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Stratification of American Ginseng Seed: Embryo Growth and Temperature

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Abstract□Freshly harvested American ginseng (*Panax quinquefolium* L.) seeds were stratified at two locations over each of three years. Seed development and temperature in the stratification boxes were investigated until the seed was removed 12 months later and direct-seeded in the field. During stratification and seeding (14 months) three embryo stages were identified. In Stage I of 250 days (Sept. to mid-May) embryo length increased from about 0.5 to 1.0 mm; in Stage II of 100 days (mid-May to late Aug. when seeded) length increased to 2.0 mm and in Stage III (late Aug. to late Nov.) length increased to 5.3 mm. Exocarp split width could also be placed in three stages. Changes in embryo length correlated with embryo : endosperm length ratio. Instron compresseion tests showed that the exocarp softened rapidly in late Stage II and throughout Stage III. The stratification box temperatures at all depths (10, 25 and 50 cm) never exceeded −2℃ even when the air temperatures dropped to −13℃ and were, therefore not damaging to the seeds.

Key words□Embryo dormancy, embryo length, *Panax quinquefolium*, temperature.

Introduction

American ginseng is propagated by seed.[1] In commercial practice ginseng seed is harvested in August or September, placed in a stratification box for about 12 months, and then direct-seeded into raised beds. Germination takes place the following spring, some 18 to 22 months after seed harvest.[2] Little is known about the dormancy-controlling mechanisms of ginseng seed.

Harvested ginseng seeds have immature embryos that grow during the necessary after-ripening period.[3-5] The cool-warm-cool temperatures corresponding to winter-summer-winter over the 18~22 months stratification are necessary for embryo growth and maturation and seed germination.[3,5] The second cool period may release the embryo from endogenous dormancy.[5]

Germination of Oriental ginseng (*Panax ginseng* C.A. Meyer) can be achieved in ≈ 8 months and Proctor *et al.*[6] suggested that this technology might be adapted for American ginseng. Before this is attempted however, more should be known about seed changes during stratification. The objective of this study was to examine and compare seed development in the stratification boxes for 12 months at two locations in each of 3 years.

Materials and Methods

The experiments were carried out at 2 farms in each of 3 years ('91~'92, '92~'93 and '93~'94). Since the seed handling was similar at each farm in each year common procedure for one year is described. The fruit (berries) were hand-harvested in late August, mechanically depulped and the seeds washed, with those floating being discarded. These commercial grade seeds were surface sterilized with a 37% formalin solution and then mixed with washed (about 15% moisture) mortar sand (1 vol seed/2 vol sand) using a cement mixer. The mortar sand consisted of 96.8% sand, 2.0% silt and

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1.2% clay by weight; the sand was 0.3% very coarse, 12.1% coarse, 46.1% medium, 25.3% fine, and 13.0% very fine. The seed-sand mixture was tipped into a buried wooden stratification box in early September. The dimensions of the box were 2.7 m× 1.5 m and 0.5 m deep. The box had a screen mesh bottom to exclude rodents and to permit drainage.

The top of the box protruded about 5 cm above the soil surface. The box was filled with the seed/sand mixture to about 10 cm from the top, a screen mesh was placed on this and the rest of the box filled with sand. During the filling of the box thermistors were inserted into the seed/sand mixture at 10, 25 and 50 cm down from the surface in the center of the box and attached to a datalogger (model LI-1000; LI-COR, Lincoln, Neb.) placed in a well-ventilated shelter 1.2 m above the soil surface. Air temperature was measured adjacent to the data logger. All temperatures were recorded hourly and values discussed are daily means except where stated otherwise. Comparison of mean daily air temperatures for each year at each of the 2 farms with those measured at the standard weather stations at Simcoe (42° 52′, 80° 16′) and Delhi (42° 52′, 80° 33′) showed that they were highly correlated ($r>0.99$). The two farms were 10 km apart and were within 20 km of the weather stations. The stratification boxes were shaded with wooden lath as is done in plant growing.[1)]

