Willow Decline in Rocky Mountain National Park: Examining the Interactions of Drought, Ungulate Browsing, Sapsuckers, and *Cytospora* Fungal Infection

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We are examining willow decline at both a landscape and valley-wide scale and have identified three primary objectives to identify the causes, extent and timing of the decline. Research began in spring 2009 and this phase of the research program will end in late 2012. We initiated this research in the Kawuneeche Valley, where dieback is widespread, utilizing three existing ungulate exclosures, Colorado River trailhead (CRT), Gaskil and Green Mountain (GM), and expanded during the summer of 2010 to include sites in Endo Valley, where dieback is currently limited. This interim report reviews our research objectives and the progress/results achieved during 2011 in answering each question.

1. Timing and extent of willow dieback – in RMNP and throughout the Colorado Rocky Mountains

To investigate when the dieback occurred within the Kawuneeche Valley, we compiled a series of aerial photographs (1937, 1969, 1987, 1996, 1999, 2001, 2005 and 2008). Photos were scanned and geo-rectified (when necessary). The study area was divided into three segments, north, middle and south, based on roads. RMSE for each rectification of the entire valley was less than 2m and each valley segment was then adjusted to obtain a RMSE of 1m. A 2x2 meter grid of points was created for the entire study area. One percent of these points were randomly chosen for each of the three segments, resulting in 4431, 9813, and 11,453 points for the north, middle and southern sections, respectively. Each point was analyzed each year for the presence or absence of a willow individual or individuals. The numbers of willow present points were compared for each sampled year. We ground-truthed 138 randomly selected points in the summer of 2011. We had 76% accuracy, with 22% errors of omission (due to short willows) and 2% errors of commission. Preliminary analysis indicates that a sharp decline in willow presence points occurred from 2001 and 2005 (Figure 1). The willow counts will be analyzed using generalized linear models to examine the effects of ungulate browsing, climate and stream flow on willow presence.

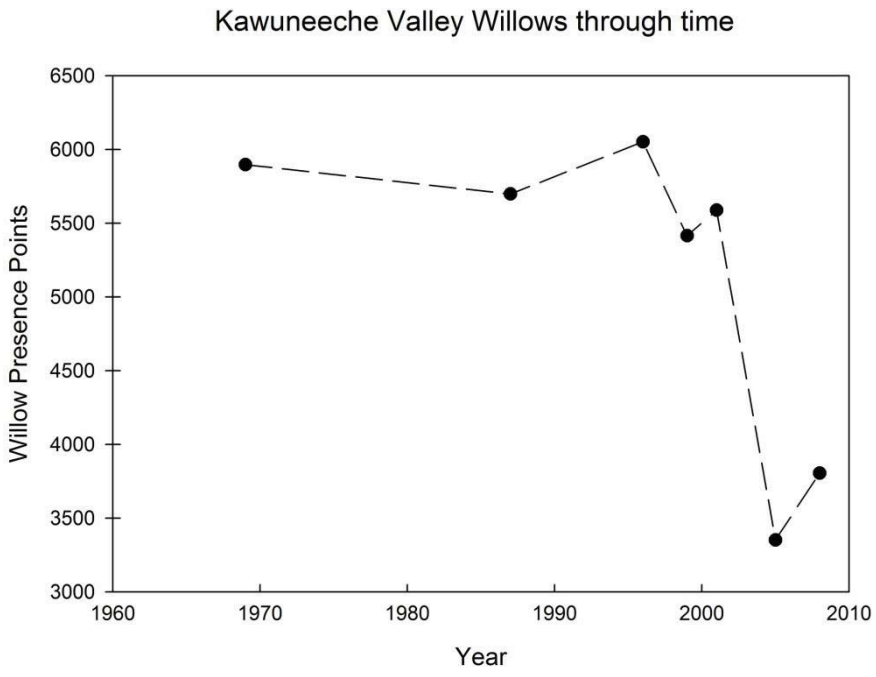

Figure 1: Counts of willow presence points from aerial photo analysis in the Kawuneeche Valley. 1937 is not included in this graph because photos only covered the northern portion of the valley.

