

## AN ABSTRACT OF THE THESIS OF

Brandon Alveshere for the degree of Master of Science in Sustainable Forest Management presented on June 9, 2017

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Abstract approved:

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Jared M. LeBoldus

River impoundment by dams, along with other human driven changes, has threatened the health of riparian forests in the northern Great Plains for much of the last century. Two major concerns in the region are the impediment of natural recruitment of plains cottonwood (*Populus deltoides* Marsh. subsp. *monilifera* (Ait.) Eckenw.) following dam construction on the Missouri River, and the impact of pathogens on stressed or weakened trees. Two independent studies were performed to look at these forest health issues. In one study, methods of establishing plains cottonwood were compared along the Missouri River in North Dakota. Three treatments: tree planting, planting with five-foot tree shelters, and planting with five-foot tree shelters and weed barrier fabric, all proved to be effective after two years. As a general trend, height, height to live crown, and caliper increased with treatment complexity, suggesting improved vigor and growth rate with more intensive treatments. In the second study, riparian woodlands in North Dakota, South Dakota, and Nebraska were surveyed to determine the ecological role of a prevalent root disease-causing fungus, *Armillaria*. The fungus was present in 78 of 101 surveyed stands, and was observed in a

pathogenic role (i.e. caused root disease) 71% of the time. Results of logistic regression suggested that the odds of observing a pathogenic interaction were greater at sites that had clearly flooded; the same was true with increases in percent clay and basal area ha<sup>-1</sup>. Host DBH, the presence of competitive grasses, and stumps and snags hectare<sup>-1</sup> had no significant effect on the ecological role of *Armillaria* in surveyed stands.

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Riparian Forest Health on the Northern Great Plains

by  
Brandon Alveshere

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APPROVED:

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Major Professor, representing Sustainable Forest Management

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Head of the Department of Forest Engineering, Resources, and Management

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Brandon Alveshere, Author

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## Chapter 1: General introduction

### Introduction

*Riparian forests on the plains:* The northern Great Plains, located in central North America, is a region that was historically dominated by grasslands. Nonetheless, several important forest types occur within the region. These include riparian, montane, upland, Black Hills pine, and pine savanna (Barker and Whitman 1988), which together account for approximately 1% of the total land area (Rumble and Gobeille 2001). Riparian forests occupy much of this 1% and are widely distributed throughout the region. Properly functioning riparian ecosystems provide valuable ecological services such as stream bank stabilization and regulation of nutrient and sediment flows (Broadmeadow and Nisbet 2004). They also support a wealth of biodiversity in prairie regions. For example, at least 71 avian species utilize cottonwood forests along the Missouri River alone, and many of these species are obligate woodland users (Rumble and Gobeille 2004).

Since European settlement, much of the region's land area has been converted for agricultural purposes (Dixon et al. 2015; Johnson 1992) and/or invaded by introduced species. Floodplain forests on impounded (dammed) rivers have been further impacted by streamflow management, which continues to drive compositional changes in vegetation (Johnson 1998; Johnson et al. 2012). Estimates show that between 1892 and 2006, riparian forestland decreased by 49%, shrubland by 52%, and grasslands by 61% along the impounded upper Missouri River alone (Dixon et al. 2012).

Relatively few species of native trees occupy the riverbanks of the northern Great Plains. Common species along major rivers include cottonwood (*Populus deltoides*

Marsh), green ash (*Fraxinus pennsylvanica* Marsh.), box elder (*Acer negundo* L.), American elm (*Ulmus americana* L.), and peachleaf willow (*Salix amygdaloides* Anders.). In some areas, Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) and eastern redcedar (*Juniperus virginiana* L.) have also moved into the floodplain, but the composition of riparian forests is typically hardwood dominated. Cottonwood and *Salix* spp. are the primary constituents in early successional forests of the region, while species such as green ash, box elder, and American elm, typify later successional forest types (Dixon et al. 2012; Johnson et al. 1976, 2012; Wilson 1970).

The region's climate is continental, with four distinct seasons and highly variable precipitation and temperatures over the course of a single year. Precipitation falls primarily as rain during the growing season from April-September (Barker and Whitman 1988), but dry spells with little-to-no moisture are not uncommon by mid-summer. Although localized conditions vary across the region, extreme low temperatures in winter can fall below -51° C, while summer highs sometimes exceed 43° C (Barker and Whitman 1988).

***Existing literature (cottonwood restoration):*** Five riparian *Populus* species occur naturally in North America (Braatne et al. 1996). These species, generically referred to as poplar, are fast-growing pioneers of bare riparian sites (Rood et al. 2003b). A single, sexually mature tree can produce over 25 million seeds per year (Braatne et al. 1996). Seeds are surrounded by many fine hairs, giving them a cotton-like appearance. This feature allows them to float on air currents after being released from female catkins. Seed dispersal coincides with receding flood waters (Braatne et al. 1996; Johnson et al. 2012), which is important because establishment of the species is associated with moist, bare

mineral substrate and continuous access to surface moisture during the first week of growth (Braatne et al. 1996; Segelquist et al. 1993).

Plains cottonwood (*Populus deltoides* Marsh. subsp. *monilifera* (Ait.) Eckenw. is the primary cottonwood subspecies in most of the northern Great Plains; however, the range of eastern cottonwood (*Populus deltoides* Marsh.) extends into the eastern limits of the region as well. Cottonwoods are stream dependent in regions of low precipitation (Braatne et al 1996; Rood et al. 2003b), such as those found in the northern Great Plains. On floodplains of the prairies, plains cottonwood is a pioneer and its natural recruitment has been linked to flooding (Johnson et al. 1976; Bradley and Smith 1986).

Existing literature specific to riparian cottonwood restoration in the northern Great Plains is limited. An ongoing study along the Missouri River north of Bismarck is comparing several methods of establishing cottonwood, but one year of data collection remains (*Personal communication*, C. Stange and W. Duckwitz). To the author's knowledge, the only other study on riparian cottonwood restoration in the region was done by a student at South Dakota State University ca. 1970. However, a report of the findings was not accessible.

Other cottonwood establishment studies in the Great Plains (e.g. Geyer and Atchinson 2002; Geyer et al. 2008) focused primarily on comparing weed control methods in dryland plantings. Much of the research pertaining to cottonwood recruitment and losses has instead emphasized hydrologic and ecophysiological concerns associated with lower cottonwood recruitment and losses of cottonwood forest. There is general consensus that without the restoration of ecological processes such as high peak flows and dynamic geomorphology, cottonwood forests on impounded meandering rivers are

likely to continue to decline in area (Bradley and Smith 1986; Dixon et al. 2012, 2015; Johnson et al 2012). However, it has been suggested that planting could be used in combination with other efforts to restore cottonwood habitat in the region (Johnson 1992; Dixon et al. 2012).

Streamflow management on the Missouri has led to dramatic reductions in sedimentation, erosion, and seasonal peak flows, which collectively have resulted in major ecological changes for riparian habitat downstream (Dixon et al. 2012; Johnson 1992). Meandering rivers, like the Missouri, require sedimentation and erosion to maintain their dynamic state, a characteristic which frequently exposes areas of fresh alluvium that can be colonized by pioneer species such as cottonwood and willow (Johnson 1992). In the specific case of Garrison Dam, accretion and erosion dropped to an estimated 1% and 25% of pre-dam rates, respectively, in years following the dam's completion (Johnson 1992). Altered sediment dynamics have resulted in reduced sandbar formation downstream of dams; area of unvegetated sandbars declined by an estimated 93% on just the upper two-thirds of the Missouri from the dam building era of the 1950s to 2006 (Dixon et al. 2012). In combination, reduced peak flows and altered sedimentation regimes ultimately lead to less suitable habitat for pioneer species such as cottonwood (Johnson 1992).

Peak flows are important to floodplain dynamism and have been directly tied to cottonwood recruitment (Bradley and Smith 1986; Johnson 1992). High peak flows (i.e. flooding) benefit riparian cottonwood by increasing soil moisture and depositing fresh alluvium for colonization (Johnson et al. 1976). High peak flows have also been reported

to kill higher proportions of invasive tree species in floodplains compared to native cottonwood (Dixon et al. 2015), reducing interspecific competition.

In the arid western U.S., reintroduction of periodic high flows via prescribed reservoir releases has led to varying levels of improvement in Fremont cottonwood (*Populus fremontii*) regeneration (Rood et al. 2003a; Shafroth et al. 2017). On the banks of the regulated Truckee River in Nevada, Fremont cottonwood seedling regeneration was observed following prescribed high flows six years in a row (Rood et al. 2003a). Recruitment along the river was often substantial, with seedling densities frequently greater than 500 stems m<sup>-2</sup> (Rood et al. 2003a). In contrast, experimental high flows were not as successful in stimulating recruitment of Fremont cottonwood along the Colorado River delta (Shafroth et al. 2017). Shafroth et al. (2017) reported that by the conclusion of their study, native *Salix* and cottonwood each established within only one of the 19, 20-meter belt transects surveyed, while non-native *Tamarix* seedlings established within 14 transects. Several hydrologic factors were believed to have contributed to the relatively low recruitment of native species observed in this study, including insufficient flow duration, peak magnitude, and volume (Shafroth et al. 2017). Poor availability of seed on some sites, competition from and exclusion by existing vegetation, and inadequate growing season soil moisture were also expected to have impacted recruitment of target species in the study (Shafroth et al. 2017).

There is evidence that flooding along the Missouri River in 2011 killed higher proportions of invading eastern redcedar and Russian olive than naturally occurring plains cottonwood (Dixon et al. 2015). It is plausible, then, that prescribed flows of some threshold duration and volume may stimulate cottonwood germination and growth, while



simultaneously killing extant species of concern. While prescribed high flows show promise as a tool for stimulating cottonwood regeneration, few success stories exist and further study is necessary prior to widespread implementation.

***Existing literature (Armillaria):*** Forest pests and diseases may be less widely studied in the northern Great Plains than in many other regions due to the general lack of forest industry in prairie landscapes; however, pests still pose a threat to the region's forest resources. Dutch elm disease, caused by the fungi *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*, was first introduced to North America around 1927 and has since resulted in the destruction of hundreds of millions of elms (Brasier 2001), including most of the American elm that were once abundant both in the region and throughout much of the eastern U.S. An introduced beetle, the emerald ash borer (*Agrilus planipennis* Fairmaire; further "EAB") was recently identified in the city of Omaha (L. Stepanek, *personal communication*), which lies in eastern Nebraska on the periphery of the northern Great Plains region. EAB, which attacks and kills healthy and stressed ash trees, has already annihilated tens of millions of ash across much of eastern North America (Johnson et al. 2012). The entrance of EAB to the plains would be catastrophic both ecologically and economically, as ash is abundant in riparian areas and has been overplanted in many of the region's cities and towns.

While exotic pests, like those discussed above, tend to have a dramatic effect on the landscape, the influence of endemic ones is often less visible to the casual observer. However, naturally occurring root rot pathogens, including species of *Armillaria*, have proven particularly destructive in some regions. In lodgepole pine stands of Alberta, *Armillaria* root rot is a primary driver of mortality in young trees (Mallett 1990). In the

interior west of the U.S., mortality from *Armillaria* can be > 25% in some conifer stands (Wargo and Shaw 1985). With the information available at present, it would be impossible to quantify the economic or ecological impact that *Armillaria* has on the northern Great Plains region. However, *Armillaria* plays a role in carbon cycling (Baumgartner et al. 2011) and in the creation of habitat for wildlife (Van der Kamp 1991). Thus, it is likely to have some benefit in ecosystems as well.

*Armillaria* is a genus and complex of fungi known as the causal agent of *Armillaria* root disease. However, fungi within the genus often play a saprophytic role (e.g. degrade dead stumps) and may grow epiphytically on living hosts. The genus is distributed throughout the world and occurs on hundreds of host species (Kallas et al. 2003; Shaw and Kile 1991). Prior to 1979, all *Armillaria* isolates in North America were classified as *Armillaria mellea* (Vahl:Fr) Kummer s.l. (McLaughlin 2001; Anderson and Ullrich 1979). In 1979, Anderson and Ullrich concluded that *A. mellea* in North America was rather a complex of ten biological species. The ecology and distribution of these species have been the focus of many studies since that time (McLaughlin 2001).

While *Armillaria* does produce mushrooms and basidiospores, they play a minor role in the spread of the fungus (Baumgartner et al. 2011). Rather, *Armillaria* typically grows through the soil as a rhizomorph (sheathed aggregation of hyphae), which then penetrates through host bark using degradative enzymes and mechanical force (Baumgartner et al. 2011; Thomas, 1934; Zeller 1926). Mycelial fans are produced at the tip of the rhizomorph to forage for nutrients in the cambium and secondary xylem (Baumgartner et al. 2011). Large roots and stumps can sustain the fungus for 15-30 years

(Klutsch 2012), a trait important to the ecology and management of *Armillaria* in commercial forest stands.

