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GRADUATE COLLEGE

THE IMPACT OF NURSE PLANTS AND MICROCLIMATE  
ON SPATIAL AND TEMPORAL TRENDS OF *JUNIPERUS VIRGINIANA* L.  
SEEDLINGS IN AN OKLAHOMA GRASSLAND

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THE IMPACT OF NURSE PLANTS AND MICROCLIMATE  
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## Abstract

*Juniperus virginiana* L. (eastern red cedar) is a species of interest throughout North America as a result of its rapid encroachment into historic grasslands, but the effects of nurse trees on *J. virginiana* seedling recruitment and survival patterns have not been characterized. Understanding spatial (in relation to nurse trees) and temporal (across seasons) seedling dynamics will not only contribute to our understanding of how this species is so successful in habitats generally unfavorable to woody plants, but will also aid in efforts to eradicate it from pastures and grasslands. *J. virginiana* has expanded into much of Kessler Atmospheric and Ecological Field Station (KAEFS) in central OK, a region characteristic of the southern Great Plains. We tracked the emergence and mortality dynamics of more than 1700 *J. virginiana* seedlings underneath and just outside the canopies of 173 potential nurse trees, and collected corresponding environmental and microclimate data. We found significantly more seedlings directly underneath *J. virginiana* canopies than outside the dripline. Survival was greater than mortality in each census period, and on average mortality occurred in the smallest seedlings regardless of location. *J. virginiana* tree gender, height (m), canopy area (m<sup>2</sup>), DBH (cm), and age were all significantly related to seedling presence and abundance, with larger, female trees associated with the greatest number and density of seedlings. Microclimate conditions were significantly drier, cooler, and darker underneath tree canopies than in open environments, and tree characteristics indicative of stand density and tree position within the canopy were significantly correlated with the degree of microclimate modifications. However, specific microclimate variables could not be quantitatively linked to, nor were they useful predictors for, seedling spatial dynamics. This study marks the first time *J. virginiana* seedling dynamics have been characterized in a southern Great Plains grassland by tracking native cohorts spatially and temporally without manipulation.

Key words: eastern red cedar; *Juniperus virginiana*; seedlings; mortality; emergence; microclimate; encroachment dynamics

## Introduction

The Great Plains comprise a vast area: a mixture of prairie, steppe, and grasslands west of the Mississippi River and east of the Rocky Mountains (USDA Forest Service 2020). The southern Great Plains grasslands cover parts of Oklahoma, Kansas, New Mexico and Texas, these grass-dominated biomes are both a global and a local source of rich ecological and economic value (Engle *et al.*, 2007; Campbell 2019; USDA Forest Service 2020). In the United States, the Great Plains ecosystems are a transition zone between the eastern deciduous forests and the western desert, providing surface runoff and groundwater recharge (Zou *et al.*, 2018). The vegetation serves as an anchor and a filter: securing the soils from the strong winds, and allowing the ground the means by which to absorb water in rainfall events (White *et al.*, 2000; USDA Forest Service 2018). Grasslands are utilized as rangeland for a variety of domestic animals, cropland, and geological services such as mineral, oil, and gas extraction (White *et al.*, 2000; USDA Forest Service 2018). They also serve as ideal places for recreation, such as hiking and birding (White *et al.*, 2000; USDA Forest Service 2018). The lands are also home to native plant and animal species – many of which are endangered or in danger of becoming so – as well as historic and cultural sites (White *et al.*, 2000; USDA Forest Service 2018).

Over time, these rich, diverse landscapes were plowed to create fields, the native bison were replaced with domestic stock, and woody species were introduced as wind-breaks (White *et al.*, 2000; Engle *et al.*, 2007; USDA Forest Service 2018). The traditional combination of frequent fires and water limitation resulted in grasslands remaining free of woody plant influence; however, anthropogenic effects (limiting fire frequency and modifying ecosystems) resulted in a shift – historically inhospitable, grasslands became a resource untapped to woody plants (Briggs *et al.*, 2002). Woody species began increasing in range due to a complex combination of environmental changes such as increased fire control, livestock overgrazing, and climate change (Schmidt and Stubbendieck 1993; Briggs *et al.*, 2002; Farjon 2013). The growth of woody species into areas where they were traditionally absent (woody plant encroachment) represents the expansion in range of species historically confined to a particular area (Engle *et al.*, 2007). Woody plant encroachment results in shifting ecosystem dynamics: altering resource availability, shifting carbon storage from below to aboveground, and increasing competition between native species and those newer species attracted to the shelter and forage provided by shrubs and trees (Briggs *et al.*, 2002; Van Auken 2005; Engle *et al.*, 2007; Van Auken 2009; van

Els *et al.*, 2010; McKinley and Smith 2011; Blair *et al.*, 2014; Zou *et al.*, 2015; Davis *et al.*, 2018). In Oklahoma, *Juniperus virginiana* is a species of particular concern, as it is rapidly replacing grasslands (Norris *et al.*, 2001; Wang *et al.*, 2018; Zou *et al.*, 2018). As of 2010, it was demonstrated that juniper trees had taken over 130,000-ha of grasslands in Oklahoma, with a 4,800-ha year<sup>-1</sup> rate of increase (Wang *et al.*, 2018). Anadón *et al.* (2014) investigated the impact of woody plant encroachment on livestock production on rangelands in the USA. They quantified the negative impact that encroachment had on livestock production – a 1% increase in tree cover resulted in an overall decrease of 0.57 reproductive cows per km<sup>2</sup> in the United States (Anadón *et al.*, 2014).

A long-lived evergreen, the trees grow in a conical form with branches growing up and outward (Smith 2011). Dense, compact canopies result in the area immediately under and around the tree being shielded from the most extreme environmental conditions by intercepting radiation, modifying temperatures, ameliorating wind speed effects, increasing humidity, and decreasing soil temperatures (van Els *et al.*, 2010; Davis *et al.*, 2018). *J. virginiana* are dioecious, reaching sexual maturity around 10 years old; male and female cones are formed at different times of the year (male cones in the fall and female cones in the spring) (Johnsen and Alexander 1974; Lawson 1990; Smith 2011). Extensive shallow roots coupled with deep taproots allow this organism to establish and thrive in a variety of habitats ranging from open fields to dense woodlands, and along an altitudinal gradient from 1 m up to 1000 m above sea level (Lawson 1990; Smith 2011; Farjon 2013).

Ecologically, *J. virginiana* encroachment into grasslands has severe impacts on nutrient resources and changes biomass and carbon storage from primarily belowground to aboveground (Briggs *et al.*, 2002; McKinley and Van Auken 2005; Van Auken 2009; van Els *et al.*, 2010; Blair *et al.*, 2014; Zou *et al.*, 2015; Davis *et al.*, 2018). Thriving in disturbed habitats and as an aggressive competitor, *J. virginiana* has been shown to encroach and convert an open grassland into a closed-canopy forest in approximately 40 years (Briggs *et al.*, 2002). Rapid rates of encroachment and grassland conversion result in the alteration of grassland characteristics and productivity, as *J. virginiana* can significantly affect not only the understory composition, but potentially the overstory composition as well (Briggs *et al.*, 2002; Van Els *et al.*, 2010). The evergreen canopies create deep litter layers, altering the physical and chemical properties of the soil, which has been shown to decrease understory richness, allowing growth of only shade-

tolerant, hardy species and *J. virginiana* seedlings (Van Els *et al.*, 2010; Zou *et al.*, 2015; personal observation).

Horncastle *et al.* (2004) and Holthuijzen *et al.* (1986) identified birds as the main dispersal mechanism for the female cones, resulting in a random dispersal pattern with decreasing with increasing distance from cone-bearing trees. Seeds tend to accumulate along fence lines and near forest edges – where birds perch after feeding (Holthuijzen *et al.*, 1986); however, significant accumulation of seeds does not occur, as seed fullness and viability decline exponentially over time, resulting in short-lived seed banks (Holthuijzen and Sharik 1984). Cone crops also accumulate underneath mature trees, as well as throughout open grasslands, and their success in spatially diverse locations raises questions regarding the mechanisms by which the seedlings are able to establish and succeed. Joy and Young (2002) quantified the extent to which nurse plants modify the environment in which seedlings are found. Focused on facilitation in harsh environments associated with primary succession, the importance of solitary trees to establishment and dispersal of mid-successional woody seedlings was determined to be significant (Joy and Young 2002). Mature trees increased bird dispersed seed arrival (species abundance was relative to tree size), moderated edaphic characteristics and microclimate, and generally facilitated greater woody species abundance and richness in their immediate vicinity, findings which were corroborated with other *Juniperus* species in other environments (Joy and Young 2002; Wayne and Van Auken 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005). The majority of published literature focuses on mature tree characteristics and effects; however, very few studies have focused specifically on seedling dynamics, and fewer still on *J. virginiana* seedlings.

It has been demonstrated that *J. virginiana* seedlings had a greater survival rate when transplanted to plots that were grazed and then fenced – this was attributed to the indirect effect of grazing: a decrease in plant competition (Schmidt and Stubbendieck 1993). McKinley and Van Auken (2005) quantified a significant three-way interaction between canopy, light level, and water availability on *J. ashei* J. Buchholz seedlings in *Juniperus* woodlands. Using a manipulative study, the researchers quantified a positive seedling response to increased light levels, but only when combined with additional water and nutrients (McKinley and Van Auken 2005). There is a complex relationship present – the mature trees shade the seedlings, facilitating growth in dry conditions, but also limiting growth in wet conditions through

competition for light (McKinley and Van Auken 2005). Statistically seedling density and mortality can be related to the location of the seedlings in relation to mature tree canopies, with the greatest density and lowest mortality being directly underneath the mature tree canopy, and the lowest density and greatest mortality (100%) in an open grassland (Van Auken *et al.*, 2004).

Published literature to date has not specifically addressed *J. virginiana* seedling establishment or success in relationship to mature tree canopies and microclimate effects. The literature has also not established whether *J. virginiana* mature tree canopies facilitate seedling establishment and growth to the same degree as other *Juniperus* species (Wayne and Van Auken, 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005). Essentially, the seedling dynamics of *J. virginiana* are largely unknown. It is the purpose of this thesis to investigate the following question: what abiotic and biotic factors facilitate or restrict *Juniperus virginiana* seedling survival and growth in a southern Great Plains grassland?

The first chapter of this thesis focuses on the spatial and temporal dynamics of *J. virginiana* seedlings. Physical characteristics, density, mortality, and emergence rates of seedlings are reported seasonally over the course of a year. The relationship of mature tree canopies to seedling dynamics and the degree to which interactive effects are present is examined. The second chapter further analyzes the relationships identified in chapter 1. Microclimate effects on seedling temporal and spatial dynamics are ascertained, as well as the extent of the effect of mature canopies on the microclimate. Relationships are established and analyzed to determine whether microclimate effects are indirect or direct. This research will present novel findings for a native Oklahoma species, characterizing *J. virginiana* seedling dynamics and contributing to the literature regarding woody plant encroachment.

## **Chapter 1:** Temporal and spatial trends of *Juniperus virginiana* seedlings in an Oklahoma grassland

### **Introduction**

The global value of grasslands, and an ever-growing human population, is driving increasing interest in land management practices that can maximize food production, while still promoting a multitude of other functions such as carbon sequestration and storage (Yang *et al.*, 2019). A combination of anthropogenic activities and climate change is resulting in increased rates of change in grasslands, where historically herbaceous communities are becoming home to woody plant species (Briggs *et al.*, 2002). Grassland regeneration is disrupted by the lack of fires, and woody species are gradually encroaching into areas where they were historically destroyed by frequent fires (this movement is referred to as woody plant encroachment) (Briggs *et al.*, 2002; World Wildlife Fund 2019). Both Engle *et al.* (2007) and Ganguli *et al.* (2008) identified the expansion of woody plants as the driving factor in the shifting dynamics and decline in habitat quality in southern Great Plains grasslands. A species of particular interest in the south-central part of North America is *Juniperus virginiana* (family Cupressaceae) (Farjon 2013). Common throughout the eastern United States, its range has been artificially extended as a result of reforestation, shelter belts, and wildlife planting (Johnsen and Alexander 1974; Lawson 1990; Smith 2011). The species is increasing its density, cover, and biomass in areas it historically thrived, as well as expanding into grasslands (Van Auken 2009).

Holthuijzen *et al.* (1987) described eastern red cedar as an early successional species – large cone crops at early ages facilitate rapid colonization of open habitats – and a nonconforming pioneer plant species, with a high cone removal rate (61%) and a short seed dormancy period (~1 growing season), unlike other pioneer species. Historically, the limited dormancy of the seeds was most likely an evolutionary adaptation, as extended dormancy would not have been a positive factor in the environments *J. virginiana* was limited to (rock outcrops, etc.). In open grasslands and abandoned pastures these factors all combine to make the species a successful encroacher, with large cone crops, diverse dispersal mechanisms, and physiological adaptations (Holthuijzen *et al.*, 1987). It has been demonstrated that the canopies have varying effects in rainfall events, with smaller trees funneling water to the areas immediately underneath and surrounding the tree, creating areas with high concentrations of water and nutrients, and

larger trees contributing significantly to canopy interception (Smith 2011; Zou *et al.*, 2015). With dense, compact canopies, the area immediately under and around the tree is shielded from the most extreme environmental conditions by intercepting radiation, resulting in modified temperatures as well as ameliorating wind speed effects, increasing the humidity, and decreasing soil temperatures (van Els *et al.*, 2010; Davis *et al.*, 2018). The canopies also create deep litter layers, altering the physical and chemical properties of the soil, which has been shown to decrease understory richness, allowing growth of only shade-tolerant, hardy species and *J. virginiana* seedlings (van Els *et al.*, 2010; Zou *et al.*, 2015; Biral *et al.*, 2019; personal observation).

Ecologically, *J. virginiana* encroachment into grasslands has severe impacts on nutrient resources and changes biomass and carbon storage from primarily belowground to aboveground (Briggs *et al.*, 2002; McKinley and Van Auken 2005; Van Auken 2009; van Els *et al.*, 2010; Blair *et al.*, 2014; Zou *et al.*, 2015; Davis *et al.*, 2018). Outcompeting native grassland species, *J. virginiana* alters the plant and animal communities, creating niches for invasive plants and encroachment of other species (van Els *et al.*, 2010; Schmidt and Stubbendieck 1993; Engle *et al.*, 2007; Smith 2011). Thriving in disturbed habitats and an aggressive competitor, *J. virginiana* has been shown to encroach and convert an open grassland into a closed-canopy forest in approximately 40 years (Briggs *et al.*, 2002). Rapid rates of encroachment and grassland conversion result in the alteration of grassland characteristics and productivity, as *J. virginiana* can significantly affect not only the understory composition, but also has the potential to affect overstory composition as well (Briggs *et al.*, 2002; van Els *et al.*, 2010).

While the characteristics and consequences of *J. virginiana* have been and are currently being studied in an effort to aid land managers in preventing encroachment, little is known about the dynamics of seedling establishment and growth. McKinley and Van Auken (2005) demonstrated in a manipulative study with *Juniperus ashei* seedlings that the most important factor in growth and survival was light, with a significant three-way interaction with water and nutrient availability. It has been hypothesized that the microclimate effects of larger canopies facilitate initial establishment and growth of seedlings, but over time, as the seedlings grow and begin requiring additional resources, the relationship becomes more competitive than facilitative (Van Auken *et al.*, 2004). It is also possible that canopy buffering is necessary in drier

conditions, but becomes an obstacle to seedling growth in wet conditions (McKinley and Van Auken 2005).

The dynamic interactions between seedling establishment and spatial and environmental factors have been shown to be important for successful establishment of other juniper species (Van Auken *et al.*, 2004), but never specifically investigated for *J. virginiana*. Therefore, it is the purpose of this study to investigate the temporal and spatial trends in *J. virginiana* seedling dynamics in an encroached grassland. Our study sought to answer the following questions: i) how does seedling presence, emergence, and mortality vary seasonally; ii) how do seedling dynamics vary spatially in proximity to a nurse tree; and iii) what nurse plant characteristics are most related to seedling dynamics?

## **Methods**

### *Research Site*

This research study was conducted at Kessler Atmospheric and Ecological Field Station (KAEFS) in Purcell, OK (34.982928 lat. and -97.519809 long.). A 360-acre (146 ha) research and education facility owned by the University of Oklahoma, the property is characteristic of the southern Great Plains rural landscape. With a 15-year mean annual air temperature of 16.11°C (January min. = -2.22°C; July max. = 38.89°C) and total annual precipitation of 918.72 mm, the property sits within the Washita River drainage basin and is bisected by the Finn Creek watershed (Mesonet 2020). Soils are of the Nash-Lucien complex (Xu *et al.*, 2013).

With diverse vegetation throughout the property, slopes and hilltops are primarily dominated by grassland species, while creeks and lowlands are predominantly riparian woodlands (Kessler 2019). Tallgrass prairie on lower slope areas and upland slope areas exhibit mixed communities of species. Scattered upland areas are dominated by *Quercus stellata* (post oak) and *Q. marilandica* (blackjack oak), species native to the Cross Timbers forest of central and eastern Oklahoma. The western portion of KAEFS has been left unmanaged for 50+ years, resulting in *J. virginiana* encroachment due to fire suppression, lack of grazing, or any other type of land management. These regions exhibiting *J. virginiana* encroachment dynamics are characteristic of the southern Great Plains and offer an opportunity to monitor and quantify encroachment dynamics (Kessler 2019; Buthod and Hoagland 2016).

### *Plot Establishment*

The research was conducted along a transect demonstrating varying levels of *J. virginiana* encroachment – moving from an enclosed forest to a transition zone then into the open grassland. Three parallel transects were established in order to determine replication across time and space, as well as to capture varying encroachment dynamics. Five plots were established on each transect, each approximately 40 m in diameter and approximately 75 m apart (from center point to center point) for an encroachment survey. Each transect has approximately the same plot-level characteristics, with three plots in the grassland, one plot in the transition zone between grassland and forest, and one plot in the closed forest. This design roughly captured the relative proportions of these different vegetation communities within the study site. The center point of each plot was marked digitally using a handheld GPS (Garmin eTrex Vista® H, Olathe KS); the boundaries of the plot were marked in four or more areas using 4' wooden stakes to ensure trees tagged fell within the study area (Figure 1).

### *Tree Selection and Characterization*

All *J. virginiana* trees with a measurable diameter at breast height (DBH) (1.3 m and taller) within each plot in each transect were tagged with a unique identifying number and their location within the plot digitally tagged with the GPS. We tagged 328 *J. virginiana* trees across the 15 plots. A random subset of these trees was selected to be included in the study. Using a random number generator in Microsoft Excel® (Excel version 2019), 173 of the tagged *J. virginiana* trees were selected using a pre-determined criterion of no more than 15 trees per plot (maximum number of *J. virginiana* identified in a single plot = 65; minimum number identified in a single plot = 3). For plots with fewer than 15 *J. virginiana*, all tagged trees were automatically included. Using this selection criterion, transect 1 had 46 study trees, transect 2 had 65 study trees, and transect 3 had 62 study trees.

### *Seedling Sampling*

The area under each *J. virginiana* was divided into eight sections based on compass directions. The subdivision of the area covered by the study tree canopy was done in order to increase the accuracy of seedling observations, as *Juniperus* spp. are known to have large numbers of seedlings underneath their canopies (Van Auken *et al.*, 2004; personal observation).

The subdivision was based on compass direction and randomized, so each study tree was assigned a directional octet in order to account for any environmental differences influenced by direction. Of the 173 study trees, 30 had an octet in the ENE direction, 16 were ESE, 24 were NNE, 22 were NNW, 21 were SSE, 21 were SSW, 23 were WNW, and 16 were WSW. Octet boundaries were marked so that the extent of the study area was clear. In order to capture any differences in seedling dynamics outside the study tree canopy, the octet extended up to 1 m outside the dripline of the study tree.

