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Extended Stratification of North American Ginseng Seed

John T.A. Proctor[#] and Audra Stechyshyn-Nagasawa*

*[#]Department of Plant Agriculture and *Department of Environmental Biology,*
University of Guelph, Guelph, Ontario, N1G 2W1, Canada

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Abstract : The North American ginseng (*Panax quinquefolius* L.) seed crop varies from year to year. The ability to hold stratified seed for a year would ensure continuity of seed supply and no interruption in production cycles. Seed drying and rehydration protocols at room temperature (21±2°C) were developed. These protocols and seed storage at 4 ±1°C and 35%, or variable, relative humidity (RH) allowed the holding of stratified seed for one year and then establishment of the following five treatments in field plots: Trt.1 : dried 2005 stratified seed (seed harvested Fall 2004) held at 4°C and at variable humidity ; Trt.2 : 2006 stratified seed planted directly into the field; Trt.3 : 2005 stratified seed dried in October 2005 and held at 4°C and 35% RH ; Trt.4 : 2005 stratified seed held in moist sand from October to December 2005 at room temperature (21±2°C) and then in December dried and held at 4°C and 35 % RH; Trt.5 : 2005 stratified seed held in moist sand from October to December 2005 at room temperature and then in December dried and held at -12°C. Seedling emergence was best in Trts. 2 and 4 with 67.3 and 65.1% respectively which is similar to the industry expected rate of 68% after regular stratification. Seedling growth was similar in Trts. 2 and 4 with root dry weights of 172 and 159 mg respectively in mid-August. Therefore, if holding stratified seed in August/September for one year is desired, the seed can be placed in moist sand until December and then dried and stored at 4°C and 35% RH. These seed can be planted in the following August/September and will germinate and grow in the following year to give an acceptable crop.

Key words : *Panax quinquefolius,* propagation, seed dormancy

INTRODUCTION

North American ginseng (*Panax quinquefolius* L.) is a slow growing perennial herbaceous plant that is cultivated under artificial shade for its highly valued fleshy root and seeds.[1,2] Seeding is the principal method of propagating ginseng, yet little is known about seed after-ripening, stratification, and germination. [3] In North America, ginseng seeds are after-ripened in stratification boxes buried outdoors, or aboveground in controlled environments after they are collected in August/September.[1,4] These seed will not germinate and grow until the second spring following harvest (18-22 months). Although stratification of North 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.[5] Mature stratified seed at seeding in late August falls into Stage III and has an embryo length of about 2 mm which increases in the soil to about 5.3 mm by late November.

In some growing seasons in southern Ontario, as in 2005 when it was hot and dry, ginseng flowers and seed heads dry up. This results in a reduced seed crop (green seed) for stratification. Should there be a surplus of stratified seed at the same time as the reduced green seed crop then the stratified seed could be held over for one year and then planted. This would ensure a continuity of seed supply and no interruption in production cycles. However, we do not know how to hold stratified seed for one or more years.

Possibly the stratified seed could be dried down and held at low temperature and relative humidity as is done for Asian ginseng [6] and for many other seeds.[7] Therefore, the objective of this work was to develop a protocol for drying stratified ginseng seed, storing them at low temperature and humidity, rehydrating them, and seeding into the field to evaluate their germination and growth.

[#]To whom correspondence should be addressed.
(Tel) (519) 824-4120, ext. 53446; (Fax) (519) 767-0755
(E-mail) jtprocto@uoguelph.ca

MATERIALS AND METHODS

Stratified seed were purchased from a grower in September 2005. These seeds had an embryo length of 3.15 mm and 25% of the seed were cracked. Some of the seeds were dried to constant weight at room temperature and held at 4°C and variable relative humidity (RH), or 35% RH. Other seed lots were placed in moist sand at room temperature or in cold storage at 4°C and the embryo allowed to grow to 5 mm and for more seed to become cracked. These seed were either held at 4°C and 35% RH, or were frozen and held at - 12°C. Details of the five treatments of these seeds for field plot establishment are given below.

