DISSERTATION

RIPARIAN WILLOW DECLINE IN COLORADO: INTERACTIONS OF UNGULATE

BROWSING, NATIVE BIRDS, AND FUNGI

Submitted by

Kristen Mannix Kaczynski

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2013

Doctoral Committee:

     Advisor: David Cooper

     William Jacobi
     Alan Knapp
     David Merritt

ABSTRACT

RIPARIAN WILLOW DECLINE IN COLORADO: INTERACTIONS OF UNGULATE

BROWSING, NATIVE BIRDS, AND FUNGI

Willows (*Salix* spp.) are critical components of Rocky Mountain riparian ecosystems. They pro-

vide food for ungulates and beavers; habitat for resident and migratory bird populations, and am-

phibians; and are integral components of the structure and function of montane riparian ecosys-

tems. In Rocky Mountain National Park (RMNP), willows form the dominant riparian shrub com-

munity. However, willow decline over the past 17 years has led to a dramatic change in riparian

ecosystems in RMNP, resulting in the conversion of a tall willow community to a community dom-

inated by short willows, with cascading effects on habitat for beaver and migratory and resident

songbirds. Research on willow decline has focused primarily on the effects of ungulate browsing

and altered hydrologic regimes controlled by beaver populations. However, damage from sapsuck-

ers [woodpeckers] and *Cytospora chrysosperma* fungal infection are interacting with these known

stressors.

My dissertation research investigates willow decline using a multifaceted approach and covers

three main topics: 1. The biotic and climatic factors contributing to the willow decline; 2. The

spatial and temporal dynamics of willow decline; and 3. The effect of altered water tables and

increased temperatures on *Cytospora* fungal infection and willow production. My research pro-

vides a comprehensive new understanding of the dynamics of willow decline in RMNP that can

be applied to riparian sites throughout the Rocky Mountain ecoregion. My first study explains the,

previously unidentified, interaction of sapsucker wounding, *Cytospora* fungal infection and ungu-

late browsing in the decline of the riparian ecosystem. My second study demonstrated that the

increase in moose populations explained the sharp decline in willows that occurred between 2001 and 2005. Past climate, such as the droughts of the early 2000s, was not the main driver in the decline. Finally, my third study found that willow stems are highly susceptible to fungal infection and my experiment demonstrated that once *C. chrysosperma* is present on a wound, it will form enlarging cankers under a wide range of environmental conditions. Results from my dissertation research support the conclusion that willow decline is more strongly driven by biotic, rather than climatic stressors. This new understanding of the interactions resulting in willow decline will allow land management agencies to develop more effective restoration strategies.

ACKNOWLEDGMENTS

I am extremely grateful to everyone who has helped me along the way. I could not have done this research without the assistance of many people. First and foremost I would like to thank my advisor, David Cooper, for the inspiration for this project. He took me in after I came knocking on his door as an orphan graduate student. He saw my potential as a researcher and gave me the confidence I needed to continue pursuing a PhD. He spent many days with me in the field and countless hours reviewing drafts. I'd also like to thank Bill Jacobi for introducing me to the exciting world of fungal pathogens, and the rest of my committee members: David Merritt, Monique Rocca and Alan Knapp for guidance along the way.

This work would not have been successful without the support of Rocky Mountain National Park. The Resources Management staff, specifically Judy Visty, Jeff Connor and Ben Bobowski, have helped every step of the way, from logistical support to providing funding for this research. Lindsay Colgrave helped with logistics for my "greenhouse" experiment. I would also like to thank Cheri Yost and Paul McLaughlin for reserving camp sites for me at Timber Creek. Ron Thomas assisted me with all of the GIS data. Finally, thanks to the Interpretive staff, the VIPs and all the rangers who have retold this story of the willow decline.

This project involved a lot of field data collection and it would not have been possible without the help of many people. Thank you Andrew Carlson for being a phenomenal field assistant. I really appreciate all your hard work, insightful comments and especially all the campfire pizzas. Thanks to Scott Rashid who volunteered his time helping mist net red naped sapsuckers. I would also like to thank Arden Leeman, Jennifer Jones, Julie Kray, Kelsey Feller, Becky Brice, Derek Schook, Whitney Mowll, Andrea Borkenhagen, Shinichi Asao, Emily Everall, and volunteers at RMNP

for helping me pot willows, measure stems, estimate dieback, and for keeping me company in the field.

I would like to thank Jacob Snelling for spending days with me in the lab, patiently teaching me how to pipet and extract DNA. Ned Tisserat also helped with sequence identification. Thanks to Gerard Adams for sharing his *Cytospora* sequences with me. Thank you Tamla Blunt for always being available to mix antibiotics for my PDA++ plates.

Thank you to Tom Hobbs, Kristin Marshall, Lindsay Reynolds, David Merritt and Erin Schliep for many discussions on analyses and help with R coding. Kirk Oldham of the Colorado Division of Parks and Wildlife provided me with data on elk and moose population numbers.

I'd like to thank Rick Knight for providing me many opportunities (6 semesters for NR420!) to improve my teaching.

I'd like to thank my friends and colleagues in the Graduate Degree Program in Ecology, especially Jamie Fuller, Kerry Byrne, and Hannah Wilbur. The Cooper lab, past and current, has provided so much support and I especially thank Kristin Marshall, Julie Kray, Jennifer Jones, Lindsay Reynolds, Andrea Borkenhagen, Sarah Bisbing, Ed Gage, Katie Driver, Derek Schook, and Covey Potter. Thank you Jessica Salo and Seema Sheth for all the support, for many great conversations and for being great accountability partners. Finally, thanks to my running partners: Jessica Salo, Erin Schliep, Patty York, Kristin Marshall and all of the Pretty hot Doctors and team Supersaurus.

Finally, I would like to thank my family and my friends, especially my mentor Meryl Rose, for supporting me through all the ups and downs.

TABLE OF CONTENTS

LIST OF TABLES

1 Introduction

Plant communities are dynamic, resilient and can resist low-level stresses. Under climate change scenarios, stressors can interact in novel ways across spatial scales and in extreme cases can result in the dieback and decline of individuals and populations of plant species (Peters et al., 2007). Unlike large disturbances such as wildfires and blowdowns, that can have immediate altering effects on a community, species decline typically is a slow process, occurring over years to decades.

Species declines

Forest species declines have been studied throughout the world and their linkages to climate change, particularly widespread droughts, make it a critical topic for research (Anderegg et al., 2012b; Allen et al., 2010; van Mantgem et al., 2009). Each species decline may be triggered by different processes and have interacting stressors. A landscape wide dieback of the Hawaiian tree *Metrosideros polymorpha* was first thought to be an epidemic disease or insect outbreak (Akaski and Mueller-Dombois, 1995). However, dieback was due initially to poorly drained soil substrates on volcanic surfaces and related to climatic perturbations, such as higher annual rainfall. In contrast, the culminating stressors in many tree species declines are saprogens, or forest pathogens, such as fungi. Fungi are present at low abundance in most forest ecosystems and are thought primarily to affect stressed plants (Castello et al., 1995; Desprez-Loustau et al., 2006). The decline of *Austrocedrus chilensis* (called 'mal del ciprés') occurred over several decades in Patagonia and was partially due to poor soil drainage that allowed the pathogen *Phytophthora austrocedrae* to flourish (La Manna et al., 2008). However, the onset of this decline was linked to climate and geologic events (Cali (1996) in La Manna et al. 2008). Similarly, the widespread, rapid decline of *Populus tremuloides* stands Colorado was most common in low density mature stands on southern

exposures at low elevations (Worrall et al., 2008). The initial stress was thought to be drought that occurred in conjunction with higher ambient temperatures. Once the stand was exposed to these stresses, fungal infection and insect attacks killed trees causing stand decline (Worrall et al., 2008).

Dieback and decline of shrubs has been little studied compared with trees, even though shrubs dominate important ecosystems throughout the world. Dieback of two desert shrubs was caused by drought stress, however older shrubs in well developed soils experienced less dieback than juvenile shrubs in weakly developed soils (Hamerlynck and McAuliffe, 2008).

Willows and riparian ecosystems

Riparian ecosystems comprise a small percentage of western North American landscapes, yet provide important ecosystem functions, including streambank stabilization, moderation of water temperature, and habitat for many species of amphibians, reptiles, insects and mammals (Naiman et al., 1993). Willows (*Salix* spp.) are the dominant overstory in many riparian areas throughout North America and their stand structure and canopy characteristics govern habitat quality for neotropical and resident birds (Olechnowski and Debinski, 2008). Tall willows, 2.5 to greater than 3.0 m in height, are essential components of western riparian ecosystems. More than 80% of breeding migratory birds use riparian areas for at least part of their life cycle (Knopf et al., 1988), as well as almost 75% of all vertebrate species (Bunnell and Dupuis, 1993), resulting in diverse biota sharing resources. Willows are phreatophytes and rely on groundwater as their primary water source. Dieback and decline of riparian willow communities has been documented throughout the western US and in particular the Rocky Mountains (Peinetti et al., 2002; Limb et al., 2003; Marshall, 2012). The decline is characterized by tall (>2 m) dead stems with live stems averaging < 1 meter

2

in height. This is the first research examining multiple stressors and fungal infection resulting in the decline of a phreatophytic shrub dominated riparian community.

Forest pathogens

Forest pathogens (insects and fungus) are thought to primarily affect individuals with low vigor or those that are genetically inferior (Castello et al., 1995). Some non-native forest pathogens affect all individuals, such as the chestnut blight that eliminated the American chestnut (*Castanea dentata* (Marsh.) Borkh.) tree from its native range (Beattie and Diller, 1954). However, native forest pathogens have evolved with forest communities and can be highly abundant, yet not affect healthy individuals. While pathogens can be the single, ultimate cause of a forest individual or species' death, the pathogens that are often implicated in tree decline are known as 'secondary action organisms', affecting species that are predisposed to a stress (Manion and Lachance, 1992). Three types of stressors have been described: predisposing, inciting and contributing factors (Manion and Lachance, 1992). Predisposing factors occur over long periods of time and include climate change, soil changes, or plant aging. Inciting factors are of shorter duration and include drought effects, late spring frost, or insect damage. Contributing factors weaken an already stressed plant and ultimately kill it, for example canker and root decay fungi.

Plant-animal interactions

Fungi and pathogens can produce high numbers of spores or offspring. Dispersal methods include wind and rain and common carriers such as birds and mammals (Tainter and Baker, 1996). Birds carry fungal spores on their feathers and feet, and a few researchers have documented the effectiveness of migratory birds in transporting spores (Warner and French, 1970; Evans and Prusso, 1969). Birds carried spores of Chestnut Blight, some in high densities (Heald and Studhalter, 1914). The

3

numbers of spores carried by a bird may be linked to weather events. For example, spores are released after rains and spore numbers on birds are highest at these times. Spore density of some fungal species on house sparrows was strongly positively correlated with the intensity of wind gusts, while others were strongly correlated with temperature or humidity (Hubalek, 1976).

Willow decline in Rocky Mountain National Park

Willows are a critical component of Rocky Mountain riparian ecosystems and in Rocky Mountain National Park (RMNP) they are the dominant woody vegetation providing the structure and function of riparian ecosystems. However, willow decline over the past two decades has led to a dramatic change and willow canopies have decreased by an average of 65% at the headwaters of the Colorado River (Figure 1.1). Many montane riparian and alpine willow communities within RMNP are so degraded that cascading effects are occurring through adjacent ecosystems.

Several factors could be contributing to willow decline in RMNP, some related to water stress. Beavers use willows to build dams on streams that flood riparian areas. The hydrologic effects of beaver dams on floodplains are profound but the almost complete loss of tall willow stems has limited dam building and beaver no longer can inhabit the Colorado River valley within RMNP, leading to the drying of floodplain further contributing to willow decline (Westbrook et al., 2006). In addition to beaver absence, lowered water tables caused by the diversion of water by the Grand Ditch is altering floodplain dynamics (Woods, 2000). Another possible stress is temperature induced drought, which has been implicated in widespread tree mortality throughout the western United States (van Mantgem et al., 2009; Anderegg et al., 2012*a*). This could be caused by decreased snowpack and earlier snowmelt reducing water availability in late summer, resulting in

4

drought stress. Woody plants respond to reduced water availability with physiological and/or morphological adjustments, including loss of leaves and branches, or whole plant death.

Herbivory is also contributing to willow decline. For example, ungulates browse heavily on willows (Beyer et al., 2007; Singer et al., 1998). Willows comprise the majority of moose diet and during the summer greater than 90% of their diet is willow (Dungan and Wright, 2005). In addition to browsing, red-naped sapsuckers remove bark to consume calorie rich willow sap. These sap wells are also used by other bird species and insects, such as hummingbirds and ants (Ehrlich and Daily, 1988; Daily et al., 1993).

The interaction of these biotic and climatic stressors may reduce willow stem resistance to *Cytospora chrysosperma* infections which can kill weakened stems. The life cycle of *C. chrysosperma,* which has been understood for nearly a century (Long, 1918), has two stages: a sexual (*Valsa sordida*) stage and a more common asexual (*C. chrysosperma*) stage. Spores are transported by wind, rain, insects, and birds (Tainter and Baker, 1996). *C. chrysosperma* requires physical damage to the stem before infection can occur. Once present on a wound, the fungus can quickly grow beneath the bark and cambium, blocking flow in the xylem and phloem, killing the stem (Biggs and Davis, 1983). When a plant is predisposed to a stressor such as drought induced water stress, temperature stress, ungulate browsing or sapsucker sap well creation, a fungal infection is more likely to kill the wounded stem (Kepley and Jacobi, 2000; McIntyre et al., 1996). Research on *Populus tremuloides* has identified a significant positive relationship between drought stress and fungal infection (McIntyre et al., 1996). Fungal infection can occur during the growing season or the winter when plants are dormant. During the growing season plants are more likely to fight off infections by

shutting off growth above the wounded site on the stem. In winter, dormant plants cannot fight the fungal infection.

Willow decline is a complex multi-scale ecological issue. Large spatial scales include climate change, drought, and the possibility of fungal infection due to the presence of spores. Small spatial scale issues include localized drought (due to the absence of beavers), overbrowsing by ungulates, and wounding of stems by sapsuckers. This research investigates willow decline using a multi-faceted approach and covers three main topics: 1. The biotic and climatic factors contributing to the willow decline; 2. The spatial and temporal dynamics of willow decline; and 3. The effect of altered water tables and increased temperatures on *Cytospora* fungal infection and willow production. Each topic is addressed in the following three studies and together provide a comprehensive new understanding of the dynamics of willow decline in RMNP that can be applied to riparian sites throughout the Rocky Mountain ecoregion. The first study examines the interactions of ungulate browsing, sapsucker wounding, fungal infection and drought. Using twelve year old moose exclosures at the headwaters of the Colorado River, I propose a new understanding of a mechanism for the dieback of tall willow stems. The second study uses GIS to examine the long temporal scale and epicormic shoot analysis to examine the short temporal scale of the dieback. I also examined the spatial extent of the dieback throughout the northern Colorado Rockies by sampling willows along randomly selected stream reaches. Finally, for the third study I performed a manipulative experiment on *Salix monitcola* investigating the interactions water stress and warming air temperatures, as predicted under climate change scenarios, on *Cytospora* fungal infection.

Understanding the mechanisms that have and may still be contributing to willow decline is imperative for the conservation of riparian ecosystems. Results will inform resource managers on

the timing and causes of this decline and will identify concepts for use in riparian recovery and

restoration efforts.
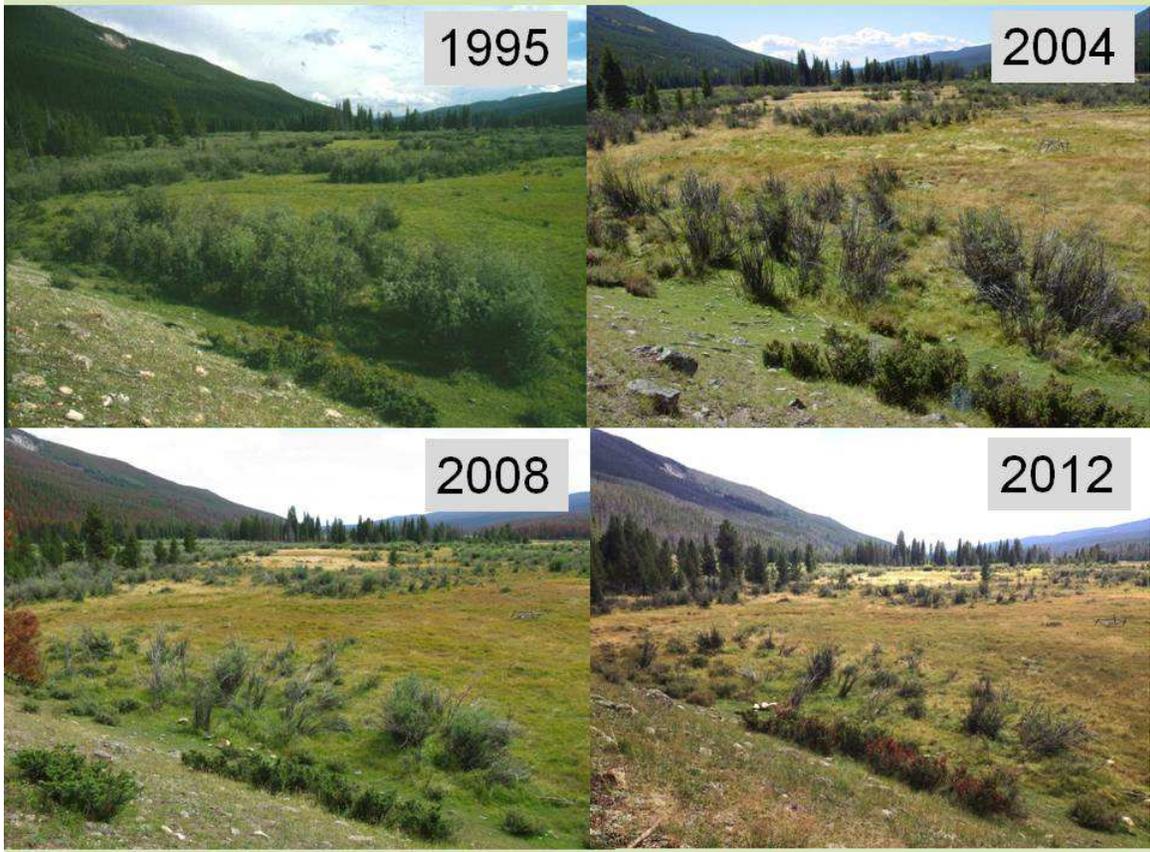
Figure 1.1: Willow dieback in the Kawuneeche Valley, headwaters of the Colorado River, in Rocky Mountain National Park. Photos were taken between July and August each year. Photos by David Cooper and Kristen Kaczynski

2 Interactions of native birds, herbivores and fungi facilitate decline of riparian vegetation

2.1 Introduction

Disturbances at multiple spatial and temporal scales occur in all ecosystems and most communities are resistant or resilient to these events (Pickett and White, 1985). Landscape scale disturbances such as crown fires, windstorms, and insect and disease outbreaks have immediate effects on many species and can alter ecosystem structure and function in lasting ways. Fires in Yellowstone National Park in 1988 burned 3213 km^2 and dramatically changed plant community composition and cover, shifted the distribution of wildlife, and altered streamflow and nutrient cycling (Knight and Wallace, 1989; Turner et al., 2003). Subsequent disturbances affect landscapes in different ways. For example a recently burned forest may be less susceptible to bark beetle infestation (Bebi et al., 2003; Veblen et al., 1994). While large high intensity disturbances are critical ecological processes, smaller spatial scale, low intensity disturbances and their interactions may produce similar lasting effects on community composition, structure and function.

In the past two decades riparian willow (*Salix* spp.) populations have experienced dramatic dieback in the Sierra Nevada, California (California Forest Pest Council, 2003) and many regions of the Rocky Mountains, including Montana (Limb et al., 2003), Wyoming (Wolf et al., 2007; Bilyeu et al., 2008), and Colorado (Peinetti et al., 2002). In Yellowstone National Park, the tall riparian willow stands, with abundant beaver dams, high water tables and willows that averaged 2.5 to 4.5 m in height, has been converted into short willow stands due to heavy browsing. Willows now average < 1 m tall, and are inadequately sized for use by beavers (*Castor canadensis* Kuhl) and the lack of dams has created drier valleys with grass dominated vegetation

9

(Wolf et al., 2007). The interactions of small scale disturbances, such as ungulate browsing and water table decline due to the absence of beavers, has led to a dramatic alteration of willow stature and population in certain regions. However these factors do not explain willow decline throughout the West (Alstad et al., 1999; Bilyeu et al., 2008; Singer et al., 1998; Zeigenfuss et al., 2002; Wolf et al., 2007).

