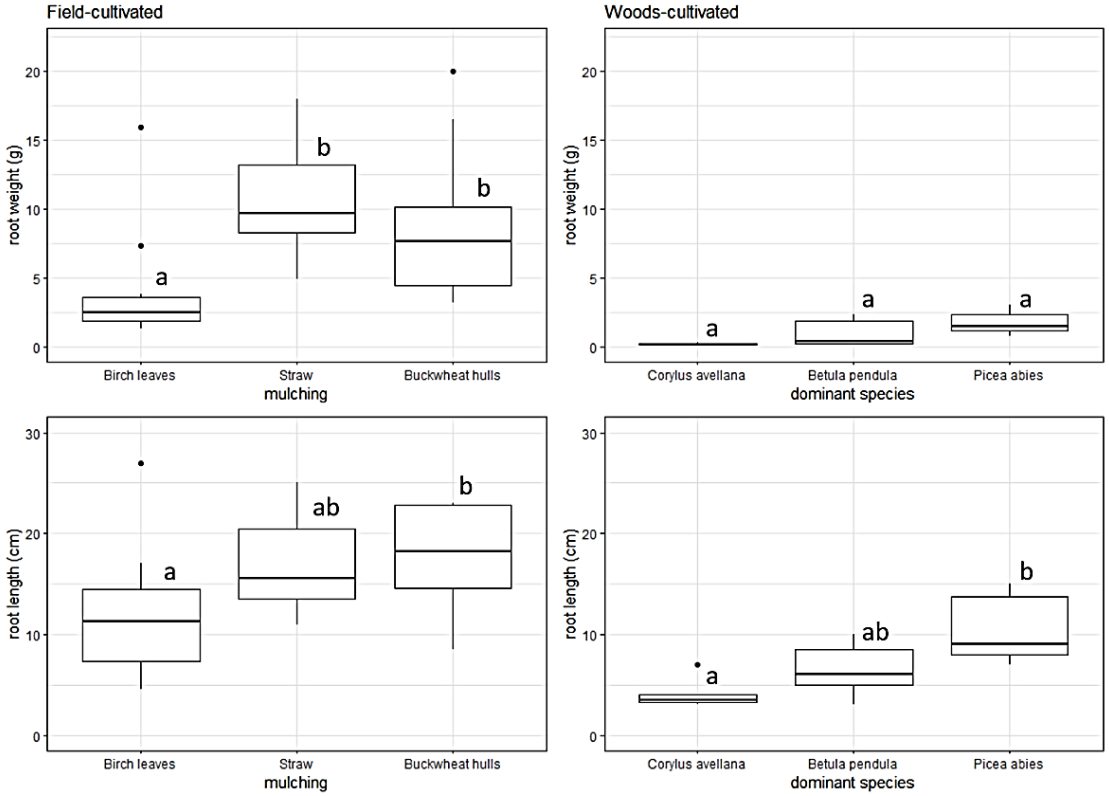

Figure 1. Boxplot of average fresh root length and weight of woods - cultivated and field - cultivated *P. quinquefolius.* Experimental variants marked with the same letter do not differ according to ad - hoc *Tuckey test* ($p > 0.05$).

The cultivation method and growing conditions are the key factors of root weight at harvest. Four years of cultivation is the minimum period required to obtain proper yield (Court et al., 1996). Oliver (1998) states that the average root weight for commercial harvesting after four years is 14 g; therefore, neither field cultivation nor woods cultivation produced sufficient root weight to meet this requirement. According to Li and Wardle (2002), American ginseng root weight increased with age (Li & Wardle, 2002), indicating that at least five years of cultivation is required in the Nordic - Baltic region to achieve the target root weight. Field cultivation yields the larger root weight, making it the more suited method for growing ginseng in Latvia.

Root length and weight were both affected by treatments within each agroecosystem. Mulch application on ginseng beds in field conditions is recommended as a common practice to mimic forest conditions and ensure more stable moisture and temperature conditions in the soil (Olson, 2014). Chopped straw is the most common mulch used by ginseng growers (Roy et al., 2011). The availability of materials and their impact on plant development, on the other hand, must be considered at each specific site. Buckwheat hulls, birch leaves, and reed straws were selected in the study based on their local availability.

The type of mulch used in field cultivation had a significant impact on root weight and length. Straw and buckwheat hull cover resulted in significantly longer root length and weight compared to birch leaf mulch and is therefore recommended for ginseng bed mulching. Ginseng cultivation was also tested in three woods with different dominant species. American ginseng naturally grows mainly in Northern American forests that are associated with *P. quinquefolium*, - *Arisaema triphyllum,* and *Acer saccharum* (Turner & McGraw, 2015). In this study three different Latvian forests with major cover species of *Betula pendula*, *Picea abies* and *Corylus avellana* were selected.

The average fresh root weight of cultivated ginseng in woods was very low - only 0.1–1.6 g - and did not differ significantly between forest types, but root length was greater in woods dominated by *Betula pendula* and *Picea abies* (Fig. 1).

This could be explained by a decrease in available nutrients in the soil, particularly phosphorus. One of the reasons for developing smaller roots is the lack of phosphorus (Choi et al., 2007). Dry conditions throughout the growing season in forest systems also contributed to lower root weight. This was most likely caused by precipitation being absorbed by the forest canopy.

