

ERIGENIA

Number 29
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Number 29, Spring 2025

The Illinois Native Plant Society Journal

The Illinois Native Plant Society is dedicated to the conservation and study of the native plants and vegetation of Illinois.

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ERIGENIA is named for *Erigenia bulbosa* (Michx.) Nutt. (harbinger-of-spring), one of our earliest blooming woodland plants and a species that serves as a promise of things to come. The first issue was published in August 1982.

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COVER ILLUSTRATION: Drawings of *Erigenia bulbosa* on the front cover and inside back page by Paul Nelson.

JOURNAL DESCRIPTION

ERIGENIA is a peer-reviewed journal of the Illinois Native Plant Society. We invite the submission of original articles on the botanical resources of Illinois and adjacent states. Topics accepted include the following:

TAXONOMY of vascular plants, mosses, algae, lichens, and fungi

FLORISTICS and NOTEWORTHY COLLECTIONS (complete with voucher specimen citations)

ECOLOGY of species, communities, and their biotic and abiotic interactions

RESTORATION AND MANAGEMENT of natural areas

NATURAL HISTORY of Illinois including geology and biogeography

ETHNOBOTANY of native plants

HORTICULTURE as it relates to native plants in restored or cultural environments

BIOGRAPHICAL REVIEWS of botanists and explorers

BOOK REVIEWS of botanical interest

IN MEMORIAM: Memoria for botanists and conservationists who have passed

INSTRUCTIONS FOR AUTHORS - GENERAL GUIDELINES

Authors may submit material in an e-mail attachment to erigenia.editor@gmail.com. Manuscripts must be submitted as a Microsoft Word© document. Use Times New Roman 12-pt font, double line spacing,

1-inch margins, left-side alignment, number each page, and use line numbering. Include any tables on separate page(s) at end of document. Place figure captions at the end of the Word document. Figures must be separate files with figure number in file name. Tables and illustrations must fit in a 7 x 9 inch area. Retain copies of each file.

TITLE PAGE - For each author, list affiliation, street address, and e-mail addresses; for corresponding author, include telephone number and/or email address.

ABSTRACTS - All papers should include an abstract with up to 250 words. The ABSTRACT should state goals, principal results, and major conclusions of the paper.

TAXONOMIC NAMES - Cite the botanical nomenclature utilized in the manuscript or, alternatively, include taxonomic authority for each taxon. Common names should be referenced following first use of a scientific name; thereafter, scientific or common names can be used alone if done so consistently. Lists of species in tables and appendices need only to include scientific name.

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IN MEMORIAM

ROBERT H. MOHLENBROCK (B: 26 SEPTEMBER 1931 – D: 5 NOVEMBER 2024)

John B. Taft, editor

The world has lost a botanical giant with the death of Dr. Robert H. Mohlenbrock at age 93. Dr. Mohlenbrock committed his long and productive life to the study of vascular plants with a particular focus on the flora of Illinois (Figure 1). A native and lifelong Illinoian born and raised in Murphysboro, his interest in botany was early on with timely encouragement by a high school instructor. By the age of 26, he had earned his doctorate from Washington University, specializing in plant systematics and floristics, and began a 34-year career as a professor of Botany at Southern Illinois University in Carbondale, serving as department chairperson for nearly half that time. During these academic years he fostered a keen interest in botany for hundreds of students who enrolled in classes he taught and he received numerous awards acknowledging him as an outstanding teacher. His lab was often bustling with botanical researchers where a total of 25 students earned PhDs and 68 earned Masters degrees under his mentorship. Dr. Mohlenbrock's impact is particularly magnified when considering that many of these students became leaders in systematics and especially in the study and protection of natural areas. A prolific author, Dr. Mohlenbrock published 81 books and nearly 700 scientific and popular articles extending his influence far beyond the classroom. Perhaps foremost among these are his field guides and illustrated flora series for the vascular flora of Illinois, enabling botanists throughout the state to have resources for plant identification. For the field botanist, determining species identifications using the field guide was like having Dr. Mohlenbrock along in your daypack. I wore out many copies of the editions over the years. Particularly relevant to botanical study and appreciation for the flora of Illinois, the Illinois Native Plant Society (originally the Southern Illinois Native Plant Society) and this journal were conceived in Dr. Mohlenbrock's lab with the first issue of *Erigenia* published in 1982. Dr. Mohlenbrock helped the journal get off to a good start contributing about 40% of the articles in the first ten volumes. Botanists and

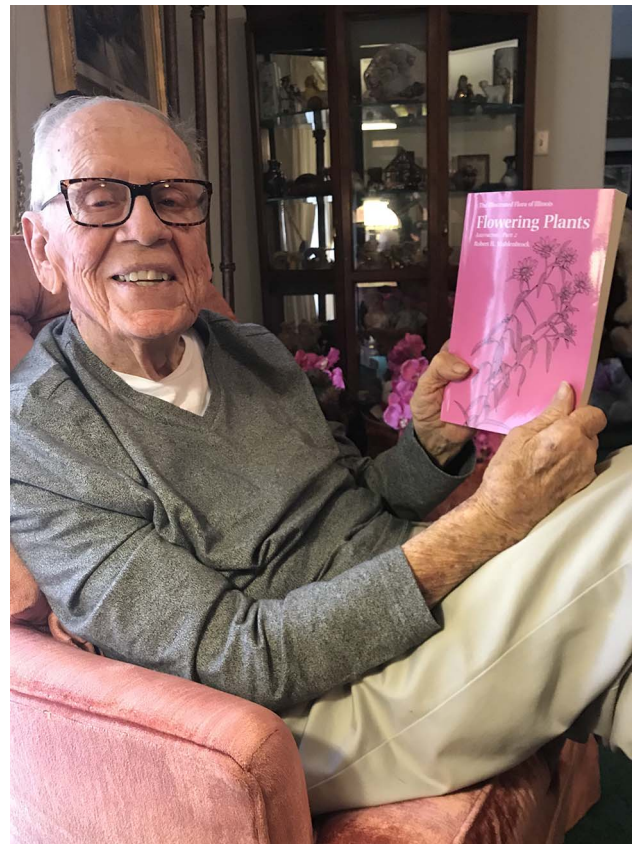


Figure 1. Dr. Robert H. Mohlenbrock in March 2020 holding a copy of the recently published Asteraceae Volume 2 for the Illustrated Flora series. Photo by Chris Benda.

plant enthusiasts everywhere, particularly Illinois, owe a major debt of gratitude for the tremendously impactful legacy of Dr. Robert H. Mohlenbrock's contributions toward the study, conservation, and appreciation of botanical resources.

HARRIS BRALEY PARKS' "THE TREES OF MACOUPIN COUNTY, ILLINOIS"

William E. McClain^{1,*} and John E. Ebinger²

ABSTRACT: The size, abundance, habitat, and distribution of 54 tree species, 51 natives and 3 adventives, within Macoupin County, Illinois are described from a manuscript completed by H. B. Parks in 1900 while a student at Blackburn College in Carlinville, Illinois. A copy of this unpublished manuscript was obtained from the Science Library of Blackburn College, and a review of the text determined that annotations for ten tree species were missing. The remaining text describes the distribution and habitats of tree species at a time when old-growth forest remnants were still present. Exceptionally large trees representing several species, and native stands of tulip tree (*Liriodendron tulipifera* L.) are reported; however, two taxa, swamp chestnut oak (*Quercus michauxii* Nutt.) and ground juniper (*Juniperus communis* L.), remain undocumented from Macoupin County. Impacts upon forests by household uses of wood for heating, cooking, farming, building, and light industry, such as wagon manufacturing, are described. The greatest loss of old-growth forest is attributed to railroad construction from 1850 to 1895, a process that occurred throughout Illinois during the last half of the nineteenth century. The entire text of the Parks manuscript is presented in the appendix.

INTRODUCTION

Harris Braley Parks (1879-1958) was born on 10 June 1879 to William Stewart Parks and Sarah (Cowdry Braley) Parks in Carlinville, Macoupin County, Illinois. He attended the local schools before beginning studies in the Preparatory School (high school) of Blackburn College in Carlinville in 1893, earning a Bachelor of Science Degree in 1900 (Alex 1959). Parks farmed in Illinois from 1900 to 1903, and in South Dakota from 1903 to 1907 before becoming an instructor and superintendent of the Sheldon Jackson Indian School in Sitka, Alaska from 1907 to 1911. In 1912 he accepted the head position of the Science Department at Palmer College in Albany, Missouri, but left for College Station, Texas in 1917 where he served as an entomologist with the Texas A & M Extension Service. He was named Chief of the Division of Apiculture at what is now Texas A & M University in 1927, becoming curator of the Museum and Botanist of the Tracy Herbarium at this institution in 1945, eventually retiring in 1949.

Parks published several papers during his professional career, including "Valuable Plants Native to Texas" (Parks 1937), a "Catalogue of the Flora of Texas" (Cory and Parks 1937), and "The Fauna and Flora of the Big Thicket" (Parks and Corey 1938). One manuscript, "The Trees of

Macoupin County, Illinois", written while a student at Blackburn College in Carlinville in the late 1890s, was never published (Parks 1900). This article remained relatively unknown until 1928 when Parks shared the data with Illinois forester Robert Barclay Miller, a member of the Illinois Department of Conservation, and one of the authors of "Forest Trees of Illinois, How to Know Them" (Fuller et al. 1928).

Macoupin County in west-central Illinois has an area of 552,960 acres (223,796 ha) and lies within the Carlinville Section of the Western Forest-Prairie Division (Schwegman 1973). The original vegetation consisted of a mixture of forest and prairie, with forest occupying ridge tops, hillsides, portions of bottomlands, and dry, relatively flat uplands. Prairie was present in the northern and southern parts of the county and the Macoupin Creek Bottoms. The topography varies from nearly level, poorly drained, former prairie sites in the north to dissected, rolling, forested hills in the south. The creek bottoms, especially those along Macoupin Creek, are wide, sometimes exceeding a mile (1.6 kms). The climate is continental, with an average winter temperature of 31° F (–1° C) and an average summer temperature of 75° F (24.0° C). The growing season averages 175 days, and average annual precipitation is 38.6 inches (98.0 cm), most of which falls as rain from April through September (Tegeler 2004).

Most soils within Macoupin County developed from wind-blown loess with smaller units derived from glacial drift, alluvium, or bedrock residuum. Prairie, forest, and forest-prairie transition vegetation influenced soil development on uplands, and wet prairie-wet forest vegetation influenced soil development in bottomlands

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(Tegeler 2004). Approximately 401,300 acres (162,400 ha) of prairie and 148,300 acres (60,015 ha) of forest were present in Macoupin County in the early 1800s (Iverson et al. 1989). Nearly all prairie has since been converted to agriculture and the extent of forest vegetation reduced to about 85,000 acres (34,398 ha) since the study of Parks (1900). Only 118 acres (47.8 ha) of this total represent high quality forest (Iverson et al. 1989). The forest remnants of today are greatly modified from their original composition as various tree species have increased or decreased in abundance due to diseases and logging practices. The purpose of this article is to publish the annotated list of tree species of H. B. Parks for Macoupin County, Illinois (Parks 1900), and to create awareness of tree sizes and their values within old-growth forest communities once present in west-central Illinois.

METHODS

We reviewed the manuscript of H. B. Parks (1900) and updated the taxonomy of all tree species to correspond with Mohlenbrock (2014). Scientific names used by Parks are enclosed in parenthesis for species with taxonomic changes since 1900. We also added family names and arranged the annotations alphabetically by family, genus, and species. We made efforts to locate an original copy of the Parks' manuscript and his voucher specimens deposited in the Blackburn College Herbarium at the completion of his study. Additional herbaria searches were made at the University of Illinois (ILL), Illinois Natural History Survey (ILLS), and the Illinois State Museum (ISM). Metric equivalents were developed for all measurements. The complete manuscript of Parks with the additions mentioned above is included in the appendix.

Field surveys were conducted to locate extant specimens of tulip tree (*Liriodendron tulipifera* L.) in the southern part of Macoupin County. The southern half of Brushy Mound, the large mound that gives the township its name, was searched and interviews were conducted with current and former residents. Portions of the Macoupin Creek Bottoms were searched to locate specimens of swamp chestnut oak (*Quercus michauxii* Nutt.). Interviews were also conducted with researchers regarding these two tree species included in Parks (1900) in attempts to verify them as indigenous to Macoupin County. Neither species has been verified as a part of the flora of Macoupin County.

RESULTS

We found an introduction, a numbered list of species, and numbered annotations corresponding to the list of taxa included in the Parks' manuscript, "Trees of Macoupin County, Illinois". We found pages missing from the manuscript that included annotations for ten species included in the list of species cited by Parks: *Amelanchier arborea* (Michx. F.) Fern. (Juneberry, shadbush), *Cornus florida* L. (flowering dogwood), *Crataegus calpodendron* (Ehrh.) Medic. (woolly haw), *Crataegus crus-galli* L. (cock-spur thorn), *Crataegus mollis* (Torr. & Gray) Scheele (red haw),

Malus coronaria (L.) Mill. (wild sweet crab apple), *Prunus americana* Marsh (wild plum), *Prunus serotina* Ehrh. (wild black cherry), *Juniperus communis* L. (juniper), and *Sambucus nigra* L. (elderberry). Also missing from the manuscript was a map referenced by Parks delineating forest boundaries in the late 1890s. The remaining text describes, in various levels of detail, 54 woody plants regarded as trees by Parks that were present in Macoupin County in the late 1890s.

Neither a copy of the complete, original manuscript or a retyped version could be located at Blackburn College. We also determined the occurrence of two devastating fires, University Hall in 1927 and Robertson Hall in 1959, that greatly affected the resources of the biology department. Both buildings were destroyed along with extensive collections and archival materials. We were unable to locate any specimens collected by Parks in the current herbarium of Blackburn College, the University of Illinois (ILL), the Illinois Natural History Survey (ILLS), or the Illinois State Museum (ISM). Specimens dating to the late 1800s were absent in the Blackburn College Herbarium despite a known history of plant collecting by botanists at this institution during this time.

