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HABITAT AND DEMOGRAPHY OF THE OZARK CHINQUAPIN (CASTANEA OZARKENSIS) AT ROARING RIVER STATE PARK IN BARRY COUNTY, MISSOURI

Danielle Evilsizor Pittsburg State University, devilsizor@gus.pittstate.edu

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HABITAT AND DEMOGRAPHY OF THE OZARK CHINQUAPIN (*CASTANEA OZARKENSIS*) AT ROARING RIVER STATE PARK IN BARRY COUNTY, MISSOURI

A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Degree of Master of Science

Danielle F. Evilsizor

Pittsburg State University

Pittsburg, Kansas

December 2023

HABITAT AND DEMOGRAPHY OF THE OZARK CHINQUAPIN (*CASTANEA OZARKENSIS*) AT ROARING RIVER STATE PARK IN BARRY COUNTY, MISSOURI

Danielle F. Evilsizor

APPROVED:

Thesis Advisor __

Dr. Neil Snow, Department of Biology

Committee Member __

Dr. Andrew George, Department of Biology

Committee Member __

Dr. Catherine Hooey, Department of History, Philosophy, and Social Science

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HABITAT AND DEMOGRAPHY OF THE OZARK CHINQUAPIN (*CASTANEA OZARKENSIS*) AT ROARING RIVER STATE PARK IN BARRY COUNTY, MISSOURI

An Abstract of the Thesis by Danielle Evilsizor

The Ozark chinquapin, *Castanea ozarkensis* Ashe, is a chestnut tree with a range concentrated in the Interior Highlands of North America. Like other North American members of *Castanea*, it was reduced from an overstory tree to an understory shrub by the invasive chestnut blight fungus (*Cryphonectria parasitica* [Murrill] M.E. Barr) during the early $20th$ century. However, relatively little is known about the habitat of this species or its health and reproductive capability post chestnut blight. Chapter one of this study analyzed the habitat of this species through a random forest species distribution model (SDM) to predict where it might grow. Based on the values of the Area Under the Curve and other statistics, this model predicted the species' current distribution in the study area reasonably accurately. The results of the SDM showed that elevation and slope are the most important habitat variables for the Ozark chinquapin. Additionally, the associated flora is indicative of dry, acidic, chert woodlands. This SDM and future habitat studies will enable resource agencies to narrow their survey areas and inform where Ozark chinquapins might be reintroduced as chestnut blight resistant technology develops. In Chapter II, we evaluated the health and reproductive status of the Ozark chinquapin at Roaring River State Park, approximately seventy years after the arrival of the blight to the central United States. While numerous studies have documented the response of the American chestnut (*C. dentata*) to chestnut blight, little is known about the health and reproductive status of the Ozark chinquapin post-blight. This study aimed to assess the

factors that influence the probability of chestnut blight infection and to evaluate which variables predict reproduction in an Ozark chinquapin population in the southcentral Missouri Ozarks. We used generalized linear models and AIC model selection to examine potential factors that affect probability of reproduction or blight infection. Models showed that Ozark chinquapin reproduction was related to the maximum stem height of the tree and past fire frequency. Additionally, stem height, the presence of deer damage, and the amount of time since the area was last burned increased blight infection. Our findings suggest that the Ozark chinquapin is capable of reproduction in certain situations but is susceptible to chestnut blight through deer damage and the burn time interval, opening the door to further research to better inform conservation efforts for this blight-stricken tree.

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ABSTRACT

The Ozark chinquapin, *Castanea ozarkensis* Ashe, is a chestnut tree with a range concentrated in the Interior Highlands of North America. Like other North American members of *Castanea*, it was reduced from an overstory tree to an understory shrub by the invasive chestnut blight fungus (*Cryphonectria parasitica* [Murrill] M.E. Barr) during the early $20th$ century. However, relatively little is known about the habitat of this species. This study analyzed the habitat of the Ozark chinquapin through a random forest species distribution model (SDM) to predict where it might grow. Goodness of fit tests indicated that the model performed reasonably well at predicting the distribution within the study area. The results of the SDM suggested that elevation and slope are the most important habitat variables for predicting occurrence of the Ozark chinquapin. Additionally, the associated flora was indicative of dry, acidic, chert woodlands. This SDM and future habitat studies will enable resource agencies to narrow their survey areas and inform where blight resistant Ozark chinquapins might be introduced.

INTRODUCTION

The Ozark chinquapin's distribution is centralized in the Interior Highlands of North America with outlying populations in Louisiana, Mississippi, and historically in Alabama (Kartesz, 2023; Fig. 1.1). Originally described by Ashe (1923) at the species level, it was later described as a variety of the Allegheny chinquapin – *Castanea pumila* var*. ozarkensis* (Ashe) G.E. Tucker (1975). However, differences in morphology, habitat, range, and higher levels of genetic diversity differentiate *C. ozarkensis* from *C. pumila* (Dane et al., 1999; Shaw et al., 2012; Spriggs and Fertakos, 2021).

Like other members of *Castanea*, the Ozark chinquapin has been impacted by chestnut blight (*Cryphonectria parasitica*), which was first detected in eastern North America in 1904 (Paillet and Cerny, 2012; Rigling and Prospero, 2018). Originally from Asia, chestnut blight was introduced to North America through imported infected chestnut trees (Rigling and Prospero, 2018). Within fifty years of the arrival of *C. parasitica*, the entire North American distribution of the American chestnut species (*Castanea dentata*) was reduced from canopy trees to the understory (Rigling and Prospero, 2018). By the 1950s, *C. parasitica* had spread to the Interior Highlands, infecting *Castanea pumila* and *Castanea ozarkensi*s (Paillet and Cerny, 2012).

In Missouri, *Castanea ozarkensis* is found only in nine counties (Fig. 1.1) and is ranked "State Imperiled" by the Missouri Natural Heritage Program (2023). Before the arrival of the blight, all chestnut species were of high economic importance to the timber industry and were an important food source for wildlife and indigenous and foreign settlers (Holmes et al., 2009). The removal or catastrophic decline of an ecologically prominent tree species can have significant ecological impacts on woodland structure,

nutrient cycling, and loss of shelter and food for a variety of wildlife, irrespective of our ability to document such changes (Holmes et al., 2009). For long-term conservation efforts to be successful, basic habitat data are needed to better understand the Ozark chinquapin's abiotic and biotic interactions (Dane et al., 1999). However, relatively little is known about the ecology of the Ozark chinquapin in Missouri.

The most detailed account of its habitat and associated species states that *C. ozarkensis* is found on the dry, upper chert ridges of oak-hickory forests in southwestern Missouri in acidic, non-calcareous soil with an accumulation of leaf mold (Steyermark, 1963). It is commonly found with *Nyssa sylvatica* Marshall, *Cornus florida* L., *Quercus velutina* Lam., *Quercus marilandica* Münchh, *Quercus stellata* Wangenh, *Carya texana* Buckley, *Carya tomentosa* Nutt., and *Carya ovata* (Mill.) K. Koch, while the understory flora is composed of *Vaccinium* and other acidic-soil dependent species (Steyermark, 1963). It is uncommon in southernmost Missouri but can be found on acidic soils of mesic to dry upland forests and rocky, wooded slopes (Yatskievych, 2013; Fernald, 1970). Another description characterizes the habitat of *C. ozarkensis* as deciduous forests 150 – 600 meters in elevation (Nixon, 1997). A study by Paillet and Cerny (2012) near the border of Missouri and Arkansas documented *C. ozarkensis* in upland forests on the Boone Limestone formation, which has a significant amount of chert residuum and a pH of 4.5 – 6.0. The Ozark chinquapin was uncommonly found on sandstone soils (Paillet and Cerny, 2012).