Following the reclassification of *Armillaria mellea* (Vahl:Fr) Kummer s.l in North America into ten biological species (Anderson and Ullrich 1979), studies have documented *Armillaria* ecology and species compositions in many provinces of Canada (McLaughlin 2001) and in parts of the western (Baumgartner and Rizzo 2001; J. Blodgett, *personal communication*) and eastern (Blodgett and Worrall 1992a, 1992b; Brazee and Wick 2009, 2011) United States. However, as a general trend, *Armillaria* spp. have been studied only in novel ecosystems within the prairies of the northern Great Plains and information is widely lacking for most of the land area in the region. *Armillaria ostoyae* has been studied and described in discrete areas such as the Black Hills of South Dakota (Kallas 1997; Kallas et al. 2003; Klutsch et al. 2012) and *Armillaria gallica* from the Niobrara Valley Preserve in Nebraska (Kim and Klopfenstein 2011), but these ecosystems are not typical of most of the region. Research in the prairie provinces of Canada (Alberta, Saskatchewan, Manitoba) has focused on *Armillaria* in subalpine and boreal forests, and has excluded the riparian forests in the rest of the region (Mallett 1990). Based on current literature, only three species of *Armillaria* are known to occur in or very near the northern Great Plains region: *A. ostoyae*, *A. gallica*, and *A. sinapina*. Of these, *A. gallica* is the only species that has been documented outside of the Black Hills or the subalpine and boreal forests at the outer limits of the region. To the author's knowledge, characterization of *Armillaria* populations to species level in the riparian forests of the northern Great Plains has not been documented.

Research on *Armillaria* spp. has shown that members of this group of saprophytes and pathogens can range in terms of the ecological niche they occupy. *Armillaria gallica*, *A. ostoyae*, and *A. sinapina* are all known to cause butt rots and root rots in the northeastern U.S. (Brazee and Wick 2009). In Nebraska, Kim and Klopfenstein (2011) described *A. gallica* only as an epiphyte of trees, but the species is also known to occur as an aggressive pathogen, a weak pathogen, and a saprophyte (Brazee and Wick 2009). In the prairie provinces of Canada, *A. ostoyae* was pathogenic on all hosts it was found in association with, including both hardwood and coniferous species (Mallett 1990); it is also the principle cause of *Armillaria* root disease on Ponderosa pine (*Pinus ponderosa*) in the Black Hills (Kallas et al. 2003; Klutsch et al. 2011). It is uncertain what ecological role *A. sinapina* has in the subalpine and boreal forests at the extreme northwestern edge of the northern Great Plains, which is the only area where it is known to occur near the region. The species can kill and cause butt-rot in trees in North America (McLaughlin 2001), and occurred much more frequently in deciduous hosts than in coniferous ones in the prairie provinces (Mallett 1990). Based on this relative affinity *A. sinapina* has shown for angiosperms in central North America, it is plausible that this species could be a component of the *Armillaria* spp. complex in the northern Great Plains. Furthermore, the species is known to occur in the states of Massachusetts (Brazee and Wick 2009, 2011) and New York (Blodgett and Worrall 1992a, 1992b), which sit at latitudes within the southern half of the range occupied by the northern Great Plains.

Statistical analysis has been used to determine where *Armillaria* or its associated root disease are likely to occur based on site characteristics (Kallas et al. 2003; Klutsch et al. 2012; Wiensczyk et al. 1997). Wiensczyk et al. (1997) used the sigmoidal growth

function and nonlinear regression to show that infection levels in stands of northwestern Ontario increased with total stump basal area, but decreased as clay content in the C horizon increased. Using regression analysis, Kallas et al. (2003) demonstrated that occurrence of *A. ostoyae* in the Black Hills increased with elevation and decreased with slope. In another study in the Black Hills, Klutsch et al. (2012) used regression analysis to show that incidence of *Armillaria* root disease was positively correlated with elevation and precipitation, but negatively correlated with slope and total basal area. Thus, in the Black Hills, data generally show that higher elevation sites with less slope are favorable for disease. Although this demonstrates replicability, neither elevation nor slope are likely to vary in floodplains across the region.

## **Objectives**

The main objectives of this thesis are: (1) to test methods for restoring riparian cottonwood stands along the Missouri River in North Dakota; and (2) to summarize the ecology of *Armillaria* spp. in riparian forests of North Dakota, South Dakota, and Nebraska. To meet these objectives, we conducted two studies.

In the first study, the goals were to determine: (i) whether survival would differ among treatments of varying cost and set-up time; (ii) if height, height to live crown, and caliper would differ among the treatments; (iii) if treatments with tree shelters would be less susceptible to deer browse than those without shelters; and (iv) if tilling to expose bare mineral soil prior to seed drop would result in improved cottonwood recruitment. Collectively, meeting these goals would fulfill objective (1) by indicating which treatments can be used to establish plains cottonwood along the Missouri River in North Dakota.

The primary goals of the second study were to (i) characterize site and host associations of *Armillaria* fungi; (ii) determine if evidence of recent flooding correlated with a greater likelihood of *Armillaria* occurring and causing root disease in surveyed stands; and (iii) identify the species of *Armillaria* in riparian forests of the northern Great Plains. Meeting these goals will fulfill objective (2) by providing basic information about the role of *Armillaria* in the region's riparian forest stands.

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## **Chapter 2: Methods for establishing *Populus deltoides* along the Missouri River in south-central North Dakota, USA**

### **Introduction**

In North America, natural regeneration and establishment of plains cottonwood (*Populus deltoides* Marsh. subsp. *monilifera* (Ait.) Eckenw.) is impacted by anthropogenically driven shifts in riparian ecology. Factors contributing to the reduction of cottonwood regeneration on reaches during the last century relate directly to streamflow management, but areas of mature floodplain forest have also been lost to cutting (Johnson et al. 1976) and/or converted for agricultural purposes (Dixon et al. 2015; Johnson 1992). Damming in particular has led to ecological changes along many rivers throughout North America (Nilsson and Berggren 2000). These changes include inundation of upstream vegetation by reservoirs (Dixon et al. 2012, 2015; Johnson et al. 1976), reduction in the magnitude of peak flows (Johnson 1992), and reduction of downstream sediment loads, leading to downcutting (Rood et al. 2003).

In the northern Great Plains of North America, about 23,000 hectares of forestland along the Missouri River were inundated following the completion of Garrison Dam (Johnson et al. 2012). Reductions in peak flows have led to reduced seasonal flooding and eliminated erosion and sedimentation. These processes are essential for the creation of suitable habitat for riparian cottonwood (Johnson 1992). Furthermore, downcutting downstream of Garrison Dam has led to lowered riverbed elevation on roughly the first 80 kilometers (km) of the 166 km remnant stretch of the Missouri River studied. It is hypothesized that this has also lowered groundwater levels along incised stretches of the river (Johnson et al. 2012). The cumulative impact of these changes has

resulted in impaired cottonwood regeneration along the Missouri River, where plains cottonwood is frequently the most abundant tree species on the floodplain.

Concerns about the long-term survival of cottonwood in the Great Plains were first reported by Johnson et al. (1976), who noticed low frequencies of small diameter cottonwood in stands along the Missouri River (seedling frequency = 0%; sapling frequency = 2.4%). In 2008, re-measurement of 88% of these stands indicated a continued trend toward larger average diameter trees (Johnson et al. 2012). These findings support the hypothesis that cottonwood regeneration is insufficient for stand replacement (Johnson et al. 1976). Over time, this will likely result in a shift in species composition of existing cottonwood stands toward a late successional forest type, most commonly comprised of green ash (*Fraxinus pennsylvanica* Marsh.) and box elder (*Acer negundo* L.) (Johnson et al. 2012).

Like many pioneer tree species, plains cottonwood exhibits some level of intolerance to shading and establishes best on bare mineral substrates; thus, cottonwood seedlings are typically found in greater abundance on areas of floodplains and sandbars where light is plentiful and interspecific competition is low. Prior to dam construction (1881-1945) historical rates of sediment deposition along the Missouri River were estimated to be 111 ha/yr (Johnson 1992), which is believed to have provided sufficient habitat for plains cottonwood regeneration. Following construction of the Garrison Dam on the Missouri River, sediment deposition decreased to 1.3 ha/yr (1969-1979). Over the same time period, erosion rates also decreased from 93 ha/yr to 21 ha/yr (Johnson 1992). This resulted in much greater rates of erosion than deposition, which contributed to reductions in suitable downstream habitat for cottonwood seedlings (Johnson 1992).

Similarly, the area of unvegetated sandbars saw a 96% decline over much of the upper Missouri between 1892 and 2006 (Dixon et al. 2012). Dixon et al. (2012) estimated that only 10% of the stands they surveyed were younger than 25 years of age in 2006, suggesting low rates of natural recruitment. Prior to European settlement, nearly half of the floodplain forests were in young age classes (<40 years) (Johnson 1992).

While replanting as part of a management strategy for regenerating cottonwood forest along the Missouri River has been recommended (e.g. Dixon et al. 2012; Johnson 1992); to our knowledge, no published study has tested or compared different methods of cottonwood restoration. As a result, the primary objective of this study was to assess the efficacy and economic feasibility of four methods of establishing *P. deltoides* along the Missouri River in south-central North Dakota. The specific objectives for the study were to determine: (1) whether or not survival would be different among treatments of varying cost and set-up time; (2) if height, height to live crown, and caliper would be significantly different among the treatments; (3) if treatments utilizing tree shelters would be less susceptible to browsing than those without shelters; and (4) if tilling to expose bare mineral soil before seed drop would result in improved cottonwood recruitment.

## **Materials and Methods**

***Plant propagation:*** Trees planted in this study were propagated in a greenhouse from 10 cm long dormant cuttings. Plant material was collected from 3.5-year-old trees near the Graner Bottoms site during the winter of 2015. Cuttings were planted into SunGro Professional Mix number 8 growing medium (SunGro Horticulture Ltd., Agawam, MA) in cone-tainers (Ray Leach SC10 Super Cone-tainers; Stuewe and Sons, Inc., Tangent, OR). Prior to planting, cuttings were soaked for approximately 48 h in tap

water. Initially, the growing medium was amended with 12 g of 15-9-12 (N-P-K) Nutricote slow-release fertilizer with micronutrients (Scotts Osmocote Plus; Scotts Company Ltd., Marysville, OH). This was supplemented on a bi-monthly basis with 20-20-20 (N-P-K) liquid fertilizer (Scotts Peters Professional; Scotts Company Ltd., Marysville, OH). Trees were watered as needed. Rooted cuttings (approx. 2,200) grew in the greenhouse for four months before being cut back so that only a few healthy buds remained. The trees were then stored at 4° C until they were transported to their respective sites for outplanting.

***Site selection:*** Two sites along the Garrison Reach of the Missouri River in south-central North Dakota were selected in the spring of 2015. The first site was located in Schmidt Bottoms (lat. 46.685080, long. -100.762396) and the second site in Graner Bottoms (lat. 46.653435, long. -100.710408) (Figure 2.1). The Schmidt Bottoms site had been used to grow supplemental food for wildlife since 2012. The Graner Bottoms site had been planted to alfalfa in 2004, but had been left idle since 2008. Both sites were established within 500 meters of mature cottonwood to ensure that a seed source was available nearby. The soil type was similar at each site: Havrelon loam was the primary soil in Schmidt Bottoms, while Graner Bottoms was classified mainly as Havrelon fine sandy loam (NRCS 2016a). Havrelon series soils are classified as Typic Ustifluvents (NRCS 2016b).

***Experimental design:*** At each site, the experimental design was a randomized complete block design with 3 blocks. Each treatment and untreated control occurred once per block. The treatments were: tree planting (T), tree planting with tree shelters (TS), tree planting with tree shelters and synthetic weed barrier fabric (TSF), and conventional

tillage (CT); a control, which was only mowed prior to initial planting, was also included in each block. The area of each treatment within a block was 21.34 m x 21.34 m (70 ft x 70 ft). (Figure 2.2)

***Site preparation:*** In April of 2015 vegetation at both sites was mowed to a height of approximately 10 cm with a brush mower. In May, an ATV with a sprayer attachment was used to apply the non-selective herbicide glyphosate (Monsanto Company; St Louis, MO) to plots according to manufacturer instructions to minimize competing vegetation. Glyphosate was applied to all plots except for the control in each block (Fig. 2). Trees were planted by hand using a dibble bar on May 27<sup>th</sup> and 28<sup>th</sup> of 2015. A total of 49 trees were planted in each treatment at a 3.05 m spacing. A border row of rooted cottonwood cuttings was planted around each trial. The sites were replanted mid-June to replace trees that had not survived the original planting. Synthetic weed barrier fabric (DeWitt Sunbelt Woven Ground Cover 5 x 600 ft rolls, Sikeston, MO) was installed by hand, and secured with 25.4 cm anchor pins (DeWitt Anchor Pins, Sikeston, MO). The pins were inserted approximately every 0.5 meters along both edges of the fabric. Additional pins were used to secure the ends. Tree shelters were 1.5 m tall (Tree Pro tree protectors, West Lafayette, IN) and were installed between late June and early July 2015. Support stakes for the tree shelters were made from 1.83 m lengths of 1.9 cm schedule 40 PVC pipe. All tree shelters were removed during a final survey in 2016. For CT treatments, tillage was accomplished using a small tractor and tilling implement following the start of seed dispersal in mid-June of 2015.

