

## Notes and Discussion

### Lodgepole Pine Seed Germination Following Tree Death from Mountain Pine Beetle Attack in Colorado, USA

**ABSTRACT.**—Cones of lodgepole pine (*Pinus contorta* var. *latifolia*) are often serotinous, releasing their seeds from closed cones under heat from fire. Stand-replacing fires in predominantly serotinous stands can thus be expected to result in a strong regeneration response. After large-scale mortality caused by mountain pine beetle (*Dendroctonus ponderosae*), however, the seeds in serotinous cones may remain on the dead trees for a number of years, impacting germination and viability. We tested seeds collected from living and beetle-killed serotinous stands to determine whether they remain viable after tree death, and whether germination rates were affected by cone age. There was no significant difference in percent germination from the living stand vs. the dead stand. While there was a significant relationship between cone age and percent germination, cones that were 21–25 y still had >30% germination. We conclude that post-beetle regeneration likely will not be limited by viable seed availability in stands with serotinous cone-bearing trees.

#### INTRODUCTION

The mountain pine beetle (*Dendroctonus ponderosae*) has recently reached epidemic population levels in the United States and Canada (Raffa *et al.*, 2008). In Colorado, the State Forest Service estimates that over 600,000 ha have been affected during the current outbreak, a scale unprecedented in recorded history. Lodgepole pine (*Pinus contorta* var. *latifolia*), the tree most affected in the outbreak, usually regenerates in large numbers following stand-replacing fire. An important regeneration mechanism is the production of serotinous cones, which remain closed until stimulated by the heat of the fire to open and release their seeds (Tower, 1909; Clements, 1910). Lodgepole pine vary greatly in the proportion of serotinous and non-serotinous cones (*e.g.*, Schoennagel *et al.*, 2003), but many of the stands affected by the current outbreak in Colorado are composed of predominantly serotinous trees; *e.g.*, a recent survey of lodgepole pine stands on the west side of Rocky Mountain National Park found a mean serotiny per stand of 63% (C. Aoki, pers. obs.). In the absence of fire, large numbers of beetle-killed trees will remain on the landscape, with their seeds still tightly held within the cones. What will this mean for the future regeneration of these stands across the landscape?

Unlike a fire, in which seeds are released by heat in a single event, a large-scale mortality event caused by beetles will result in a slower release of the seeds. The cones will open over a number of years, either through radiant heat in the canopy or by absorbing heat near the ground as limbs break off and fall (Tower, 1909; Lotan, 1964). A key question, then, is whether or not seeds held in serotinous cones remain viable for years after the tree has died. Early studies indicated that in some instances, seeds could survive a number of years on a dead tree, or even separated from the tree (Sargent, 1880; Tower, 1909; Mills, 1915). Mirov (1946) showed that lodgepole pine seeds kept in cold storage for over nine years still maintained high germination. However this has not been evaluated in a controlled experiment utilizing seeds remaining in the canopy of standing dead trees. Will stands dominated by serotinous lodgepole pine have the viable seed needed for regeneration over the years following the beetle outbreak?

To test this question, we conducted an experiment using serotinous trees from the current beetle outbreak in Rocky Mountain National Park, Colorado, comparing germination in serotinous cone seeds between living trees and trees killed in the current outbreak, as well as between cones located on younger vs. older portions of branches. Knowing whether seed germination declines over time in dead trees will help us to understand the future regeneration possibilities for these stands with extensive overstory mortality.

Before we began our study, we also needed to determine an effective method of determining cone age from sampled branches. Previous studies requiring cone aging used bud scale scars or branch whorls to determine age (*e.g.*, Hellum and Barker, 1981; Benkman *et al.*, 2003). However, bud scale scars are often not visible beyond the initial years of a stem's growth, and branch whorl morphology on old trees is often highly variable. Most morphological studies of pine stem growth and its relation to cone growth (*e.g.*, Shaw, 1914; Franklin and Callaham, 1970; Van Den Berg and Lanner, 1971) have been

conducted on young stems, making it difficult to know whether it was reasonable to count one year for each whorl of branches or cones in older stems or branches. These previous studies describe lodgepole pine as a multi-nodal species, which can produce more than one whorl of branches or cones per year. No study has yet documented how to use these whorls for aging once the stem or branch has matured.