The objective of this study was to describe the habitat requirements of *C. ozarkensis* and to develop a species distribution model (SDM) to predict where else it could occur. Species distribution models are increasingly used to predict potential

occurrences and the distributions of species. Species distribution models typically increase detection rates and reduce the cost and time of surveying for rare species by narrowing the search area (Buechling and Tobalkse, 2011; Edwards et al., 2005; Engler et al., 2004). SDMs can identify potential critical and sensitive habitats, predict where plants may spread, and project future distributions under climate change scenarios (Araújo et al., 2005; Araújo et al., 2002; Buechling and Tobalske, 2011; Zhang et al., 2020). Species distribution models identify the environmental and resource gradients that are associated with the occurrence of the target species and use these gradients to predict the current or future distributions (Guisan and Zimmerman, 2000).

The SDM used in this analysis was a random forest model. Random forest is an ensemble machine learning technique, meaning that many different models are trained on a subset of bootstrapped data (data sampled with replacement; Berhane et al., 2018). Each model is a decision tree that uses randomly sampled environmental data to split the nodes of the tree (Liaw and Wiener, 2002). The decision trees then "vote" or give a probability estimate of whether the target species is present or absent in a particular location (Liaw and Wiener, 2002). The decision trees then are averaged to obtain the final model and predictions (Liaw and Wiener, 2002). Finally, the model is tested on the remaining data to determine the model performance (Garzón et al., 2006). Random forest was chosen for this analysis due to its ability to outperform other modelling techniques and its ability to rank variables by their importance (Garzón et al., 2006; Buechling and Tobalske, 2011; Berhane et al., 2018; Mi et al., 2017). Additionally, random forest is robust to nonparametric data, nonlinear relationships, correlated variables, and model overfitting

due to random selection of the predictor variables and its ensemble technique (Breiman, 2001).

In addition to the SDM, we examined the associated plant species of the Ozark chinquapin to create a plant community description. The community description and SDM may help agencies find and assess the health of unknown populations of *C. ozarkensis*, as well as guide where new trees could be introduced as the engineering of blight resistant chinquapins continues to advance.

Figure 1.1: Distribution map for *Castanea ozarkensis* in North America *(*Kartesz J.T., 2023).

METHODS

Study Area

Roaring River State Park (hereafter RRSP; Fig. 1.1 & 1.2) occupies 1950 ha in the White River watershed in Barry County, Missouri (36.5857° N, 93.8371° W; Fig. 1.1). Its geology comprises Mississippian limestone and Jefferson City dolomite, resulting in a highly dissected terrain (MDNR, n.d.). The elevation ranges from 300 – 446 m (980 – 1,463 ft). Climatic data from 1991 to 2020 indicate the annual average precipitation as ca. 120 cm (47 in). August is the hottest month, with an average temperature of 24.8° C (76.6 $^{\circ}$ F), whereas January is the coldest month, with an average of 0.78°C (33.4℉) (National Weather Service, n.d.). The ecological diversity of RRSP includes a system of glades, woodlands, forests, and riparian communities (C. Crabtree, Missouri Department of Natural Resources, pers. comm., 2023). The dominant woodland type is oak-hickory (*Quercus-Carya*) with *Vaccinium* spp.*, Acer rubrum* L*., Cornus florida,* and *Cercis canadensis* L*.,* and other species in varying quantities occupying the understory. Oak-pine woodlands, which were heavily logged in the early $20th$ century, still occur in the western portion of the park (Nigh and Schroeder, 2002). Sporadic signs of past logging are evident with cut trunks and skid trails, most likely occurring before the land was acquired as state property.

Indigenous American occupation of the region during the early 19th century included Kickapoo, Shawnee, Delaware, Osage, and Cherokee tribes, who hunted wild animals and managed vegetation through fire (Blansett, 2010; Nigh and Schroeder, 2002). After 1830, when the area was permanently settled by subsistence farmers, the Ozarks were fire-suppressed, logged, and grazed heavily by cattle and hogs (Jacobson and Primm, 1997; Nigh and Schroeder, 2002). Today, the Missouri Department of

Natural Resources manages RRSP through prescribed fire, manual removal of Eastern Red Cedar (*Juniperus virginiana* L*.*) from glades, and herbicide application (C. Crabtree, Missouri Department of Natural Resources, pers. comm., 2023).

Ozark Chinquapin Surveys

During the first field season, we surveyed RRSP using the intuitive meander survey technique (also known as intelligent meandering, random meandering, targeted, directed, or species-at-risk surveys) which is commonly used to survey rare plants (Nelson, 1985; Pennsylvania Department, 1992; Ministry of Environment, 2018; ANPC, 2012; Whiteaker, et al., 1998). The intuitive meandering surveying technique was chosen over transects or other randomized surveying techniques because a primary objective of this study was to locate and map the locations of as many Ozark chinquapins as possible to create a habitat description and help in conservation planning. This surveying strategy covers a larger area of land than transects and is more efficient at targeting specific plants (Ministry of Environment, 2018; Wisconsin DNR, 2015). Intuitive meandering, used routinely by plant taxonomists doing extensive or intensive floristic surveys (e.g. Legler, 2010; Pryer et al., 2019), employs known facts about distributional tendencies of plants and their habitats with alert surveying of the participants, and is believed to maximize the chances of encountering species that occur infrequently in a given area (Alba et al., 2021). The intuitive meandering survey technique was also beneficial given the steep topography and large area of Roaring River State Park (ANPC, 2012; Ministry of Environment, 2018; Whiteaker, et al., 1998). We surveyed RRSP regardless of Ozark chinquapin habitat suitability for the purpose of collecting absence points around the park where Ozark chinquapins were not growing. A GPS was used to record the intuitive

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meander route to show search effort as well as to collect absence points (Appendix I; Guisan et al., 2006).

To choose the presence and absence locations used in the random forest model, a 30-meter buffer was established around every Ozark chinquapin location, with the assumption that trees inside the buffer were not independent. We then generated random points from the GPS routes such that points were outside of the 30 m buffers, and at least 30 m apart. Thus, all random points represented absence locations that were independent and visually confirmed to lack chinquapins. This approach generated 368 presence points and 623 absence points (Appendix I & II).

Associated Species

An important aspect of understanding habitat relationships is to determine what other species reliably co-occur with the Ozark chinquapin. In addition to documenting the Ozark chinquapin locations, associated plant data were collected from around 201 randomly sampled Ozark chinquapins. We sampled a one-meter square for vascular plants and their percent covers at a one-meter distance from the base of the Ozark chinquapin in the four cardinal directions. Additionally, we identified the woody tree species within a five-meter radius of the base of the Ozark chinquapin and estimated their height into four cover classes. A value of 1 was given to trees that were $0 - 2$ m $(0 - 7$ ft) tall. A value of 2 was assigned to trees that were $2 - 6$ m $(8 - 20$ ft) tall. A value of 3 was given to trees that were $6 - 12$ m $(21 - 40$ ft) tall and a value of 4 was assigned to trees greater than 12 m ($> 41 \text{ ft}$). For herbarium vouchers, one individual each of graminoids and herbaceous taxa were removed entirely, given the importance of having underground parts and basal leaves for identification. For woody species, we removed approximately

30 cm of branch length for herbarium specimens. Data fields (e.g., locality, geocoordinates, date, etc.) followed DarwinCore standards, as implemented by the Sperry Herbarium, where the voucher specimens were deposited (Sperry Herbarium, 2023).

Environmental Variables

A ten-meter resolution digital elevation map (DEM) available from the Missouri Spatial Data Information Service (MSDIS; http://www.msdis.missouri.edu) was transformed into a raster representing slope (degrees) and incoming solar radiation (Watt hours) for Missouri's approximate growing season from April 15 to October 15. The variable 'tree canopy cover' was a 30-m resolution raster from the National Land Cover Database (NLCD) that gives percent tree canopy cover in 2021 for each raster cell (USDA Forest Service; n.d.). This NLCD layer was later converted to a 10-m resolution using ArcGIS Pro (version 3.0.2; ESRI, 2023). Additionally, aspect (derived from the 10 m DEM) and a geology raster (MSDIS) were used in the descriptive analysis.