Riparian areas comprise a small percentage of western North American landscapes, but provide important ecosystem functions, including streambank stabilization, stream water temperature moderation and habitat for many species of amphibians, reptiles, insects and mammals (Naiman et al., 2005). More than 80% of breeding migratory birds use riparian areas for at least part of their life cycle (Knopf et al., 1988), as well as almost 75% of all vertebrate species (Bunnell and Dupuis, 1993), resulting in diverse biota sharing resources. Localized disturbances such as annual flooding and herbivory by wild ungulates or livestock are spatially variable yet can shape riverine landscapes. Peak flows in snowmelt driven rivers occur in early summer and periodically scour banks, erode vegetation, and deposit sediment and nutrients onto floodplains (Naiman and Decamps, 1997). Riparian species are adapted to flood events and many are biologically linked to them, having seed dispersal timed to the period after peak flows when suitable bare and wet mineral soil habitat for seedling establishment is abundant (Gage and Cooper, 2005). Beavers utilize woody riparian vegetation as a food source and their dam building along stream reaches with tall willows and other species of woody plants can control local water tables, and moderate the hydrologic effects of climate (Westbrook et al., 2006). Woody riparian plants, a primary food source for native ungulates, can persist under conditions of low to moderate browsing intensities, however higher levels of browsing can alter stand structure, negatively affecting species that rely

10

on tall plants (Baker et al., 2005*a*; Olechnowski and Debinski, 2008). Shorter willow stems provide insufficient nesting and breeding habitat for many species of migratory birds. In the Yellowstone region willow stands with stems averaging 1.5 m tall supported twice the number of avian species and individuals as willow stands averaging 0.66 m tall (Baril et al., 2011; Olechnowski and Debinski, 2008).

Red naped sapsuckers (*Sphyrapicus nuchalis* Baird, hereafter sapsucker) create nest cavities in aspen (*Populus tremuloides* Michx.) trees infected with stem decay fungus (*Phellinus tremulae* (Bondartsev) Bondartsev & P.N. Borisov) (Daily et al., 1993). Sapsuckers also create distinctive bark wounds, called sap wells, on aspen and tall, large diameter willow stems that they use to feed on calorie rich sap (Figure 2.1). The wells are kept open through repeated visits during their breeding season. Sapsuckers may be considered keystone species in a sapsucker-willow-aspen-stem decay fungus community (Daily et al., 1993). Their cavities provide nesting sites for other birds and mammals, including tree and violet green swallows and squirrels, and sap wells are used by many species of mammals, birds and insects (Ehrlich and Daily, 1988; Daily et al., 1993). Sapsuckers use stems larger than 1 cm in diameter because short or thin willow stems are too narrow for sapwell formation. If tall willows are absent, biodiversity decreases due to the cascading effect on willow sap reliant species (Daily et al., 1993).

Plant communities affected by multiple disturbances may experience species declines involving insects or fungal pathogens. Native forest pathogens have evolved with tree species and are thought to selectively eliminate individuals with low vigor or genetic inferiority (Castello et al., 1995; **?**). While pathogens can be the single and ultimate cause of a trees death, most pathogens implicated in tree declines are 'secondary action organisms', affecting individuals that are

predisposed or repeatedly exposed to stress (Manion and Lachance, 1992). Widespread aspen decline in the western United States has been linked to extreme drought, above average temperatures, high ungulate herbivory, fungal pathogens and insect attacks (Worrall et al., 2008). An ongoing dieback of narrowleaf alder (*Alnus incana* (L.) Moench ssp. *tenuifolia* (Nuttall) Breitung), a common riparian tree in western North America, is linked to high maximum summer temperatures and an epidemic of *Cytospora* canker (*Valsa melanodiscus*, anamorph *Cytospora umbrina*) (Worrall, 2009). The interactions of multiple disturbance processes on the dieback of these two important western North American forest species have led to changes with far reaching ecological implications such as displaced bird communities (Griffis-Kyle and Beier, 2003) and altered nutrient cycling (Nossov et al., 2011; Huang and Anderegg, 2012).

In this study I examine the relative influences of ungulate browsing, sapsucker wounding and fungi in riparian willow decline by addressing two primary questions: 1. Is ungulate browsing the trigger for willow community collapse or are multiple interacting small scale disturbances required to trigger the collapse? and 2. Once the community collapse is initiated is the dieback maintained solely by ungulate browsing?

2.2   Materials and Methods

Study Area Description

Rocky Mountain National Park (RMNP), located in Colorado, USA, covers 108,000 ha and ranges from 2240 to 4345 m elevation. Long term climate stations are located in Grand Lake (2554 m) on the western side and Estes Park (2347 m) on the eastern side of RMNP. The average minimum temperature in Grand Lake and Estes Park in January is -16.5ºC and -9.2ºC and the

average maximum temperature in July is 24.6ºC and 25.7ºC. Average precipitation is 64 cm and 40 cm, with most falling as snow. Average annual snowfall in Grand Lake is 350 cm and Estes Park is 177 cm (Western Regional Climate Center).

Vegetation is largely conifer forest at lower elevations and alpine tundra above 3450 m. The lowest elevation forests are dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex C. Larson) and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.). Higher elevation forests are dominated by subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Englemann spruce (*Picea engalmanii* Parry ex Engelm.) and aspen. The river valleys are dominated by tall willows (*Salix monticola* (Bebb), *S. geyeriana* (Andersson), *S. planifolia* (Pursh) and *S. drummundiana* (Barratt ex Hook.)), that average 2.5 - 4.5 m in height, and wet meadow (*Carex aquatilis* (Wahlenb.) dominated) communities.

Elk (*Cervus canadensis* Erxleben) and moose (*Alces alces* Gray) are common throughout RMNP. Elk populations have increased since the early 1900's due to the extirpation of wolves, while moose were introduced into Colorado in the late 1970's. Both elk and moose browse on willows, and willows comprise 79% of moose summer diet (Dungan and Wright, 2005). The current elk population on the east side of RMNP appears to have little seasonal migration and is managed through a culling program initiated in 2008. West side elk population numbers are unknown (Therese Johnson, personal communication). Moose began overwintering on the west side of RMNP during the early 1980s. Current moose population is unknown, but was estimated in 2003 that 61 to 66 moose reside in the Colorado River valley (Dungan, 2007). Moose have also become established on the Eastern side of RMNP.

Beaver in RMNP have experienced a large population decline (Mitchell et al., 1999). More than 200 colonies with more than 1800 individuals occurred in 1940 (Packard, 1947), however a 2009 park-wide survey found beaver present at only 17% of sites where they were found historically (Scherer et al., 2011).

Sapsucker populations have not been sampled in RMNP. Sapsucker populations have remained constant in Colorado from 1968 - 2010, with an average annual trend estimate of +2.77% (95% Credible Interval: +1.00 - +4.47) (Sauer et al., 2011).

Headwaters of the Colorado River

The Kawuneeche Valley study area encompasses 12.5 km of the Colorado River headwaters within RMNP (40°22'N and 105°51'W). This valley varies from approximately 400 to 1600 m wide. Historically, the hydrologic regime was beaver influenced, with dams along the Colorado River diverting up to 70% of flow onto the floodplain (Westbrook et al., 2006). However, within the last decade, beaver activity has declined and few beavers are present. Tall riparian vegetation is dominated by *Salix monticola*, *S. geyeriana*, *S. drummondiana*, meadows by *Deschampsia cespitosa* ((L.) P.Beauv.) and *Calamagrostis canadensis* ((Michx.) P. Beauv.), and peat-accumulating fens by *S. planifolia* and *Carex aquatilis*. Hillslope vegetation is dominated by *Pinus contorta*, *Picea engelmannii* and *Abies lasiocarpa*.

Endo Valley

Endo Valley is a willow dominated river valley on the eastern side of RMNP (40°24'N and 105°39'W; 2624m). The study area is 0.25 km². Beaver are present creating large dams that span the Fall River and smaller dams on side channels. Vegetation is dominated by *Salix monticola*, *S.

14

geyeriana, *S. planifolia*, with an herbaceous layer dominated by *C. aquatilis* and some non-native species that are actively managed.

Willow Survey

I utilized three 2500 m^2 exclosures, with 2.5 m tall fences, that were established in the Kawuneeche Valley in 1997 to exclude ungulate browsing(Zimmerman, 1995). Five groundwater monitoring wells were installed in randomly located sites inside and outside of each exclosure for a total of 30 wells. Around each well, six tall willows species were randomly selected, tagged, and monitored from 2009-2011. Five randomly selected plants were also chosen around three long term monitoring groundwater wells and four temporary staff gauges in Endo Valley (n = 35). These plants were measured and manipulated from 2009 – 2011. Plant nomenclature follows Weber and Wittmann (2001).

The effects of browsing were analyzed on 180 willow plants, 30 inside and 30 outside each exclosure in the Kawuneeche Valley from 2009 to 2011. An additional 35 willows were tagged in Endo Valley and monitored in 2010 and 2011. Browsing intensity was assessed in 2011 along a 0 - 100% scale, at 10% intervals for each plant. I measured the tallest stem to determine overall plant height and recorded flowering status and sex of each plant in 2010 and 2011. To determine current annual growth (CAG) stem length and diameter were measured on a subset of stems and live stems were counted using the methods of Bilyeu et al. (2007). Fifty to 60 stems of the three dominant species (*Salix geyeriana*, *S. monticola* and *S. planifolia*) were collected to develop quantitative relationships between stem length (cm), diameter (mm) and biomass (g) using regression analysis. There were no significant differences in biomass:length and biomass:diameter among species, therefore they were pooled for analysis. The regression relating stem length to biomass

15

was used to determine CAG ($r^2 = 0.92$, biomass:diameter, $r^2 = 0.91$).

$$biomass = 0.637 * (stemlength) + 3.6675 \qquad (2.1)$$

$$biomass = 0.3324 * (stemdiameter) + 1.3024 \qquad (2.2)$$

Water relations

Xylem pressure potentials

Predawn (23:00 to 03:00 hrs) xylem pressure potential (Ψ_{xp}) was measured on current year twigs using a Scholander-type pressure chamber (PMS instruments, Corvallis, Oregon). Sixty plants, 30 inside and 30 outside of two exclosures were measured during three periods (end of June, mid-July, early August) and all tagged plants in Endo Valley during the summer of 2010.

Depth to groundwater

Depth to groundwater was measured biweekly during the growing season in bore holes in 2009 and in fully slotted 4 cm outside diameter PVC wells during 2010 and 2011 growing seasons.

Fungal infections and stem wounding

Willow stem sections were collected that had fruiting fungal pycnidia in cankered areas of the stem. Stems were placed in humidity chambers to force spore production and spores were cultured on 1/4 strength potato dextrose agar (PDA) ++ media (with antibiotics Streptomycin sulfate (0.1g/5ml of H2O) and Chloramphenicol (0.1g/2.5 ml ethanol) per 500 ml of liquid media). I performed DNA analyses on eight of the isolates and identified them as *Cytospora*

chrysosperma (Pers.:Fr.)Fr. (anamorph; *Valsa sordida* (Nitschke), teleomorph) as matched to

unpublished *Cytospora chrysosperma* sequences (G.C. Adams, Department of Plant Pathology,

Michigan State University).

Wounding experiment

Artificial wounds were created to determine the susceptibility of willow stems to naturally

dispersed *Cytospora* spp. spores. The season when infection occurs is important as some

pathogens can be active when their host species are dormant (Hinds, 1985). Sixty stems were

wounded in late July 2009 and 60 in early December 2010, for a total of 120 stems inside and

outside of exclosures. A vegetable peeler was used to create uniform stem wounds approximately

2 cm long, 7 mm wide and deep enough to remove the bark to the cambium. Wounds were

created at 2/3 of the total stem height above the ground and covered approximately 25% of the

stem circumference. In June 2010 stems were examined for the presence of *Cytospora* spp.

pycnidia, assessed as live, dead, or dead above the wound but alive below wound, and compared

with 60 control stems.

Sapsucker wounded stems

A random selection of stems naturally wounded by sapsuckers within ungulate exclosures in the

Kawuneeche Valley during 2009 (n=59) and 2010 (n=55) were tagged. The species wounded,

length of the wound, stem diameter at the wound, and height of the stem were assessed during the

year of wounding. The following growing season, I recorded whether the stem was alive, dead, or

dead above the wound but alive below the wound, epicormic sprout development, and

presence/absence of *Cytospora* sp. based on pycnidia.

17

Inoculations

Cytospora isolates used for inoculations were collected from infected stems and cultured on full strength potato dextrose agar (PDA) in the laboratory. In the field, I created four circular 8 mm diameter wounds approximately 15 cm apart on opposing sides of 14 randomly selected unbrowsed stems (*S. drummundiana, S. planifolia, S. monticola, S. geyeriana*) located within one exclosure in September 2010. The first and third wounds were inoculated with sterile PDA, while the second and fourth wounds were inoculated with one isolate of *Cytospora* sp. Vertical canker length was measured in December 2010 and again when stems were harvested in July 2011. Fungi were cultured from all cankers and grown on ¼ PDA ++ and DNA analysis was performed on a subset of recultured fungi.

Fungi carried by sapsuckers

I located active sapsucker nests, all of which occurred in aspen trees, near the study areas in June 2010 and 2011. I captured and sampled four birds in 2010 and five birds in 2011 using mistnets erected at each cavity. Sampling of bird pairs occurred after eggs hatched but prior to young fledging, because movement in and out of the nest is nearly constant at that time. The beak and feet of each bird were wiped with sterile cotton swabs that were then placed into sterile test tubes with 0.2 ml of deionized (DI) water and processed the day of collection. To ensure that I cultured all biota, each swab was streaked four times across two ¼ PDA++ plates. After streaking, swabs were placed in 0.5 ml of DI water and the solution was then poured onto an additional two plates. Plates were left at room temperature for 6 weeks. After growth, I visually identified fungal genera that were separated on individual plates to fruit. Plates were stored at 6.5º C temperature after

18

fruiting to cease growth. DNA analysis was performed on fungal cultures that resembled *Cytospora* sp.

DNA analyses

DNA analyses were performed to determine the species of *Cytospora* on samples recultured from inoculations and on sapsucker fungal swabs. I used Easy DNA™ kit Genomic DNA isolation (Invitrogen corp) methods (protocol #3) to extract DNA from 10 day old fungal cultures. Polymerase chain reaction (PCR) amplification techniques were performed using Internal Transcribed Sequence (ITS) universal primers 1 and 4, as these primers enhance amplification of fungi specific sequence regions (White et al., 1990). Sequencing of the samples was performed at Proteomics and Metabolomics Facility at Colorado State University. The resulting sequences were matched in GeneBank and to known *Cytospora* sp. sequences from Dr. Gerald Adams (Department of Plant Pathology, Michigan State University).

Analyses

Log transformed stem height and CAG were analyzed using generalized linear mixed models (GLMM) for each growing season, with ungulate exclosure as a random effect. Fixed effects tested in each model included browsed versus unbrowsed and depth to water table in late July. Endo Valley data (2010 - 2011) and 2011 Kawuneeche Valley data included a browsing intensity class variable. I used ANOVA to test for differences in CAG among species, with a Tukey's HSD to adjust for unequal group sample sizes. All analyses were done using R v2.9.2 (R Development Core Team, 2008), with packages nlme, lmmfit, and MASS.

19

Predawn xylem potential was analyzed using generalized linear repeated measures mixed models. Ungulate exclosure was modeled as a random effect, and July depth to water table, stem height, species, browsed or unbrowsed, and sex were fixed effects. I also tested for possible 2 way interactions. Stem death due to sapsucker wounding was modeled using logistic regression. Variables tested included in the models were stem height, length of wound, diameter at the wound, and species. I investigated possible 2 way interactions. ANOVA models were used to compare inoculated and control canker sizes from inoculation experiment. I used a maximum likelihood-based model selection framework to evaluate competing generalized linear models, using corrected Akiake's Information Criteria (AICc) (Burnham and Anderson, 2002) to compare generalized linear models. AICc difference values and weights provided a measure of the strength of competing models. I examined the amount of deviance explained by the model compared with the null model to determine model strength and reported this percentage as the amount of variance explained.

2.3   Results

Browsing, Willow Heights and Current Annual Growth

Unbrowsed willow stems averaged 170% taller than browsed willows ($p < 0.01$) (Table 2.1) and browsed willows, regardless of browsing intensity, were shorter than unbrowsed willows (Figure 2.2). Browsing was the best predictor of willow height in both 2010 and 2011 (Table 2.2). Depth to groundwater for study sites ranged from >160 cm deep to near the soil surface during the growing season yet was not a statistically significant predictor of stem height in any study year ($p = 0.856$) or any site and was similar in browsed and unbrowsed areas in the Colorado River valley ($p = 0.536$).

CAG was greater for unbrowsed than browsed stems in the Colorado River valley ($p < 0.01$) in all years and was higher in Endo than Kawuneeche Valley (Table 2.2). CAG was not correlated with browsing intensity ($R^2 = 0.06$, $p = 0.36$; Figure 2.3) in Endo Valley, while in the Kawuneeche Valley, CAG decreased as browse intensity increased ($R^2 = 0.20$, $p = 0.14$; Figure 2.3). Stem height (model 1) and height and browse intensity (model 2) best predicted 2011 annual growth in the Kawuneeche Valley (Table 2.3), explaining 43 to 53% of variation. Depth to water table in mid summer and species identity were not predictors of CAG in either year. Only 3% of browsed plants flowered, thus the sex of most plants could not be identified or used as a model variable, whereas 93% of unbrowsed plants flowered and could be sexed. In Endo Valley, the best growth model for 2011 included a combination of the variables browsing, species, and sex (Table 2.3), and no statistical difference in CAG existed between male and female plants ($p = 0.15$). The top two models included browse class from the previous growing season (Table 2.3).

Drought Stress

Predawn xylem pressures for all willows averaged -0.13 MPa, indicating little or no water stress, and were not significantly different between sampling periods ($p = 0.32$) or between browsed and unbrowsed plants in the Kawuneeche Valley ($p = 0.82$). Xylem pressures did differ among the three sampling periods in Endo Valley ($p < 0.01$). The best model included current seasonal precipitation total (beginning in May) and previous day precipitation.

Stem Wounding by Sapsuckers

Sapsuckers utilized all tall willow species in the study area and selected stems that ranged from 7.5 to 24.4 mm ($n = 115$) in diameter and averaged 13.8 mm. Sapsuckers wounded stems

averaged 237 cm tall and ranged from 137 to 365 cm. All wounded stems were inside ungulate exclosures because all stems outside exclosures were of insufficient diameter (mean 7.5 mm; n = 90) or height (mean 81 cm) for sapsucker use (Figure 2.4). Epicormic shoots formed below the wound on 69% and 68% of stems in 2009 and 2010 (Figure 2.1). Wounding likely caused stress on the apical meristem leading to lateral bud expansion. Stem mortality occurred above sapsucker wounds on 47% and 62% of stems in 2009 and 2010. Wound length was the best predictor of stem mortality in logistic regression models. For stems with wounds two cm long (β_0 in the model), an increase in wound length of 1 cm increased odds of death from 0 to 0.30. Stems that died above the wound had visible *Cytospora* infection rates of 93% and 100% in 2010 and 2011. Sapsucker wounds were located an average of 82 cm above the ground surface, and stem death triggered by sapsucker wounding produced live stems averaging 85 cm tall, which was not significantly different than stem heights maintained by ungulate browsing (mean = 81 cm; p = 0.63).

Twenty three of 120 experimentally wounded stems died by June 2010, including 11 from winter wounding, 10 from summer wounding and 2 control stems. Stems did not exhibit signs of *Cytospora* infection, suggesting that a single wounding event did not replicate sapsucker wounding produced by repeated visits to keep the wounds open and producing sap.

Cytospora Fungal Infection

Cytospora chrysosperma inoculated cankers averaged 18 mm in length (sd 8 mm) and were significantly larger than controls that averaged 11 mm long after three months (p < 0.01, $\eta_l^2 =$ 0.45). Fifty seven percent of inoculated wounds had canker expansion. Reisolations from six of eight cankers were identified as *C. chrysosperma*, indicating that *C. chrysosperma* infected *Salix*

spp. resulting in stem death. Two control wounds had canker growth from a species other than *C. chrysosperma* and these were not analyzed.