Little information was found in the partial manuscript that described the methods used by Parks in his surveys. He defines a tree as a perennial woody plant having a straight trunk whose diameter is greater than two inches (5.1 cm) at a height of six inches (15.2 cm) above the soil in the introduction but does not provide additional explanation on how tree heights and large trunk diameters were determined. He describes searching for tree species within all creek bottoms and their bluffs throughout Macoupin County, suggesting a thorough survey.

DISCUSSION

Data on tree heights and diameters from the late 1800s from old-growth forests in Illinois are scarce. One of the best-known publications from this time describes gigantic trees within the forests of the lower Wabash and White rivers of Illinois and Indiana (Ridgeway 1882). While the Wabash forests were exceptional and unique, especially for the eastern United States, large trees once characterized old-growth forests throughout the nation, including Macoupin County, Illinois (Brugam et al. 2016). Their removal, especially during the period 1850 to 1890, left scattered tracts of original forest plus many widely dispersed, large trees considered defective by lumbermen.

Initially, the removal of trees during the early part of the nineteenth century was limited mostly to household, farm, and small business uses (See Appendix). Tulip tree and hickory (*Carya* spp.) wood was used to make wagons and cooperage hoops, oaks were used for heating, cooking, and building, and Ohio buckeye (*Aesculus glabra* Willd.), a light, strong wood, proved ideal for oxen yokes. Basswood (*Tilia americana* L.), sycamore (*Platanus occidentalis* L.), tulip tree, silver maple (*Acer saccharinum* L.), and elms (*Ulmus americana* L. and *U. rubra* L.) were

planted extensively for shade. Collectively these uses, coupled with forest clearing for agriculture, slowly reduced the extent of the original forests, but their impacts do not compare in magnitude to the rapid removal of huge swaths of old-growth forest by railroad companies. Crews of the Alton and Sangamon Railroad (later the Chicago and Alton) cut vast amounts of timber for ties, bridges, fuel, and buildings. One Macoupin County sawmill supplied this railroad with 500,000 ties cut from oaks (McClain 2012). It was a process repeated during the late 1800s throughout Illinois as railroads crisscrossed the state, resulting in the removal of vast amounts of old-growth forest. Individuals must now diligently search for trees that match the dimensions cited by Parks

A recent survey for large-sized trees in Macoupin County, conducted approximately one hundred years following the study of Parks, identified large white, bur, and post oaks, sycamores, cottonwoods, and other species (Mahan 2002). This study, like that of Parks (1900), investigated all creek bottoms within the county. Large trees were rare and found on sites not readily accessible to lumbermen. Many of these trees were equal in size and height to those reported by Parks (Mahan 2002). One difference between these two studies is the scarcity of old-growth forest remnants like those present at the time of European settlement. Only 118 acres (47.8 ha) of old-growth forest remains in all of Macoupin County (Iverson et al. 1989). Large trees are now less dominant on the landscape due to additional forest clearing and timber management practices that discriminate against large, hollow, and open-grown trees.

The loss of big trees within forest communities has had dramatic detrimental impacts upon wildlife. The demise of large, hollow trees, including sycamores, eliminated nesting and roosting sites for a variety of bird species such as swallows and owls. Small forest remnants are unsuitable nesting habitat for many migratory bird species. More recently, diseases and insect infestations have had devastating effects upon several tree species. Elms (*Ulmus americana*, *Ulmus rubra*), once common in Macoupin County, are less prominent now due to Dutch elm disease (*Ophiostoma ulmi* [Buisman] Nannf. Nairn) and butternut (*Juglans cinerea* L.) has been virtually eliminated due to a canker disease (*Ophiognomonia clavigneti-juglandacerarum* [Nair, Kostichka & Kuntz] Broders & Boland). The emerald ash borer (*Agrilus planipennis* Fairmaire) is currently infesting ash (*Fraxinus*) trees throughout the county, greatly reducing their numbers. The absence of wildland fire has favored an increase in abundance of species like sugar maple (*Acer saccharum*) at the expense of fire-tolerant oaks (McClain et al. 2006). The advent of exotic, invasive species, such as garlic mustard (*Alliaria petiolata* (Bleb.) Cavara & Grande.) and Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim.), has altered the understory of many woodlands, making them unsuitable for ground-nesting birds such as whippoorwills and ovenbirds (McClain 2011).

Parks prepared voucher specimens for all tree species included in his study. However, collections dating to the 1800s, including those made by Parks and botany professor

W. E. Andrews, were conspicuously absent from the Blackburn College Herbarium. It is thought that the herbarium was destroyed when University Hall, the main campus building, burned to the ground (Carlinville Democrat 1927). A second fire on 10 November 1959 destroyed Robertson Hall, the next location of the biology department. The losses included the Taylor Museum, a 21,000 fossil specimen collection, Native American artifacts, and numerous archival materials (Emory 2019). These fires effectively severed the past from the Blackburn College Biology Department.

The loss of voucher specimens makes it impossible to confirm the identities of three species reported by Parks that are currently undocumented as part of the native flora of Macoupin County (Mohlenbrock and Ladd 1978). One species is tulip tree, reported by Parks from the Macoupin Creek Bottoms, Brushy Mound south of Carlinville, and a grove southwest of Plainview. We believe his identification of this tree is correct due to its unmistakable leaf shape and flowers. This statement is supported by the observation of wild growing tulip trees approximately two feet in diameter south of Carlinville near Brushy Mound (Larry Mahan, pers. com. 2 October 2022). The nativity of the other two species is much less certain. Parks describes the exceptional trunk diameters and heights of swamp chestnut oak (*Quercus michauxii*) trees and their extensive use by the railroad but does not define a locality or habitat. Swamp chestnut oak occurs in southern Illinois but is absent in the northern two-thirds of the state. Its nativity to Macoupin County, in the absence of voucher specimens, remains uncertain, and no trees were found by Mahan (2002) who surveyed the entire county. The habitats of this tree, wet bottomland forests like those once present along Macoupin Creek, have all been destroyed. Parks used the scientific name *Quercus prinus* for this tree and separated it from yellow chestnut oak (*Q. muhlenbergii*) based on size and the resistance of swamp chestnut oak wood to decay.

Another species not known from Macoupin County is *Juniperus communis*, a sprawling shrub currently restricted to the sand dunes of Lake Michigan in northeastern Illinois (Mohlenbrock 2014). The sand dune habitat required by this plant is not present in Macoupin County, causing us to believe that the inclusion of this species in the Macoupin County Flora is incorrect. The only juniper species recorded for the county, which still grows naturally, is eastern red cedar (*Juniperus virginiana* L.). This is the taxon most likely observed by Parks in Macoupin County.

We are unlikely to witness tree sizes in the future like those described by Parks. He includes several woody species, known today as small shrubs, that attained remarkable sizes. Individuals of smooth sumac (*Rhus glabra* L.) reached heights of 25 feet (7.6 m) with trunks large enough for firewood. Lance-leaved buckthorn (*Rhamnus lanceolata* Pursh.), with heights of 30 feet (9.1 m) and trunk diameters of six inches (15.2 cm), grew along Macoupin Creek. Wafer ash (*Ptelea trifoliata* L.) specimens with heights greater than 20 feet (6.1 m) and trunks with diameters greater than four inches (10.2 cm) grew along Honey Creek.

The sizes listed for several trees are equally impressive. A specimen of sassafras, known mostly as a small tree today, had a diameter of five feet (1.5 m) and a height of nearly 150 feet (45.7 m). Diameters of four (1.2 m) to six feet (1.8 m) and heights of 50 (15.2 m) to 60 feet (18.2 m) were reported for American and red elms, including one American elm near Macoupin Station that had a diameter of six feet (1.8 m) at a height of eight feet (2.4 m) above the ground. A sycamore east of Carlinville, damaged in a storm, had a hollow top five feet (1.5 m) in diameter at a height of sixty feet (18.2 m). White oaks four feet (1.2 m) in diameter and seventy-five feet (22.9 m) in height were common and post oaks up to five feet (1.5 m) in diameter were scattered throughout pastures. The largest trees were those of swamp chestnut oak, a species whose nativity in Macoupin County remains questionable. Individuals of this tree, having immense diameters of eight (2.4 m) to ten feet (3.0 m), were cut to furnish lumber for the construction of the Alton and Sangamon Railroad in the 1850s (McClain 2012).

Parks mentions, in correspondence with R. B. Miller, that additional species could be added to the manuscript "Trees of Macoupin County". Absent from his list are redbud (*Cercis canadensis* L.), and green ash (*Fraxinus lanceolata* Borkh.). Redbud is common along forest edges and fence rows and green ash is found along the creeks of the county. Several invasive exotic tree species are now naturalized in Macoupin County, including callery pear (*Pyrus calleryana* Decne.), white poplar (*Populus alba* L.), and mimosa (*Albizia julibrissin* Durazz.). These trees may be found along roads, fences, and disturbed sites, and have been increasing in abundance and distribution within the state in recent years (McClain et al. 2012; Mohlenbrock 2014; White et al. 2005).

We consider the tree heights and diameters described by Parks to be remarkable. It is our hope that his study will create interest and efforts to increase the number and distribution of large trees representing many species within our forest remnants. Their presence would provide enjoyment, enhance wildlife habitat, and create educational opportunities. His study may also encourage the reconstruction of certain communities, such as bottomland pin oak forests, that once characterized portions of the Macoupin Creek floodplain.

LITERATURE CITED

- Alex, A.H. 1959. Obituary of Harris Braley Parks. *Journal of Economic Entomology* 52(2):362.
- Brugam, R.B., P. Kilburn, and L. Lueking. 2016. Pre-settlement vegetation of Greene, Jersey, and Macoupin counties along the Prairie/Forest Border in Illinois. *Transactions of the Illinois State Academy of Science* 109:9-17.
- Carlinville Democrat. 1927. Disastrous Fire at Blackburn. No. 52., 23 August 1927, p 1. Lumpkin Library, Blackburn College, Carlinville, Illinois.
- Corey, V.L. and H.B. Parks. 1937. Catalogue of the Flora of Texas. *Texas Agricultural Experimental Station Bulletin* 550. College Station, Texas.
- Emory, T. 2019. The Robertson Hall Fire of 1959. The Bengil Post, 5 November 2019. www.thebengilpost.com
- Fuller, G.D., W.R. Mattoon, R.B. Miller, and E.E. Nuuttila. 1928. Forest Trees of Illinois, How to know them: A pocket manual describing their most important characteristics. 3rd ed. State of Illinois, Department of Conservation, Springfield.
- Iverson, L.R., R.L. Oliver, D.P. Tucker, P.G. Risser, C.D. Burnett, and R.G. Rayburn. 1989. Forest Resources of Illinois: An Atlas and Analysis of Spatial and Temporal Trends. *Illinois Natural History Survey Special Publication 11*. Champaign, Illinois.
- Mahan, L.P. 2002. In search of large trees. Production Press Inc., Jacksonville, Illinois.
- McClain, W.E. 2011. Amur honeysuckle, the perfect invasive. *Illinois Audubon* 318:11-17.
- McClain, W.E. 2012. Early railroads: The high cost of progress. *Illinois Audubon* 320:20-22.
- McClain, W.E., V.L. La Gesse, and J.E. Ebinger. 2006. Dynamics of species composition and importance from 1965-1998 in Baber Woods Nature Preserve, Edgar County, Illinois: evidence of the effects of fire suppression. *Castanea* 71(4):312-320.
- McClain, W.E., P.M. McClain, and J.E. Ebinger. 2012. Naturalized mimosa (*Albizia julibrissin* Durazz., Fabaceae) in Illinois. *Castanea* 77(3):231-234.
- Mohlenbrock, R.H. 2014. Vascular Flora of Illinois, 4th ed. Southern Illinois University Press, Carbondale, Illinois.
- Mohlenbrock, R.H. and D.M. Ladd. 1978. Distribution of Illinois Vascular Plants. Southern Illinois University Press. Carbondale.
- Parks, H.B. 1900. The Trees of Macoupin County, Illinois. Typed, unpublished manuscript presented before and printed by the Science Club, Blackburn University, Carlinville, IL.
- Parks, H.B. 1937. Valuable Plants Native to Texas. Texas Agricultural Experimental Station Bulletin 551. College Station, Texas.
- Parks, H.B. and V.L. Corey. 1938. Flora and Fauna of the Big Thicket. Sam Houston State Teachers College. Huntsville, Texas.
- Ridgeway, R. 1882. Notes on the native trees of the lower Wabash and White river valleys in Illinois and Indiana. *Proceedings of the United States National Museum* 49-83.
- Schwegman, J.E. 1973. Comprehensive Plan for the Illinois Nature Preserves System, Part 2: The Natural Divisions of Illinois. Illinois Nature Preserves Commission, Rockford.
- Tegeler, R.A. 2004. Soil Survey of Macoupin County, Illinois. United States Department of Agriculture, Natural Resources Conservation Service, and Illinois Agricultural Experiment Station. United States Government Printing Office, Washington, D.C.
- White, J., W.E. McClain, and J.E. Ebinger. 2005. Naturalized Callery Pear (*Pyrus calleryana* Decne.) in Illinois. *Transactions of the Illinois Academy of Science* 98(3&4):123-130.

APPENDIX

Annotated list of tree species for Macoupin County, Illinois by H. B. Parks, prepared during 1895-1900 while a student at Blackburn College, Carlinville, Illinois. The annotations for each species are from the manuscript of Parks with very minor editing to provide greater clarity.

The Trees of Macoupin County, Illinois

An annotated list of the larger woody plants which occur in this county, together with notes on their former occurrence and history of removal and replacement.

By
H. B. Parks
Carlinville, Illinois
Presented before and printed by the Science Club
Blackburn University
1900

The Trees of Macoupin County

The most marked feature of any landscape is its trees, either singly, in groups, or as the forest's vast extent. To grow a forest cover takes hundreds of years. In these years of growth, the lesser plants, the birds, the animals, the insects, and even the fishes gradually adapt themselves to the new and agreeable surrounding and nature finds a balance, where for countless seasons there is little change save in the replacement of dead individuals by a similar number of the same kind. When man arrives with his axe and plow, a year will see the destruction a dozen centuries work. Nature is suddenly thrown out of tune and a barren, plague-stricken landscape is the result.