***Data collection:*** Tree mortality was evaluated in T, TS, and TSF treatments during September 2015. Cottonwood recruitment in control and CT plots was estimated

using five 1 m<sup>2</sup> quadrats. Quadrats were haphazardly placed throughout each CT and control plot. The number of living cottonwood seedlings was tallied for each quadrat.

A final survey of both sites was conducted in September of 2016. Survival, height, height to live crown, caliper, and damaging agents (e.g. *Melampsora* rust, deer feeding) in the T, TS, and TSF treatments were recorded. The height to live crown measurement was taken along the main stem, beginning at the soil line and terminating at the base of the first living branch, lateral shoot, or lateral bud. Calipers were measured approximately 15 cm from the ground surface. Trees were assumed to have been browsed if the leader or one or more branches had been severed. In the control and CT plots, plains cottonwood, green ash, and box elder seedlings were tallied as described above.

**Data analysis:** All statistical analyses were conducted in ‘R’ version 3.3.2 (R Core team 2016) and statistical significance was assessed at  $\alpha = 0.05$ . The effects of T, TS, and TSF treatments on survival, height, height to live crown, caliper, and browsing were compared using mixed models and the lme() function in the ‘nlme’ package (Pinheiro et al. 2017). The initial model used in all analyses was:  $Y_{ijk} = \mu + S_i + B_j + T_k + S_i*B_j + S_i*T_k + B_j*T_k + S_i*B_j*T_k + \epsilon_{ijk}$ . Where, S denotes site, B denotes block, T denotes treatment, and i, j, and k signify the site (1-2), the block (1-3), and the treatment (1-5), respectively. The terms  $S_i*B_j$ ,  $S_i*T_k$ , and  $B_j*T_k$  signify interactions between site and block, site and treatment, and block and treatment, respectively. The term  $S_i*B_j*T_k$  represents the interaction of site, block, and treatment. The term  $\epsilon_{ijk}$  denotes the experimental error of the i<sup>th</sup> site, the j<sup>th</sup> block, and the k<sup>th</sup> treatment. Block was the only random effect in the model; all other parameters were fixed.

Model selection was conducted using Likelihood ratio chi-squared tests. The order of testing was determined based on default p-values from the full model; larger values were tested first. Unequal variances were modeled as necessary. Subsequently, multiple comparisons among treatment means for each response variable were conducted using the 'lsmeans' package (Lenth 2016). P-values were adjusted for multiple comparisons using a Tukey correction. Survival data from Schmidt and Graner Bottoms were analyzed separately, due in part to a site effect in the model when sites were combined. More importantly, sites were analyzed separately to evaluate differences in the proportions of surviving trees in the T treatment at each site. The effects of the T, TS, and TSF treatments on height, height to live crown, and caliper were compared in the same fashion, but data from the two sites were combined for this analysis because, overall, variances were closer to equal compared with separating data by site.

Mean recruitment between the CT treatment and the control were compared separately for cottonwood, green ash, and box elder using Welch's t-tests. The t-tests were conducted on pooled data from both sites ( $n = 6$  for each treatment). The effect of treatment on browsing was analyzed using a mixed model and the procedure outlined above.

***Cost analysis:*** The cost of planted treatments was estimated by calculating the total cost of materials used. To estimate the cost per tree for T, TS, and TSF treatments, the combined costs of greenhouse rental and the materials purchased specifically for each treatment were considered. Total greenhouse costs associated with each tree were determined by dividing the total cost of greenhouse rental for the five months preceding out planting of stock by the total number of trees initially planted (1,702 trees). For TS



and TSF treatments, tree shelter costs and tree shelter, fabric, and anchor pin costs were factored into cost per tree estimates, respectively.

## Results

Model selection for tree survival resulted in the same parameters at each of the experimental sites. Both models contained only parameters for block and treatment. The final model for height included site, block, treatment, and the interactions of site by treatment and block by treatment. The final model for height to live crown was the same as the initial model. The only parameters in the final model for caliper were site, block, treatment, and the interaction of site by treatment. The final models used to compare treatment effects are presented in Table 2.1.

In 2015, survival was 98% for T, 97% for TS, and 91% for TSF treatments in Graner Bottoms. At the Schmidt Bottoms site, mean survival was 90% for the T treatment, 95% for the TS treatment, and 99% for the TSF treatment (Figure 2.3). Mean recruitment estimates for cottonwood in 2015 were 6,000 trees per hectare (tph) in CT plots and 333 tph in control plots (Table 2.2.)

In 2016, mean survival in Schmidt bottoms was 61% in the T treatment, 90% in the TS, and 98% in the TSF (Figure 2.3). Comparisons of the mean survival between treatments indicated differences among T and TS ( $p = 0.027$ ), and T and TSF ( $p = 0.010$ ), where the planting only treatment (T) had lower mean survival than both the TS and TSF treatments. Mean survival was similar between TS and TSF treatments ( $p = 0.578$ ). In 2016, the mean survival in Graner Bottoms was 87% in T treatments, 93% in TS treatments, and 88% in TSF treatments (Figure 2.3); there were no differences in survival

among T and TS ( $p = 0.368$ ), T and TSF ( $p = 0.981$ ), and TS and TSF ( $p = 0.450$ ) treatments.

Differences ( $p < 0.001$ ) in mean height and height to live crown were observed among T, TS, and TSF treatments. Mean caliper values were significantly different among T and TSF treatments ( $p < 0.001$ ), and between TS and TSF treatments ( $p < 0.001$ ), but not between T and TS treatments ( $p = 0.085$ ) (Table 2.4). The TSF treatment produced the greatest mean height, height to live crown, and caliper, and the T treatment produced the lowest.

Differences in recruitment between CT and control treatments were not detected for cottonwood ( $p = 0.363$ ), green ash ( $p = 0.246$ ), or box elder ( $p = 0.094$ ) in 2016. Mean recruitment estimates for the CT treatment were 11,667 tph for green ash, 1,000 tph for box elder, and 2,000 tph for cottonwood. For the control, estimated mean recruitment was 25,667 tph for green ash; 10,333 tph for box elder; and 0 tph for cottonwood (Table 2.2).

***Damaging agents:*** The most common types of damage to cottonwood in this study were *Melampsora* rust (*Melampsora* spp.), bark rub, browsing, and girdling. The mean proportion of trees browsed was 0.14 in the T treatment, 0.02 in the TS treatment, and 0.00 in the TSF treatment. Differences in browsing were significant between T and TS ( $p = 0.007$ ), and T and TSF ( $p = 0.003$ ), but not between TS and TSF treatments ( $p = 0.773$ ). At the time of data collection, no trees were girdled severely enough to cause visible symptoms of stress in the crown. An overall summary of damaging agents is presented in Table 2.3.

## Discussion

**Site differences:** Land use history and competing vegetation differed between the two sites, potentially influencing treatment effects. The most recent crop in Schmidt Bottoms had been a diverse mix containing twelve species that had been planted in 2014. When the Schmidt Bottoms site was surveyed for mortality in September of 2015, many trees in the T plots had become lodged along with forage oats (*Avena sativa* L.) that had volunteered on the site. The volunteer oats appeared to have caused extensive shading of lodged trees contributing to the nearly significant lower overall survival that was observed in the T at this site (61%) compared to the T at Graner Bottoms (87%) in 2016 ( $p = 0.059$ ). Differences observed in survival between T and TS ( $p = 0.027$ ), and T and TSF ( $p = 0.010$ ) treatments in Schmidt Bottoms, but not in Graner Bottoms, were likely due to mortality caused by lodging and shading in the T treatments.

The Graner Bottoms site had received a thin layer (approx. 1.5-2 cm, on average) of sand deposition across much of the study area following inundation during a major flood event in 2011. Schmidt Bottoms flooded during the same time period, but no sand deposition was evident. At the time of planting in 2015, much of the Graner Bottoms area had become colonized by invasive plant species such as Russian thistle (*Salsola kali* L.), smooth brome (*Bromus inermis* Leyss), and Canada thistle (*Cirsium arvense* (L.) Scop.). Competing vegetation in Graner Bottoms was sparse in comparison to that of Schmidt Bottoms.

When trials were surveyed in September of 2016, competing herbaceous vegetation was abundant in nearly every plot. Common weeds at the Schmidt Bottoms site included Canada thistle, mares tail (*Conyza canadensis* (L.) Cronquist), and Russian

thistle, but Canada thistle was the most abundant and grew in dense thickets. In the Graner Bottoms trial, sweet-clover (*Melilotus officinalis* (L.) Lam.), Kentucky bluegrass (*Poa pratensis* L.), and Canada thistle were the most prevalent weeds. At both sites, the weed canopy was roughly the same height as the tree shelters (1.5 m); however, the sweet clover in Graner Bottoms sometimes exceeded this height. The Canada thistle that covered much of the trial area in Schmidt Bottoms was extremely dense and little light was able to penetrate the weed canopy. The sweet clover in Graner Bottoms had a more open architecture, which must have allowed light to reach developing seedlings underneath. Thus, shading may also have contributed to differences in cottonwood survival in T treatments at the two sites ( $p = 0.059$ ).

**Treatments:** Treatment impacts on height, height to live crown, and caliper were clear in this study. As a general trend, the response of the three parameters increased with increasing treatment cost (Table 2.4). The only exception to this trend was caliper, which differed significantly among T and TSF and TS and TSF treatments only. (Table 2.4). The significant difference in mean caliper among T and TSF ( $p < 0.001$ ) but not T and TS ( $p = 0.085$ ) treatments implies that extended weed control (e.g. synthetic weed barrier fabric) may have a more noticeable effect than tree shelters on caliper diameter growth in plains cottonwood. In their 2011 study, Kabba et al. (2011) demonstrated that weed competition reduced nitrogen uptake in hybrid poplars (*Populus deltoides* x *Populus x petrowskyana* var. Walker) by nearly tenfold compared to a weed free control. For the same hybrid, they observed increases as great as 100% in root collar diameter where weed control was maintained for the duration of the study (Kabba et al. 2011).

Treatment impacts on survival were less clear. The lodging and shading issues at Schmidt Bottoms discussed earlier likely resulted in significant differences in survival among T and TS ( $p = 0.027$ ), and T and TSF ( $p = 0.010$ ) treatments. In contrast, no significant differences were observed for survival among treatments in Graner Bottoms. Generally, these results suggest that the use of 1.5 m tree shelters increased cottonwood survival by reducing competition from some weed species, but not others. The TSF had no impact on survival at either Schmidt Bottoms ( $p = 0.578$ ) or Graner Bottoms ( $p = 0.450$ ), indicating that long-term weed control (e.g. weed barrier fabric) may not be necessary for cottonwood establishment in this area.

The T treatment was the least expensive of the three planting treatments tested at an estimated cost of \$0.47 (U.S. Dollars; further “USD”) per tree. Low cost and minimal labor make this treatment ideal on sites where competing vegetation is minimal and juvenile trees will be able to compete for sunlight and nutrients. However, certain species of competing vegetation (e.g. Canada thistle and field oats) were believed to have negatively impacted survival. Additionally, there may be advantages in growth rates associated with more costly treatments (Table 2.4). In general, trees grown without tree shelters or weed barrier fabric appeared to be less vigorous compared with trees grown using TS and TSF treatments. The differences in growth rates are believed to have been largely a function of light availability in the TS treatments and light, water, and nutrient availability in the TSF treatments. Furthermore, browsing damage was relatively low and only 14% of trees in the unprotected T treatment were affected between the two sites.

Planting with 1.5 m tree shelters (TS) was estimated to cost \$4.24 (USD) per tree. This was approximately nine times more expensive than the T treatment. Trees in

Schmidt bottoms were better able to survive the lodging and shading issues in TS treatments than in T treatments ( $p = 0.027$ ). At both sites, it was observed that tree shelters prevented trees from being shaded by tangled masses of competing vegetation. As a result, we hypothesize that the treatment effect was a function of both the tree shelters preventing the young trees from becoming lodged and of light being able to reach cottonwood leaves through the opening in the top of the tree shelters. It should be noted; however, that most incidences of bark rub and girdling occurred from stem abrasion against exposed edges along the tops of tree protectors.

The TSF treatment was estimated to cost \$6.54 (USD) per tree. The TSF treatment was the most expensive, but also provided the greatest mean response in height, height to live crown, and the only significant increase in caliper ( $p < 0.001$ ) of the three treatments (Table 2.4). As a result, TSF may be best suited for situations where relatively few trees need to be established quickly from juvenile planting stock. Biodegradability of tree shelters and synthetic fabric are often a concern when using these items for conservation and/or restoration purposes; therefore, it may be of value to explore the use and efficacy of more readily biodegradable products for weed control and tree protection. At the conclusion of our study, no noticeable deterioration of tree shelters or weed barrier fabric was evident.