Eleven fungi/yeast/bacteria species were identified in cultures from sapsucker beaks and feet. Six cultures had fruiting bodies of C*ytospora* sp. and were sequenced for identification. Four were identified as *Cytospora chrysosperma*, three from sapsucker feet and one from a beak. *C. chrysosperma* was found on 1/3 of the captured birds, indicating that sapsuckers are a possible vector for *Cytospora* dispersal.

2.4   Discussion

The dieback and collapse of tall willow communities in RMNP is driven by multiple interacting low intensity stressors, including repeated sapsucker wounding, fungal infection and ungulate browsing of epicormic shoots. Short willows lack the structure suitable for use by beaver and songbird populations (Naiman and Decamps, 1997; Baril et al., 2011). While browsing by elk and moose are maintaining willows in a short stature, our results indicate browsing was not the process that triggered willow decline.

Role of Sapsuckers

Sapsucker use of willow stems has been recognized in North America for more than a century (McAtee, 1911). In western Colorado, Ehrlich and Daily (1988) reported that sapsucker impacts on willow stems were "severe, and probably greater than that of any other herbivore that feeds on willows in the summer". They observed that 72% of willow shrubs had severely damaged stems and within a shrub, an average 28% of stems were damaged and 98% of damaged stems were dead (Ehrlich and Daily, 1988). Recent sapsucker damage was apparent, however, approximately

23

five years after their death stems had lost their bark and the evidence of sapsucker wounding. In RMNP 47% and 62% of stems wounded by sapsuckers in 2009 and 2010 were dead the following growing season, indicating that high mortality can result from sapsucker wounding. While sapsucker wounds are necessary, wounds alone are not sufficient to kill most willow stems. However, wounds are the entry point for the fungal spores. Sapsucker initiated stem dieback reduced willow heights to less than 1 m, a height similar to that maintained by ungulate browsing (Figure 2.4). An important difference between RMNP study sites and the Gunnison River headwaters sites investigated by Ehrlich and Daily (1988) was the low ungulate browsing in their sites (Floyd, personal communication, 2012). Willow stems died at their study site, yet the formation and growth of epicormic shoots allowed stems to regain their original height, facilitating the long-term persistence of tall willow communities. In contrast, resprouting epicormic shoots in RMNP are browsed by ungulates creating and maintaining a short willow community.

Willows compartmentalize sapsucker wounds by altering the growth of cells around the wound and creating a barrier to slow or prevent the spread of disease or decay (Biggs et al., 1984). However sapsuckers regularly visit and open wounds to access sap and overcome the willow healing processes. Our one-time stem wounding experiment produced little stem dieback likely due to the stem healing these wounds. This clarified the importance of repeated wounding to the process of stem death. Erhlich and Daily (1988) found that artificially created sap wells that were not continuously maintained yielded no sap and sap wells not repeatedly visited by sapsuckers dried up.

Fungal infection

As early as 1918, Long (1918) suspected that *Cytospora chrysosperma* infected willow stems. The life cycle of *C. chrysosperma* has two stages, a sexual (*Valsa sordida*) and a more common asexual (*Cytospora chrysosperma*) stage. Spores are transported by wind, rain, insects (Tainter and Baker, 1996) and birds. *Cytospora* spp. are know to be facultative wound pathogens that require physical damage to the bark, such as a sapsucker well, before infection can occur. Once present on a wound, *Cytospora* fungi grow through the phloem and block sap flow killing the stem above the wound (Biggs and Davis, 1983). Drought stressed plants are thought to be more prone to canker fungal infection (McIntyre et al., 1996; Kepley and Jacobi, 2000). However, I found that 57% of wounded and inoculated stems with canker expansion had low predawn xylem pressure potentials throughout the summer. Thus drought stress was not a precursor for *C. chrysosperma* infection on the study willow species.

Cytospora chrysosperma infects aspen and has been implicated in their widespread decline in the western US (Worrall et al., 2008), however the linkage between sapsuckers and *C. chrysosperma* on willows has not previously been documented. Sapsuckers preferentially create cavity nests in aspens, often in close proximity to willow stands and utilize willows throughout their breeding season (Daily et al., 1993). While *C. chrysosperma* spores can be wind and rain dispersed, sapsuckers can also facilitate their dispersal from aspen to willow through numerous trips from their nests to willows. Migratory birds are known to carry fungal spores on their feathers and feet and appear to be effective transport vectors (Evans and Prusso, 1969; Warner and French, 1970). For example, in the eastern US, birds carried chestnut blight spores in high numbers (Heald and Studhalter, 1914), and the number of fungal spores on house sparrows was positively correlated

with the intensity of wind gusts and the ratio of fungal genera was highly correlated with temperature and humidity (Hubalek, 1976).

Role of browsing in maintaining willow height

In the absence of predators, ungulate populations have increased through time in RMNP and other areas with unmanaged ungulate populations producing detrimental effects on riparian communities (Peinetti et al., 2002; Singer et al., 1998; Zeigenfuss et al., 2002). Peinetti et al. (2002) concluded that willow decline in RMNP was "clearly associated with an increase in elk abundance". Ripple and Beschta (2004) suggested that large elk populations triggered willow decline on Yellowstone National Park's northern range and the reintroduction of wolves restored the natural balance allowing willows to increase in height. However, in many locations in the northern range ungulate browsing intensity is still high and willow heights have not recovered (Bilyeu et al., 2008; Marshall, 2012).

Willow stems greater than 200 to 250 cm tall are thought to be above the reach of browsing elk and moose (Keigley et al., 2002). The tallest single willow stem outside of exclosures in the Kawuneechee Valley was 170 cm tall, and the average was only 82 cm indicating that the entire population of unexclosed willows is within the reach of browsers. Only 3% of willows outside of ungulate exclosures were not browsed in 2011. Although willows are resilient to ungulate browsing, CAG was significantly higher on unbrowsed stems in all years, and annual browsing has reduced their productivity and maintains their short stature (Singer et al., 1998).

While ungulate browsing can maintain short willows, browsing likely interacted with other factors to create and maintain short willow stands. Baker et al. (2005*a*) proposed that where

beavers cut willow stems nearly to the ground, ungulate browsing removed resprouting shoots to maintain short stature willows. Here I have demonstrated that in the absence of beavers, sapsuckers facilitated *Cytospora* infection can also result in short willows.

Role of Drought

Predicted warmer average annual temperatures and earlier peak streamflows in the future (Stewart et al., 2005) could influence willow growth and physiology. However, willow communities with active beaver dams rarely experience prolonged drought because willows are phreatophytes, using groundwater as their water source. Westbrook et al. (2006) found that in the extreme low stream flow years of 2002 and 2004, 15% and 21% of their floodplain study area was inundated late in the summer by beaver dams, and much of the study area had water tables close to the soil surface maintained by these dams. In contrast, overbank flooding in 2003, the fifth highest flow year in the 55 year Colorado River record (1956 - 2011), inundated only 10% of the study area and persisted for just 3 to 7 days. The high flow washed out beaver dams on the main river channel and the water table during most of the summer was deeper than in 2002 and 2004, producing larger areas of dry soils than in the drought years. Beavers have not been present in the upper Colorado River valley since 2005 (D.J. Cooper, personal observation) because the short willows, created by sapsucker/*Cytospora* interactions and maintained by ungulate browsing, do not provide suitable food or dam building material.

Alternate hypothesis for decline of willow populations

This research indicates that the interactions of sapsuckers, *Cytospora* and ungulate browsers collectively contributed to willow decline. No one or two single factors were explained willow

height or mortality in isolation. Sapsuckers create and maintain open wounds that allow

Cytospora infection to kill stems above the sap wells. Epicormic shoots that form below the

sapsucker wounds determines the fate of these stands: if shoots are browsed by ungulates, short

stature willows result and are maintained but if they escape browsing then tall willow stands can

regenerate. These processes can explain the large scale dieback of tall willows in RMNP, and

likely willow stands throughout western North America. Many tall dead willow stems in the

study areas are much taller than the browse limit of ungulates, retain their tall dead stems, yet due

to bark decay, the presence of sapsucker wells or *Cytospora* pycnidia cannot be identified. My

research suggests that sapsuckers wounded these stems resulting in their death, and leaving

telltale tall, dead yet unbrowsed stems.

Sapsuckers, *Cytospora*, ungulates and beaver are components of functioning willow ecosystems

in the Rocky Mountains (Figure 2.5). Under a scenario of low to moderate browsing, the majority

of willows have tall stems, beavers have access to willows suitable for food and dam building and

sapsucker use is dispersed. *Cytospora* spp. infect and kill wounded or stressed stems, but

epicormic shoots grow to full height maintaining a tall willow community. Under high browsing

pressure, the majority of willow stems are of short stature, unsuitable for beavers and sapsucker

use is highly concentrated on willows with larger diameter and taller stems, exacerbating the

dieback, and producing widespread drought on the floodplain. The loss of epicormic shoots to

browsing produces a bottleneck in the long-term maintenance of willow dominated riparian

ecosystems.

A diverse group of species rely on riparian ecosystems for survival and yet their combined low

intensity disturbances have led to the decline of the willow community. Our understanding of

these interactions can allow land management agencies to develop appropriate and effective restoration strategies. Long term recovery and survival of a willow community could require a reduction in ungulate browsing, by either animal population reduction or fencing. Only these actions will allow epicormic shoots to grow to fully regain height, support beaver, maintaining a tall willow community and conserving the high biodiversity supported by these ecosystems.

Table 2.1: Comparison of the mean of willow height (cm) and annual growth (g/plant) for 2009, 2010 and 2011. Standard errors of the mean are in parentheses. The column 'mean' is the averages over the sample years. Plants in the Kawuneeche Valley outside exclosures are browsed and plants inside are unbrowsed. Each plants in Endo Valley is browsed at a different level of intensity (0 to 100%). Endo Valley willows were not measured in 2009. Sample sizes for heights: Kawuneeche Valley - Outside, n = 30; Kawuneeche Valley - Inside, n = 30; Endo Valley, n = 35. Sample size is 15 for each location and year of production.

Location	height (cm)			mean (cm)	annual growth (g/plant)			mean (g/plant)
	2009	2010	2011		2009	2010	2011	
Kawuneeche Valley - Outside Exclosure	84 (3)	82 (3)	81 (3)	82 (2)	67 (10)	63 (15)	81 (16)	71 (8)
Kawuneeche Valley - Inside Exclosure	191 (6)	216 (7)	219 (6)	209 (4)	254 (52)	315 (54)	316 (45)	301 (29)
Endo Valley	–	299 (13)	307 (12)	303 (7)	–	404 (56)	511 (90)	457 (32)

Table 2.2: Model comparisons based on AICc for explaining willow stem height. The number of parameters in the model are 'k'. Model weight is the Akiake weight and represents the relative likelihood of the model. The variance explained is the amount of variance explained over the null model. All models for the Kawuneeche Valley included exclosure as a random effect. Water table is the July depth to the water table (cm). Browsed is a binary variable for Kawuneeche Valley and a class variable for Endo Valley (0 - 100% scale, at 10% intervals for each plant). *Cytospora* is a class variable (0 - 100% scale, at 10% intervals for each plant). Models for 2011 data were similar, therefore only 2010 are shown here.

model ranking	k	AICc	$\triangle$AICc	model weights	variance explained
2010 Kawuneeche Valley					
Browsed	2	108.69	0	0.99	0.69
Browsed + water table	3	121.89	13.2	0.001	0.71
Browsed * water table	4	135.76	27.07	0	0.71
water table	2	298.43	189.74	0	0.005
2010 Endo Valley					
Browse intensity + *Cytospora*	3	380.14	0	0.67	0.57
Browse intensity * *Cytospora*	4	382.62	2.48	0.19	0.58
Browse intensity	2	383.51	3.36	0.13	0.50
Browse intensity + *Cytospora* + dieback + water table + species + sex	7	389.29	9.14	0.007	0.64

Table 2.3: Model comparisons based on AICc for explaining willow annual growth (g) in 2011. The number of parameters in the model are 'k'. Model weight is the Akiake weight and represents the relative likelihood of the model. The variance explained is the amount of variance explained over the null model. All models for the Kawuneeche Valley included exclosure as a random effect. Height is height of tallest stem on an individual plant in cm. Species and sex are categorical variables. 'Browsed in 2010' is a binary variable and browse intensity is a class variable, 0 - 100% scale, at 10% intervals for each plant in 2011 (for the Kawuneeche Valley or Endo Valley) or 2010 (for Endo Valley). *Cytospora* 2011 is a class variable (0 - 100% scale, at 10% intervals for each plant).

model ranking	k	AICc	ΔAICc	model weight	variance explained
2011 Kawuneeche Valley					
height	2	147.54	0	0.49	0.43
height + browse intensity	3	148.45	0.91	0.31	0.53
height + browse intensity + browsed in 2010	4	149.86	2.32	0.15	0.53
browse intensity	2	152.24	4.7	0.05	0.49
2011 Endo Valley					
browse intensity in 2010 + browse intensity 2011 + species + sex	5	84.69	0	0.30	0.52
browse intensity in 2010 + species + sex	4	84.74	0.05	0.29	0.47
browse intensity in 2010 + species	3	86.03	1.34	0.15	0.41
browse intensity in 2011 + *Cytospora* 2011 + species + sex	5	86.36	1.66	0.13	0.49

Figure 2.1: Fresh red-naped sapsucker (*Sphyrapicus nuchalis*) wounding on willow stem (left). Only willows exclosed from browsing were sufficiently tall and of large diameter for sapsucker use. During the year of wounding, epicormic shoots initiate below the wound (right).
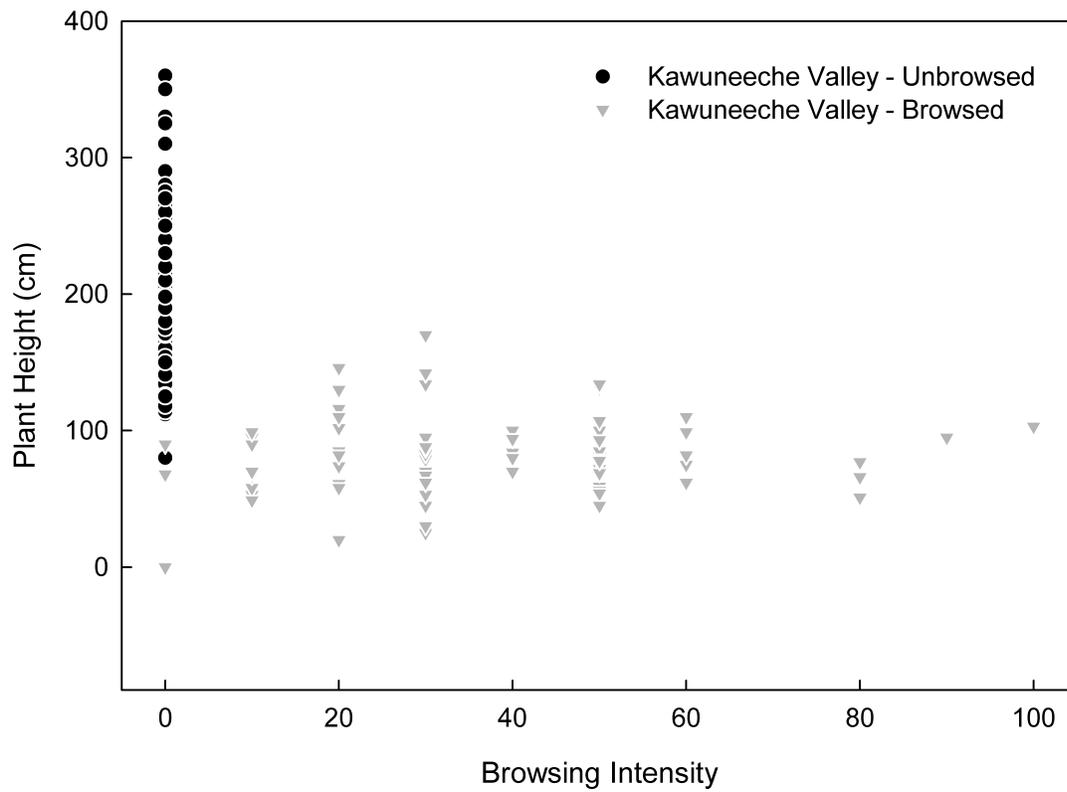
Figure 2.2: 2011 browse intensity (0 - 100% scale, at 10% intervals for each plant) and willow plant height (cm) of individual willows in the Kawuneeche Valley. Triangles are browsed plants and circles are unbrowsed (inside exclosure).
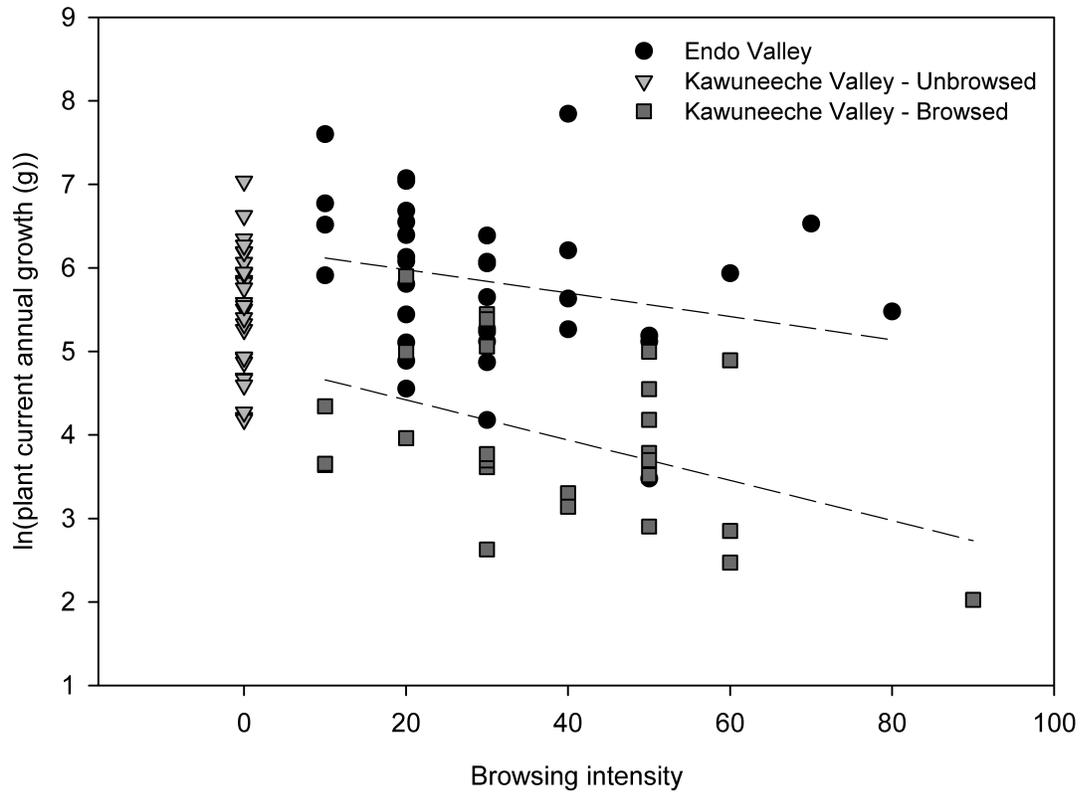
Figure 2.3: Browse intensity (0 - 100% scale, at 10% intervals for each plant) and current annual growth (g) in 2011 (natural log scale) for willow plants in Endo (circles) and Kawuneeche Valleys (inside exclosure: inverted triangles; outside exclosure: squares). Plants inside exclosures are un-browsed. Annual growth is not related to browsing intensity in Endo Valley (6.26-0.014*browsing intensity (R^2 = 0.06; p = 0.36)) and demonstrates a gradual decline in the Kawuneeche Valley (4.90-.024*browsing intensity (R^2 = 0.20; p = 0.14))
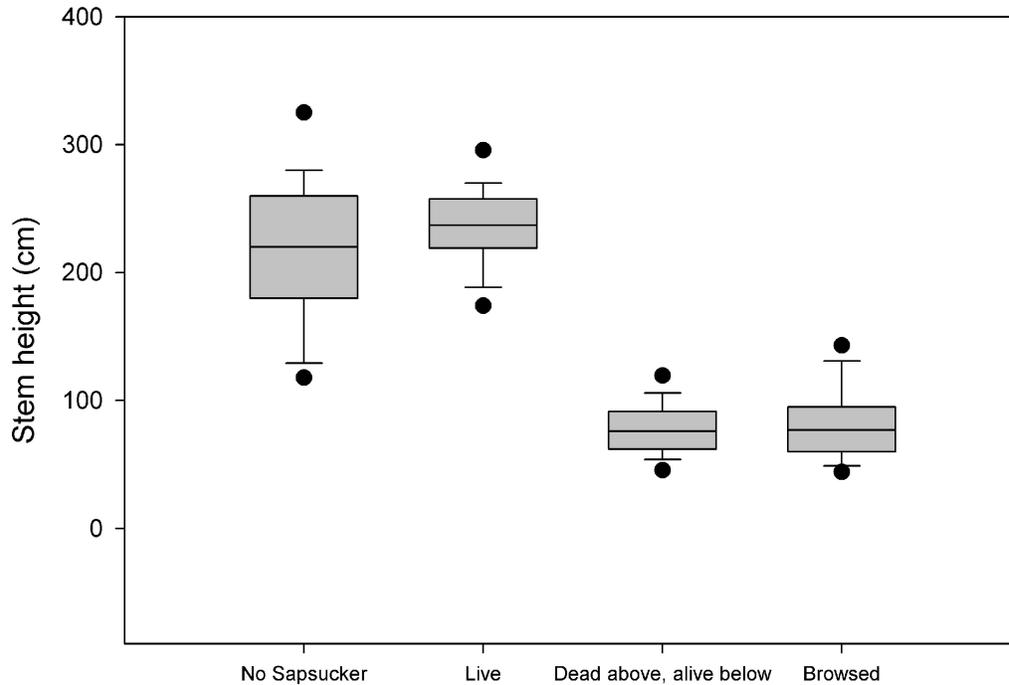
Figure 2.4: Comparison of heights of willows stems. Box plots are the median, 10th, 25th, 75th, and 90th percentiles, with 5% and 95% outliers. 'No sapsucker' are live, unbrowsed stems. 'Live' are sapsucker wounded stems which did not experience stem death above the wound. 'Dead above, alive below' are 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. No sapsucker and live stems are not significantly different ($p = 0.13$) and dead above, alive below and browsed stems are not significantly different ($p = 0.94$). These two sets of stems are significantly different from one another ($p < 0.01$).
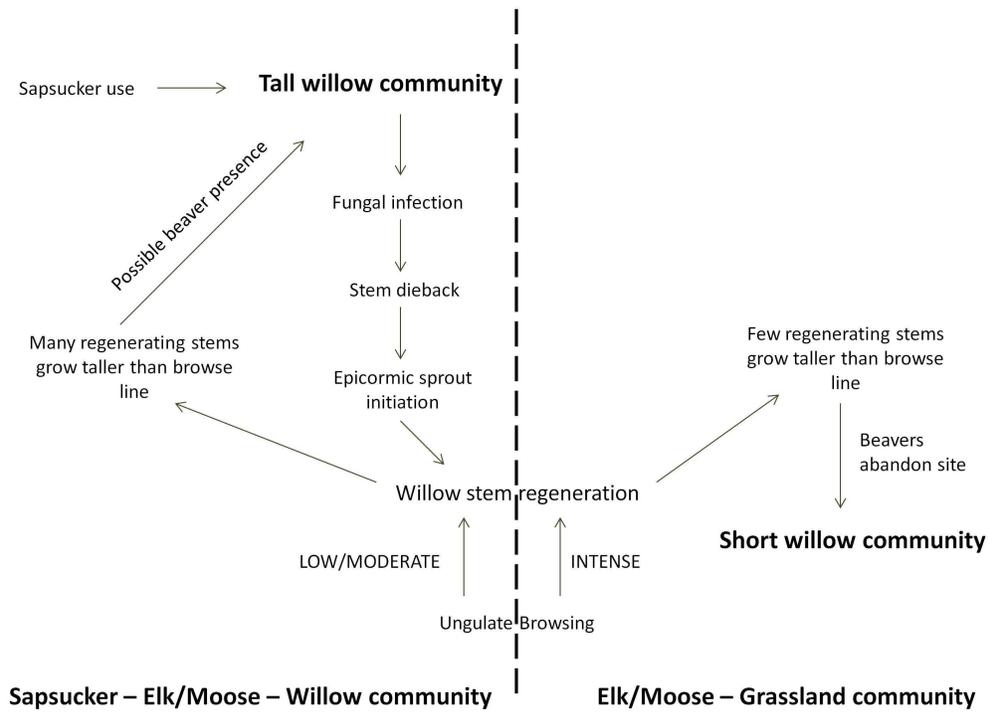
Figure 2.5: Conceptual diagram describing the relationships among ungulate browsing, beaver occupation, sapsucker use and fungal infection on willow communities.