Data Analysis

The locations of all 964 Ozark chinquapins were analyzed to determine habitat range. Ecological site descriptions were used in the habitat analysis (https://edit.jornada.nmsu.edu/catalogs/esd). The mean relative abundance of the associated plant species were calculated by averaging the percent cover of an individual species across all the quadrats, and then dividing that number by the total number of plant species found. The universalFQA.org website was used to derive physiognomy statistics and the mean conservatism value of the plants surveyed in the quadrats (Ladd and Thomas, 2015).

Species Distribution Model

The randomForest package within program R version 4.3.0 was used to create the SDM (Liaw and Wiener, 2002). The dataset was divided into a training dataset containing 80% of the data and a testing dataset containing the final 20% of the data. For the species distribution model, a cross-validation test was performed to test different parameters with the optimal number of base models being 700. The number of variables at each split in the tree (*mtry*) ranged from 1 to 4, with the optimal being 2 variables. We evaluated the accuracy of the model using the accuracy value, area under the receiver operating characteristic curve (AUC), and the Kappa coefficient (Allouche et al., 2006; Monserud and Leemans, 1992). A final probability predictive map was created by having each decision tree give a value of probability occurrence which were than averaged together for each cell in the map.

RESULTS

By the end of the first field season, we located 964 individual Ozark chinquapins (Fig. 1.2). Ozark chinquapins in RRSP occurred primarily on the Reeds Spring Formation $(n = 781)$ and Pierson Limestone $(n = 122; Fig. 1.3)$. The mean elevation was 405 m above sea level with a range of $362 - 445$ m (Fig. 1.4A). Percent tree canopy cover averaged 90%, with a range from $67 - 96%$ (Fig. 1.4B). Additionally, chinquapins were found on a mean slope of 25 degrees with a range of 3 degrees – 43 degrees (Fig. 1.4C). Most Ozark chinquapins were located on northwest (28%), west (24%), and southwest (20%) facing slopes (Fig. 1.5). The two most populated ecological sites were the Chert Exposed Backslope Woodland (54%) and the Chert Protected Backslope Forest (23%; Fig. 1.6).

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We identified a total of 167 vascular plant species in the quadrats surrounding the sampled Ozark chinquapins. The physiognomy included 30 tree species, 17 shrubs, 7 vines, 83 forbs, 19 grasses, 8 sedges, and 3 fern species, with a mean conservatism ranking of 4.4. Out of the 52 families represented, Asteraceae was the most represented family with 23 species (Appendix III). Fabaceae ($n = 21$), Poaceae ($n = 19$), and Rosaceae $(n = 10)$ were the next most represented families (Appendix III). From the quadrat data, *Hylodesmum nudiflorum* L. (Nakedflower Ticktrefoil), *Vaccinium pallidum* Aiton (Lowbush blueberry), *Sassafras albidum* (Nutt.) Nees (Sassafras), and *Parthenocissus quinquefolia* Planch*.* (Virginia creeper) occurred with the greatest frequency (Table 1.1). *Quercus alba* (White Oak), *Amelanchier arborea* (F. Michx.) Fernald (Serviceberry), *Quercus velutina* (Black Oak), and *Acer rubrum* (Red Maple) occurred in approximately half of the sampled quadrats. *Vaccinium pallidum* had the greatest mean relative abundance, totaling 18.8% of the total associated plant community (Table 1.1). *Hylodesmum nudiflorum* totaled 14% of the total associated plant community, and *Quercus alba* totaled 6.9% of the total associated plant community.

Within the 5-meter radius around the sampled Ozark chinquapins, *Quercus alba*, *Quercus velutina*, *Quercus rubra* (Red Oak), and *Pinus echinata* Mill. (Shortleaf Pine) were the most abundant canopy trees greater than 12 meters tall (Table 1.2). *Quercus alba*, *Amelanchier arborea*, *Quercus velutina*, *Carya tomentosa* (Mockernut hickory), *Acer rubrum*, *Juniperus virginiana* (Eastern Red Cedar), *Sassafras albida*, and *Nyssa sylvatica* (Black Gum) were the most abundant species in the mid story of $6 - 12$ meters tall (Table 1.2).

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The final species distribution model had an AUC value of 0.80, a kappa statistic of 0.59, and an accuracy value of 0.80. The variables used in the SDM were ranked in importance by the mean decrease Gini impurity as well as the Mean Decrease Accuracy. The variables were ranked as elevation (highest importance), slope, tree canopy, and then solar radiation (lowest importance; Fig. 1.7) and were used to create the final predictive species distribution map (Fig. 1.8).

Figure 1.2. Elevation map of Roaring River State Park (polygon) in Barry County, Missouri with purple dots representing 964 known Ozark chinquapin locations from a 2021 survey.

Figure 1.3. Frequency of occurrence of the Ozark chinquapin in Roaring River State Park, Barry County, Missouri with respect to geologic formation, in order from highest elevation to lowest elevation: Burlington-Keokuk Limestone, Reeds Spring Formation, Pierson Limestone, Kinderhookian Series, and Jefferson City Dolomite.

Figure 1.4. Frequency of occurrence of the Ozark chinquapin in Roaring River State Park, Barry County, Missouri with respect to: A) Elevation; B) Percent Tree Canopy; and C) Slope.

Barry County, Missouri with respect to aspect.

Figure 1.6. Frequency of Ozark chinquapin occurrence in various ecological sites in Roaring River State Park, Barry County, Missouri. Ecological Sites are categorized based on geology, soil, and plant communities (USDA NRCS, n.d.).

Table 1.1 Plant species found in quadrats sampled within one meter of the 201 sampled Ozark chinquapins with frequency (the percent of sampled Ozark chinquapins it occurred near) and the mean relative abundance (the percent composition of the species in the overall sampled plant community). Full table in Appendix IV.

Plant Species	Relative Frequency	Mean Relative Abundance
Hylodesmum nudiflorum	81.0%	14.0%
Vaccinium pallidum	80.0%	18.8%
Sassafras albidum	62.0%	5.1%
Parthenocissus quinquefolia	60.0%	2.0%
Quercus alba	56.0%	6.9%
Amelanchier arborea	53.0%	2.8%
Quercus velutina	51.0%	3.1%
Acer rubrum	49.0%	2.7%
Vitis vulpina	39.0%	0.6%
Amphicarpaea bracteata	39.0%	3.6%
Carya ovata	35.0%	3.3%
Carex cephalophora	31.0%	3.2%
Galium aparine	31.0%	0.8%
Cunila origanoides	28.0%	0.8%
Carya tomentosa	28.0%	1.6%
Carex blanda	24.0%	1.9%
Danthonia spicata	23.0%	0.4%
Nyssa sylvatica	23.0%	1.0%
Quercus rubra	22.0%	0.5%
Dichanthelium dichotomum		
var. barbulatum	21.0%	0.5%
Vitis aestivalis	20.0%	1.1%
Prunus serotina	19.0%	0.5%
Vaccinium stamineum	18.0%	2.7%
Lonicera flava	17.0%	0.6%
Castanea ozarkensis	17.0%	0.7%
Galium arkansanum	17.0%	0.1%
Hylodesmum glutinosim	17.0%	2.0%
Dichanthelium ashei	14.0%	0.3%
Fraxinus americana	14.0%	1.0%
Juniperus virginiana	14.0%	0.5%
Brachyelytrum erectum	14.0%	0.7%
Ulmus rubra	14.0%	0.2%
Lespedeza hirta	13.0%	0.4%

Viburnum rufidulum	13.0%	0.1%
Antennaria parlinii	12.0%	0.5%
Carex hirsutella	12.0%	0.4%
Ulmus alata	12.0%	0.5%
Hieracium gronovii	11.0%	0.3%

Table 1.2. Relative abundance within the height classes of the tree species found within a 5-meter radius of the 201 sampled Ozark chinquapins. Trees with 0.0% across all height classes were present but were less than 0.1% abundance and less than 2 meters tall.