All *J. virginiana* selected to be in the study were surveyed in order to characterize the study trees as well as identify relationships between tree characteristics and seedling dynamics. Spatial measurements of the octet underneath the study tree were also taken so that seedling dynamics could be examined using area as a metric. The study trees were surveyed between seedling censuses, the survey began in September of 2018, and was completed by the following June. The following data was recorded: a photo of the tree, the gender [M (male), F (female), UK (unknown)], height (m), height to canopy (cm), DBH (cm), diameter at base (cm), and canopy diameter (m) (measured N-S, then E-W, values averaged and used to calculate Canopy Area). Canopy light exposure (CLE) was also noted at each tree. This is an integer value (0 – 5) indicating the number of sides which would have more than 30% of live foliage directly exposed to light if the sun were directly above the tree (Bechtold 2003). We also estimated tree age based on relationships established at the site [ $12.776 + (0.535 \cdot \text{DBH}) + (1.219 \cdot \text{CLE}) + (0.703 \cdot \text{Canopy Area})$ ] (Giddens and McCarthy, unpublished data).

Based on the age estimates, a fourth gender category was established – reproductively immature (RI) – to capture the trees which had not yet reached reproductive maturity (~10 years old) (Lawson 1990; Johnsen and Alexander 1974). The number of trees with an unknown gender is driven by growth conditions. It has been demonstrated that open grown trees and trees at the edge of stands produce cones at earlier ages as a result of more sun exposure, as the length of the juvenile period depends on interacting effects of chronological age, physiological conditions, and environmental influences (Krugman *et al.*, 1974).

### *Seedling Censuses*

Seedling censuses were taken four times over the course of this 12-month research study. The first census was taken May – June, 2018 (Summer 2018); second December, 2018 – January

2019 (Winter 2018/19); third March, 2019 (Spring 2019); fourth September 2019 (Summer 2019). The censuses were irregular due to time and site access constraints. All seedlings within the octet of a given study tree (underneath the tree canopy and up to 1 m outside the dripline) were tagged with a unique number using a write-on metal tag. The tag was secured to the ground as close as possible to the seedling using a 4" barn nail spray-painted red for visibility. Some tags were attached using a 4" black zip-tie, primarily for seedlings larger than 30 cm tall. At the first census all seedlings were tagged within the study area (octet) underneath each *J. virginiana* and assigned to the first cohort of this study.

The date tagged, height (cm), number of branches, basal diameter (mm), and location in relationship to the study tree (U = under study tree canopy; O = outside study tree dripline, in the open; C = outside study tree dripline under external canopy type) were recorded. Height was measured with a ruler, and basal diameter was measured using digital calipers (Pittsburgh 6-in Digital Calipers). Subsequent censuses were done in order to track the survival and mortality of the first cohort, as well as capture and track subsequent cohorts. Seedlings were marked as "present" (surviving seedlings), "absent" (seedlings which died or were absent since previous censuses), or "new" (seedlings which emerged since the previous census). If a previously tagged seedling could not be found it was marked as "unknown", and in the event of two consecutive unknown categorizations the seedling was considered absent (Van Auken *et al.*, 2004). Less than 0.4% of tagged seedlings throughout the entire study were marked "unknown".

### *Data Analysis*

Data was analyzed using R statistical software (v. 3.6.2; R core team 2019) in RStudio. Total seedling count, density, percent mortality, and percent emergence were compared within and across censuses by location in relationship to the study tree canopy. Repeated measures analysis of variance (ANOVA) was used to analyze seedling mortality ("absent" seedlings) and emergence ("new" seedlings) across censuses and location. Model validity was checked using histograms of the residuals, q-q plots, and residuals vs fitted plots. Percent mortality and emergence was arcsine square root transformed. Tests were considered significant when the p values were less than 0.05. Multivariate analysis of covariance (MANCOVA) was utilized to examine relationships between seedling dynamics and the study trees. Student's t-tests were used to identify when and where significant differences occurred. Changes in seedling dynamics

across censuses and location (plot and location in relationship to the study tree, U, C, and O) were analyzed using repeated measures ANOVA with census, plot, and location as response variables, and percent mortality or emergence as the dependent variable. Comparison tests between seedling dynamics and census, plot, and location were done using Tukey's Honestly Significant Difference test. Decision trees were generated using study tree characteristics and Summer 2019 seedling data. Study tree characteristics were analyzed using Pearson's correlation and common variables above 0.8 were removed. The Party package in R was used to generate the decision trees (Hothorn *et al.*, 2006). Environmental conditions documented by the Washington Mesonet site (located at Kessler, less than 1 mile from the study area) were requested in order to analyze changes in air temperature, solar radiation, and cumulative rainfall throughout the course of the study (Mesonet 2020). The data was analyzed in order to determine if seasonal differences influenced seedling dynamics.

## **Results**

### *Study Tree and Seedling Characteristics*

Three transects were established for replication; both tree number and seedling abundance varied at the transect and plot level. Transect 1, with the fewest study trees (46), contained the largest trees on average as well as more than half of the total seedlings tagged (Table 1; Appendix). Plots 3, 4, and 5 contributed the most seedlings to the study, with plot 4 alone outnumbering transect 2 and 3 (Appendix). Average seedling density per tree was greatest for plots 3 and 4 in every census ( $> 3.8$  seedlings/m<sup>2</sup> in the octet). Thus, plot was included as a factor in subsequent analyses in order to account for spatial disparities. The number of seedlings found in each plot ranged from 1 to 572. Seedling characteristics varied in each plot, with basal diameter ranging from  $<1$  mm to 22.27 mm and height from  $<1$  cm to 180 cm (Appendix).

### *Seasonal Trends in Presence, Emergence, and Mortality*

The initial census in Summer 2018 recorded a total of 1274 seedlings present throughout the entire study area. This number declined through the winter (2018/19) and spring (2019) then increased to 1360 by the following summer (2019) (Appendix), an increase of 7%. Percent survival was significantly greater than mortality in each census, regardless of location (all  $p < 0.03$ ). Based on repeated measures ANOVA, both seedling mortality and emergence differed

significantly across censuses (all  $p < 0.01$ ), but not across plots (all  $p > 0.57$ ). Each census yielded different mortality and emergence dynamics: mortality was significantly greater ( $p < 0.05$ ) than emergence in Winter 2018/19, emergence and mortality were not significantly different ( $p = 0.59$ ) in Spring 2019; and Summer 2019 yielded an emergence which was significantly greater ( $p < 0.01$ ) than mortality (Appendix). Across censuses, emergence rates were significantly higher in Summer 2019 than either of the previous censuses (31.3% vs 3.4% and 1.2%; all  $p < 0.0003$ ; Appendix). Mortality rates were similar in Winter 2018/19 and Summer 2019 ( $p = 0.13$ ), but were significantly lower in Spring 2019 ( $p < 0.01$ ; Appendix).

### *Seedling Dynamics in Relation to Nurse Tree Canopies*

We assessed seedlings underneath study tree canopies, as well as in the area extending 1 m beyond study tree canopy driplines, in order to explore seedling dynamics underneath and outside nurse tree canopies. Seedling mortality, emergence, and survival, as well as seedling count and density, all differed based on seedling position relative to study trees (Table 2 and 5). The area outside the study tree canopy dripline was classified as either being open (O) or covered by an adjacent canopy type (C). Across all censuses, the majority of seedlings were present underneath the study trees (U), with the lowest number of seedlings present in the open (O), and seedlings outside the dripline under an adjacent tree canopy (C) falling somewhere between the two (Table 2). The density of seedlings by location (U, C, and O) in relationship to the study tree also varied significantly (all  $p < 0.004$ ). The final census yielded an average seedling density which was highest in U, with 1554% more seedlings than in O and 147% more than in C. Seedling density was 570% higher in C than O (Table 2).

Seedlings under an adjacent tree canopy (C) were either under evergreen canopy (*J. virginiana*), deciduous canopy, or a combination of the two. In order to determine whether the type of canopy impacted seedling numbers, we divided C into different canopy cover types (deciduous, evergreen, or both). Data from Summer 2019 was used in order to maximize the number of adjacent canopy cover types which had seedlings present. Deciduous cover mean seedling density was 0.82 seedlings/m<sup>2</sup>, evergreen (*J. virginiana*) was 3.69 seedlings/m<sup>2</sup>, both was 2.07 seedlings/m<sup>2</sup>, and the mean seedling density for the open (O) was 0.48 seedlings/m<sup>2</sup> (included for comparison; Figure 2). Adjacent *J. virginiana* canopies had significantly more

seedlings than deciduous canopies ( $p < 0.02$ ) and the open ( $p < 0.01$ ), but seedling densities were not statistically different between the deciduous, both, and open categories (all  $p > 0.13$ ).

Height (cm) and basal diameter (mm) distribution for seedlings that survived to the end of the study were normalized and compared across seedling locations to determine if there were differences in size of seedlings underneath and outside nurse tree canopies (Figure 3). The majority of seedlings, regardless of location, fell into the lower categories of basal diameter, with most in the 0–2 mm range. Height had a wider spread, with most seedlings U and C standing 3–6 cm tall, while O seedlings tended to be larger, ranging from 9 to 15 cm tall (Figure 3).

Over the course of the study an overall increase in total seedlings and seedling density was observed for all locations (Table 2). Seedling numbers increased by 6%, 12%, and 8% for U, C, and O respectively. Although these increases are fairly similar, seedling numbers were more stable in O than U or C, due to mortality and emergence patterns. Mortality (dead seedlings/m<sup>2</sup>) in U was significantly greater than in O ( $p < 0.003$ ) in every census (Table 2; Figure 4; Appendix). Emergence data (new seedlings/m<sup>2</sup>) yielded no significant location density differences except for Summer 2019, where emergence rate in U was significantly higher than in O ( $p < 0.05$ ; Table 2; Appendix). Repeated measures ANCOVA showed that, for mortality, seedling location and sampling area (m<sup>2</sup> area underneath canopy or outside the dripline) were highly significant ( $p < 0.008$ ) interactive variables: as the area sampled (U or O) increased so did the number of seedlings that died. There were no sampling area effects on mortality in C; the mortality remained constant regardless of the area sampled. Seedling emergence exhibited a different relationship: location and area sampled were not significant ( $p > 0.5$ ), only census appeared as an explanatory variable ( $p < 0.0001$ ).

For each census, the average size of seedlings that died and those that survived were compared in order to determine if seedling characteristics (measured when initially tagged) explained mortality patterns (Table 3). Seedlings that were dead at the Winter 2018/2019 census were similar in size (height and basal diameter; all  $p > 0.24$ ) to those that were dead in the Spring 2019 census. However, seedlings in U and C that were dead in the Summer 2019 census were significantly larger (all  $p < 0.01$ ) than those that died in previous censuses. Size dynamics for mortality in O across censuses were not significantly different (all  $p > 0.15$ ), with seedlings of similar size dying in each census. Average height and basal diameter of surviving seedlings

remained similar (all  $p > 0.09$ ) across censuses. Seedlings that survived were significantly (all  $p < 0.02$ ) larger than seedlings that died within each census and location.

Examining location dynamics within each census yielded varying results. There were no significant differences (all  $p > 0.06$ ) in average height or basal diameter of dead seedlings between locations (U, C, or O) in Winter 2018/19 or Summer 2019. However, in Spring 2019, average basal diameter of dead seedlings in C was significantly greater ( $p = 0.02$ ) than in U [the remaining location comparisons, C vs O and U vs O, were not significantly different (all  $p > 0.2$ )]. Height of dead seedlings did not vary by location (all  $p > 0.17$ ). Characteristics of surviving seedlings varied by location, according to census. Surviving seedlings in Winter 2018/19 were significantly taller and had larger basal diameters (all  $p < 0.033$ ) in C than in U and O, and seedlings in U were significantly taller ( $p < 0.005$ ) but not significantly larger ( $p > 0.06$ ) than seedlings in O. The same height trends were observed in Spring and Summer 2019 (all  $p < 0.04$ ), but surviving seedlings in C were significantly larger (all  $p < 0.018$ ) than seedlings in U and O, and U seedlings were significantly larger ( $p = 0.014$ ) than seedlings in O.

There were no differences (all  $p > 0.2$ ) identified in the environmental variables measured at the Washington Mesonet site (air temperature, solar radiation, and cumulative rainfall) between years (2018 and 2019) or individual seasons (e.g. fall 2018 vs fall 2019); the only significance identified was between different seasons (e.g. summer 2018 vs fall 2018; all  $p < 0.01$ ; Table 4).

### *Correlating Nurse Plant Characteristics with Seedling Dynamics*

*J. virginiana* study trees vary in size and age, with heights between 1.40 m and 10.2 m, DBHs from 0.20 cm to 30.8 cm, canopy areas of 0.38 m<sup>2</sup> to 84.95 m<sup>2</sup>, and estimated ages from 8.26 to 75.86 years old (Table 1). This large range in characteristics allowed us to assess correlations between study trees and seedling dynamics. Study tree gender, height (m), canopy area (m<sup>2</sup>), DBH (cm), and age were all significantly related to seedling presence and abundance (all  $p < 0.03$ ) using repeated measures ANOVA. Figure 5 illustrates the density of seedlings in the octet (U and C and/or O) at each study tree in relationship to tree characteristics, separated by gender. For each variable separately, seedling density under female trees was positively correlated with height, canopy area, DBH, and age (all  $p < 0.01$ ; adjusted R<sup>2</sup> values ranging from

0.2 – 0.3), while for other gender categories no significant relationships were evident (all  $p > 0.07$ ; Figure 5).

Study tree and seedling data from Summer 2019 were used to create decision trees in order to predict the presence and number of seedlings at study trees (Figure 6). Characteristics which were highly correlated ( $> 0.8$ ) to one another included age and DBH, and as a result these variables were not included in the decision tree models. Summer 2019 data was used for these models because it represents the maximum number of seedlings present. Tree height was the most significant variable for predicting seedling presence ( $p < 0.001$ ). For trees less than or equal to 3.7 m in height, there was a ~50% chance the tree would have seedlings while for trees greater than 3.7 m in height the chance of seedlings increased to above 80%. For predicting the actual number of seedlings for a given tree, canopy area, height, and gender were all selected by the model (all  $p < 0.0031$ ; Figure 6), with canopy area the most significant variable ( $p < 0.001$ ). For trees with a canopy area greater than 19.63 m<sup>2</sup> female trees had an average of 50 or more seedlings present; if the tree belonged to any other gender category the average was near 0 with a maximum of <25 (gender  $p = 0.003$ ). For trees with canopy areas less than or equal to 19.63 m<sup>2</sup>, if the height ( $p < 0.001$ ) was less than (or equal to) 6.5 m, the average number of seedlings was near 0 with a maximum range of ~25 for all gender categories ( $p < 0.001$ ). If the height was greater than 6.5 m the average for seedlings was closer to 10 with a range of ~50.

Decision trees were also generated to predict mortality and emergence (Figure 7). Predicting whether seedlings would die involved a combination of canopy area ( $p < 0.001$ ), location in relation to the tree (U, C, and O) ( $p < 0.001$ ), and CLE ( $p = 0.015$ ). Canopy areas greater than 15.21 m<sup>2</sup> had a high (above 70%) mortality rate in U, but a low (<20%) mortality rate in C and O. Canopies less than or equal to 15.21 m<sup>2</sup> had no mortality in O, and mortality for U and C was driven by CLE: with one side of the tree exposed to light there was ~30% chance of mortality, trees with any other level of light exposure had ~10% chance of seedling mortality. Predicting seedling emergence was more complex, involving location ( $p < 0.001$ ), height ( $p < 0.001$ ), canopy area ( $p = 0.002$ ), and gender ( $p = 0.017$ ). Emergence in O was <10% likely, with no additional influencing factors. Emergence in U and C was driven first by height: for trees less than or equal to 4.4 m, larger canopy areas yielded the greatest (~40%) emergence rates, while smaller canopy areas yielded ~15% emergence rates. For trees greater than 4.4 m in height,

emergence in C was ~20%, while emergence in U was dictated by gender: female and male trees had ~80% chance of emergence, while the other gender categories were closer to 40%.

## Discussion

In order to characterize the factors influencing encroachment in a southern Great Plains grassland, our study captured *J. virginiana* seedling and tree dynamics both temporally, over the period of a year, and spatially, in relation to nurse trees. We identified seasonal and location-dependent seedling dynamics, with seedling presence, emergence, and mortality following similar trends across space [under our study trees (U), under an adjacent *J. virginiana*/deciduous canopy (C), and in the open (O)] and time (censuses 1 – 4). Describing seedling success based on proximity to study trees allowed us to identify significant (all  $p < 0.004$ ; Table 2, Summer 2019 data) differences in dynamics in each location and provide insight into the effects of nurse plants, both evergreen (*J. virginiana*) and deciduous. Seedling data was correlated to study tree characteristics such as gender and size [height (m), canopy area (m<sup>2</sup>), DBH (cm)] to such a degree that we were able to craft models, which could be used to predict seedling presence and abundance (as well as mortality and emergence) underneath and within 1 m adjacent to *J. virginiana* trees.

### *Study Tree and Seedling Characteristics*

Study tree and seedling differences across plots were most likely long-term effects of *J. virginiana* encroachment dynamics. Literature indicates some directionality in *J. virginiana* encroachment patterns, but this may vary depending on spatial scale (Castellano and Boyce 2007; Wang *et al.*, 2018). The study trees in plots 3, 4, and 5 were larger, older trees which had been present at Kessler a greater amount of time than other trees selected for inclusion in this study. It has been demonstrated that the viability of *J. virginiana* seeds decreases exponentially over time, as well as the fact that seedlings do not tend to emerge from the litter of trees less than 20 years old (Holthuijzen and Sharik 1984; Holthuijzen *et al.*, 1987). While the impact of the seed bank would be minimal, greater seed input from older trees over time, compared to plots with younger organisms, would ultimately result in comparatively greater emergence rates (Holthuijzen *et al.*, 1987). Many of the largest trees in transect 1 are located along a fence line. These are most likely a byproduct of avian dispersal and may be one of the locations of earliest

encroachment into the site, as birds tend to move in a directional pattern and fence lines have been identified as prominent gathering places and, consequently, locations of greater seed input (Holthuijzen *et al.*, 1987). Additionally, tree size (height, canopy width, and basal diameter) has been directly related to abundance of bird-dispersed woody species, indicating a preferential selection of larger trees by birds (Joy and Young 2002). Therefore, the fact that transect 1 contains both the largest trees (on average) and the most seedlings is attributable to the fact that larger trees are statistically more likely to be female with large numbers of seedlings present (Figure 6, B). Seedling size varied across plots and locations, most likely a direct effect of variables described above – increasing seed inputs with increasing size of female *J. virginiana* and seed dispersal mechanisms (Holthuijzen *et al.*, 1987).

#### *Seasonal Trends in Presence, Emergence, and Mortality*

Van Auken *et al.* (2004) reported a total of 87% mortality of *J. ashei* seedlings in 1 m<sup>2</sup> quadrats just outside a mixed woodland canopy dripline in the first two growing seasons of the study, along with low/no emergence. Our maximum observed mortality outside the dripline of the study trees (O) was 11.11% along with a maximum emergence rate of 11.39% (Table 2). Differences in reported trends may be attributable to different *Juniperus* species, the time-frames in which the data was collected, or environmental differences between San Antonio, TX, and Kessler, OK. *J. ashei* has a much more limited range than *J. virginiana* (Johnsen and Alexander 1974), thereby utilizing different physiological traits in response to different climate conditions. Van Auken *et al.* (2004) reported on data collected over 2 years (1994 – 1995) and our study spanned 12 months (2018 – 2019); the reported minimum monthly mean temperature for the study location in TX was higher, and the maximum monthly mean temperature was lower, than those recorded at the Washington Mesonet site at Kessler, OK. Difference in range of temperatures throughout the study had a significant negative effect on seedling survival (Wayne and Van Auken 2002; Van Auken *et al.*, 2004).