In 2011, we identified 5 willow dominated sites within RMNP (Kawuneeche Valley, Endo Valley, Moraine Park, Wild Basin and Hallowell Park) and 16 sites outside of RMNP. Sites outside of RMNP were randomly selected using GIS. We randomly selected among riparian sites dominated by willows located on public land, within 1km of a road, with a low gradient slope, between 7500 and 9000 ft in elevation (Figure 2). At each site, we chose a random starting location to walk a transect with 10 point center quarter sample plots. At each plot, we sampled 4 willows (resulting in 40 willows per site). We recorded species, % dieback, % browse, % *Cytospora*, and number of stems affected by sapsuckers. Preliminary analysis has shown that dieback, browsing and *Cytospora* is greater within RMNP than for sites outside RMNP.

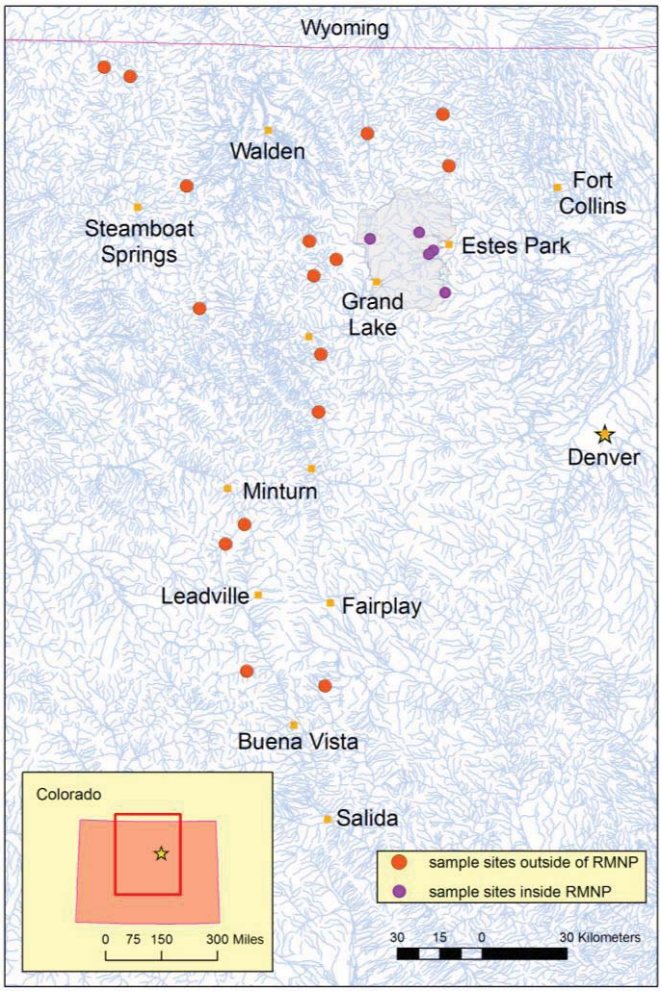

Figure 2: Locations sampled for willow decline inside and outside of RMNP

We also collected, mounted and sanded an additional 58 willow stem sections from both the Kawuneeche Valley to determine the timing of the dieback. The timing of individual stem death can be identified using the age of epicormic shoots, which appear when the main stem is stressed or dying. Epicormic stem ages will be used to create a willow stem dieback chronology, which may help us identify whether the dieback can be correlated with large scale climate drivers, such as the drought of 2002.

2. Factors contributing to willow stem dieback

We initiated a factorial experiment examining the effects of warming, drought and *Cytospora* on willow stems (Figure 3). We paired warming and ambient treatments to create 3 blocks. In each warming treatment we manipulated water levels to create a drought stressed and a well watered treatment. Within each water treatment, we wounded and inoculated 4 stems. We created 3 wounds on each stem, one control wound 'inoculated' with sterile agar, and two wounds inoculated with two different isolates of *C. chrysosperma*. Our total sample size was 96 *S. monticola* stems. We monitored temperatures in each treatment using Hobo temperature loggers and monitored soil moisture in each pot using a handheld TDR probe. We measured photosynthetic capacity using a Li-Cor portable

photosynthesis system at two periods during the summer. All plants were harvested at the end of the experiment in early September 2011, to measure aboveground and belowground annual net primary production. *Cytospora* will also be reisolated from wounds that demonstrated canker growth.