Figure 1.7. Variable importance plot generated by the randomForest package. This shows the model variable importance by the Mean Decrease in Accuracy as well as the Mean Decrease Gini Impurity.

Figure 1.8. Random forest species distribution model predicting the probability of Ozark chinquapin presence in Roaring River State Park (black polygon), Barry County, Missouri.

DISCUSSION

In a post chestnut blight landscape, there is a need for further research to document the ecology of the Ozark chinquapin throughout its range. In this study, we described the habitat of the Ozark chinquapin and its associated plant community and created a species distribution model to aid in locating this species for future research efforts.

The random forest SDM for the predicted habitat of the Ozark chinquapin had AUC and Kappa values within the threshold indicating a good model (Monserud and Leemans, 1992). Solar radiation was the least influential variable in the model, most likely because Ozark chinquapins were found on all aspects, therefore experiencing varying amounts of solar radiation. According to the mean tree canopy cover percentages, Ozark chinquapins were found in more heavily wooded areas. This may not represent historical tree canopy percentages as fire suppression has allowed for expansion of forests in the early $20th$ century (Beilmann and Brenner, 1951) and chestnut blight suppresses the growth of the Ozark chinquapin, allowing other trees to dominate the canopy (Paillet and Cerny, 2012). Elevation and slope were the most important environmental variables to the model's predictive power. This predictive map can be used to support search efforts on further surveys at RRSP and the surrounding areas for the Ozark chinquapin.

Elevation is directly correlated with geology at RRSP. In order from highest elevation to lowest elevation, the geology of the ridgetops in RRSP is Burlington-Keokuk limestone, then the Reed Springs formation, the Pierson Formation, and the Kinderhookian series (MDNR, n.d.; Fig. 1.3). The lowest geological layer is the Jefferson City Dolomite which is Ordovician instead of Mississippian like the above layers in

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RRSP (MDNR, n.d.). The Ozark chinquapin mostly occurred in the Mississippian geology with one outlier on the Jefferson City Dolomite (Fig. 1.3). The elevation range on which Ozark chinquapin occurrence is concentrated corresponds with the Reed Springs and Pierson formations. These layers are fossiliferous and cherty limestones with the Reed Springs formation often composed of more than 50% chert (Thompson, 1979).

Based on the slope of most Ozark chinquapins being 20% or greater, as well as the elevation range and geologic associations of the tree, Ozark chinquapins mainly occurred on the upper chert ridges, but rarely on the ridgetops of RRSP. This corroborates Steyermark's (1963) assertion that they occur on dry, upper chert ridges. The Ozark chinquapins also occurred mainly on Northwest, West, and Southwest aspects, which are drier than Northeast, East, and South aspects. The elevation range of the Ozark chinquapin at RRSP (300 – 446 meters) falls within the range stated by Nixon (1997; 150 – 600 meters).

The associated ground flora and tree species were typical of dry, chert upland woodlands (Nelson, 1985). Missouri land management agencies have classified the state into ecological site (ES) classifications based on soil, landform, and vegetation (USDA NRCS, n.d.). Currently, RRSP has 15 different ecological sites within its borders (USDA NRCS, n.d.; Appendix V). For the Ozark chinquapin, the dominant ecological sites were the Chert Exposed Backslope Woodland (CEBW) and the Chert Protected Backslope Forest (CPBF) (Fig. 1.6). These habitats are most like Nelson's Dry-Mesic Chert Woodland classification (Wallace & Young, n.d.; Nelson, 1985). Chert Exposed Backslope Woodlands are found on steep southern and western slopes with a canopy of $65 - 85%$ tree cover (Wallace & Young, n.d.). The open tree canopy creates a diverse

understory flora with species like *Carex muehlenbergii* Willd.*, Carex albicans* Willd. Ex Spreng.*, Carex cephalophora* Muhl ex. Willd.*, Bromus pubescens* Muhl. ex Willd.*, Schizachyrium scoparium* (Michx.) Nash*, Solidago ulmifolia* Muhl. ex Willd.*, Solidago nemoralis* Aiton*, Desmodium glabellum* (Michx.) DC.*, Hylodesmum nudiflorum, Helianthus hirsutus* Raf.*, Asclepias quadrifolia* Jacq.*,* and *Vaccinium pallidum* (Appendix IV). The dominant tree canopy species for this ecological site were *Quercus marilandica, Q. velutina, Q. stellata, Q. alba, Carya texana, Pinus echinata,* and *Sassafras albidum*, and many others (Wallace $& Young$; Appendix IV). All of these species were found in the quadrats or tree plots around the Ozark chinquapins sampled at RRSP. The plant species listed in the ES description that were absent at RRSP within the quadrats were: *Carex pensylvanica* Lam*., Elymus hystrix* M.E.Jones*, Desmodium marilandicum* Darl*., Tradescantia virginiana* L*., Silene virginica* L*., Cardamine concatenate* (Michx.) O.Schwarz*, Parthenium integrifolium* L*., Callitriche terrestris* Raf*., Liatris squarrosa* (L.) Willd*., Liatris cylindracea* (Michx.)*, Desmodium obtusum* (Muhl. ex Willd.) DC*., Desmodium ciliare* (Muhl. ex Willd.) DC*., Corylus americana* Walter*, Amorpha canescens* Pursh*, Solidago nemoralis* Aiton*,* and *Echinacea purpurea* (L.) Moench.

Chert Protected Backslope Forests were on steep northern and eastern aspects with a 15 –70% slope (Wallace & Young). This habitat has a dense canopy cover of 90 – 100%, which makes it cooler and moister than the Chert Exposed Backslope Woodland (Wallace & Young). The dominant trees are *Quercus alba, Q. rubra, Q. velutina, Carya ovata, Pinus echinata, Acer rubrum, and Acer saccharum* Marshall. The dominant understory flora are *Carex digitalis* Willd*., Muhlenbergia sobolifera* Trin*., Danthonia spicata* (L.) P.Beauv. ex Roem. & Schult*., Bromus pubescens, Hylodesmum nudiflorum,*

Symphyotrichum anomalum (Engelm.) G.L.Nesom*, S. patens* (Aiton) G.L.Nesom*, Solidago ulmifolia, Maianthemum racemosum* (L.) Link*, Uvularia grandiflora* Sm.*, Botrichium virginianum* (L.) Sw.*, Polystichum acrostichoides* (Michx.) Schott*, Asplenium platyneuron* (L.) Britton, Sterns & Poggenb.*, Lindera benzoin* (L.) Blume*, Amelanchier arborea, Amphicarpaea bracteatea* (L.) Fernald*, Monarda bradburiana* Beck*, Asclepias quadrifolia*, and many others (Wallace & Young; Appendix IV). All of these listed species were found during the botany sampling around the Ozark chinquapins at RRSP. While these (and many other) associated plant species were found near the Ozark chinquapins, all are widely distributed in the Ozarks (Kartesz, 2023), so the presence of these species does not necessarily indicate ideal Ozark chinquapin habitat. The plant species in the ES description that were not found during the botany sampling at RRSP were: *Desmodium cuspidatum* (Muhl. ex Willd.) DC. Ex G.Don*, Verbesina helianthoides* Michx.*, Claytonia virginica* L.*, Cypripedium parviflorum var. parviflorum, Erythronium albidum* Nutt.*, Hepatica nobilis* Schreb.*, Hydrastis canadensis* L.*, Phlox divaricate* L.*, Podophyllum peltatum* L.*, Trillium sessile* L.*, Cystopteris protrusa* (Weath.) Blasdell*,* and *Smilax glauca* Walter.