Seed cracking and embryo length. At intervals during the various experiments seed samples, usually 4 samples each of 20 seeds, were taken. If the endocarp was cracked this was noted. To measure embryo length the endocarp was removed, the endosperm was split to reveal the embryo and its length measured microscopically.

Seed drying and rehydration. Seed, or its parts (endocarp and endosperm plus embryo) was dried by placing them in aluminum weighing dishes and allowing them to dry at room temperature (21±2°C) and relative humidity (25±5%, or as stated in the text). Air flow over the drying seed was 0.05 (range 0.03 to 0.10) m/s as measured with a Model 8345/8346 Air Velocity Meter, TSI Incorporated (1998). In some instances seed samples were oven-dried at 104°C to remove most of the seed moisture. Percentage moisture content was determined periodically during drying and calculated as fresh weight minus dry weight divided by fresh weight and multiplied by 100.

To determine rehydration of seeds during imbibition, water uptake was measured intermittently over a three day period. During rehydration, individual seeds were weighed and placed on moist mortar sand in a Petri dish. There were 4 Petri dishes each with 10 seeds. At sampling time the seed were removed from the Petri dish, surface dried with tissue paper, weighed and returned to the dish. Percentage water uptake was calculated as the amount of water taken up relative to the original weight of the seed.

Seed storage facilities were developed at two locations: one in Guelph and one with the co-operating grower. At Guelph a Constant Temperature Control Limited cooler (Weston, Ontario, Canada) was set up at 4±1°C and 35% RH. The inside dimensions of the cooler are 2.48 m wide x 3.06m deep x 2.4 m high. The relative humidity of 35% in the cooler was established and maintained with a Munters M90L Dehumidifier (Munters Inc., Toronto, Ontario, Canada). The storage facility at the grower's farm was a modified portion of a small barn where the temperature was held at 3.8±2°C and the relative humidity was not regulated.

Seed germination potential in the field was assessed for the five treatments (see below) by burying about 200 seeds of each treatment in cheese-cloth bags at about 3.5 cm deep in the soil and covering with about 6cm of wheat straw. Embryo growth and seed germination were measured in late November 2006 and early May 2007.

Field plots were established in October 2006 at the co-operating grower's farm in Waterford. The five treatments established in the field were as follows: Trt.1: dried 2005 stratified seed (seed harvested Fall 2004) held at 4° C and at variable humidity; Trt.2: 2006 stratified seed planted directly into the field; Trt.3: 2005 stratified seed dried in October 2005 and held at 4°C and 35% RH; Trt.4: 2005 stratified seed held in moist sand from October to December 2005 at room temperature (21±2°C) and then in December dried and held at 4°C and 35% RH; Trt.5: 2005 stratified seed held in moist sand from October to December 2005 at room temperature and then in December dried and held at -12°C. The experiment was set up as a randomized complete block with four blocks. Each block was 22 m long and 0.75m wide (half-bed width).

Dry seed were rehydrated for 24 hours (see Fig. 3), surface dried so that they would not stick in the seeder, and then seeded into a commercial field at a rate of 112 kg /ha with a Planet Junior seeder. At this seeding rate and an expected 70% germination, plant stand would be about 150 plants/m^2. The settled straw mulch depth ranged from 7 to 10cm. Seedling emergence and plant counts in four, one-m^2 plots in each of the 5 treatments were made on June 21, 2007. Seedling harvests were carried out on August 21, 2007.

Four replicates each of 10 seedlings in each treatment were harvested and stem length, root diameter and length, leaf area, and dry weight at 80°C of the leaves, stems, and roots were measured.

The experiments on seed drying and rehydration were carried out at least twice. 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.). In Figs 1 to 4 the

standard errors are less than the diameter of the symbols.