Figure 3: One replication of the effects of warming, drought stress and *Cytospora* on willows experiment

Willows in both Endo and Kawuneeche Valleys have been compared over the sampled years (2009 – 2011) using variables such as height (Table 1) and annual net primary production (Table 2). Production was measured using the methods of Bilyeu et al. (2007). Willow height inside exclosures is significantly taller than outside. Presence or absence of browsing is the strongest predictor of willow heights in the Kawuneeche Valley, with depth to water table and species not contributing to the best model. Annual net primary production is significantly higher inside exclosures compared with outside. In the Kawuneeche Valley, annual production was best predicted by presence or absence of browsing, with depth to water table, species, and stem height not contributing to the best model.

Table 1: Comparison of willow heights in Endo Valley (EV) and inside (Kaw - I) and outside (Kaw - O) exclosures in the Kawuneeche Valley. Decrease in height is a percent decrease between inside and outside exclosures.

	2009	2010	2011
EV		299cm	307cm
Kaw - I	191cm	217cm	219cm
Kaw - O	84cm	82cm	81cm
Difference in height	56%	62%	63%

Table 2: Comparison of willow annual production (in kg/plant) in Endo Valley (EV) and inside (Kaw - I) and outside (Kaw - O) exclosures in the Kawuneeche Valley. Decrease in ANPP is a percent decrease between inside and outside exclosures.

	2009	2010	2011
EV		404	511
Kaw - I	254	315	316
Kaw - O	67	63	81
Difference in ANNP	74%	80%	76%

We analyzed additional samples of fungi cultured from dead and dying willow stems in RMNP using DNA to determine the genus and species of fungus potentially killing the willows. The DNA sequences match sequences of *Cytospora chrysosperma* (Pers.:Fr.) Fr., the same species associated with aspen decline.

We inoculated 14 stems inside the CRT exclosure in September 2010. Measurements of the wound's length and width were made to determine the spread of the infection were made in mid-December 2010 and June 2011. Canker growth was significantly larger on inoculated wounds than on controls ($p < 0.01$). We successfully re-isolated *Cytospora* from the cankers, indicating that the *Cytospora* we inoculated with did create the canker.

3. Sapsuckers and *Cytospora* infection

We observed that willow stems with sapsucker sap wells were dead above the wound, and many dead stems displayed signs of *Cytospora* infection, including fruiting bodies. Sapsuckers preferentially choose larger diameter willow stems, which more often occurred inside exclosures. We reassessed each tagged stem (n = 59 in 2009 and n = 58 in 2010) in 2011 to assess for death and death with signs of *Cytospora*. Stem death due to sapsucker was best predicted by the length of the wound.

Table 3: A. Sapsucker wounded stems in 2009 (n = 59) and B. Sapsucker wounded stems in 2010 (n = 55)

A	2009	2010
Epicormic sprouts	--	69%
Dead stems	0%	47%
Cytospora (on dead stems)	0%	93%

B	2010	2011
Epicormic sprouts	68%	--
Dead stems	0%	62%
Cytospora (on dead stems)	0%	100%

We observed that sapsuckers not only contribute to potential *Cytospora* infection, but also play a role in reduction of stem heights (Figure 4). Sapsuckers reduced the heights of willow stems to the same average height as stems browsed by ungulates.