Although the ecological site descriptions for both the Chert Protected Backslope Forest and Chert Exposed Backslope Woodland state that the soils are not acidic (Wallace & Young), several of the flora species sampled are acidic-soil indicators. *Vaccinium pallidum, V. arboretum* Marshall*, V. stamineum* L.*, Pinus echinata, Quercus marilandica, Amelinchior arboreum, Dichanthelium linearifolium* (Scribn.) Gould*, Dichanthelium werneri* (Sribn.) Mohlenbr.*, Carex umbellata* Schkuhr ex Willd.*,* and *Ionactis lineariifolia* (L.) Greene are acidic-soil species (A. Braun, Missouri Department on Natural Resources, pers. comm., 2023). These acidic-soil indicators corroborate results from Paillet and Cerny (2012) that found Ozark chinquapins on acidic soils, and Steyermark's (1963) assertion that acidic loving species were found in the understory of Ozark chinquapins. Additionally, chert has a slightly acidic pH (Clark, 2023) that may influence soil chemistry if the limestone has eroded. Finally, Ozark chinquapins were found in Low-Base ecological sites (Low-Base Chert Upland Woodland, Low-Base Chert Exposed Backslope Woodland, Low-Base Chert Protected Backslope Woodland; Fig. 1.6; Appendix V), which do have acidic subsoils (Wallace and Young, n.d.).

Continued research is needed to fully understand the habitat requirements and ecology of the Ozark chinquapin. This research should be expanded to include the whole range of the Ozark chinquapin to understand why this species is concentrated in Arkansas, with only a few counties in southwestern Missouri. Even though the Ozarks encompass a large part of Missouri, and the associated plant species identified in this study are common throughout the Ozarks, the distribution of the Ozark chinquapin is comparatively narrow. Studies should focus on the outlying populations in Alabama, Louisiana, and Mississippi to see whether habitat characteristics are similar in those locations to the core population. With a larger study area, climatic variables such as humidity, precipitation, temperature, and seasonal differences between those variables could be included in the SDM and may better explain the current range of this tree. Additionally, soil chemistry and geology data across a larger range could better inform the habitat requirements of the Ozark chinquapin.

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CONCLUSION

This study demonstrates that a random forest species distribution model was successful at predicting the occurrence of the Ozark chinquapin in Roaring River State Park. The most informative habitat variables were elevation and slope, which showed the Ozark chinquapin mainly occurring on upper chert ridges and on the Reeds Spring geological formation and the Pierson formation. The associated flora was indicative of acidic, dry, chert upland woodland habitat and common throughout the Ozarks. The continued study of the habitat of the Ozark chinquapin could better explain why the Ozark chinquapin has a narrow range in Missouri and inform land managers where to survey for this tree. Additional information could also indicate where new Ozark chinquapins could be planted as the technology to mitigate chestnut blight advances.

CHAPTER II

ANALYSIS OF OZARK CHINQUAPIN (*CASTANEA OZARKENSIS*) REPRODUCTION AND CHESTNUT BLIGHT INFECTION AT ROARING RIVER STATE PARK, BARRY COUNTY, MISSOURI

ABSTRACT

The Ozark chinquapin, *Castanea ozarkensis* Ashe, is a chestnut tree whose range is concentrated in the Interior Highlands of North America. Like other North American members of *Castanea*, the Ozark chinquapin was reduced from an overstory tree to an understory shrub by the invasive chestnut blight fungus (*Cryphonectria parasitica* [Murrill] M.E. Barr) during the early $20th$ century. While numerous studies have documented the response of the American chestnut (*C. dentata*) to chestnut blight, little is known about the Ozark chinquapin's health and reproductive status post-blight. Approximately seventy years after the arrival of the blight to the central United States, this study aimed to assess the factors that influence the probability of being infected by chestnut blight and to determine which variables best predict the reproductive status of the Ozark chinquapin in south-central Missouri. We used generalized linear models and AIC model selection to examine potential factors that affect probability of reproduction or blight infection. Models showed that Ozark chinquapin reproductive status was related to the maximum stem height of the tree and how many times that area experienced fire.
Additionally, stem height, the presence of deer damage, and the amount of time since the area was last burned increased blight infection. Our findings suggest that the Ozark chinquapin is capable of reproduction in certain situations but is susceptible to chestnut blight through deer damage and the burn time interval, opening the door to further research projects to better inform conservation efforts for this blight-stricken tree.

INTRODUCTION

The Ozark chinquapin (*Castanea ozarkensis* Ashe) is centralized in the Interior Highlands (Fig. 1), with outlying populations in Louisiana, Mississippi, and Alabama (Kartesz, 2023). Originally described by Ashe (1923) at the species level, it was later considered to be a variety of the Allegheny chinquapin – *Castanea pumila* var*. ozarkensis* (Ashe) G.E. Tucker (1975). However, differences in morphology, habitat characteristics, and higher levels of genetic diversity differentiate *C. ozarkensis* from *C. pumila* (Dane et al., 1999; Shaw et al., 2012; Spriggs and Fertakos, 2021).

Similar to other chestnuts in North America, the Ozark chinquapin has been impacted by chestnut blight (*Cryphonectria parasitica* [Murrill] M.E. Barr), which arrived in America in the early 1900s (Paillet and Cerny, 2012). Originally from Asia, the fungus was introduced to North America through imported trees (Rigling and Prospero, 2018). Within fifty years of the arrival of chestnut blight, the entire North American distribution of the American chestnut species (*Castanea dentata*) was reduced from a dominant canopy tree of the Eastern Deciduous Forest to the understory (Rigling and Prospero, 2018). By the 1950s, chestnut blight had spread to the Interior Highlands, infecting the Allegheny chinquapin (*Castanea pumila* [L.] P. Mill.) and the Ozark chinquapin (Paillet and Cerny, 2012).

Chestnut blight enters the tree through wounds on the bark. The mycelium of the fungus spreads through the bark and cambium, killing the host's cells by secreting digestive enzymes and oxalic acid, eventually girdling the tree (Rigling and Prospero, 2018; Zhang et al., 2013). Because the fungus only affects the tree above ground, individuals infected with the blight often sprout epicormic shoots from the root collar, giving individuals a shrub-like appearance and enabling them to survive after the main trunk dies (Rigling and Prospero, 2018). However, new epicormic sprouts eventually become infected by chestnut blight, which is dispersed by wind and rain (Guerin et al., 2001).

Castanea ozarkensis is found only in nine counties in Missouri (Fig. 2.1) and is ranked S2 (State Imperiled) by the Missouri Natural Heritage Program (2023). Before the arrival of the blight, all chestnut species were considered economically important to the North American timber industry and were an important food source for wildlife and indigenous and foreign settlers (Holmes et al., 2009).

Relatively little is known about the demography of the Ozark chinquapin (e.g., Paillet, 2002; 2012), in contrast with the numerous studies of *C. dentata* throughout its range (Anagnostakis, 1987; Laport et al., 2020; Tindall et al., 2004). The Ozark chinquapin, which has greater genetic diversity than the American chestnut (Dane et al., 1999) despite its narrower overall geographic distribution, is worthy of research and conservation for this reason alone, in addition to its ecological and economic values (Holmes et al., 2009). The American chestnut rarely reaches reproductive maturity before being killed by the blight and is viewed by some as functionally extinct (Stephenson et al., 1991; Paillet, 2002; 1982). However, little is known about the Ozark chinquapin's

reproductive status and whether it reaches maturity (Paillet, 1993; 2002). The goal of this study was to document the size of the Ozark chinquapin population in Roaring River State Park in southwestern Missouri. Additionally, we assessed the number of trees infected by chestnut blight and identified factors that influenced blight infection and Ozark chinquapin reproduction.