We addressed three questions: (1) Can branch and cone whorls be used to reliably age cones? (2) Does germination of seeds of a given age differ between live trees and trees dead at least 3 y after beetle attack? (3) Does seed germination differ in older vs. younger serotinous cones?

#### STUDY AREA AND METHODS

Two sites were selected on the western side of Rocky Mountain National Park (ROMO), where the current outbreak is underway. Instrumental climate data are available for approximately the last 70 y, from a station located very close to our southernmost site. Average annual precipitation was 503 mm, average Jan. minimum temperature was  $-16.5$  C, and average Jul. maximum temperature was  $24.6$  C (Colorado Climate Center, 2010). To collect samples from trees that had been dead for the longest period of time, we selected a lower-elevation site near ROMO's western entrance, Harbison Meadows Picnic Area ( $40^{\circ}17'N$ ,  $105^{\circ}50'W$ , 2661 m, uneven ages ranging from approximately 180 to 280 y). Within the first year following an attack, the dead tree's needles turn red. These needles subsequently drop off, with nearly 100% needle loss occurring between 2 and 3 y post-attack (British Columbia Ministry of Forests, 1995). Nearly all the overstory trees at Harbison Meadows had been killed by beetles, and most had lost all their needles, so the seeds on these trees have been on dead branches for at least 3 y, possibly longer. We selected Timber Lake Trailhead ( $40^{\circ}24'N$ ,  $105^{\circ}51'W$ , 2716 m, even-aged stand of approximately 110 y), also on the west side of ROMO, as the site for sampling living trees. A few trees in this area showed evidence of beetle attack, but most appeared healthy. In Sept. 2007, ten representative canopy trees were felled by chainsaw at each location, for a total of 20 trees. Individual branches were then harvested from the upper third of each individual. The harvested limbs were stored in a cool, dry basement for approximately 5 mo.

Our first task was to determine the ages of the cones along the branches (our methodological question 1). To do this, we cut apart several samples, and finely sanded a cookie taken from between branch or cone whorls (Fig. 1). The annual rings in these cookies were then counted under a dissecting scope to determine the age of the nearest adjacent cone. We made detailed observations of the relationship between whorl morphology and the age of the branch at that point, to determine whether the whorls provided accurate aging for the cones themselves.

Once we were confident in our ability to correctly identify the number of years per whorl (*see* Results below), we removed the cones and placed them in 4 five-year age bins: 6–10 y, 11–15 y, 16–20 y and 21–25 y. (To ensure proper dating on each individual sample, a cookie was taken and ring-counted from the cut end of all samples, and if the branch's tip was missing, a cookie was also taken and counted from the top end, thus assuring proper cone aging between the two ends.) The cones were prechilled in a refrigerator at 3 C for 5 wk (Tanaka, 1984). We then heated the cones for 24 h in an oven at 60 C, an average temperature to completely open serotinous cones without inhibiting their germination response through overheating (Clements, 1910; Perry and Lotan, 1977; Knapp and Anderson, 1980; Johnson and Gutsell, 1993). Seeds that were clearly empty (*i.e.*, those that could be easily crushed between the fingers and were thus comprised of a seed coat with no interior) were removed, and the remainder were de-winged and divided into lots of up to fifty seeds per age bin per tree. Table 1 shows the distribution of seeds by individual tree and age class. Each lot was placed in a petri dish containing two filter paper circles, then dusted with the fungicide Captan (50 percent), and wetted with 5 ml of water (Abouguendia and Redmann, 1979). The dishes were covered and placed in a germinator with alternating light and temperature (27 C for 8 h in the light, 20 C for 16 h in the dark (Knapp and Anderson, 1980; Tanaka, 1984). The filter paper was re-wetted as needed throughout the experiment. Seeds with the radicle protruding at least 1 mm were considered germinated.

