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 questions 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 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 questions and the progress/results achieved thus far in answering each question.

1. When did the dieback occur and how widespread is dieback in RMNP?

In 2010, 19 Rocky Mountain Network wetland monitoring sites were visited that are willow dominated. Sites were located between 2549-3357 m elevation. Sites with the highest proportion of dieback were located in the Kawuneeche Valley, North Inlet and La Poudre Pass. *Salix planifolia* was the most common willow species encountered. All sites showed signs of browsing, except one near Dream Lake. *Cytospora* infections on willows were identified at 10 sites, however in low percentages. The low proportions of *Cytospora* could have been due to the timing of the surveys and the lack of fruiting bodies for identification. Once willow stems die and bark is lost, positive identification of *Cytospora* infection is much more difficult.

We have also collected, mounted and sanded willow stem sections from both the Kawuneeche Valley and Endo Valley to determine the timing of the dieback. The timing of individual stem death can be identified using the age of epicormic sprouts, which appear when the stem is stressed or dying. The 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. What factors influence whether a willow stem will die due to *Cytospora* infection?

It is important to understand whether a *Cytospora* infection could be initiated simply by a wound on a stem. During 2009 we wounded 60 stems in July and 60 stems in December, both inside and outside of exclosures within the Kawuneeche Valley. Our hypothesis is that wounds created during the winter would be more likely to trigger stem death because dormant stems cannot fight a possible infection. Wounds were created using a vegetable peeler and were located at 2/3 of the stem height, as measured from the base of the stem. In June 2010 the status of experimentally infected stems was analyzed. Only 23 of the 180 stems had died – 11 from the summer wounding, 10 from the winter wounding and two controls. These preliminary findings suggest that willow decline is not due simply to wounding.

We analyzed whether summer drought stress could be a factor contributing to the decline of willows. During three time periods during the summer of 2010 (end of June, mid July, early August) we

sampled willow stems along a hydrologic gradient within Endo Valley, as well as willow stems inside and outside of the CRT and Gaskil exclosures. Predawn xylem pressure potential measurements were made using a Scholander type pressure chamber. Xylem pressures are negative, with values close to zero demonstrating minimal drought stress. In addition we monitored ground water levels using existing wells or staff gages throughout the summer. Ground water levels decreased throughout the summer at most sites, however, drought stress levels remained constant and low throughout the summer (Table 1 and Table 2).

Table 1: Average percent browsed and water pressure potentials (in MPa) in Endo Valley (6 sites, 5 plants at each site). Saturated soil can have a pressure potential of 0 MPa and overnight plants can rehydrate to equilibrate their pressure potential to that of the soil.

Site	% browsed	7/1/2010	7/26/2010	8/12/2010
S164	68	-0.17	-0.18	-0.15
S43	34	-0.21	-0.21	-0.10
S58	40	-0.22	-0.16	-0.10
S65	52	-0.19	-0.18	-0.11
W41	80	-0.19	-0.20	-0.05
W43	26	-0.19	-0.13	-0.08
W46	36	-0.27	-0.15	-0.10

Table 2: Heights (with standard deviations), in 2009 and 2010, and water pressure potentials (in MPa) both inside and outside of exclosures within the Kawuneeche Valley.

	Height 2009 (cm)	Height 2010 (cm)	6/30/2010	7/27/2010	8/13/2010
CRT					
Inside	225 (52)	258 (55)	-0.12	-0.23	-0.06
Outside	93 (31)	84 (25)	-0.12	-0.22	-0.07
Gaskil					
Inside	181 (37)	205 (38)	-0.06	-0.19	-0.12
Outside	75 (31)	71 (28)	-0.07	-0.23	-0.09
GM					
Inside	164 (56)	182 (65)	-	-	-
Outside	78 (31)	88 (31)	-	-	-

Willows will be compared over the sampled years using variables such as height (Table 2). All willows growing inside exclosures gained height from 2009 to 2010, whereas individuals outside of exclosures decreased in height at two sites and increased height at one site. Height was a variable which predicted whether a stem would die from a sapsucker wound (see question 3 below).

We analyzed a small sample 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 most closely resembled *Cytospora chrysosperma*, the same species of *Cytospora* known to affect aspen. More DNA

analyses will be done during 2011 on additional samples of cultured fungi to clarify species identification.