Total seedling numbers fluctuated from census to census (Appendix), decreasing through the winter and into the spring, then increasing in the summer. It was reported for *J. ashei* that the majority of seedling emergence occurred in the late fall, over winter, and in the first months of spring (Van Auken *et al.*, 2004). While our study recorded some new seedlings in the late summer through winter months, we observed the greatest emergence rates between Spring and

Summer 2019 (31.3%; all  $p < 0.0003$ ; Appendix). These differences are attributable to the different phenology of *J. virginiana* and *J. ashei*: the majority of *J. virginiana* seed germination has been described as occurring in the early spring, 1 – 2 years after dispersal, during February and March, while *J. ashei* seeds germinate in the fall and winter (Johnsen and Alexander 1974; FEIS 2003). Emergence and overall seedling numbers were much more dynamic for U and C as compared to O, which had overall less emergence and less mortality (Table 2). Mortality patterns revealed lower mortality and higher emergence for O as compared to U and C in the winter, whereas in the summer O continued to demonstrate lower mortality rates, but also lower emergence. This is most likely a combined result of decreased seed inputs (Holthuijzen *et al.*, 1987; Horncastle *et al.*, 2004) and increased environmental stressors outside the canopy dripline (Van Auken *et al.*, 2004; McKinley and Van Auken 2005; van Els *et al.*, 2010; Zou *et al.*, 2015; Davis *et al.*, 2018; Biral *et al.*, 2019).

Environmental data from the nearby (< 1 mile) Mesonet site yielded no significant variations (all  $p > 0.2$ ) between years (2018 vs 2019) or individual seasons across years (Spring 2018 vs Spring 2019; Mesonet 2020). Lack of significant differences indicates similar environmental conditions in a given season in each of the two years.

### *Seedling Dynamics in Relation to Nurse Tree Canopies*

In a study of *J. ashei* at a woodland-grassland edge, the majority of seedlings (96%) were present underneath the woodland canopy, ~2% of the seedlings were along the canopy edge in the grassland, and ~2% were 5 m outside the woodland canopy dripline (Van Auken *et al.*, 2004). Our research demonstrated similar seedling dynamics for *J. virginiana*, with 94% of the seedlings underneath a canopy and 6% of the seedlings outside the dripline in the open grassland. Seedling density Summer 2019 was significantly greater in U than in C or O (all  $p < 0.004$ ; Table 2), which was expected as a result of increased seed input and predicted higher survival (Holthuijzen *et al.*, 1987; Horncastle *et al.*, 2004). Adjacent *J. virginiana* facilitated greater seedling densities ( $p < 0.02$ ) compared to adjacent deciduous canopies, likely a combination of additional seed input and preferential perch selection by avian dispersers (Holthuijzen *et al.*, 1987; Joy and Young 2002). Seedling densities were not significantly different (all  $p > 0.13$ ) for external canopy types comprised of only deciduous or a combined deciduous/*J. virginiana* canopy, suggesting that, while shading effects from both deciduous and evergreen sources would

be invaluable for growing seedlings, the evergreen canopies provide better conditions for success.

Seedling mortality in U and O showed similar rates for smaller trees, but increased with increasing sampling area. As larger sampling areas were associated with larger trees, this is most likely the result of a greater number of seedlings being present. Underneath the tree canopy this would result in greater competition for resources, and outside the canopy there would be increased competition due to the presence of other plant species, and little to no microclimate buffering as the seedlings grew. Emergence was similar for all three locations (U, C, and O) at smaller sampling areas, with the density of seedlings emerging in U increasing with increased area underneath the tree canopy, and C and O densities remaining relatively constant regardless of area. Increased density of new seedlings underneath *J. virginiana* study trees compared to outside the dripline was most likely a combination of greater seed inputs as well as microclimate buffering, creating an ideal location for emergence (Van Auken *et al.*, 2004; McKinley and Van Auken 2005; van Els *et al.*, 2010; Zou *et al.*, 2015; Davis *et al.*, 2018; Biral *et al.*, 2019).

Average seedling size was different in U, C, and O, with seedlings in C significantly larger (all  $p < 0.035$ ). There were no significant differences (all  $p > 0.10$ ) in seedling size for those which survived across the censuses, but there were significant differences in size for seedlings which died (all  $p < 0.006$ ). Mortality dynamics did not significantly change (all  $p > 0.24$ ) until Summer 2019, when seedlings U and C that died were significantly larger (all  $p < 0.006$ ) than those that died in the two previous censuses. The summer months caused greater stress to seedlings U and C compared to the winter months, most likely due to a combination of increased temperatures and drought conditions (Joy and Young 2002; Wayne and Van Auken 2002; Van Auken *et al.*, 2004). Greater overall mortality in U and C compared to O may be attributable to both greater seedling densities and smaller seedling sizes – increased competition for resources and diminished physical characteristics would cause larger numbers of seedlings to die when conditions became unfavorable (Van Auken *et al.*, 2004; McKinley and Van Auken 2005). It appears that nurse plant effects are limited in their range, effective only until a certain tipping point, at which point the environmental inputs will overwhelm buffering effects. Seedling size for mortality O was not significantly different across censuses (all  $p > 0.15$ ), indicating more consistent death rates and environmental effects, as the seedlings were not buffered in any way. We expected seedlings in the open category to be smaller overall due to

less favorable growth conditions; however, their greater height may be explained by competition for light with the surrounding grasses and forbs. Greater emergence in U and C may skew distribution toward smaller sizes, therefore it is not possible to make conclusions about growth rates vs age influence.

#### *Correlating Nurse Plant Characteristics with Seedling Dynamics*

*J. virginiana* gender, height, canopy area, DBH, and age were all significant factors related to seedling presence and abundance. Height, canopy area, DBH, and age were highly correlated with one another (Pearson's product-moment correlation  $> 0.7$ ), as age is a calculated value which includes DBH and canopy area, and all three measured characteristics relate to tree size. Total seedling density for the octet (U and C and/or O) was significantly related (all  $p < 0.01$ ) to tree characteristics for female trees, while our other gender categories were not (all  $p > 0.07$ ). Female trees had higher seedling counts and densities compared to males and trees with unidentified gender, and were overwhelmingly larger and older. Female trees are the source of seeds, resulting in a larger seed shadow underneath and in the area surrounding them than elsewhere, therefore this finding was not entirely unexpected (Holthuijzen *et al.*, 1987; Joy and Young 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005).

The use of *J. virginiana* characteristics to create models for seedling presence and abundance indicates that it is statistically highly probable that: trees larger than 3.7 m tall will have seedlings, and that greatest number of seedlings will be present for female *J. virginiana* with large canopies (the number of seedlings increasing with tree size) (Joy and Young 2002). Predicting mortality and emergence rates was less straight-forward than predicting presence and abundance. Mortality was dependent upon canopy area size, location in relationship to the study tree (U, C, or O), and canopy light exposure (CLE) (Figure 7, A). Emergence was dependent upon location (U, C, or O), tree height, canopy area, and gender (Figure 7, B). Overall, greater mortality occurred underneath larger, female *J. virginiana*, where emergence was also high and seedling numbers were greater, both factors increasing competition pressures (McKinley and Van Auken 2005). These illustrated relationships may be a useful tool for land managers and owners looking for a place to begin when working to control *J. virginiana* encroachment, as they provide a condensed overview of tree characteristics which can assist with identifying encroachment points of convergence.

## Conclusion

This study demonstrated the seasonal patterns presence, emergence, and mortality of *J. virginiana* seedlings in a southern Great Plains grassland, capturing dynamics over the course of a year. These fluctuations are strongly influenced by the seedling's proximity to, and the size of, a nurse tree, with seedlings underneath *J. virginiana* canopies exhibiting greater densities, emergence, and mortality rates. In order to determine whether the nurse plant effects evident in this study are directly guiding seedling success, or indirectly affecting seedlings through microclimate buffering, additional data and analysis is needed.

Statistically significant relationships have been identified between *J. ashei* seedling emergence and mortality and monthly temperatures and precipitation (Van Auken *et al.*, 2004). Inversely related to rainfall and directly related to temperature, highest mortality was observed during warm, dry months, and emergence was typically observed during the coolest months following rainfall events, with decreasing mortality from 1 m outside the canopy edge into full woodland cover (Van Auken *et al.*, 2004). It would be informative to have similar information for *J. virginiana*. Quantifying the effect of the tree canopies on the microclimate as well as microclimate effects on seedling dynamics would provide the necessary information to further investigate whether there is a direct or indirect relationship between trees and seedling dynamics (Joy and Young 2002). This data would be a key contribution to the growing body of knowledge, and need for management techniques, for *J. virginiana*.

## Management implications

Properly addressing the environmental influences of woody plant encroachment has been a topic of concern for some time, and the majority of the published literature focuses on tree effects (Norris *et al.*, 2001; Briggs *et al.*, 2002; Van Auken 2009; van Els *et al.*, 2010; Smith 2011; Anadón *et al.*, 2014; Limb *et al.*, 2014; Zou *et al.*, 2015; Zou *et al.*, 2018; Wang *et al.*, 2018; Biral *et al.*, 2019). The efficacy of different management methods has been addressed (Schmidt and Stubbendieck 1993; Van Auken 2009; Smith 2011; Limb *et al.*, 2014), and the various merits of each method compared. Limb *et al.* (2014) found that plant community composition was strongly affected by the removal of *J. virginiana*, with a significant negative

linear relationship between herbaceous biomass and canopy cover. Sites in which *J. virginiana* was removed returned to historical composition and productivity within 5 years regardless of the % canopy cover (80% cover was the maximum observed in this study) (Limb *et al.*, 2014). Restoration of native species has been shown to be possible due to the native seed bank, in which viable seeds remained mostly intact for around 40 years following woody plant encroachment (D'Souza and Barnes 2008). Seed banks are supplemented by uneven suppression, where native species persist in canopy gaps throughout encroached areas; all these factors combine to make restoration possible following the complete removal of *J. virginiana* (Limb *et al.*, 2014).

Ganguli *et al.* (2008) demonstrated that *J. virginiana* seedling survival and growth increased with species richness and diversity in a tallgrass prairie. Based on our findings, it would be beneficial for more research to target the seedling stage of *J. virginiana*, as better understanding seedling dynamics will contribute to land management, providing information necessary to proactively work to prevent woody plant encroachment rather than dealing solely with larger trees. Specifically, targeting all *J. virginiana* trees greater than 3.7 m in height (Figure 6, A), and female trees regardless of size (Figure 6, B), allows for focused destruction of both trees and the seedlings they were facilitating, removing above-ground competition as well as eliminating new growth. This method would remove the largest of the trees, as well as eliminate the largest portion of the seedling population, slowing encroachment and allowing for restoration opportunities. Chances of restoration decrease with the amount of time *J. virginiana* is present, as the native seed bank eventually loses viability (Limb *et al.*, 2014). Soil water and other limiting environmental factors may also affect recovery time; therefore, it is imperative to better understand the interacting factors which influence woody plant encroachment and grassland restoration (Limb *et al.*, 2014).

## **Chapter 2: *Juniperus virginiana* canopies and the microclimate: investigating the extent of the relationship between nurse plants and seedlings**

### **Introduction**

As a result of anthropogenic effects and climate change, the dynamics of grasslands are rapidly evolving (Schmidt and Stubbendieck 1993; Briggs *et al.*, 2002; Engle *et al.*, 2007; Boval and Dixon 2012). Climate change, decreased fire frequency, overgrazing, and the planting of shelter belts all contribute to habitat fragmentation and degradation, decreasing grassland habitat quality and ultimately resulting in rapid rates of woody plant encroachment (Briggs *et al.*, 2002; Engle *et al.*, 2007; Smith 2011; Felton and Smith 2017). Historically, species rich ecosystems have been understood to be more resistant to encroachment; however, recent literature is demonstrating the opposite relationship (Ganguli *et al.*, 2008; Felton and Smith 2017). Species richness and diversity are not decreasing woody plant encroachment and success, but may be positively correlated (Ganguli *et al.*, 2008). The biodiversity of the southern Great Plains grasslands, combined with changing environmental conditions, are lending themselves to rapid replacement; in Oklahoma this is predominantly the work of a native species, *Juniperus virginiana* (Briggs *et al.*, 2002; Smith 2011; Farjon 2013; Kartesz 2015). A member of the family Cupressaceae, *J. virginiana* is commonly called eastern red cedar (Farjon 2013). A dioecious, evergreen species, the tree canopy has a dense, conical shape, the total area of which varies depending on where the tree is growing (Smith 2011). Sexual maturity is reached around 10 years old, and with a high cone removal rate, a relatively short seed dormancy, and physiology which allows it to thrive in diverse ecological habitats, *J. virginiana* is extremely successful at exploiting niches wherever possible (Johnsen and Alexander 1974; Holthuijzen and Sharik 1984; Holthuijzen *et al.*, 1987; Lawson 1990; Briggs *et al.*, 2002).

The presence of *J. virginiana* shifts grassland biomass allocation from primarily below- to aboveground; modifies carbon and nitrogen sequestration patterns; affects the availability of resources such as light, water, and nutrients; and causes shifts in plant and animal species – all of which negatively impact plants and animals native to the southern Great Plains by causing dramatic shifts in resource availability (Schmidt and Stubbendieck 1993; Norris *et al.*, 2001; Briggs *et al.*, 2002; McKinley and Van Auken 2005; Van Auken 2009; van Els *et al.*, 2010; Smith 2011; Blair *et al.*, 2014; Zou *et al.*, 2015; Davis *et al.*, 2018; Biral *et al.*, 2019).

Vegetation cover and richness in the understory decreases with increasing *J. virginiana* canopy cover – the C<sub>4</sub> grasses and forbs typical of grasslands are noticeably absent close to and underneath *J. virginiana* canopies, where only C<sub>3</sub> shade tolerant forbs and woody species are found to thrive (Briggs *et al.*, 2002; van Els *et al.*, 2010). The broader influences of *J. virginiana* on grassland ecosystems over time are well established; however, the degree to which microclimate modifications occur and how this may influence seedling establishment and success is not well understood.

It is widely acknowledged that trees act as buffers for understory environments, shielding them from fluctuating environmental extremes and modifying the microclimate (Joy and Young 2002; Van Auken *et al.*, 2004; Van Auken 2009; van Els *et al.*, 2010; Davis *et al.*, 2018). Establishment of woody plants affects surface and soil dynamics. Increased litter input and decreased light availability have been identified as factors that likely affect herbaceous plant presence and productivity underneath the canopy (Biral *et al.*, 2019). Joy and Young (2002) identified solitary trees as beacons to birds and small animals, organisms key in seed dispersal; as focal points with increased water and nutrient availability; and as providing a degree of protection from herbivory. The adult canopies filter light and moderate temperature levels: affecting vapor pressure deficit (VPD), raising minimum temperatures, and lowering maximum temperatures (Joy and Young 2002; Van Auken 2009; Davis *et al.*, 2018; chapter 1). In forested regions of the NW United States it was demonstrated that the magnitude of buffering was positively related to solar radiation – for low canopy cover there was decreased buffering with increased solar radiation, and for high canopy cover there was increased buffering with increased solar radiation (Davis *et al.*, 2018). In *J. virginiana*, it has been documented that the air temperature closest to the trunks was cooler than in the open, as was soil temperature, and volumetric soil moisture increased with increasing distance from the trunk (van Els *et al.*, 2010; Biral *et al.*, 2019). Such microclimate buffering creates more moderate conditions which may be beneficial to seedling establishment, as the number of seedlings is significantly higher closest to larger nurse plants (Holthuijzen and Sharik 1984; Holthuijzen *et al.*, 1986; Joy and Young 2002; Wayne and Van Auken 2002; McKinley and Van Auken 2005).

Using data from *J. ashei* seedlings it has been demonstrated that under the tree canopy, ideal conditions exist for seedling germination and establishment (Jackson and Van Auken 1997; Van Auken *et al.*, 2004; McKinley and Van Auken 2005). Van Auken *et al.* (2004) identified a

significant inverse relationship between *J. ashei* seedling emergence and temperature, with more seedlings emerging during cooler months, and a significant relationship between the number of emergences per month and the monthly mean temperature and rainfall. McKinley and Van Auken (2005) used a manipulative study to identify a significant 3-way interaction between canopy cover, light, and water availability to the seedlings; light alone was not a significant factor in seedling survival. Schmidt and Stubbendieck (1993) identified precipitation as a significant factor in seedling survival, with greater precipitation resulting in higher initial and long-term seedling survival. It was also determined that seedling establishment and survival was dependent upon beneficial weather conditions: microclimate effects provided significant advantages to seedlings when they included increased water availability and decreased temperatures (Schmidt and Stubbendieck 1993; McKinley and Van Auken 2005). In a coastal habitat, Joy and Young (2002) studied woody seedlings underneath *J. virginiana* and demonstrated that the microclimate was significantly different from the open dunes. Light levels were significantly reduced, air and soil temperatures were lower with less variation, and a thick litter layer facilitated decreased water evaporation and increased soil water availability, all of which factored into the likelihood of seedling survival (Joy and Young 2002).

Immediately underneath *J. virginiana* trees is the highest density of seedlings and saplings (McKinley and Van Auken 2005; chapter 1) and their survival is driven by a combination of abiotic and biotic factors which may shift as they grow, where facilitation as seedlings becomes competition as saplings (Jackson & Van Auken, 1997; Joy and Young 2002; Horncastle *et al.*, 2004; McKinley and Van Auken 2005). It has been demonstrated that seedling establishment and survival increases when in closer proximity to a tree (Joy and Young 2002; Van Auken *et al.*, 2004). It is well established that the *Juniperus* tree has a significant effect on the microclimate directly underneath and immediately surrounding the canopy (Jackson and Van Auken, 1997; Joy and Young 2002; Horncastle *et al.*, 2004; Van Auken *et al.*, 2004; McKinley and Van Auken 2005; van Els *et al.*, 2010) but the degree to which this buffering directly or indirectly affects seedling dynamics has not been established.

Nurse plant effects and microclimate effects have been studied in *Juniperus* species; however, these dynamics have not been characterized for *J. virginiana* in a Great Plains environment (Schmidt and Stubbendieck 1993; Jackson and Van Auken, 1997; Joy and Young 2002; McKinley and Van Auken 2005). The rapid expansion of this species demands a greater

understanding of the factors driving their success (Briggs *et al.*, 2002). Although seedling dynamics for different *Juniperus* species have been characterized, the extent of the interaction between seedlings and the nurse tree has yet to be directly described (Schmidt and Stubbendieck 1993; Jackson and Van Auken, 1997; Joy and Young, 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005; chapter 1). This study aims to determine the extent of nurse plant effects on microclimate, and resulting microclimate effects on seedlings, in order to capture and describe whether seedling facilitation is a direct or indirect phenomenon, and how that relationship is contributing to the successful encroachment of *J. virginiana*. We aim to answer the following questions for a southern Great Plains grassland: i) what is the effect of *Juniperus virginiana* canopies on microclimate; ii) do microclimate effects promote seedling growth and survival; and iii) is there a direct or indirect relationship between the nurse tree and seedling dynamics?

