# Viability of European bird cherry (*Prunus padus* L.) seed after two-year retention in traps along the Chester and Campbell Creek Trails, Anchorage, Alaska

**Submitted by:**

Alaska Natural Heritage Program, University of Alaska Anchorage
L. Flagstad, H. Cortés-Burns, E. Johnson, L. Simpson, A. Brownlee
707 A Street
Anchorage, Alaska 99501

**Submitted to:**

Municipality of Anchorage
Parks and Recreation
City Hall
632 West 6th Avenue
Anchorage, Alaska 99501

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## Background

The Alaska Natural Heritage Program recently concluded a study of the distribution, demography and reproductive ecology of the invasive tree species, European bird cherry (*Prunus padus* L.) for the Municipality of Anchorage (Cortés-Burns and Flagstad 2010). This work was conducted over two field seasons (2008, 2009) and focused on populations along the Chester and Campbell Creek Trails in Anchorage, Alaska.

As part of this study, seed-traps that had been set up along both greenbelts in 2008 were revisited in 2009, when part of the seed was collected and tested for viability using a tetrazolium biochemical test (AOSA 2007). We found that, after one winter, 79% of the seeds tested remained viable (Cortés-Burns and Flagstad 2010). This percent viability is within range for similar studies: Gordon and Rowe (1982) reported 74% viability of *Prunus padus* seed after one year, and Grisez (1974) recorded an 85% average germination rate of *Prunus padus* seeds after one year.

To determine whether any of the seeds that remained in the 2008 traps were still viable two years later, AKNHP revisited and re-sampled the traps in 2010. The results of this second round of testing are presented herein and will help guide management of *Prunus padus* infestations in Anchorage and similar riparian habitats and provide direction for any future seed viability testing for this species.

## Methods

Burlap seed traps set up along the Chester and Campbell Creek Trails in summer 2008 were sampled in July 2010 to collect two-year-old *Prunus padus* seeds for viability testing. (Seed had already been collected from these traps in 2009 to assess the viability of one-year old seeds). The 2009 findings are summarized in the results section of this report and detailed in Cortés-Burns and Flagstad (2010); also see Cortés-Burns and Flagstad (2010) for the description of the study area, plot location and seed trap construction. Only twelve seeds remain in traps after the 2010 collection: two at plot BCT 02.11D, on the Chester Creek trail, and ten at plot BCT 17.4A, on the Campbell Creek trail (Figure 1). Plots where no seeds remained (BCT 01, 03-16, 18-20) were dismantled; aluminum marker tags were cut from trees, and pegs securing the burlap seed traps were removed from the ground. If necessary, these plots can be relocated from their GPS coordinates and plot photos (see Appendices III and VIII, Cortés-Burns and Flagstad 2010).

Seed viability testing was conducted at the Ecosystems and Biomedical Lab on the University of Alaska Anchorage campus. Viability was assessed by tetrazolium staining in accordance with the procedure developed for seeds of species belonging to the Rosaceae family, *Prunus* genus, by the Association of Official Seed Analysts (2007). This biochemical test differentiates live from dead seeds based on the activity of respiration enzymes in the seeds.

Figure 1. View from south (left photo) and north (right photo) of plot BCT 17 (N 61.17152$^{o}$, W -149.87222$^{o}$).

Figure 2. Endocarp cracking along seam using a small vice (AOSA 2007).

Seeds were prepared for staining by soaking them in lidded plastic vials of deionized (DI) water for 12 hours at room temperature (20-25°C). After 12 hours, the endocarp was cracked and removed using a pair of needle-nose pliers. Three of the 37 seeds selected for testing cracked quickly, causing the embryo to split in half or become slightly crushed. For this reason, a small vice would be a more effective tool for cracking the endocarp, as it would allow greater control of the pressure applied (Figure 2). Eight of the 37 seeds selected for testing were already split in half when the endocarp was removed. Split embryos could still be stained, despite the occasional separation of the cotyledon and radicle. Seeds that were completely rotten (black, moldy, gooey) when the endocarp was removed were not tested for viability with tetrazolium, but were simply counted as 'unviable'. Seeds with minimal necrosis were tested.

Figure 3. External view of endocarp, left. Endocarp and seed, middle. Seed coat showing nick at distal end, right (AOSA 2007).

After the endocarp was removed, the seed coat was nicked using a small razor blade at the distal end (Figure 3, 4). It is important to take care with this step as it is necessary to fully nick the seed coat, without cutting into the embryo. Nicked seeds were transferred back to their vials and soaked in fresh DI water for 48 hours at 20-25°C; water was changed every 24 hours. We recommend that in future testing efforts, the seeds soak for a full three days to facilitate removal of the seed coat and nucellus from the embryo (Figure 5). The seed coat and nucellus were removed using two pairs of tweezers. Again, care must be taken in this step to avoid damaging the embryo.