While it is commonly known that forest - grown ginseng roots are more desirable on the market, they require longer time to produce - at least six years (Olson, 2014). As a result, we can conclude that a four-year period is insufficient to obtain commercially valuable *Panax quinquefolium* roots in *Corylus avellana*, *Betula pendula*, or *Picea abies* - dominated forests in Latvia. Furthermore, additional investments in improving soil composition, additional irrigation or preselecting forests with better summer moisture regime, and growing for longer seasons are suggested for the cultivation of American ginseng in woods.

P. quinquefolius roots are known to contain six characteristic active substances - ginsenosides Rg1, Re, Rb1, Rc, Rb2 and Rd. A total of 26 ginsenosides were separated and identified in analyzed samples by comparison of their retention behavior, among them 3 ginsenosides Rg1, Re and Rb1 were identified by commercially available external standards.

Six main ginsenosides (Rg1, Re, Rb1, Rc, Rb2 and Rd) were quantified by linear regression equations of standard curves, while all other ginsenosides were quantified by Rb1. All these ginsenosides were found in ginseng roots cultivated in Latvia (Table 3). All tested saponins in this study belong to the dammarane type ginsenosides. According to Zhang (2014), structurally, dammarane - ginsenosides are classified into protopanaxadiol (PPD) (Rb1, Rc, Rb2 and Rd) and protopanaxatriol (PPT) types (Rg1 and Re), according to the different positions of aglycones linked to the parent nucleus structure. The changes of PPT-/PPD - type ratio result in variance of the potency of bioactivity and further alter the overall quality of ginseng roots (Shan et al., 2014). Zhang et al. (2014) described variation of ginsenoside heterogeneity in different tissues - leaves,

rhizomes, and main roots. He found that the alteration of the proportions of the two dammarane types of ginsenosides may change along with years of cultivation. Ginsenoside - Rc and Rg1 show relatively higher changing ratios during the period from 1- to 13-year-old main root samples.

Table 3. Comparison of ginsenosides in woods cultivated and field cultivated 4 year oldginseng roots

Cultivation type	Ginsenoside composition (% on dried basis)						Sum of Rg1 and Rb1, %	Sum of all ginsenosides, %
	Rg1*	Re	Rb1	Rc	Rb2	Rd		
	Field cultivated with different mulching							
Buckwheat	0.10a	0.62a	0.52a	0.06a	0.16a	0.13a	0.62a	2.42a
Birch leaves	0.11b	0.70a	0.59b	0.07a	0.07b	0.13a	0.70b	2.02b
Straw	0.03a	0.63b	0.60b	0.08a	0.21a	0.23b	0.63a	3.05c
Assinewe et al. (2003)	0.25	1.75	1.88	0.36	0.13	0.48	2.13	4.85
	Wood - cultivated with different dominant canopy species							
Corylus avellana	0.45a	0.84a	0.38a	0.13a	0.09a	n.a.	0.84a	3.09a
Picea abies	0.15b	0.51b	0.36b	0.11a	0.09a	0.04a	0.51b	1.62b
Betula pendula	0.11b	0.68c	0.57b	0.11a	0.13b	0.06a	0.68ab	2.25c
Assinewe et al. (2003)	0.94	1.42	2.81	0.42	0.09	0.29	3.75	5.78

* Under each of the growing systems, ginsenoside content indicated with the same letter in the same column do not differ according to Tuckey test ($p > 0.05$).

In Canada, Assinewe et al. (2003) found no significant difference in ginsenoside content between four-year wood - cultivated and field - cultivated ginseng roots. We also observed similar results in total ginsenosides content in both wood and field cultivated ginseng in our study. However, unlike Assinewe (2003), the total content of ginsenosides in our study did not reach the 4% threshold set by US Pharmacopeia. However, the sum of Rg1 and Rb1 exceeded the 0.4% threshold set by European Pharmacopeia for *Panax ginseng* roots.

The variance in saponins could be due to harvesting data, growth conditions, or other factors. The highest concentration of ginsenosides was found to be Re and Rb1.

These findings are partially consistent with those of other studies (Lim et al., 2005; Qu et al., 2009). According to the abovementioned authors research, ginsenoside Rb1 is the most abundant and Re is the second most abundant ginsenoside in roots. Re > Rb1 > Rd> Rg1 = Rb2 > Rd was the overall relative abundance of the six ginsenosides.

The amount of ginsenosides in the roots increases as the ginseng becomes older (Court et al., 1996; Schlag & McIntosh, 2006). The levels of accumulated ginsenosides increase rapidly in the first four years of growth, then remain steady for the next four years, with the fifth year of cultivation representing a critical transitional period in the plant's life cycle (Zhang et al., 2014).

As a result, a longer period of cultivation is recommended for ginseng, both in field and forest growing conditions, to accumulate the required amounts of ginsenosides in Latvian growing conditions and to reach sufficient root weight.

CONCLUSIONS

Cultivating American ginseng in raised beds in field settings under wooden board roofing resulted in higher yields and root lengths than woods - cultivated ginseng. Mulching had a noticeable positive effect on ginseng growth. Straw and buckwheat hull mulches are more preferable than birch leaves.

The results of this study indicate that cultivation of *P. quinquefolius* is feasible in Northern European conditions at latitude 57°N, but that more than four years of cultivation is recommended to ensure acceptable yields and ginsenoside content.

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