RESULTS AND DISCUSSION

Seed cracking and embryo length

The seeds that were purchased in early October 2005 and used for Trts. 3, 4 and 5 had a mean embryo length of 3.15 mm and 25% of the seed were cracked. At the time of field planting in October 2006 most seed were cracked. Seeds in Trt.1 had the longest embryos at 4.68 mm and by December 15 in moist sand in the cooler at 4°C had grown to 5.5 mm and 70% of the seed germinated (Fig. 1). By December 15 seed in Trt.5 were soft and rot was extensive suggesting that similar conditions might be occurring in the field planting. Embryo measurements in this treatment were discontinued. We have found previously that seed that have been frozen do not establish well in the field.[8)]

Embryos in Trts. 2, 3 and 4 grew in a linear manner from 3.2 mm in October 2006 to 5.5 mm in April 2007 (Fig. 1). The 2006 stratified seed embryos (Trt. 2) grew rapidly to mid-February by which time 75% had germinated. Over the next 2 months this germination increased to 87%. Although embryos in Trts. 3 and 4 achieved a maximum length similar to Trt.1 only 4% of the seed in Trt.4 germinated. These data for Trts. 1, 2, 3 and 4 suggested that in the field, seeds in Trts. 1 and 2 would germinate but seeds in Trts. 3 and 4 might germinate slowly or be delayed.

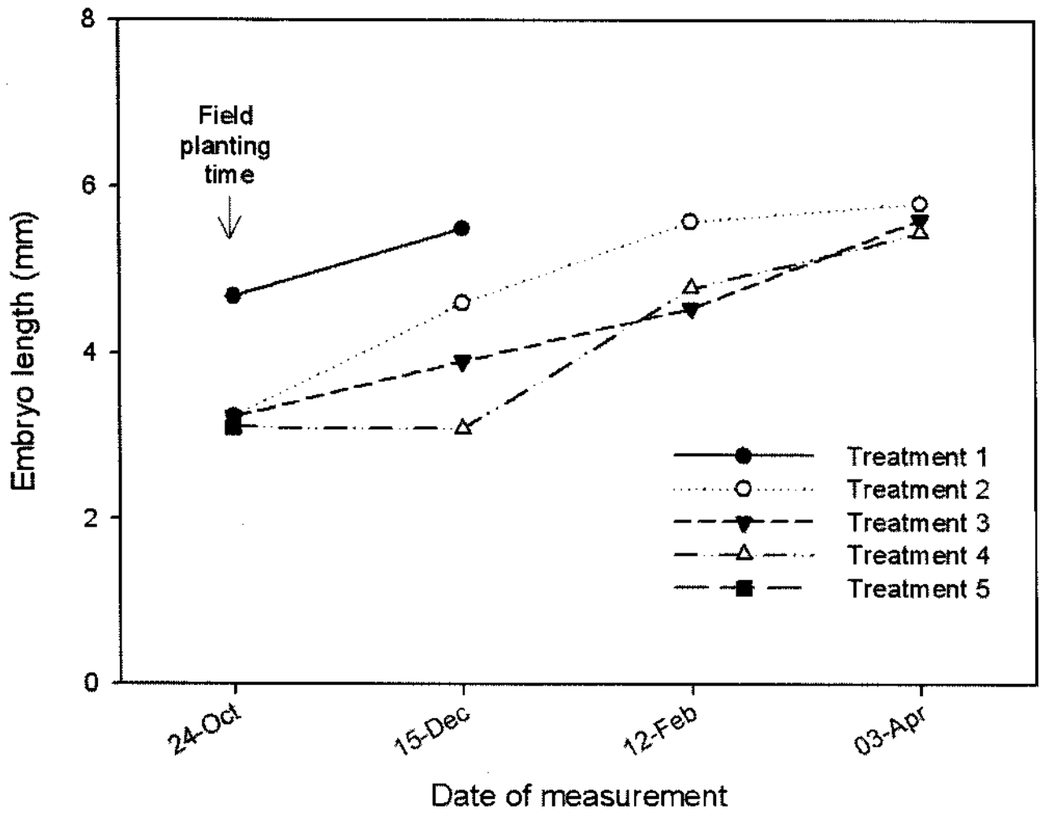

Fig. 1. Embryo length at field planting time in 2006 in each of the five treatments (see Fig. legend) and at three times over the following 6 months. Seed were held in moist sand at 4°C.