The CT treatment coincided with the start of seed fall and did not appear to be a reliable technique for restoring cottonwood in this study. No significant differences in recruitment were detected between tilled plots and controls in 2015 ( $p = 0.176$ ) or 2016 ( $p = 0.363$ ). Our initial stocking rate in the planted treatments was 1,077 trees per hectare, while the CT treatment and the control averaged 6,000 and 333.3 tph, respectively, at the

end of the 2015 growing season. At the conclusion of the study, the CT treatment averaged 2,000 tph and the control averaged 0 tph. While the average number of trees per hectare was greater in CT treatments than planted ones throughout the study, establishment was inconsistent and cottonwood seedlings were only observed in one of the six 0.046 hectare tilled plots across both sites during the final survey in 2016. Assessing the CT treatment using a larger area of floodplain may produce different results. The spatial distribution of seeds following their release from mother trees is highly stochastic, so providing a larger area of disturbance may better capture the efficacy of the CT treatment option.

Estimates made in 2016 on box elder and green ash recruitment were compared to estimates from 1969 and 1970 reported in Johnson et al. (2012). Across 34 forested sites in the Garrison Reach, the average recruitment of green ash and box elder was 6.6 and 1.0 seedlings  $\text{m}^{-2}$ , respectively in 1969-70 (Johnson et al. 2012). In our tilled plots ( $n = 6$ ), average recruitment was 1.2 seedlings  $\text{m}^{-2}$  for green ash and 0.1 seedlings  $\text{m}^{-2}$  for box elder. Average recruitment in the control plots ( $n = 6$ ) was 2.6 seedlings  $\text{m}^{-2}$  for ash and 1.0 seedlings  $\text{m}^{-2}$  for box elder. While this comparison appears to suggest a decrease in both green ash and box elder recruitment over time, it should be noted that data from Johnson et al. (2012) were generated from existing stands, while new estimates were made using relatively open floodplain areas with mature ash and box elder nearby.

While the results of this study indicated that irrigation is not necessary for adequate survival when establishing cottonwood from rooted cuttings, supplemental moisture may have improved recruitment in the CT treatment. Cottonwood seeds only remain viable for 7-14 days under typical environmental conditions; however, following

wetting, the duration of viability is reduced and seeds may only survive for two to three days (Braatne et al. 1996). Cottonwood seed was observed in CT plots shortly after they were tilled in mid-June of 2015, but in some areas seed desiccated in direct sunlight. It is plausible that a greater proportion of seed could have survived if the site were irrigated at regular intervals during germination and early establishment.

### **Considerations and conclusions**

The two aspects of experimental design most limiting to scope of inference in this study were the short study duration and the small geographic area within which both trials were located. Trials were only monitored for two growing seasons following planting. Other cottonwood establishment studies in the Great Plains lasted for three (Geyer and Atchison 2002) and five (Geyer et al. 2008) years; however, mean heights for cottonwood trees in our treatments ranged from 0.69 to 2.78 meters at the conclusion of the study, justifying the use of the word “establishment.” Although it is not known how survival may have differed if data were collected after one or more additional growing seasons, we hypothesize that survival would have been generally reduced due to the increased potential for natural or anthropogenic disturbances.

The straight-line distance between the two sites was approximately 5.3 km (3.3 mi), which limits the geographic area to which the results of this study can be directly applied; however, differences were evident even between the closely located sites used in this study because of competing vegetation and land use history. This supports the idea that restoration needs to be tailored to individual sites.

Trends observed in this study were similar to those observed in previous research conducted in the Great Plains region. Atchison and Geyer (2002) reported a general



increase in survival with extended weed control for plains cottonwood from their weed competition studies in Kansas. Planting into live sod resulted in the lowest survival of 1-0 cottonwood seedlings after three years (27.8%), while 71.4% of seedlings survived in the treatment utilizing sunbelt weed barrier fabric (Atchison and Geyer 2002). Height and stem diameter trends were similar between our study and previous work.

In conclusion, the T, TS, and TSF methods described in this manuscript were successfully used to establish plains cottonwood along the Missouri River in North Dakota. All four of the specific objectives outlined in the introduction were also addressed by this study. (1) survival was only different between T and TS, and T and TSF treatments in Schmidt Bottoms. The T treatment in Schmidt Bottoms had a survival rate of 61%, which equates to approximately 657 tph after two full growing seasons. As a point of reference, the average density of cottonwood stands along the Garrison Reach reported by Johnson et al. (1976) was substantially lower at 191.3 tph. (2) The height, height to live crown, and caliper of trees in TSF plots were significantly greater than those in TS and T plots. Height and height to live crown were greater for trees in the TS treatment than for trees in the T treatment, but calipers of trees in the two treatments did not differ significantly. (3) Trees were less susceptible to browsing where tree shelters were used, but even unsheltered trees had low levels of browsing damage. (4) By the conclusion of the study, only one of the six CT plots had cottonwood seedlings. Although there was a higher estimated density of surviving trees in the CT treatment than in planted treatments, individual seedlings were dispersed irregularly and colonized too little of the tilled area for the treatment to be considered effective. Future research targeting cottonwood restoration using irrigation and/or larger tracts of tilled alluvium

may provide valuable insights on restoring cottonwood forest without the need of growing or purchasing planting stock. For the time being, restoration of cottonwood forest using rooted stock and methods such as those detailed in this manuscript may be the most effective.

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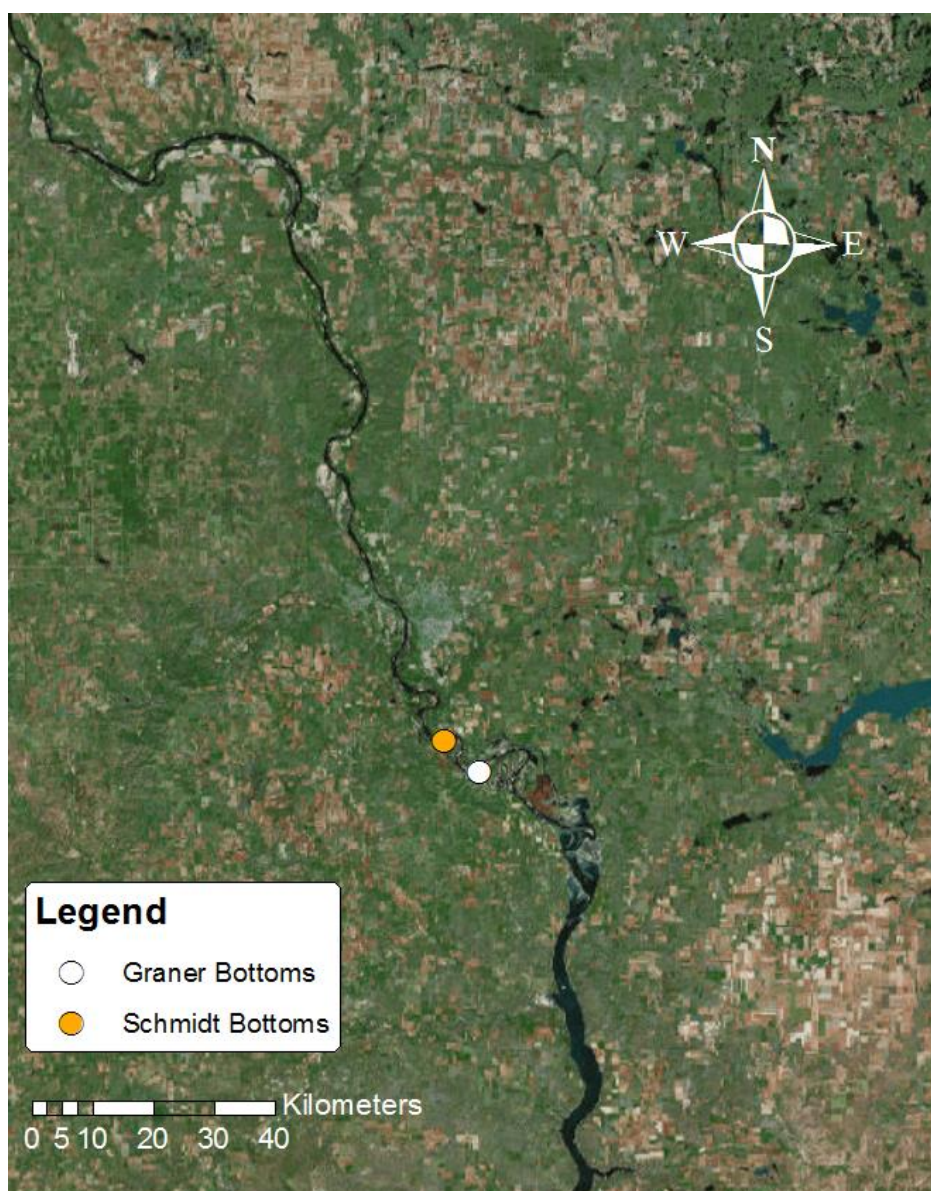


Figure 2.1: Locations of cottonwood restoration trials along the Missouri River in south-central North Dakota (2015). Graner Bottoms is located at (lat. 46.653435, long. -100.710408) and Schmidt Bottoms at (lat. 46.685080, long. -100.762396).

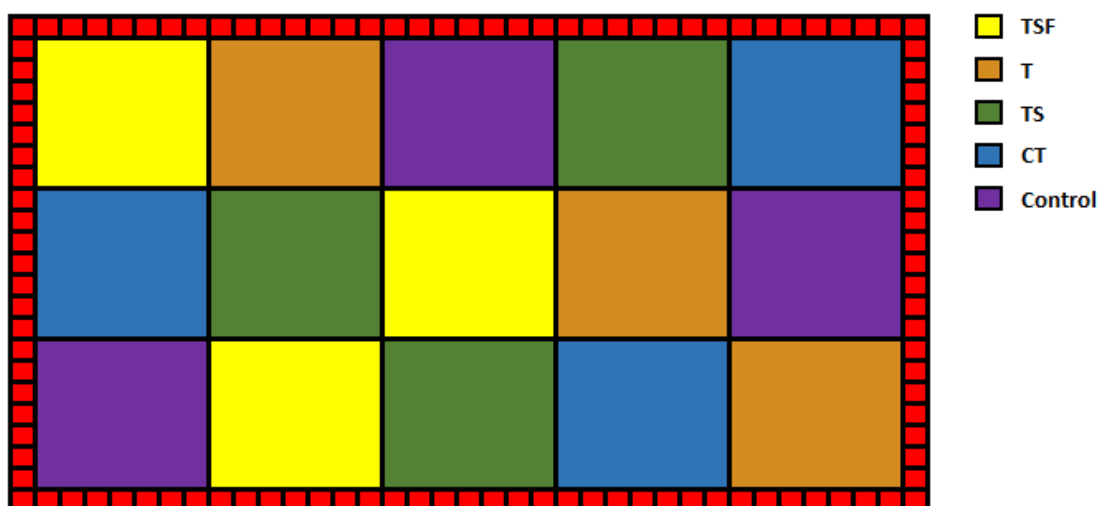


Figure 2.2: General layout of cottonwood restoration trials along the Missouri River in south-central North Dakota (2015). Red boxes outlining the trial represent a single border row of *P. deltoides*. Acronyms displayed in the legend correspond to tree planting (T), tree planting with tree shelters (TS), tree planting with tree shelters and synthetic weed barrier fabric (TSF), and conventional tillage (CT). Control plots were only mowed once, and that was prior to planting.

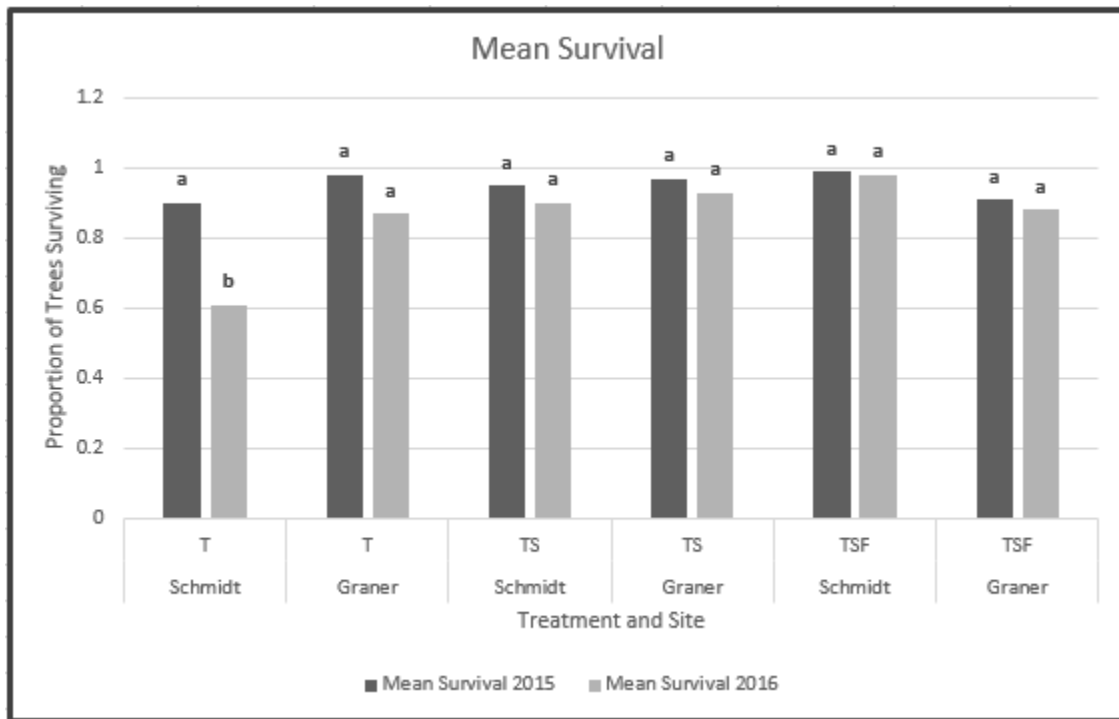


Figure 2.3: Mean proportions of surviving cottonwood seedlings by treatment and site at cottonwood restoration trials along the Missouri River in south-central North Dakota.

