Controlled-temperature, Aboveground Stratification of North American Ginseng Seed

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SUMMARY. Freshly harvested, immature (green) seeds of north american ginseng (*Panax quinquefolius* L.) were stratified for 12 months either traditionally in buried wooden boxes outdoors, or in plastic pails in a controlled environment room [$3 \pm 0.2^\circ\text{C}$ ($37.4 \pm 0.11^\circ\text{F}$)], $85\% \pm 5\%$ relative humidity) for about 9 months followed by about 3 months at $20 \pm 2^\circ\text{C}$ ($69.8 \pm 1.1^\circ\text{F}$). Embryo growth in Stage II (mid-May to late August when direct seeded) was more rapid [0.016 versus 0.009 mm·d⁻¹ (0.00062 versus 0.00035 inches/day)] under controlled-temperature conditions. Seedling emergence rate did not vary between treatments. Root dry weight (economic yield) was similar for seedling, 2, 3, and 4-year-old plants whether grown from traditionally or controlled-temperature stratified seed. Controlled-temperature stratification of north american ginseng seed is an acceptable alternative to traditional outdoor, in-ground stratification.

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North american ginseng, a perennial herbaceous plant grown for its fleshy root and seeds (Proctor, 1996; Proctor and Bailey, 1987), is propagated by seed (Proctor and Louttit, 1995).

Traditionally, ginseng seed harvested in August or September, is placed in a wooden stratification box for about 12 months and then direct-seeded into raised beds. Seeds germinate the following spring, some 18 to 22 months after seed harvest.

A number of problems in ginseng stand establishment have been linked to the seed stratification procedure. For instance, Proctor and Louttit (1995) reported premature seed germination (up to 10%) in the seed box after only 8 months of stratification, a condition likely due to fluctuating temperatures. These premature seedlings die and associated fungi may be potential pathogens that might colonize other seed in the box. Also, ginseng seed harbor pathogenic fungi and seed can be colonized during stratification despite fungicidal treatment of seed (Tianyi and Weiqun, 1992; Ziezold et al., 1998). Seepage of water from adjacent soil into stratification boxes may introduce unwanted fungi.

Because of these problems stratification of ginseng under controlled-temperature conditions and aboveground has been suggested as a viable alternative. The objective of this study was to compare the traditional method of seed stratification with a controlled-temperature, above-ground method.

Materials and methods

Two experiments were carried out at a Winco Canadian Ginseng Ltd., farm at Vanessa, Ontario, from September 1992 to October 1997 (Expt. 1) and from September 1993 to September 1997 (Expt. 2). Since seed handling was similar in each experiment, the common procedure is described and related dates are given. The fruit (berries) were hand-harvested in late August/early September in 1992 for Expt. 1 and in 1993 for Expt. 2. The berries were mechanically depulped, surface sterilized with formaldehyde and mixed with sand following traditional practices (Oliver, 1998; Ontario Ministry of Agriculture, Food and Rural Affairs, 1999; Proctor and Louttit, 1995).

Four stratification treatments were established. The seed-sand mixture was placed in the buried wooden stratification box on 10 Sept. 1992 (Expt. 1) and 21 Sept. 1993 (Expt. 2) where it remained until 23 Aug. 1993 and 23 Aug. 1994, respectively. This traditional in-ground stratification was considered the control. For aboveground indoor stratification (treatments 2, 3, and 4), nine samples, each of 10-L (0.35-ft³) plastic pails of seed-sand mixture, were taken randomly at the time of box filling and held at $3 \pm 0.2^\circ\text{C}$ ($37.4 \pm 0.11^\circ\text{F}$) for three different time periods: treatment 2 until 15 Apr., treatment 3 until 1 May, and treatment 4 until 15 May in each of the 2 years. There were three samples (pails) in each treatment. At the end of each

Fig. 1. Growth of ginseng seed embryo during stratification in the traditional in-ground seed box; and in the controlled temperature [$3 \pm 0.2^\circ\text{C}$ ($37.4 \pm 0.11^\circ\text{F}$)] cooler for 9 months (days of stratification and corresponding dates are shown) and room temperature [$21 \pm 2^\circ\text{C}$ ($69.8 \pm 0.11^\circ\text{F}$)] for 3 months; 25.4 mm = 1.0 inch.

cold [3 ± 0.2 °C (37.4 ± 0.11 °F)] stratification period the pails were moved to room temperature [21 ± 2 °C (69.8 ± 1.1 °F)] and held until seeding on 23 Aug.