3 Biotic factors are more important than climate in the decline of riparian willows in Colorado

3.1 Introduction

Forest tree species declines have been studied throughout the world and have become a focus due to linkages with climate change, in particular widespread drought events (Bigler et al., 2007; van Mantgem et al., 2009; Anderegg et al., 2012*a*). Declines are thought to be caused by multiple interacting abiotic and biotic factors, including soil moisture levels and chemistry (Hamerlynck and McAuliffe, 2008; La Manna and Rajchenberg, 2004), ambient air temperatures (Worrall, 2009; D'Amore and Hennon, 2006), herbivory (Zeigenfuss et al., 2002), and insect and fungal pathogens (Desprez-Loustau et al., 2006; Worrall et al., 2008; Eschtruth and Battles, 2008). Species declines can be a slow process, taking years to decades and may go undetected for long periods of time (Manion and Lachance, 1992).

Riparian sites comprise a small percentage of North American landscapes and in proportion to their area in a watershed, perform a greater number of biologic functions than their adjacent uplands (National Research Council, 2002). These include streambank stablization, water temperature moderation, nutrient retention and cycling and habitat for many species of amphibians, reptiles, insects and mammals (Naiman et al., 2005). Almost 75% of all vertebrate species rely on riparian sites (Bunnell and Dupuis, 1993) and greater than 80% of breeding birds use riparian areas for at least part of their life cycle (Knopf et al., 1988), resulting in diverse biota sharing resources. Salicaceae (poplars and willows) is the dominant plant family along boreal and temperate rivers throughout North America (Malanson, 1993).

Riparian areas comprise less than 1% of the northern Rocky Mountain landscape (Skagen et al., 1998). Willows (*Salix* spp.) are the dominant riparian vegetation at higher elevations in the Rocky Mountains and throughout the western United States. The integrity of riparian systems in the Rocky Mountains is dependent on tall willows, ranging from 2.5 - 4.5 m in height. However many tall willow communities have declined to form stands of short stature willows < 1m in height (Peinetti et al., 2002; Limb et al., 2003). In addition to a reduction in stature, there is also a large decrease in plant volume.

Landscape wide climatic and local scale biological factors have been implicated in willow decline (Peinetti et al., 2002; Perry et al., 2012). Decreased snowpack results in lower average and earlier peak streamflows, which can cause growing season drought stress (Perry et al., 2012). However, climatic factors may be mitigated by local scale beaver activity. Beavers dam waterways with tall willows, trapping fine sediment and inundating the floodplain, allowing willows to have access to water later in the growing season. Willows are phreatophytes and rely on groundwater as their primary water source. In years of extreme drought, such as 2002 and 2004 in Colorado, floodplain inundation due to beaver dams led to shallower groundwater tables than in years with higher than average peak flows and no beaver dams (Westbrook et al., 2006). Riparian sites where beavers have been absent for many decades may be unsuitable for beaver return due to stream incision, leading to a conversion from a riparian shrubland to a grassland (Wolf et al., 2007). Ungulates, primarily elk (*Cervus canadensis* Erxleben) and moose (*Alces alces* Gray), utilize riparian willows as a food source (Hobbs et al., 1981; Kufeld and Bowden, 1996). Willow growth is stimulated by winter browsing, where even high intensity browsing can result in 100% regenerative growth the following growing season (Marshall, 2012). High ungulate browsing

maintains short stature willows and has negative effects on other species, reducing songbird breeding and nesting habitat (Baril et al., 2011). Red naped sapsuckers (*Sphyrapicus nuchalis* Baird, hereafter red naped sapsucker) also contribute to willow decline by creating sap wells that cause death of the apical meristem and dieback of the top of the stem due to *Cytospora chrysosperma* fungal infection (Kaczynski dissertation, Chapter 2). Resprouting epicormic shoots are easily accessed by ungulates, maintaining a short willow community.

Determining the timing of plant species declines can be aided by repeated analysis of aerial photos (Peinetti et al., 2002). In addition, the use of dendrochronology in aging tree death and dates of disturbance events is common (Schweingruber, 1988), but identifying the age of stem death is difficult with multi-stemmed shrubs. Stems on an individual plant grow at different rates and there are no dendrochronological methods or signals in the growth rings to know when a dead stem died. However, stems have a compensatory response to disturbances where individual stems produce epicormic sprouts from lateral meristems when the apical a meristem is damaged. This mechanism for regrowth after fire or herbivory will occur during the year of disturbance and is very common on willows (Stromberg and Rychener, 2010; Zvereva and Kozlov, 2001). The aging of epicormic sprouts can be used to determine years of damage and dieback to the apical meristem and provide insight into the timing and cause of stem stress.

In this paper I address two primary research questions: 1. How have climate and biotic factors influenced the timing of willow dieback within Rocky Mountain National Park (RMNP)?; and 2. How widespread is the dieback of willows in the southern Rocky Mountains? To examine the temporal scale of the dieback, I used historic aerial photographs and aged epicormic shoots to determine broad and local scales of the willow dieback at the headwaters of the Colorado River in

RMNP. I examined the spatial scale of willow dieback by surveying willow stands throughout the Colorado Rocky Mountains.

3.2 Materials and Methods

STUDY AREA

The Southern Rocky Mountain ecoregion in Colorado ranges from 1140 to 4400 m in elevation. Streams are dominated by snowmelt runoff, with peak flows occurring from mid May through early June. Forests are dominated by *Pinus contorta* (Dougl. ex Loud. var. *latifolia* Engelm.), *Picea engelmannii* (Parry ex Engelm.) and *Abies bicolor* (A. Murray), with large patches of *Populus tremuloides* (Michx.) typically found on slopes above riparian valleys. Riparian valleys are dominated by populations of the tall willows *Salix geyeriana* (Andersson) and *S. boothii* (Dorn) and short willows *S. wolfii* (Bebb) and *S. planifolia* (Pursh). Herbaceous vegetation consists of sedges and grasses. Elk and moose browsing is prevalent and cattle grazing can also occur on land outside of RMNP. Beaver are common on streams with suitable habitat and relics of their presence, such as beaver ponds, are often found decades after beaver have left an area.

The valley study area encompasses 12.5 km of the Colorado River headwater within Rocky Mountain National Park (RMNP) (40°22'N and 105°51'W). The site is a broad valley with widths ranging from approximately 0.4 km at the narrowest to 1.6 km at the widest portion. The total area is approximately 10.3 km^2 with a mean elevation of 2686 m. Streamflow is dominated by snowmelt with periodic thunderstorms in July and August. The USGS Baker Gulch gauging station (USGS 09010500) record contains streamflow measurements from 1953 to the present. Mean annual precipitation is 640 mm with 42% falling as snow at the Phantom Valley SNOTEL

station (CO05J04S, elevation 2750 m) and 885 mm with 84% falling as snow at the Lake Irene

SNOTEL station (CO05J10S, elevation 3260 m). The long-term mean December and July air

temperatures in the valley are -9.6°C and 12.4°C. Historically, the local valley hydrologic regime

was beaver driven, with dams along the Colorado River diverting up to 70% of the flow to the

floodplain (Westbrook et al., 2006). However, within the last decade, there has been a decline in

beaver activity due to very low numbers of resident beaver populations (NPS personal

communication) and a lack of suitable tall willows. Vegetation in the valley includes riparian

shrublands dominated by *Salix monticola* (Bebb), *S. geyeriana*, *S. drummondiana* (Barratt ex

Hook.), wet meadows, dominated by *Deschampsia cespitosa* ((L.) P.Beauv.) and *Calamagrostis*

canadensis ((Michx.) P. Beauv.), and peat-accumulating fens dominated by *S. planifolia* and

Carex aquatilis (Wahlenb.). Hillslope vegetation is dominated by *Pinus contorta*, *Picea*

engelmannii and *Abies lasiocarpa*. Plant nomenclature follows Weber and Wittmann (2001).

Temporal Dynamics of Dieback

Aerial Photo Analysis

Aerial photos for the years 1969, 1987, 1996, 1999, and 2001 were scanned at 1200 dpi (Table

3.1). All images were true color and flown in late August or early September, with the exception

of National Agriculture Imagery Program (NAIP) imagery. Each NAIP image, 2005 and 2011,

was taken during the leaf on period of growing season. Four band color imagery (color + IR) was

used in 2008.

I created photo mosaics of each year and rectified each mosaic to the 2005 NAIP imagery using

ArcGIS 9.3. The valley bottom was delineated into three segments: north, middle and south,

based on roads that bisected the valley. Root mean squared error (RMSE) for each rectification of

the entire valley was less than 2 m and each valley segment was then adjusted to obtain a RMSE

of 1 m. All images were resampled using cubic convolution and stretched using standard

deviations of n = 4. I created a 2 x 2 m grid of points for the entire study area and 1% of these

points were randomly selected for sampling in each of the three segments, resulting in 4431,

9813, and 11,453 points for the north, middle and southern sections. Each point was analyzed

each year for the presence or absence of a willow individual or individuals. I compared the

number of willow present points for each sample year.

I ground truthed the accuracy of willow presence and absence detection during the summer of

2011 using a double blind survey of 130 randomly selected points from 2008 aerial photo

analysis. Accuracy was 76%. Omission errors were 20%, due to short willows that could not be

discerned from the tall herbaceous vegetation, while only 4% were commission errors.

I estimated parameters using Bayesian change point Poisson regression,

$$Y_{ijkl} \sim Poisson(\mu_{ijkl})$$

$$log(\mu_{ijkl}) = \alpha_{t<cp,\ t>cp} + \beta_i \ldots$$

where α is estimated before (t < cp) and after (t > cp) a change point. Models were fit using

JAGS within R (Su and Yajima, 2012). I ran three chains simultaneously for 35000 iterations,

after a burn-in of 5000 iterations to guarantee convergence. I standardized all predictor variables

(x_i - xbar / sd) and tested for main effects, to avoid overfitting the model. Climatic variables

included average stream flow, average stream flow one and two years prior, peak stream flow,

peak stream flow one and two years prior, total water year precipitation and one year prior, total

snowfall accumulation and growing degree days (GDD). I used a threshhold of 5º C to calculate

GDD to obtain an estimate of growing season length. Peak and average stream flows (88%), snow

depth and peak (75%) and average (80%), and snow depth and precipitation (86%) were highly

correlated, therefore only one measure of each was used in each model. Elk population data for

the E8 Game Management Unit (GMU), which includes the western part of RMNP, were obtained

from the Colorado Division of Parks and Wildlife. No data are available for moose populations in

specific GMUs, therefore the number of hunting tags allowed per year in each GMU was used as an

estimate of moose populations. The number of tags is determined by hunter success, antler spread,

non-hunter mortality, and illegal mortality (Kirk Oldham, Colorado Division of Parks and Wildlife,

personal communication). Biological variables included in the model were elk population in the

year of the aerial photo and one year prior, the number of moose hunting tags issued in the year of

the photo and one year prior, and the presence or absence of beaver. I fit the full model, dropped

terms when estimated coefficients were centered on zero and refit the reduced model to obtain

final parameter estimates. I used non-informative priors for all parameters: normal priors for all

regression coefficients and gamma priors for precisions (inverse of variance). Models were ranked

using Deviance Information Criterion (DIC) (Link and Barker, 2010). I evaluated model fit using

posterior predictive checks (Gelman and Hill, 2007) by calculating a test statistic from observed

and simulated data from the model. A Bayesian P-value (PB), the probability that the simulated

data are more extreme than the observed data, for the mean (PB-mean), standard deviation (PB-

SD) and coefficient of variation (PB-CV) was used to evaluate model fit. A model shows lack-of-fit

if PB is close to 0 or 1 (Gelman and Hill, 2007).

Dating initiation years of epicormic sprouts

Aerial photo analysis only allowed me to examine presence and absence of willows, therefore I developed a novel method to more closely examine the alteration of tall to short willows. Seventy seven stems were collected throughout the valley study area in 2010 and 2011. All stems had a sapsucker wound, apical meristem dieback and epicormic shoots below the stem wound. I cut the epicormic shoot at the base and mounted, sanded and aged the year of initiation. One outlier was removed from the data set because it was not representative of the population as a whole. The shoot initiated in 1994 and detectability of epicormic shoots decreases sharply as the age of the epicormic shoot increased because of high ungulate browsing of epicormic shoots.

I compared years of epicormic shoot emergence to long-term precipitation and temperature records at nearby weather stations (Phantom Valley Snotel site and Grand Lake RAWS station) and to stream flow data from the Baker gulch gauge on the Colorado River to identify large scale trends in landscape wide stressors. There was slight overdispersion of the counts therefore I corrected standard errors by modeling the variance as μ x φ, where μ is the mean and φ the dispersion parameter. I estimated parameters using Bayesian methods and ranked models using DIC (Link and Barker, 2010).

$$Y_{ijkl} \sim Poisson(\mu_{ijkl})$$

$$log(\mu_{ijkl}) = \beta_0 + \beta_1 + \beta_2 + \varphi$$

Models were fit using JAGS within R (Su and Yajima, 2012). I ran three chains simultaneously for 50000 iterations, after a burn-in of 5000 iterations. I standardized all predictor variables (x_i - xbar / sd) and tested two-way interactions. Climatic variables included total water year precipitation (October - September), total snowfall accumulation, peak and average streamflow during the year of epicormic sprout development and in the previous year, and growing degree days. Biological variables included elk population in the year of and one year prior to the epicormic shoot, and the number of moose hunting tags issued in the year of one year prior to the initiation of the epicormic shoot. Site specific sapsucker data were not available, however populations have remained constant in Colorado from 1968 - 2010, with an average trend estimate of +2.77% (95% Credible Interval: +1.00 - +4.47) (Sauer et al., 2011).

Willow measurements

I utilized three ungulate exclosures established in 1997 within the study valley to compare volumes (m^3) of randomly selected unbrowsed willows. Browsed willows were measured in plots randomly located within 250 meters of the exclosure. I measured height, length and width of 90 unbrowsed and 90 browsed willows during 2009 and 86 unbrowsed and 80 browsed willows in 2011. Spherical volume was approximated by multiplying plant height, length and widths.

Spatial extent of dieback throughout northern Colorado Rocky Mountains

I chose a set of possible study locations outside of RMNP, within the Colorado Rocky Mountain region based on valleys which were similar to the headwaters of the Colorado within RMNP (Figure 3.1). Sites were selected in ArcGIS based on six criteria: 1.) elevation between 2280 and 2990 m, 2.) along a perennial stream, 3.) within 700 m of a road, 4.) located on US Forest Service

46

land, and 5.) less than 20% slope, as determined using a digital elevation model (DEM), and 6.) greater than 5 km from another point. *Salix* sp. populations were verified at each of the 150 points using Google Earth (Google, Inc.) and 17 sites were randomly selected from the 59 containing willows. In addition, five valley sites were sampled within RMNP. Sites were sampled in summer 2011. I walked a transect and stopped at 10 random locations approximately 100m apart to assess the willows using a point-center quarter vegetation sampling method (Cottam et al., 1953). I collected data on species, presence of sapsucker wells, % stems with *Cytospora* infection and dieback, and length, height and width of four willows at each location, resulting in 40 willows per transect, therefore 880 plants from 22 sites were analyzed.

I performed exploratory path analysis in a maximum likelihood estimation framework to determine which variables were most important in predicting willow dieback in Colorado. Path analysis allows for the testing of direct and indirect effects on multiple explanatory variables (McCune and Grace, 2002). I used AMOS (Analysis of Moment Structures) software for analysis (SPSS). Endogenous, or dependent, variables included percent dieback, percent browsing intensity, percent of stems with *Cytospora* and overall plant volume. Percents were transformed for analyses using a logit transformation, $y_i = ln((x_i + \varepsilon)/[1 - x_i + \varepsilon])$ where x_i is percent and ε is equal to minimum non-zero proportion of x (0.1) (Warton and Hui, 2011). Exogenous, or independent, variables included topographic wetness index, aspect, slope, sub basin (USGS HUC 8 watersheds), presence of beaver, number of stems with sapsucker wounding, summer and winter elk and moose browsing concentrations, and 2010 moose and elk population estimates normalized by data analysis unit (DAU) area, comprised of between one and seven game management units (GMU) of varying sizes. Models were tested and modified to increase explanatory power.