Table 2.1. A summary of the final models used for statistical analyses. Final models were used to draw conclusions about cottonwood restoration methods tested at two sites along the Missouri River in south-central North Dakota.

Mean Response	Final Model*
Survival - Schmidt	$Y_{jk} = \mu + R_j + T_k + \epsilon_{jk}$
Survival - Graner	$Y_{jk} = \mu + R_j + T_k + \epsilon_{jk}$
Height	$Y_{ijk} = \mu + S_i + R_j + T_k + S_i * T_k + R_j * T_k + \epsilon_{ijk}$
Height to Live	$Y_{ijk} = \mu + S_i + R_j + T_k + S_i * R_j + S_i * T_k + R_j * T_k + S_i * R_j * T_k$
Crown	$+ \epsilon_{ijk}$
Caliper	$Y^{ijk} = \mu + S_i + R_j + T_k + S_i * T_k + \epsilon_{ijk}$

\* In final models, S denotes site, R denotes replicate (block), T denotes treatment, and i, j, and k signify the  $i^{\text{th}}$  site (1-2), the  $j^{\text{th}}$  replicate (1-3), and the  $k^{\text{th}}$  treatment (1-5), respectively.

Table 2.2. Seedling recruitment of native riparian tree species in control and tilled (CT) plots at two study sites along the Missouri River in south-central North Dakota. Estimates are presented in number of stems per hectare.

Species	Tillage (CT) 2015	Control 2015	Tillage (CT) 2016	Control 2016
<i>P. deltoides</i>	6,000	333a	2,000	0
<i>F. pennsylvanica</i>	N/A	N/A	11,667	25,667
<i>A. negundo</i>	N/A	N/A	1,000	10,333

Table 2.3: Summary of damaging agents for all trees planted using the T (tree planting), TS (tree planting with tree shelters), and TSF (tree planting with tree shelters and synthetic weed barrier fabric) treatments at two sites along the Missouri River in south-central North Dakota.

Damaging Agent	# Affected (SB) <sup>1</sup>	% Affected (SB) <sup>1</sup>	# Affected (GB) <sup>2</sup>	% Affected (GB) <sup>2</sup>
<i>Melampsora</i> rust	357	97.81	392	99.75
Bark rub	100	27.40	72	18.32
Browsing	24	6.58	9	2.29
Girdling	24	6.58	6	1.53

\*Number Affected columns indicate how many trees still living during the final survey had each problem. Percent Affected columns indicate what percent of the living trees were afflicted with the respective problem.

<sup>1,2</sup> The acronyms SB and GB correspond to Schmidt Bottoms and Graner Bottoms (trial locations) respectively.

Table 2.4. Mean values for height, height to live crown, and caliper at two (combined data) cottonwood restoration trials along the Missouri River in south-central North Dakota. Values are for the three treatments in which rooted cuttings were planted.

Treatment	Mean Height (m)	Mean Crown (m)	Mean Caliper (cm)
Only Trees (T)	0.69a*	0.27a	0.505a
Trees & Shelters (TS)	1.27b	0.385b	0.595a
Trees, Shelters, & Weed Barrier (TSF)	2.78c	0.775c	1.725b

\* Letters a, b, and c appearing next to mean values indicate significant differences between treatments. For values followed by the same letter, differences were not significant.

## Chapter 3: Distribution and ecology of *Armillaria* in riparian forests of the northern Great Plains

### Introduction

Globally, *Armillaria* is known to affect hundreds of plant species (Kallas et al. 2003; Shaw and Kile 1991), ranging from giant sequoia (*Sequoiadendron giganteum* (Lindle.) Buchholz) (Baumgartner and Rizzo 2001) to the potato (*Solanum tuberosum* L.) (Gregory 1985). This fungus is primarily known as the causal agent of *Armillaria* root disease; however, it also behaves as a saprophyte (e.g. by degrading logs and dead stumps) and can grow symbiotically on living hosts without causing an infection. Basidiocarps (mushrooms) and basidiospores are thought to play a minor role in the spread of this pathogen (Baumgartner et al. 2011). Instead, *Armillaria* typically grows through the soil as rhizomorphs (sheathed aggregations of hyphae), which eventually come into contact with a host. Rhizomorphs can then enter through host bark using degradative enzymes and mechanical force (Baumgartner et al. 2011; Thomas, 1934; Zeller 1926), where they subsequently produce mycelial fans to forage for nutrients in the cambium and secondary xylem (Baumgartner et al. 2011). Furthermore, *Armillaria* can survive in the absence of living hosts for 15-30 years by colonizing remnant large roots and stumps (Klutsch 2012), a trait important to the management of *Armillaria* root disease in commercial forest stands.

*Armillaria* spp. have been well studied in forest dominated areas such as the northeastern (e.g. Blodgett and Worrall 1992a, 1992b; Brazee and Wick 2011) and northwestern (e.g. Banik et al. 1996; Ferguson et al. 2003) United States. In contrast, little information exists on these fungi in the northern Great Plains region, where trees are



generally less common on the landscape. *Armillaria ostoyae* (Romagnesi) Herink. has been reported as a pathogen in the Black Hills of South Dakota (Kallas 1997; Kallas et al. 2003; Klutsch et al. 2012) and *Armillaria gallica* as an epiphyte in the Niobrara Valley Reserve in Nebraska (Kim and Klopfenstein 2011). However, these limited observations may not be representative of the ecology or species composition of *Armillaria* across this region.

The primary ecological role of *Armillaria* varies substantially between the mostly deciduous forests of the eastern U.S. and the coniferous forests of the western U.S. (Wargo and Shaw 1985). In the east, the fungus is often observed in a facultative, or secondary, role as a pathogen that infects stressed and weakened trees (Wargo and Shaw 1985). In western coastal forests *Armillaria* is most likely to incite root disease in young trees (<25 years), while it attacks and often kills otherwise healthy conifers of any age in the interior west (Wargo and Shaw 1985).

Hardwoods such as plains cottonwood (*Populus deltoides* Marsh. subsp. *monilifera* (Ait.) Eckenw.), green ash (*Fraxinus pennsylvanica* Marsh.), box elder (*Acer negundo* L.), and *Salix* spp. typically dominate riparian forests of the northern Great Plains. The only conifers observed in floodplains of the region are Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) and eastern redcedar (*Juniperus virginiana* L.). Both species are typically found in upland sites and only invade bottomlands if unmanaged. The susceptibility of *Juniperus* spp. to *Armillaria* in the northern Great Plains is not known, but eastern redcedar has been reported as a host for *A. tabescens* in Virginia (Schnabel et al. 2005). Due to the general dominance of hardwoods over

conifers in the region, it is logical that the ecology of *Armillaria* in riparian forests of the northern Great Plains may be similar to that of *Armillaria* in eastern hardwood forests.

Over the last half-century, studies (e.g. Johnson 1998; Johnson et al. 1976, 2012) have shown that dam construction along major rivers of the northern Great Plains have resulted in extensive changes to the ecology of existing riparian habitat. Impoundment of rivers has directly led to reduced peak flows and sediment loads, as well as changes in the timing of seasonal flows (Dixon et al. 2012; Johnson 1992). Losses of flooding on the landscape have been tied to reduced growth rates in plains cottonwood and some other riparian tree species (Johnson et al. 1976), suggesting trees may be stressed for moisture and/or nutrients. Extensive flooding occurred along the Missouri River and many of its tributaries in the spring of 2011. In some areas, land adjacent to the river was inundated for as long as three months (Dixon et al. 2015), and flooding occurred during the growing season when trees' root systems generally require the most oxygen. Although the vast majority of mature riparian trees survived the flooding (Dixon et al. 2015), the long-term impacts of this event remain unclear. We hypothesize that the lack of oxygen associated with flooding was stressful to surviving riparian trees, so it is probable that *Armillaria* and other facultative pathogens will have become more aggressive in these floodplain ecosystems.

This study was conducted to examine the role of *Armillaria* in the riparian forests of the northern Great Plains, where long-term moisture stress followed by recent flooding has predisposed these ecosystems to attack by facultative pathogens. The primary objectives of the study were to (1) characterize the site and host species associations of *Armillaria* fungi; (2) determine if evidence of recent flooding correlated with a greater

likelihood of *Armillaria* occurring and causing root disease in surveyed stands; and (3) identify the species of *Armillaria* in the region.

## **Materials and Methods**

**Field Surveys:** *Armillaria* surveys were conducted on publicly owned lands in North Dakota, South Dakota, and Nebraska that supported riparian forest stands at least 0.4 hectares in area. These lands included parks and protected areas managed by municipal, county, state, and national government entities. Sites were identified using a variety of geoinformation software including ArcMap 10 (ESRI ©1995-2016), Google Maps (Google ©2015), and Google Earth (Google ©2015).

Parameters of interest and collection methods for the surveys described below were adapted from Blodgett and Worrall (1992a, 1992b). A forest stand > 0.4 ha in area with stumps, snags, and/or trees exhibiting crown dieback or other stress symptoms was located. One hour was spent searching for signs of *Armillaria* (rhizomorphs or mycelial fans). This was done by digging around root collars and major roots of stumps, snags, and severely stressed trees. When stems exhibited sunken or necrotic lesions at the base, small areas of bark were peeled back with a knife to check for mycelial fans. If *Armillaria* was found on a stump or snag, up to five stressed trees in the surrounding area were searched for evidence of root and butt rot in the manner previously described. Rhizomorphs and/or infected wood samples were collected from the first living tree found to have a root or butt rot. If *Armillaria* was not observed causing disease in a tree that was still living, a snag, stump, or living tree hosting the fungus, but with no observed infection was collected from instead. This was done to reduce the chances of missing a pathogenic interaction in an area of the stand where the fungus was present.

At each site where *Armillaria* was found, a 15 by 15 m square plot was established around the tree from which fungal samples were collected. Species, tree condition (root rot; butt rot; stump/snag; and epiphyte), crown position, diameter at breast height, percent live crown, and major damages were recorded for the selected host at each site. Root rot was characterized by any infection of living host vascular tissue in which a live-dead margin was observed adjacent to invading *Armillaria*. Butt rot was recorded whenever one or more rhizomorphs were found growing through decaying wood (specifically, white rot). If *Armillaria* mycelium was observed colonizing only a dead stump or snag and no pathogenic interaction was observed, the interaction went into the stump/snag category. Where rhizomorphs were found attached to tree roots, but no infections or mycelium were observed, *Armillaria* was classified as an epiphyte. It should be noted, however, that where *Armillaria* was not observed in a pathogenic capacity, it is possible that isolates were instead facultative parasites not currently infecting a host.

Host conditions were then grouped to separate isolates by ecological role (i.e. saprophyte or pathogen) within the forest based on observed interactions between isolates and host species. *Armillaria* isolates collected from infected root or other vascular tissues were categorized as pathogenic, while those acquired from stumps, snags, areas decayed by butt rot, or healthy tree tissue (epiphytes) were classified as having a primarily saprophytic role in the ecosystem.

Sites for which flood damage had been recorded were compared to those for which it was not. Evidence of flood damage included features such as the presence of adventitious roots, discoloration of bark from extended contact with water, large amounts

of washed in debris (e.g. driftwood,) and significant sediment deposition. Only sites with obvious signs of flooding were noted as having been flooded.

Cover types of surveyed forest stands were determined visually based on the dominant tree species. Cottonwood stands often sustained one or more smaller tree species such as green ash (*Fraxinus pennsylvanica* Marsh.), box elder (*Acer negundo* L.), mulberry (*Morus* spp.) eastern redcedar, and Rocky Mountain juniper, and these species were collectively grouped as “minor” components. Seventeen stands had three or more predominant species and were pooled into a “mixed hardwoods” category.

Species and diameter at breast height (DBH) of trees within each plot were also recorded, but only for trees measuring at least 8 cm DBH. DBH was later used to estimate basal area per hectare for living trees of all species at each site. Basal area for measured trees in each plot was calculated using the standard equation  $BA = \frac{\pi}{4 \times 10,000} (DBH^2)$ . For each plot, basal areas of individual trees were added together and the sum scaled up from the 225 m<sup>2</sup> plot size to one hectare.

In each plot, fifteen 8 cm<sup>2</sup> O-horizon (dead plant material and duff) samples were taken from the forest floor, and fifteen mineral soil samples were taken to a depth of 8 cm. These were collected in an approximately even distribution across each plot in order to make two composite samples, one O-horizon and one mineral. Samples were used to determine the range of edaphic characteristics supporting *Armillaria*. An 8 cm<sup>3</sup> soil sample was also taken at each of the fifteen soil collection locations within the plot and inspected for rhizomorphs. The proportion of soil samples that contained rhizomorphs was recorded for each plot.