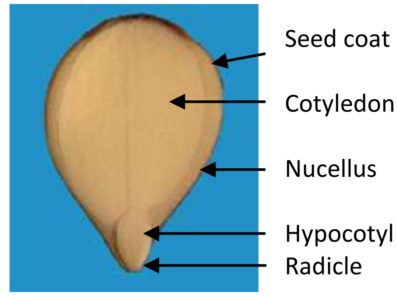


Figure 4. Embryo cross-section (AOSA 2007).

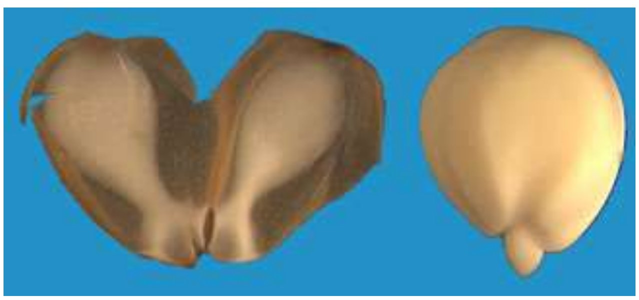

Figure 5. Seed coat and nucellus, left. Embryo, right (AOSA 2007).

A 1% tetrazolium solution was made using 1 g of 2,3,5-triphenyl tetrazolium chloride (TTC) powder dissolved in 99 mL DI water (99 g massed). Embryos were placed back in their vials and immersed in the 1% TTC solution for two hours in a water bath heated to 30-35°C. After two hours, seeds were assessed for viability. Vials, with TTC solution and seeds inside, were collected as chemical waste.

This biochemical test differentiates live from dead tissue based on color. Respiring tissue of the embryo stains red, whereas non-respiring tissue of the embryo does not stain (remains white, Figures 6, 7).

A seed is considered **viable** if:

- The radicle is completely stained (an unstained distal tip is acceptable)
- The hypocotyl is completely stained
- The cotyledons are completely stained, or if pervading necroses, less than 1/3 of the distal end of the cotyledons remains unstained, or if superficial necroses, less than 1/2 of the distal end of the cotyledons, or the area near the seed center remains unstained

A seed is considered **unviable** if:

- More than the distal tip of the radicle remains unstained
- The hypocotyl is less than completely stained
- The basal half of the cotyledons are less than completely stained, or if pervading necroses, more than 1/3 of the distal end of the cotyledons remain unstained, or if superficial necroses, more than 1/2 of the distal end of the cotyledons remains unstained

Figure 7. Example of tetrazolium seed viability test results. This test differentiates live, respiring seeds (red) from dead, non-respiring seeds (white) based on color.

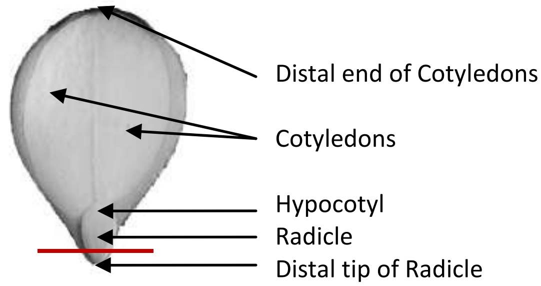


Figure 6. Cross-section of seed with the distal tip of the radicle delineated. The radical develops into the root, the hypocotyl develops into the stem and the cotyledons develop into the leaves.

## Results

Of the 40 traps established in 2008, only 20 were relocated in 2010. Of these, only ten (10) contained two-year-old Prunus padus seeds. A total of 82 two-year-old seeds were counted in the traps. Twelve (12) of the 82 seeds were placed back in their traps. Of the 70 seeds removed, 33 were discarded without biochemical testing because they were completely rotten and were therefore considered unviable. Tetrazolium testing was carried out on the remaining 37 seeds. Results indicate that 27% of the 70 seeds collected in 2010 were viable (Table 1).

There is a marked decrease in seed viability from one to two-year old seeds; 79% of the 119 one-year old seeds tested in 2009 were viable, in contrast with 27% in 2010. Viability of the one-year old seeds ranged from 56 to 100 percent; due to the smaller sample size, viability of two-year old seeds ranged from 0 to 100 percent. Finally, when comparing the viability of seeds collected from a given trap in both 2009 and 2010, we find a decrease in percent viability for all plots except BCT 07 (Chester Creek), where 100% of seed was viable in both 2009 (n=3) and 2010 (n=1).