47

3.3 Results

Onset of the decline

Willow cover in the Kawuneeche Valley decreased by an average of 40% between 2001 and 2005 (Figure 3.2). Bayesian Poisson regression models with a change point performed better than models not including a change point (Table 3.2). Change points occurred between 2001 and 2005 for all models. The best model included the number of moose hunting tags issued and snow accumulation, and had a a DIC weight of 0.95, indicating 95% of the weight of evidence favored this model over all other candidate models (Table 3.2). Posterior predictive checks for the best model indicated good fit as the simulated data was consistent with actual observations (model 1: PB-mean = 0.50, PB-SD = 0.50, and PB-CV = 0.50). As moose hunting tags increased, holding snow accumulation constant, the presence of willows decreased and as snow accumulation increased, holding moose hunting tags constant, willow presence increased (Table 3.3). The number of moose hunting tags issued in a given year was the strongest variable tested (Table 3.3).

Epicormic shoot analysis – finer scale examination of the decline

Sixty nine percent of epicormic shoots initiated between 2002 and 2005. This indicates the year when disturbance to the stem affected the apical meristem. The timing coincided with the sharp decline in willow cover in the valley measured using aerial photographs (Figure 3.3). Since 2005 a decrease occurred in the number of epicormic sprouts initiated because all formerly tall willow stems in the study area have died back. Thus, epicormic sprout formation is no longer possible on most willow plants in the study area. The top three models describing the initiation of epicormic sprouts, all with a ΔDIC <5 indicating all three models should be considered (Table 3.4). No

models included interactions. In all models, as the length of the growing season increased, the number of epicormic sprouts increased, when holding other variables constant. GDD was the strongest variable in all models (Table 3.4). The second variable in the model is either current year elk numbers, current year moose hunting tags, or previous year elk numbers. Elk population numbers had a negative effect on epicormic sprout initiation. As elk numbers increased in the current year or previous year, the number of epicormic sprouts decreased, holding GDD constant. Moose hunting tags had a positive effect. As hunting tags increased, indicating more moose, the number of epicormic sprouts increased. Parameter estimates for moose and previous year elk population have credible intervals that overlap with 0, making these estimates less important in the model.

Willow volumes

In 2009 and 2011 willow volume averaged 2.80 m^3 and 3.90 m^3 for unbrowsed plants and 0.53 m^3 and 0.51 m^3 for browsed plants. The volume of browsed plants was 19% and 14% of unbrowsed plants in 2009 and 2011 (Figure 3.4). The volume of unbrowsed plants increased over the two years, while it decreased for browsed plants, resulting in a willow community volume that is 14% of pre-2005 volume structure.

How widespread is the dieback in the Southern Rocky Mountains

Willow dieback is highly variable in the southern Rocky Mountains but on average is higher within RMNP than in areas outside RMNP. There was little variation in dieback levels outside of RMNP (mean: 0.19, 95% Credible Interval:[0.18, 0.21], n = 17) and high variation within RMNP (mean: 0.41, 95% Credible Interval: [0.36, 0.47], n = 5).

Exploratory path analysis demonstrated that few landscape or biological predictors explained dieback throughout the southern Rocky Mountains, however browsing, dieback, *Cytospora*, and overall volume were linked (χ^2 = 12.431, df = 5, CFI = 0.985, RMSEA = 0.042) (Figure 3.5). Willow volume varied by subbasin. Population estimates of moose and elk did not explain browsing intensity. Browsing intensity had a moderate effect on dieback. Dieback and the number of sapsucker wounded stems had a moderate effects on percentage of stems with *Cytospora*. Willow volume was moderately affected by *Cytospora*, because larger plants were more likely to have stems with dieback attributed to *Cytospora*.

3.4   Discussion

The structure of the willow community at the headwaters of the Colorado River has changed dramatically. The community is dominated by short stature willows that have experienced an 86% decline in the crown volume compared with what was present prior to 2005. This change of willow stand structure has had cascading effects through the riparian ecosystem, from the loss of migratory bird and beaver habitat, to the drying of soils through the valley (Westbrook et al., 2006). While both climatic and biotic factors have been implicated in vegetation declines (Worrall et al., 2008; Worrall, 2009), biotic factors, such as ungulate browsing, rather than historic and current climatic factors, explained more of the short and long term dynamics of the willow dieback in the Colorado River headwaters.

Landscape drivers of willow stem dieback

The most important factors explaining the landscape level dieback at the Colorado River headwaters are increasing moose population and total snow accumulation. In the absence of predators, moose populations have increased steadily since their introduction into Colorado in the late 1970's.

Moose have a negative effect on willow presence, and as moose populations have increased, willow cover decreased. Snow depths > 80 cm have been shown to hinder moose movement (Hundertmark et al., 1990; Poole and Stuart-Smith, 2006). Therefore, in years with higher snow accumulation, browsing on willows was decreased.

The droughts of 2002 and 2004 did not initiate willow dieback in the study area. This may have been because beaver were active in the valley until 2005 (D.J. Cooper, personal observation) and their damming of the Colorado River kept water tables high and mitigated the effects of drought. Westbrook et al. (2006) found that in low stream flow years of 2002 and 2004, 15% and 21% of their floodplain study area was inundated late in the summer and much of the study area had a water table near the soil surface. In contrast, 2003 was the fifth highest flow year in the 55 year Colorado River record (1956 - 2011), and flooding inundated only 10% of the study area and persisted for just 3 to 7 days. The high 2003 flow also washed out beaver dams on the main river channel and the short duration flooding resulted in a deeper summer water table than occurred in 2002 and 2004, producing larger areas with dry soil than in the drought years.

Local drivers of willow stem dieback

Prior to 2001, willow communities were stable for at least the 40 years of air photo record, throughout the upper Colorado River valley. Aging the initiation dates of epicormic shoots identified the years when willow stems experienced stress to the apical meristem resulting in stem dieback. It is likely that willow stem dieback and epicormic shoot development is a natural process in healthy willow stands, as sapsuckers have always been integral parts of these riparian ecosystems and drivers of stem dieback. However, increased ungulate browsing after 2001 led to a change in the processes shaping willow ecosystems. Historically, stems that died were replaced

51

by epicormic shoots that reached the full height each species of *Salix* attains (Kaczynski dissertation, chapter 2). However, because dead willow stems lose their bark, the older stems leave no historical record of the timing of their death. In addition, ungulates browse the epicormic shoots, preventing them from attaining full height, producing plants that are entirely of short stature.

Erhlich and Daily (1988) found that dead willow stems lost their bark after about five years, and following bark loss no definitive cause of stem death can be identified. Despite the lack of available data on epicormic shoot initiation prior to 2001, a sharp decline in shoots occurred since 2006. I suspect this is due to the loss of tall willow stems in the study area and the loss of willows suitable for sapsucker foraging. All models explaining the recent willow stem dieback included growing season length, where a longer growing season triggered more epicormic shoot initiations (Table 4). Moose had a positive effect on epicormic shoot initiation, likely because moose are the primary herbivores in the study area, are resident throughout the year and populations are steadily increasing. Elk had a negative effect, as elk migrate to higher elevations during the summer and the increase in moose populations has likely lessened the effect of elk.

Patterns of dieback across the Colorado Rockies

There was a wider range of willow dieback in RMNP while there was little variation outside RMNP. Inside RMNP, elk and moose have little seasonal movement and occupy some valleys in large numbers, while they are nearly absent from others. Outside RMNP, hunting is prevalent and ungulate movement is greater and behavior is more active. Percent *C. chrysosperma* infection on an individual plant was explained by the percent dieback on the plant. Signs of a *C. chrysosperma* infection, pycnidia and fruiting bodies, will only be visible on a dead stem, and

although live stems may be infected, the infection is usually not detected. Population estimates for elk and moose per GMU were not specific enough for on the ground estimates of dieback and browsing intensity. The number of sapsucker wounded stems helped explain some of the variation in percent *C. chrysosperma.* Previous research has supported this connection, where *Cytospora* infection rates on sapsucker wounded stems were 93 and 100% during two sample years (Kaczynski dissertation, chapter 2).

Implications for Management

A diverse group of mammals, birds and amphibians rely on tall willow riparian ecosystems for survival. Dieback of willows in the greater Colorado Rocky Mountain ecosystem has had cascading effects through the entire riparian ecosystem, including a reduction in songbird species richness and abundance (Olechnowski and Debinski, 2008), beaver populations (Baker et al., 2005*a*), and amphibians (Scherer, 2010). The continuing loss of willows could result in a dramatic alteration, as occurred in Yellowstone National Park, where willow stands transitioned from an elk-beaver-willow state, where tall willows thrive under low to moderate browsing, to an elk-willow grassland, where high intensity browsing has resulted in short willows and drier soils (Wolf et al., 2007).

Willow dieback at the headwaters of the Colorado River is tied to biotic variables rather than climate and is much higher inside than outside of RMNP. In the absence of apex predators and hunting, ungulate populations have increased and have become more stationary. This has likely resulted in the indirect effect of beaver population decline, due to lack of suitable tall willows. In the absence of any management action, willow populations will continue to decline. Annual willow growth on browsed individuals steadily decreased between 2009 and 2011 and unbrowsed willows were 324% more productive than browsed willows (Kaczynski dissertation, chapter 2).

In addition, only 2% of browsed willows flowered in 2010, compared with 92% of unbrowsed willows, decreasing the likelihood of any willow regeneration, even if suitable sites were available (n = 80 browsed, n = 85 unbrowsed, Kaczynski, unpublished data). Flowering occurs on shoots that initiated the previous year, therefore when browsing intensity is high, many shoots do not even survive one year. However, willows are resilient and short, highly browsed stems released from browsing will produce taller stems within one year (Baker et al., 2005*b*). Large exclosures will allow for willows to regain height and the potential for reproduction and excluding ungulates is a first step to restoring riparian willow communities.

Table 3.1: Year, type and resolution of aerial imagery used in photo analysis. #CIR = Color Infrared. *composite imagery: small areas of the valley which were not photographed at the 0.38m resolution were flown at 1.5m resolution and were mosaicked.

Year	Type	Resolution (m)
1969	Color	0.45
1987	Color	0.43
1996	Color	0.57
1999	Color	0.21
2001	Color	0.38, 1.5*
2005	Color	1.0
2008	CIR#	0.35
2011	Color	1.0

Table 3.2: Model comparisons ranked by Deviance Information Criterion (DIC). Model weight (wt$_{\text{DIC}}$) represents the relative likelihood of the model. ΔDIC is the change in DIC between the model and the highest ranking model. All highest ranking models included a change point ($\alpha_{1(t<cp)}$, $\alpha_{2(t>cp)}$), which occurred between 2001 and 2005 for all models. Variables in the model included moose, which are the number of hunting tags issued for the Colorado Division of Wildlife game management unit number 18, and is a surrogate for actual moose population estimates; total water year snow accumulation; and elk population estimates for game management unit number 18.

model	DIC	ΔDIC	wt$_{\text{DIC}}$
($\alpha_{1(t<cp)}$, $\alpha_{2(t>cp)}$) + moose + snow accumulation	100.7	0	0.95
($\alpha_{1(t<cp)}$, $\alpha_{2(t>cp)}$) + moose	107.4	6.7	0.03
($\alpha_{1(t<cp)}$, $\alpha_{2(t>cp)}$) + moose + elk	109.2	8.5	0.01
moose + snow accumulation	197.3	96.6	—

Table 3.3: Parameter estimates for the highest ranking Bayesian Poisson regression model explaining willow presence through aerial photo analysis. Model included a change point between 2001 and 2005, with estimates before the change point modeled as, $\alpha_{1(t<cp)}$, and estimates after the change point modeled as $\alpha_{2(t>cp)}$. Also included in the model are total water year snow accumulation (cm), and the number of moose hunting tags distributed for Colorado Division of Wildlife game management unit 18 (a surrogate for moose population estimate). The rate of decline is the regressor slope. All estimates are standardized. Change point estimates (α) are on a log scale.

variable	estimate	CI_{lower}	CI_{upper}
$\alpha_{1(t<cp)}$	9.120	8.975	9.265
$\alpha_{2(t>cp)}$	8.628	8.489	8.766
snow accumulation	-0.042	-0.070	-0.014
moose	0.275	0.180	0.369
rate of decline	-0.102	-0.133	-0.070

Table 3.4: Bayesian Poisson models with an overdispersion factor that explain the year of epicormic shoot initation. β_0 is the intercept term; β_1 is the estimate for growing degree days (GDD), a measure of growing season length; β_2 in model one is previous year elk population estimates in Colorado Division of Wildlife game management unit 18 (CDOW GMU 18), in model 2 current year moose population for CDOW GMU 18 (estimated by moose hunting tags), and model 3 current year elk population estimates for CDOW GMU 18. Upper and lower 95% credible intervals are in parentheses. φ is the dispersion parameter. All estimates are standardized.

models	β_0	β_1	β_2	φ	DIC
GDD + prev elk + φ	1.480 [0.810, 2.040]	0.764 [-0.095, 1.669]	-0.399 [-1.289, 0.447]	3.021 [0.373, 7.738]	65.8
GDD + moose + φ	1.481 [0.84, 2.009]	0.759 [0.049, 1.512]	0.519 [-0.258, 1.329]	3.998 [0.428, 11.949]	67.3
GDD + elk + φ	1.477 [0.884, 1.960]	0.632 [0.079, 1.222]	-0.511 [-1.104, 0.050]	7.983 [0.516, 23.315]	68.2
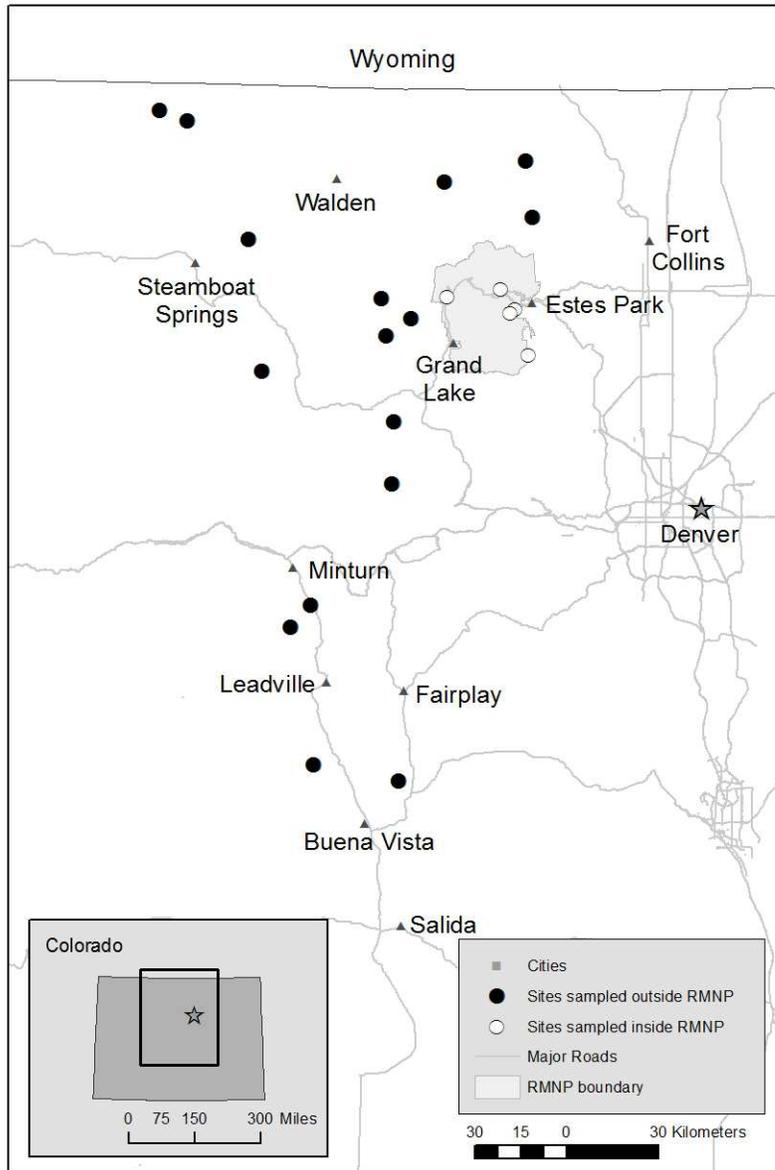
Figure 3.1: Locations of randomly selected willow dieback sample sites throughout the Colorado Rocky Mountain region. Black circles indicate sites outside of Rocky Mountain National Park and white circles are sites inside Rocky Mountain National Park.
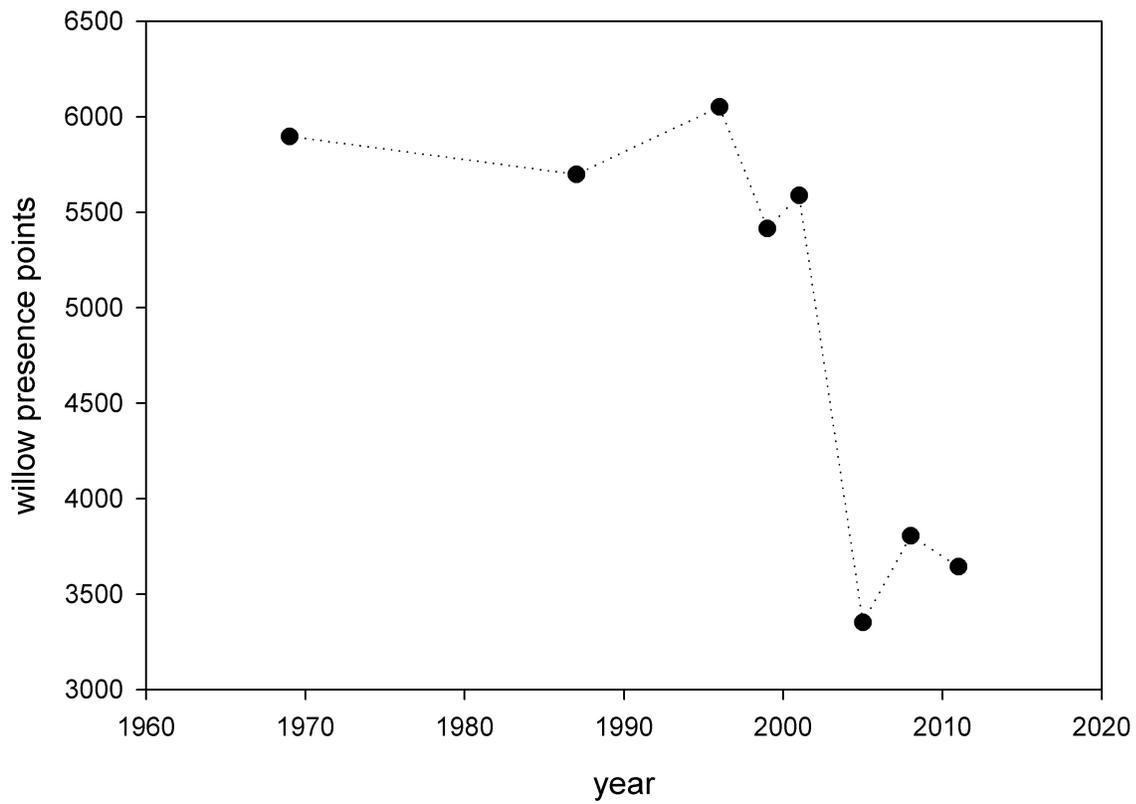
Figure 3.2: Willow presence points through time in the Kawuneeche Valley in Rocky Mountain National Park. Each point represents the total number of willow presence points from the aerial photo analysis.
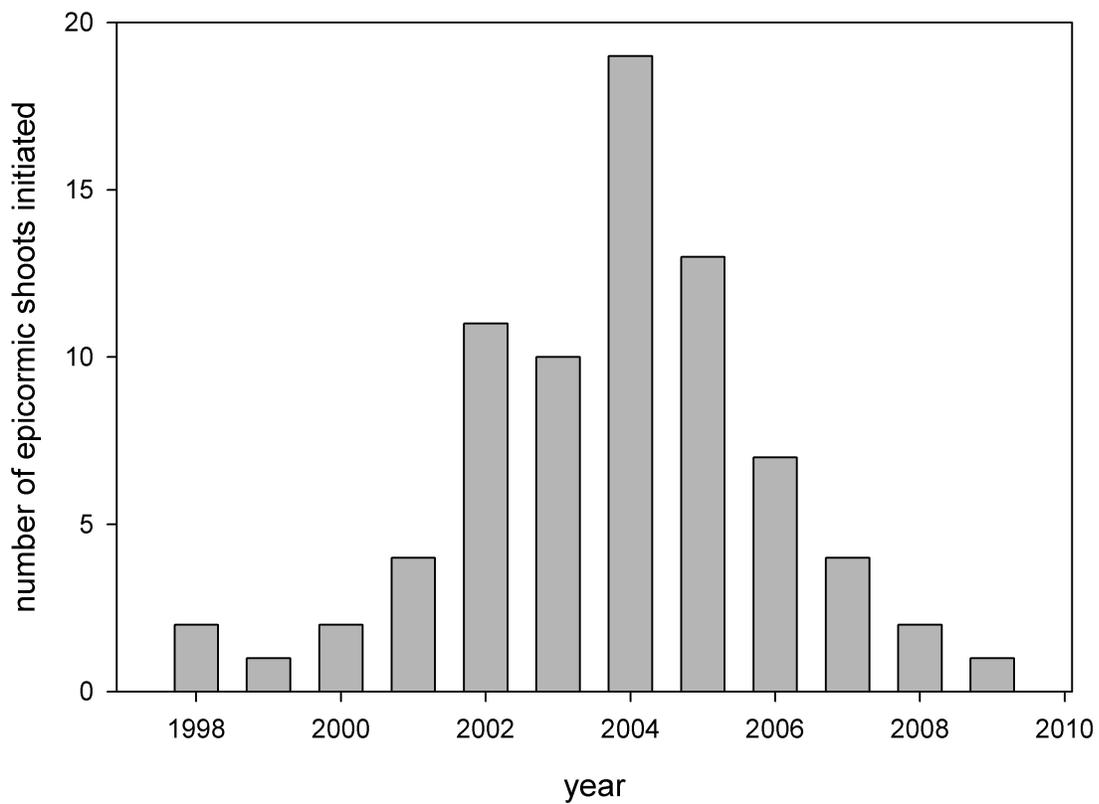
Figure 3.3: Years of epicormic shoot intiation as determined by dendrochronology. The presence of epicormic shoots demonstrates disturbance to the apical meristem and gives insight into years when stems were experiencing stress. Stems (n=77) were collected throughout the Colorado River headwater study area in Rocky Mountain National Park.
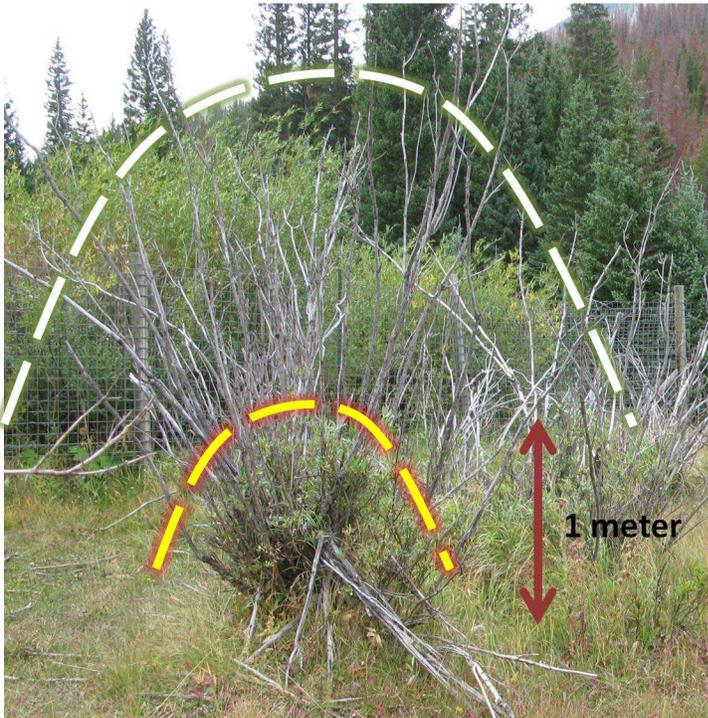
Figure 3.4: Change in willow shrub volume (m^3). Dashed line displays approximate height based on tall dead stems. Dash-dot line displays the actual height in 2009, approximately 1.25 m. Willows have lost approximately 86% of their original volume, based on the presence of the tall dead stems. Tall live willows in background are growing inside an ungulate exclosure and are not browsed.
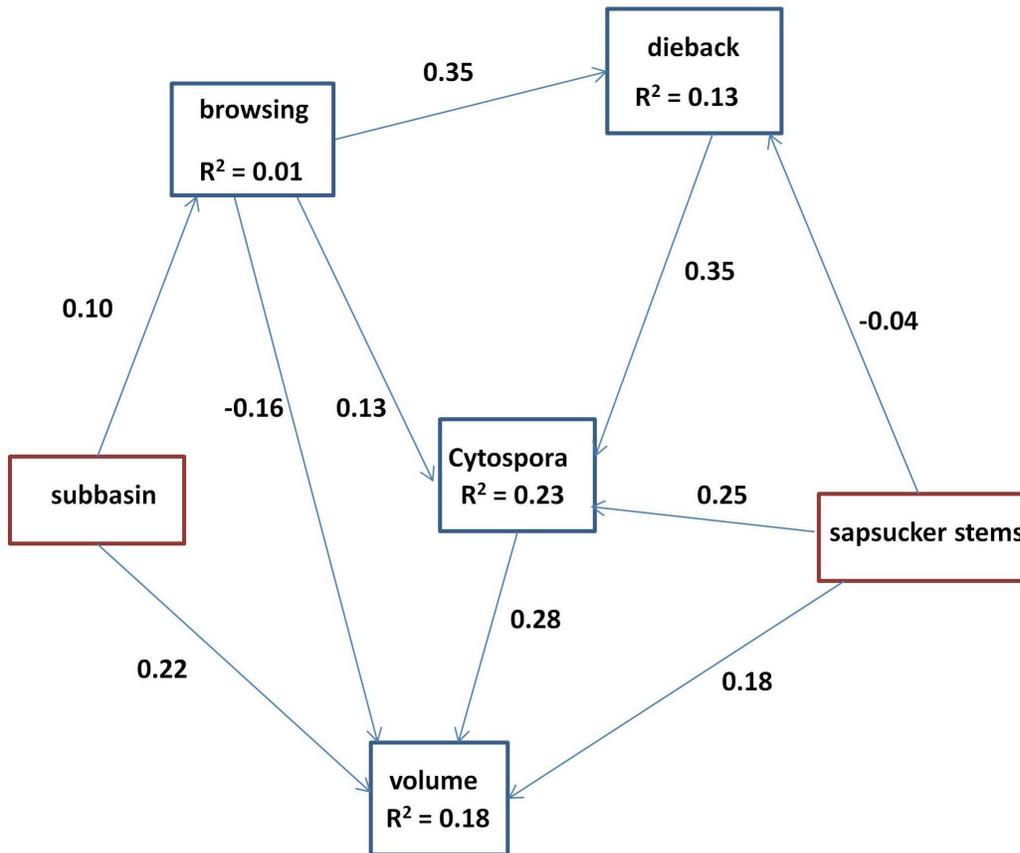
Figure 3.5: Structural equation model demonstrating relationships among browsing, cytospora, dieback and volume of willows. Endogenous, or variables that are caused by one or more variables, are in blue, and exogenous, or variables that cause other variables, are in red. *Cytospora*, browsing and dieback are percents and were logit transformed for this model. Subbasin, as defined by US Geologic Survey Hydrologic Unit Code, and the number of sapsucker wounded stems are categorical variables. R^2 values on endogenous variables display the amount of variation explained by the paths directed into it. Path coefficients along each arrow are standardized partial regression coefficients.