Germination percentages were compared using logistic regression, with tree status (living/dead) and age bins as predictor variables. We selected logistic regression due to the binary nature of our germination data (germinated/did not germinate), as well as the ordered nature of the age class bins. Logistic regression ensures that model results are bounded by 0 and 1, and accounts for the fact that the

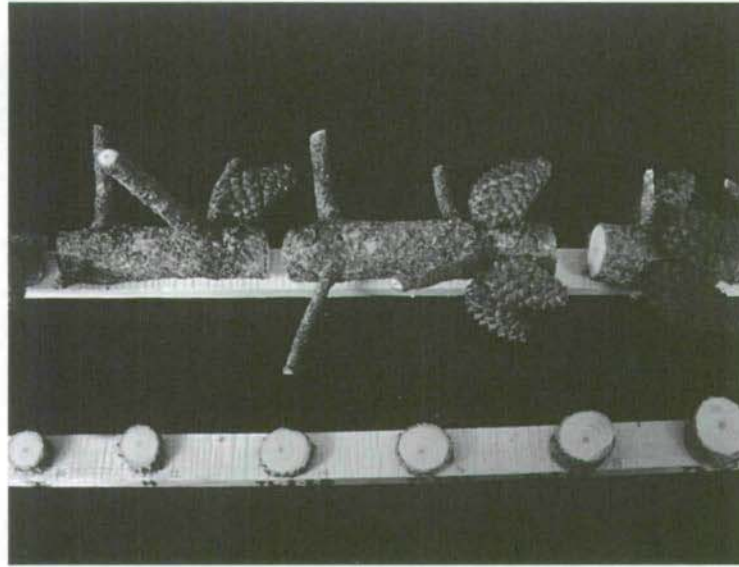


FIG. 1.—Branch fragment shown (above) with mounted and sanded cookies (below). In this example, the fragment was sectioned every two whorls

errors are not normally distributed (Hosmer and Lemeshow, 2000). Analysis was performed using R statistical software (R Core Development Team, 2009). The lowest age bin (0–5 y) was eliminated due to the large number of missing branch tips from the dead trees whose crowns tended to shatter upon hitting the ground.

#### RESULTS

*Question 1: Cone ages and branch morphology.*—We identified two distinct morphologies, one in which the branches stemmed upward at an acute angle to the main stem, and did indeed represent one year per whorl, and a second one in which the branches stemmed in multiple directions from the branch, sometimes appearing to represent two whorls. These rings also represented just a single year, though they often could appear to represent two (Fig. 2).

The two morphologies were distinct enough from one another that it was almost always possible to distinguish them on the branch and assign ages correctly.

TABLE 1.—Number of seeds by site, per tree and age class

Site	Age Class	Tree Number										Total
		1	2	3	4	5	6	7	8	9	10	
<i>Dead Trees</i>	6–10	50		50	50	50	50		50	47		347
	11–15	32	11	50	50	50	50	31	50	50		374
	16–20	50	50		50		50	50	50	18		318
	21–25		50		21				32			103
<i>Living Trees</i>	6–10	35	50	50	50	31	50	50	25	14	50	405
	11–15	29	50	45	50	50	50	50	35		42	401
	16–20		50						50	50		150
	21–25		50						25	37		112

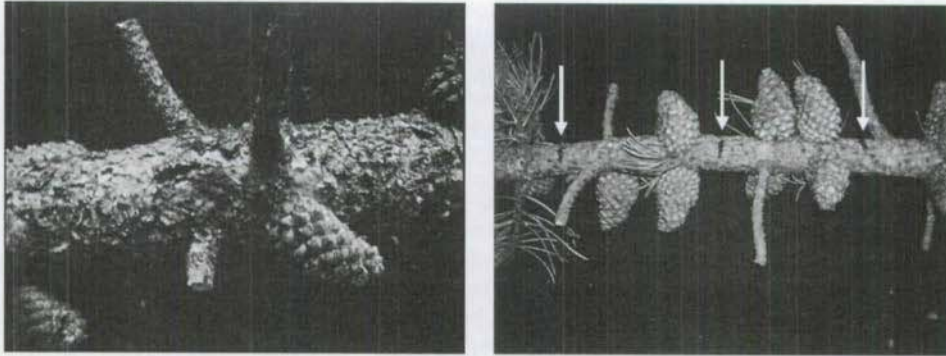


FIG. 2.—Two examples of “double whorls” representing one year each. In the photo on the right, years are marked with arrows

*Question 2: Seed germination in dead vs. living trees.*—Seeds from the dead trees germinated at an average of 53%, compared with 58% from the living trees (Fig. 3a). This difference was not statistically significant ( $P = 0.64$ ).