At intervals (see Fig. 1) during the stratification period 4 samples of 20 seeds were removed from the box at 15~30 cm from the surface using a soil probe. If the exocarp was cracked, crack width was measured. The exocarp was then removed and endosperm width and length, and embryo length were measured. Early in the stratification process the exocarp had to be softened by soaking the seeds in 2.5% NaOH for 20 minutes to facilitate removal.[7)] Compression force, a measure of seed hardness, was determined with an Instron Universal Testing Instrument (Model 4204, Instron Corp., Canton, Mass.). Each seed (10 per rep.) was compressed between flat plates at 10 mm·min ^{1}. On contact with the seed the first detected event was exocarp cracking followed by endosperm crushing after which the moving plate was reversed. The compression force (N) reported here was for exocarp cracking.

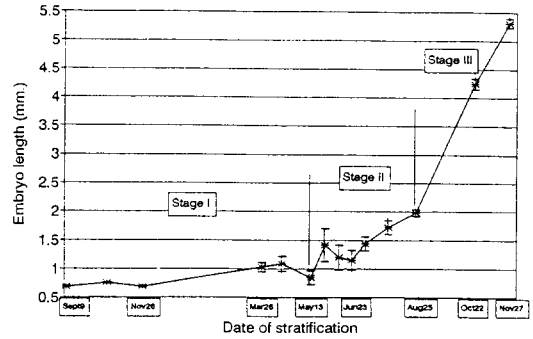

Fig. 1. Changes in ginseng seed embryo length during stratification. Data are the mean of 3 years. Vertical bars represent SE.

All statistical analyses were carried out using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program package (SAS Institute, Cary, N.C.)

Results and Discussion

During stratification 3 embryo growth stages were identified (Fig. 1). There was no effect of location or year so the data were combined. Stage I was 250 days (Sept. to mid-May) during which embryo length increased from about 0.5 to 1.0 mm (0.002 mm day^{-1}). Stoltz and Snyder[5)] reported little embryo growth in the first 90 days in seeds stratified outdoors in Lexington, Kentucky. Exocarp splitting was first observed in some seeds in late January (around day 125), but like embryo length increased only slightly by day 250.

Stratification box temperatures (mean of 3 depths) during stage I decreased from about 15℃ to about 1℃ (Sept. to Dec., about 100 days), remained constant at about 1℃ for about 100 days, and then in the next 50 days increased to about 10℃ (Fig. 2). Although air temperatures dropped to −13℃ stratification box temperatures never went below −2℃. Over the three winters of the study the lowest air temperatures occurred from about mid-Dec. to the end of Feb. with the lowest minimum air temperature being −35℃ on Feb. 10, 1994. During this period the seed box temperature never went below

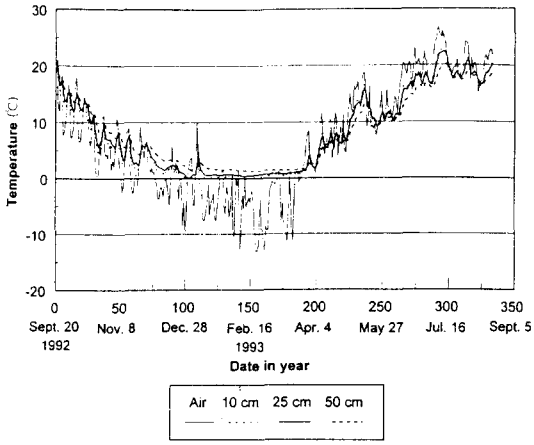

Fig. 2. Chagnges in air and stratification box temperatures at 3 depths (10, 25 and 50 cm) during stratification from Sept. '92 to Sept. '93. Temperatures for other years were similar.

−2℃.

In controlled freezing tests with non-germinated ginseng seeds, Proctor and Lee[8], showed that seed damage did not occur until temperatures were below −137℃. Therefore, nongerminated seeds in Stage I were not injured by the low temperatures of −2℃ in the stratification box. Snow cover had little effect on temperatures in the seed box. Probably this is because most boxes have a top layer of 5~10 cm of sand which may mimic snow cover. Tan and Layne[9] reported that snow cover in a peach orchard maintained soil temperatures around 0 to −2℃ comparable to that shown in Fig. 2.

