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URBAN, JONATHAN S. APRIL 2012. Effect of Cold Stratification period on the Germination and Seedling growth of *(Vaccinium corymbossum)* seeds. Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The pre-sowing treatment and germination portion of blueberry seeds were conducted at the Seed Laboratory and seedling emergence study was studied at the Pomology Nursery of Benguet State University, from November 2011 to March 2012 to assess the effect of stratification treatments, ascertain the best durations of stratification treatment in breaking dormancy of blueberry seeds and to determine the different ways of enhancing seed germination.

Results revealed that the various durations of cold storage of blueberry seeds had significantly affected seed germination and seedling emergence. Seedlings resulting from the seeds subjected to nine days cold storage had enhanced seed germination that resulted to higher percentage of seed germination and development of more normal seedlings. Likewise, seeds subjected to nine days cold storage resulted to taller seedlings.

RESULTS AND DISCUSSION

A. Laboratory Germination Test

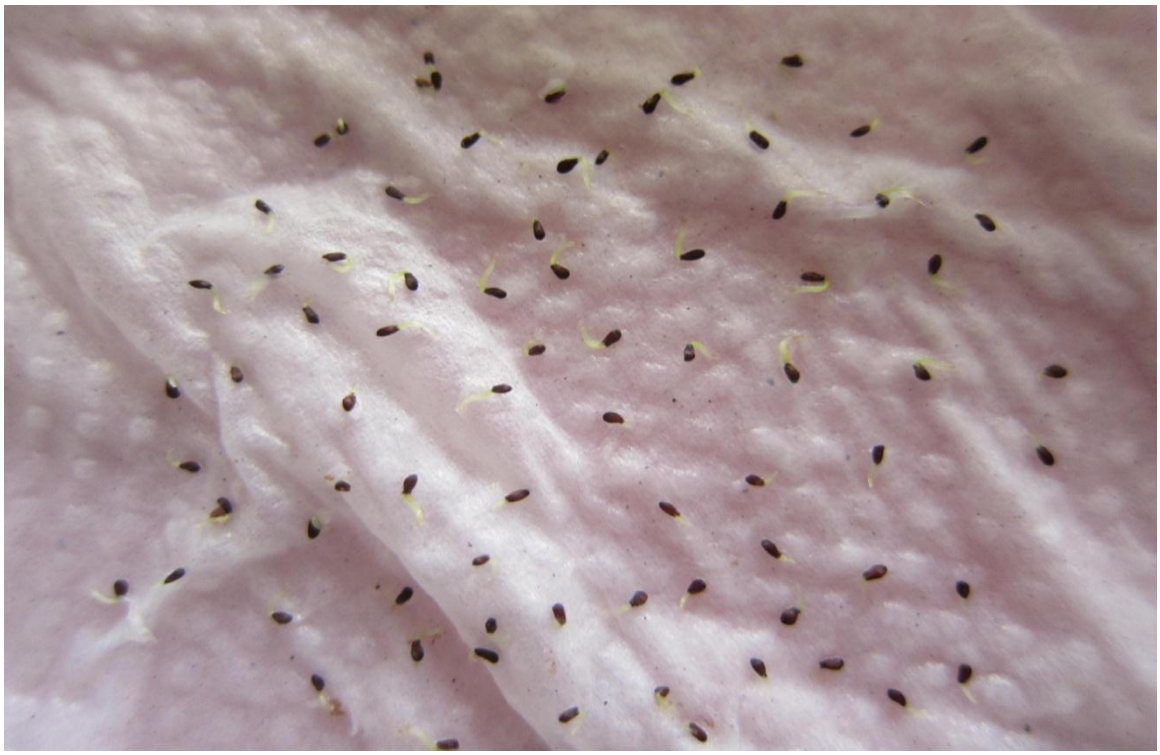

Figure 1. Counted seeds germinated and removed from the petri dishes

Percentage of Seeds Germinated

As shown in Table 1, there were highly significant differences on the percentage of seedling emergence among the different treatments as affected by the various cold storage durations. It was observed that blueberry seeds subjected to nine days cold storage attained the highest percentage of seedling emergence with a mean of 96 % followed by seeds subjected in cold storage duration for fifteen, six and twelve days having a mean of 93.33, 92 and 88.67% respectively, but were statistically comparable with each other. On the other hand, seeds that were not subjected to cold storage had the lowest percentage of seedling emergence with a mean of 69.33%.