Seed drying

Ginseng seed drying involves convectional-diffusive dehydration of the whole seed. If the seed is cracked, as was the usual case here, water moves by diffusion from inside the endosperm to the air-endocarp/endosperm interfaces and then by convection to the moving air. Drying of ginseng seed follows an exponential curve (Fig. 2). Similar exponential drying curves for ginseng roots have been reported ; for a recent discussion of this see Martynenko et al.[9)] A stratified cracked ginseng seed weighs about 0.055 g and at room temperature and 40±5% relative humidity has about 58% dry matter and dries down to about 9% moisture content (dry basis) in about 72 hours. Whole cracked stratified seed and seed embryos plus embryos dried down similarly in about 72 hours (Fig. 2). Whole cracked seed dried to 58.5% of the initial seed weight and the endosperms plus embryos to 55.7%. Seed coats dried very quickly achieving constant weight in about 5 hours (Fig. 2). This suggests that the seed coat moisture was on the coat surface and evaporated quickly, whereas the moisture in the endosperm plus embryos had to move by diffusion and then by evaporation.

Seed rehydration

Seeds started to absorb water as soon as they were placed on the moist sand (Fig. 3). Water uptake was very rapid in the first 5 hours, about 35% of the initial seed weight. After this time the water uptake rate slowed, reaching 60% in about 24 hours. After 48 hours, at about 70% uptake, the rate slowed and leveled off at about 80%