Temperatures in the seed box and pails were measured with thermistors attached to dataloggers LI-1000; LICOR, Lincoln, Neb.) and recorded hourly. In the box the thermistors were inserted into the seed-sand mixture at 10, 25, and 50 cm (3.9, 9.8, and 19.7 inches) from the surface in the center of the box; thermistors were placed in the center of three of the pails.

Moisture content in all treatments was measured gravimetrically periodically during stratification and maintained at 10% to 12%. At intervals (see Fig. 1) during the stratification period 4 samples each of 20 seeds were removed from each of the four stratification treatments. The exocarp of the seed was removed and embryo length was measured.

On 23 and 24 Aug. in 1993 and 1994, seeds were sown at a rate of 90 kg·ha⁻¹ (80.3 lb/acre) with a Stanhay seeder in a randomized complete block design with 4 blocks. The amount of seed available from stratification allowed seeding of about 130 m² (1416 ft²) in each block.

Seedling emergence counts in four 1-m² (10.8-ft²) plots in each treatment in each block were made each spring at 2 to 5 d intervals until maximum values were obtained. Seedlings were considered emerged when they appeared through the straw mulch. The sigmoidal curve of seedling emergence (Fig. 1) was divided visually into two parts: stage I consisted of a rapid emergence phase (to about day 20) while stage II was where final emergence was attained and remained stable thereafter. The stage I emergence rates were compared by analyzing the emergence percent per day using linear regression. In stage II final emergence was compared using the statistical analysis system (SAS) program package (SAS Institute Inc., 1988).

Seedling harvests in both experiments were carried out in mid-August by excavating randomly 10 seedlings in each treatment in each block. Leaflet area, and the dry weight at 80 °C (176 °F) of the leaflets, stem and root were measured. An additional 10 seedling roots from each treatment in each block were excavated to measure dry

weight in October after leaflet senescence. Two-year-old root weights in both experiments were determined subsequently in October as for seedlings.

At the end of each experiment all roots in 1 m² in each treatment in each block were hand dug in October, washed free of soil, and weighed. All data were analyzed, and treatment means separated using Duncan's multiple range test at $P = 0.05$, using the SAS package (SAS Institute Inc., 1988).

Results and discussion

EMBRYO GROWTH. Embryo growth in the two stratification environments followed a similar two-step pattern (Fig. 1) in agreement with Proctor and Louttit (1995). Embryo growth did not vary with removal date so data from the three dates were combined. For the first 270 d (September to May, Stage I) embryo length increased slightly from about 0.65 to 0.8 mm [0.0005 mm·d⁻¹ [0.025 to 0.031 inch (0.000019 inches/d)]]]. In Stage II (May to seeding in August) embryo growth was significantly more rapid in controlled-temperature [0.7 to 2.0 mm = 0.016 mm·d⁻¹ (0.027 to 0.078 inch = 0.00062 inches/d)] than normal stratification [0.7 to 1.4 mm = 0.009 mm·d⁻¹ (0.027 to 0.055 inch = 0.00035 inches/d)]. The faster embryo growth rate in the controlled-temperature was probably due to higher

temperature, 21 versus 15.2 °C (69.8 versus 59.4 °F) in the seed box. Mean monthly temperatures at 25 cm (9.8 inches) in the seed box were 9.4, 13.5, 18.5, and 19.6 °C (48.2, 56.3, 65.3, and 67.3 °F) May to August respectively, similar to typical seed stratification box temperatures shown in Proctor and Louttit (1995).