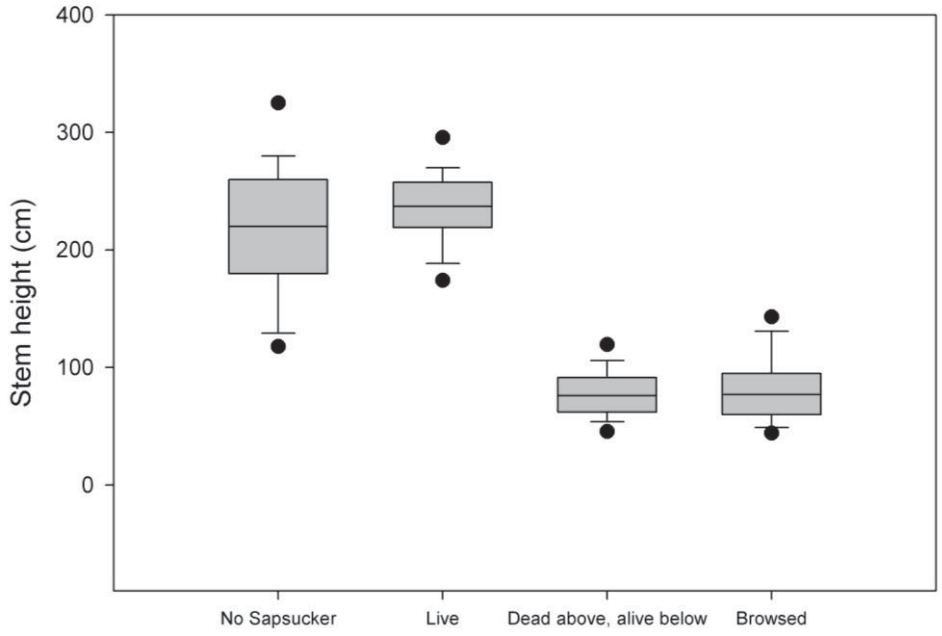

Figure 4: Comparison of heights of willows stems. Box plots are the median, 10th, 25th, 75th, and 90th percentiles, with 5% and 95% outliers. 'No sapsucker' are live, unbrowsed stems. 'Live' are sapsucker wounded stems which did not experience stem death above the wound. 'Dead above, alive below' are sapsucker wounded stems in which the portion of the stem above the wound had died within one year of the initial wounding. 'Browsed' stems are stems which were browsed by ungulates, not wounded by sapsuckers.

In addition to wind and rain splash dispersal of *Cytospora* spores, we are also interested in possible biological vectors of *Cytospora* dispersal, especially sapsuckers. We continued our mist netting of sapsuckers during the summer of 2011. We identified active sapsucker nests throughout the eastern side of RMNP and targeted these nests in late-June for bird capturing. We set up mist nets outside nesting holes and birds were caught as they returned to or left the nest. We swabbed beaks and feet of each bird. Five birds (3 males and 2 females from 4 nests) were tagged, measured, and swabbed. In 2010 we captured four birds. Swabs were stored in sealed test tubes with 5ml of sterilized distilled water and brought to the lab. Swabs were used to inoculate agar plates in four separate streaks. Two additional plates were created after the swabs were rinsed in 1ml of water and this water was poured onto plates. Fungi were grown for 2 months, and then separate plates were created for each distinctive looking culture. Clean cultures were created of each culture for eventual identification. Based on

fruiting bodies, we identified cultures which most resembled *Cytospora* for DNA analysis. Four of the nine samples that we analyzed were positively identified as *C. chrysosperma*. We found *C. chrysoperma* on three of the nine individual birds. One individual had fungi on both its beak and feet and 3 other individuals only had the

4. Additional Accomplishments

The co-PI Kristen Kaczynski also received a National Park Service George Melendez Wright Climate Change Fellowship (2011 – 2012) this past year ($19,684).

In addition to data collection, the co-PI Kristen Kaczynski has given 7 presentations on this research:

- Front Range Student Ecology Symposium, Colorado State University (Feb 2011)
- RMNP – Resource Stewardship Day – oral presentation (Feb 2011)
- Guest lecture – Conservation Leadership through Learning hydrology course (CSU) (March 2011)
- Society of Wetland Scientists, Denver Chapter Speaker Series (April 2011)
- RMNP - interpretive staff training (June 2011)
- RMNP – Science Behind the Scenery talk (July 2011)
- Forest and Rangeland Stewardship Department seminar, Colorado State University (Oct 2011)
- RMNP Research Conference (March 2012, anticipated)
- Ecological Society of America annual conference (Aug 2012, anticipated)

Literature Cited:

Bilyeu, D. M., D. J. Cooper, and N. T. Hobbs. 2007. Assessing impacts of large herbivores on shrubs: tests of scaling factors for utilization rates from shoot-level measurements. Journal of Applied Ecology **44**:168-175.