Table 1. Percentage of seeds germinated

TREATMENT	MEAN (%)
Control (no cold storage)	69.33[b]
Three days cold storage	74.67[b]
Six days cold storage	92[a]
Nine days cold storage	96[a]
Twelve days cold storage	88.67[a]
Fifteen days cold storage	93.33[a]

Means with the same letters are not significantly different at 5% level by DMRT

Median Germination Times.

Table 2 shows the median germination times (G_{50}) of the stratified seeds. Statistical results showed that seeds subjected in cold storage for nine days accelerated earlier germination with a mean of 456.67 hours followed by the seeds subjected to cold storage for fifteen and six days cold storage having a mean of 462.67 and 486.67. It was further followed by the seeds subjected to cold storage for twelve days with a mean of 496 hours. Seeds that were not subjected to cold storage had the longest duration to germination with a mean of 536 hours but were comparable to the seeds subjected to three days cold storage only.

Table 2. Median germination times.

TREATMENT	MEAN (HOURS)
Control (no cold storage)	536[a]
Three days cold storage	535.33[a]
Six days cold storage	486.67[bc]
Nine days cold storage	456.67[c]
Twelve days cold storage	496[b]
Fifteen days cold storage	462.67[bc]

Means with the same letters are not significantly different at 5% level by DMRT

Median Spread of Germination Times.

The median spread of germination times (G_{90}-G_{10}) as affected by different durations of cold storage is presented in Table 3. Apparently, seeds stored for cold storage durations for fifteen days had 550.40 hours to show median spread of germination times followed by the seeds subjected to cold storage durations for twelve, nine, six and three days showing median spread of germination times with a mean of 537.07, 505.73, 488.03 and 449.33 hours, respectively having means that are highly significant with the other treatments against the unstratified seeds.

Table 3. Median spread of germination times.

TREATMENT	MEAN (HOURS)
Control (no cold storage)	332.68[f]
Three days cold storage	449.33[e]
Six days cold storage	488.03[d]
Nine days cold storage	505.73[c]
Twelve days cold storage	537.07[b]
Fifteen days cold storage	550.4[a]

Means with the same letters are not significantly different at 5% level by DMRT

B. Greenhouse Emergence Test

Figure 2. Overview of the experimental area

Number of Days From Sowing to
Initial Seed Emergence

Seed emergence as affected by different cold storage durations was determined based on the number of days from sowing to the time the seed had emerged on the soil surface (Table 4). Statistical results showed that nine days cold storage enhanced earlier emergence of blueberry with a mean of 53.67 days from sowing which was highly significant as compared to the other treatments. It was followed by the seeds subjected to six, twelve, fifteen and three days of cold storage with a mean of 55, 56, 56.67 and 59.33 days respectively. The seeds that were not subjected to cold storage were the last to emerge with a mean of 68 days.

These findings collaborate with the earlier observations of Paing (1980) that cold storage shortened the period from sowing to germination of garden pea. Weaver (1974) mentioned that pre-treated seeds in medium of carefully controlled temperature is conditioned for rapid germination. However, many seeds placed in an environment considered adequate for germination still do not germinate because of some internal limitations and unfavorable environmental conditions (Devlin, 1977). Furthermore, Daubenmire (1974) explained that the physiological explanation of this conditioning effect is still unknown. In some seeds, low temperature may function to render the seed coat more permeable to gasses and other substances.

Table 4. Number of days from sowing to initial seed emergence

TREATMENT	MEAN (DAYS)
Control (no cold storage)	68[a]
Three days cold storage	59.33[b]
Six days cold storage	55[bc]
Nine days cold storage	53.67[c]
Twelve days cold storage	56.67[bc]
Fifteen days cold storage	56[bc]

Means with the same letters are not significantly different at 5% level by DMRT

Percentage Survival (%)

As shown in Table 5, there were highly significant statistical differences on the percentage survival of seedlings among the different treatments as affected by various cold storage durations. It was observed that blueberry seeds subjected to nine days storage attained the highest percentage of seedling survival with a mean of 88 % which is statistically different to the seeds that were not subjected to cold storage with a mean of 56.67 % which is also highly significantly different with the rest of the treatments.