EMERGENCE. The first seedlings emerged about mid-May and the canopy was fully established by early June. The sigmoidal relationship of seedling emergence with time over the four treatments is shown in Fig. 2. Based on seeding rate and the area seeded the calculated 100% seedling emergence would have been 260 seedlings/m² (24.2 seedlings/ft²). A final emergence of 68% in the control treatment (traditional stratification) is similar to the accepted ginseng industry expectation. This low percentage emergence is attributed to poor quality, ungraded and diseased seed (in spite of formalin treatment) before stratification (Ziezold et al., 1998).

The rate of seedling emergence in Stage I did not differ between treatments and the overall mean was 13 seedlings/d in Expt. 1 (Fig. 2). Similar results were obtained in Expt. 2 but the rate was 8.8 seedlings/d (data not shown). The cooler spring in 1995 probably accounts for the slower emergence rate in Expt. 2. We reported above that embryo length was greater at seeding time in seeds given con-

Fig. 2. Seedling emergence curves from May to August 1994 and corresponding days from planting (Expt. 1) of seed stratified under i) traditional in-ground conditions (control) and ii) controlled-temperature conditions with three seed removal dates.

Table 1. Effect of traditional stratification and of time of ginseng seed transfer (15 Apr., 1 May, or 15 May) from controlled temperature stratification [3 ± 0.2 °C, (37.4 ± 0.11 °F)] to warm temperature [20 ± 2 °C, (69.8 ± 1.1 °F)] on seedling growth ($6.45 \text{ cm}^2 = 1.0 \text{ inch}^2$, $28,350 \text{ mg} = 1.0 \text{ oz}$).

	Leaf area (cm ²)	Dry wt (mg)				Root:shoot ratio ^y
		Leaf	Stem	Root	Root (24 Oct.)	
Expt. 1. Measurements were taken on 11 Aug. and 24 Oct. 1994 (roots only)						
Traditional stratification	19.7 a ^z	82.5 ^{NS}	26.6 ^{NS}	274 a	388 ^{NS}	2.5 ^{NS}
Controlled-temperature stratification						
15 Apr.	18.1 b	85.9 ^{NS}	24.2 ^{NS}	281 a	376 ^{NS}	2.5 ^{NS}
1 May	18.5 ab	80.8 ^{NS}	26.5 ^{NS}	244 b	377 ^{NS}	2.3 ^{NS}
15 May	19.5 a	85.1 ^{NS}	25.9 ^{NS}	272 a	390 ^{NS}	2.4 ^{NS}
Expt. 2. Measurements were taken on 24 Aug. and 18 Oct. 1995 (roots only)						
Traditional stratification	15.9 ab	88.9 ^{NS}	26.0 b	295 ^{NS}	374 a	2.5 ^{NS}
Controlled-temperature stratification						
15 Apr.	16.9 a	90.9 ^{NS}	30.4 a	298 ^{NS}	341 ab	2.4 ^{NS}
1 May	15.0 b	82.6 ^{NS}	25.3 b	267 ^{NS}	301 b	2.5 ^{NS}
15 May	15.3 b	81.5 ^{NS}	29.9 a	270 ^{NS}	359 a	2.4 ^{NS}

^zMean separation within columns by Duncan's multiple range test, $P < 0.05$, are indicated by different letters, or nonsignificant (NS).

^yCalculated from dry weight, on 11 Aug. 1994 or 24 Aug. 1995.

trolled-temperature stratification. However, this difference did not result in a higher seedling emergence rate.

Ginseng seed used in commerce is ungraded, broadcast seeded at high rates, takes about 8 months between seed sowing and germination (Oliver, 1998) and is genetically diverse (Boehm et al., 1999). Therefore, lack of a relationship between embryo growth and seedling emergence is not surprising.