Figure 2.1. Distribution map for *Castanea ozarkensis (*Kartesz J.T., 2023).

METHODS

Study Area

Roaring River State Park (hereafter RRSP; Fig. 1.1 & 1.2) occupies 1950 ha in the White River watershed in Barry County, Missouri (36.5857° N, 93.8371° W; Fig. 2.1). The park has a highly dissected terrain with the elevation ranging from 300 – 446 m (980 to 1,463 ft). The ecological diversity of RRSP includes a system of glades, woodlands, forests, and riparian communities (C. Crabtree, Missouri Department of Natural Resources, pers. comm., 2023). The dominant woodland type is oak-hickory

(*Quercus-Carya*) with *Vaccinium* spp.*, Acer rubrum* L*., Cornus florida,* and *Cercis canadensis* L*.,* and other species in varying quantities occupying the understory. Oakpine woodlands, which were heavily logged in the early $20th$ century, still occur in the western portion of the park (Nigh and Schroeder, 2002).

Indigenous American occupation of the region during the early 19th century included Osage, Kickapoo, Shawnee, Delaware, and Cherokee tribes, who hunted wild animals and managed vegetation through fire (Blansett, 2010; Nigh and Schroeder, 2002). After 1830, the area was permanently settled by subsistence farmer and the Ozarks were fire-suppressed, logged, and grazed heavily by cattle and hogs (Jacobson and Primm, 1997; Nigh and Schroeder, 2002). Today, the Missouri Department of Natural Resources manages RRSP through the manual removal of Eastern Red Cedar (*Juniperus virginiana* L*.*) from glades, herbicide application, and prescribed fire (C. Crabtree, Missouri Department of Natural Resources, pers. comm., 2023).

Figure 2.2. Roaring River State Park in Barry County, Missouri with the location and reproductive status of 201 *Castanea ozarkensis* recorded in 2022 along with the park's burn units.

Demography Data Collection

We located individual Ozark chinquapins during the first field season by methodically walking through RRSP following the intelligent meandering technique (ANPC, 2012; Ministry of Environment, 2018; Whiteaker, et al., 1998). We used proportional stratified sampling to select a subset of individuals ($n = 201$) that were sufficiently dispersed to avoid uneven sampling (Manly and Navarro, 2014). From those 201 trees, the diameter of the root collar of each epicormic stem was measured with either a DBH tape or calipers to the nearest millimeter and the height of each stem was recorded in centimeters (Tindall et al., 2004). We recorded the presence or absence of chestnut blight on each stem, which is observable by the orange coloring of stromata, cankers on the bark, or diagnostic leaf wilting (Rigling and Prospero, 2018; Figs. 2.3A-C). Stems were assessed for their condition (alive or dead) and whether they were flowering, fruiting, or sterile (Appendix VI - VIII). Additionally, each stem was checked for deer damage caused by herbivory or antler scraping (Appendix IX). A five-meter radius was searched around the base of each tree for seedlings and chestnut burrs (Appendix X)*.* Several voucher specimens of Ozark chinquapin were collected and deposited at the T.M. Sperry Herbarium at Pittsburg State University.

Habitat Data Collection

A ten-meter digital elevation map available from the Missouri Spatial Data Information Service (http://www.msdis.missouri.edu) was transformed into a raster representing slope and incoming solar radiation for Missouri's approximate growing season from April $15th$ – October $15th$. The density of *C. ozarkensis* was calculated by counting the number of other chinquapins within a ten-meter radius from each raster cell. Each chinquapin was assigned a density value depending on the raster cell in which it

was located. Two variables were created for prescribed fire in RRSP. Fire frequency was the number of times a location with sampled Ozark chinquapins had been burned in the recorded history of the park (0, 1, 2, or 3 times). Burn time intervals established the time interval between fires $(0 - 5$ years, $6 - 15$ years, or never burned). Fire data was provided by Missouri State Parks (C. Crabtree, Missouri Department of Natural Resources, pers. comm., 2022). Each of the 201 trees were given a value for elevation, slope, solar radiation, density, burn time interval, and fire frequency, based on their location in RRSP (Tables 2.1 & 2.2).

Data Analysis

We used an information-theoretic approach to analyze the factors that influence 1) the probability of chestnut blight infection of individual chinquapin stems, and 2) the reproductive status of individual trees (Burnham and Anderson, 2004). In the first analysis, we fit binomial mixed models of the relationships between environmental factors and the presence or absence of chestnut blight infection. Epicormic stems were used as the sampling units because chestnut blight does not infect the root collar of the whole tree (Rigling and Prospero, 2018). Models included a random intercept for individual trees to account for non-independence of multiple stems on the same tree. The fixed effects included chinquapin density, the height of the stem, deer damage, and burn categories (Table 2.1). We fit and ranked models with all possible additive combinations of variables.

In the second analysis, we used binomial models to evaluate the effects of maximum stem height, proportion of blighted stems per tree, frequency of burn, elevation, slope, and solar radiation on the presence or absence of reproduction in living

stems of *C. ozarkensis* (Table 2.2). Individual trees were used as the sampling unit. We used a stepwise approach to reduce the total number of models. Two global models were compared first, one each with and without maximum stem height. Since maximum stem height was informative based on a lower AIC value, it was then included in all models except the null. Four models representing a priori hypotheses were then compared: a null model with only the intercept, a global model with all variables, a habitat model, and a stress model (that is, trees stressed with infection by blight and/or fires). The habitat model included the predictor variables maximum stem height, elevation, slope, and solar radiation (Table 2.2). The stress model included the variables maximum stem height, proportion of blighted stems per tree, and burn frequency. In both analyses, all continuous variables were z-transformed and variables with $r > 0.7$ were excluded from the same models. All models were fit and ranked using Akaike's Information Criterion (AIC) and model weights. Models containing informative variables with delta AIC <2 were considered supported (Arnold, 2010). Spatial variables were created using ARCGIS (version 3.0.2; ESRI, 2022) and models were fit and ranked in Program R (R Core Team, 2023) and with package "lme4" (Bates et al., 2015).

Table 2.1. Predictor variables used in linear models to predict infection by chestnut blight.

Table 2.2. Predictor variables used in linear models to predict reproduction by Ozark

Ϯ Parameters included in the stress model

*Parameters included in the habitat model

RESULTS

A total of 964 Ozark chinquapins were mapped in Roaring River State Park. The 201 sampled individuals included 1,091 stems; of these, 418 stems were dead (38%), 651 stems were vegetative (60%), and 22 stems were reproductive (2%). Two seedlings were found within 5 meters of the same reproductive parent tree. Burrs were found within 5 meters of the base of 10 trees (5%). The number of stems per tree ranged from 1 to 29 with a mean of 5 stems per tree. The majority of the chinquapins (91%) had ten stems or fewer per tree (Fig. 2.4A).

Reproductive stems were found on 18 trees (9%). The average height of flowering stems was greater (mean $= 719.4$ cm; range $= 158 - 1,298$ cm) than the average height of vegetative stems (mean = 125.9 cm; range = $4 - 1,557$ cm) and the average height of dead stems (mean = 101.8 cm; range = $3 - 838$ cm; Fig. 2.5A). The average root collar diameter was larger on reproductive stems (14.13 cm) than on vegetative (1.7 cm) or dead stems (1.8 cm; Fig. 2.5B). In 82% of the trees, the tallest stem was 500 cm or shorter (Fig. 2.4B). The mean number of stems on reproductive trees was 3.89 (std. dev. $= 0.2219$; n = 18), whereas non-reproductive trees had an average of 5.03 stems (std. dev. $= 0.0245$; n = 182). The mean percentage of stems per tree showing deer damage (herbivory or antler scraping) was 40 percent (Fig. 2.4C). A total of 211 stems out of 1,091 (19%) had visible signs of chestnut blight through leaf wilt, cankers, and/or the orange stromata (Fig. 2.3A and B). The average percent of blighted stems per tree was 20% (Fig. 2.4D).