## **Methods**

### *Research site*

This study was conducted at Kessler Atmospheric and Ecological Field Station (KAEFS) in Purcell, OK (34.982928 lat. and -97.519809 long.). KAEFS is 360-acre (146 ha) facility for research and education owned by the University of Oklahoma. The property is characteristic of the southern Great Plains rural landscape, with a 15-year mean annual air temperature of 16.11°C (January min. = -2.22°C; July max. = 38.89°C) and mean annual precipitation of 918.72 mm (Mesonet 2020). The property sits within the Washita River drainage basin and is bisected by the Finn Creek watershed (Kessler 2019). The soil type is a Nash-Lucien complex (Xu *et al.*, 2013).

### *Plot Establishment*

Data was gathered across a gradient of encroachment: from an enclosed forest to a transition zone then into the open grassland. Three parallel transects were established to increase replication and capture varying encroachment dynamics. Five plots were established on each transect, each approximately 40 m in diameter and approximately 75 m apart (from center point to center point). Each transect had approximately the same distribution of plot-level

characteristics, with one plot in the closed forest, one plot in the transition zone between grassland and forest, and three plots in the grassland.

The center point of each plot was marked digitally using a handheld GPS (Garmin eTrex Vista® H, Olathe KS); the boundaries of the plot were marked using 4' wooden stakes. All *J. virginiana* trees within each plot in each transect were tagged physically, using a unique identifying number, and digitally with the GPS. Study trees were identified as those having a measurable DBH (1.3 m and taller). A total of 328 *J. virginiana* were tagged throughout the 15 plots. A random subset of the tagged trees was selected to be included in the seedling study. Using a random number generator in Microsoft Excel® (Excel version 2019), 173 trees were selected using a pre-determined criterion of no more than 15 trees per plot and no fewer than the minimum number present in a given plot (maximum number of *J. virginiana* in a single plot = 65; minimum number in a single plot = 3). Using this selection criterion, transect 1 had 46 study trees, transect 2 had 65, and transect 3 had 62.

After the plots were established and the study trees were selected, the area under each study tree canopy was divided into eight sections (based on compass directions) and one octet was randomly chosen for seedling measurements. Of the 173 study trees, 30 had an octet in the ENE direction, 16 were ESE, 24 were NNE, 22 were NNW, 21 were SSE, 21 were SSW, 23 were WNW, and 16 were WSW. In order to also capture seedling dynamics outside the study tree canopy, the octet extended 1 m outside the dripline of the study tree.

### *Study Tree Characteristics*

All 173 *J. virginiana* trees were surveyed to characterize the tree dynamics and identify relationships between nurse trees and seedling dynamics. The dimensions of the octet under the study tree were also measured so that seedling dynamics could be examined on a ground area basis. Study trees were surveyed between seedling censuses, beginning in September of 2018 and completed by the following June.

The following data was recorded: a photo of the tree, the gender [M (male), F (female), UK (unknown)], height (m), height to canopy (cm), diameter at breast height (DBH) (cm), diameter at base (cm), canopy light exposure (CLE; Bechtold 2003), and canopy diameter (m) (measured N-S, then E-W, values averaged and used to calculate canopy area). We also estimated tree age based on relationships established at the site [ $12.776 + (0.535 * \text{DBH}) +$

$(1.219 * CLE) + (0.703 * \text{Canopy Area})$ ] (Giddens and McCarthy, unpublished data). Based on the age estimates for all the study trees a fourth gender category was established, reproductively immature (RI), in order to correctly categorize the trees which had not yet reached reproductive maturity (~10 years old) (Johnsen and Alexander 1974; Lawson 1990).

### *Seedlings*

Seedling censuses were conducted four times over the course of this research study. The first census was taken in May – June, 2018 (Summer 2018); the second in December, 2018 – January 2019 (Winter 2018/19); the third in March, 2019 (Spring 2019); and the fourth in September 2019 (Summer 2019). All seedlings within the octet of a given study tree (underneath the study tree canopy and 1 m outside the dripline) were tagged using a write-on metal tag. The tag was secured to the ground using a 4” barn nail spray-painted red for visibility as close to the seedling as possible. Seedlings larger than 30 cm tall were tagged using a 4” black zip-tie instead of a nail at ground-level. The area underneath the study tree canopy (U) was treated as a single location type, while the 1 m outside the dripline of the study tree canopy was classified based on the presence (or absence) of adjacent canopies. The area outside the dripline was either completely open and exposed (O) or covered by an adjacent tree canopy (C) [either deciduous, evergreen (*J. virginiana*), or a combination of the two]. At the first census all seedlings were tagged within the octet and assigned to the first cohort of this study. Date tagged, height (cm), number of branches, basal diameter (mm), and location in relationship to the study tree (U, C, O) was recorded.

Subsequent censuses were taken in order to track the mortality and survival of the first cohort, as well as to capture and track subsequent cohorts. At each census following the first, seedlings were marked as “present” (surviving seedlings), “absent” (seedlings which died or were absent since previous censuses), or “new” (seedlings which emerged since the previous census). If a previously identified seedling could not be found it was marked as “unknown”; after two consecutive unknown categorizations the seedling was considered absent (Van Auken *et al.*, 2004). Less than 0.4% of tagged seedlings throughout the entire study were marked “unknown”.

### *Environmental Data Collection*

Microclimate data was collected in Summer 2018 and Spring 2019 in order to characterize the effects of the study tree on the environment underneath and immediately outside the canopy. Up to eight trees per plot were measured; all trees were surveyed in plots with eight or fewer trees. In plots with more than eight trees, typically five trees with seedlings and three trees without were selected – this division was chosen to reflect the greater abundance of trees with seedlings in the entire study area, so that the sub-sample was representative of the nurse plant/seedling dynamics throughout the entire site. Due to natural spatial disparities, this exact ratio was not achieved in all plots: some plots had more trees with seedlings than without and vice versa, some had exactly 4 in each category. However, soil volumetric water content (VWC) (%) was not significantly different underneath or outside the canopy for trees with seedlings when compared to trees without (all  $p > 0.4$ ), so the final number of trees in each sub-category per plot was not significant.

At each selected tree, VWC (%), litter depth (cm), solar radiation (mV), and surface temperature ( $^{\circ}\text{C}$ ) were taken throughout the octet. Solar radiation in mV was converted to  $\text{W}/\text{m}^2$ . For each type of measurement, in order to capture environmental differences underneath and outside of the study tree canopy, no fewer than three and no more than seven measurements were taken in each part of the octet (for a total of 10 measurements), with “each part” being defined as U, C, or O. The number of measurements taken in each part of the octet was determined at each tree based on the size of the study area underneath the study tree compared to the area outside the dripline (e.g. if the area outside the dripline was larger than the area underneath the canopy, a greater proportion of the 10 measurements was taken outside).

VWC was taken along the rope marking the boundary of the octet using a handheld probe (Hydrosense II, Campbell Scientific, Logan, UT, USA). Five measurements were taken along each side of the octet, with no fewer than four and no more than 6 measurements taken in a given part of the octet (under the tree canopy or outside the dripline). Litter depth was measured using a wooden skewer marked at 1 cm increments; solar radiation was measured using a pyranometer (SP-215, Apogee Instruments, Logan, UT, USA); surface temperature was taken using a Traceable® Infrared Thermometer (Thomas Scientific, Swedesboro, NJ, USA). These measurements were taken randomly throughout each part of the octet. The time a given type of measurement began was recorded, as well as the time those measurements ended.

Weather data was obtained from the Washington Mesonet site (Mesonet 2020) located at KAEFS, less than 1 mile from the study area, in order to examine site-level environmental characteristics. Rainfall (mm), air temperature (°C), and solar radiation (W/m<sup>2</sup>) were obtained for the entirety of the study period in 5-minute intervals. Wind direction and speed data was also obtained, for each day over the entirety of the study period and analyzed.

### *iButton Data Collection*

Temperature loggers (iButton, Maxim Integrated, San Jose, CA, USA) were used to track soil surface temperature (°C) dynamics underneath the study tree canopy and outside the dripline in order to characterize study tree canopy buffering dynamics. Set to take measurements at 2-hour intervals, the iButtons were placed in the field for ~1 month and rotated between the three transects for 12 months, providing ~30 days of data, per transect, in each season. Transect 1 had iButtons in all five plots from Oct. 29 – Dec. 3, 2018; Feb. 8 – Mar. 6, 2019; May 9 – Jun. 6, 2019; and Aug. 12 – Sept. 3, 2019. Transect 2 had iButtons in all five plots from Dec. 5, 2018 – Jan. 8, 2019; Mar. 8 – Apr. 2, 2019; Jun. 7 – Jul. 2, 2019; and Sept. 4 – Oct. 7, 2019. Transect 3 had iButtons in all five plots from Jan. 9 – Feb. 4, 2019; Apr. 4 – May 7, 2019; Jul. 12 – Aug. 7, 2019; and Oct. 11 – Nov. 9, 2019.

For a given study tree, one iButton was placed approximately center under the tree canopy and a second iButton was placed approximately 1 meter from the first iButton – outside the dripline of the study tree canopy. Solar radiation was measured using a pyranometer (as described previously) wherever the iButton was placed, either at the time of placement or at the time of pickup, to serve as an indicator of canopy cover. iButton data was processed to obtain daily maximum, average, and minimum temperatures as well as overall maximum, average, and minimum temperatures for the entire data collection period.

### *Data analysis*

Data was analyzed using R (v. 3.6.2; R core team 2019) in RStudio. Study tree characteristics were analyzed using Pearson's correlation and for common variables above 0.8 only one was included in further analyses. Environmental data (VWC, temperature, solar radiation, and litter depth) was averaged by both entire octet and location (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the

dripline of the study tree, open to the environment). VWC data was represented as a proportion (instead of a percentage) and arcsine square root transformed for the purposes of analysis. PCAs were run on environmental data by location in order to examine spatial relationships between Summer 2018 and Spring 2019 variables. Canopy cover type influences on the microclimate C was analyzed for significance within and between each collection period, by location. Study tree characteristics and environmental data was analyzed by location using multiple linear regression. Seedling dynamics and environmental data were analyzed by location using generalized linear models. Tests were generally considered significant when  $p < 0.05$ ; however, significance of some tests was analyzed using Bonferroni's correction (original p value / number of tests performed) in order to correct for multiple comparisons of variables which were not entirely dependent or independent of one another. A correction value was calculated for each type of microclimate data (VWC, solar radiation, and temperature; all = 0.00625] as well as litter depth (0.00833), and significant correlations determined according to this standard.

## **Results**

### *Study Tree and Seedling Characteristics*

Three transects were established to capture encroachment dynamics in a grassland with a range of encroachment. Study tree dynamics varied at the transect level, with transect 1 containing the largest trees (on average) in all measured characteristics except height to canopy (distance from the ground to the first live needles) (Table 1). Pearson's product-moment correlation revealed significant ( $> 0.8$ ) correlation between tree height (m) and DBH (cm), canopy area (m<sup>2</sup>) and DBH, canopy area and age (years), and DBH and age. Consequently, only tree height and canopy area, not tree age and DBH were included in subsequent statistical analyses. There were no strong ( $< 0.8$ ) correlations between the remaining variables.

The number of seedlings found in the octet of each study tree ranged from 0 to 184 (0 - 13.27 seedlings/m<sup>2</sup>). Seedling characteristics varied, with basal diameter ranging from  $<1$  mm to 22.27 mm and height from 0.75 cm to 180 cm. Seedling presence varied greatly based on proximity to study tree. Across censuses the greatest number and density of seedlings were present underneath the study tree canopy (U) with the lowest number of seedlings present outside the dripline with no canopy shading (O); the number of seedlings outside the dripline of the study tree, but underneath an adjacent canopy (C) was between U and O (all  $p < 0.004$ ).

Survival (%) was significantly greater than mortality (%) in each census, regardless of location (Table 2; all  $p < 0.03$ ). Based on repeated measures ANOVA, both seedling mortality and emergence differed significantly across censuses (all  $p < 0.01$ ), with significantly higher mortality in U than O in every census ( $p < 0.003$ ; Table 2). Only Summer 2018 (census 1) and Spring 2019 (census 3) data are reported in Table 2, as these were the censuses which most closely aligned with the microclimate data collection periods and were therefore utilized for microclimate analyses.

### *Microclimate Dynamics – Temporal Patterns and Location Effects*

Principal component analyses of microclimate data (VWC, temperature, solar radiation) and litter depth (cm) by location (Figure 1) revealed that conditions outside the dripline of the study tree but covered by an external canopy (C) were similar to those underneath the study tree canopy (U). However, the conditions in these locations were substantially different than those outside the dripline with no canopy coverage (O). Spatial differences were primarily driven by solar radiation and Summer 2018 temperature (PCA1 explains 41.6%), addition of VWC and Spring 2019 temperature explained an additional 27.1% of the variance (PCA2; Figure 1). 2-way ANOVAs were utilized to examine each microclimate variable by season (Summer 2018 and Spring 2019), location (U, C, and O), and octet direction. Three key relationships were identified: 1) Summer 2018 was significantly different from Spring 2019 for every factor (all  $p < 0.04$ ) except litter depth ( $p = 0.39$ ); 2) location in relationship to the study tree canopy was a significant factor for the microclimate variables (all  $p < 0.006$ ) across data collection seasons; and 3) octet direction was significant for VWC [only for WNW and NNE octets ( $p = 0.049$ )], solar radiation [only for SSE and NNE octets ( $p = 0.015$ )], and litter depth [only for SSE, SSW, and NNE octets (all  $p < 0.012$ )]. Evidence of directional effects on microclimate data resulted in octet direction being included in subsequent analyses.

Microclimate variables within each data collection period (Summer 2018 and Spring 2019) were compared (U vs C vs O) to investigate spatial differences (Figure 2). Average soil VWC was significantly higher in O than C or U in each season (all  $p < 0.021$ ). VWC in U was significantly greater than in C ( $p = 0.0018$ ) in the summer, and C was significantly greater than U in the spring ( $p < 0.0001$ ). Average solar radiation was significantly greater in O in each season, followed by C, then U (all  $p < 0.002$ ). Average surface temperature was significantly (all

$p < 0.0002$ ) higher in O than C or U in Summer 2018, and U and C were not significantly different from one another ( $p = 0.37$ ); Spring 2019 yielded greater temperatures in O than U ( $p < 0.00001$ ) and greater temperatures in C than U ( $p = 0.0018$ ), but O and C were not significantly different ( $p = 0.78$ ). Average litter depth was significantly (all  $p < 0.032$ ) different between U and C in both seasons; there was no litter layer to measure in O. All microclimate variables, by location, were significantly different ( $p < 0.042$ ) between seasons (Table 3) except litter depth (all  $p > 0.20$ ) and solar radiation (W/m<sup>2</sup>) outside the dripline of the study tree in the open (O) ( $p = 0.5$ ).

Although all study trees were *J. virginiana*, adjacent canopy types varied, allowing us to explore whether evergreen (*J. virginiana*), deciduous, and mixed canopies had different effects on the microclimate. Microclimate conditions were similar across canopy types, with a few exceptions (Figure 3). The only microclimate variables which were significantly different between canopy types were VWC in Summer 2018 (deciduous vs evergreen  $p = 0.004$ ; Figure 3, A) and solar radiation in Spring 2019 (deciduous was significantly greater than both evergreen and both, all  $p < 0.029$ ; Figure 3, B). There were no other significant differences for microclimate variables within data collection seasons (all  $p > 0.06$ ). Canopy type results were also compared across seasons, Summer 2018 and Spring 2019, in order to determine how conditions changed with time under the external canopies (Table 4). VWC was significantly different in summer and spring for all canopy types (all  $p < 0.0007$ ), with higher values in each location for Spring 2019. Solar radiation was significantly different for deciduous canopies ( $p < 0.005$ ), with higher values in Spring 2019, but no other significant differences were detected (all  $p > 0.47$ ). Surface temperature was significantly lower for all canopy cover types (all  $p < 0.031$ ) in Spring 2019. Litter depth did not vary significantly (all  $p > 0.18$ ) across data collection periods.

Tree characteristics (height and canopy area) were analyzed in order to determine whether they explained the directional differences. There were no significant (all  $p > 0.061$ ) differences between tree characteristics, study area size (m<sup>2</sup>), or VWC averages for octet directions NNE and WNW. Differences were identified between NNE and SSE octets: of the two, NNE octets opened more frequently (6 compared to 1) into grassland conditions. There were no significant differences (all  $p > 0.078$ ) between tree characteristics for SSE, SSW, and NNE study areas. Mesonet data for wind direction and speed was analyzed in order to determine

the dominant wind direction, which was from SSE (Mesonet 2020). Differences in octet direction varied based on the microclimate variables being examined, and there were no statistically significant explanations based on tree and other microclimate characteristics – in order to resolve directional issues, broader environmental influences had to be considered.

Temperature loggers (iButton, Maxim Integrated) allowed for a more in-depth analysis of diurnal and seasonal patterns in temperature variation underneath (U) and outside the study tree dripline (C or O) (Figure 4). Monthly maximum temperatures were significantly cooler (all  $p < 0.36$ ) for U compared to O in the late spring and summer months (Figure 4, A). U had significantly (all  $p < 0.028$ ) lower maximum temperatures compared to C in January, March, May, and June of 2019. C was significantly (all  $p < 0.018$ ) cooler than O in both the winter and summer months. Monthly average temperatures were significantly different between U and O for nine of the 12 months (all months except January, February, and October 2019; all  $p < 0.047$ ), and C was significantly different from O for six of the 12 months (all  $p < 0.05$ ), with slightly warmer temperatures in U and C in the early winter months, and much cooler temperatures in the spring and summer months (Figure 4, B). U and C were only different in March and June of 2019, when average temperatures in U were significantly cooler (all  $p < 0.033$ ). Monthly minimums were significantly different for all three locations in December 2018 (all  $p < 0.03$ ), with U having the highest minimum and O having the lowest (Figure 4, C). U and C were both significantly (all  $p < 0.029$ ) warmer than O in November 2018, and C was significantly warmer ( $p = 0.03$ ) in August 2019 compared to U. C and O were significantly (all  $p < 0.045$ ) different in January, June, and September, 2019, with C having a higher minimum in the winter and a lower minimum in the summer.

### *Study Tree Influences on Microclimate*

Multiple linear regression analysis to link study tree characteristics with microclimate data yielded different trends for each variable (Table 5). These relationships were analyzed both at the whole octet scale, as well as separately by location (U, C, and O). Bonferroni's correction was employed in order to account for multiple comparisons of variables. Summer 2018 and Spring 2019 VWC for the octet and in U were significantly related to CLE (all  $p < 0.00001$ ); VWC in C was also significantly related to CLE, but only in the summer ( $p = 0.00013$ ; all positive correlations). Soil VWC increased with increasing CLE values, and decreased with

increased height to canopy. Spring 2019 VWC in C was not significantly related to CLE but was to height to canopy ( $p = 0.00027$ ; negative correlation), with VWC decreasing with increased height to canopy. In both seasons, solar radiation for the whole octet was significantly (all  $p < 0.0035$ ) related to CLE but there were no location effects (all  $p > 0.039$ ). The correlation to CLE was a very strong, positive relationship, with increased solar radiation tied to increased CLE values. No significant relationships appeared for temperature (all  $p > 0.043$ ), and there were no strong relationships between any of the environmental variables and tree characteristics for O (all  $p > 0.047$ ).

### *Microclimate Influences on Spatial Seedling Dynamics*

Generalized linear models using a quasipoisson distribution were utilized to examine the relationship between seedling numbers, microclimate data (VWC, temperature, solar radiation), and litter depth (cm) for each location within the octet. Our model also included important tree-level variables (height, height to canopy, CLE, and canopy area) as well as octet area ( $m^2$ ) as covariates. However, there were no significant relationships between environmental variables and seedling numbers within each location, even with the inclusion of known covariates.