It was observed further that some seeds did not emerge for reasons that cannot be explained though it may be due to chilling injury that caused the death of the embryo in the seeds during the long cold storage periods and may also be due to the not fully decomposed coco coir dust added in the media that was used. Hartman et al. (1990) stated that such result could be related to high concentration of soil solutes in the

Table 5. Percentage survival

TREATMENT	MEAN (%)
Control (no cold storage)	56.67[d]
Three days cold storage	66[cd]
Six days cold storage	78[ab]
Nine days cold storage	88[a]
Twelve days cold storage	70[bc]
Fifteen days cold storage	87[a]

Means with the same letters are not significantly different at 5% level by DMRT

upper layer of the germination media. Likewise, Handreck (1993) wrote that coco coir pith has a carbon-nitrogen ratio of 104:1 which means that it would take decades to be decomposed. The partly decomposed coco coir dust might have released some toxic substance or gas that affected the emergence of seeds.

Number of Days From Sowing
to First Appearance of Leaves

Table 6 shows highly significant differences among the different cold storage durations affecting the number of days from sowing to first appearance of leaves. Results showed that seeds stored for nine days attained the shortest days for leaf development and produced leaves within 57.33 days. This was followed by the seeds subjected to cold storage for six, fifteen, twelve and two days having a mean of 60, 60.33, 61.33 and 64.67

days respectively. On the other hand, seeds that were not subjected to cold storage had the longest days to leaf formation having a mean of 72 days.

Table 6. Number of days from sowing to first appearance of leaves

TREATMENT	MEAN (DAYS)
Control (no cold storage)	72[a]
Three days cold storage	64.67[b]
Six days cold storage	60[c]
Nine days cold storage	57.33[c]
Twelve days cold storage	61.33[bc]
Fifteen days cold storage	60.33[c]

Means with the same letters are not significantly different at 5% level by DMRT

Dao-ines (2010) found in his experiment that blueberry seeds refrigerated for one week cold storage appeared to be the best, as the seedlings attained the highest percentage of germination, tallest seedlings, complete emergence, normal seedlings and included the seedlings faster leaf to emergence.

Percentage of Normal Seedlings

Table 7 shows that there were highly significant differences observed among the different cold storage durations in terms of percentage of normal seedlings. Based on the results, blueberry seeds subjected to nine days cold storage had the highest percentage of

normal seedlings having a mean of 84.67 %. It was followed by the seeds subjected to cold storage durations for fifteen, six, twelve and three days having a mean of 82.67, 74, 64.67 and 60.67% of normal seedlings respectively. Lastly, seeds that were not subjected to cold storage had the lowest mean of 50% showing normal seedlings.

Table 7. Percentage of normal seedlings

TREATMENT	MEAN (%)
Control (no cold storage)	50[e]
Three days cold storage	60.67[d]
Six days cold storage	74[bc]
Nine days cold storage	84[a]
Twelve days cold storage	64.67[cd]
Fifteen days cold storage	82.67[ab]

Means with the same letters are not significantly different at 5% level by DMRT

Salisbury and Ross (1992) explained that pre-chilling of seeds sometimes has a strong effect on growth in addition to its breaking dormancy action. If the embryos of peach seed are exercised from their cotyledons, they will germinate without pre-chilling, but the seedlings are frequently stunted and abnormal. However, when the exercised embryos are treated with low temperature, they grow into normal seedlings. Thus, it is pre-chilling and not the presence of the cotyledons that ensures their normality.

Seedling Height (mm)

The seedling height (Table 8) of blueberry was obtained by measuring the seedlings from the base up to the tip of its secondary leaves. Results showed that seeds subjected to cold storage for nine days had the tallest seedlings having a mean of 9 mm followed by the seeds subjected to cold storage durations for six, fifteen, twelve and three days have a mean of 8.33, 7.67, 7 and 6.67 mm respectively. However, blueberry seeds that were not subjected to cold storage had the shortest height with a mean of 5.33 mm.

Table 8. Seedling height (mm)

TREATMENT	MEAN
Control (no cold storage)	5.33^{d}
Three days cold storage	6.67^{cd}
Six days cold storage	8.33^{ab}
Nine days cold storage	9^{a}
Twelve days cold storage	7^{bc}
Fifteen days cold storage	7.76^{abc}

Means with the same letters are not significantly different at 5% level by DMRT

Results showed that nine days cold storage duration of seeds prior to sowing enhanced growth of seedlings leading to taller seedlings. These findings collaborated with the findings of Dao-ines (2010) who stated that cold storage of blueberry seeds for a period of one week at 5 ^{o}C hastened seed germination, improved rate of seed emergence and promoted the development of taller seedlings.