*Question 3: Younger vs. older cones.*—Average percent germination from both sampling sites declined significantly between the 6–10 y bin, and the 21–25 y bin ( $P < 0.0001$ , Fig. 3b.). However the average germination over both sites was  $>30\%$ , even for the oldest age bin.

#### DISCUSSION

Landowners and the public see many hectares of dead trees across the landscape, and they worry that these stands may not regenerate. Previous studies of extensive mountain pine beetle mortality have shown that lodgepole pine stand structure can change substantially following beetle outbreaks (e.g., Roe and Amman, 1970; Sibold *et al.*, 2007). Moderate outbreaks where advance regeneration is present may

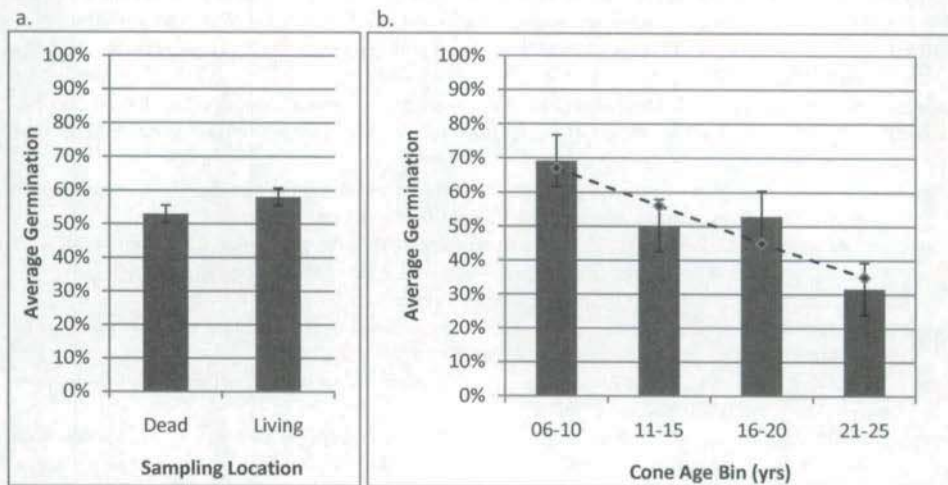


FIG. 3.—Comparison of average percent germination, showing mean and standard error. (a) dead trees vs. living trees; (b) cone age bins in years; dotted line indicates the probability of germination based on the logistic regression analysis

lead to the successional dominance of other species such as Douglas-fir (*Pseudotsuga menziesii*) or subalpine fir (*Abies lasiocarpa*), while severe outbreaks in single-story stands may lead to dominance by grasses which subsequently suppress lodgepole pine regeneration (Amman, 1977; Stone and Wolfe, 1996). However, previous studies have not addressed successional questions related to stand serotiny and canopy seed viability and germination following beetle-induced tree mortality. While non-serotinous cones release their seeds as the cones mature, leaving little seed bank for regeneration following an outbreak, serotinous cones have the potential to hold a canopy seed bank from which beetle-killed stands may regenerate. Many of the beetle-killed stands in ROMO contain a high percentage of serotinous trees. Their cones will begin to open following tree death, particularly from ground heat as the branches fall; only a few days near the soil surface are required to break the cone's resin bonds (Tower, 1909; Lotan, 1964). Our experiment showed that these standing dead serotinous trees do hold many viable seeds, even after the beetle epidemic has moved on and most of the overstory has died. Even cones that had been on the tree for up to 25 y prior to tree death contained many viable seeds. Thus, stands with a high percentage of serotinous trees may follow a different post-beetle regeneration trajectory than non-serotinous stands.

Other variables, such as quality of seed beds, competition with herbaceous and shrub species, and seed predation, may result in poor regeneration. However, our results suggest that post-beetle regeneration likely will not be limited by seed germination ability in stands with serotinous cone-bearing trees.

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