In 1815, David Coop made the first (European) settlement in Macoupin County. As has been the case with all early settlers, he chose the wooded part of the land to the prairie. Here on the side of that beautiful rise of land that now bears the name of Coop's Mound, he cut out his fields. What a change has eighty-five years have brought about! So changed is the original terrain that it is with the great difficulty that the boundaries of the old forest can be traced. Every year sees more and more of the forest cover removed and yet a hopeful note that can be heard above that of the axe is the sound of the spade setting out trees to replace those that have disappeared and to grace the prairies where none have existed before.

In 1894, W. E. Andrews, Professor of Botany at Blackburn University, Carlinville, Illinois, published in the Carlinville Enquirer a checklist of the flowering plants that he had collected in this county up to that date. In the six years that have passed since that list was printed the writer has seen acre after acre of the forest cover of this same area give way to the progress of agriculture and knows of the eradication of several species of plants from the county. The loss of such plants as the white lady's slipper (orchid), the yellow lady's slipper (orchid), wild pansy, wild hydrangea, and the wild honeysuckle are to be deplored and charged up to man's ignorance. That Prof. Andrew's record might be kept up to date and supplemented, the writer, in 1895, began the list that is herein given. He has followed the nomenclature used by Prof. Andrews and each species given was verified by Prof. Andrews. Herbarium specimens of all species recorded are deposited in the Biological Laboratory of Blackburn University. The notes on occurrence

and history, especially the boundaries of the old forests, are mostly the work of the writer, although some of them were made in connection with Prof. W. E. Andrews, and Dr. C. Robertson.

In order that this article be of convenient length, it was decided to include in it a record of those woody plants that are commonly called trees. As everyone knows, it is very hard to say where a bush ends, and a tree begins. So, for the sake of convenience, a tree, as spoken of in this list, is any woody perennial plant that attains a diameter of two inches or more six inches above the ground. The local history of the occurrence and destruction of trees, as well as the limits of the almost forgotten forest, have come in part from the old settlers and part of the files of the county newspapers. To complete this history of the woody plants, a paper on the "Shrubs and Woody Vines of Macoupin County" is in preparation.

H. B. Parks
Feb. 1, 1900

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Species Annotations

ACERACEAE

1. *Acer negundo* L. (*Negundo aceroides* Moench., boxelder) - This tree is common along the water courses. It is seldom found on the upland with the exception of one place in Barr Township where several acres (ha) of this species are present around a prairie lake. The tree does not reach a large size and has nothing to mark it as peculiar.

2. *Acer saccharinum* L. (*A. dasycarpum* Ehrh., silver, white maple) - This is the most common maple tree of the county and is the most common tree planted for shade in this section of the state. Its rapid growth, thick shade, and wonderful coloring in autumn make it desirable as an ornamental tree. Its greatest drawback is the fact that the wood is weak and in wind or ice storms will fill the streets with broken limbs. The bright red of the new branches and of the blossom which appear in February or March make this tree very conspicuous in the spring forest. The seed which are produced in large quantities germinate very readily and any abandoned field will soon show a growing maple forest. The trees very often reach an immense size and the wood is utilized for box making and stove wood. The tree is a native of the creek bottoms land, (and) is seldom found growing on uplands, unless planted.

3. *Acer saccharum* Marsh. (sugar maple) - This tree is very common in Macoupin County, but nowhere are the stands large enough to warrant the tapping for sugar. Many large specimens can be found in the Macoupin Creek bottoms. These will range from three to five feet (0.9 to 1.5 m) in diameter. Trees growing in the river (creek) bottoms are generally very tall with a central trunk. The majority of sugar maples, however, grow in isolated localities on rich hillsides. Here they form a round topped tree, which seldom reaches a height of more than fifty feet (15 m). This tree is the latest blooming of the maples, and the blossoms appear the latter part of May.

ANACARDIACEAE

4. *Rhus copallina* L. (winged dwarf sumac) - This variety of sumac is not common, but there are a few large thickets in Carlinville, South Otter, and South Palmyra Townships. It is generally found in the vicinity of prairie lakes where it very often reaches the size designated as the lower limits for trees specified in this paper. This sumac blooms later than *Rhus glabra* and the flowers are white instead of yellow. The seed clusters are much smaller and darker in color. The plant furnishes material for tanning leather, and it is reported that the species was largely killed out by the continued collecting of its new growth for dying purposes.

5. *Rhus glabra* L. (sumac) - This plant hardly deserves a place in this list. It must, however, be included as many specimens are greater than two inches (5.0 cm) in diameter at the ground. There are many thickets of this plant on both the uplands and creek bottoms in the county. On the rich black lands in the north part of the county specimens 25 feet (7.6 m) in height are not uncommon. Sections of the trunks were seen that were large enough to be used as cord wood. This tree blooms in June and

July. The flowers are followed by the beautiful flame-colored clusters of seeds that hang on all winter, furnishing food to the few birds that remain in the county throughout the winter. The small boys rely on this plant for material to make pop guns. In an early day the new shoots of his plant and the seed clusters were colored (collected) and used in the dying of leather.

ANNONACEAE

6. *Asimina triloba* (Dunal) L. (papaw) - The pawpaw is a native of the recently moved alluvial soils along the creeks of the county. There is no record of its occurrence in the upland. It will grow, however, in almost any location if it is protected while young. It has been used to good effect as an ornamental in the yards in Carlinville and Bunker Hill. Because of the cutting of the forests and the straightening of the water courses, this tree is now restricted to the overflow lands along the larger creeks. The largest specimen known stands near the bridge of the Chicago and Alton Railroad a mile (1.6 km) south of Macoupin Station. This tree will soon disappear from the county with the exception of those specimens maintained for ornament or fruit. As the seedlings must be grown in the shade and as the tree puts out no sprouts when cut off at the ground. This tree is worthy of propagation for its fruit. The fruits from different trees vary little in size but a great deal in the number of seeds per fruit and in flavor. The manager of Otwell's Nursery reports that the plants come true to seed, are easily propagated, and bear the fourth year. The blooming period is April and May.

BETULACEAE

7. *Betula nigra* L. (red birch) - The red or river birch is common only in Barr and Western Mound Townships where it is found in the flat timberland around sloughs. These swampy locations are covered with a heavy growth of this birch, cottonwood, willow, and persimmon. It is very probable that this birch was brought in by the migratory birds as its natural home seems to be along creek and river bottoms. A most careful search along the Macoupin, Cahokia, and Apple creeks failed to reveal a single specimen, yet on the uplands there are a good many acres (ha) of pure stands of this birch.

BIGNONIACEAE

8. *Catalpa bignonioides* Walt. (catalpa) - The catalpa tree occurs only in the vicinity of farms and cities and undoubtedly the trees, which seem native, came from seed of imported stock. There are a number of very large catalpas, ranging from two to three feet (0.61 to 0.91 m) in diameter, growing within the county, but these are all on old farmsteads and local history says they were planted about 1830. The majority of these old trees are hollow and while the trees are recommended for cultivation as a fence post tree, this habit of becoming hollow is very detrimental. In Hilyard Township there is a large catalpa grove. The trees are not over twenty years of age, and their origin is a number of planted trees on a farmstead in the vicinity.

CAPRIFOLIACEAE

9. *Sambucus nigra* L. (*S. canadensis* L., elder) - missing from manuscript

10. *Viburnum prunifolium* L. (black haw) - This small tree is restricted to the partially wooded uplands, especially to thickets at edges of prairies. It is one of the first plants to put out its flowers in the spring and its black fruits hang on throughout the winter. This plant is very common in the county but is rapidly disappearing as farms are being increased in size and partially wooded land is being cleared. This tree lends itself to landscape gardening, and there are several specimens of it already in cultivation in Carlinville.

CELASTRACEAE

11. *Euonymus atropurpureus* Jacq. (wahoo) - The word delicate is the only adjective that will describe this beautiful little tree. It has a true tree form including a straight upright trunk and almost horizontal branches. Its smooth, green bark, delicate green leaves, clusters of small red flowers and large red seed pods, make it an ornamental of the first class. It is found in all situations where rich soil can be found throughout the county. It is easily transplanted and responds very readily to cultivation. It blooms in May and June and the seed pods turn red in August. These are persistent and hang on throughout the winter.

CORNACEAE

12. *Cornus florida* L. (flowering dogwood) - missing from manuscript.

CORYLACEAE

13. *Carpinus caroliniana* Walt. (hornbeam) - This tree does not in any way resemble its near relative which is described below. It grows in the rich creek bottom lands, especially around sloughs, river bottoms and lakes. The smooth, dark green bark and ridged trunk of the tree give it the name of hornbeam, and they closely resemble the horn of an elk or deer. The wood has always been highly prized by farmers for use as hand spikes and hay poles. This tree would make a wonderful ornamental in localities where it can be grown.

14. *Ostrya virginiana* (Mill.) K. Koch (hop hornbeam) - This small tree, which is commonly mistaken for an elm, is found wherever the original forest remains. It grows on the points of the limestone hills where it reaches its best development. The ordinary sized tree is about eight inches (20.3 cm) in diameter and thirty feet (9.1 m) high. The fruits resemble clusters of hops and as they are light green in color make a contrast to the dark green of the leaves. This tree has been used to some extent as an ornamental in St. Louis.

CUPRESSACEAE

15. *Juniperus communis* L. (juniper) - missing from manuscript. **Note:** This is considered to be *Juniperus virginiana* L.

EBENACEAE

16. *Diospyros virginiana* L. (persimmon) - This is a common but not abundant tree in the county. It occurs in the creek bottoms and on the uplands in about the same numbers. It is a lover of damp situations as the upland localities are always near the prairie lakes. Persimmon trees growing in the lowlands are generally tall and straight trees, reaching heights of nearly 75 to 80 feet (22.8 to 24.3 m), while those on the uplands are

generally wide-spreading and seldom reach a height greater than 50 feet (15.2 m). The creek bottom trees generally bear larger fruit than those on the upland. This may be due to one of two things. The more rapid growth of the trees in the lowlands would make a tree appear much older than it really is and the size of the fruit seems to depend on the age of the tree. There is a great variation in the fruit and undoubtedly there are some strains that it would pay to cultivate. One of the trees known in the county the fruits vary from one half inch (1.3 cm) in diameter up to two inches (5 cm) in diameter. The amount of sugar contained is also extremely variable. The great variation in the fruit suggests that it would pay to cultivate some strain.

FABACEAE

17. *Gleditsia triacanthos* L. (honey locust) - The honey locust is fairly common throughout the county. It is found on the uplands and in the creek bottoms. The trees growing in the creek bottoms grow much taller than the ones on the upland and have fewer thorns. They, however, produce a greater number of seed pods. The honey locust, as it grows in the upland timber flats, is a low growing, round topped tree, protected by immense thorns. It is common in every township in the county. It is most prevalent in Brushy Mound Township than elsewhere. The tree blooms in June but has nothing to recommend it as an ornamental shade tree or for timber. The wood is weak and of little value. As the tree is almost impossible to kill by cutting off and grubbing, it is a pest to a farmer who has cut his farm out of the timber land.

18. *Gymnocladus dioica* (L.) K. Koch. (*G. canadensis* Lam., Kentucky coffee tree) - This is a rather common tree in the county. It is found only in the most densely wooded parts of creek bottoms, and then only as an occasional tree. Such a thing as a pure stand or a group of trees is unknown. The coffee tree grows to great heights, often more than a hundred feet (30.5 m). The straight, upright manner of growth, the large clusters of terminal white flowers, and huge beans make it a very beautiful ornamental where it can be induced to grow. The bean pods are about six inches (15.0 cm) in length, two inches (5.0 cm) in width, and three-fourths of an inch (2.0) in thickness. The beans are very hard and do not germinate readily. It is only after long-continued overflows of the river bottoms that young coffee trees were noted. It must be that they seeds require soaking before they will sprout. The only example of this tree growing on the uplands was observed in the white oak flats just east of Carlinville.

19. *Robinia pseudoacacia* L. (common locust) - It is doubtful that this tree is a native to the county. Almost every farmstead has a grove or lanes of this tree which were set out many years ago. There are many examples that might be considered native, but all evidence seems to point to the fact that the trees were noticed by the early settlers from the Southeast and that the trees which are seemingly native are only escapes. The waste land around almost any town in the county is very largely grown up to this species, as there is a growing demand for locust for fence and telephone posts. This species may prove a paying wood crop on the pure clay lands through the center of the county. Their longevity and masses of white flowers in June make them very beautiful ornamental trees.

FAGACEAE

20. *Quercus alba* L. (white oak) - The white oak is one of the most beautiful trees in Macoupin County. It is found in all situations, but it reaches its best development on the open hill upland.

There are still some of the original white oaks left that show the enormous size of the individuals that made up the original forest. In the hills between Carlinville and Macoupin Creek on the east are many white oaks that are four feet (1.2 m) in diameter and reach a height of 75 feet (22.9 m). The wood of the white oak has been used for dimension timber and quarter-sawn oak. The demand has been so great that there are, but few marketable trees left excepting those reserved by farmers for fence posts. This tree has an ornamental value far above any of its close relatives. It leaves are persistent and hold their color from the time of the first frost until the growing buds of the next spring force the old leaves from the trees.

21. *Quercus bicolor* Willd. (swamp white oak) - This tree grows only in the creek bottoms, and is confused by the lumbermen with the common white oak. In fact, there is no distinction made in the lumber of these two trees. Young trees of this species are sought out by the basket makers and the very best baskets on the market come from the wood of this oak. The larger specimens of this tree in the county are at the north end of Rob-Roy Lake southeast of Carlinville.