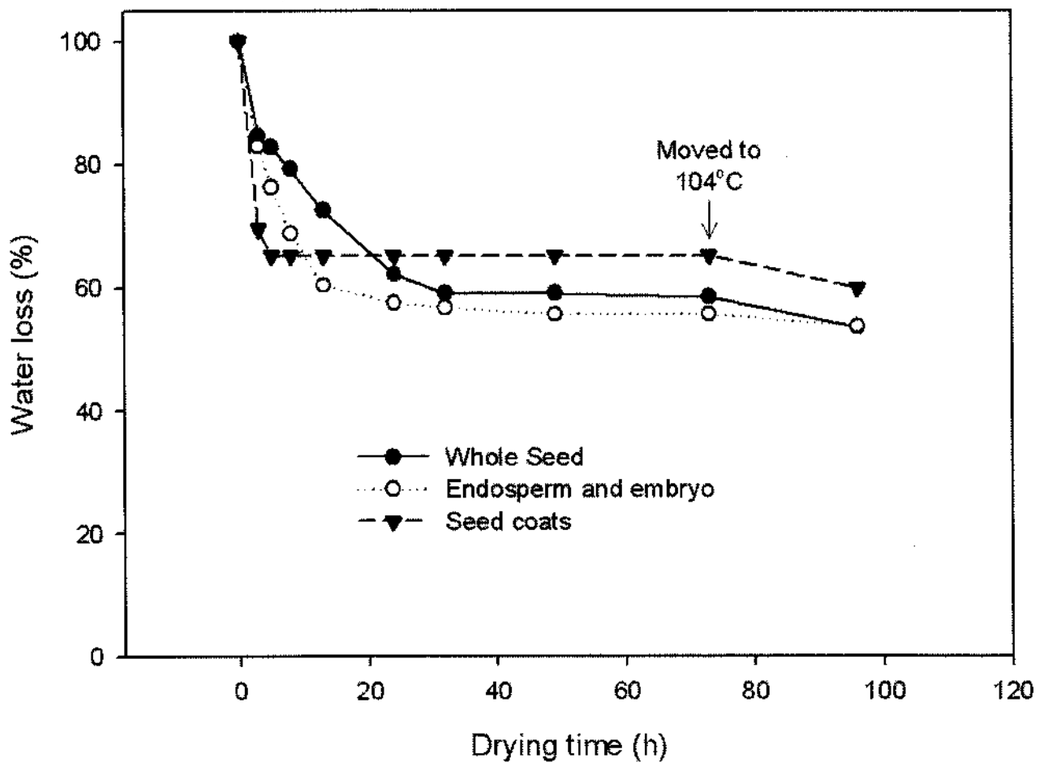

Fig. 2. Effect of room temperature drying (21±2°C, 40±5% relative humidity) on water loss (% of initial seed weight) and by drying at 104°C on whole cracked stratified North American ginseng seed and it's two components, seed coat (endocarp) and endosperm plus embryo.

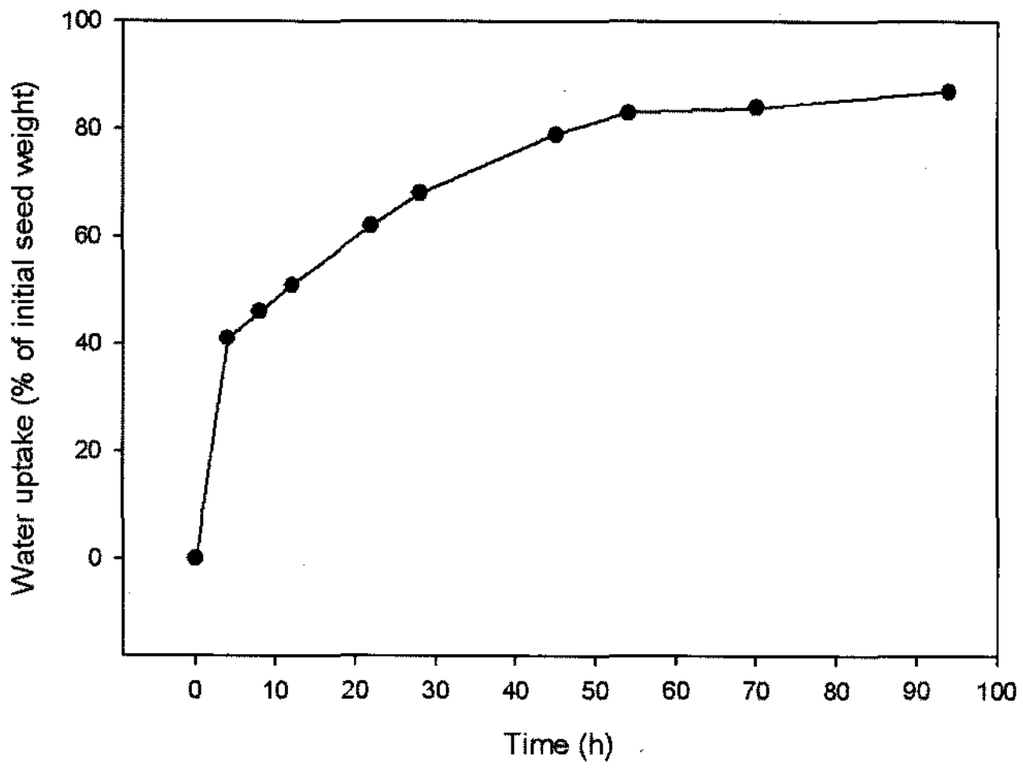

Fig. 3. Water uptake (% of initial seed weight of room dried seed) during rehydration of cracked, stratified room dried (21±2°C) North American ginseng seed.

