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Low-Temperature Storage of Immature (Green) North American Ginseng Seed for Fall Planting

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Abstract : Freshly harvested, immature (green) seeds of North American ginseng (*Panax quinquefolius* L.) were stratified for up to 3 years in plastic pails in controlled environment rooms at 5±1°C for 9 months and then 21± 2°C for 3 months (Trt. 1, regular stratification), or continuously at -2±0.2°C (Trt. 2), or continuously at 3±0.2°C (Trt.3). During stratification at -2 and 3°C embryos did not grow. On seeding in the field embryos grew rapidly and resultant seedlings were comparable to those from regularly stratified seed. Seedling emergence rate was acceptable at the industry expected rate of 68% after one year of storage, but not after two years storage when it declined to 17.5%. Seed rot was so severe in year 3 that no planting was carried out. Seedling and second year growth were similar at the three stratification temperatures; most importantly, root dry weight (economic yield) was similar. Low-temperature storage of freshly-harvested North American ginseng seed is an acceptable method for short-term retention of propagating material.

Key words : *Panax quinquefolius*, root weight, seed stratification

INTRODUCTION

North American ginseng (*Panax quinquefolius* L.) is a slow growing perennial herbaceous plant that is native to the under-story of eastern North American hardwood forests.[1] It is cultivated under artificial shade for its highly valued fleshy root and seeds.[2,3]

Seeding is still the principal method of propagating ginseng, yet little is known about seed after-ripening, stratification, and germination, particularly for American ginseng.[1] In North America, ginseng seeds are after-ripened in stratification boxes outdoors after they are collected in Aug./Sept.[1] They will not grow until the second spring following harvest (18-22 months).

Although stratification of American ginseng seed is an established practice, information about seed development and temperature in stratification boxes from box filling to 12 months later, when the seed are direct-seeded in the field is sparse. During stratification and seeding (14 months), three embryo stages have been identified.[4] In Stage I, 250 days (Sept. to mid-May), embryo length increased from about 0.5 to 1.0 mm; in Stage II, 100 days (mid-May to late Aug. when seeded), length increased to 2.0 mm and in Stage III, 90 days (late Aug. to late Nov.), length increased to 5.3 mm. Endocarp split width could also be placed in three stages. Embryo length increased with endosperm length. Instron compression tests showed that the endocarp softened rapidly in late Stage II and throughout Stage III. The stratification box temperatures at all depths (10, 20, and 50cm) never dropped below -2°C even when the air temperatures dropped to -13°C so no seed damage occurred.

Proctor *et al.*,[5] stratified freshly harvested, immature ("green") seeds of North American ginseng for 12 months either traditionally in buried wooden boxes outdoors, or in plastic pails in a controlled environment room (3±0.2°C, 85±5%RH) for approximately 9 months followed by about 3 months at 20±2°C. Embryo growth in Stage II (mid-May to late Aug. when direct seeded) was more rapid (0.016 vs. 0.009 mm/day) under controlled-temperature conditions. Seedling emergence rate did not vary between treatments. Root dry weight (economic yield) was similar for seedling, two, three and four-yr-old plants whether grown from traditionally or controlled-temperature stratified seed. Indoor controlled-temperature stratification of North American ginseng seed is an acceptable alternative to traditional outdoor, in-ground stratification as it allows control over the environment, easier seed han-

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dling, avoids premature seed germination and may have application in spring seeding.[6] The Canadian ginseng industry has widely accepted controlled-temperature, above-ground stratification [5] and incorporated it into its production systems.[2]

Ginseng seed yield varies from year to year resulting in a surplus of seed in some years. If this seed could be maintained in a dormant state for several years it would ensure an adequate supply of seed for planting. The objectives of this study were to compare long-term stratification of immature (green) seed in controlled environments at 3 and -2°C and to evaluate subsequent plant establishment and crop growth in field plantings.