22. *Quercus imbricaria* Michx. (shingle oak, laurel oak) - The shingle oak is fairly common on the rich uplands. It does not occur in pure stands but is generally associated with bur oak and white oak. The tree has a quick growth and has a central trunk. There are a few good specimens of this tree still standing, because of its quick growth and straight trunk the wood splits very easily. The pioneers and farmers have taken advantage of this fact and this tree supplies them with shingles, shakes, clapboards, and rails. And even at the present date as fast as a tree reaches a diameter of eighteen inches (45.7 cm) to two feet (61.0 cm), it is cut down and made into clapboards.

23. *Quercus macrocarpa* Michx. (bur oak) - The bur oak has likewise followed the road of the white and the post oaks. Its wood has been used largely for flooring and quarter-sawn lumber. Some very large trees can be found along the valleys of the larger creeks where they have escaped cutting because their location was such that it would be very hard to remove the logs. The acorns of this tree are highly sought after by cattle and hogs. It is very probable that it would pay anyone to plant these oaks for their acorns alone. They are very prolific yielders and commence to bear fruit during their tenth year. Mr. Theo Brown, living south of Carlinville, has several acres (ha) of land planted in bur oaks which are now fifteen years of age and are yielding six bushels of acorns per tree. He says that the land is worth more for the acorns produced than it would be if planted in corn.

24. *Quercus marilandica* Muench. (*Q. nigra* Marsh., black-jack, scrub oak) - This oak is a rare tree in the county. It is found only along the edges of prairie slews (sloughs) and the gravelly points of hills. It is never found in pure stands, but always as a single tree that presents an appearance of being more dead than alive. The lower branches are always dead and do not break from the tree but hang downward, forming a brush pile around the base of the trunk. This protection makes the blackjack oak a city of refuge for birds, squirrels, mice and rabbits. The wood is of so little value that the farmer seldom cuts these trees unless it is in his way. These lone oaks mark the boundary of the old forest line and can be found all along the edge of the now obliterated forest. The majority of oaks come readily from the seed, but for some unknown reason this oak seldom produces acorns, and the acorns will not grow.

25. *Quercus michauxii* Nutt. (*Q. prinus* L., swamp chestnut oak) - The largest trees of the original forest that covered Macoupin County belonged to this species. There still stand in the county a number of specimens that measure more than eight to ten feet (2.4 to 3.0 m) in diameter, which were left by the lumbermen because of defects. The stumps of chestnut oak cut in 1853 and 1859 are still very common. The wood does not disintegrate quickly, and the stumps are often seen almost covered by the growth of other varieties of trees. When the Chicago and Alton Railroad (originally the Alton and Sangamon Railroad) was built, a contract was made with a sawmill firm in this county for 500,000 ties sawed from chestnut oak. It was this contract, and other similar ones by the same railroad, that removed the original oak forest from the county.

26. *Quercus muhlenbergii* Engelm. (yellow oak chestnut) - The yellow or chestnut oak is found in mixed stands of trees throughout the county. Because of its straight growth, it is a favorite with the farmer for fence posts and fence rails. In the days when tanning was one of the industries of Carlinville, the bark of this oak was used in the process of the tanning of leather. Some of the finest specimens of this tree can be found on the Birk land north and east of Carlinville. Between 1885 and 1890 a hardwood lumber firm from St. Louis purchased many of the best trees of this species in the vicinity of Carlinville. The logs were shipped to St. Louis and were sawed up into flooring. In the early day many of these trees went for railroad ties and railroad timbers.

27. *Quercus palustris* Muench. (swamp oak, Spanish oak or pin oak) - This oak is not well known. Where it does grow, it occurs in pure stands. It is found only in the creek valleys, especially in swampy places where it occurs in pure stands. It is a rapidly growing tree, with a central trunk and often attains a height of a hundred feet (30.5 m) or more. There was formerly considerable acreage of this tree in the county but from 1885 to 1895 almost every group of this species was cut out for pilings by the railroad companies. These companies hired men to seek good stands of this oak and wherever it was found, the timber was bought and removed. The removal of these forests from swampy localities gave the owners the opportunity to drain the land and the former swampy Spanish oak forests are now bottomland farms.

28. *Quercus rubra* L. (red oak) - This oak is found only on the uplands and in some places on the rich side hills leading down to the deeper creek valleys. This oak grows to a medium-sized tree and in late years has been cut for dimension timber especially for the making of floors for bridges and culverts. In the western part of the county this oak surpasses all others and pure stands of red oak are very common in Western Mound, Barr, and Scottville townships.

29. *Quercus stellata* Wagh. (post oak) - This oak is common on all the upland sections of the county. It is seldom encountered in the creek valleys. Like the white oak, the lumbermen have removed the entire first crop of this tree. Only a few specimens of the original forest remain, and these are generally defective trees. The farmer is also responsible for the destruction of this oak as he looks upon it as the source of his best fence posts. There are numerous examples of this tree five feet (1.5 m) in diameter in many of the wooded pastures in the county.

30. *Quercus velutina* Lam. (*Q. coccinea* Wang. Var. *tinctoria* Gray, black oak, Spanish oak) - The black oak is a very common tree in the black uplands near the edges of the prairies. It is also found in the creek bottoms where it makes a much larger tree.

The acorns make up a large part of the "mast" so frequently spoken of as hog feed. The wood from this tree is very heavy and easily split, making excellent stove wood. Most of the larger trees have been removed to satisfy the demands of the kitchen.

HIPPOCASTANACEAE

31. *Aesculus glabra* Willd. (Ohio buckeye) - The buckeye is a very common tree in all the creek bottoms in the county. One or two specimens are known growing on the uplands. This tree grows to a very large size in thick growths of timber, but singly it forms a round-topped tree seldom reaching more than fifty feet (15.2 m) in height. The wood is soft and is now used for no purpose but firewood. In the pioneer days, however the wood was used extensively for ox yokes, as it is light and strong. The buckeye, as the seed of this tree is called, are said to be poisonous and many farmers have cut out these trees from their pastures because of the fear that their cattle might be poisoned. This species will maintain itself through long periods of time, as it sprouts readily from the cut-off stem and the seed will germinate in most any kind of soil.

JUGLANDACEAE

32. *Carya cordiformis* (Wangenh.) K. Koch (*Carya amara* Nutt., bitternut or pignut) - The bitternut or pignut is common in both creek bottoms and uplands. The fruits vary in size, thickness of hull, and bitterness. Some of these trees produce fruits that are edible while the majority produce fruits that are extremely bitter. The trees vary also in their habit of growth, some of them being tall with a central stalk and others much branched. It is very probable that the trees included within this species could be divided into three or four legitimate species, as this tree produces fruit within shell, large amounts of meat, and in great quantities. It offers a better prospect for improvement by the horticulturists than the other species of hickory.

33. *Carya illinoensis* (Wangenh.) K. Koch. (*C. olivaeformis* Nutt., pecan) - The pecan is found only in the western edge of the county. There are a number of large trees in Chesterfield Township near the bridge of the Chicago, Peoria, and St. Louis Railway. From that point down the creek to the edge of the county there are many groves of this tree.

34. *Carya laciniosa* L. (*C. sulcata* Nutt., kingnut, big shellbark) - The big shellbark or king nut is restricted to the lower creek bottoms. It is quite common along Honey, Macoupin, and Apple Creeks. These trees grow to an immense size and produce large amounts of fruit. The nuts which range from two to three inches in length (5.0 to 7.5 cm) are of little use as they contain so little meat, and the shell is so very hard. The wood of this tree is not as good as that of *Carya ovata* for wagon building, but nevertheless most of these trees have been cut out for wagon timber. Differing from *Carya ovata*, this species does not easily replace itself while *Carya ovata* will sprout out from the stump and comes readily from seed. In fact, where a mixed stand of hickory and oak are removed, the forest is replaced by a pure stand of hickory. This condition can be seen in many places along the Macoupin Creek hills east of Carlinville.

35. *Carya ovata* L. (*C. alba* Nutt., shellbark hickory) - The shellbark hickory is the best known and the most popular tree in this county. It is a tree of the uplands where it grows to a height of from 50 to 100 feet (15.2 to 30.5 m) and a diameter of three or

four feet (0.9 to 1.2 m). Just after the Civil War, when Carlinville was the center of manufacture for wagons and buggies, a large amount of this hickory was cut to furnish material for wagon building. Much of the second growth hickory has been cut for hop-poles. In many parts of the county the cutting of these poles is quite a paying occupation during the winter months. The nuts from this tree are of fine flavor and have a fairly thin shell. They are in great demand by the produce men who ship them to the St. Louis market. The production per tree is not large but there are many individual trees that it would pay to propagate from as their fruits are thin-shelled and large.

36. *Carya tomentosa* Nutt. (mockernut) - The mockernut, or white-heart hickory, also called sour land hickory, is very common in the oak flats in all sections of the county. It is never found in creek bottoms or in rich land. It presents a ragged appearance and seldom reaches a height greater than thirty feet (9.1 m). The fruits are quite large in size but are almost solid wood. The timber of this tree generally goes to the cord-wood pile. It was formerly a member of the sour land association of trees that has been removed from much of the flat land of this county.

37. *Juglans cinerea* L. (white walnut, butternut) - The butternut is not a common tree. It is restricted to the creek bottoms and even there is found only in isolated localities. The tree seldom grows to a height of more than fifty feet (15.2 m) and a diameter of three feet (0.9 m). It is not much sought after by the lumber men, and one is at a loss to understand just why it has disappeared from our flora. The nuts are eagerly sought for and bring about twice the price of the black walnut. As nearly as can be found, Macoupin Creek is the north boundary of the occurrence of the butternut in this part of the state.

38. *Juglans nigra* L. (black walnut) - The black walnut was at one time one of the most plentiful and largest of the forest trees of this county. Because of the beauty and usefulness of its wood, it has been sought out until hardly a tree of any dimensions is left. It is very evenly scattered over the county in the upland and creek bottoms. A number of very large trees which are hollow or otherwise disfigured have been left by the lumbermen. There is one tree on the Taggart Land east of Carlinville that is six feet (1.8 m.) in diameter and is broken off about fifty feet (15.2 m) from the ground, apparently during a storm in 1857. Since the black walnut has been reduced in numbers there has developed quite a market for its fruits. The nuts find ready sale on the produce market and to the nurseryman.

LAURACEAE

39. *Sassafras albidum* (Nutt.) Nees (*S. officinale* Ness., sassafras) - Sassafras is very common throughout the county as a bush. As a tree, it is more rare. It is a species growing on the uplands and generally along the edges of the prairies. It is very persistent and when once cut off it sends up a large number of sprouts which seem impossible to grub out. A patch of sassafras sprouts will persist twenty-five or more years of intense cultivation. As the sassafras trees grow in locations that have mostly been cut off, the large trees are a thing of the past. There are, however, a number of immense sassafras trees within the county. Near Miles Station there is standing a specimen of this species which is five feet (1.5 m) in diameter and must be nearly 150 feet (45.7 m) high. This tree is all that remains of the former forest, the trees having all been cut away and this noble specimen left. The only reason that it exists is that it is the corner tree of the township. The

white flowers appear in April and are followed by the red berries with their spicy-like odor in July and September.

MAGNOLIACEAE

40. *Liriodendron tulipifera* L. (yellow poplar, Indiana poplar, tulip tree) - This beautiful tree occurs very frequently as a cultivated tree in the yards at Carlinville. There are now no native tulip trees near this city as the Macoupin Creek seems to have been the northern and western limit of this tree. There were numerous large tulip trees in the Macoupin and Honey Creek valleys prior to the general clearing of this section by the sawmill men between 1857 and 1865. Poplar, as the wood of the tulip tree was called, was in great demand by the wagon makers, the cabinet makers, and the pattern makers. There exists in Carlinville numerous pieces of furniture made from this wood, the origin of which was the wooded side of Brushy Mound. All that remains of this once common tree is at present a few molding stumps and scattering trees that remain as ornamentals around the farmsteads. In southwest Macoupin County, however, there are still standing a number of tulip trees in their native groves. In 1898 the writer visited one of these groves located five miles (8.05 km) southwest of Plainview. There were about six acres (2.4 ha) of tulip at this place. The tree is a quick growing symmetrical one and is covered in May with yellow tulip-like flowers that rival the garden tulip.

MORACEAE

41. *Maclura pomifera* L. (Osage orange, hedge) - This tree is not a native of the county. Popular tradition says the first hedge plants were introduced in 1857. The "hedge fever" kept up until about 1875 when the farmers realized that a fence could never be made from Osage orange. In many places hedges that were not cared for grew into trees and the seed from these trees had been scattered by birds and water, so that now Osage orange is a rather common tree in the creek valleys. Osage orange is now common in the creek valleys. Because of the recent introduction of this species, no large trees are known. Some few ten or twelve inches (25.4 to 30.5 cm) in diameter can be seen in the neighborhood of old farmsteads.

42. *Morus rubra* L. (red mulberry) - The red mulberry, like the persimmon, is not a widely disappearing tree in this county. The small boy can be depended upon to give full information as to the location of every tree as during June and July these trees receive his daily visits in quest of fruit. It is to be supposed that the mulberry has been very largely reduced in numbers as the majority of the larger ones have been cut for fence posts. This tree grows more often on the upland than in the river bottoms, although some fine examples can be seen near Dickinson Bridge east of Carlinville.

OLEACEAE

43. *Fraxinus americana* L. (white ash) - The white ash is a rare tree in Macoupin County. Such a thing as a clump or a pure stand of this species does not exist. It is generally found in oak woods on the tops of the highest ridges. There is a tradition of the county that at one time there were several large ash trees which were cut out between 1850 and 1870 for use for

wagon material. There is, however, no direct evidence of this fact.

PLATANACEAE

44. *Platanus occidentalis* L. (sycamore) - The sycamore is very common along the larger creeks of the county but no upland specimens growing naturally are known along the creeks. It reaches an immense size, but as it increases in size, the majority of these immense trees become hollow from top to bottom. There is one tree on the Walker Land east of Carlinville that is sixty feet (16.7 m) in height but has a hollow five feet (1.5 m) in diameter at the top where the tree is broken off. For many years this tree has been the home of chimney swallows. Their nests are placed on a side of the cavity generally at a height of about twelve feet (3.6 m) from the ground. This tree has been utilized for a shade tree to such an extent that is very well scattered all over the county on the upland.