**Soil analysis:** Texture, pH, and percent organic matter of mineral soils as well as pH and percent organic matter of O-horizon soils were analyzed in the lab. Composite O-horizon samples were air-dried, homogenized, and split repeatedly until an appropriately sized sub-sample was produced. Sub-samples of organic composites were then ground using a coffee grinder (Proctor Silex; Glen Allen, VA). Composite mineral soil samples from each site were air-dried, pulverized with a rubber mallet, and passed through a 2mm sieve. Soil texture of mineral samples was evaluated using the hydrometer method (Gee and Bauder 1986).

Soil organic matter content of both mineral and organic samples was estimated using the loss-on-ignition method (Nelson and Sommers 1996). The pH of mineral soil samples was determined using the 2:1 method with deionized water (Thomas, 1996), and organic pH was determined using a modified version of the same protocol. In the modified pH protocol, approximately 15 ml of loosely packed, unground, homogenized organic material was mixed with 20 ml of de-ionized water; the solution was allowed to rest at room temperature for fifteen minutes before being re-agitated and measured with a pH probe (Mettler-Toledo, LLC; Columbus, OH).

***Armillaria isolations:*** Isolations were made from rhizomorphs and mycelial fans collected at field sites. Mycelial fans were carefully removed from within bark and phloem using sterile forceps before being plated. Prior to being plated, rhizomorphs were cut into approximate 1 cm lengths and surface sterilized. Surface sterilization was accomplished by submerging the sectioned rhizomorphs in a 1.05% NaOCl solution for five minutes and then in a 20% ethanol solution for one minute. Mycelium and surface sterilized rhizomorph sections were plated on malt extract agar amended with benomyl

and streptomycin (1.5% malt extract (Difco Laboratories Inc.; Sparks, MD), 1.7% Bacto agar (Difco Laboratories Inc.; Sparks, MD), 0.005% Benlate (50% benomyl; DuPont chemical company; Wilmington, DE), and 0.002% streptomycin sulfate (Sigma Chemical Co.; St. Louis, MO). Streptomycin was added following autoclaving. Plated isolates were allowed to grow for one to six weeks, until pure *Armillaria* mycelium could be removed and transferred to a new culture. Sub-cultured isolates were then grown in the dark on malt extract agar (1.2% maltose, 0.3% dextrin, 0.2% glycerol, 0.08% peptone, 1.5% agar; Difco Laboratories Inc.; Sparks, MD) with a square sheet of cellophane placed on the surface prior to sub culturing the *Armillaria*. This allowed mycelium to be harvested without agar for DNA extraction. After 2 to 3 weeks, mycelium was scraped into microfuge tubes and frozen for DNA extraction.

**DNA extraction:** Genomic DNA (gDNA) was extracted using the protocol and buffer of Winton and Hansen (2001). Frozen tissue was homogenized in a beadmill, and CTAB (cetyltrimethylammonium bromide) extraction buffer was added to each sample; the samples were then incubated at 65°C for 1 h. Incubation was followed by a chloroform extraction, and isopropanol was used to precipitate DNA.

**PCR amplification:** A roughly 1000 base-pair (bp) portion of the Translation Elongation factor *1-α* (*tef1-α*) gene was amplified and sequenced in order to identify the species of *Armillaria* collected at each site. Polymerase chain reaction (PCR) was conducted using a total volume of 58.5 µl, which included 8 µl template, 34 µl nuclease-free water, 5 µl 10X Combination Reaction Buffer (Mg free) (a balanced KCl/NH<sub>4</sub> buffer), 4 µl 2.5mM DNTP, 1.5 µl ARMEF-983F, 1.5 µl ARMEF-2218R, 1 µl Hot-Start DNA polymerase, and 3 µl 25M MgCl<sub>2</sub>. The 10X Combination Reaction Buffer and

MgCl<sub>2</sub> were provided with the Hot-Start DNA polymerase (Apex Bioresearch Products; Houston, TX). Reactions were carried out using a 2720 Thermal Cycler (Applied Biosystems Inc.; Foster City, CA). The reaction conditions were: 95° C for 15 minutes, 30 cycles of 94° C for 30 seconds, 57° C for 30 seconds, 72° C for 1 minute 30 s, and 72° C for 7 minutes. The PCR-product was sequenced on a 3730 DNA Analyzer (Applied Biosystems Inc; Foster City, CA) using the following primers: ARMEF-983F, ARMEF-2218R, ARMEF-R2, ARMEF-F3, ARMEF-RI2, and ARMEF-FI2 (Table 3.1).

***Species identification:*** A subset of 22 isolates was identified. Staden Package (Bonfield et al. 1995) was used to first orient raw DNA reads from sequencing and to generate files for use in BioEdit (Hall 1999). Sequences were aligned to one another and edited using BioEdit. Bases at positions with more than one nucleic acid read were changed according to IUPAC nucleotide codes. The consensus sequence from each respective isolate was then compared to *tef-1α* amplicon sequences from GenBank. The species reported in the results correspond to the species of highest percent identity in BLAST, where coverage was at least 97%.

***Data analysis:*** Two separate analyses were conducted to determine: (i) if certain site characteristics could predict the presence/absence of *Armillaria* (occurrence); and (ii) how these and additional parameters impacted the ecological role of *Armillaria* when it was present in a stand. Explanatory variables for both analyses were chosen because they were biologically relevant to the questions of interest. P-values were taken directly from model output in R, and mean estimates were back-transformed for reporting. Where variables were used in more than one model, p-values reported in the text are from the model having the lowest AIC (best fitting).



In the first analysis, logistic regression was used to estimate the effect of stumps and snags  $\text{ha}^{-1}$ , presence/absence of competitive grasses, and flooding on the odds of observing *Armillaria* in a stand (occurrence). Two models were used to model occurrence; one included flooding and grass, and the other included the variables flooding and snags and stumps  $\text{ha}^{-1}$ . In addition to modeling, a Welch two-sample t-test was used to compare mean DBH of all trees in plots where *Armillaria* was or was not found, and an F-test to compare variances of the same data.

In the second analysis, conducted specifically on sites where *Armillaria* was found, logistic regression was used to test the effects of basal area  $\text{hectare}^{-1}$ , host DBH, snags and stumps  $\text{ha}^{-1}$ , presence/absence of competitive grasses, percent clay content, and flooding on the ecological role of *Armillaria* (pathogen/saprophyte). Four models were used for this purpose, and the pairings of explanatory variables in each model were as follow: stumps and snags  $\text{ha}^{-1}$  and flooding, grasses and flooding, percent clay and flooding, and basal area  $\text{ha}^{-1}$  and host DBH. Models used in these analyses are summarized in Table 3.2.

All logistic regressions were conducted using the “logistf” package (Heinze and Ploner 2016) in ‘R’ version 3.3.2 (R Core team 2016). The “firth=TRUE” link was used when separation of the data was evident. Separation occurs when maximum likelihood estimates for odds ratios approach zero or  $\infty$  (Heinze 2006). For example, the phenomenon is observed where zero occurrences are observed in one of two categories defined by a dichotomous covariate (Heinze 2006). Separation is common when the number of observations in a category is small.

## Results

**Field surveys:** In total, 101 stands were surveyed during 2015 and 2016.

*Armillaria* was found in 78 (77%) of these stands. Pathogenic interactions were recorded in 55 stands, and *Armillaria* was observed only in a saprophytic and/or epiphytic capacity in 23 stands. *Armillaria* was most often associated with cottonwood ( $n = 21$ ), green ash ( $n = 19$ ), and box elder ( $n = 12$ ), which together accounted for 67% of the total observed hosts. These forest types were also the most commonly sampled. Thirty-seven percent of stands were dominated by cottonwood and 22% were dominated by green ash (*Fraxinus pennsylvanica* Marsh.) or box elder (*Acer negundo* L.) (Table 3.3). Green ash and box elder were included as components of the mixed hardwood cover type as well, which included 17% of the sites. *Armillaria* was found in association with at least 13 host species and incited root disease in at least 12 of those species (Table 3.4). The location of each collection site and the ecological condition in which each corresponding isolate was found are displayed in Figure 3.1.

***Armillaria* and flooding:** In total, 30 of the sampled sites had noticeable evidence of flooding. Of these sites, *Armillaria* was found at 19. Flooding did not affect the odds of finding *Armillaria* after accounting for stumps and snags per hectare ( $p = 0.831$ ), or grass ( $p = 0.855$ ).

Pathogenic interactions between *Armillaria* and a host were observed in 17 flooded stands, while *Armillaria* was found only as a saprophyte or epiphyte in two flooded stands. *Armillaria* was not found in 11 of the flooded sites, which accounted for nearly half (48%) of the total sites where *Armillaria* was not observed. Flooded sites where the fungus was found were 0.18 times more likely to have *Armillaria* acting

pathogenically than unflooded sites, after accounting for stumps and snags hectare<sup>-1</sup> ( $p = 0.038$ ). Observing *Armillaria* as a pathogen was 0.12 times more likely where flooding occurred after accounting for clay ( $p < 0.001$ ). Of the three models containing flooding, the one with clay was the best fitting with an AIC of -65.42.

**Cover and soil analysis:** Basal area ranged from 5.8 to 119 m<sup>2</sup> hectare<sup>-1</sup>, host DBH from 8.4 to 109.9 cm, and snags and stumps hectare<sup>-1</sup> from 0 to 577.8. Mean basal area was 42.6 m<sup>2</sup> h<sup>-1</sup> in stands where *Armillaria* was pathogenic, 26.5 m<sup>2</sup> h<sup>-1</sup> in stands where it was saprophytic, and 29.1 m<sup>2</sup> h<sup>-1</sup> where it was not found (Table 3.5). After accounting for host DBH, increases in basal area ha<sup>-1</sup> significantly increased the odds of observing a pathogenic interaction ( $p = 0.007$ ). Mean host DBH was 37.6 cm for non-pathogenic interactions and 41.7 where pathogenic interactions were observed, but host DBH did not influence the odds of observing a pathogenic interaction within a stand ( $p = 0.559$ ) after accounting for basal area hectare<sup>-1</sup>. Neither the odds of finding *Armillaria* ( $p = 0.098$ ) nor of observing a pathogenic interaction ( $p = 0.135$ ) changed as the number of snags and stumps hectare<sup>-1</sup> increased.

*Armillaria* was found at all 20 of the sites with competitive grasses including smooth brome (*Bromus inermis* Leyss.), Kentucky bluegrass (*Poa pratensis* L.), and reed canary grass (*Phalaris arundinacea* L.). The fungus was pathogenic in 15 of these stands and saprophytic or epiphytic in five. After accounting for flooding, there was no evidence that competitive grasses affected the odds of observing *Armillaria* ( $p = 0.990$ ) or of observing a pathogenic interaction ( $p = 0.613$ ). On average, mineral soils were high in sand ( $\bar{X} = 48.7\%$ ) and silt ( $\bar{X} = 46.6\%$ ), and low in clay ( $\bar{X} = 4.8\%$ ) where *Armillaria* was

found. After accounting for flooding, each percent increase in clay increased the odds of observing a pathogenic interaction by 0.85 times ( $p < 0.001$ ).

**Species identification:** Twenty-two isolates were identified as *Armillaria gallica* Marxmuller & Romagn. Of these isolates, 17 were observed causing a root rot, and only five were observed exclusively as saprophytes. Three isolates were from Nebraska, seven from South Dakota, and twelve from North Dakota (Figure 3.1). *A. gallica* was associated with six species of living hosts and snags of five host species. Live hosts included cottonwood (*P. deltoides*), box elder (*Acer nugundo*), green ash (*F. pennsylvanica*), silver maple (*A. saccharinum*), American elm (*U. americana*), and white mulberry (*M. alba*). Dead hosts consisted of cottonwood, slippery elm (*U. rubra*), American linden (*T. Americana*), box elder, and green ash.

## Discussion

**Field surveys:** *Armillaria* was found at 78 of 101 sites surveyed. Riparian forest stands on publicly owned lands were uncommon in the western halves of South Dakota and Nebraska, limiting the number of sites in those two states (see Figure 3.2). Surveys along the Little Missouri River and Upper Missouri River in western North Dakota instead provided much of the information about *Armillaria* in the western half of the study area. Along the Little Missouri, *Armillaria* was causing root disease every time it was observed (7 observations over 11 sites). The same trend was evident in northeastern and north central North Dakota (4/5 and 6/6 observations, respectively). In contrast, ecological roles of *Armillaria* were exclusively saprophytic where the fungus was observed in the Sheyenne National Grasslands (5/5) of southeast North Dakota.

*Armillaria* was observed in all roles along the Missouri River (24 total observations) (Figure 3.2).

Riparian areas were dominated by hardwoods at all 101 surveyed sites. However, in some areas, eastern redcedar and Rocky Mountain juniper were abundant in the understory. Over two field seasons and three U.S. states, *Armillaria* was never observed colonizing either species. To our knowledge, neither species has been shown to be susceptible to *Armillaria* in the northern Great Plains. Western Juniper (*Juniperus occidentalis* Hook.) has been reported as a host for *Armillaria mellea* in California (Baumgartner et al. 2010), and *J. squamata* and *J. Virginiana* as hosts for *A. tabescens* in South Carolina and Virginia, respectively (Schnabel et al. 2005), but there is a general lack of literature on *Armillaria* and *Juniperus* spp. in North America.