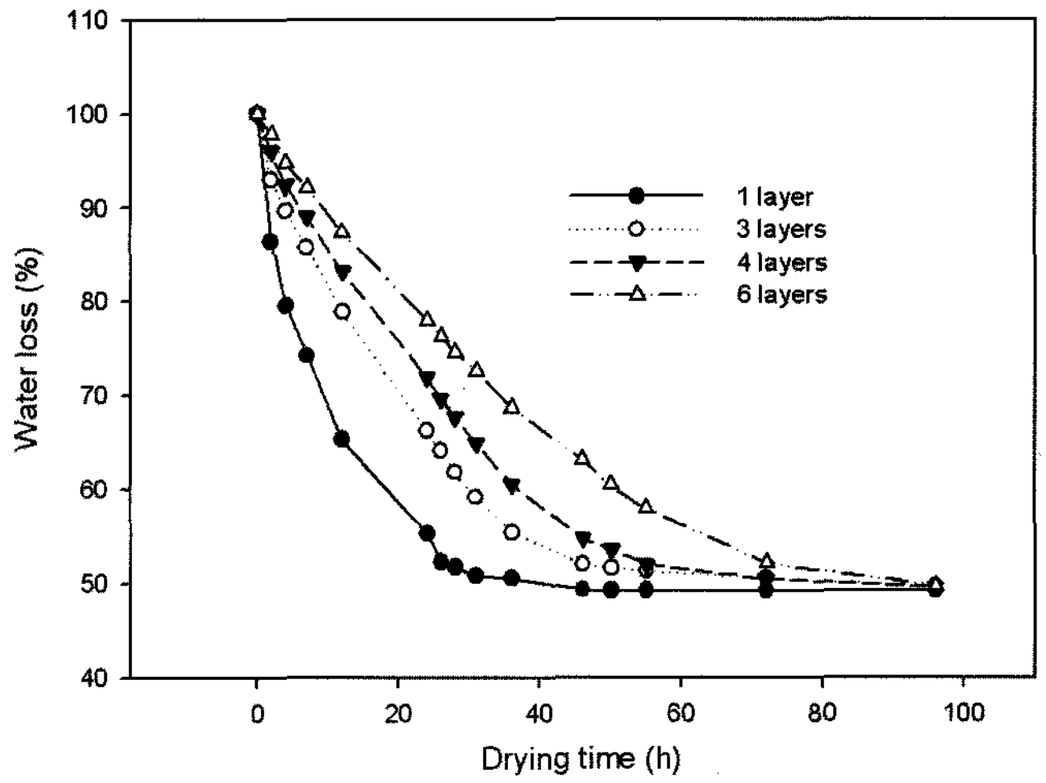

Fig. 4. Water loss (% of initial seed weight) during drying of four different seed layers (loading rates) of North American ginseng seed at 21±2°C and 40±5% relative humidity.

moisture content. These seed appeared to be fully imbibed. Water uptake was more rapid than water loss during drying – compare Figs. 3 and 2.

This pattern of water uptake follows the triphasic model of Bewley and Black, p.151[7] particularly for Phases I and II. Phase I is characterized by rapid water uptake regardless of the state of the seed, and by the start of metabolism. In Phase II water uptake slows but metabolic activities are enhanced in preparation for radicle emergence in Phase III. Radicle emergence was not observed in these seed indicating that some inherent properties of the seed prohibited radicle emergence and germination. Since these seed eventually germinated it is suggested that a required cold period had to be met.

Multi-layer drying

The fastest drying was for the single layer of seeds, equivalent to a loading rate of 1.85 kg/m^2 (Fig. 4); the slowest drying was for the 6 layers of seed, equivalent to a loading rate of 10.7 kg/m^2. Based on the data in Figure 4 the estimated drying time to 50% loss of moisture from the seed was 6, 16, 21 and 27 hours for 1, 3, 4 and 6 layers of seed respectively. Also, the approximate time for a single layer of seeds to dry was two days whereas it was 4 days for 6 layers of seed. These data suggest that in seed when growers make a decision about seed drying they must be aware that seed loading rate will influence the rate of drying and the time to complete drying.

Seed germination potential in the field

In November 2006, only seeds in Trt. 1 had germinated in the cheese-cloth bags in the soil suggesting, in relation to the 2006 stratified seed, that this might be premature germination and these seedlings might not persist in the soil. In May 2007 germination varied greatly between seed treatments. In Trt. 1 only 5% of the seed germinated. Many seeds had empty shells (endocarps) and there was considerable seed rotting. The stratified seed planted in October 2006 (Trt. 2) had 78% germination and the seedlings looked green, strong and healthy. Trts. 3 and 5 each had only 5% germination, while the December dried (Trt. 4) seed had 50% germination. In summary, the most successful of the five seed treatments was the fall 2006 planted stratified seed (78% germination) and the December 2005 dried seed (50% germination) suggesting that seed of these two treatments would perform best in the field plantings.