Number of Leaves Per Plant

Regarding the number of leaves of blueberry seedlings per plant, it was shown in Table 9 that there were no significant statistical differences among the different cold storage durations with regards to the number of leaves per plant. However, numerical results revealed that seedlings from the seeds subjected to nine days cold storage had the highest number of leaves with a mean of 3. Whereas, seeds that was not subjected to cold storage had the lowest number of leaves developed with a mean of 2.

Table 9. Number of leaves per plant

TREATMENT	MEAN
Control (no cold storage)	2[a]
Three days cold storage	2.33[a]
Six days cold storage	2.67[a]
Nine days cold storage	3[a]
Twelve days cold storage	2.33[a]
Fifteen days cold storage	2.67[a]

Means with the same letters are not significantly different at 5% level by DMRT

Seedling Vigor Index

The results in Table 10 showed that there were no significant statistical differences observed among the different treatments used in the study affecting the seedling vigor of

blueberry. However, numerical results showed that seedlings from the seeds subjected to nine days cold storage exhibited excellent growth.

Tocnang (2009) observed that guapple subjected to nine days cold storage promoted excellent growth of seedlings. Likewise, Banagen (2008) noted that storing coffee seeds under cold temperature influenced seedling vigor. Cold stratification enhanced faster growth which may explain the performance of coffee seeds subjected to cool treatments as having seedlings that are more vigorous as compared to the unstratified seeds.

Table 10. Seedling vigor rating

TREATMENT	MEAN
Control (no cold storage)	3[a]
Three days cold storage	3.33[a]
Six days cold storage	3.33[a]
Nine days cold storage	3.67[a]
Twelve days cold storage	3.33[a]
Fifteen days cold storage	3.33[a]

Means with the same letters are not significantly different at 5% level by DMRT

Rating	Description	Remarks
4	Most vigorous	excellent growth with dark green leaves
3	Vigorous	good growth with green leaves
2	Less vigorous	slightly growth with light green leaves
1	Poor	poor growth with yellow leaves

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The laboratory germination test of blueberry seeds as affected by cold storage durations were conducted at Seed Laboratory and seedling emergence and seedling growth of blueberry seeds was done at the Pomology Project Nursery, Benguet State University, La Trinidad, Benguet from November 30, 2011 to March 11, 2012. The blueberry seeds were subjected to low temperature with an average of 5 ^{o}C at various periods from three days to fifteen days.

A. Laboratory Seed Germination Test.

Results showed that there were highly significant statistical differences observed among the various cold storage durations used. Nine days cold storage of blueberry seeds showed the best performance among all the treatments that enhanced highest seed emergence with a mean of 96 % compared to the unstratified seeds having the lowest percentage of seed germination with a mean of 69.33%. Concerning median germination times (G_{50}), storing blueberry seeds at nine days considerably accelerated germination compared with the rest of the other treatments and the unstratified seeds resulted with highly significant differences to the other treatments. As for the median spread of germination times (G_{90}- G_{10}), it was observed that cold storage of blueberry seeds for nine days markedly improved uniformity of seeds germination due to the statistically similar responses with that of the unstratified seeds.

B. Greenhouse Seed Emergence Test.

Results showed that there were highly significant differences observed among the various cold storage durations used. Nine days cold storage of blueberry showed the best performance among all treatments that enhanced earlier seed emergence with a mean of 53.67 days and shorter days to develop true leaves with a mean of 57 .33 days. Furthermore, seeds subjected to cold storage for nine days had the tallest seedlings having a mean of 9 mm. Likewise, the same duration of cold storage attained the highest percentage of seed emergence having a mean of 88 % and also had the highest percentage of normal seedlings with a mean of 84.67 % which is highly significant with the other treatments.

With regards to the number of leaves and seedling vigor, statistical results showed that there were no significant differences among the different cold storage durations used. However, numerical results showed that seedlings from seeds stored for nine days had more number of leaves and more vigorous seedlings as compared to other treatments.

Conclusion

Based on the results of the study, subjecting blueberry seeds to cold storage for nine days before sowing hastened germination (G_{50}) and showed the best results. It was also found that cold storage of blueberry seeds for a period of nine days at $5^{o}C$ markedly improved uniformity of germination (G_{90}-G_{10}) and enhanced faster germination and seed emergence as well as higher percentage of normal seedlings and vigorous seedling growth.

Recommendation

Based on the results, it is recommended that cold storage of blueberry seeds for a period of nine days at 5 ^{o}C prior to sowing should be done in order to enhance faster and uniform seed germination and seed emergence as well as to promote taller seedlings, development of more leaves and vigorous seedling growth.

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