***Armillaria and flooding:*** *Armillaria* was more likely to be found as a pathogen at sites with evidence of flooding after accounting for clay ( $p < 0.001$ ). In some stands, long-term inundation would have deprived roots of oxygen for weeks or months, thereby weakening existing trees and predisposing them to attack from facultative pathogens. Moisture stress from inundation has been shown to increase susceptibility to *Armillaria* in some woody species (Popoola and Fox 2012), and may have had a similar effect on riparian tree species of the northern Great Plains.

Flooding may also have led to an increase in total volume of colonizable substrate on-site by killing individual trees unable to tolerate long periods of inundation. Dead stumps and snags, though dependent on age and species, are easily colonized by *Armillaria* and serve as sources of inoculum. Increases in inoculum could have led to faster spread of the fungus and therefore more interactions between *Armillaria* and

weakened or otherwise susceptible trees. A greater number of interactions between hosts and *Armillaria* would have made it more likely to observe a pathogenic interaction within a stand. Dixon et al. (2015) reported greater mortality in eastern redcedar and Russian olive than cottonwood following flooding along the Missouri, but did not report on common species such as green ash and box elder that may also have suffered higher mortality than cottonwood. While *Armillaria* was not observed on either Russian olive or eastern redcedar in our study, increased mortality in green ash and box elder, which were both susceptible to *Armillaria* in the region, may have facilitated faster spread of the fungus through stands.

**Cover and soil characteristics:** While basal area hectare<sup>-1</sup> was not a reliable indicator for the occurrence of *Armillaria*, increases in basal area were associated with increases in the odds of observing a pathogenic interaction ( $p = 0.007$ ). Basal area did not allow us to distinguish between young stands with many small trees and old stands with few, large trees. However, diameter class distributions for stands where *Armillaria* was present and those where it was not suggest differences between the two groups of sites (Figure 3.3). Stands in which *Armillaria* was found had larger mean diameter trees ( $\bar{X} = 24.8$  cm) than stands where it was not observed ( $\bar{X} = 20.6$ ;  $p < 0.001$ ). An F-test was performed on diameter data from both stands, and the variances were different ( $p < 0.001$ ). It is therefore reasonable that an increase in basal area due to the presence of larger trees could have increased the odds of finding *Armillaria*.

Diameter growth in trees naturally declines with age (Fritz 1966; Johnson et al. 1976), suggesting decreased plant vigor in older trees. Thus, stands of older and larger

trees may have been less able to resist infection compared to stands with younger, more vigorous trees.

Host DBH ranged greatly, from 8.4 cm to 109.9 cm, indicating that *Armillaria* affects trees of all diameter classes in the northern Great Plains. Hosts with diameters less than 40 cm at breast height made up 62% (48 of 78 trees) of the host population, suggesting at most a slight trend toward preference for smaller diameter trees as hosts. While this may seem contradictory, it is possible that *Armillaria* was found more often in stands with larger trees because they were disturbed less recently, but exhibits a slight preference for smaller hosts within those stands. Trees with suppressed or intermediate crown positions within the canopy are generally smaller in diameter and stressed, which also supports this notion.

After accounting for flooding, the odds of finding *Armillaria* did not change as the number of snags and stumps hectare<sup>-1</sup> increased ( $p = 0.098$ ). However, both the species and age of snags and stumps may affect *Armillaria*'s ability to colonize them. For example, *Armillaria* was never found on *Juniperus* spp. of any condition. As a result, a potentially higher incidence of *Juniperus* stumps and snags may have reduced the probability of finding *Armillaria* in some stands. The species and ages of stumps and snags were not assessed during surveys.

Mature cottonwood stands, especially those in decline, typically have open canopies allowing full sun to reach many parts of the understory. This environment is suitable for invasive grasses, which may compete with trees for space, water, and nutrients. Johnson et al. (2012) reported that between 1969 and 2008, the relative cover of smooth brome increased from 5.4% to 34.4% in study sites along the Missouri River.

They also noted that reed canary grass was absent in 1969, but had invaded about one-third of the sites to varying extents by 2008. We hypothesized that stands with competitive grasses would have a greater proportion of stressed trees. However, results from our analysis provided no evidence that the presence of competitive grasses influenced the ecological role of *Armillaria*. It is interesting to note that *Armillaria* was present in every stand with grass as an understory component.

Increases in percent clay content of mineral soil increased the odds of observing a pathogenic interaction ( $p < 0.001$ ). All sites were established along watercourses with minimal aspect (aspect was not recorded) with percent clay content of 19.2% or less. McLaughlin (2001) found that *A. gallica*, the only species we have identified, showed no strong preference for soil type; however, fewer than 11% of his isolates came from clayey sites. Wiensczyk et al. (1997) found that *Armillaria ostoyae* infection levels on black spruce (*Picea mariana* (Mill.) BSP) decreased as C horizon clay content increased, but reasoning behind this result was uncertain. It was suggested, however, that because rhizomorphs are generally more abundant in upper horizons, higher percent clay content in the C horizon was more likely to be improving host vigor than pathogen virulence (Wiensczyk et al. 1997). Neither example seems to indicate the same relationship between *Armillaria* and clay observed in riparian forests of the northern Great Plains, though neither study was restricted to riparian areas.

One possible explanation is that *Armillaria* may have directly benefitted from the greater moisture holding capacity generally associated with increases in percent clay. Greater moisture retention is conducive to fungal growth, and thus may have provided the fungus with a competitive advantage over riparian trees. While trees may also have



benefitted from increases in available water capacity and nutrients associated with increases in percent clay, the top 8 cm of the soil profile may not have been utilized by tree roots to the same extent as the fungus. Rood et al. (2011) showed that the median root depth of *P. deltoides* on the St Mary river in Alberta was 90 cm, which is far below the maximum depth of our soil cores. In contrast, rhizomorphs and live-dead margins associated with root infections were observed almost exclusively within the top 20 cm of the soil profile during our field surveys.

***Armillaria gallica in the northern Great Plains:*** The subset of isolates identified as *A. gallica* were primarily pathogenic in their respective ecosystems (17/22 isolates). These isolates were collectively associated with six species of living hosts and snags of five host species. Eight of the 22 isolates were collected from flooded sites suggesting no distinct association between flooding and occurrence. Of the flooded sites, *A. gallica* was pathogenic at six and saprophytic at two. However, this ratio is subject to change as more isolates are identified. These results suggest that *A. gallica* has a wide host range, acts as a pathogen and saprophyte, and exhibits no major preference for sites that were or were not flooded in the region.

Mineral and O horizon pH were not used in analyses, but provided additional information about the adaptability of *Armillaria* and *A. gallica*. The mean pH of mineral soils and O horizons at sites where *A. gallica* was found were  $\bar{X} = 7.62$  and  $\bar{X} = 7.05$ , respectively. These are much higher than the means observed for any of the five species of *Armillaria* identified by Blodgett and Worrall (1992b) in New York state. There, *A. gallica* was associated with sites having both the highest average mineral pH (4.6) and organic pH (4.6) (Blodgett and Worrall 1992b). Though *A. gallica* was associated with

acid soils in New York State, it also showed a preference for higher pH mineral and organic soils than did the other four *Armillaria* species described. Furthermore, in Ontario, McLaughlin (2001) showed that *A. gallica* exhibited a preference for calcareous soils, which are characteristically high in calcium carbonate. Collectively, these results suggest that *A. gallica* tolerates a wide soil pH range and may exhibit a preference for more basic soils than other *Armillaria* species.

## Conclusion

*Armillaria* is widely distributed in riparian forests of North Dakota, South Dakota, and Nebraska, where it acts pathogenically, saprophytically, and epiphytically on many species and genera of hardwood trees. Within the study area, *Armillaria* was never found on a single coniferous host. Stands where *Armillaria* occurred had larger mean diameter trees than stands where the fungus was not found ( $p < 0.001$ ), but hosts of all diameters  $\geq 8$  cm were equally susceptible to infection.

Results indicated that flooding in the northern Great Plains relates to host stress and aggressiveness of *Armillaria* in riparian forests. In this study, interactions between *Armillaria* and hosts were more likely to be pathogenic following flooding. Flooding is believed to have increased host stress, as many surveyed sites were along the Missouri River and its tributaries, some of which experienced months of inundation. Furthermore, flooding likely exacerbated an ongoing problem of forest stress in the region, as decreases in flooding associated with streamflow management have been tied to reductions in soil moisture and growth of cottonwood and other riparian tree species (Johnson et al. 1976). Thus, many riparian trees may already have been experiencing

stress at the time of flooding, potentially making them even more susceptible to infections caused by *Armillaria*.

Finally, in this study, *A. gallica* had a wide host range, was observed as both a pathogen and a saprophyte, and was identified in all three U.S. states surveyed. The lack of information about individual species of *Armillaria* limited our ability to make comparisons between ecological roles at the species level. However, results from this study provide further evidence of the adaptability of *A. gallica* already described by others (e.g. Blodgett and Worrall 1992a, 1992b; Brazee and Wick 2009, 2011; McLaughlin 2001).

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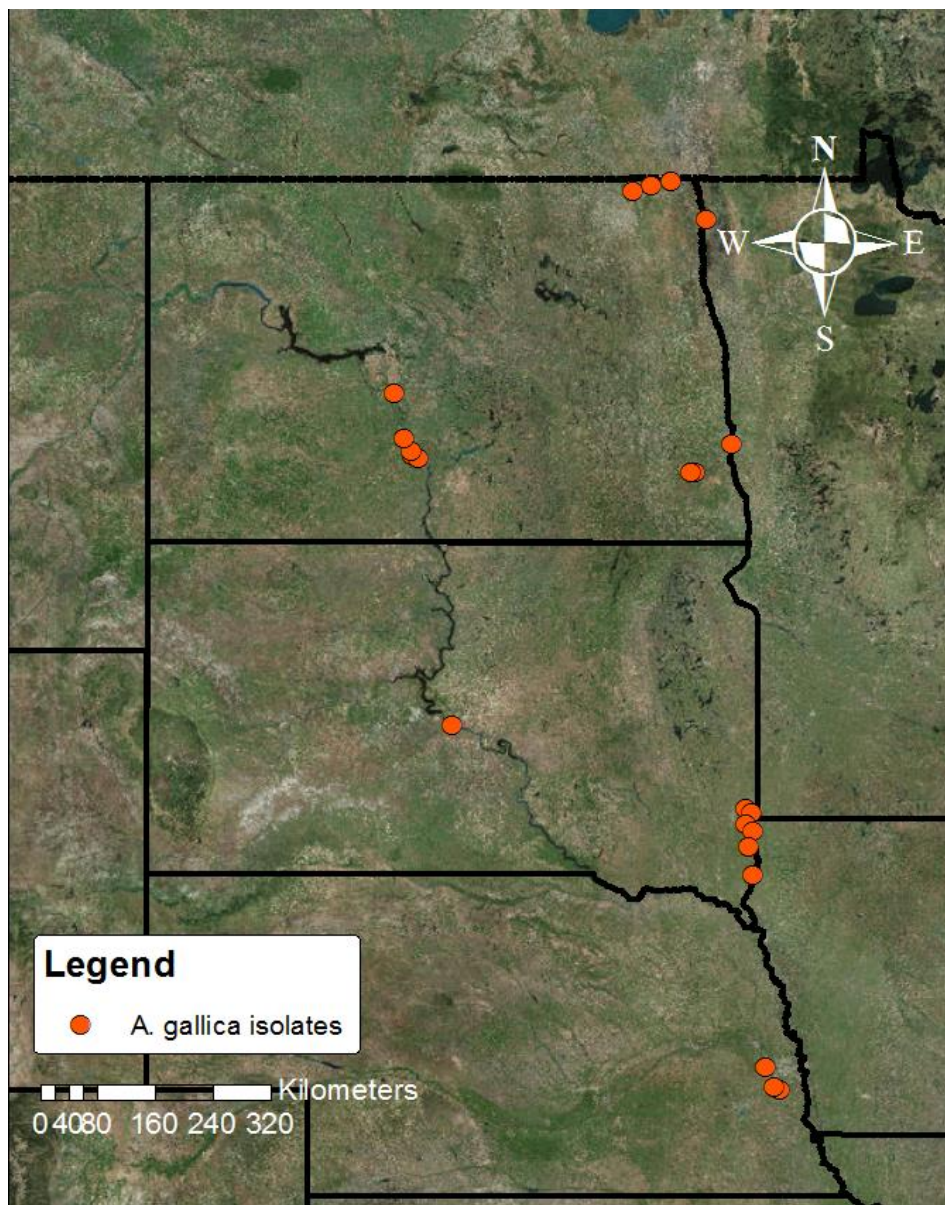


Figure 3.1: Geographic distribution of known *A. gallica* isolates in North Dakota, South Dakota, and Nebraska