Additional analyses were done using location as a covariate, to capture relationships between environmental variables and seedling numbers across locations. This regression approach allows for the estimation of separate parameters quantifying the effect of different values of discrete categories such as gender and location; location parameters were estimated for U, C, and O, and gender parameters were estimated for female, male, unknown, and immature. Census 1 seedling densities were compared to Summer 2018 microclimate data, and census 3 seedling densities were compared to Spring 2019 microclimate data (Table 6). Census 1 seedling density was significantly related to temperature (positive parameter;  $p = 0.0028$ ), location (U only; positive parameter;  $p = 6.32E-06$ ), canopy area (positive parameter;  $p = 0.0013$ ), and gender (male and unknown; negative parameters; all  $p < 0.0011$ ). There were also some significant interactive effects between temperature and solar radiation (negative parameter;  $p = 0.030$ ), and solar radiation and litter (positive parameters;  $p = 0.031$ ). Census 3 seedling density was significantly related to location (U only; positive parameter;  $p = 0.00040$ ), canopy area (positive parameter;  $p = 0.011$ ), and gender (male and unknown; negative parameters; all  $p < 0.0062$ ). There were no interactive effects. In this analysis, a significant positive parameter

for U indicates greater seedling densities underneath the study tree canopies as compared to C and O. The female trees were represented as the baseline for comparison, therefore the negative parameters for male and unknown genders indicate that male and unknown trees having significantly fewer seedlings compared to females.

Decision trees were created to explore mortality and emergence using the same variables as in Chapter 1, but with the addition of microclimate data and the removal of characteristics that were significantly correlated with the microclimate variables (Figure 5). These models were created to explore the nature of microclimate and seedling relationships. The exclusion of significant tree level parameters identified in chapter 1 eliminates important explanatory variables, resulting in less robust models; therefore, the models in chapter 1 remain the best for predictive purposes. Summer 2018 microclimate data was used, as the summer conditions were the best index for comparing to Summer 2019 seedling census.

The decision tree for modelling seedling emergence included tree height ( $p < 0.001$ ) and solar radiation ( $p = 0.023$ ). At a height of less than or equal to 4.4 m seedlings were only ~10% likely to emerge, and at heights of greater than 4.4 m, solar radiation was the primary driver of emergence, with greater (~30%) emergence in light levels less than or equal to 188.3 W/m<sup>2</sup>, and lower (~18%) emergence in light levels greater than 188.3 W/m<sup>2</sup> (Figure 5, B). Seedling mortality was driven solely by canopy area ( $p < 0.001$ ), with lower (<10%) mortality underneath canopies less than or equal to 15.21 m<sup>2</sup>, and greater (~35%) mortality underneath canopies greater than 15.21 m<sup>2</sup> (Figure 5, A). The chapter 1 decision tree for seedling mortality contained canopy area, location, and CLE, while the seedling emergence tree contained location, height, canopy area, and gender (chapter 1, Figure 7). Removing characteristics correlated to the microclimate variables (CLE, height to canopy, and location) eliminated significant predictors which were not readily replaced.

## Discussion

The effects of *J. virginiana* on the southern Great Plains ecosystem include not only the loss of native plant species, but also shifting microclimate variables (Briggs *et al.*, 2002; van Els *et al.*, 2010; Biral *et al.*, 2019). We identified significantly lower soil VWC (%) and temperatures (°C) underneath *J. virginiana* canopies compared to outside the dripline, as well as greatly decreased solar radiation (W/m<sup>2</sup>) and increased litter depth (cm). These changes

remained constant across multiple seasons, with the microclimate under evergreen canopies significantly different from the open conditions for every metric. Although study trees changed the microclimate underneath their canopies, greater seedling success could not be quantitatively linked to any specific microclimate changes. Increased seedling numbers underneath the canopy may be driven by multiple variables, resulting in our inability to isolate singular relationships.

### *Study Tree and Seedling Characteristics*

*J. virginiana* tree and seedling dynamics across our study site are most likely the result of parent tree age and dispersal. The berry-like cones of female trees are primarily dispersed by birds and small animals, carried across fields to fence lines, solitary trees, and the forest edge in varying magnitudes. The cones are dropped by dispersers and by wind and gravity in the immediate vicinity of the parent plant (Holthuijzen *et al.*, 1987; Horncastle *et al.*, 2004). As our first transect was established along a fence line at KAEFS, the presence of larger, older trees and the increased abundance of seedlings is most likely due to avian dispersal and their preferential use of fences and larger trees as perches, as well as the fact that larger trees are statistically more likely to be female and therefore have more seedlings (Holthuijzen *et al.*, 1987; Joy and Young 2002; chapter 1).

Census 1 and 3 were the primary focus of analysis in this chapter due to their temporal proximity to the microclimate data collection periods. However, temporal matching (or accounting for possible lags) between seedling numbers and microclimate data is not critical, as the main goal of this analysis was to explore spatial patterns in microclimate, study trees, and seedlings, not to draw conclusions about temporal trends.

### *Microclimate Dynamics – Temporal Patterns and Location Effects*

Seasonal (Summer 2018 and Spring 2019) differences in microclimate were expected, as summer and spring have different temperature (Figure 4), solar radiation, and precipitation dynamics (Mesonet 2020). Location effects overall indicated differences between U, C, and O, with significant interactions varying based on measurement type and season (Figure 2). Directional effects were not consistent for all directions or microclimate variables, and there were no significant explanations from tree characteristics; in order to resolve directional issues, broader environmental influences had to be considered.

ANOVA results indicated that VWC was significantly different ( $p = 0.049$ ) between NNE and WNW octets, but there were no significant differences (all  $p > 0.061$ ) attributable to tree characteristics, octet area, or VWC averages between the two when directly compared. Solar radiation was significantly different ( $p = 0.015$ ) between NNE and SSE octets. We expect that increased solar radiation along the south side of *J. virginiana* may have increased the total canopy area (of study trees and other trees). When the conditions outside the canopy for trees with NNE and SSE octets were analyzed, they had the same number of adjacent evergreen (*J. virginiana*) and combined (evergreen and deciduous) canopy types; however, seven SSE octets had deciduous canopies and only one was completely open, whereas NNE had four deciduous and six open. More open measurements in the NNE octets would result in higher solar radiation values for NNE, while fewer open measurements would have significantly lowered the values for SSE. Litter depth was significantly different between SSE, SSW, and NNE octets (all  $p < 0.012$ ). Data from the Washington Mesonet site revealed that the predominant wind directions were from the south (south, SSE, SE, and ESE) for the entirety of our study (Mesonet 2020). This wind directionality would predominantly affect the south-side of the *J. virginiana*, potentially increasing rates of foliage loss, and thereby increased litter depth. There were no significant differences (all  $p > 0.078$ ) in tree characteristics which could otherwise explain these directional effects.

The microclimate underneath *J. virginiana* trees (U) was significantly different from the open in every respect (all  $p < 0.021$ ), with a lower VWC, solar radiation, and temperature in both Summer 2018 and Spring 2019. These findings agree with what has been previously described for *Juniperus* and other woody species (Joy and Young 2002; Van Auken *et al.*, 2004; Van Auken 2009; van Els *et al.*, 2010; Davis *et al.*, 2018; Biral *et al.*, 2019). The dense evergreen canopy shields the area underneath the tree to such a degree that surface temperatures are modified (Joy and Young 2002; Van Auken 2009; Davis *et al.*, 2018). Decreased VWC underneath the canopy is most likely a result of canopy interception, and the resulting evaporation of moisture from the canopy, and precipitation redistribution (van Els *et al.*, 2010; Zou *et al.*, 2015).

The area underneath the study tree canopy (U) was always a single location type (evergreen *J. virginiana* canopy), while the 1 m outside the dripline was either completely open and exposed (O), or covered by an adjacent tree canopy (C) [either deciduous, evergreen (*J.*

*virginiana*), or a combination of the two]. The C category types offered a chance to examine the effects of different canopy types on *J. virginiana* seedling presence. The only significant (all  $p < 0.03$ ) differences were for Summer 2018 VWC and Spring 2019 solar radiation (Figure 3). Average soil VWC was significantly greater under adjacent deciduous trees than under adjacent *J. virginiana*, which was to be expected as *J. virginiana* have been demonstrated to have modified soil water content due to rainfall interception, increased litter depth, and decreased solar radiation (van Els *et al.*, 2010; Zou *et al.*, 2015). Spring 2019 deciduous solar radiation was significantly greater than evergreen and both – which was most likely a byproduct of the fact that deciduous canopies were beginning to leaf-out and had not yet reached full cover, thereby resulting in increased radiation measurements. It is worth noting that, when compared, conditions directly underneath our study trees vs adjacent *J. virginiana* microclimate conditions were never different (all  $p > 0.12$ ), so that the effect of the study tree canopy and adjacent *J. virginiana* canopies were the same.

Across seasons, deciduous VWC and solar radiation were significantly greater in Spring 2019, while surface temperature decreased, indicative of decreased leaf area for shading and mirroring the cooler temperatures of the spring as opposed to the summer. Evergreen and both VWC were also significantly greater in Spring 2019, with decreased surface temperatures, but solar radiation did not change, likely due to the evergreen nature of the *J. virginiana* (Joy and Young 2002; Van Auken 2009; Davis *et al.*, 2018). Litter depth did not significantly change (all  $p > 0.19$ ) for any of the canopy types from Summer 2018 to Spring 2019.

The temperature loggers (iButtons) provided a long-term look at the temperature buffering effects of *J. virginiana* canopies. The study tree canopies moderated the microclimate, raising the minimum temperatures and lowering the maximum temperatures. Intra-annual variability in temperature was greatest in the open (O) and least underneath the canopy (U). Outside the canopy dripline (O) represented the most extreme conditions, demonstrating the highest temperatures in the summer and the lowest temperatures in the winter. Underneath the study tree canopy (U) exhibited the greatest microclimate buffering, with the warmest temperatures in the coldest months, and coolest temperatures in the warmest months. Temperatures under an external canopy (C) fell between U and O in almost every month, and when this alignment did not hold true it was closer to U than O. It has been demonstrated, also using iButton data, that soil surface temperatures increased with increasing distance from the *J.*

*virginiana* trunk, and that there were significant differences in surface temperatures according to the time of year (Biral *et al.*, 2019).

### *Study Tree Influences on Microclimate*

Multiple linear regression analysis revealed different relationships between study tree characteristics and microclimate variables (Table 5). Soil VWC was positively correlated to CLE and negatively correlated to height to canopy, both of which are strongly affected by tree density. Greater CLE indicates fewer neighboring trees and results in a smaller height to canopy; greater height to canopy indicates a greater density of trees and lower CLE. Increased CLE, and therefore decreased tree density, also corresponds to a decreased competition for soil water. Likewise, increased height to canopy would correspond to increased competition for soil water as well as greater canopy interception of rainfall. Solar radiation was positively correlated to CLE, but only at the octet level, there were no significant relationships for U, C, or O. Lack of significance by location is likely due to lack of variation in each location. All U measurements took place under *J. virginiana* canopies, which do not significantly vary from one another even in different seasons ( $p = 0.44$ ; Table 4), and all C data was collected under canopy types which would yield similar results as well, thereby yielding no significant trends in either location; likewise, conditions in the open remained that way for the course of the study, so no physical barriers affected radiation values, resulting in similar measurements across locations ( $p = 0.5$ ; Table 3). Temperature was not significantly related to study tree characteristics, either at the octet level or by location.

Litter depth (cm) was positively correlated to both tree height (m) and height to canopy (cm), and negatively correlated to CLE, with one or more of these relationships evident in both seasons (Summer 2018 and Spring 2019) for the whole octet, U, and C. Overall, litter depth (cm) was greatest in the forested plots (plots 5, 10, and 15) due to the strong deciduous influence – the litter composition throughout the octet was almost exclusively deciduous, not needle litter as was typically found in areas with fewer deciduous trees (personal observation). A positive correlation to height to canopy (cm), and negative correlation to CLE, is primarily driven by data collected in forested plots, as greater height to canopy was observed for trees growing in dense stands, where competition for light resources (lower CLE) causes the lower limbs to self-prune. A positive correlation to tree height (m) is most likely representative of both greater needle

production, resulting in greater total needle drop, and the fact that taller trees tended to be in dense clumps, so they had litter layers influenced by adjacent evergreen and deciduous litter (personal observation).

It has been demonstrated that litter depth and type has varying effects on seedling dynamics underneath *Juniperus* trees (Joy and Young 2002; van Els *et al.*, 2010; Biral *et al.*, 2019). Litter depth decreases with increasing distance from the tree trunk, affecting soil moisture dynamics, decreasing soil temperatures, and modifying the soil chemical composition – all of which contribute to modifying seedling dynamics (Joy and Young 2002; van Els *et al.*, 2010; Biral *et al.*, 2019). Litter type has been shown to have a very physical effect on seedling dynamics, affecting the germination of small and large seeds (Myser 1994; Yager and Smeins 1999). While the accumulation of evergreen needle litter is detrimental for many species, as evident by the reduction in plant richness underneath *Juniperus* canopies (Yager and Smeins 1999; van Els *et al.*, 2010), it appears these conditions are ideal for *Juniperus* seedlings (Wayne and Van Auken 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005).

Relationships between microclimate and study trees were not primarily driven by characteristics related to tree size (height and canopy area), but metrics (CLE and height to canopy) which are influenced by tree density and the stand conditions a given tree is experiencing. Although the degree of microclimate modification is not linked to tree size, rather to the density of trees and the position of tree relative to others, larger trees do have a larger area of microclimate influence. When studying microclimate data, examining the characteristics of the nurse plant or an individual tree was not enough, we must also account for the characteristics of the broader environment in which the trees grow. The presence of a *J. virginiana* alone is enough to make drastic changes, driving shifts in ecosystem function and productivity (Schmidt and Stubbendieck 1993; Norris *et al.*, 2001; Briggs *et al.*, 2002; McKinley and Van Auken 2005; Van Auken 2009; van Els *et al.*, 2010; Smith 2011; Blair *et al.*, 2014; Zou *et al.*, 2015; Davis *et al.*, 2018; Biral *et al.*, 2019).

### *Microclimate Influences on Seedling Spatial Dynamics*

The lack of direct relationships between seedlings and microclimate within a given location (in U, C, or O) may be the result of the individual areas having small ranges of values for certain variables. Analysis using location as a covariate revealed some patterns of

significance for microclimate variables, but they were not consistent. Underneath the tree, where the temperatures are influenced by increased humidity (van Els *et al.*, 2010; Davis *et al.*, 2018), there was a slight positive correlation of temperature to seedling density, but that does not necessarily mean this relationship is true for all locations as O had significantly (all  $p < 0.004$ ) lower densities than C and U and experienced greater temperatures (all  $p < 0.0002$ ).

Temperature appearing as a significant variable in Summer 2018 and failing to appear in Spring 2019 may be the byproduct of higher temperatures (Figure 2) and greater temperature differences (Figure 4) between locations, making effects more detectable in summer. Some interactive effects were present between microclimate variables; however, they were largely driven by U canopy patterns.

It has been demonstrated in *J. ashei* seedling and microclimate analyses that light, soil temperature, and soil moisture are highly important characteristics for seedling survival, as lower light levels, decreased temperature, and increased soil moisture alleviate stress in the shade (Wayne and Van Auken 2002). Of these, the most important variable was light, but ultimately it was concluded that increased mortality in low-light conditions suggested that the microclimate was not always directly benefitting the seedling (McKinley and Van Auken 2005). While direct comparisons are not possible, due to physiological differences between these two species, as well as location and climate differences between Oklahoma and Texas, it is interesting to note that similar results appeared in our study. The conditions are very different underneath and outside the dripline of the study tree canopies for both *J. ashei* and *J. virginiana* (Joy and Young 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005; van Els *et al.*, 2010; Biral *et al.*, 2019; chapter 1). Microclimate modifications may significantly affect seedlings at the smallest stage, facilitating a protective environment for new emergence, but it has been hypothesized that as the seedlings grow the buffering effects may become detrimental (Wayne and Van Auken, 2002; Van Auken *et al.*, 2004; McKinley and Van Auken, 2005). Consistent with this, we observed high mortality and emergence rates underneath the *J. virginiana* canopy; inconsistent with this, mortality U was primarily observed in the smallest height (cm) and basal diameter (mm) categories of seedlings, not the largest. It may be that increased mortality in larger seedlings would have been observed over a longer period of time, or it may be that competition between seedlings is more intense than expected.

Seedling emergence, mortality, and survival dynamics are strongly related to study tree characteristics but have not yet been directly linked to microclimate data. Issues connecting microclimate variables to seedling data could be partially due to data collection methodology. Our microclimate data was collected over a range of dates and times resulting in variability for temperature and solar radiation measurements and adding noise which may be complicating efforts to make connections. Collecting the microclimate data over a shorter period of time could contribute to reducing variation in measurements and provide a more robust data set for analysis, however this would be logistically very difficult due to the number of sample locations. Additionally, it has been demonstrated that the interaction of multiple variables (water, nutrients, and light) were significant for seedling success under *J. ashei* (McKinley and Van Auken 2005). We may have missed significant relationships due to the inherent correlation between microclimate variables, or failure to capture additional explanatory variables.

The decision trees revealed little additional information regarding relationships between seedlings and the microclimate. The seedling mortality model was not improved at all, and the removal of significant variables correlated to environmental data decreased the predictive ability of the model to a singular variable. Similarly, the emergence model was restricted by the removal of location as a variable, only tree height was able to explain the main association of the variables to new seedlings, and solar radiation appeared as a secondary explanatory variable, replacing location, gender, and canopy area as seen in Chapter 1, Figure 7, B. The significance of solar radiation serves as a proxy for those three variables, as increased solar radiation is associated with larger canopy areas and location in relation to the canopy. While these microclimate changes could not be quantitatively linked to seedling numbers, it is evident that larger *J. virginiana* facilitate more seedlings as well as changing the microclimate, both factors which are contributing to the changes seen in grasslands with woody plant encroachment.

## **Conclusion**

This study demonstrated that the microclimate relationship to *J. virginiana* tree characteristics is complex to a degree that we cannot characterize based on the data we collected. There were significant differences in microclimate along a location gradient in relation to the study tree, but it appears that the degree of microclimate modification was more affected by broader spatial patterns (e.g. tree density and position of study tree relative to other trees) than

any singular tree characteristic. The relationships were more nuanced than we were able to identify and describe. Although microclimate was very different (cooler, darker, drier) underneath study trees, the microclimate data we collected was not directly or consistently related to seedling presence, and did not add significant explanatory power to decision tree models, with the only microclimate variable [solar radiation ( $\text{W}/\text{m}^2$ )] appearing in the emergence model, likely as a proxy for the underneath (U) canopy location. It appears that the relationship between the nurse tree and seedlings is significantly more direct than indirect. The trees have a significant influence on seedling presence, mortality, and emergence rates, both spatially and temporally (chapter 1), most likely the result of increased seed input directly below the *J. virginiana* canopies (Holthuijzen *et al.*, 1987; Joy and Young 2002; Van Auken *et al.*, 2004; McKinley and Van Auken 2005), and a complex suite of microclimate effects. While the microclimate buffering is significant, it is not strongly influenced by tree size but stand characteristics (as indicated by CLE and height to canopy). We may conclude that the *J. virginiana* seedlings are directly influenced by the presence and characteristics of a nurse plant, but that their dynamics could not be linked to the degree of spatial variations in microclimate conditions at this time.