The best supported model for infection by chestnut blight included the height of the stem, presence of deer damage, and the burn time intervals (0 to 5 years, 6 to 15

years, or never burned) with a model weight of 33% (Table 2.3, Fig. 2.7). The taller the stem, the greater the chance of being blighted, with a stem 100 cm tall being 9% likely to show blight symptoms, while a stem 1,200 cm tall had a 92% chance of being blighted (Fig. 2.6A). Deer damage positively increased the chance of infection, with deer damaged stems being 35% more likely to be infected by blight (Fig. 2.6B). The burn year intervals showed that the unit that had not been burned for 6 to 15 years (the Natural Area burn unit) had a 95% increased chance of blight infection than in the units that last experienced fire 0 to 5 years ago (Roger's Hollow, Ketchum Hollow West, Eagle's Nest, the wildfire, and the overlapping burn unit; Fig. 2.6C). The unit last burned within 6 to 15 years (the Natural Area burn unit) was also 37% more likely to show blight symptoms than the individuals in the parts of the park that had never experienced fire (Fig. 2.6C).

The best supported model for the reproduction of the Ozark chinquapin was the stress model, which consisted of the variables maximum stem height, proportion of blighted stems per tree, and the frequency of burns (0, 1, 2, or 3 times), with a model weight of 85% (Table 2.3). The most informative parameters for predicting tree reproduction from the stress model were maximum height and frequency of burns (Table 2.4). The greater the maximum height of the tree, the greater the chance of reproduction with the probability of reproduction at 9% for stems 500 cm tall, and 91% for stems 1,200 cm tall (Fig. 2.7A). Additionally, the Ozark chinquapins that have never experienced fire over RRSP's recorded history were 99% more likely to reproduce than the trees in units that have been burned three times (Fig. 2.7C).

Figure 2.3. Signs of chestnut blight A) dead leaves (leaf wilt), B) orange stromata (fungal fruiting bodies), C) a canker, observed on Ozark chinquapins in Roaring River State Park in 2022 (photographs by Danielle Evilsizor).

Figure 2.4. Distribution of *C. ozarkensis* in RRSP with respect to A) stem count B) maximum stem height C) proportion of deer damaged stems and D) proportion of blighted stems.

Figure 2.5. Mean stem height (A) and mean root collar diameter (B) among blighted, unblighted, dead, reproductive, and vegetative Ozark chinquapin stems with standard error bars.

Table 2.3. Ranked (Δ AIC) models estimating the probability of chestnut blight infection and reproduction on stems of *C. ozarkensis* in RRSP. The null (intercept-only) model is included. K represents the number of variables in the model.

Response Variable	Model	$\bf K$	$\triangle AIC$	Model Weight
Chestnut Blight Infection	$Height + Decr + BTI$	6	0.0	0.33
	$Height + BTI$	5	1.0	0.20
	$Height + Der$	$\overline{4}$	1.8	0.13
	$Height + Decr + BTI + Density$	7	2.0	0.12
	$Height + Density + BTI$	6	3.0	0.07
	Height	3	3.2	0.07
	$Height + Density + Decr$	5	3.7	0.05
	$Height + Density$	$\overline{4}$	5.1	0.03
	BTI	$\overline{4}$	76.2	< 0.001
	$BTI + Deer$	5	76.5	< 0.001
	Density + BTI	5	78.2	< 0.001
	$Density + Decr + BTI$	6	78.4	< 0.001
	Deer	3	80.5	< 0.001
	Null	$\overline{2}$	80.7	< 0.001
	Density + Deer	$\overline{4}$	82.4	< 0.001
	Density	3	82.6	< 0.001
Ozark chinquapin reproduction	Stress Model	$\overline{4}$	0.0	0.85

	Global Model		3.5	0.15
Ozark chinquapin reproduction continued	Habitat Model	5	14.2	< 0.001
	Null Model		49.6	< 0.001

Table 2.4. Estimated coefficients for the top models for chestnut blight infection and

Ozark chinquapin reproduction models in RRSP.

Figure 2.6. Predictive plots based on the top blight model showing the effects of A) stem height, B) deer damage, and C) number of years burned before sampling, on chestnut bight infection in the Ozark chinquapins at RRSP. Error bars represent 95% confidence intervals.

Figure 2.7. Predictive plots based on the top reproductive model showing the effects of A) maximum stem height, B) proportion of stems with blight, and C) burn frequency on Ozark chinquapin reproduction at RRSP. Error bars represent 95% confidence intervals.

DISCUSSION

During this study, we found that certain variables influenced the probability of both chestnut blight infection and Ozark chinquapin reproductive status. The top chestnut blight infection model showed that stem height, the presence of deer damage, and the amount of time since last burned best predicted chestnut blight infection. Increasing stem height increased the probability of chestnut blight infection. The trees that had been burned 6 to 15 years prior to sampling were more likely to be blighted than the trees burned 0 to 5 years prior to sampling and the trees that had never been burned. Additionally, the presence of deer damage increased the likelihood of blight infection. Ozark chinquapin reproduction was more likely in taller stems and in areas of RRSP that were less frequently burned.

In Canda, Tindall et al. (2004) found that chestnut blight infection in the American chestnut was more evident in taller stems than in smaller stems. A taller stem indicates an older tree that has had more time to be exposed to trunk damage and to the fungus. Additionally, this study shows that the presence of deer damage through herbivory or trunk scraping increased the probability of chestnut blight infection. Since chestnut blight requires a fresh wound to infect the tree, the deer damage could be an entrance to the blight, increasing infection rates (Zhang et al., 2013; Rigling and Prospero, 2018). Damaged or dying bark caused by drought, fire, cutting, and galls of the chestnut gall wasp can serve as an entrance to *Castanea* spp. tissue (Rigling and Prospero, 2018).

The burn time intervals showed that the sections of RRSP that were last burned 6 to 15 years prior to sampling (the Natural Area burn unit) had a greater likelihood of

blight infection than the sections that were burned 0 to 5 years prior to sampling and the sections that have never been burned. It is not certain why the Natural Area burn unit has the highest likelihood of blight. One explanation is that the differences in habitat between those burn units influenced the presence of chestnut blight. The west portion of RRSP (which has never been burned) is unique to the park and considered "low-base woodlands" due to the prevalence of short-leaf pine

(https://edit.jornada.nmsu.edu/catalogs/esd; Appendix V, Fig. 2.2). More than half of the trees that had never experienced fire were in this west section- the oak-pine woodland. In contrast, the Natural Area burn unit (6 to 15 years) and the Roger's Hollow, Ketchum Hollow West, Eagle's Nest, the Wildfire, and the Overlapping burn units (0 to 5 years) had few pine trees and are oak-hickory dominated.

Tindall et al. (2004) found that litter depth had a positive relationship with the presence of virulent cankers in the American chestnut. While it is not known precisely how long chestnut blight spores are viable for in soil, they were able to survive drying in a lab setting for an average of 81 days (Heald and Gardner, 1914). Although the spores were able to withstand drying for that length of time, moisture is important for chestnut blight's reproduction (Heald and Gardner, 1914; Rigling and Prospero, 2018), and presumably the spores could survive longer in a moist environment. Oak litter can have more fungal and bacterial colonies and retain moisture longer than pine litter (Witkamp, 1966). The difference in chemical composition, nutrients, surface area, and decomposition rates between oak-hickory litter and pine litter could create a microhabitat more conducive to chestnut blight ecology and reproduction. Even though the Roger's Hollow, Ketchum Hollow West, Eagle's Nest, the Wildfire, and the Overlapping burn

units (0 to 5 years) are also oak-hickory dominated like the Natural Area unit (6 to 15 years), the more recent prescribed fire would result in less accumulated leaf litter, potentially decreasing the survival of fungal spores in the leaf litter or creating a different microclimate around the trees.