4 Role of potential future climate change on willow dieback

4.1 Introduction

Climate driven species declines and mortality events have occurred across the globe (Allen et al., 2010). The declines have altered ecosystem processes, including nutrient cycling and energy and water fluxes, resulting in cascading effects through ecosystems (Anderegg et al., 2012*b*). Climate related stressors have been shown to decrease a plant's ability to defend against diseases (Boyer, 1995; Ayres, 1984). For example, drought is recognized as an inciting factor with pathogens (Desprez-Loustau et al., 2006; Manion and Lachance, 1992) and climate change has increased the risk of pathogen outbreaks (Harvell et al., 2002; Garrett et al., 2006; Burdon et al., 2006). Widespread aspen (*Populus tremuloides* Michx.) decline in western North America has been linked to extreme drought, above average temperatures, fungal pathogens and insect attacks (Worrall et al., 2008; Anderegg et al., 2012*a*; Michaelian et al., 2011). Dieback of narrowleaf alder (*Alnus incana* (L.) Moench ssp. *tenuifolia* (Nuttall) Breitung), a common riparian tree in western North America, is caused by high maximum summer temperatures and an epidemic of *Cytospora* canker (*Valsa melanodiscus*, anamorph *Cytospora umbrina*) (Worrall, 2009). The dieback of these two important western North American forest species, linked to climatic events, has produced far reaching ecological effects, such as displaced bird communities (Griffis-Kyle and Beier, 2003; Losin et al., 2006) and altered food webs (Bailey and Whitham, 2003).

Large scale dieback and decline of riparian willow communities has been documented throughout the Rocky Mountains over the the past two decades (Limb et al., 2003; Marshall, 2012; Peinetti et al., 2002). In Rocky Mountain National Park, Colorado (RMNP) willows have tall (2-3 m) dead stems, while most live stems average 0.5-1.0 m in height. The dead stems are the result of a

complex interaction among sapsuckers, *Cytospora chrysosperma* ((Pers.:Fr.) Fr. anamorph; *Valsa sordida* (Nitschke)), teleomorph) fungal infection, and ungulate browsing (Kaczynski dissertation, chapter 2). The fundamental difference between riparian willow decline compared with upland species declines, such as aspens, is that riparian willows are phreatophytes and utilize shallow groundwater as a water source (Johnston et al., 2011), while upland plants rely on precipitation driven soil moisture. Drought stressed plants can experience hydraulic failure when the water column in the xylem conduit cavitates, resulting in a loss of conductivity. For example, aspen branches exhibit 50% loss of conductivity between -1.1 and -2.5 MPa (Hacke et al., 2001), while willows similarly exhibit 50% loss of conductivity between -1.57 and -2.18 MPa (Johnston et al., 2007). A lack of a reliable source of water can make aspens more susceptible to drought. However, drought stress can occur in willows and develops from two processes. One is low precipitation that limits shallow soil water availability and ground water recharge due to low streamflow. The second is the absence of beavers that limits local flooding and ground water recharge. In the Rocky Mountains, peak stream flows occur in response to snowmelt, and low flows occur if there is a low water content snowpack (Cayan, 1996). In addition, with the interaction of warming temperatures and reduced snow pack, peak stream flows may occur earlier in the growing season, leading to low late summer flows (Stewart et al., 2005; Clow, 2010). Beavers utilize woody riparian vegetation as a food source and dam building material and where suitable tall willows and other woody plant species occur they control local scale water tables, and moderate the hydrologic effects of climate (Westbrook et al., 2006), allowing willows to access shallow groundwater later in the growing season.

Increasing air temperatures and earlier peak streamflows have been documented throughout the Rocky Mountains (Clow, 2010; McGuire et al., 2012) and under projected climate change scenarios, will continue to change. Mean annual temperature is projected to increase by 2.5ºC in middle elevation zones in the Rocky Mountains (Bradley et al., 2004), which can lead to soil warming and drying. More precipitation is falling as rain during the winter months, reducing snowpack formation and resulting in lower summer stream flows (Knowles et al., 2006). These changes can result in increased drought stress on plants, particularly where beavers are absent.

Field and greenhouse studies have been used to show that woody plants exposed to warming (Bitty et al., 2004) or weakened by drought stress and subsequently wounded (Guyon et al., 1996; Kepley and Jacobi, 2000), are more susceptible to pathogen infection. My research examined the effects of warmer summer air temperatures and drought stress on *Salix monticola* (Bebb), the most common and abundant tall riparian willow species in the region. I was interested in how these climate change stressors affect above and belowground growth and leaf level stomatal conductance and I investigated how these multiple stressors influence the susceptibility of willows to *Cytospora* fungal infection. I used a manipulative field experiment to answer three questions: 1. Are drought stressed plants more susceptible to fungal infection?, 2. Are willows in warmer air temperatures more susceptible to fungal infection?, and 3. How does *Cytospora* fungal infection affect aboveground and belowground biomass?

4.2   Materials and Methods

The experiment was conducted in Rocky Mountain National Park (RMNP), Colorado (40.33° N , 105.60° W; elevation: 2620 m). *Salix monticola* stems were collected while dormant during the winter of 2010-2011. Stems were kept in cold storage until April, 2011, when they were planted

in a mixture of potting soil (Premier Horticulture Pro-mix BX Mycorrhizae) and sand, to create a sandy loam texture soil that is similar to floodplain soils in the study area (Westbrook et al., 2006). Each stem was planted in a 2.83 L (10 cm x 36 cm) tree pot (Stuewe and Sons, Tangent, OR, USA). Stems were grown in the Colorado State University greenhouse in Fort Collins, CO (40.58° N, 105.08° W; elevation: 1525 m), hardened in mid-May to acclimate to ambient temperatures and transported to RMNP in early June 2011. Eight pots were put into each of twelve 64 gallon rubber bins (N = 96 willow stems). The experiment was conducted from June through August, 2011, when willow stems were harvested.

Experimental Design

I used a split split plot experimental design (Figure 4.1): temperature was a whole plot factor, water level was a subplot factor, and inoculations were a subsubplot factor. I built three passive warming shelters using clear poly vinyl plastic, which were paired with three ambient temperature controls. All plots were covered with clear plastic corrugated roofing (Suntuf) to eliminate precipitation inputs (Figure 4.2). Wooden posts were used as corner supports. An automated vent (Univent automatic opener) opened or closed to maintain warming shelter temperatures 2 - 3° C above ambient. Temperatures were recorded hourly in the warming treatment and ambient control (n = 6) using Hobo H8 units (Onset) (Figure 4.3). Within each temperature, two water level treatments were used to simulate a shallow (well watered treatment) and deep water table (drought treatment) . Water levels were controlled with holes in the rubber bins that limited water depth. The deep water table bins had holes drilled approximately 3 cm from the bin bottom, while the high water table bins had holes drilled 20 cm from the base. Plants were watered from the bottom twice weekly. Bins were filled until water flowed out the holes. Soil moisture (volumetric water content (VWC))

was measured biweekly with a 12 cm TDR probe (Campbell Hydrosense). VWC between drought and watered differed significantly throughout the experiment ($p < 0.001$; Table 4.1).

Fungal Isolates

Two *Cytospora chrysosperma* isolates collected from RMNP willows were used in the experiment. Isolates were grown in the laboratory on 1/4 strength potato dextrose agar (PDA). They were then transferred to petri dishes containing full strength PDA and grown in the laboratory at 23º C for one week prior to inoculation into the study willows.

Inoculations

Willows were grown under full treatment conditions for seven days prior to inoculation. Four stems in each bin were randomly selected for inoculation. Stems were wiped with 95% ethanol prior to creating three 5 mm diameter circular wounds. The two lower wounds were inoculated with *C. chrysosperma*, while the top wound was a control inoculated with sterile agar. Wounds were wrapped in parafilm for seven days after inoculation. Vertical canker growth was measured in late August 2011. Canker size was compared between both main effects and interactions using ANOVA.

Plant Responses

Current annual aboveground biomass (AG) and belowground biomass (BG) were compared between treatments. To determine AG, new shoot length and diameter were measured on all stems. Fifty five stems were collected to develop quantitative relationships between shoot length (cm), diameter (mm) and biomass (g) using regression analysis. The linear regression relating stem length to biomass was used to determine AG ($r^2 = 0.94$, biomass:diameter, $r^2 = 0.86$). Mass was trans-

formed to linearize the data and achieve homogeneity of variance, where $ln(biomass_i + 0.85) = 0.0188 * length_i - 0.1245$. Small shoots infrequently had negative estimates of mass and were assigned a mass of 0.01 g. BG was measured by drying and weighing roots. All BG biomass was 0 at the start of the experiment.

Stomatal conductance was measured in mid August using a Li-Cor 6400XT with PAR held constant at 1500 micromol / m^2s.

Treatment main effects and interactions on BG, AG:BG ratio (using post-treatment AG production), and canker growth were analyzed using ANOVA with a random effect for replicate. Effect size was also computed when there was a significant treatment effect. Final AG was analyzed using ANCOVA, with pre-treatment AG as a covariate. All analyses were completed using Rx64 v2.15 (R Development Core Team, 2008) using the nlme package. Stomatal conductance was investigated only for main effects, as it was measured once through the experiment.

4.3   Results

Warming/Ambient and Drought/Well watered treatment effects

Warmed plant BG was 30% greater than ambient BG (p = 0.012; ES = 0.065) (Table 4.2). However, BG was not significantly different between low and high water tables (p = 0.313), and there was no interaction between temperature and water levels (p = 0.191).

Post-treatment AG was not significantly different between low and high water table (p = 0.35), or warming and ambient temperature treatments (p = 0.283), when accounting for pre-treatment AG. There was also no interaction between water levels and temperature on AG (p = 0.878).

The ratio of AG:BG was not significantly different between ambient and warming treatments (p = 0.0525), nor water levels (p = 0.227). The interaction of water level and temperature was also not significant (p = 0.086).

Plants with low water tables had lower stomatal conductance than plants in high water table treatments, 0.10 mol H_2O/m^2s compared with 0.14 mol H_2O/m^2s, demonstrating that the treatment had the desired effect on willows (p = 0.0031; ES = 0.0896) (Table 4.2). Stomatal conductance did not differ between plants in the warming treatment and control (p = 0.768).

Effect of drought and warming on *Cytospora* fungal infection

Cankers formed by *C. chrysosperma* averaged 45 mm and 52 mm in length on the middle and bottom wound and were significantly larger than control wound sizes, which averaged 11 mm (wound 1 vs. control: p < 0.000; wound 2 vs. control: p < 0.000). There were no significant differences in canker lengths between the two isolates (t = 0.266, p = 0.791) or the wound position (t = -1.959, p = 0.054), therefore the isolate and wound number effects were combined (Table 4.3). There was no significant difference between canker lengths in warming and ambient (p = 0.594), low and high water tables (p = 0.346) or the interaction of temperature and water level (p = 0.72). Stomatal conductance averaged 0.11 mol H_2O/m^2s in leaves on inoculated plants, but was not significantly lower in control plant leaves, 0.13 mol H_2O/m^2s (p = 0.085) (Table 4.2).

Cytospora **effects on AG and BG**

Cytospora inoculations had no effect on either AG (p = 0.149) or BG (p = 0.768). Examining BG, the interaction between temperature and inoculation (p = 0.371), water level and inoculation (p = 0.576) and the three way interaction, between water level, temperature and inoculation, (p = 0.563)

were not significant. AG, when accounting for pre-treatment biomass, was not significantly differ-ent when examining the interaction between water level and inoculation (p = 0.549), temperature and inoculation (p = 0.443) or a three way interaction (p = 0.626). Inoculations had no effect on AG:BG (p = 0.892). The interaction of water level and inoculation (p = 0.597), temperature and inoculation (p = 0.508) and the three way interaction (p = 0.387) were all not significant.

4.4   Discussion

Willow dieback in the Rocky Mountains has been linked to biotic factors including ungulate browsing, sapsucker wounding and *Cytospora* fungal infection (Kaczynski dissertation, chapter 2). Drought, due to the absence of beavers and decreased streamflows, both of which lower flood-plain water table depths, and increased air temperatures, may impose increased stress on willow stems in the future. Plants exposed to drought or increased air temperature have been shown to have increasing susceptibility to pathogen outbreaks. However, I found that after inoculation, wil-lows in low water table and warming treatments had large *Cytospora* canker growth. Canker size was not significantly different than those occurring on willows with high water tables and control environments. Therefore, water availability and temperature did not have a significant treatment effect compared with the controls.