## Conclusion

The ecological impacts of *Juniperus* species encroaching into grasslands are well established (Briggs *et al.*, 2002; Engle *et al.*, 2007; Ganguli *et al.*, 2008; Smith 2011). The resulting shift in dynamics and factors which significantly influence woody plant encroachment success has been documented in some *Juniperus* species, but not all (Van Auken *et al.*, 2004; McKinley and Van Auken 2005), and not all facets have been studied. It was the purpose of this study to characterize the abiotic and biotic effects influencing *J. virginiana* seedling success in a southern Great Plains grassland, in order to fill a gap in the literature regarding this native species which is of such ecological and economical significance (White *et al.*, 2000; Briggs *et al.*, 2002; Engle *et al.*, 2007; Ganguli *et al.*, 2008; Boval and Dixon 2012; Anadón *et al.*, 2014). Chapter 1 addressed the seasonal variations in seedling presence, emergence, and mortality; the spatial variations in seedling dynamics in proximity to a nurse tree; and which nurse tree characteristics were significantly related to seedling success. Chapter 2 examined the specific relationships driving spatial dynamics by identifying the effects of *J. virginiana* trees on the microclimate, describing the interactions between seedlings and the microclimate, and demonstrating the direct relationships present.

Seedling dynamics temporally (over the course of a year) and spatially (in relation to nurse trees) varied. Total seedling numbers decreased through the winter and into the spring, then increased over the summer with the emergence of the spring cohort (Johnsen and Alexander 1974; FEIS 2003; Holthuijzen *et al.*, 1987). We observed lower mortality and greater emergence outside the dripline of the study trees than expected based on the performance of *Juniperus* species in other studies (Wayne and Van Auken 2002; Van Auken *et al.*, 2004). Seedlings underneath the canopy and outside the dripline, under an external canopy type were much more dynamic than those seedlings which were completely exposed, which had lower mortality and emergence overall. We identified significant differences in seedling dynamics by location and insight into effects of nurse plants, both evergreen and deciduous. 94% of the *J. virginiana* seedlings tagged and tracked in this study were directly underneath the study tree canopy or under an adjacent canopy type (*J. virginiana* and/or deciduous), the remaining 6% were within 1 m of the study tree dripline. Significantly greater (all  $p < 0.02$ ) seedling densities underneath *J. virginiana* suggested that increased seed input directly resulted in greater seedling establishment and success. Increased mortality and emergence were also evident under *J. virginiana* canopies.

Emergence rates were likely the result of increased seed inputs, and mortality was significantly affected by the high number of seedlings in the smallest sizes, making them more vulnerable (Holthuijzen *et al.*, 1987). The summer 2019 census documented increased emergence and increased mortality in all locations, with seedlings in the smaller size classes dying and larger seedlings overall persisting.

The effect of the study tree canopies on the microclimate was significant, but not linked directly to tree size. Instead, it appears that broader dynamics are responsible for the degree of microclimate effects; CLE and height to canopy (cm) are variables which are indicative of the stand density – with greater CLE and less height to canopy for trees in the open, and lower CLE and greater height to canopy for trees in thickly forested areas. We identified significant differences in the microclimate underneath the canopy and outside the dripline, in the open, for every variable in each season. The use of temperature loggers (iButtons) to track temperature patterns in different spatial proximity to the nurse plant revealed the buffering capabilities of the dense, evergreen *J. virginiana* canopies, with warmer temperatures in the coldest months, and cooler temperatures in the warmest months. We anticipated a priori that the effect of the study tree on seedling dynamics would be more indirect than direct, facilitating a beneficial microclimate which would then facilitate seedlings. Although the study trees had a very direct and measurable effect on seedling dynamics, and a more indirect influence on microclimate, seedling dynamics could not be quantitatively linked with any specific microclimate changes.

We created models which predicted seedling presence and abundance as well as mortality and emergence for *J. virginiana* seedlings in a southern Great Plains grassland. Seedling presence and abundance were significantly related to *J. virginiana* gender, height (m), canopy area (m<sup>2</sup>), DBH (cm), and age (years). Larger, older trees were statistically more likely to be female and to have higher seedling counts and densities. Study tree characteristics were used to create predictive models, identifying key characteristics linked to seedling dynamics and presenting them in a clear and concise manner – these relationships may be useful for land managers when working to address *J. virginiana* encroachment. Addition of microclimate variables to these models did not improve them, therefore we do not encourage their use for predictive purposes.

This research was conceived in order to meet a need for additional information regarding *J. virginiana* seedling dynamics in the natural habitat, without site or seedling manipulation

(Schmidt and Stubbendieck 1993; McKinley and Van Auken 2005). This marks the first time *J. virginiana* seedling dynamics have been characterized in a southern Great Plains grassland by tracking native cohorts spatially and temporally. Specific relationships between evergreen and deciduous nurse trees and *J. virginiana* seedlings were isolated and described, and *J. virginiana* tree characteristics were used to model and predict seedling dynamics. The use of microclimate data to examine and attempt to explain spatial patterns of seedlings in relationship to a nurse plant yielded few significant explanatory variables, resulting in the conclusion that the highly significant effects of the study trees on seedling dynamics may be overshadowing any microclimate-driven relationships, or that the relationships may be more complicated than we can address here.

The management implications of this study are clear. To address *J. virginiana* encroachment one should begin in the most open areas and entirely remove readily identifiable female trees – this will decrease seed inputs to the immediate vicinity and surrounding land (Johnsen and Alexander 1974; Holthuijzen and Sharik 1984; Holthuijzen *et al.*, 1987; Lawson 1990; Briggs *et al.*, 2002), as well as expose previously hidden seedlings (which should also be destroyed). Focus should then move to the remaining trees which are larger relative to the others present – these are older and statistically likely to have more seedlings (chapter 1). We advise leaving the smaller trees and extremely wooded areas for last: they do contribute to microclimate modification (chapter 2) and may be facilitating seedlings (chapter 1), but they are not contributing as significantly as the larger trees, as are less likely to have reached sexual maturity or have large numbers of associated seedlings (Krugman *et al.*, 1974; chapter 1). Following mechanical removal, regular maintenance will need to be on-going, removing all emerging *J. virginiana* as they get large enough to see. Ideally, livestock should not be immediately returned to the land, but if necessary they should be prevented from over-grazing, as this decreases herbaceous biomass and causes soil disturbance, both of which open niches for encroachment (Van Auken 2004; Anadón *et al.*, 2014). As native grass biomass returns, over time, frequent burning regimes can then be reliably used to control woody plant growth (Van Auken 2004).

Moving forward, we have demonstrated that it is possible to accurately identify and track large numbers of *J. virginiana* seedlings in relationship to a nurse plant. Our study could be improved in a number of ways, including extending the study area farther past the dripline of study tree canopies and tracking seedling dynamics over a longer period of time and with greater

frequency. We were not able to make separate observations regarding seedling growth rates and age, it would have been ideal to measure the seedlings at each census, so as to track growth patterns. Closely monitoring seedling growth could provide the data necessary to create models which would establish a better cut-off point between seedlings and saplings, so as to make data analysis more focused on true seedling dynamics without being skewed by larger, better established organisms. It would also be beneficial to collect the microclimate data over a much shorter period of time, so as to eliminate the variation in measurements such as temperature and solar radiation. This would allow better characterization of the spatial variation in the microhabitat in which these seedlings were found. Finally, it would be beneficial to collect long-term microclimate data, similar to the temperature data we collected, in order to analyze temporal as well as spatial changes.

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Chapter 1: Tables and Figures



Transect and Plot Layout, KAEFS, OK

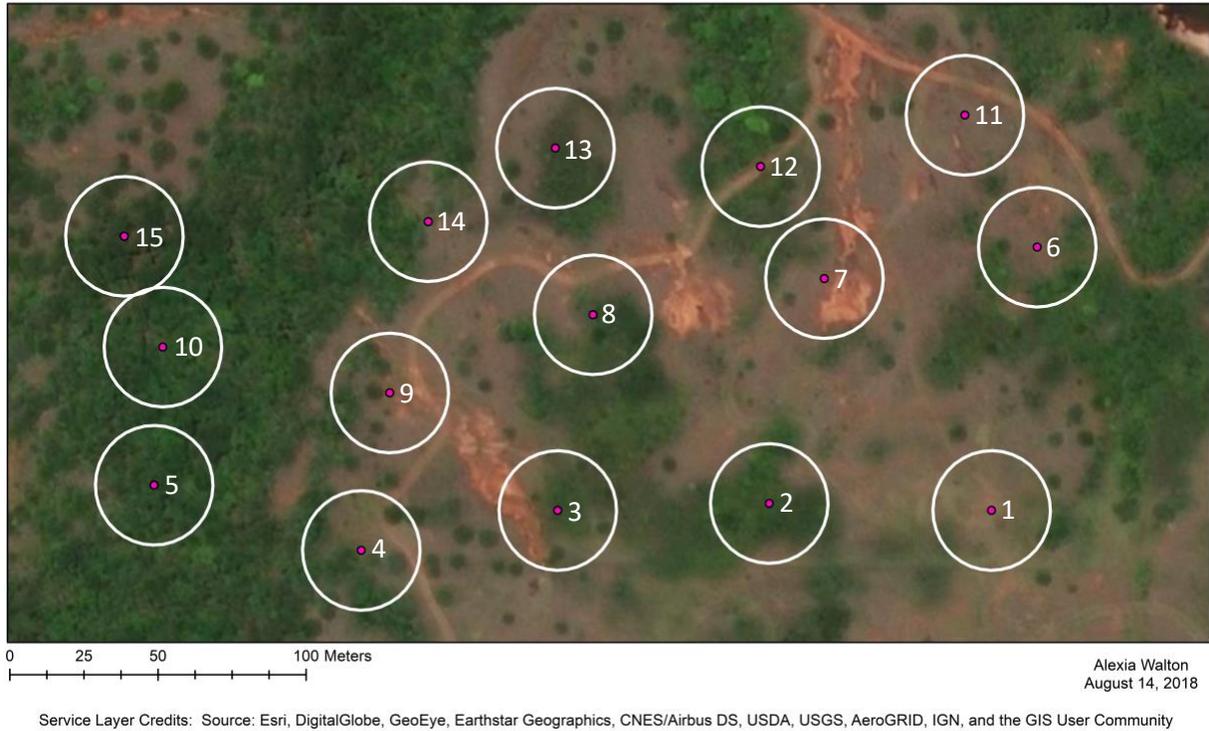


Figure 1: Transect and plot layout at the study site, Kessler Atmospheric and Ecological Field Station (KAEFS), Purcell, OK. Three parallel transects (transect 1 closest to the bottom), each with 5 plots. Plot center points are represented by the pink point, and the outer boundary the white circle. Each transect had one plot in a thickly forested area (5, 10, and 15), one plot along the transition zone from closed canopy to grassland (4, 9, and 14), and the remaining plots moved further into the encroached grassland.

Transect	Study trees	Height (m)	DBH (cm)	Canopy area (m <sup>2</sup> )	Age (years)
1	46	4.51 (1.50 – 10.20)	8.79 (0.48 – 29.40)	14.35 (1.46 – 84.95)	24.27 (8.26 – 75.86)
2	65	4.19 (1.60 – 9.50)	7.03 (0.20 – 23.80)	9.19 (0.57 – 37.59)	20.87 (8.46 – 43.08)
3	62	3.21 (1.40 – 8.00)	5.80 (0.50 – 30.80)	8.36 (0.38 – 50.90)	19.10 (8.95 – 61.46)

Table 1: *Juniperus virginiana* study trees data by transect: average height (m), diameter at breast height (DBH) (cm), canopy area (m<sup>2</sup>), and age (years) (and range).

Census Period	Summer 2018			Winter 2018/19			Spring 2019			Summer 2019		
Location	U	C	O	U	C	O	U	C	O	U	C	O
Total seedlings	966	234	74	873	213	79	849	204	81	1017	263	80
Seedling density (m <sup>2</sup> )	2.28	0.78	0.16	2.12	0.73	0.18	2.08	0.71	0.18	2.59	0.89	0.19
Mortality (%)				11.9	12.82	5.41	3.89	4.25	1.27	17.95	17.33	11.11
Emergence (%)				2.52	4.23	11.39	1.18	0.49	3.70	31.66	36.50	10.0

Table 2: Spatial and temporal patterns of *J. virginiana* seedlings across census periods. Total seedling counts reflect the sum of seedlings counted as new and present in a given census in a given location in relationship to the *J. virginiana* study tree canopy (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the dripline of the study tree, open to the environment). Seedling density represents the average density in a given location in a given census period. Percent mortality is based on the number of seedlings absent since the prior census (100 - % survival, where % survival was the seedlings present in a given census divided by seedlings present in the previous census, multiplied by 100). Percent emergence is based on the number of new seedlings in a given census (new seedlings divided by total seedlings present in a given census, multiplied by 100).

	Winter 2018/19				Spring 2019				Summer 2019			
	Census	Location			Census	Location			Census	Location		
		U	C	O		U	C	O		U	C	O
Mortality	A	A	A	A	A	A	A	A	B	B	B	A
Height	3.50	3.46	3.50	4.75	3.75	3.74	3.59	5.10	5.63	5.71	5.07	6.96
Basal Diameter	0.27	0.28	0.20	0.39	0.30	0.32	0.21	0.50	0.58	0.59	0.50	0.69
Survival												
Height	15.36	14.64	19.73	11.56	15.47	14.85	19.69	11.18	17.09	16.31	22.50	11.99
Basal Diameter	1.79	1.68	2.40	1.34	1.80	1.70	2.39	1.28	1.99	1.87	2.73	1.42

Table 3: Average seedling height and basal diameter by census period. Values represent average for a given census (Census), as well as averages for seedlings in relationship to the *J. virginiana* study tree canopy (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the dripline of the study tree, open to the environment). Significant (all  $p \leq 0.005$ ) seedling mortality differences are indicated with uppercase letters. No significant differences (all  $p \geq 0.11$ ) were present across seedling survival.

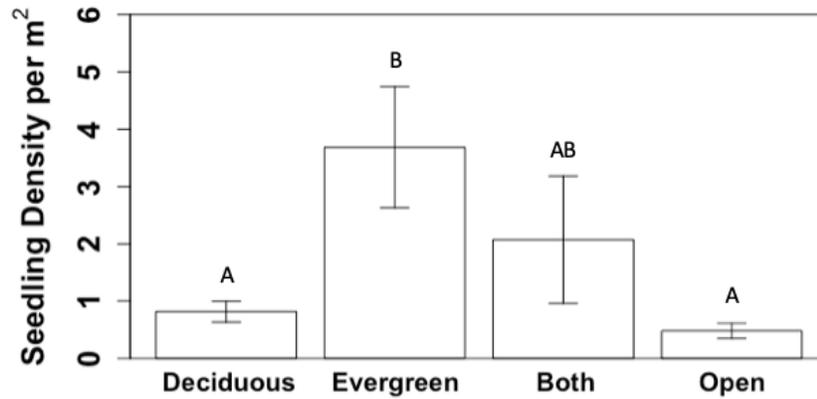


Figure 2: Canopy cover type analysis results. Averages for seedling density under external canopy types are illustrated here. Significantly different ( $p < 0.01$ ) variables are indicated by uppercase letters.

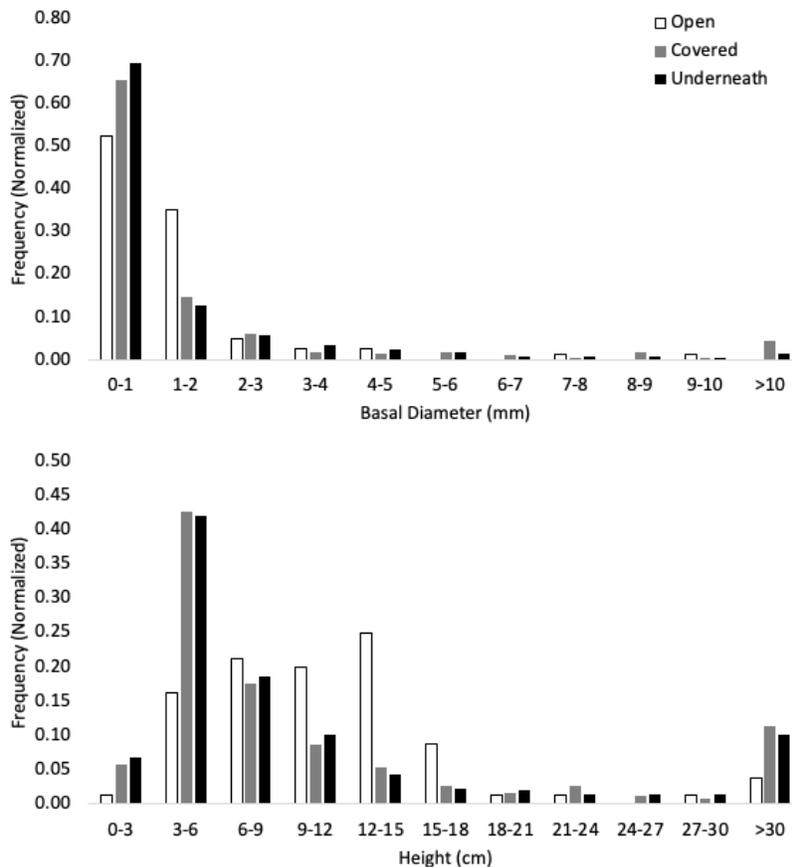


Figure 3: Normalized frequency of seedling height (cm) and basal diameter (mm) for *J. virginiana* seedlings which survived throughout the study, by location [black bars (underneath) = underneath study tree canopy; gray bars (covered) = outside the dripline of the study tree, covered by canopy; white bars (open) = outside the dripline of the study tree, open to the environment].

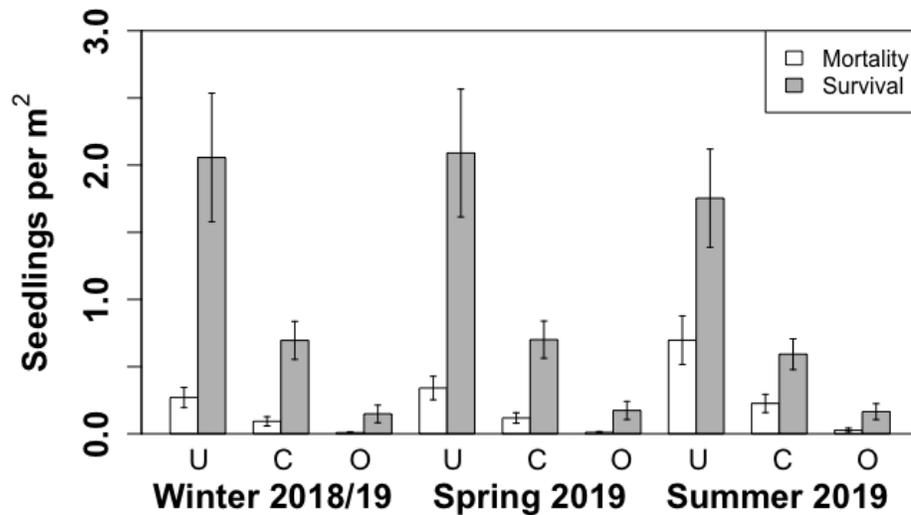


Figure 4: Seedling survival (white bars) and mortality (gray bars) numbers expressed as density by location in relation to the *J. virginiana* study tree (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the dripline of the study tree, open to the environment).

	Air Temperature (°C)	Solar Radiation (W/m <sup>2</sup> )	Rainfall (mm)
2018			
Winter	4.47 (-13.33 – 24.81)	115.83 (0 – 769.33)	224.90
Spring	16.48 (1.67 – 30.37)	250.67 (0 – 1149)	643.35
Summer	26.75 (15.56 – 37.96)	283.63 (0 – 1239)	447.86
Fall	15.28 (0.56 – 30.37)	141.08 (0 – 1015)	691.11
2019			
Winter	4.67 (-8.15 – 20.74)	104.45 (0 – 837.33)	442.74
Spring	15.20 (-2.22 – 27.96)	216.64 (0 – 1172.33)	1004.13
Summer	26.19 (14.63 – 37.59)	282.47 (0 – 1244)	434.97
Fall	16.24 (0.74 – 31.67)	173.75 (0 – 978)	474.33

Table 4: Environmental conditions for the Washington Mesonet site. Air temperature (°C) and solar radiation (W/m<sup>2</sup>) daily averages (and range), and cumulative rainfall (mm), for the seasons within the 2018 – 2019 study period. Data retrieved from Oklahoma Mesonet Data (Mesonet.org, 2020).