RHAMNACEAE

45. *Rhamnus lanceolata* Pursh. (buckthorn) - This large bush reaches the dimensions of a tree along the bluffs of Macoupin Creek. Specimens six inches (15.2 cm) in diameter and thirty feet (9.1 m) high are not uncommon. It is a little-known plant because of its inconspicuous yellow flowers which occur in May and June. It has nothing to mark it out as distinct from many of the varieties of bushes occurring as under-brush in the river bottoms. Very few people have a name for it. During the early fall it is covered with very small, red berries which are attractive to migrating birds. The finest specimen of this plant seen is in Brushy Mound Township on Honey Creek, near the wagon bridge over that stream. If one does not look closely, this tree is easily mistaken for a large cherry tree.

ROSEACEAE

46. *Amelanchier arborea* (Michx. F.) Fern. (*A. canadensis* Torr. & Gray, serviceberry) - missing from manuscript.

47. *Crataegus calpodendron* (Ehrh.) Medic. R (wooly haw) - missing from manuscript.

48. *Crataegus crus-galli* L. (cock-spur thorn) - missing from manuscript.

49. *Crataegus mollis* (Torr. & Gray) Scheele (*C. coccinea* L. var. *mollis* Torr. & Gray, red haw) - missing from manuscript.

50. *Malus coronaria* (L.) Mill. (wild sweet crab apple) - missing from manuscript.

51. *Prunus americana* Marsh. (wild plum) - missing from manuscript.

52. *Prunus serotina* Ehrh. (wild cherry) - missing from manuscript.

RUTACEAE

53. *Ptelea trifoliata* L. (wafer ash) - The only reason that this plant is included in this list is the existence of several specimens growing along Honey Creek which have a diameter of more than four inches (10.1 cm) and a height of better than 20 feet (6.1 m). The ordinary height of this shrub is less than six feet (1.8 m). It occurs very commonly in damp locations along the river (creek) bottoms and is found to some extent in a dwarfed form on the

uplands. The blooms which are not showy appear in April and May and the seed pods which are extremely ornamental are developed by the first of August. As these are persistent and do not fall off until the next spring, this bush should be used as an ornamental in all landscape work.

54. *Zanthoxylum americanum* Mill. (prickly ash) - The prickly ash is a rather common shrub or tree on the uplands of the county. It is generally found in a mixed stand of oaks and hickories. The largest specimens are seldom more than twenty feet (6.1 m) high and have a diameter of less than four inches (10.1 cm). This tree has no commercial value but will make an ornamental tree that is very attractive. It is covered with small yellow flowers during April and May, and these are followed by bright red berries during the summer. During the wintertime these berries open and the shiny black seeds add color to the plant the year round. This tree comes as near being a bright leafed evergreen as can be found in the county. It does not occur in any great quantities and is nowhere found in pure stands. From the peculiarity of its occurrence, one is led to suspect that the seeds are scattered by birds.

SALICACEAE

55. *Populus deltoides* Marsh. (*P. monilifera* Ait., cottonwood) - The cottonwood is not a forest forming tree. It occurs as individual specimens all over the county. It is just as much at home at the edge of the prairie pond, miles from other timber, as it is along the edges of our largest creeks. It is rather hard to say just where it reaches its best development. Along Macoupin Creek there many specimens five and six feet through (1.5 to 1.8 m) and 150 feet high (45.7 m), and yet specimens the same size can be found in the prairie townships. In swampy places along the creeks, it is not uncommon to find a pure stand of cottonwoods of a few acres (ha) in extent. Of these groups, one or two overtop the rest which die from the common fungus disease which attacks cottonwoods and willows. The cottonwood is the tree beloved by wild animals and birds. Its large trunk and great branches are generally hollow or partially so. These cavities become the home of many species of small animals and birds. Owls and buzzards find in these tree cavities that make them permanent homes because of the height to which the trees grow and the inaccessible treetop of the cottonwood becomes the nesting place of the larger hawks.

56. *Populus tremuloides* Michx. (aspen) - The trembling aspen is a very rare tree in Macoupin County. While it may occur elsewhere, the only grove of this species known is about three miles (4.8 km) north and east of Carlinville where a strip of these trees about a half mile (0.80 km) by a hundred yards (91.4 m) in dimension lie between the oak forest and the prairie. This peculiar island of trees has been long known and many young trees have been dug from it to be used as ornamentals in Carlinville. None of the specimens in this island are more than six inches (15.2 cm) in diameter nor have a height of over thirty feet (9.1 m). This leads one to believe that this island came from some parent tree that has disappeared at a very recent time.

57. *Salix amygdaloides* Anderss. (peach-leaved willow) - This is the willow tree that is found along the edges of the prairie sloughs and artificial ponds. The tree has a central trunk with a smooth bark and grows to a height of sixty to seventy feet (18.2 to 21.3 m). It is generally found in combination with cottonwood

and has been used to a considerable extent for planting purposes. There are a number of these trees used for ornamentals in the City of Carlinville.

58. *Salix nigra* Marsh. (black willow) - This is the common willow tree found along the creek bottoms throughout the county. It has a rough bark and a branched trunk. It seldom reaches a height of over 40 feet (12.1 m) and a diameter of two to three feet (0.6 to 0.9 m). The wood is very soft and the tree rots easily causing the older specimens to break off near the ground. Because of its growing along the creek banks, the tree seldom reaches its full growth as it is either washes away or is cut away by the farmers in removing the brush from the creek banks. The wood has no other use than that of stove wood.

SIMAROUBACEAE

59. *Ailanthus altissima* (Mill.) Swingle. (*A. glandulosus* Desf., tree-of-heaven) - This tree is not a native to the county and was introduced as an ornamental shade tree a great many years ago. Some of these original trees have reached a diameter of three feet (0.9 m) or more. One of them, located on East Main Street in Carlinville, is forty inches (101.6 cm) in diameter and has a height of 70 feet (21.3 m). This tree is able to maintain itself and seedling trees are found over much of the county as the seed is wind borne as well as carried by water. The tree has a bad habit of sending out root sprouts. This, in connection with the odor which comes from bruised leaves, has not made it a favorite as a shade tree. It is now cut out as a weed in the streets and alleys of the cities where it was once cultivated. Where one wishes a highly ornamental tree and is ready to care for the land where it is grown, one can find no better than this tree. It is of quick growth; has large showy pinnate leaves, and the clusters of flowers and seeds make an ornamental that cannot be overlooked.

STAPHYLEACEAE

60. *Staphylea trifolia* L. (bladdernut) - This plant is neither a tree nor a bush. It is about half between a tall bush and a vine. The straight stems often reach a diameter of three inches (7.6 cm) and a length of 30 to 40 feet (9.1 to 12.1 m). It grows in thick stands of timber and its trunk must be supported by the surrounding trees. Where it grows by itself, it has more the character of a bush and seldom reaches a height of over five or six feet (1.5 to 1.8 m). While the plant is young it has straight, light green stems striped with white, and its pale green leaves make it a beautiful ornamental. Clusters of cream-colored flowers which appear in April and May are very beautiful and are attractive to butterflies. These are followed in mid-summer by three cornered wafer-like pods which are persistent until frost. These pods are three-celled with one hard seed to the cell. The seeds break loose in the cell and when the pod is moved, they make a rattling sound. The school children call these pods "cat bells". A tradition says that the slender new shoots of this tree were used by the Indians for arrow shafts. One may believe this as the wood is straight and tough.

TILIACEAE

61. *Tilia americana* L. (basswood) - This tree is a common resident of the county and blooms during the latter part of May and early part of June. It is found growing in the uplands and creek

bottoms. It is very common along Macoupin Creek in Shaw's Point Township and in Brushy Mound along Honey Creek. As the timber from this tree does not occur in quantities and the wood is soft, these trees were left by the men that cut off the original forest. Some of our largest trees are of this species. During the fifties, when the English settlement was made in the southwestern part of the county, these people set out many groves and avenues of this tree which was so abundant along the creeks, and which reminded them of the linden of old England. These trees under the care of the farmers have produced some magnificent specimens. The finest example of this early plant can be seen at the home of H. N. Challacombe in Chesterfield Township. This tree is of quick growth but of long life, and as it is a native of the county, should receive the attention of anyone wishing to plant shade trees.

ULMACEAE

62. *Celtis occidentalis* L. (hackberry) - The hackberry or sugarberry is not very common in the county. It is found along the larger creek bottoms and occasionally on the uplands but does not occur in numbers sufficient to be a familiar tree. It grows to a large size and is very prolific in the production of berries. As these fruits are persistent, this tree becomes the feeding grounds for many of the migrating birds during autumn. There is a marked difference between the trees growing on the upland and those in the river (creek) valley. The river valley (creek) specimens have a fairly smooth bark, while those growing on the upland have a very heavily ridged bark, showing ridges sometimes two inches (5.0 cm) in depth.

63. *Ulmus americana* L (white elm) - The white elm reaches its perfection along the Macoupin Creek Bottoms. Specimens six feet (1.8 m) in diameter and 150 feet (45.7 m) are not uncommon. There is one tree near the bridge across Macoupin Creek between Macoupin Station and Plainview that measures six feet (1.8 m) in diameter eight feet (2.4 m) from the ground. This tree is found throughout the county and is on the increase. It is one of the first trees to put in its appearance along fence rows or abandoned fields. Its rapid manner of growth makes it very popular as a tree for planting. It is very probable that this tree is the most common of all of the trees occurring within this county.

64. *Ulmus rubra* L. (*U. fulva* Michx., slippery elm) - The slippery elm is one of the earliest blooming elms which occur in this county. It is very easily distinguished from the American elm by its short, heavy-set trunk. The diameter of the trunk is often four or five feet (1.2 to 1.5 m), but the height of the tree is rarely more than 50 to 60 feet (15.2 to 18.2 m). Because of the remarkable toughness of the wood, this elm has been spared in many cases. Many trees are now standing because of the inability of the farmer to work up the trunk and branches into stove wood. The tree is well known because of its glutinous bark. In the vicinity of most towns there are certain of these trees which have become famous as the producers of slippery elm bark which is the delight of the small boy and of some value to the medical profession. The slippery elm is one of the most common trees in the county. The larger specimens are found in the creek bottoms, but the greatest number are found in the open uplands.

CHANGES IN STRUCTURE AND COMPOSITION OF WOODY STRATA AT KARCHER'S POST OAK WOODS NATURE PRESERVE AFTER TWENTY YEARS

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ABSTRACT: The woody vegetation of 16-ha Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois, was examined in September 2020 in a 100 m × 300 m and an adjacent 50 × 200 m vegetation sampling area in mature, second-growth timber. Previous studies of this portion of the site had been conducted in 2000 and 2009. In 2000, prior to any recent management, overall density of trees ≥10.0 cm diameter at breast height (dbh) was 378.1 trees/ha. *Carya* spp. (hickory) density was 255.0 trees/ha and *Quercus stellata* Wagh. (post oak) density was 78.3 trees/ha. Hickory density in the 10.0–19.9 cm diameter class was 169.4 trees/ha (66.4% of total hickory stems). Prescribed burning was conducted on 28 March 2000, 22 March 2001, 22 October 2003, 10 March 2009, and 22 February 2020. Mechanical thinning of subcanopy hickory was conducted in 2014. In 2020, overall tree density was 264.2 trees/ha, about 70% of baseline. Hickory density was reduced to 197.5 trees/ha while the hickory density in the 10.0–19.9 diameter class was reduced to 85.6 trees/ha. Natural mortality reduced post oak density to 41.7 trees/ha and its presence in all classes from seedling through 29.9 cm dbh was minimal. Other oak species were present only as minor components of the forest. These data suggest the forest is shifting from post oak to hickory with oak species as lesser associates.

INTRODUCTION

Quercus stellata Wagh. (post oak) forests were once prevalent on the uplands of south-central Illinois (Telford 1926). The post oak forests occurred primarily on Illinoian-age soils that were heavy, acidic, low in organic matter and had clay subsoil. These soils frequently contain an argillic horizon with low permeability that retards the downward percolation of water, creating a perched water table directly above the claypans at a depth that can range from several centimeters to as much as two meters, often resulting in excessive drying and slow forest growth rates. Telford (1926) reported that it could take post oaks 100 years to reach 35 cm in diameter and up to 300 years for them to reach 76 cm. The tree density within the post oak forests could be quite high with Telford (1926) reporting an average of 575 trees/ha in the forests he sampled; however, the species diversity was often low, with post oak accounting for 73.8% of the trees sampled and

scrub oak, *Carya* sp. (hickory), *Quercus velutina* Lam. (black oak) and *Q. imbricaria* Michx. (shingle oak) as lesser associates. *Quercus alba* L. (white oak) was often associated with post oak on better drained sites with undulating topography and a deeper argillic horizon while *Q. marilandica* Münchh. (blackjack oak) occurs with post oak in flatwoods with shallow A horizon (IDNR 2017).

The Illinois Natural Areas Inventory (INAI) recognizes only 22 sites totaling less than 300 ha (762 acres) as Grade A or B post oak woodland or southern flatwoods with relatively little disturbance (IDNR 2022). Of those sites, 19 totaling 256 ha are southern flatwoods. Karcher's Post Woods Nature Preserve and a privately-owned site in Saline County are the only post oak dominated sites recognized by the INAI as forest or woodland. These sites are distinguished from flatwoods by their undulating topography, greater depth of the A horizon to the argillic horizon, and canopy cover that ranges from 60–80%.

Study Site Description

Karcher's Post Oak Woods Nature Preserve is located in Dahlgren Township (T4S R5E; 38.207355N, –88.618197W) about eight miles northwest of McLeansboro, Hamilton County, Illinois in the Mount Vernon Hill Country Section of the Southern Till Plain Natural Division of Illinois (Schwegman 1973). This natural division is characterized by nearly flat to gently rolling topography that was mostly timbered before European settlement, though extensive prairie inclusions were present. Four prairies were located within 4 km of

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the preserve with the largest, about 5 km² in size, described as a wet prairie by the General Land Office surveyors (GLO field Notes, Vol. 76, Illinois State Archives). The GLO notes generally described the area in the immediate vicinity of the preserve as “land flat and wet” and “land thinly timbered with post oak, otherwise prairie with scattered groves of timber.”