Tindall et al. (2004) found more blight symptoms when hickory trees were dominant than with other dominant associated woody species. Several species of trees can be alternate hosts for chestnut blight, such as oaks and maple (Rigling $&$ Prospero, 2018; Mooij, 1997). Increased blight infection in the Natural Area unit could result directly from the associated tree species being alternate hosts, or indirectly based on shared ecological conditions between the trees in that unit and chestnut blight.

The top reproduction probability model for the Ozark chinquapin included maximum stem height, proportion of blighted stems per tree, and burn frequency. The taller the stem, the greater the chance of reproduction. Stem height and root collar diameter in reproductive stems (both blighted and unblighted) were much taller and larger than in vegetative and dead stems. Chestnut blight slows tree growth by restricting the flow of nutrients from the roots and the leaves and kills most stems before they reach reproductive height (Paillet, 2002). While chestnut blight usually prevents the Ozark chinquapin from reaching reproductive height, there is documentation of canopy gaps enabling chinquapin growth and flower production (Paillet, 2002; Paillet and Cerny, 2012). Those trees that can reach reproductive height seem to be able to reproduce regardless of chestnut blight infection (Fig. 2.5A&B). However, the Ozark chinquapin is self-incompatible and requires other nearby reproductive trees for pollination (Paillet, 2002). Historical accounts recorded that mature Ozark chinquapins reached heights of up

to 18 to 20 meters and diameters of up to 1 meter (Paillet and Cerny, 2012). Today, they rarely get larger than 3 meters in height and 10 cm in diameter (Paillet and Cerny, 2012). This drastic change from an overstory tree to an understory shrub and the differing amounts of sunlight received by those two forms likely has an impact on the reproductive success of the Ozark chinquapin*.*

Although the proportion of blighted stems per tree was in the top model for reproduction, this variable was not particularly meaningful and did not show a discernable pattern with Ozark chinquapin reproduction (Table 2.4; Fig. 2.7B). However, burn frequency did influence the probability of reproduction, decreasing by almost 100% between the trees that have never been burned, and the trees that had been burned three times over the history of the park (Table 2.4; Fig. 2.7C). Because reproduction is so closely linked to stem height and diameter (Fig. 2.5A and B; Fig. 2.6A), an intense fire could damage or kill the stems, requiring the tree to send up new sprouts and start completely over. Additionally, fire damage could serve as an entry to chestnut blight infection, inhibiting the growth of the stem or killing it completely (Rigling and Prospero, 2018). It is interesting to note that chestnut blight infection probability was lowest in the burn areas that experienced fire within the last five years compared to the units that have never had fire and units last burned 6 to 15 years ago (Fig. 2.6C). Fire almost assuredly decreases leaf litter to some extent around the tree or kill fungal spores outright, suppressing the spread of fungal spores. However, this is potentially countered by the negative relationship between fire and Ozark chinquapin reproduction.

Continued research is needed to fully understand the factors that influence chestnut blight infection and Ozark chinquapin reproduction. Research on habitat

variables like microclimates (humidity, temperature, etc.), leaf litter depth and soil pH could aid in understanding the conditions needed for chestnut blight to grow and reproduce. Additionally, a long-term study looking at the effects of fire frequency on both chestnut blight infection and Ozark chinquapin reproduction could inform land management decisions and bring further insight into the potential role of fire in chinquapin conservation. Further research is also needed to learn what factors help stems reach a reproductive height. During this study, we anecdotally observed that Ozark chinquapins in canopy gaps seemed more likely to be reproductive. A study that compared canopy densities at reproductive trees and non-reproductive trees could help determine how sunlight exposure influences reproduction. Finally, comparative studies looking at the probability of chestnut blight infection and Ozark chinquapin reproduction should be conducted throughout its range.

CONCLUSION

This study demonstrated that environmental variables influenced the probability of chestnut blight infection, such as taller stem height, the presence of deer damage, and the trees last experiencing fire 6 to 15 years before sampling. We also found that increasing stem height and decreasing fire frequency were factors that influenced Ozark chinquapin reproduction. The continued study of this tree and the factors that influence its health and reproduction could inform future land management decisions. Creating environments that are more hospitable to *C. ozarkensis* reproduction would benefit the population of this blight-altered tree species.

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APPENDIX

Appendix I. Elevation map of Roaring River State Park, Barry County, Missouri with the locations of 964 Ozark chinquapins with GPS tracks to show survey effort. Absence locations for the species distribution model were later pulled from the GPS tracks.

Appendix II. Elevation map of Roaring River State Park, Barry County, Missouri with both the presence and absence points of the Ozark chinquapin used in the random forest species distribution model. There are 368 presence points and 623 absence points with a 30-meter minimum distance between the point
Family	Number of Species	Family cont.	Number of Species
Asteraceae	23	Geraniaceae	
Fabaceae	21	Moraceae	1
Poaceae	19	Oleaceae	1
Rosaceae	10	Ophioglossaceae	1
Cyperaceae	8	Oxalidaceae	
Fagaceae	6	Phrymaceae	L
Juglandaceae	5	Pinaceae	1
Rubiaceae	5	Polygonaceae	1
Anacardiaceae	4	Rhamnaceae	1
Violaceae	4	Santalaceae	1
Apiaceae	3	Saxifragaceae	
Caprifoliaceae	3	Urticaceae	1
Ericaceae	3	Verbenaceae	1
Sapindaceae	3		
Ulmaceae	3		
Vitaceae	3		
Celastraceae	\overline{c}		
Cornaceae	$\overline{2}$		
Euphorbiaceae	$\overline{2}$		
Lamiaceae	$\overline{2}$		
Lauraceae	$\overline{2}$		
Liliaceae	\overline{c}		
Ranunculaceae	\overline{c}		
Smilacaceae	$\overline{2}$		
Acanthaceae	1		
Annonaceae	1		
Araceae	1		
Aristolochiacea			
Asclepiadaceae			
Aspleniaceae	1		
Balsaminaceae			
Betulaceae			
Brassicaceae			
Campanulaceae			
Caryophyllacea			
Clusiaceae			
Cupressaceae			
Dryopteridaceae			
Ebenaceae	L		

Appendix III. Plant families represented in the quadrat data at Roaring River State Park, Barry County, Missouri.

Appendix IV. Full table of plant species found in quadrats sampled within one-meter of the 201 sampled Ozark chinquapins with frequency (the percent of sampled Ozark chinquapins the species occurred near), the mean relative abundance (the proportion of the species to the overall plant community sampled in the quadrats), and if the species is present (X) in the species list of the Ecological Sites found in Roaring River State Park in Barry County, Missouri.

Appendix V. Ecological Sites of Roaring River State Park with locations 964 Ozark chinquapins surveyed in 2022.

Appendix VI. Vegetative Ozark chinquapin branch. (Picture by Danielle Evilsizor).

Appendix VII. Ozark chinquapin flowering branch. (Picture by Danielle Evilsizor).

Appendix VIII. Ozark chinquapin immature fruiting branch. (Picture by Danielle Evilsizor).

Appendix IX. Antler damage to Ozark chinquapin trunk. (Picture by Danielle Evilsizor).

Appendix X. Ozark chinquapin burrs in leaf litter. (Picture by Danielle Evilsizor).