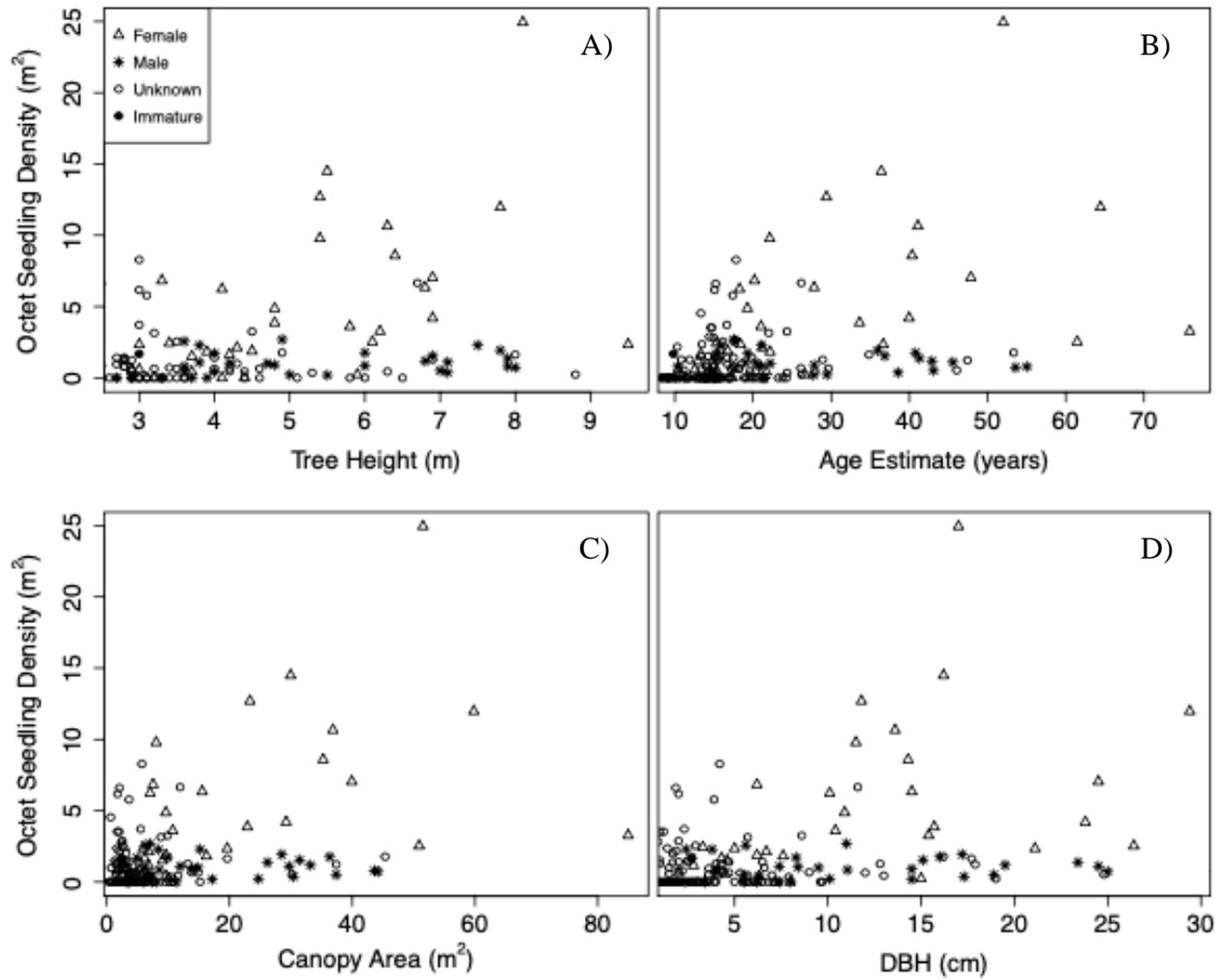


Figure 5: Octet seedling density (entire study area at a given tree) against *J. virginiana* study tree characteristics: A) tree height (m), B) age estimate (years), C) canopy area ( $m^2$ ), and D) DBH (cm). Female trees are represented by open triangles ( $\Delta$ ), male trees are represented by the asterisk (\*), unknown trees are represented by open circles ( $\circ$ ), and reproductively immature trees are represented by filled circles ( $\bullet$ ).

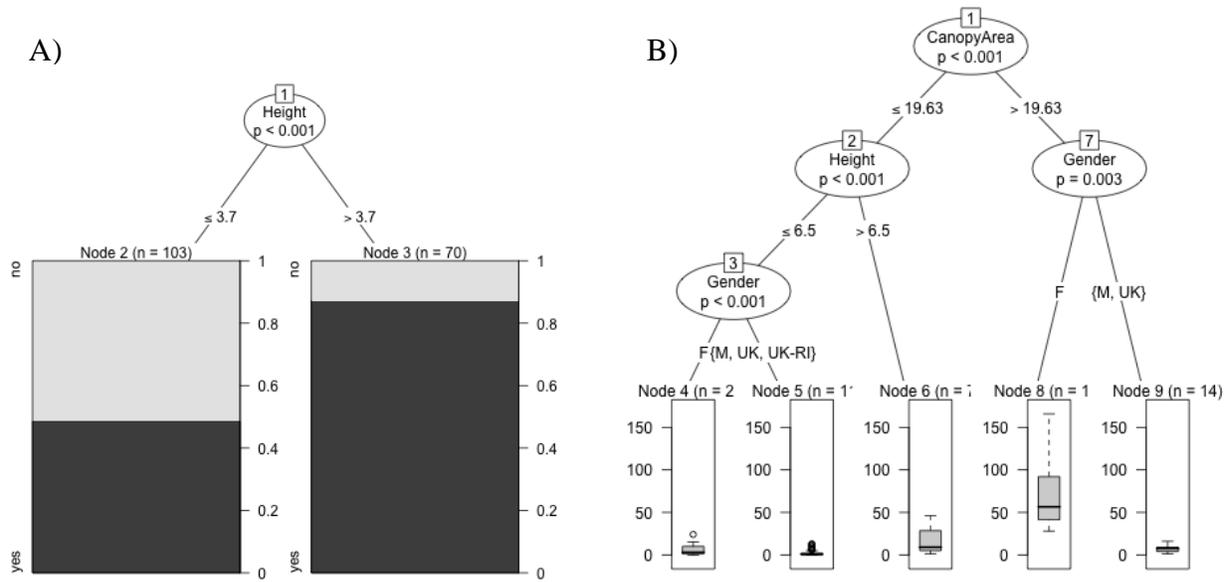


Figure 6: Decision trees: A) predicting seedling presence at a given *J. virginiana* tree and B) predicting the number of seedlings present at a given *J. virginiana* tree. Height is in meters (m), canopy area is in square meters (m<sup>2</sup>).

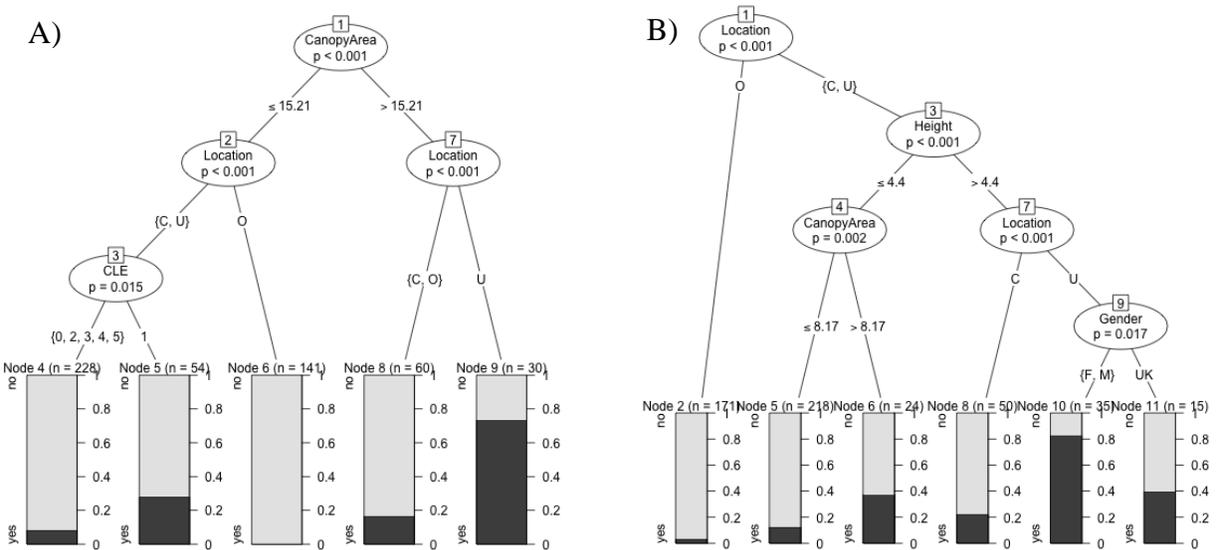


Figure 7: Decision trees: A) predicting seedling mortality at a given *J. virginiana* tree and B) predicting seedling emergence at a given *J. virginiana* tree. Height is in meters (m), canopy area is in square meters (m<sup>2</sup>).

## Chapter 2: Tables and Figures

Transect	Study trees	Height (m)	DBH (cm)	Crown Size (m <sup>2</sup> )	Age (years)	CLE	Height to Canopy (cm)	Octet Area (m <sup>2</sup> )	Underneath the Canopy (m <sup>2</sup> )	Outside the Dripline (m <sup>2</sup> )
1	46	4.51 (1.50 – 10.20)	8.79 (0.48 – 29.40)	14.35 (1.46 – 84.95)	24.27 (8.26 – 75.86)	2.53 (0 – 5)	31.63 (0 – 230)	3.48 (0.80 - 19.88)	1.70 (0.07 - 14.68)	1.78 (0.73 - 5.20)
2	65	4.19 (1.60 – 9.50)	7.03 (0.20 – 23.80)	9.19 (0.57 – 37.39)	20.87 (8.46 – 43.08)	2.14 (0 – 5)	33.37 (0 – 260)	2.82 (0.74 - 8.56)	1.23 (0.05 - 5.29)	1.59 (0.68 - 3.28)
3	62	3.21 (1.40 – 8.00)	5.80 (0.50 – 30.80)	8.36 (0.38 – 50.90)	19.10 (8.95 – 61.46)	2.18 (0 – 5)	23.63 (0 – 135)	2.40 (0.57 - 11.45)	1.00 (0.02 - 7.60)	1.40 (0.55 - 3.85)

Table 1: Study tree characteristics by transect. Number of mature *J. virginiana* trees, average tree height, diameter at breast height (DBH), canopy area, tree age, canopy light exposure (CLE), height to canopy, study area, area underneath the study tree canopy, and area outside the study tree (and range).

Census Period	Summer 2018				Spring 2019			
	Octet	U	C	O	Octet	U	C	O
Total seedlings	1274	966	234	74	1134	849	204	81
Seedling density (m <sup>2</sup> )	1.53	2.28	0.78	0.16	1.42	2.08	0.71	0.18

Table 2: Spatial and temporal patterns of *J. virginiana* seedlings across census periods. Total seedling counts reflect the sum of seedlings counted as new and present in a given census in a given location in relationship to the *J. virginiana* study tree canopy (Octet = entire octet, underneath and up to 1 m outside the study tree dripline; U = underneath study tree canopy; C = outside the dripline of the study tree, covered by an adjacent canopy; O = outside the dripline of the study tree, open to the environment). Seedling density represents the average density in a given location in a given census period.

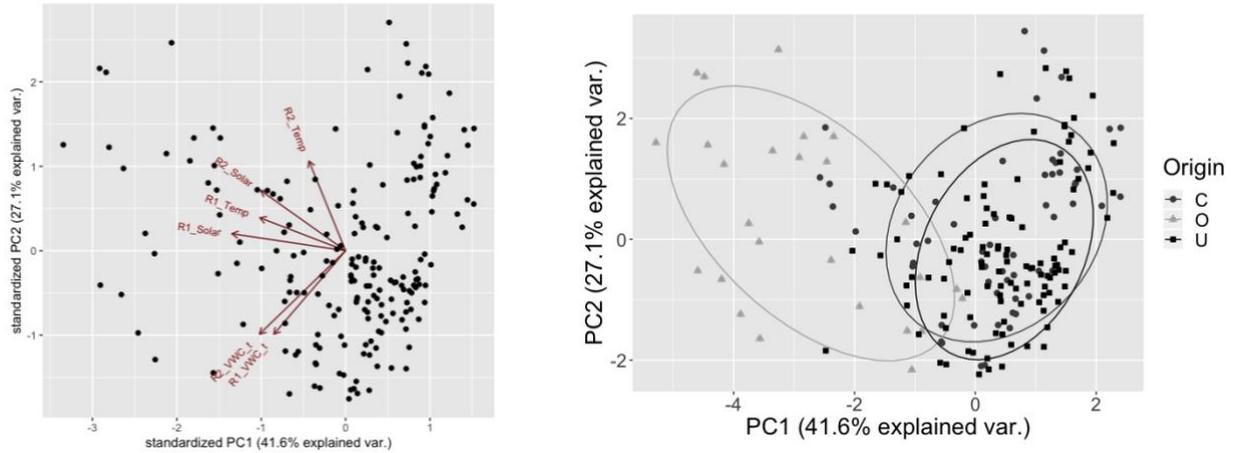


Figure 1: Principal component analysis of microclimate variable averages [Summer 2018 and Spring 2019; soil volumetric water content (VWC; %), temperature ( $^{\circ}\text{C}$ ), solar radiation ( $\text{W}/\text{m}^2$ )] in relationship to the study tree (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the dripline of the study tree, open to the environment).

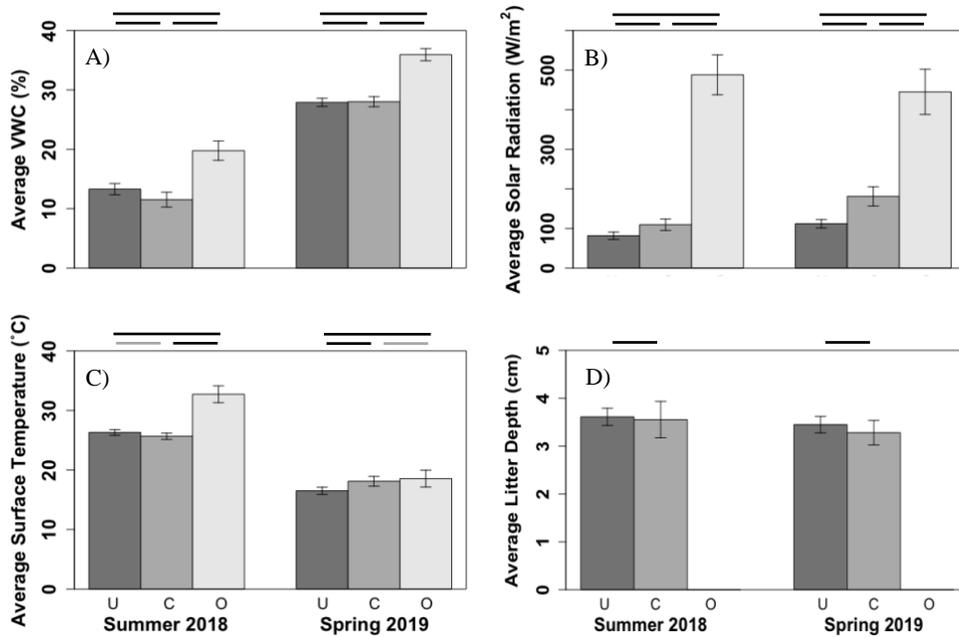


Figure 2: Microclimate data by location and collection period (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by an adjacent canopy; O = outside the dripline of the study tree, open to the environment). Bar graphs of average A) soil volumetric water content (VWC; %); B) solar radiation ( $\text{W}/\text{m}^2$ ); C) soil surface temperature ( $^{\circ}\text{C}$ ); and D) litter depth (cm), with standard errors. Litter depth was 0 in the open location. The solid bars above each panel indicate relationship between locations within the collection period. Black indicates significant relationships; gray indicates non-significant relationships. Results represent both paired t-tests (U vs C and U vs O) and un-paired t-tests (C vs O).

	Summer 2018 vs Spring 2019		
	Underneath	Covered	Open
VWC %	< 2.2E-16	< 2.2E-16	1.277E-11
Litter depth (cm)	0.21	0.28	-
Solar Radiation (W/m <sup>2</sup> )	0.041	0.0049	0.5
Surface Temperature (°C)	< 2.2E-16	5.206E-11	1.202E-08

Table 3: T-tests (paired) comparing Summer 2018 and Spring 2019 microclimate data within each location (Underneath = underneath study tree canopy; Covered = outside the dripline of the study tree, covered by and adjacent canopy; Open = outside the dripline of the study tree, open to the environment). Comparison could not be made in the open condition due to absence of a litter layer.

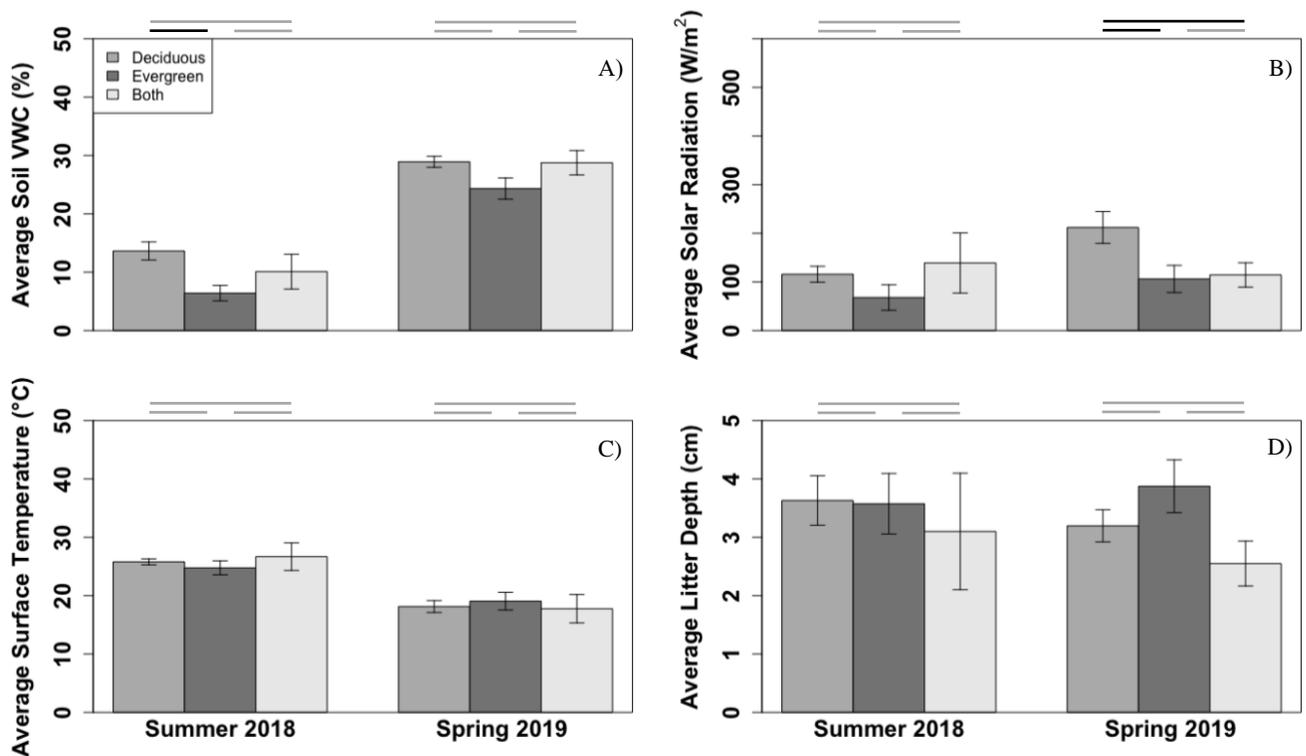


Figure 3: Summer 2018 and Spring 2019 microclimate data by canopy type outside the study tree dripline. Bar graphs of average A) soil volumetric water content (VWC) (%); B) solar radiation (W/m<sup>2</sup>); C) soil surface temperature (°C); and D) litter depth (cm), with standard errors. Solid bars above each panel indicate relationship between locations within the collection period. Black indicates significant relationships; gray indicates non-significant relationships. Results represent un-paired t-tests.