Drought and warming effects on Cytospora fungal infection

The link between drought stress and susceptibility to fungal infection has been documented for many tree species (Desprez-Loustau et al., 2006). Drought stressed plants are typically more prone to canker fungal infection (McIntyre et al., 1996; Kepley and Jacobi, 2000). However, I found no treatment effect and drought did not make *S. monticola*, the most abundant tall willow in the southern Rocky Mountains, more susceptible to *C. chrysosperma* infection. Inoculations

produced cankers that were not significantly different in shallow and deep water table treatments, suggesting that *S. monticola*, and likely other tall willow species, were highly susceptible to *C. chrysosperma* infection, irrespective of the environmental stresses imposed by my experiment. I previously showed that 57% of wounded and field inoculated willow stems with canker expansion had low predawn xylem pressure potentials throughout the summer, demonstrating that plants not experiencing drought stress were also susceptible to fungal infection (Kaczynski dissertation, chapter 2). Siberian alder (*Alnus viridis* (Chaix) DC. ssp. *fruticosa* (Rupr.) Nyman) was also susceptible to *C. umbrina* at both high and low water stress levels (Rohrs-Richey et al., 2011). While aspen and other upland species may exhibit increased rates of fungal infection with drought stress (McIntyre et al., 1996), this response may not occur in all species.

Increased air temperatures have been implicated in stem infection with *C. umbrina* that caused the rapid decline of narrow-leafed alder (Worrall, 2009). Experimental warming appears to produce plant responses similar to drought stress, but responses are highly species specific and cannot be generalized (Bitty et al., 2004). My study is the first to examine experimental warming and *Cytospora* infection. A 2-3º C increase in temperature did not increase the susceptibility of willow to *C. chrysosperma* infection when compared to controls as all inoculated wounds had significantly larger cankers than control wounds.

Inoculations reduced stomatal conductance by 18% on the study willows, however, canker growth had no significant effects on AG, BG or stomatal conductance. Drought stressed Siberian alders inoculated with *Cytospora* had a 40% reduction in stomatal conductance compared with controls (Rohrs-Richey et al., 2011). I observed in the field that although the initial effects of a *Cytospora* infection are limited, infected stems typically die prior to the subsequent growing season (Kaczyn-

ski dissertation, chapter 2). If the length of this experiment were extended, there likely would have been greater *Cytospora* effects on AG and BG.

The high susceptibility of willows to *Cytospora* infection may be due to it's multi-stemmed life form. Rather than fight the fungal infection, the plant may limit resources to the infected stem and resprout a new stem from the base. This is in contrast to tree species, where an infection on a single bole can lead to the entire plant death.

Belowground biomass and current annual growth

Belowground biomass was significantly different between the warming treatment and ambient control. Warming increased root biomass, regardless of water level. Warming experiments conducted in many ecosystem types have also found increased belowground biomass as temperatures increased (Gill and Jackson, 2000; Pendall et al., 2004; Wu et al., 2011). As air temperatures rise, soil temperatures will also rise (Schlesinger and Andrews, 2000). Root growth is positively correlated with soil temperature, when variables such as soil nutrients and water, are controlled (Pregitzer et al., 2000). In Alaska, warming experiments on *Salix rotundifolia* produced a 25% increase in belowground biomass compared with ambient temperatures (Hollister and Flaherty, 2010).

Although initial root growth increases with warming, root turnover also increases, resulting in lower root biomass over time (Zhou et al., 2012; Pregitzer et al., 2000). This experiment was conducted in one growing season, therefore, it is unclear the long term patterns in root growth will be. On a longer time scale experiment I would expect plants in the warming treatments to have lower root biomass than ambient controls. Lower root biomass over time could have important implica-

tions for carbon cycling and net ecosystem production, including effects on microbial communities (Norby and Jackson, 2000).

There was no significant difference in AG or the ratio of AG:BG among treatments. Short term warming experiments on other woody species, such as *S. rotundifolia*, have also failed to demonstrate changes in ANPP or annual growth (Hollister and Flaherty, 2010). Warming has a positive effect on AG, consistent across ecosystem types, and longer duration warming experiments have shown a more pronounced positive effect (Wu et al., 2011). A 10 year field experiment in Yellowstone NP has shown that higher water tables result in greater willow biomass, with significant effects of water table depth on plant growth within three years (Marshall, 2012; Bilyeu et al., 2008).

Climate change, *Cytospora chrysosperma*, and willows

Cytospora chrysosperma is a native species of fungus and it has long been known to infect willows (Long, 1918). Warming and drought, consistent with what is predicted under climate change scenarios, may have little effect on the rate of infection by this species. My experiment demonstrated that once *C. chrysosperma* is present on a wound, it will form enlarging cankers under a wide range of environmental conditions. Biological drivers, such as *Cytospora* infection, ungulate browsing and sapsucker wounding, appear to be more important than climatic drivers in willow decline in the Rocky Mountains (Kaczynski dissertation, chapters 2 and 3). This is likely because as phreatophytes with a perennial water source, willows are partially buffered from climate processes, like soil water availability (Johnston et al., 2011). Because *Cytospora* infection can result in stem death (Kaczynski dissertation, Chapter 2), regeneration of willow stems after infection is essential for maintenance of a tall, intact riparian willow ecosystem. Resource managers can ensure willow regeneration by understanding and managing the uncontrolled effects of ungulate browsing.

Table 4.1: Means of main effects for treatments and controls, for aboveground biomass at the end of the experiment (AG-post), belowground biomass (BG) and the ratio of aboveground to belowground biomass (AG:BG). Stomatal conductance was measured in late July, 2011. No interactions were significant. 95% confidence interval in parentheses. ** significantly different at 0.01 level; * significantly different at 0.05 level.

	AG-post (g)	BG (g)	AG:BG	stomatal conductance (mol H_2O/m^2s)
Inoculated	8.30 (+/- 1.18)	24.38 (+/- 3.44)	0.39 (+/- 0.13)	0.11 (+/- 0.02)
Control	6.31 (+/- 0.87)	25.16 (+/- 3.85)	0.39 (+/- 0.07)	0.13 (+/- 0.02)
Warming	7.14 (+/- 1.02)	28.04 (+/- 3.94)*	0.31 (+/- 0.06)	0.12 (+/- 0.02)
Ambient	7.47 (+/- 1.11)	21.50 (+/- 3.07)*	0.46 (+/- 0.14)	0.12 (+/- 0.02)
Drought	6.87 (+/- 0.70)	23.22 (+/- 3.71)	0.44 (+/- 0.14)	0.10 (+/- 0.02)**
Watered	7.72 (+/- 1.32)	26.1 (+/- 3.56)	0.34 (+/- 0.06)	0.14 (+/- 0.02)**

Table 4.2: Mean canker length (in mm) from *Cytospora chrysosperma* inoculation per main effect. Canker length was measured vertically and including the 10 mm circular wound. All cankers from inoculated wounds were significantly larger than controls (p < 0.0001). 95% confidence intervals are in parentheses.

	canker length (mm)	control (mm)
Warming	51 (+/- 11.67)	11 (+/- 0.27)
Ambient	48 (+/- 11.19)	10 (+/- 1.85)
Drought	51 (+/- 11.58)	10 (+/- 0.30)
Watered	52 (+/- 11.18)	11 (+/- 1.56)

	Ambient		Warming	
Shallow water table	Inoculated	Not inoculated	Inoculated	Not inoculated
Deep water table	Inoculated	Not inoculated	Inoculated	Not inoculated

Figure 4.1: Experimental design of one paired treatment. Temperature (ambient and warmed) was the whole plot factor. Water table depth (shallow or deep) was the subplot factor. *Cytospora chrysosperma* inoculation was the subsubplot factor. There were 3 paired treatments total. Total sample size was 96 willow stems.

Figure 4.2: One replication of paired experimental treatments: Ambient temperature in front, passive warming shelter in plastic sheeting behind. Hobo (Campbell Scientific) temperature sensor hanging in center.
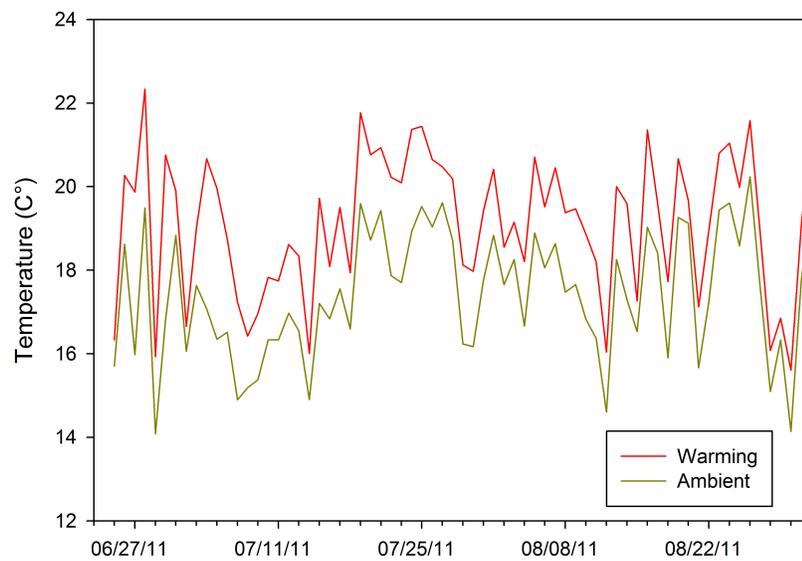
Figure 4.3: Daily mean temperature differences between warming treatment (red) and ambient control (green). Means are averaged for the three warming treatments and three ambient controls.
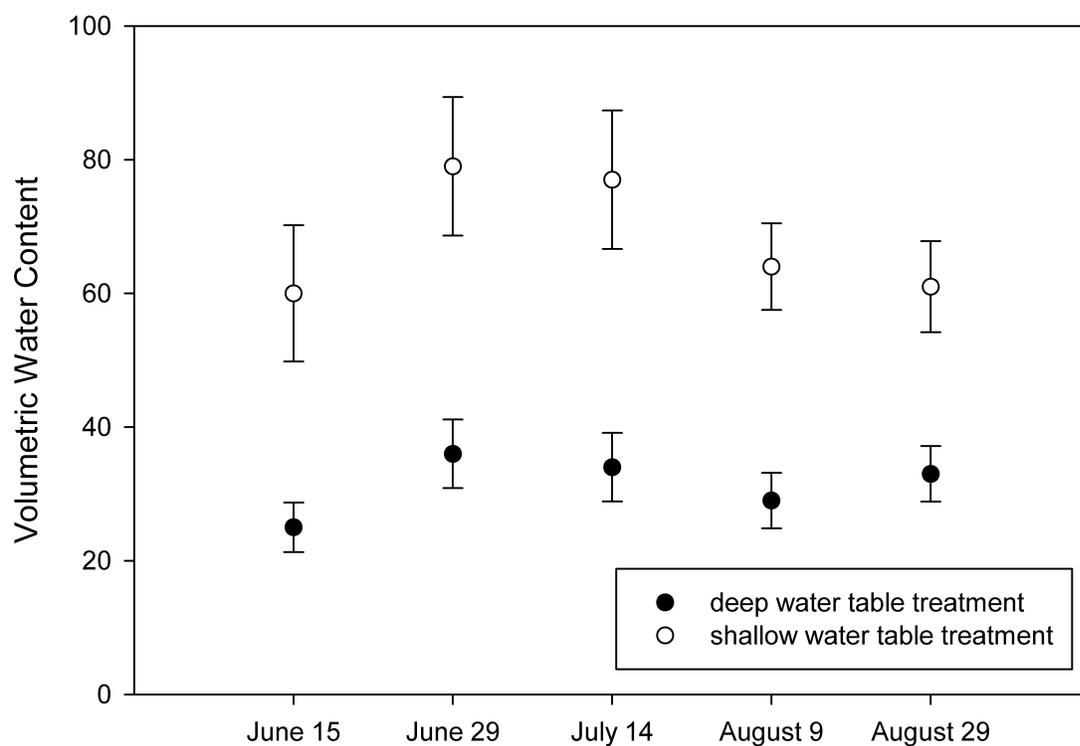
Figure 4.4: Average soil moisture (volumetric water content) at 12 cm in shallow (well watered) and deep (drought) treatments with 95% confidence intervals. Shallow and deep water table soil moisture is significantly different on all dates ($p < 0.001$).

5 Synthesis

My dissertation research examined willow decline within Rocky Mountain National Park (RMNP). I applied a multifaceted approach to investigate the roles of climatic and biotic stressors. I used observational field studies of willow dieback to determine the relative influences of biotic and climatic stressors (Chapter 2); GIS to examine the long temporal scale and epicormic shoot analysis to examine the short temporal scale of the dieback (Chapter 3); and a manipulative field experiment investigating the interactions of climate change stressors on *Cytospora* fungal infection (Chapter 4). All studies supported the conclusion that willow decline is more strongly driven by biotic, rather than climatic stressors. Although most of this research took place within RMNP, the findings are applicable to riparian sites throughout the Rocky Mountain region.

Unbrowsed willow stems, inside ungulate exclosures for 12 years at the onset of this research, were integral to understanding the mechanisms of the dieback, as these sites are some of the few places in RMNP that have tall willows suitable for sapsucker use. Chapter 2 explained the, previously unidentified, interaction of sapsucker wounding, *Cytospora* fungal infection and ungulate browsing in the decline of the riparian ecosystem. I confirmed that *Cytospora chrysosperma* can form enlarging cankers on willow stems by performing field inoculations and DNA analyses. The major finding from Chapter 2 was that inside exclosures, sapsucker wounded and *Cytospora* infected stems die, yet epicormic shoots initiating below the wound allow for regeneration and maintenance of a tall willow community. This is in stark contrast to stems outside of exclosures, where, due to the high intensity browsing, sapsucker wounded stems can not regenerate, resulting in a conversion and eventual maintenance of a short willow community. The interaction of beavers and ungulate

browsing has been proposed as a cause of riparian willow decline (Baker et al., 2005*a*) and, in the absence of beavers, my finding provides an alternative explanation.

Biotic factors explained the initiation of the willow decline, as demonstrated in Chapter 3. I used two methods to investigate the timing of the decline: aerial photo analysis and a novel method aging epicormic shoot initiation. The increase in moose hunting tags, a surrogate for moose population numbers, explained the sharp decline in willow presence between 2001 and 2005. The initiation of epicormic shoots, a compensatory response to disturbance to the apical meristem, coincided with the sharp decline in willow cover throughout the headwaters of the Colorado River. Shoot initiation was also best explained by the increase in moose population numbers.

The introduction of moose into Colorado by the Colorado Division of Parks and Wildlife has been very successful. Two groups of twelve moose were introduced northwest of RMNP in 1978 and 1979. Populations grew and began to overwinter in the Kawuneeche Valley at the headwaters of the Colorado River in RMNP in the early 1980's. There are no current population estimates, however one study in 2003 estimated that there were between 61-66 resident moose within the valley (Dungan, 2007). While moose populations are declining in parts of the northern Rocky Mountains (Smith et al., 2011), in Colorado, populations are increasing and expanding. In the absence of natural predators, moose populations have increased by 17% over the past five years throughout the state (Colorado Division of Parks and Wildlife). Willows comprise the majority of moose diet, specifically during the summer when 90% of their diet is willow (Dungan and Wright, 2005). Moose browsing not only affects above ground production, but has also been demonstrated to lower annual fine root production, with browsed willows producing a statistically significant 31% fewer fine roots over 3 years compared with unbrowsed plants (Ruess et al., 1998).

My finding that increasing moose population is one of the primary factors leading to the decline of willow populations at the headwaters of the Colorado River should be an alarm to resource managers as moose are expanding east of the Continental Divide into some of the last intact tall riparian willow stands in RMNP (eg. Wild Basin).

Climate change factors, such as drought and increasing air temperatures, did not have a significant effect on *Cytospora* fungal infection, as described in Chapter 4. Willow stems are highly susceptible to fungal infection and my experiment demonstrated that once *C. chrysosperma* is present on a wound, it will form enlarging cankers under a wide range of environmental conditions. Contrary to other studies on *Cytospora*, willows do not need to be predisposed to drought or temperature stress for canker expansion to occur.

My research has demonstrated that climatic variables were not the main drivers in the decline. Across western North America in the early 2000's prolonged drought was a cause of landscape wide dieback of upland tree species, including aspen (Michaelian et al., 2011), Douglas fir and white fir (Kane, 2012), and piñon pine (Breshears et al., 2005). However, previous research demonstrated that the presence of beaver dams and active beaver populations can mitigate the effects of climate, lessening the effects of two large drought events in Colorado (2002 and 2004) (Westbrook et al., 2006). Beaver dams along the Colorado River allowed for greater extent, longer duration and deeper surface inundation of the floodplain when compared with overbank flooding that occurred during peak flows. Beaver dams also altered groundwater flows. With the decline of willow communities, beavers are no longer active in many parts of the park, and drought events in the future could interact with biotic stressors and lead to further decline, and the possible extirpation of willows.

Forest tree species declines have been studied throughout the world and have become a focus due to linkages with climate change, in particular widespread drought events (Bigler et al., 2007; van Mantgem et al., 2009; Anderegg et al., 2012*a*). Declines are thought to be caused by multiple interacting abiotic and biotic factors, including soil moisture levels and chemistry (Hamerlynck and McAuliffe, 2008; La Manna and Rajchenberg, 2004), ambient air temperatures (Worrall, 2009; D'Amore and Hennon, 2006), herbivory (Zeigenfuss et al., 2002), and insect and fungal pathogens (Desprez-Loustau et al., 2006; Worrall et al., 2008; Eschtruth and Battles, 2008). A fundamental difference between riparian willow decline compared with upland forest tree species declines is that willows are phreatophytes and utilize shallow groundwater as their primary water source (Johnston et al., 2011), while upland plants rely on precipitation driven soil moisture. Drought stressed plants can experience hydraulic failure when the water column in the xylem conduit cavitates, resulting in a loss of conductivity. For example, aspen branches exhibit 50% loss of conductivity between -1.1 and -2.5 MPa (Hacke et al., 2001), while willows similarly exhibit 50% loss of conductivity between -1.57 and -2.18 MPa (Johnston et al., 2007). A lack of a reliable source of water can make aspens more susceptible to drought, as overnight recovery of Ψ_{leaf} is limited to the amount of water in the soil. Willows demonstrate fast overnight recovery from low water potentials during the day, sometimes exhibiting a higher Ψ_{leaf} than $\Psi_{soil_{60cm}}$ during predawn measurements, suggesting that their roots are relying on a deep water source (Foster and Smith, 1991).

Management Implications

Riparian ecosystems throughout RMNP have changed dramatically over the past two decades (Figure 1.1). Willows are surviving in the valleys, but their stature is greatly reduced and their function in the riparian system has been altered. The impact of ungulate browsing has not only had con-

sequential effects on current willow populations, the high intensity of browsing has resulted in extremely low rates of flowering, decreasing the likelihood of willow regeneration, even if suitable sites are available. In addition, short statured willows do not provide habitat for migratory and resident songbirds, nor are they suitable for use by beaver and as a result, songbird populations are changing and beaver are now absent from many riparian valleys. An understanding of the interactions resulting in willow decline can allow land management agencies to develop appropriate and effective restoration strategies. Long term recovery and survival of a willow community will require a reduction in ungulate browsing, by either animal population reduction or fencing. Willows are resilient and removing browsing may be enough to allow tall stems to regenerate and promote flowering. Even short willows will regenerate if roots have access to ground water (Johnston et al., 2011). Only these actions will allow epicormic shoots to regain height, support beaver, maintaining a tall willow community and conserving the high biodiversity supported by these ecosystems.

Literature Cited

Akaski, Y., and D. Mueller-Dombois. 1995. A landscape perspective of the Hawaiian rain forest dieback. Journal of Vegetation Science **6**:449–464.

Allen, C. D., A. K. Macalady, H. Chenchouni, D. Banchelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. T. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.-H. Lim, F. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heart-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management **259**:660–684.

Alstad, K., J. Welker, S. Williams, and M. Trlica. 1999. Carbon and water relations of Salix monticola in response to winter browsing and changes in surface water hydrology: an isotopic study using 13 C and 18 O. Oecologia **120**:375–385.

Anderegg, W. R., J. A. Berry, D. D. Smith, J. S. Sperry, L. D. Anderegg, and C. B. Field. 2012*a*. The roles of hydraulic and carbon stress in a widespread climate-induced forest dieback. Proceedings of the National Academy of Sciences **109**:233–237.

Anderegg, W. R., J. M. Kane, and L. D. Anderegg. 2012*b*. Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change page doi:10.1038/nclimate1635.

Ayres, P. 1984. The interaction between environmental stress, injury, and biotic disease physiology. Annual Review of Phytopathology **22**:53–75.

Bailey, J. K., and T. G. Whitham. 2003. Interactions among elk, aspen, galling sawflies and insectivorous birds. Oikos **101**:127–134.

Baker, B. W., H. C. Ducharme, D. C. Mitchell, T. R. Stanley, and H. P. Peinetti. 2005*a*. Interaction of beaver and elk herbivory reduces standing crop of willow. Ecological Applications **15**:110–118.