MATERIALS AND METHODS

This experiment was carried out at Winco Ginseng Ltd., farms near Delhi, Ontario starting in September 2001. Seeds were obtained from fruit (berries) hand-harvested in early September 2001. The berries were mechanically depulped, surface sterilized with formaldehyde and mixed with mortar sand following traditional practices.[2,4]

Three stratification treatments were established. The seed-sand mixture was placed in above-ground stratification boxes held in a controlled environment room (5±1°C, 85±5% relative humidity and then 21±2°C for 3 months)[5] This above-ground stratification was considered the control (Trt. 1). For the other two stratification treatments (Trt. 2 and Trt. 3), two sets of nine samples of 10L plastic pails of seed-sand mixture were taken randomly at the time of filling of the above-ground stratification boxes. Nine of these pails were held at -2±0.2°C (Trt. 2) and the other 9 pails at 3±0.2°C (Trt. 3). At three different dates, August 8, 2002, August 21, 2003 and August 15, 2004 one pail of seed from each treatment was seeded into a commercial field at a rate of 90 kg /ha with a Stanhay seeder. The exceptions were that the controls (Trt. 1) in 2003 and 2004 were of 11 month stratified seed taken from above-ground stratification boxes.

At intervals during the stratification period 4 samples each of 20 seeds were removed from each of the 3 stratification treatments. The endocarp of the seed was removed and the embryo length measured.

Seedling emergence and plant counts in four, one-m^2 plots in each treatment were made on the first planting on September 17, 2003 and on September 15, 2004, and on the second planting on September 15, 2004 and on September 15, 2005. Seedling harvests were carried out for the first two plantings on September 17, 2003 and on September 15, 2004. Two-year-old plants were harvested from the first planting on September 15, 2004 and from the second planting on September 15, 2005. Four replicates each of 10 seedlings, and two-year-old plants, were harvested and stem length, leaf area, and dry weight at 80 degrees C of the leaves, stems, and roots were measured.

All data were analysed, and treatment means separated using Duncan's multiple range test at P=0.05, using the Statistical Analysis System package (SAS Institute Inc., Cary. N.C.).

RESULTS AND DISCUSSION

Embryo growth

Embryo length in the controls increased in the first year (Sept./2001 to Aug./2002, planting time 1) from 1.03 to 2.12 mm (Table 1); embryo length of about 2 mm is expected at seeding time.[4] Further embryo lengthening would have taken place in the field in Fall 2002 and the seedlings emerged and grew in the field in 2003 – see Table 2 for harvest data of these seedlings on September 17, 2003. Embryo length of the "green" seed at both stratification temperatures remained unchanged, range 0.68 to 0.70 mm, over the first year (Table 1, Sept.5/2001 to Aug. 8/2002). However, once planted in the field embryo growth was rapid, seedlings emerged the following spring and growth in all treatments was similar – see Table 2 for harvest data for September 17, 2003.

Embryo length for controls at planting time 2 (Aug.21/

Table 1. Changes in ginseng seed embryo length at three stratification temperatures over three years of the experiment.

Treatment (Trt.) and stratification temperature (°C)		Embryo length (mm)				
		Sept. 5, 2001	Oct. 29, 2001	Aug. 8, 2002 Planting time 1	Aug. 21, 2003 Planting time 2	Aug. 15, 2004 Planting time 3
Trt. 1	Control (5/21)[z]	1.03	1.68	2.12	2.07	2.10
Trt. 2	-2°C	0.70	0.69	0.69	0.80	0.80
Trt. 3	3°C	0.70	0.69	0.68	0.68	0.88

[z]Samples of control seeds on Sept. 5/2001, Oct. 29/2001 and Aug. 8/2004 were from the same seed lot. Samples on Aug. 21/2003 and Aug. 15/2004 were 11 month stratified seed from above-ground stratification boxes.

Table 2. Effect of stratification temperature (Control, -2 and 3°C) and planting time 1 (seeded August 8, 2002) and time 2 (seeded August 21, 2003) on seedling and two-year-old plant number and growth.