	Summer 2018 vs Spring 2019		
	Deciduous	Evergreen	Both
VWC %	4.851E-14	3.452E-08	0.0006
Litter depth (cm)	0.20	0.48	0.67
Solar Radiation (W/m <sup>2</sup> )	0.0042	0.44	0.74
Surface Temperature (°C)	1.516E-07	0.011	0.03

Table 4: T-test (paired) comparing Summer 2018 and Spring 2019 microclimate data by canopy type outside the study tree dripline. Deciduous = deciduous canopy; Evergreen = *J. virginiana* canopy; Both = combined *J. virginiana* and deciduous canopy.

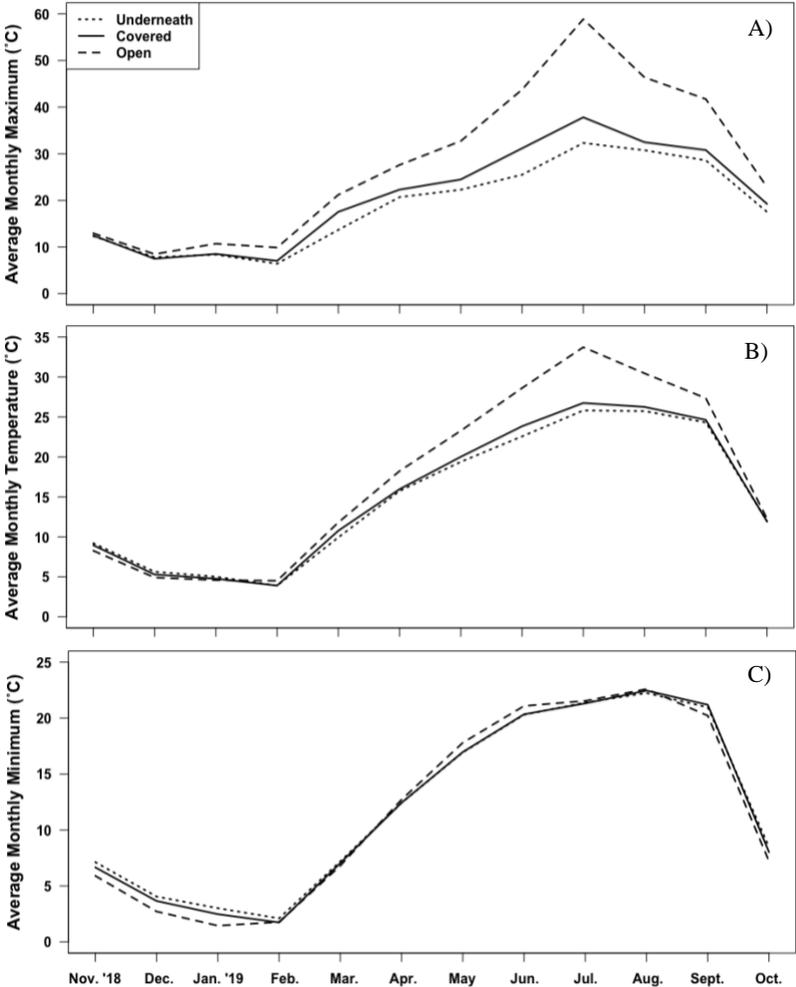


Figure 4: Monthly temperatures (°C) by location (Underneath = underneath study tree canopy; Covered = outside the dripline of the study tree, covered by adjacent canopy; Open = outside the dripline of the study tree, open to the environment). A) average monthly maximums, B) average monthly temperatures, and C) average monthly minimums.

Season and Level of Comparison	Study Tree Characteristic	VWC (%)			Solar Radiation (W/m <sup>2</sup> )			Temperature (°C)			Litter Depth (cm)		
		Coeff.	SE (±)	p-value	Coeff.	SE (±)	p-value	Coeff.	SE (±)	p-value	Coeff.	SE (±)	p-value
<u>Summer 2018</u>													
Whole Octet	Height (m)	-0.010	0.0093	0.26	-13.99	10.68	0.19	-0.42	0.43	0.33	0.42	0.11	<b>0.00019</b>
	Canopy Area (m <sup>2</sup> )	0.0011	0.0012	0.37	-0.83	1.39	0.55	-0.036	0.055	0.52	-0.0095	0.014	0.51
	Height to canopy (cm)	-0.0011	0.00044	0.017	-0.94	0.51	0.066	-0.024	0.02	0.23	0.011	0.0052	0.031
Underneath	CLE	0.032	0.0065	<b>3.81E-06</b>	30.08	7.48	<b>0.00011</b>	0.36	0.3	0.24	-0.208766	0.077	<b>0.0079</b>
	Height (m)	-0.012	0.0096	0.22	-4.67	7.09	0.512	-0.16	0.38	0.67	0.43	0.12	<b>0.00052</b>
	Canopy Area (m <sup>2</sup> )	0.0012	0.0013	0.33	-0.42	0.92	0.65	-0.061	0.05	0.22	-0.0088	0.016	0.58
Covered	Height to canopy (cm)	-0.00093	0.00046	0.043	-0.38	0.34	0.26	-0.02	0.018	0.28	0.0052	0.0057	0.37
	CLE	0.031	0.0067	<b>1.32E-05</b>	5.92	4.97	0.24	0.11	0.27	0.67	-0.32	0.085	<b>0.00033</b>
	Height (m)	-0.013	0.013	0.30	-13.95	10.17	0.18	-0.27	0.39	0.5	0.58	0.27	0.039
Open	Canopy Area (m <sup>2</sup> )	-0.00033	0.0021	0.88	0.86	1.34	0.52	-0.0087	0.052	0.87	-0.068	0.057	0.24
	Height to canopy (cm)	-0.0013	0.00051	0.017	-0.61	0.44	0.17	-0.015	0.017	0.39	0.028	0.0096	<b>0.0058</b>
	CLE	0.048	0.012	<b>0.00013</b>	14.08	9.18	0.13	-0.29	0.36	0.43	-0.38	0.26	0.16
Spring 2019	Height (m)	0.0018	0.021	0.93	31.62	56.09	0.58	-1.21	1.57	0.45			
	Canopy Area (m <sup>2</sup> )	0.0026	0.0031	0.42	-6.5	8.54	0.45	0.19	0.24	0.43			
	Height to canopy (cm)	0.0014	0.0035	0.69	0.55	5.55	0.92	0.024	0.15	0.88			
Whole Octet	CLE	-0.0021	0.02	0.92	-30.3	44.3	0.50	-1.076	1.1	0.34			
	Height (m)	-8.56E-03	4.64E-03	0.068	-1.59	12.54	0.90	0.75	0.49	0.13	0.33	0.095	<b>0.00068</b>
	Canopy Area (m <sup>2</sup> )	-2.28E-05	6.05E-04	0.97	-1.19	1.63	0.47	-0.042	0.064	0.51	-0.0028	0.012	0.82
Underneath	Height to canopy (cm)	-7.50E-04	2.20E-04	<b>0.00091</b>	-0.57	0.59	0.34	0.019	0.023	0.42	0.0071	0.0045	0.12
	CLE	1.52E-02	3.25E-03	<b>9.34E-06</b>	26.38	8.79	<b>0.0034</b>	-0.45	0.35	0.19	-0.2	0.067	<b>0.0028</b>
	Height (m)	-9.33E-03	5.16E-03	0.074	8.49	8.27	0.31	0.77	0.46	0.092	0.3	0.12	0.014
Covered	Canopy Area (m <sup>2</sup> )	-4.67E-05	6.72E-04	0.95	-1.34	1.08	0.22	-0.043	0.059	0.47	-0.0016	0.016	0.92
	Height to canopy (cm)	-5.85E-04	2.44E-04	0.018	0.6	0.39	0.13	0.029	0.022	0.18	0.00026	0.0058	0.97
	CLE	1.47E-02	3.62E-03	<b>9.09E-05</b>	1.57	5.8	0.79	-0.65	0.32	0.044	-0.33	0.085	<b>0.00020</b>
Open	Height (m)	-0.0065	0.0064	0.32	1.53	16.82	0.93	0.9	0.59	0.13	0.23	0.17	0.20
	Canopy Area (m <sup>2</sup> )	0.00041	0.001	0.69	1.05	2.22	0.64	0.0071	0.078	0.93	-0.017	0.036	0.65
	Height to canopy (cm)	-0.00098	0.00025	<b>0.00027</b>	-0.99	0.73	0.18	0.025	0.026	0.34	0.027	0.0062	<b>8.69E-05</b>
Spring 2019	CLE	0.013	0.0058	0.026	31.93	15.19	0.040	-0.21	0.53	0.7	-0.2	0.17	0.24
	Height (m)	0.0038	0.01036	0.72	-9.054	62.84	0.89	1.17	1.5	0.44			
	Canopy Area (m <sup>2</sup> )	-0.00041	0.0015	0.79	-4.29	9.56	0.66	-0.17	0.23	0.47			
Spring 2019	Height to canopy (cm)	0.0018	0.0017	0.29	-6.54	6.22	0.30	-0.055	0.15	0.71			
	CLE	0.014	0.0097	0.18	-35.2	49.62	0.48	-2.17	1.05	0.048			

Table 5: Multiple linear regression analysis results for microclimate data and study tree characteristics. Analyses were done at the whole octet scale and for each canopy position (Underneath = underneath the study tree canopy; Covered = outside the dripline of the study tree, covered by an adjacent canopy; Open = outside the dripline of the study tree, open to the environment) separately. Bonferroni's correction was employed in order to correct for multiple comparisons of variables. A correction value was calculated for each type of microclimate data [VWC (%), solar radiation (W/m<sup>2</sup>), and temperature (°C) all = 0.00625; litter depth (cm) = 0.00833], significant p-values according to this standard are bolded.

Covariates	Census 1 Seedling Density			Covariates	Census 3 Seedling Density		
	Coeff.	SE (±)	p-value		Coeff.	SE (±)	p-value
VWC (%)	-23.329	30.718	0.45	VWC (%)	-5.798	5.971	0.33
Temperature (°C)	0.139	0.046	<b>0.0028</b>	Temperature (°C)	0.031	0.362	0.93
Solar Radiation (W/m <sup>2</sup> )	0.233	0.125	0.064	Solar Radiation (W/m <sup>2</sup> )	-0.012	0.050	0.81
Litter Depth (cm)	-0.500	1.271	0.69	Litter Depth (cm)	0.118	1.572	0.94
Location - O	-1.316	0.704	0.062	Location - O	-1.305	0.886	0.14
Location - U	1.883	0.412	<b>6.32E-06</b>	Location - U	1.857	0.521	<b>0.00040</b>
Height (m)	0.144	0.078	0.065	Height (m)	0.104	0.096	0.28
Canopy Area (m <sup>2</sup> )	0.029	0.009	<b>0.0013</b>	Canopy Area (m <sup>2</sup> )	0.023	0.009	<b>0.011</b>
Gender - M	-2.053	0.412	<b>9.09E-07</b>	Gender - M	-1.761	0.503	<b>0.00051</b>
Gender - UK	-0.970	0.293	<b>0.0010</b>	Gender - UK	-0.997	0.362	<b>0.0061</b>
Gender - UK-RI	-3.293	2.113	0.12	Gender - UK-RI	-2.658	2.002	0.18
VWC:Temperature	0.914	1.384	0.51	VWC:Temperature	0.531	0.730	0.47
VWC:Solar Radiation	-0.316	0.288	0.27	VWC:Solar Radiation	0.033	0.090	0.72
Temperature:Solar Radiation	-0.013	0.006	<b>0.030</b>	Temperature:Solar Radiation	0.000	0.002	0.85
VWC:Litter	12.587	12.306	0.31	VWC:Litter	3.625	3.858	0.35
Temperature:Litter	-0.048	0.057	0.40	Temperature:Litter	-0.030	0.121	0.80
Solar Radiation:Litter	0.093	0.043	<b>0.031</b>	Solar Radiation:Litter	0.015	0.029	0.60
VWC:Location - O	23.836	30.854	0.44	VWC:Location - O	6.646	6.887	0.33
VWC:Location - U	23.035	32.736	0.48	VWC:Location - U	-2.256	8.601	0.79
Temperature:Location - O	-0.103	0.076	0.18	Temperature:Location - O	0.042	0.372	0.91
Temperature:Location - U	0.145	0.082	0.08	Temperature:Location - U	-0.177	0.544	0.74
Solar Radiation:Location - O	-0.245	0.128	0.06	Solar Radiation:Location - O	-0.012	0.065	0.85
Solar Radiation:Location - U	-0.196	0.230	0.40	Solar Radiation:Location - U	-0.010	0.194	0.96
Litter:Location - O	NA	NA	NA	Litter:Location - O	NA	NA	NA
Litter:Location - U	2.92	2.00	0.15	Litter:Location - U	-2.93	3.34	0.38

Table 6: Generalized linear model analysis results for seedling density, microclimate data, and significant study tree characteristics with location as a covariate (U = underneath the study tree canopy; C = outside the dripline of the study tree, covered by an adjacent canopy; O = outside the dripline of the study tree, open to the environment). Significant p-values are bolded.

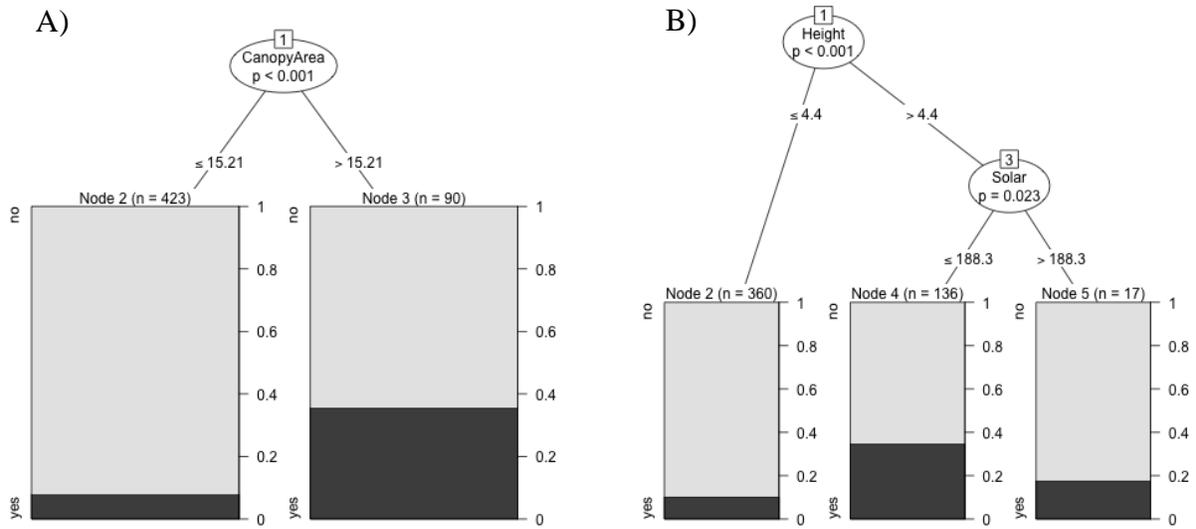


Figure 5: Decision trees: modeling A) seedling mortality and B) seedling emergence at a given *J. virginiana* tree influenced by the nurse plant and microclimate variables. Canopy area is in square meters (m<sup>2</sup>), height is in meters (m), “Solar” represents solar radiation (W/m<sup>2</sup>).

## Appendix

*J. virginiana* seedling data for the entire study area. Total number of seedlings, as well as average number of seedlings per *J. virginiana* study tree, by transect and plot. Average (and range) of measured characteristics. The range of seedling branches was 0 – 30+ for each transect; the range of branches was 0 – 28 for plot 13, and 0 – 30+ for the remaining plots.

Location	Total seedlings	Average per Tree	Basal diameter (mm)	Height (cm)
Transect 1	1006	30	1.23 (<1 – 20.63)	11.47 (0.75 – 122.5)
Plot 1	1	1	0.76	4.50
2	3	2	0.41 (<1 – 0.48)	5.00 (4 – 6)
3	319	40	0.55 (<1 – 11.9)	6.09 (1 – 91)
4	572	41	1.73 (<1 – 20.63)	15.15 (0.75 – 122.5)
5	111	12	0.63 (<1 – 9.48)	8.15 (2 – 106)
Transect 2	466	9	1.08 (<1 – 22.16)	9.69 (1 – 139)
Plot 6	147	16	0.65 (<1 – 7.81)	6.65 (1 – 90)
7	69	7	1.96 (<1 – 22.16)	15.06 (1 – 137)
8	23	4	1.49 (<1 – 5.01)	10.54 (2 – 46)
9	133	10	1.37 (<1 – 16.47)	11.83 (1 – 139)
10	94	4	0.59 (<1 – 8.83)	7.27 (2 – 94)
Transect 3	290	8	1.58 (<1 – 22.27)	14.03 (2 – 180)
Plot 11	46	9	1.77 (<1 – 22.27)	16.26 (2 – 158.50)
12	78	9	2.61 (<1 – 22.26)	21.16 (2 – 180)
13	47	7	0.53 (<1 – 2.56)	5.90 (2 – 20)
14	58	7	1.30 (<1 – 16.44)	11.72 (2 – 123.5)
15	61	9	1.18 (<1 – 11.97)	11.68 (2 – 94.5)

Temporal patterns of *J. virginiana* seedlings across censuses. Total seedling counts reflect the sum of seedlings counted as new and present in a given census. Percent mortality is based on the number of seedlings absent since the prior census (100 - % survival, where % survival was the seedlings present in a given census divided by seedlings present in the previous census, multiplied by 100). Percent emergence is based on the number of new seedlings in a given census (new seedlings divided by total seedlings present in a given census, multiplied by 100). Significant relationships ( $p \leq 0.03$ ) within each census indicated by lowercase letters, across censuses indicated by uppercase letters.

Census period	Summer 2018	Winter 2018/19	Spring 2019	Summer 2019
Total seedlings	1274	1165	1134	1360
Mortality (%)		11.7 aA	3.8 aB	17.4 aA
Emergence (%)		3.4 bB	1.2 aB	31.3 bA

Percent emergence and percent mortality by location in relationship to the study tree (U = underneath study tree canopy; C = outside the dripline of the study tree, covered by canopy; O = outside the dripline of the study tree, open to the environment). Percent mortality is based on the number of seedlings absent since the prior census (100 - % survival, where % survival was the seedlings present in a given census divided by seedlings present in the previous census, multiplied by 100). Percent emergence is based on the number of new seedlings in a given census (new seedlings divided by total seedlings present in a given census, multiplied by 100).