Table 1. Number and percent viability of one (left) two-year-old (right) *Prunus padus* seeds collected from seed traps along the Chester (plots 001-010) and Campbell (plots 011-020) Creek trails in Anchorage, Alaska.

| Plot Number (Plot.Transect.Meter) | # seeds found in '09 | # seeds left in traps in '09 | # seeds tested in '09 | # seeds viable in '09 | % viability in '09 | # seeds found in '10 | comments | # seeds left in traps in '10 | # seeds discarded due to rotting in '10 | # seeds tested in '10 | # seeds viable in '10 | % viability in '10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BCT-001.1.11D** | 26 | 23 | 3 | 3 | 100 | 3 | all cracked | 0 | 0 | 3 | 1 | 33 |
| **BCT-001.2.4A** | 52 | 42 | 10 | 9 | 90 | 1 | rotten | 0 | 0 | 1 | 0 | 0 |
| **BCT-002.1.4A** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-002.2.11D** | 25 | 22 | 3 | 3 | 100 | 9 | found 9, left 2, 6 rotting, 1 cracked | 2 | 0 | 7 | 1 | 14 |
| **BCT-003.1.11D** | 238 | 213 | 25 | 18 | 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-003.2.4A** | 235 | 215 | 20 | 18 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-004.1.4A** | trap missing | trap missing | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-004.2.11D** | 2 | 2 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |

(Table 1 cont'.)

| Plot Number (Plot.Transect.Meter) | # seeds found in '09 | # seeds left in traps in '09 | # seeds tested in '09 | # seeds viable in '09 | % viability in '09 | # seeds found in '10 | comments | # seeds left in traps in '10 | # seeds discarded due to rotting in '10 | # seeds tested in '10 | # seeds viable in '10 | % viability in '10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BCT-005.1.11D** | 0 | 0 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-005.2.4A** | 13 | 12 | 1 | 1 | 100 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-006.1.4A** | 0 | 0 | 0 | 0 | 0 | 0 | trap destroyed | 0 | 0 | 0 | 0 | 0 |
| **BCT-006.2.11D** | 0 | 0 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-007.1.11D** | 30 | 27 | 3 | 3 | 100 | 1 | looked good, trap has large hole | 0 | 0 | 1 | 1 | 100 |
| **BCT-007.2.4A** | 28 | 25 | 3 | 3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-008.1.4A** | 259 | 234 | 25 | 14 | 56 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-008.2.11D** | 42 | 38 | 4 | 3 | 75 | 1 | seed shriveled, trap with holes | 0 | 0 | 1 | 0 | 0 |
| **BCT-009.1.11D** | 1 | 1 | 0 | 0 | 0 | 0 | trap had large holes | 0 | 0 | 0 | 0 | 0 |
| **BCT-009.2.4A** | 1 | 1 | 0 | 0 | 0 | 0 | trap had large holes | 0 | 0 | 0 | 0 | 0 |
| **BCT-010.1.4A** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-010.2.11D** | 22 | 20 | 2 | 2 | 100 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |

(Table 1 cont'.)

| Plot Number (Plot.Transect.Meter) | # seeds found in '09 | # seeds left in traps in '09 | # seeds tested in '09 | # seeds viable in '09 | % viability in '09 | # seeds found in '10 | comments | # seeds left in traps in '10 | # seeds discarded due to rotting in '10 | # seeds tested in '10 | # seeds viable in '10 | % viability in '10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BCT-011.1.11D** | 0 | 0 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-011.2.4A** | 18 | 17 | 1 | 1 | 100 | 7[1] | 1 seed shriveled, 6 looked good, (2 look like 2009 seeds) | 0 | 0 | 7 (2 look like 2009 seeds) | 3 (5 if we include the 2 2009-seeds) | 43 |
| **BCT-012.1.11D** | 0 | 0 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-012.2.4A** | 11 | 10 | 1 | 1 | 100 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-013.1.4A** | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-013.2.11D** | 45 | 40 | 5 | 4 | 80 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-014.1.11D** | 4 | 4 | 0 | 0 | 0 | 3 | 2 cracked, 1 looked good | 0 | 0 | 3 | 2 | 67 |
| **BCT-014.2.4A** | 16 | 15 | 1 | 1 | 100 | 5 | 4 rotten, 1 looked good | 0 | 4 | 1 | 1 | 25 |
| **BCT-015.1.4A** | 53 | 48 | 5 | 4 | 80 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-015.2.11D** | trap missing | trap missing | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |

[1] The seed trap at plot BCT 17 contained at least two (and maybe four) one-year-old seeds. The surfaces of the suspect seeds were shiny, chestnut-colored and showed minimal wear, whereas surfaces of two-year-old seeds were typically dull or matte and grayish-brown-colored. All four of the suspect seeds tested viable. Two (the shiniest) of these seeds are not included in the totals, thus reducing the percent viability from 33 to 27%.