Soils of the study site are Bluford silt loam and Belknap silt loam (Web Soil Survey 2020). Bluford silt loam is a deep, nearly level, somewhat poorly drained soil that formed in loess over mixed loess and drift. The seasonal water table is 15 to 95 cm below the surface. Belknap silt loam is a somewhat poorly drained, occasionally flooded soil that developed on silty alluvium. It is associated with the intermittent stream that meanders through the east-central portion of the preserve. The seasonal water table is 30 to 110 cm below the soil surface. The climate is characterized by hot, humid summers and cold winters. Average annual precipitation is 105 cm. Annual frost-free growing days average 184 days.

The preserve has an undulating topography that slopes gently from southwest to northeast with elevation ranging from 134 m to 141 m above sea level. The far east-central portion of the preserve contains 3 ha of old field and 2 ha of young forest with the trees generally less than 20 cm diameter at breast height (dbh). The western portion of the nature preserve contains 11 ha of mature old- or second-growth timber. An intermittent stream flows easterly near the southern boundary of the preserve, turns northward at the west edge of the young timber, then east along the north edge of that timber and the old field. The 16-ha nature preserve was purchased by the Illinois Audubon Society in 1998 and dedicated as an Illinois Nature Preserve in November 1999.

Previous Work

The preserve is included in a 43-ha stand of timber that was purchased by Augustus Karcher in 1875. Within the stand and adjacent to the nature preserve is an 8-ha stand of timber that was harvested in 1996. Prior to harvest, the structure and composition of the stand was very similar to the nature preserve (McClain and Ebinger 2003). Cross sections from the stumps of harvested trees were analyzed to determine the age of the trees and the fire history of the stand (McClain et al. 2010). That study indicated the stand experienced three distinct periods: (1) a fire era from 1776-1850 when fires were of landscape scale with a fire return interval of 1.97 years; (2) a fire-free period from 1851-1884 which coincided with a period of rapid settlement of Hamilton County and (3) a second fire period from 1885-1996 with generally lower intensity fires confined to woodlots and a fire return interval of 1.44 years. Fires occurred nearly annually from 1904 and 1972. The exception being a fire-free period from 1957 through 1963. Three post oak cohorts were identified: 211-224 years old, 137-151 years old, and 104-115 years old. Post oak recruitment declined considerably after the last cohort and was replaced in more recent times

by hickory, *Prunus serotina* Ehrh. (wild black cherry), black oak, and *Sassafras albidum* (Nutt.) Nees (sassafras).

Prior to management of the nature preserve, a thick layer of leaf litter covered the forest floor, herbaceous vegetation was sparse, few oak seedlings were present, and hickories dominated the subcanopy strata. Prescribed burning to reduce the leaf litter was conducted on 28 March 2000, 22 March 2001, and 22 October 2003. Subsequent burns were conducted on 10 March 2009 (Edgin 2009) and 22 February 2020. The prescribed fire in March 2009 was of moderately high intensity while the February 2020 burn was of very low intensity with a patchy distribution. Prescribed burning was attempted in the spring of 2015 and 2017, but ground conditions were not favorable so the burns were cancelled. Thinning to reduce subcanopy hickory density and shading was conducted in 2014.

There were two primary goals with this study. The first was to examine the seedling, sapling and overstory strata of the old- or mature second-growth forest to verify the perceived diminishing status of post oak within various strata of the stand. A second goal was to determine whether the removal of subcanopy hickory and the re-introduction of fire promote post oak recruitment.

METHODS

Overstory Sampling

A 100 m × 300 m vegetation sampling area was established in a north-south orientation in the mature second-growth timber in the summer of 1999. In 2000, an addition 50 × 200 m area was added immediately adjacent to the east side of the 100 m × 300 m area. To facilitate the location of the area for future studies, the corners of the study area and the interior grid were marked with sections of galvanized sign post driven into the ground. The exterior boundaries were marked at 25 m intervals and the interior grid was marked at 50 m intervals. Sampling was conducted in by dividing the 4.0 ha area into 64 25 m × 25 m quadrats using string and all living and dead-standing trees ≥ 10.0 cm dbh were identified and their diameters recorded. From these data, tree density (trees/ha), basal area (m²/ha), relative density, relative dominance, importance value (IV) and average diameter were determined for each taxon. Determination of IV follows the procedure used by McIntosh (1957) and is the sum of relative density and relative dominance with total possible score of 200. Dead-standing tree density (trees/ha), basal area (m²/ha), and average diameter were determined for each taxon.

Woody Understory

Within the 4.0 ha study area, the woody understory composition including large saplings (≥2.5 cm and <10.0 cm dbh), small saplings (≥50 tall cm and <2.5 cm dbh) and seedlings (<50 cm tall) was sampled using stratified random plots located at 20-m intervals along five north-south transect lines which corresponded to the 25, 50, 75, 100 and 125 m locations along the north and south

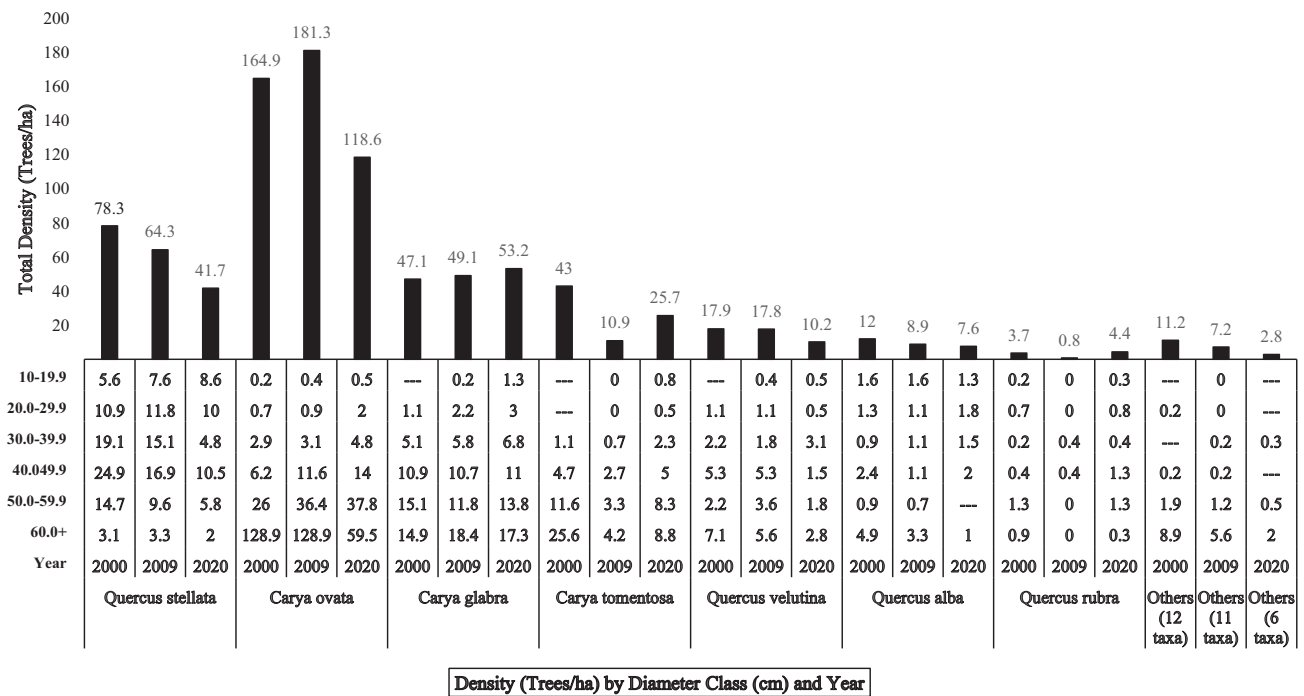


Figure 1. Density (stems/ha) by diameter class and total stem density for overstory species in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020. Diameter classes shown in 10-cm intervals (lower left margin).

exterior lines of the study area. A random numbers table was used to determine the distance (m) from the transect line to the center of each stratified plot. Odd-numbered plots were located on the right side of the transect line; even-numbered on the left. Large saplings were recorded in 0.01ha plots ($n = 60$). Small saplings were recorded in 0.001ha plots ($n = 60$). Seedlings were recorded in 0.0001 ha plots ($n = 300$) with four additional 0.0001 ha circular plots located 7 m from the center of each plot in each of the four cardinal compass directions. Density (stems/ha) was determined for each taxon in each understory category. Initial sampling was conducted in summer 2000. The sample was replicated in September 2009 and October 2020. Botanical nomenclature follows Mohlenbrock (2002).

RESULTS

Overstory

The 2000 overstory sample included 19 taxa with an overall tree density of 378.1 trees/ha and a total basal area of 23.24 m²/ha (Figures 1 and 2). Post oak and *Carya ovata* (Mill.) Koch (shagbark hickory) were co-dominants with IV's of 67.0 and 63.2, respectively (Table 1). *Carya glabra* (Mill.) Sweet (pignut hickory) was third with an IV of 25.6 followed by *C. tomentosa* (Poir.) Nutt. (mockernut hickory). Post oak was most prevalent in the 20.0-59.9 cm diameter classes while hickories were most abundant in the 10.0-29.9 cm diameter classes where they accounted for 82.9% of the trees. Black oak and white

oak were lesser associates having densities of 17.9 and 12.0 trees/ha, respectively. *Quercus rubra* L. (red oak) was a minor component with a density of only 3.7 trees/ha. Twelve additional taxa had a combined density of 11.2 trees/ha, total basal area of 0.311 m²/ha and were most prevalent in the 10.0-19.9 cm diameter class. Among those taxa, wild black cherry (3.1 trees/ha), *Ulmus americana* L. (American elm, 2.7 trees/ha), and sassafras (2.0 trees/ha) had a tree density ≥ 1.0 trees/ha. *Celtis occidentalis* L. (hackberry), *Cornus florida* L. (flowering dogwood), *Diospyros virginiana* L. (persimmon), *Fraxinus americana* L. (white ash), *Morus rubra* L. (red mulberry), *Quercus bicolor* (swamp white oak), shingle oak, blackjack oak, and *Ulmus rubra* Muhl. (slippery elm) were represented by only one or two trees.

The 2009 sample included 18 taxa with an overall density of 340.3 trees/ha a decline of 37.8 trees/ha from 2000 (Figure 1, Edgin 2009). Post oak density declined from 78.3 to 64.3 trees/ha with all the decrease occurring in the 20.0-49.9 cm diameter classes as post oak density shifted toward the larger diameter classes. Hickory remained most abundant in the 10.0-29.9 diameter classes though their overall density declined by 13.7 trees/ha. Black oak remained relatively unchanged in both density and distribution among diameter classes. White oak density declined slightly, but was evenly distributed among all diameter classes, though in very low numbers. Red oak was present only in the 30.0-49.9 diameter classes. Eleven additional taxa, each with only one or two trees, had a

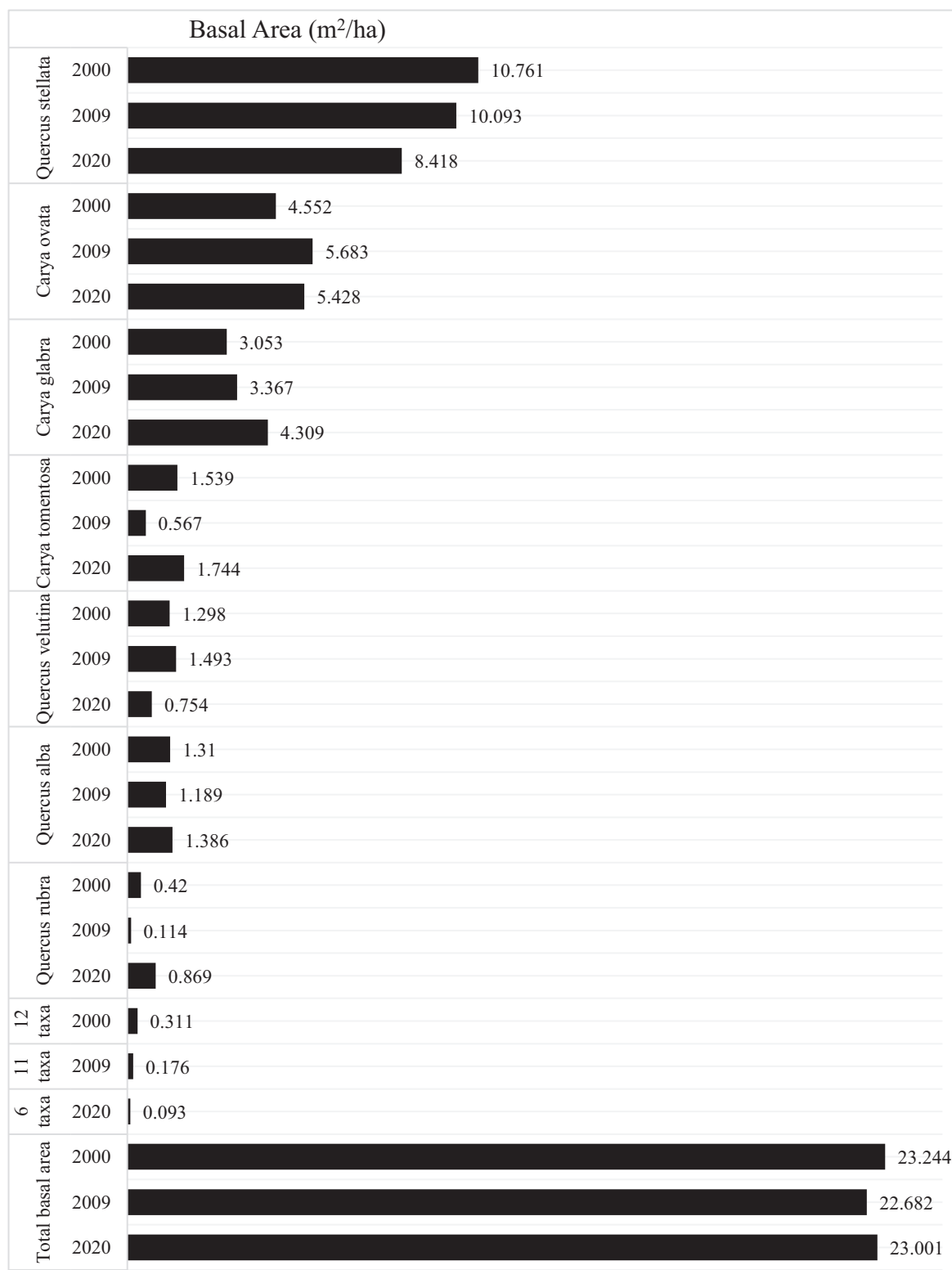


Figure 2. Basal area (m²/ha) for woody overstory species in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020.