Finally, we collected *Salix monticola* stems from Endo Valley for a greenhouse study to begin in Spring 2011. This experimental design is still in the planning stages, but will likely include inoculations of stems which are growing at varying water table depths. We may also include a warming/ambient treatment as well.

3. Do sapsuckers create disturbances that trigger *Cytospora* infection?

Sapsuckers create distinctive patterns of bark removal to consume calorie rich willow sap. These sap wells are also used by other species, including hummingbirds and ants (Ehrlich and Daily 1988, Daily et al. 1993). We observed that willow stems with sapsucker sap wells were dead, 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. Utilizing the three exclosures within the Kawuneeche Valley, we identified 59 stems wounded by sapsuckers in the summer of 2009 and 55 in the summer of 2010. During the summer of 2010, sapsuckers were not using the most southerly exclosure (GM). For each wounded stem we identified the species of *Salix* and measured the length of the wound, diameter of stem at the wound, height of the stem, height to the wound, and presence of epicormic sprouts, dieback and *Cytospora* sp. We assessed stems in Aug 2009, June 2010, August 2010, June 2011, and August 2011.

Preliminary analyses have been completed on stems wounded in 2009. In August 2009, none of the wounded stems were dead or showed signs of *Cytospora* infection. In June 2010, 41% of the stems were dead above the wound and alive below, with 33% of the dead stems showing signs of *Cytospora* infection. In addition, 69% of the wounded stems had epicormic sprouting, indicating that the stem was experiencing a stress, or dieback. In August 2010, 48% of the stems were dead, with 93% of the dead stems showing signs of a *Cytospora* infection. Factors which were strong predictors of a possible stem death due to sapsucker wounds included: length of the wound (longer wounds led to higher probabilities of death) and stem height (shorter stems had higher probabilities of death). We will examine stems wounded in 2009 and 2010 again in June 2011 and August 2011.

We inoculated 14 random sapsucker wounded stems to determine if sapsucker wounded stems were more likely to become infected (exhibit canker growth) with *Cytospora* spp. than control stems. We used a 4mm cork borer to create 4 wounds per stem, a control wound with agar and an inoculated wound, both above and below a sapsucker wound (or above and below a randomly selected location approximately half of the height of the stem). Wounds were located 15 cm apart and staggered 90 degrees apart around the stem. Inoculations occurred in early 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 will be re-measured in June 2011. Preliminary data analysis indicated that canker growth occurred in sapsucker wounded and control stems inoculated with *Cytospora* while stems inoculated with a control showed minimal to no canker growth, and this difference is significant ($p < 0.01$).

In addition to wind and rain splash dispersal of *Cytospora* spores, we were also interested in possible biological vectors of *Cytospora* dispersal, especially sapsuckers. Two species of sapsuckers occur in RMNP, red-naped (*Sphyrapicus nuchalis)* and Williamson's (*S. thyroideus*). Red-naped sapsuckers nest primarily in aspen (*Populus tremuloides*), which may be affected by *Cytospora chrysosperma* (McIntyre et al. 1996, Kepley and Jacobi 2000). During two periods of the summer of 2010, with the help of RMNP volunteers, birds were mist-netted. In early June 2010 mistnets were set up within the CRT exclosure, and Wilson's warblers were the most common bird captured. In late June, before the young fledged, we targeted nesting holes of sapsuckers in aspen which were in close proximity to willow stands in Horseshoe Park and Endo Valley. Mistnets were set up outside nesting holes and birds were caught as they returned to or left the nest. Nest holes were up to eight feet above each trees base. Four birds (two pairs) were tagged, measured, and swabbed. Swabs were stored in sealed test tubes with 5ml of sterilized distilled water and brought to the lab (Farris et al. 2004). 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. To compare sapsuckers with controls, we swabbed non-sapsuckers as well as used open air plates. Open air plates were put out once an hour for 20 minutes to monitor airborne pathogens. Plates have not yet been analyzed.

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

- RMNP research conference - poster presentation (Feb 2010)
- Front Range Student Ecology Symposium (at Colorado State University) – poster presentation (Feb 2010)
- RMNP - interpretive staff training (June 2010)
- RMNP - Thursday night presentation (July 2010)
- Ecological Society of America annual conference - oral presentation (August 2010)
- RMNP – Resource Stewardship Day – oral presentation (Feb 2011, anticipated)
- Front Range Student Ecology Symposium – oral presentation (Feb 2011, anticipated)

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