Lee *et al.*[3] found that optimal temperatures for embryo growth and development varied with different stages of embryo growth. Initial and middle stages of embyro growth were optimal at 15℃. Although stratification box temperatures were about 15℃ in September they declined rapidly to around 0℃ and were conducive only to minimal (not optimal) embryo growth (Fig. 1) but did lead to initiation of exocarp cracking. Compression force was 100 N (± 7=SE) at the start of Stage I, declined slightly to 81 N (± 11) by late stage II, and then declined linearly to 36 N (± 2) at the end of Stage III (Table 1).

In stage II of embryo growth (100 days from mid-

Table 1. Changes in exocarp split width and seed hardness during Stages II and III (see Fig. 1) of seed stratification in 1992

Date	Exocarp split width (mm)	Seed hardness as measured by compression force (N)
May 13	0.05± 0.01[a]	79± 9
May 27	0.29± 0.11	89± 7
June 10	0.18± 0.08	−
June 23	0.14± 0.07	81± 11
July 6	0.29± 0.05	87± 12
July 29	0.32± 0.05	77± 10
August 5	0.27± 0.05	64± 6
October 22	1.00± 0.04	−
Novermber 27	1.18± 0.03	36± 2

[a] ± Standard error.

May to late August) embyro length increased from 1 to 2 mm (0.01 mm day $^{-1}$) (Fig. 1). This embryo growth occurred mainly in the cotyledons, with the radicles growing only slightly. Temperature in the box during this period increased from about 10 to around 20℃ (Fig. 2) and could be considered nearly optimal for embryo growth at this stage as suggested by Lee *et al.*[3] Exocarp split width paralleled embryo growth increasing from 0.05 mm to 0.3 mm over the 100 day, stage II period (Table 1). Embryo : endosperm length ratio correlated with embryo length in agreement with Jo *et al.*[10] In late May or early June of some years early seed germination in the seed box occurred after only 8 months of stratification. This was observed in early June, 1992 and the rapid embryo growth after May 13 (Fig. 1) may be an indicator of this potential.

To assess the magnitude of this event we extracted 8 samples from one of the stratification boxes. An average of 4.5% of the seeds were growing (range 0 to 8%) and an additional 2.5% had large exocarp splits indicating the potential to germinate. We suspect, from observations of time of filling of the seed boxes by growers, that premature germination is related to stratification box temperature in the fall. In the fall, the seed box is warmer than air and takes time to cool down (Fig. 2). If the seed box is filled when its temperature is still around 15℃ then probably the seed stratification process is hastened. To avoid this some growers hold their

seed in cold storage until later in September when they transfer it to the seed box. Holding the seed in cold storage likely puts the seed into dormancy.

Other support for our proposal is that in Korea ginseng seed is stratified outdoors from mid-July to mid-November, then seeded, and germinates the following spring (≈ 8 months).[6] This short stratification period is possible because monthly air temperatures in Seoul, Korea are approximately 6, 5, 4 and 2℃ higher in Aug., Sept., Oct., and Nov., respectively than in Simcoe, Ontario.[11]

Stage III of embryo growth (late Aug. (day 350) to late Nov.) was the most rapid with length increasing linearly from 2 to 5.3 mm (0.03 mm day^{-1}) (Fig. 1). Exocarp split width increased to about 1.2 mm (Table 1). As in stage II, embryo growth was mostly in the cotyledons with radicle protrusion being evident only towards the end of the period. During this period seed were sampled from the field since seeding took place on Sept. 4. Our data differs from that of Stoltz and Snyder[5] who reported a 60 day period (day 300 to 360) of no embryo growth. In summary, ginseng seeds undergo relatively little change in the first 9 or 10 months of stratification. In the next 4 or 5 months changes in embryo length, exocarp splitting and softening are dramatic. These changes leave the seed ready to germinate after an additional 5 month obligatory cold period.

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