Treatment (Trt.) and stratification temperature (°C)		Plant number (m^2)	Stem length (mm)	Leaf area (cm^2)	Dry wt. (mg)		
					Leaf	Stem	Root
Seedling growth, planting time 1 (measurements taken on September 17, 2003)							
Trt. 1	Control	207 a	13.5 b	21.3 NS	80 NS	30 b	316 NS
Trt. 2	-2	126 c	13.1 b	22.1 NS	86 NS	28 b	303 NS
Trt. 3	3	156 b	16.4 a	21.3 NS	85 NS	33 a	290 NS
Seedling growth planting time 2 (measurements taken on September 15, 2004)							
Trt. 1	Control	175 a	14.3 a	23.2 a	92 NS	31 NS	442 NS
Trt. 2	-2	37 b	12.1 c	22.1 ab	101 NS	29 NS	424 NS
Trt. 3	3	25 b	13.1 b	20.6 b	93 NS	29 NS	397 NS
Two-year-old plant growth, planting time 1 (measurements taken on September 15, 2004)							
Trt. 1	Control	194 a	168 b	151 b	502 b	122 b	2081 b
Trt. 2	-2	134 b	167 b	188 a	675 a	142 a	2543 a
Trt. 3	3	131 b	182 a	175 a	627 a	144 a	2505 a
Two-year-old plant growth, planting time 2 (measurements taken on September 15, 2005)							
Trt. 1	Control	146 a	181 a	193 a	750 b	155 a	3473 b
Trt. 2	-2	32 b	166 a	212 a	945 a	147 a	4860 a
Trt. 3	3	30 b	172 a	172 b	773 b	136 b	3984 b

[z]Mean separation within columns within planting time/plant age by Duncan's multiple range test, P=0.05, is indicated by different letters, or non-significant, NS

2003) and planting time 3 (Aug.15/2004) were similar, 2.07 vs 2.10 mm (Table 1). Embryo length of "green" seed held at -2 and 3°C until planting times 2 and 3 changed little, range 0.68 to 0.88 mm (Table 1) since the start of stratification. Seedlings from planting time 2, like those in planting time 1, grew similarly in all treatments (see Table 2, harvest data for September 15, 2004).

Sometimes premature germination of ginseng seed occurs during the stratification process.[4] No premature germination was observed in this green seed held at either -2 or 3°C over the 3 years of stratification.

Plant counts and growth

Based on the seeding rate and the area seeded the calculated 100% seedling emergence would have been 260 seedlings/m^2. Emergence for controls from the two first planting times was 80% (207) for time 1 and 67% (175) for time 2 (Table 2). This variation is an indication of year to year variation in seed crops and growing conditions. The accepted ginseng industry seedling emergence rate is 68%. Seedling emergence from the two low temperature treatments was 61% (126) and 75% (156) for -2 and 3°C respectively (Table 2), a mean of 68% which is the accepted ginseng average. Holding the seed an additional year gave very low emergence rates of 21% (32) and 14% (25) for -2 and 3°C respectively (Table 2).

When the seeds were prepared for planting in year 3 seed rot exceeded 50% in both Trt. 2 and 3 so the seed were not planted in the field.

There were few differences in growth between the seedlings within the two planting times (Table 2). The economic yield (root dry weight) was higher for planting time 2 than 1 (means of 421 mg and 303 mg respectively). Again, this difference is likely a reflection of growing location and seasonal growing conditions.

The two-year-old plant emergence for controls from planting time 1 was similar to seedling emergence (75% (194) vs 80% (207)) [Table 2] but lower in planting time 2 (71% (175) vs 56% (146)). Emergence of two-year-old plants in planting time 1 in Trts 2 and 3 was similar to that of seedlings at 68% but very low, 21%, in planting time 2 (Table 2). There were few differences within the two planting times between the growth of the plants in the second year as found with seedlings (Table 2). Root growth was dramatic in the second year, particularly for planting time 2 where root weight increase was about ten-fold, from a mean of 421 mg to 4106 mg (Table 2).

These data show that green seed can be stored at low temperature and successfully used for crop establishment. However, the increasing amount of seed rotting with time suggests that pathogen-free seed and clean stratification sand must be used. Seed surface sterilization and a seed

treatment with an appropriately registered fungicide e.g. Senator® PSPT (thiophanate methyl), which awaits registration in Canada, should increase seed germination. Another approach to the long-term storage of ginseng seed for a reliable source of planting stock has been suggested by Lee *et al.*[7] They showed that stratified, cracked seeds of Korean ginseng (*Panax ginseng* C. A. Meyer) could be dried and stored at 5°C and 30% relative humidity. Seed so stored retained viability and had 73.5% germination after 7 years. Unfortunately, the seed was not field tested for crop establishment. This approach with Korean seed needs to be evaluated using North American stratified seed. In addition, the seed should be planted into the field and evaluation of seedling emergence and subsequent plant growth and performance carried out.

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