Baker, B. W., H. P. Peinetti, and M. B. Coughenour. 2005*b*. Resilience of willow stems after release from intense elk browsing. Rangeland Ecology and Management **58**:575–581.

Baril, L. M., A. J. Hansen, R. Renkin, and R. Lawrence. 2011. Songbird response to increased willow (Salix spp.) growth in Yellowstone's northern range. Ecological Applications **21**:2283–2296.

Beattie, R. K., and J. D. Diller. 1954. Fifty years of Chestnut Blight in America. Journal of Forestry **52**:323–329.

Bebi, P., D. Kulakowski, and T. T. Veblen. 2003. Interactions between fire and spruce beetles in a subalpine rocky mountain forest landscape. Ecology **84**:362–371.

Beyer, H. L., E. H. Merrill, N. Varley, and M. S. Boyce. 2007. Willow on Yellowstone's northern range: Evidence for a trophic cascade? Ecological Applications **17**:1563–1571.

Biggs, A., and D. Davis. 1983. Histopathology of cankers on Populus caused by Cytospora chrysosperma. Canadian Journal of Botany **61**:563–574.

Biggs, A., W. Merrill, and D. Davis. 1984. Discussion: Response of bark tissues to injury and infection. Canadian Journal of Forestry Research **14**:351–356.

Bigler, C., D. G. Gavin, C. Gunning, and T. T. Veblen. 2007. Drought induces lagged tree mortality in subalpine forest in the Rocky Mountains. Oikos **116**:1983–1994.

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.

Bilyeu, D. M., D. J. Cooper, and N. T. Hobbs. 2008. Water tables constrain height recovery of willow on Yellowstone's northern range. Ecological Applications **18**:80–92.

Bitty, R. A., S. Gusewell, and J. Harte. 2004. Response of plant pathogens and herbivores to a warming experiment. Ecology **85**:2570–2581.

Boyer, J. 1995. Biochemical and biophysical aspeces of water deficits and the predisposition to disease. Annual Review of Phytopathology **33**:251–274.

Bradley, R. S., F. T. Keimig, and H. F. Diaz. 2004. Projected temperature changes along the American cordillera and the planned GCOS network. Geophysical Research Letters **31**:L16210, doi:10.1029/2004GL020229.

Breshears, D. D., N. S. Cobb, P. M. Rich, K. P. Price, C. D. Allen, R. G. Balice, W. H. Romme, J. H. Kastens, M. L. Floyd, J. Belnap, J. J. Anderson, O. B. Myers, and C. W. Meyer. 2005. Regional vegetation die-off in response to global-change-type drought. Proceedings of the National Academy of Sciences **102**:15144–15148.

Bunnell, F., and L. Dupuis, 1993. Riparian habitats in British Columbia: Their nature and role. *in* Proceedings, Riparan Habitat Management and Research, Kamloops, BC. Fraser River Action Plan.

Burdon, J. J., P. H. Thrall, and L. Ericson. 2006. Dynamics of disease in plant communities. Annual Review of Phytopathology **44**:19–39.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: a practical information-theoretic approach. Springer, New York, New York, USA.

California Forest Pest Council, 2003. Forest pest conditions in California - 2003. URL `http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5346904.pdf`.

Castello, J. D., D. J. Leopold, and P. J. Smallidge. 1995. Pathogens, patterns, and processes in forest ecosystems. Bioscience **45**:16–24.

Cayan, D. R. 1996. Interannual climate variability and snowpack in the Western United States. Journal of Climate **9**:928–948.

Clow, D. W. 2010. Changes in the timing of snowmelt and streamflow in Colorado: A response to recent warming. Journal of Climate **23**:2293–2306.

Cottam, G., J. T. Curtis, and B. W. Hale. 1953. Some sampling characteristics of a population of randomly dispersed individuals. Ecology **34**:741–757.

Daily, G. C., P. R. Ehrlich, and N. M. Haddad. 1993. Double keystone bird in a keystone series complex. Proceedings of the National Academy of Sciences **90**:592–594.

D'Amore, D., and P. Hennon. 2006. Evaluation of soil saturation, soil chemistry, and early spring soil and air temperatures as risk factors in yellow-cedar decline. Global Change Biology **12**:524–545.

Desprez-Loustau, M.-L., B. Marcais, L.-M. Nageleisen, D. Piou, and A. Vannini. 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science **63**:597–612.

Dungan, J., 2007. Activity patterns, foraging ecology, and summer range carrying capacity of moose in Rocky Mountain National Park, Colorado. Master's thesis, University of Idaho, Moscow, Idaho, USA.

Dungan, J. D., and R. G. Wright. 2005. Summer diet composition of moose in Rocky Mountain National Park, Colorado. Alces **41**:139–146.

Ehrlich, P. R., and G. C. Daily. 1988. Red-naped Sapsuckers feeding at willows: possible keystone herbivores. North American Birds **42**:357–365.

Eschtruth, A. K., and J. J. Battles. 2008. Deer herbivory alters forest response to canopy decline casued by an exotic insect pest. Ecological Applications **18**:360–376.

Evans, R. N., and D. C. Prusso. 1969. Spore dispersal by birds. Mycologia **61**:832–835.

Foster, J. R., and W. K. Smith. 1991. Stomatal conductance patterns and environment in high elevation phraetophytes of Wyoming. Canadian Journal of Botany **69**:647–655.

Gage, E. A., and D. J. Cooper. 2005. Patterns of willow seed dispersal, seed entrapment, and seedling establishment in a heavily browsed montane riparian ecosystem. Canadian Journal of Botany **83**:678–687.

Garrett, K., S. Dendy, E. Frank, M. Rouse, and S. Travers. 2006. Climate change effects on plant disease: genomes to ecosystems. Annual Review of Phytopathology **44**:489–509.

Gelman, A., and J. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge.

Gill, R. A., and R. B. Jackson. 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytologist **147**:13–31.

Griffis-Kyle, K. L., and P. Beier. 2003. Small isolated aspen stands enrich bird communities in southwestern ponderosa pine forests. Biological Conservation **110**:375–385.

Guyon, J., W. Jacobi, and G. A. McIntyre. 1996. Effects of environmental stress on the development of Cytospora canker in Aspen. Plant Disease **80**:1320–1326.

Hacke, U. G., V. Stiller, J. S. Sperry, J. Paittermann, and K. A. McCulloh. 2001. Cavitation fatigue. Emolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiology **125**:779–786.

Hamerlynck, E., and J. McAuliffe. 2008. Soil-dependent canopy dieback and plant mortality in two Mojave Desert shrubs. Journal of Arid Environments **72**:1793–1802.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risk for terrestrial and aquatic biota. Science **296**:2158–2162.

Heald, F., and R. Studhalter. 1914. Birds as carriers of the chestnut blight fungus. Journal of Agricultural Research **11**:405–422.

Hinds, T. E., 1985. Diseases. Pages 87–106 *in* N. V. DeByle and R. P. Winokur, editors. Aspen: Ecology and management in the western United States, volume USDA Forest Service General Technical Report. Rocky Mountain Forest and Range Experiemtn Station, Fort Collins, CO.

Hobbs, N. T., D. L. Baker, J. E. Ellis, and D. M. Swift. 1981. Compostion and quality of elk winter diets in Colorado. The Journal of Wildlife Management **45**:156–171.

Hollister, R. D., and K. J. Flaherty. 2010. Above- and below-ground plant biomass response to experimental warming in northern Alaska. Journal of Applied Vegetation Science **13**:378–387.

Huang, C.-Y., and W. L. Anderegg. 2012. Large drought-induced aboveground live biomass losses in southern Rocky Mountain aspen forests. Global Change Biology **18**:1016–1027.

Hubalek, Z. 1976. Seasonal distribution of fungi on house sparrows. Trans. Br. mycol. Soc. **66**:509–516.

Hundertmark, K. J., W. L. Eberhardt, and R. E. Ball. 1990. Winter habitat use by moose in southeastern Alaska: Implications for forest management. Alces **26**:108–114.

Johnston, D. B., D. J. Cooper, and N. T. Hobbs. 2007. Elk browsing increases aboveground growth of water-stressed willows by modifying plant architecture. Oecologia **154**:467–478.

Johnston, D. B., D. J. Cooper, and N. T. Hobbs. 2011. Relationships between groundwater use, water table, and recovery of willow on Yellowstone's northern range. Ecosphere **2**.

Kane, J. M., 2012. Patterns and processes of tree mortality in montane forests of northern Arizona. Ph.D. thesis, Northern Arizona University, Flaggstaff, Arizona.

Keigley, R. B., M. R. Frisina, and C. W. Fager. 2002. Assessing browse trend at the landscape level Part 1: Preliminary steps and field survey. Society for Range Management **24**:28–33.

Kepley, J., and W. R. Jacobi. 2000. Pathogenicity of Cytospora fungi on six hardwood species. Journal of Arboriculture **26**:326–333.

Knight, D., and L. Wallace. 1989. The Yellowstone Fires: Issues in landscape ecology. BioScience **39**:700–706.

Knopf, L., Fritz, R. R. Johnson, T. Rich, F. B. Samson, and R. C. Szaro. 1988. Conservation of Riparian Ecosystems in the United States. The Wilson Bulletin **100**:272–284.

Knowles, N., M. D. Dettinger, and D. R. Cayan. 2006. Trends in snowfall versus rainfall in the Western United States. Journal of Climate **19**:4545–4559.

Kufeld, R., and D. Bowden. 1996. Movements and habitat selection of Shiras moose (Alces alces shirasi) in Colorado. Alces **32**:85–99.

La Manna, L., S. Matteucci, and T. Kitzberger. 2008. Abiotic factors related to the incidence of the Austrocedrus chilensis disease syndrome at a landscape scale. Forest Ecology and Management **256**:1087–1095.

La Manna, L., and M. Rajchenberg. 2004. The decline of Austrocedrus chilensis forests in Patagonia, Argentina: soil features as predisposing factors. Forest Ecology and Management **190**:345–357.

Limb, R., C. B. Marlow, and B. Jacobson. 2003. What causes willow dieback? Rangelands **25**:14–17.

Link, W., and R. Barker. 2010. Bayesian Inference with Ecological Applications. Academic Press, New York, NY.

Long, W. 1918. An undescribed canker of poplars and willows caused by Cytospora chrysosperma. Journal of Agricultural Research **13**:331–343.

Losin, N., C. H. Floyd, T. E. Scheitzer, and S. J. Keller. 2006. Relationship between aspen heartwood rot and the location of cavity excavation by a primary cavity-nester, the Red-naped Sapsucker. Condor **108**:706–710.

Malanson, G. 1993. Riparian Landscapes. Cambridge University Press, Cambridge.

Manion, P. D., and D. Lachance. 1992. Forest Decline Concepts. The American Phytopathological Society, St. Paul, MN.

Marshall, K. N., 2012. Wolves, elk and willows: Alternate states and transition thresholds on Yellowstone's northern range. Ph.D. thesis, Colorado State University, Fort Collins, CO.

McAtee, W., 1911. Woodpeckers in relation to trees and wood products. Technical report, US Department of Agriculture.

McCune, B., and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design.

McGuire, C. R., C. R. Nufio, M. D. Bowers, and R. P. Guralnick. 2012. Elevation-dependent temperature trends in the Rocky Mountain Front Range: Changes over a 56- and 20-year record. PLOS ONE **7**.

McIntyre, G. A., W. R. Jacobi, and A. W. Ramaley. 1996. Factors affecting Cytospora canker occurence on Aspen. Journal of Arboriculture **22**:229–233.

Michaelian, M., E. H. Hogg, R. J. Hall, and E. Arsenault. 2011. Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. Global Change Biology **17**:2084–2094.

Mitchell, D., J. Tjornehoj, and B. Baker, 1999. Beaver populations and possible limiting factors in Rocky Mountain National Park, 1999. Technical report, U.S. Geological Survey, Midcontinent Ecological Science Center Unpublished Report.

Naiman, R., and H. Decamps. 1997. The ecology of interfaces: Riparian zones. Annual Review of Ecology and Systematics **28**:621–658.

Naiman, R., H. Decamps, and M. E. McClain. 2005. Riparia: Ecology, Conservation, and Management of Streamside Communities. Academic Press, New York, NY.

Naiman, R. J., H. Decamps, and M. Pollock. 1993. The role of riparian corridors in maintaining regional biodiversity. Ecological Applications **3**:209–212.

National Research Council, editor. 2002. Riparian areas: Functions and strategies for management. National Academy Press, Washington DC.

Norby, R. J., and R. B. Jackson. 2000. Root dynamics and global change: seeking an ecosytem perspective. New Phytologist **147**:3–12.

Nossov, D. R., T. N. Hollingsworth, R. W. Ruess, and K. Kielland. 2011. Development of Alnus tenuifolia stands on an Alaskan floodplain: patterns of recruitment, disease and succession. Journal of Ecology **99**:621–633.

Olechnowski, B. F., and D. M. Debinski. 2008. Response of songbirds to riparian willow habitat structure in the greater Yellowstone ecosystem. The Wilson Journal of Ornithology **120**:830–839.

Packard, F. M. 1947. A survey of the beaver population of Rocky Mountain National Park, Colorado. Journal of Mammology **28**:219–227.

Peinetti, H. P., M. A. Kalkhan, and M. B. Coughenour. 2002. Long-term changes in willow spatial distribution on the elk winter range of Rocky Mountain National Park. Landscape Ecology **17**:341–354.

Pendall, E., S. Bridgham, P. J. Hanson, B. Hungate, D. W. Kicklighter, D. W. Johnson, B. E. Law, Y. Luo, J. P. Megonigal, M. Olsrud, M. G. Ryan, and S. Wan. 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist **162**:311–322.

Perry, L. G., D. C. Anderson, L. V. Reynolds, S. M. Nelson, and P. B. Shafroth. 2012. Vulnerability of riparian ecosystems to elevated CO_2 and climate change in arid and semiarid western North America. Global Change Biology **18**:821–842.

Peters, D. P., B. T. Bestelmeyer, and M. G. Turner. 2007. Cross-scale interactions and changing pattern-process relationships: Consequences for system dynamics. Ecosystems **10**:790–796.

Pickett, S., and P. White. 1985. The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, New York, NY.

Poole, K. G., and K. Stuart-Smith. 2006. Winter habitat selection by female moose in western interior montane forests. Canadian Journal of Zoology **84**:1823–1832.

Pregitzer, K., J. King, A. Burton, and S. Brown. 2000. Responses of tree fine roots to temperature. New Phytologist **147**:105–115.

R Development Core Team, 2008. R: A Language and Environment. R Foundation for Statistical Computing. URL `http://www.R-project.org`.

Ripple, W. J., and R. L. Beschta. 2004. Wolves, elk, willows, and trophic cascades in the upper Gallatin Range of Southwestern Montana, USA. Forest Ecology and Management **200**:161–181.

Rohrs-Richey, J. K., C. P. Mulder, L. M. Winton, and G. Stanosz. 2011. Physiological performance of an Alaskan shrub (Alnus fruticosa) in response to disease (Valsa melanodiscus) and water stress. New Phytologist **189**:259–307.

Ruess, R. W., R. L. Hendrick, and J. P. Bryant. 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan Tiaga forests. Ecology **79**:2706–2720.

Sauer, J. R., J. E. Hines, J. E. Fallon, K. L. Pardieck, J. D. J. Ziolkowski, and W. A. Link., 2011. The North American Breeding Bird Survey, Results and Analysis 1966 - 2010. Version 12.07.2011. Technical report, USGS Patuxent Wildlife Research Center, Laurel, MD.

Scherer, R. D., 2010. Monitoring amphibian populations and the status of wood frogs and boreal chorus frogs in the Kawuneeche Valley of Rocky Mountain National Park. Ph.D. thesis, Colorado State University.

Scherer, R. D., B. Baldwin, J. Connor, and B. R. Noon, 2011. Occupancy of beaver (Castor canadensis) in Rocky Mountain National Park: the second field season.

Schlesinger, W. H., and J. A. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry **18**:7–20.

Schweingruber, F. H. 1988. Tree rings: Basics and applications of Dendrochronology. D. Reidel Publishing Company, Dordrecht, Holland.

Singer, F. J., L. C. Zeigenfuss, R. G. Cates, and D. T. Barnett. 1998. Elk, Multiple Factors, and Persistence of Willows in National Parks. Wildlife Society Bulletin **26**:419–428.

Skagen, S., C. Melcher, W. Howe, and F. Knopf. 1998. Comparative use of riparian corridors and oases by migrating birds in southeast Arizona. Conservation Biology **12**:896–909.

Smith, M. A., S. Kilpatrick, B. Younkin, L. Work, and D. Wachob. 2011. Assessment of crucial moose winter habitat in Western Wyoming. Alces **47**:151–162.

Stewart, I. T., D. R. Cayan, and M. D. Dettinger. 2005. Changes toward earlier streamflow timing across western North America. Journal of Climate **18**.

Stromberg, J., and T. Rychener. 2010. Effects of fire on riparian forests along a free-flowing dryland river. Wetlands **30**:75–86.

Su, Y.-S., and M. Yajima. 2012. A package for running jags from R. version 0.03-06 URL `http://cran.r-project.org/web/packages/R2jags`.

Tainter, F., and F. Baker. 1996. Principles of Forest Pathology. John Wiley & Sons, Inc., New York.

Turner, M., W. Romme, and D. Tinker. 2003. Surprises and lessons from the 1988 Yellowstone fires. Frontiers in Ecology and the Environment **1**:351–358.

van Mantgem, P. J., N. L. Stephenson, J. C. Byrne, L. D. Daniels, J. F. Franklin, P. Z. Fule, M. E. Harmon, A. J. Larson, J. M. Smith, A. H. Taylor, and T. T. Veblen. 2009. Widespread increase of tree mortality rates in the western United States. Science **323**:521–524.

Veblen, T. T., K. S. Hadley, E. M. Nel, T. Kitzberger, M. Reid, and R. Villalba. 1994. Disturbance regime and distturbance interactions in a Rocky Mountain subalpine forest. Journal of Ecology **82**:125–135.

Warner, G. M., and D. French. 1970. Dissemination of fungi by migratory birds: survival and recovery of fungi from birds. Canadian Journal of Botany **48**:907–910.

Warton, D. I., and F. K. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology **92**:3–10.

Weber, W. A., and R. C. Wittmann. 2001. Colorado Flora: Eastern Slope. 3rd edition edition. University Press of Colorado, Niwot, Colorado.

Westbrook, C. J., D. J. Cooper, and B. W. Baker. 2006. Beaver dams and overbank flood influence groundwater-surface water interactions of a Rocky Mountain riparian area. Water Resources Research **42**:W06404.1.

White, T., T. Bruns, S. Lee, and J. Taylor, 1990. PCR protocols a guide to methods and applications, Chapter amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics, pages 315–322 .

Wolf, E. C., D. J. Cooper, and N. T. Hobbs. 2007. Hydrologic regime and herbivory stabilize an alternative state in Yellowstone National Park. Ecological Applications **17**:1572–1587.

Woods, S. W., 2000. Hydrologic effects of the Grand Ditch on streams and wetlands in Rocky Mountain National Park, Colorado. Master's thesis, Department of Earth Resources, Colorado State University, Fort Collins, Colorado, Fort Collins, CO.

Worrall, J. J. 2009. Dieback and mortality of Alnus in the Southern Rocky Mountains, USA. Plant Disease **93**:293–298.

Worrall, J. J., L. Egeland, T. Eager, R. A. Mask, E. W. Johnson, P. A. Kemp, and W. D. Sheppard. 2008. Rapid mortality of Populus tremuloides in southwestern Colorado, USA. Forest Ecology and Management **255**:686–696.

Wu, Z., P. Dijkstra, G. W. Koch, J. Penuelas, and B. A. Hungate. 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology **17**:927–942.

Zeigenfuss, L. C., F. J. Singer, S. A. Williams, and T. L. Johnson. 2002. Influences of herbivory and water on willow in elk winter range. The Journal of Wildlife Management **66**:788–795.

Zhou, X., S. Fei, R. Sherry, and Y. Luo. 2012. Root biomass dynamics under experimental warming and doubled precipitation in a tall grass prairie. Ecosystems **15**:542–554.

Zimmerman, M., 1995. Proposal for a study of moose herbivory on willow communities in Colorado.

Zvereva, E., and M. Kozlov. 2001. Effects of pollution-induced habitat disturbance on the response of willows to simulated herbivory. Journal of Ecology **89**:21–30.