Table 1: Relative density (Rel. Den.), relative dominance (Rel. Dom.), importance value (IV) and mean diameter (cm) for woody overstory species in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020.

Species	Year	Rel. Den.	Rel. Dom.	I.V.	Mean Diam. (cm)
<i>Quercus stellata</i>	2000	20.7	46.3	67.0	39.9
	2009	18.9	44.6	63.5	42.4
	2020	15.8	36.6	52.4	45.2
<i>Carya ovata</i>	2000	43.6	19.6	63.2	17.3
	2009	53.2	25.2	78.4	18.3
	2020	44.8	23.6	68.5	22.2
<i>Carya glabra</i>	2000	12.4	13.2	25.6	26.6
	2009	14.4	14.8	29.2	26.7
	2020	20.1	18.7	38.8	28.9
<i>Carya tomentosa</i>	2000	11.3	6.6	17.9	19.6
	2009	3.2	2.5	5.7	24.0
	2020	9.7	7.6	17.3	27.2
<i>Quercus velutina</i>	2000	4.7	5.6	10.3	27.8
	2009	5.2	6.6	11.8	29.4
	2020	3.9	3.3	7.2	39.2
<i>Quercus alba</i>	2000	3.2	5.6	8.8	32.4
	2009	2.6	5.2	7.8	36.1
	2020	2.9	6	8.9	45.7
<i>Quercus rubra</i>	2000	1.0	1.8	2.8	33.7
	2009	0.2	0.5	0.7	40.3
	2020	1.7	3.8	5.5	31.2
Others (12 taxa)	2000	3.1	1.3	4.4	
Others (11 taxa)	2009	2.3	0.6	2.9	
Others (6 taxa)	2020	1.1	0.4	1.5	
Totals	2000	100	100	200	
	2009	100	100	200	
	2020	100	100	200	

combined density of 7.2 trees/ha, total basal area of 0.176 m²/ha and were most prevalent in the 10.0-19.9 cm diameter class.

The 2020 sample included only 13 taxa and overall tree density declined by 76.1 trees/ha from the previous sample to 264.2 trees/ha (Figure 1). Post oak density fell to 41.7 trees/ha with most of its decline occurring in the 20.0-49.9 cm diameter classes. Thinning conducted in the winter of 2014 reduced overall hickory density in 10.0-19.9 cm diameter class from 151.5 to 85.6 trees/ha. White oak had a slight decline but was still evenly distributed among diameter classes. Black oak density decreased from 17.8 to 10.2 trees/ha with most of the decline occurring in the 10.0-39.9 cm diameter classes. Red oak density increased between 2009 and 2020 with the increase occurring in the smaller diameter classes. Among the lesser associates, over the 20-year period, wild black cherry density decreased from 3.7 to 0.0 trees/ha and sassafras decreased from 2.0 to 1.3 trees/ha while red mulberry, hackberry, white ash and shingle oak remained unchanged with only one or two trees present in both samples. American elm, blackjack oak, flowering dogwood, persimmon,

slippery elm and swamp white oak, which were present as one or two trees in 2000 were not encountered in 2020 and one *Quercus palustris* Münchh. (pin oak) was present in 2020, but not 2000.

Dead-Standing Trees

Density among all dead-standing trees dropped over the course of 20 years from 27.7 trees/ha to 18.1 trees/ha (Table 2). However, post oak dead-standing tree density increased from 10.7 trees/ha to 13.3. Its total basal area increased from 1.2 to 2.3 m²/ha and the average diameter increased from 34.6 to 40.3. Among dead-standing trees, post oak was the only taxon to experience an increase in both density and average diameter. Density of dead-standing black oak, shagbark and pignut hickory all decreased while their average diameters increased.

Large Saplings

Twelve species with a total stem density of 414.2 stems/ha occurred in the 2000 sample (Table 3). Shagbark hickory (300.0 stems/ha) and mockernut hickory (40.6 stems/ha) accounted for 82.2% of the total. Black oak,

Table 2: Density (stems/ha), basal area (m²/ha), and mean diameter (cm) of the dead-standing trees in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020.

Species	Density (Trees/ha)			Basal Area (m ² /ha)			Mean Diameter (cm)		
	2000	2009	2020	2000	2009	2020	2000	2009	2020
<i>Quercus stellata</i>	10.7	7.1	13.3	1.179	0.794	2.023	34.6	35.4	40.3
<i>Quercus velutina</i>	5.6	4.4	1.3	0.143	0.410	0.130	17.4	31.2	32.5
<i>Carya ovata</i>	4.0	3.8	0.5	0.125	0.084	0.063	17.2	15.5	38.7
<i>Carya glabra</i>	2.4	1.8	0.8	0.123	0.131	0.089	16.3	30.5	38.7
<i>Sassafras albidum</i>	1.6	1.1	1.0	0.019	0.014	0.013	20.8	13.8	12.7
<i>Carya tomentosa</i>	1.1	0.7	0.3	0.091	0.018	0.003	29.1	22.6	11.3
<i>Prunus serotina</i>	0.9	2.0	0.3	0.018	0.061	0.004	16.0	18.3	15.1
<i>Quercus alba</i>	0.7	1.8	0.3	0.022	0.136	0.014	19.7	27.3	26.3
Others/unknown	0.7	1.8	0.3	0.012	0.031	0.009	—	15.0	21.1
Totals	27.7	24.5	18.1	1.732	1.679	2.348			

sassafras and American elm were the only other taxa with densities greater than 3.1 stems/ha. Thirteen taxa with a combined density of 268.0 stems/ha were encountered in 2009 with shagbark, pignut and mockernut hickory accounting for 69.4% of the total. Black oak (42.0 stems/ha) and American elm (10.0) accounted for an additional 19.4% while *Fraxinus lanceolata* Borkh. (green ash, 6.0), and slippery elm (6.0) were the only taxa with a density greater than 5.0 stems/ha. Large sapling density decreased further in 2020 to 70.2 stems/ha. Shagbark hickory (23.4

stems/ha) and red oak (10.0) accounted for 47.6% of the total. White ash (8.4), sassafras (6.7), black oak (5.0) and green ash (5.0) accounted for an additional 35.8% and were the only other taxa with densities ≥ 5.0 stems/ha. Post oak was not encountered in any of the samples and white oak decreased steadily from 3.1 to 1.7 trees/ha. Flowering dogwood density ranged from 3.1 to 4.0 stems/ha in each of the samples despite its disappearance from the overstory category.

Small Saplings

Table 3: Density (stems/ha) of large saplings ≥ 2.5 cm dbh and <10.0 cm dbh in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020.

Species	Density (stems/ha)		
	2000	2009	2020
<i>Quercus velutina</i>	26.6	42.0	5.0
<i>Sassafras albidum</i>	18.8	4.0	6.7
<i>Carya ovata</i>	300.0	120.0	23.4
<i>Fraxinus lanceolata</i>	—	6.0	5.0
<i>Carya glabra</i>	—	52.0	3.3
<i>Diospyros virginiana</i>	3.1	2.0	—
<i>Prunus serotina</i>	1.6	2.0	1.7
<i>Ulmus rubra</i>	1.6	6.0	—
<i>Quercus alba</i>	3.1	2.0	1.7
<i>Carya tomentosa</i>	40.6	14.0	1.7
<i>Morus rubra</i>	1.6	4.0	—
<i>Ulmus americana</i>	12.5	10.0	—
<i>Celtis occidentalis</i>	1.6	—	—
<i>Quercus rubra</i>	—	—	10.0
<i>Fraxinus americana</i>	—	—	8.4
<i>Cornus florida</i>	3.1	4.0	3.3
Totals	414.2	268.0	70.2

Fifteen taxa with a total of 2,359.4 stems/ha were present in the 2000 sample with green ash and shagbark hickory accounting for 60.3% of the total (Table 4). Black oak (390.6 stems/ha), sassafras (109.4) and white oak (109.4) accounted for an additional 25.8% of the total stems. American elm and persimmon were the only other taxa with densities greater than 50.0 stems/ha. *Cercis canadensis* L. (redbud) had a stem density of 31.3 stems/ha; however, all of those stems occurred in a single plot and it was not encountered in the seedling, large sapling nor overstory sample in any other year. Only seven taxa with a total stem density of 680.0 stems/ha occurred in the 2009 sample with the reduction possibly due to repeated fires in 2001, 2003 and 2009. Black oak (220.0 stems/ha), persimmon (160.0) and sassafras (140.0) accounted for 76.5% of the total. Green ash (80.0) was the only other taxon with a stem density greater than 50.0 stems/ha. Species richness and stem density rebounded somewhat in 2020 with 13 taxa and total density of 2,450.5 trees/ha. Persimmon (600.1 stems/ha), black oak (433.4), red oak (350.1), sassafras (333.4), shagbark hickory (183.4) and green ash (183.4) were the most abundant and accounted for 85.0% of the total stems/ha. Post oak was not present in 2000 and 2009 and had a density of only 33.3 stems/ha in 2020. *Lonicera mackii* (Rupr.) Maxim. (bush honeysuckle), a non-native shrub, occurred in the sample for the first time in 2020 and had a density of 100.0 stems/ha.

Table 4: Density (stems/ha) of small saplings ≥ 50 cm tall; < 2.5 cm dbh in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020. Non-native species are in bold.

Species	Small Sapling Density (stems/ha)		
	2000	2009	2020
<i>Quercus velutina</i>	390.6	220.0	433.4
<i>Sassafras albidum</i>	109.4	140.0	333.4
<i>Carya ovata</i>	640.6	40.0	183.4
<i>Symphoricarpos orbiculatus</i>	—	20.0	—
<i>Fraxinus lanceolata</i>	781.3	80.0	183.4
<i>Carya glabra</i>	15.6	20.0	133.4
<i>Diospyros virginiana</i>	78.1	160.0	600.1
<i>Prunus serotina</i>	31.3	—	—
<i>Ulmus rubra</i>	31.3	—	16.7
<i>Quercus alba</i>	109.4	—	16.7
<i>Quercus stellata</i>	—	—	33.3
<i>Carya tomentosa</i>	15.6	—	—
<i>Morus rubra</i>	—	—	33.3
<i>Quercus imbricaria</i>	—	—	33.3
<i>Ulmus americana</i>	78.1	—	—
<i>Celtis occidentalis</i>	15.6	—	—
<i>Quercus rubra</i>	15.6	—	350.1
<i>Cercis canadensis</i>	31.3	—	—
<i>Asimina triloba</i>	15.6	—	—
<i>Lonicera maackii</i>	—	—	100.0
Totals	2,359.4	680.0	2,450.5

Seedlings

In 2000, 14 seedling species with a density of 7,156.5 stems/ha were encountered (Table 5). Shagbark hickory and *Symphoricarpos orbiculatus* Moench (coralberry), a small native shrub, were the most abundant, accounting for 36.2% and 24.0% of the total, respectively. Black oak, green ash, wild black cherry, persimmon, sassafras, white oak, and shingle oak all had densities greater than 100.0 seedlings/ha and accounted for an additional 35.8% of the total stems. Following a moderate intensity prescribed fire in 2009, species richness increased slightly to 18 taxa and total stem density increased to 18,800 stems/ha. Black oak (4,840 stems/ha), sassafras (3,840), shagbark hickory (3,680), coralberry (2,000) and pignut hickory (1,160) accounted for 82.5% of the total stem count. Persimmon, green ash, slippery elm, white oak, mockernut hickory and red mulberry and wild black cherry had densities of 120.0 to 600.0 stems/ha while post oak density was 360.0 stems/ha.

In 2020, 18 taxa had a total stem density of 18,215.0 stems/ha. Coralberry and shagbark hickory each had stem densities greater than 4,000 stems/ha and accounted for

Table 5: Density (stems/ha) of seedlings < 50 cm tall in Karcher's Post Oak Woods Nature Preserve, Hamilton County, Illinois in 2000, 2009 and 2020. Non-native taxa are in bold.

Species	Seedling Density (stems/ha)		
	2000	2009	2020
<i>Quercus velutina</i>	812.5	4,840.0	1,232.1
<i>Sassafras albidum</i>	218.8	3,840.0	1,431.9
<i>Carya ovata</i>	2,593.8	3,680.0	4,262.4
<i>Symphoricarpos orbiculatus</i>	1,718.8	2,000.0	4,428.9
<i>Fraxinus lanceolata</i>	468.8	480.0	499.5
<i>Carya glabra</i>	93.8	1,160.0	2,697.3
<i>Diospyros virginiana</i>	281.1	600.0	1,531.8
<i>Prunus serotina</i>	375.0	400.0	532.8
<i>Ulmus rubra</i>	—	440.0	33.3
<i>Quercus alba</i>	218.8	400.0	166.5
<i>Quercus stellata</i>	—	360.0	—
<i>Carya tomentosa</i>	31.3	200.0	—
<i>Morus rubra</i>	—	120.0	33.3
<i>Acer rubrum</i>	—	80.0	—
<i>Quercus imbricaria</i>	187.5	80.0	199.8
<i>Ulmus americana</i>	62.5	40.0	—
<i>Celtis occidentalis</i>	31.3	40.0	333.0
<i>Crataegus</i> spp.	—	40.0	—
<i>Quercus rubra</i>	62.5	—	499.5
<i>Lonicera maackii</i>	—	—	99.9
<i>Fraxinus americana</i>	—	—	99.9
<i>Elaeagnus umbellata</i>	—	—	66.6
<i>Cornus florida</i>	—	—	66.6
Totals	7,156.5	18,800.0	18,215.1

47.7% of the total stems. Black oak, sassafras, persimmon and pignut hickory accounted for an additional 37.8% of the total stems. Post oak did not occur in the sample. Non-native shrubs, bush honeysuckle and *Elaeagnus umbellata* Thunb. (autumn olive) had densities of 99.9 and 66.6 stems/ha, respectively, and were encountered for the first time during the 20-year study.