(Table 1 cont'.)

| Plot Number (Plot.Transect.Meter) | # seeds found in '09 | # seeds left in traps in '09 | # seeds tested in '09 | # seeds viable in '09 | % viability in '09 | # seeds found in '10 | comments | # seeds left in traps in '10 | # seeds discarded due to rotting in '10 | # seeds tested in '10 | # seeds viable in '10 | % viability in '10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BCT-016.1.11D[2]** | 1 | 1 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-016.2.4A** | 3 | 3 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-017.1.4A** | 56 | 51 | 5 | 4 | 80 | 45 | collected 45 seeds, left 10, 24 rotten, 3 cracked, 8 looked good | 10 | 24 | 11 | 9 | 26 |
| **BCT-017.2.11D** | 29 | 27 | 2 | 2 | 100 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-018.1.11D[3]** | 2 | 2 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-018.2.4A** | 8 | 8 | 0 | 0 | 0 | 7 | 5 rotten, 1 cracked, 1 looked good | 0 | 5 | 2 | 1 | 14 |
| **BCT-019.1.4A[4]** | trap buried | trap buried | 0 | 0 | 0 | 0 | grass clippings present | 0 | 0 | 0 | 0 | 0 |
| **BCT-019.2.11D** | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **BCT-020.1.11D** | trap missing | trap missing | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| **BCT-020.2.4A** | 0 | 0 | 0 | 0 | 0 | 0 | trap missing | 0 | 0 | 0 | 0 | 0 |
| | **Totals:** | 1104 | 119 | 94 | **79** | 82 | | 12 | 33 | 37 | 19 | **27** |

[2] Trap in poor condition. Seeds could have fallen out the bottom or off the sides of the trap in 2009.
[3] Trap located on a small island in high water. It is likely that some seed got washed away when visited in 2009.
[4] Grass clippings had been dumped on top of trap in 2009 (and again in 2010). This trap was excluded from all analyses in 2009.

## Management Implications & guidelines for future work

The marked decline in viability from one-year-old to two-year old *Prunus padus* seeds (79% to 27%) shown in this study suggests that seed will be minimally viable after three years. A similar study conducted in a boreal forest in Sweden found that approximately 10% of *Prunus padus* seed germinated after two winters and that the seed bank was completely depleted (by germination) within three years (Granström 1987). This corroborates our findings; we therefore conclude that *Prunus padus* seeds in Alaska will (on average) only remain viable for one to two years, and will have mostly germinated or rotted after the third winter.

However, further testing of the Chester and Campbell Creek seed is recommended to confirm the expected decline in viability and/or depletion of the seedbank through germination. Only 12 seeds remain in the traps established in 2008: two at Chester Creek trail plot 'BCT 02.11D' and ten at Campbell Creek trail plot 'BCT 17.4A'. Despite this small sample size, we recommend that all remaining seed be collected in the summer of 2011 and tested to determine the viability of three-year-old seed.

The results obtained through the 2009 and 2010 studies can be used to inform bird cherry tree management decisions. As previously recommended in Cortés-Burns and Flagstad (2010), control efforts should target fruit-producing *Prunus padus* trees first. Given the relatively low viability of seed in the soil, eliminating fruit-producing trees should greatly increase the possibility of eradicating the infestation (barring new introductions of seed by birds or other dispersal vectors). Furthermore, quantifying the viability of three-year-old seed in 2011 will help determine how long infestation sites need to be monitored for following removal of mature bird cherry trees. For example, if seeds have either germinated or are not viable after three years, then annual monitoring could be curtailed after four years providing that all seedlings and basal shoots have been eradicated from a given site.

In conclusion, the 2009 and 2010 seed viability findings will be helpful in developing control actions for *Prunus padus* within the Anchorage Bowl (or for similar infestations occurring along Chena River in Fairbanks). Efforts should focus on preventing the spread of this species further upstream along the Chester and Campbell creeks. Removal of fruit-producing trees in the more remote, outlying populations, followed by monitoring of the sites and extirpation of any new seedlings and saplings for a minimum of four years could help achieve this.

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