Seedling emergence continued through early June (Stage II) with significantly higher percentages for the 1 and 15 May removal dates (Fig. 2). However, by August, when natural leaf senescence started, there were no differences in seedling emergence between treatments.

SEEDLING YIELD. Few differences between seedlings from traditional and controlled-temperature seed stratification in both experiments were found (Table 1). The greater embryo growth rate in the controlled-temperature stratification (Fig. 1) did not result in greater growth of these seedlings. The root dry weights of 300 to 400 mg (0.0106 to 0.0141 oz) at the end of the first growing season are two to three times greater than those reported by Bailey and Stathers (1991). In Expt. 1 root dry weight on 11 Aug. was lowest for the 1 May transfer date but there were no differences on 24 Oct. mean of 383 mg (0.0135 oz). In Expt. 2 seedlings from the 1 May transfer date had a lower leaf area and stem dry weight but the greatest root dry weight on 18 Oct. Generally, the differences

in treatments were few and minor, probably reflecting the variable growth of seedlings from ungraded seed.

ROOT YIELD: 2-, 3-, AND 4-YEAR-OLD PLANTS. Root yield of 2-year-old plants was greater in controlled temperature stratification only in Expt. 1 (Table 2). There was no effect of treatment in Expt. 2, 2-year-old plants or in 3- or 4-year-old plants. This is not surprising as there were few differences in seedlings (Table 1). Root yields of 3-year-old plants are similar to those reported previously (Proctor et al., 1999).

We have demonstrated that aboveground, indoor controlled-temperature stratification of north american ginseng seed is a viable alternative to the traditional method of outdoor in-ground stratification. Some growers are now using this new technology while others have partially adapted it.

Partial adaptation may include in-ground stratification for the first 8 months (August to April) when the necessary cooler space is full of roots. After this initial period the seed-sand mixture is transferred to containers, e.g., and held in coolers at about 20 °C (68 °F) until seeding in late August or early September

Another possible application of this new technology may be in ginseng seed stratification for spring rather than fall seeding. Hovius (1996) has shown that the traditional 18 to 22 month stratification period of low-high-low temperatures can be reduced to 8 or 9 months of high [15 to 20 °C (59 to 68 °F)] temperatures for about 4 months followed by low [3 to 5 °C (37.4 to 41 °F)] temperatures for about 4 months. Stratification in containers held at room temperature (about 20 °C) for the first

Table 2. Effect of traditional stratification and of time of ginseng seed transfer (15 Apr., 1 May, or 15 May) from controlled temperature stratification (3 ± 0.2 °C [37.4 ± 0.11 °F]) to warm temperature [20 ± 2 °C (69.8 ± 1.1 °F)] on 2, 3, or 4-year-old root yield ($\text{kg} \cdot \text{m}^{-2}$) ($1 \text{ kg} \cdot \text{m}^{-2} = 1.84 \text{ lb/yd}^2$).

	Expt. 1		Expt. 2	
	Root yield (kg.m ⁻²) in year			
	2	4	2	3
Traditional stratification	1.06 b ^z	2.38 ^{NS}	1.34 ^{NS}	2.13 ^{NS}
Controlled temperature stratification				
15 Apr.	1.40 ab	2.39 ^{NS}	1.38 ^{NS}	2.15 ^{NS}
1 May	1.60 a	2.29 ^{NS}	1.26 ^{NS}	1.87 ^{NS}
15 May	1.72 a	2.14 ^{NS}	1.29 ^{NS}	1.77 ^{NS}
Mean	1.44	2.30	1.32	1.98

^zMean separation within columns by Duncan's multiple range test, $P < 0.05$, are indicated by different letters, or nonsignificant (NS).

4 months and then placement in coolers at 3 to 5 °C for 4 months would satisfy the stratification requirements for spring (May) seeding. In conclusion, indoor controlled-temperature stratification of ginseng seed is an acceptable alternative to outdoor stratification as it allows control over the environment, easier seed handling, avoids premature seed germination and may have application in spring seeding.

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