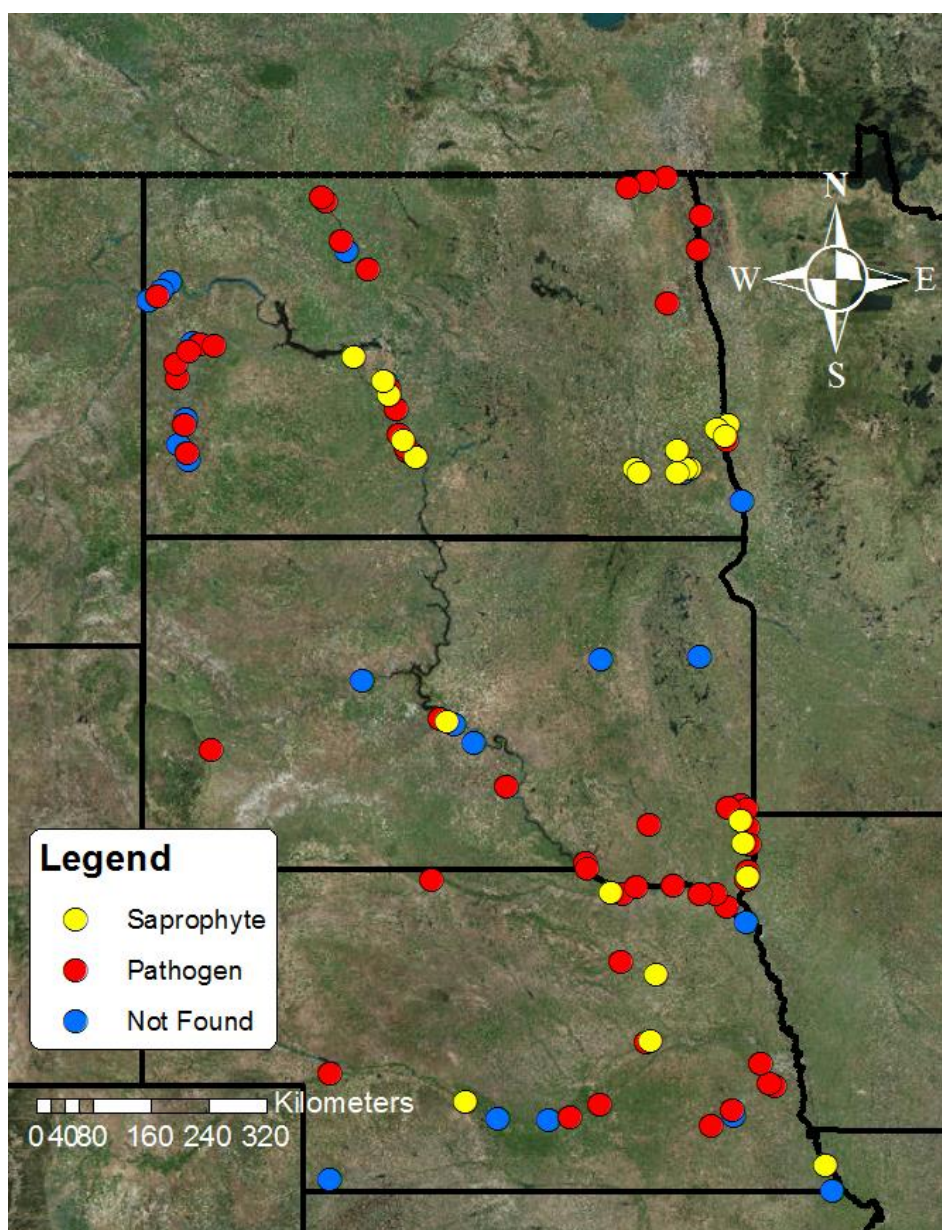


Figure 3.2: Geographic distribution of *Armillaria* isolates collected in North Dakota, South Dakota, and Nebraska in 2015 and 2016. Yellow points signify sites where *Armillaria* was observed, but not in a pathogenic capacity. Red points signify sites where *Armillaria* was observed as a pathogen, and blue points signify sites where the fungus was not found.



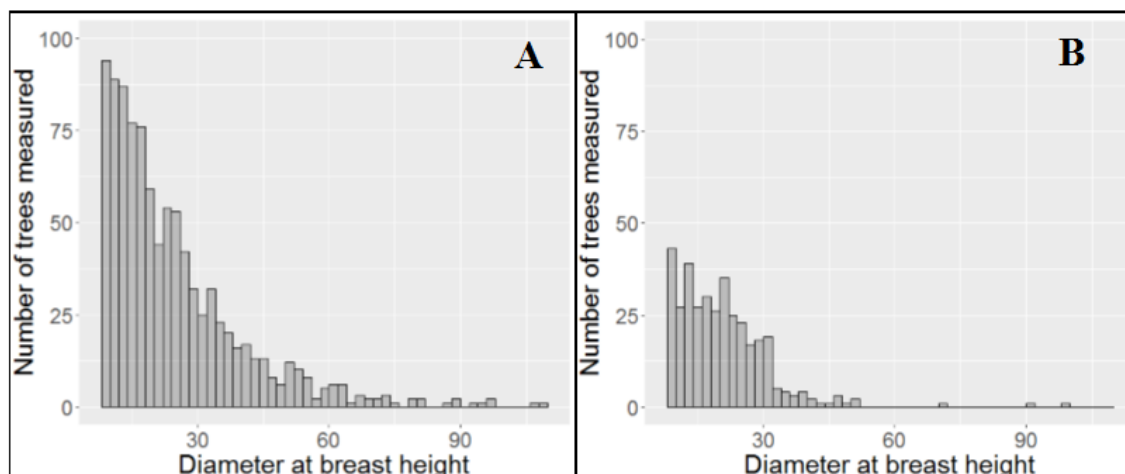


Figure 3.3: Distributions of diameter classes for trees measured at sites where *Armillaria* was (A; n = 957) and was not (B; n = 358) observed. All sites were located within North Dakota, South Dakota, and Nebraska.

Table 3.1: Nucleotide sequences of PCR primers used in the study.

Primer	Nucleotide Sequence
ARMEF-983F	5' GCY CCY GGH CAY CGT GAY TTY AT 3'
ARMEF-2218R	5' ATC ATG ACA CCR ACR GCR ACR GTY TG 3'
ARMEF-F3	5' ACGTGAYTTYATCAAGAACATG 3'
ARMEF-FI2	5' ATG CCR TGG KAY AAG GGY TGG AC 3'
ARMEF-R2	5' TAC CCG TTC GGC GAT CRA TCT 3'
ARMEF-RI2	5' GTC CAR CCC TTR TMC CAY GGC AT 3'

Table 3.2: Summary of logistic regression models used to investigate the ecology of *Armillaria* in riparian forests of the northern Great Plains.

Model*	Parameter 1 (P-value)	Estimate +/- SE	Parameter 2 (P-value)	Estimate +/- SE
Occurrence ~ Snags ha <sup>-1</sup> + Flooding	Snags ha <sup>-1</sup> (0.0984)	-0.0031 +/- 0.0019	Flooding (0.8305)	0.1234 +/- 0.5762
Occurrence ~ Flooding + Grasses	Flooding (0.8545)	-0.1037 +/- 0.5654	Grasses (0.9903)	17.6416 +/- 1458 <sup>1</sup>
Ecology ~ Snags ha <sup>-1</sup> + Flooding	Snags ha <sup>-1</sup> (0.1351)	0.0033 +/- 0.0022	Flooding (0.0384)	-1.737 +/- 0.8387
Ecology ~ Flooding + Grasses	Flooding (0.0518)	-1.5485 +/- 0.7962	Grasses (0.6128)	-0.3061 +/- 0.6048
Ecology ~ Flooding + Clay	Flooding (< 0.001)	-2.1436 +/- 1.0702	Clay (< 0.001)	-0.1623 +/- 0.1217
Ecology ~ Host DBH + Basal area ha <sup>-1</sup>	Host DBH (0.5589)	0.0074 +/- 0.0126	Basal area ha <sup>-1</sup> (0.0069)	-0.0587 +/- 0.0217

\*Occurrence and Ecology were both binary explanatory variables. Occurrence represented sites where *Armillaria* was found (1) or was not found (0), and Ecology represented the type of interaction that was observed if *Armillaria* was found (1 if pathogenic; 0 if not). Snags ha<sup>-1</sup>, Grasses, Flooding, and Clay were explanatory variables. Snags ha<sup>-1</sup> represents the estimated number of stumps and snags ha<sup>-1</sup>, Grasses the presence or absence of competitive grass species, Flooding the presence or absence of flooding, and Clay the percent clay at each site. Estimates presented in this table have not been back-transformed.

<sup>1</sup> This parameter had high standard error, and the estimate was much higher than any other observed in this study.

Table 3.3: Summary of major cover types surveyed for *Armillaria* in riparian forests of North Dakota, South Dakota, and Nebraska.

Major Cover Type*	Present	Absent	Pathogen	Saprophyte	Total (% of All Sites)
Cottonwood/minor	15	4	12	3	19 (19%)
Cottonwood	12	6	9	3	18 (18%)
Mixed hardwoods	15	2	10	5	17 (17%)
Green ash/box elder	11	1	8	3	12 (12%)
Green ash	3	3	1	2	6 (6%)
Box elder	4	1	2	2	5 (5%)

\* Twenty-four stands were excluded from this table due to small sample sizes.

Table 3.4: Summary of interactions observed between *Armillaria* and various host species in riparian forests of the northern Great Plains.

Host Species	Root Disease	Butt Rot	Stump/Snag	Epiphyte
<i>Populus deltoides</i>	15	4	5	0
<i>Fraxinus pennsylvanica</i>	13	2	3	2
<i>Fraxinus spp.</i>	0	0	1	0
<i>Quercus macrocarpa</i>	2	1	1	0
<i>Acer negundo</i>	7	4	2	2
<i>Tilia americana</i>	0	0	1	0
<i>Ulmus americana</i>	3	0	1	0
<i>Ulmus rubra</i>	0	0	1	0
<i>Acer saccharinum</i>	4	0	0	0
<i>Catalpa speciosa</i>	1	0	0	0
<i>Salix spp.</i>	4	3	0	0
<i>Morus alba</i>	3	1	0	0
<i>Morus rubra</i>	1	0	0	0
<i>Morus spp.</i>	1	1	0	0
<i>Gleditsia triacanthos</i>	1	0	0	0

Table 3.5: Mean values for estimated basal area and stumps and snags ha<sup>-1</sup> at sites surveyed for *Armillaria* in riparian forests of the northern Great Plains.

Ecology and Occur.	Basal Area (m <sup>2</sup> ha <sup>-1</sup> )	Stumps and Snags (ha <sup>-1</sup> )
Saprophyte <sup>*</sup>	26.5	166.2
Pathogen <sup>1</sup>	42.6	130.9
Present <sup>2</sup>	37.8	141.3
Absent <sup>2</sup>	29.1	191.3

<sup>\*</sup> Sites where *Armillaria* was observed, but not in a pathogenic state are represented by the term "Saprophyte."

<sup>1</sup> "Pathogen" represents sites where *Armillaria* was observed pathogenically.

<sup>2</sup> "Present" and "Absent" correspond to sites where *Armillaria* was observed and was not observed, respectively.

## Chapter 4: General conclusion

The expansive cottonwood forests of the Missouri River floodplain have decreased dramatically in area since European settlement (Dixon et al. 2012; Johnson 1992; Johnson et al. 1976). Riparian cottonwoods are of ecological significance along the Missouri (Dixon et al. 2012; Johnson et al. 1976, 2012) and throughout much of the northern hemisphere (Rood et al. 2003b). As a result, methods need to be devised to restore lost cottonwood forest and protect existing forest. While planting was a promising restoration option based on results from this study, it is not likely useful for large-scale restoration. Across seven segments of the Missouri River, Dixon et al. (2012) estimated that 435 ha of cottonwood would have to be planted each year just to offset average rates of forest losses. Thus, planting alone will be insufficient to restore large tracts of native forest, especially under continued streamflow management.

Instead, a diversified approach to cottonwood restoration using a combination of planting, prescribed flows, and floodplain vegetation management (e.g. removal of competing grasses) is likely the most viable restoration strategy for plains cottonwood. Some success has been achieved using prescribed high flows to stimulate recruitment of native cottonwood (Rood et al. 2003a). However, river hydrology and associated plant communities vary greatly, which complicates the process of developing an appropriate flow prescription. While vegetation removal (i.e. conventional tillage) was not considered a successful method of recruiting cottonwood trees in the study discussed in Chapter 2, our results indicated that larger scale studies may have more promising findings.

Though unlikely to devastate riparian forests of the region, the *Armillaria* species expected to have an influence on the northern Great Plains are all known to be pathogenic

on hardwood trees (Brazee and Wick 2009). Recent surveys of riparian forests in the region provided additional information about the ecology of *Armillaria* species, including *A. gallica*. Our research indicated that *Armillaria* was well represented in most parts of the study area, but a lack of eligible stands in the western reaches of South Dakota and Nebraska limited investigation there. *A. gallica* was associated with six host species, and *Armillaria* with at least 13. Additionally, the odds of observing *Armillaria* in a pathogenic interaction increased with flooding, percent clay, and basal area  $\text{ha}^{-1}$ . These results suggest that *Armillaria* and *A. gallica* act as facultative parasites in riparian forests of the northern Great Plains.

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