Field plots

Seedling emergence was best in Trts. 2 (2006 stratified seed) and 4 (2005 stratified seed held from October to December and then dried) with 67.3% and 65.1% respectively (Table 1). As the accepted industry seedling emergence rate is 68%, the emergence in these two treatments was good. Seedling emergence in Trts.1, 3 and 5 was very low (Table 1). Examination of seeds of Trt.1 in the field revealed empty seed coats suggesting that these seeds may have germinated after planting but failed to establish. Seeds of Trt.3 found in the soil in the field were cracked, the endosperms were firm and appeared healthy. These seed may germinate in spring 2008 after another cold

Table 1. Effect of five seed stratification treatments on seedling emergence and crop height on June 21, 2007.

Treatment	No. of seedlings emerged (m^2)	Emergence as percent of seeding rate[z]	Crop height above straw (cm)
1[y]	2.7a[x]	13	4.3 ns
2	144.0b	67.3	4.3 ns
3	2.2a	1.0	3.7 ns
4	139.4b	65.1	4.4 ns
5	5.2a	2.4	3.1 ns

[z]Seeding rate was 214 seeds/m^2
[y]See above Materials and Methods, **Field Plots** – for details of the treatments
[x]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$, is indicated by different letters, or non-significant, ns.

period. Seeds of Trt. 5 contained endosperm and protruding radicles but with extensive rot. Previously we have noted extensive rotting of seeds that have been frozen and later planted into the field.[8)]

Seedling crop height was not influenced by stratification treatment (Table 1). Adding crop height of about 4 cm to straw mulch depth of about 9 cm (see Materials and Methods above) would give a stem length of about 13 cm which is similar to our previous findings.[8)]

There were few differences in growth between the seedlings from Trts. 2 and 4 (Table 2). The most important measurement, root dry weight, was similar for the Fall 2006 stratified seed (Trt. 2, 172 mg) and the December 2005 dried seed planted in fall 2006 (Trt.4, 159 mg). Therefore, if growers wish to hold mature stratified seed in August/September they can hold the seed in moist sand until December, dry them and store at 4°C and 35% relative humidity. These seed can be planted in the following August/September and will germinate and grow in the following year to give an acceptable crop.

Another approach to ensuring an adequate supply of seed for planting and no interruption in production cycles, would be to take immature (green) seed at the start of stratification period when embryo length is about 0.5 mm (start of Stage I)[5)], place them in sand at about 12% moisture and hold them at about 3°C. In an earlier paper [8)] we showed that freshly harvested, immature (green) seeds could be successfully stratified at 3°C for one year. However, by the end of the second year of stratification, seed rot was very high because the moisture content of the sand was > 25%. Therefore, if moisture content of the sand was kept low, about 12%, seed rot might be less prevalent and a protocol for long-term stratification of immature (green) seed in controlled environments could be established. Such a protocol would avoid the necessary and costly procedure of storage at low relative humidity.

Table 2. Effect of holding time after seed stratification (0 years= Trt. 2, 1 year=Trt. 4) on seedling number and growth. Seedlings were harvested on August 21, 2007.

Seedling measurement	Holding time (years)	
	0	1
Seedling number (m^2)	144	139
Stem length (cm)	12.5	12.8
Leaf area (cm^2)	15.1	14.5
Root - diameter (mm)	6.7	6.2[z]
- length (cm)	3.1	3.7[z]
Dry weight (mg)		
Leaf	60.6	53.8[z]
Stem	21.7	21.4
Root	172	159

[z]Means across columns are significantly different at $P \leq 0.05$, t test.

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