Although the overall seedling densities were similar in 2009 and 2020, changes in the abundance of taxa were noted. Coralberry stem density, which accounted for 10.6% of the total in 2009, increased to 24.3% in 2020. Shagbark and pignut hickory density increased by 16% and 132%, respectively while black and white oak seedling densities were reduced by 25% and 40%, respectively, and no post oak were encountered in 2020.

DISCUSSION

Fire likely played a key role in determining the structure and composition of Karcher's Post Oak Woods Nature Preserve and the adjoining forest for nearly two centuries, and perhaps longer. During the early 20th

century, the scale of the fires lessened. The last of the big fires in Hamilton County occurred in the mid 1950's, creating smoke so dense that travel on the local roads was halted for several days (McClain 2000). Since that time, the fires have been much smaller in scale and confined to local woodlots. As fire frequency, and perhaps intensity, diminished, the forests began a subtle shift from post oak to hickory as the dominant species.

A similar trend has been observed in several formerly oak-dominated forests in southeast Illinois (Edgin and Beadles 2004; Edgin and Ebinger 2009) and the reduction in fire frequency is often, unsurprisingly, associated with increases in human settlement and population growth (McClain et al. 2010). Examination of increment core data from Emma Vance Woods in Crawford County, Illinois revealed the largest oak trees in that forest were between 200 and 250 years old, while the largest hickories were 150 years old, an age which corresponded with a period of rapid settlement by families moving into the area from Ohio.

Regarding the first goal of this study, to verify diminishing importance of post oak in the preserve, the status of post oak within various strata of Karcher's Post Oak Woods Nature Preserve does indeed appear to be diminishing over time. Post oak is a long-lived, fire resistant, shade intolerant species (McClain et al. 2010). Because of its longevity, post oak can persist as the dominant species in a forest for quite some time, even with low recruitment, provided its mortality is low and longevity of the competing species is much shorter than post oak (Brewer 2015). However, neither of those qualifiers exist at Karcher's. Over the course of the 20-year study, post oak density in the overstory decreased by 46.7% while the average diameter of the surviving trees increased. The decline among living overstory trees was accompanied by an increase in mortality as evidenced by the increase in density and average diameter of dead-standing trees. These data, when coupled with the relative lack of post oak recruitment in the seedling and sapling categories, do not bode well for the long-term persistence of post oak as a major component of this forest. The projection for post oak is exacerbated by the abundance of shagbark hickories, which is also a relatively long-lived tree and the most shade tolerant of the hickory species (McCarthy and Westendahl 1988).

The second goal of the study was to assess whether the removal of subcanopy hickory and re-introduction of fire promoted post oak recruitment. Prescribed fire can promote seedling recruitment which can persist for several years post fire (Taft 2020; Dems et al. 2021). However, Karcher's prescribed fire activity alone did not provide a disturbance regime sufficient to stimulate appreciable, sustained recruitment among post oak nor any other oak species. Post oak recruitment following the 2009 prescribed burn was promising with 360.0 stems/ha encountered, but those seedlings apparently did not persist. Perhaps a 2012 drought that covered much of Illinois, including Hamilton County, had a negative effect on some

seedling survivability (Refsland and Fraterrigo 2018). Precipitation was below normal in the fall 2011 and spring 2012 (Illinois Department of Natural Resources 2013; Rippey et al. 2015). For Hamilton County, abnormally low precipitation was reported in spring and early summer and drought conditions were declared on June 5. The drought conditions were categorized as severe by July 3 and extreme on August 7. Light precipitation in September eased conditions somewhat, but drought conditions or abnormally low precipitation persisted through January 2013. Annual precipitation for Hamilton County in 2012 was 40 to 50 cm below the norm of 105 cm.

While the prescribed fire in 2009 did not produce the desired post oak seedling response, it may have affected other strata as it was of sufficient intensity to substantially reduce live stem density among the small saplings and moderately reduce large sapling density. These reductions, combined with low recruitment in subsequent years, may explain the reduction in large saplings in 2020. The prescribed fire in 2020 was of low intensity and very patchy, so most of the site remained unburned. These factors may have spared the small saplings that were present in the 2009 sample allowing a substantial increase in small sapling density in 2020. Removal of the subcanopy hickories did not appear to have much influence on post oak recruitment; although not examined closely in these studies, the herbaceous ground layer appears to be more abundant and diverse.

The decline of overstory post oak is concerning and may be attributable to several factors. The largest overstory trees may have been reached the end of their natural life, or perhaps, were weakened by the drought of 2012 which hastened their demise. Ninety five percent of respondents to a 2013 Missouri survey examining rapid white oak mortality syndrome reported the drought of 2012 as a possible contributing factor (Reed et al. 2017). Drought may also make trees, regardless of age or size, more susceptible to disease (Wood et al. 2018). Another possible factor could be low mast production and acorn viability among post oaks, an area of needed further study.

If the current trends persist, the data suggest that shagbark hickory will eventually replace post oak as the dominant overstory tree with white, red and black oak as possible lesser associates. The next 20 years will likely determine if post oak will continue to be a major component of the forest. If post oak mortality continues at the rate observed over the past 10 years, this seems doubtful. Increasing the frequency of monitoring to a five-year rotation, rather than 10, may give a more precise representation of changes occurring in the forest and effects of management actions.

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LITERATURE CITED

- Brewer, J.S. 2015. Changes in tree species composition and stand structure in a mature upland oak-dominated forest reflect differences in recruitment, survival, and longevity. *Natural Areas Journal* 35(4):550–556.
- Dems, C.L., A.H. Taylor, E.A.H. Smithwick, J.K. Kreye and M.W. Kaye. 2021. Prescribed fire alters structure and composition of a mid-Atlantic oak forest up to eight years after burning. *Fire Ecology* 17:10. <https://doi.org/10.1186/s42408-021-00093-5>.
- Edgin, B. 2009. Karcher's Post Oak Woods Nature Preserve – The first ten years. *Illinois Audubon* 311:9–13.
- Edgin, B.R. and R. Beadles. 2004. Effects of prescribed burning on the woody understory at Emma Vance Woods, Crawford County, Illinois. *Erigenia* 20:59–66.
- Edgin, B.R. and J.E. Ebinger. 2009. *Carya* (hickories) in the ecotonal forests of the Illinoian Till Plain of southern Illinois. *Erigenia* 22:45.55.
- IDNR. 2022. Illinois Natural Heritage Database. <https://bionicsil.natureserve.org>. Accessed 15 July 2022.
- IDNR. 2017. The Standards and Guidelines for the Illinois Natural Areas Inventory. Natural Areas Program, Division of Natural Heritage. Springfield, Illinois.
- Illinois Department of Natural Resources. 2013. The drought of 2012 – A report of the drought response task force. <https://www2.illinois.gov/dnr/waterresources/documents/thedroughtof2012.pdf>
- McCarthy, B.C. and W.A. Westendahl. 1988. Hickory (*Carya* spp.) distribution and replacement in a second-growth oak-hickory forest of southeastern Ohio. *The American Midland Naturalist* 119(1):156–164.
- McClain, W.E. 2000. The hunts of Indian summer. *Illinois Steward* 8:19–23.
- McClain, W.E. and J.E. Ebinger. 2003. Composition and structure of a post oak woods in Hamilton County, Illinois. *Erigenia* 19:60–64.
- McClain, W.E., T.L. Esker, B.R. Edgin, G. Spyreas, and J.E. Ebinger. 2010. Fire history of a post oak woodland in Hamilton County, Illinois. *Castanea* 74(4):461–474.
- McIntosh, R.P. 1957. The York Woods. A case history of forest succession in southern Wisconsin. *Ecology* 38:29–37.
- Mohlenbrock, R.H. 2002. Vascular Flora of Illinois. Third edition. Southern Illinois University Press, Carbondale, Illinois. 490 pp.
- Reed, S.E., J.T. English, R.M. Muzika, J.M. Kabrick, and S. Wright. 2017. Characteristics of sites and trees affected by rapid white oak mortality as reported by forestry professionals. Pates 240–247 in J.M. Kabrick, D.C. Dey, B.O. Knapp, D.R. Larsen, S.R. Shifley, and H.E. Stelzer, eds. Proceedings of the 20th Central Hardwood Forest Conference. USDA Forest Service General Technical Report NRS-P-167. Newtown Square: Northern Research Station.
- Refsland, T. and J. Fraterrigo. 2018. Fire increases drought vulnerability of *Quercus alba* juveniles by altering forest microclimate and nitrogen availability. *Functional Ecology* 32:2298–2309. <https://doi.org/10.1111/1365-2435.13193>.
- Rippey, B.R. 2015. The U.S. Drought of 2012. *Weather and Climate Extremes* 10:57–64. <https://doi.org/10.1016/j.wace.2015.10.004>
- Schwegman, J.E. 1973. Comprehensive plan for the Illinois nature preserves system. Part 2. The natural divisions of Illinois. Illinois Nature Preserves Commission, Rockford, Illinois. 32 pp + map.
- Taft, J.B. 2020. Do early trends in oak barrens fire treatment predict later outcomes? Insights from three decades of vegetation monitoring. *Fire Ecology* 16:23. <https://doi.org/10.1186/s42408-020-00083-z>
- Telford, C.J. 1926. Third report on a forest survey of Illinois. *Illinois Natural History Survey Bulletin*, Vol. XVI. Article I. 102 pp.
- Web Soil Survey. 2020. Available online at <http://websoilsurvey.nrcs.usda.gov/>. Accessed October 21, 2020.
- Wood, J.D., B.O. Knapp, R.M. Muzika, M.C. Stambaugh, and L. Gu. 2018. The importance of drought - pathogen interactions in driving oak mortality events in the Ozark Border Region. *Environmental Research Letters* 13: 015004. <https://doi.org/10.1088/1748-9326/aa94fa>.

INCREASING PLANT AND MICROBE DIVERSITY ON GREEN ROOFS AS METHODS TO HELP CONSERVE NATIVE FORBS IN ILLINOIS

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ABSTRACT: Anthropogenic activities continue to alter habitats, imperiling native plant communities throughout Illinois and around the globe. Green roofs, which are rooftop habitats engineered specifically to incorporate habitat for plants, have the potential to help conserve native plant species, including those that are threatened or endangered due to habitat loss. However, green roofs are harsh habitats and successful establishment and persistence of plant populations may depend on facilitation within communities. Plant growth and survival may be supported by increasing the diversity of the plants which are selected as well and increasing the diversity of below-ground symbiotic microbes, such as arbuscular mycorrhizal fungi (AMF). Understanding the complex community interactions that promote facilitation may help predict which native species could benefit most from conservation-based green roof design. In this investigation we tested the hypotheses that increased plant and microbial diversity would increase the survival, growth, and reproduction of native forbs on green roofs. Our experiment included eight native Illinois forb species grown at three levels of diversity both with and without added AMF. By measuring survival, growth, reproduction, and AMF infection over one initial growing season, we found that facilitative and symbiotic effects are largely species-specific. Several species had increased survival when grown in diverse communities compared to monocultures and when grown in AMF-inoculated soil, but these results were inconsistent across species and may have been due to AMF species other than the one used for our experiment. Future understanding of the factors that contribute to population establishments and persistence will aid in designing green roofs to maximize their conservation potential for native Illinois plant species.

INTRODUCTION

Anthropogenic activity threatens native plant populations and communities, both around the world and in Illinois (Aronson et al. 2014; Hallfors et al. 2020). Some plant species with advantageous traits may flourish in the Midwest despite these pressures (Swanston et al. 2018), but between 0.17% and 42.5% of plant species are predicted to become extinct within the next century (Zettlemoyer et al. 2019). Biodiversity at all levels will be impacted, with a high risk of homogenization in affected areas (Cao and Natuhara 2020). The reduction or extirpation of native plant populations increases homogenization, which further threatens biodiversity and can result in extinction cascades where many species are lost in a very short period (Cao and Natuhara 2020). To prevent extinction cascades, reconciliation ecology helps promote local biodiversity by informing the design of novel ecosystems in urban areas (Hobbs et al. 2009; Pouso et al. 2020).

One system that is increasingly contributing to restoration ecology in urban environments is shallow-soil extensive green roofs. Extensive green roofs (hereafter “green roofs”) are typically installed to provide ecosystem services, such as trapping stormwater, insulating buildings to reduce energy costs, and counteracting the urban heat island effect, among many others (Orbendorfer et al. 2007; Francis and Lorimer 2011; Kowarik 2011; Xie et al. 2018). Green roofs can also potentially provide habitat to local native plant species that have lost habitat elsewhere (Armstrong 2009; Aloisio et al. 2019). Although not the typical motivation behind installing green roofs, they can be planted with local native forbs and grasses to promote biodiversity (Aguar et al. 2019), for example, by providing food and habitat for important pollinators (Williams et al. 2014; Ksiazek-Mikenas et al. 2018). Finding ways to support native plants on green roofs is therefore important to promoting overall urban biodiversity (Cook-Patton and Bauerle 2012).

Despite the theoretical potential of urban green roofs to support native plants, ensuring their survival is difficult in practice. Plants on extensive green roofs are exposed to harsh environmental factors such as high drought caused by shallow soils and intense solar radiation caused by all-

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day sunlight (Dunnett and Kingsbury 2004; Orbendorfer et al. 2007). While non-native *Sedum* species are typically used in green roof designs due to their drought-resistant properties, requests for native plants have increased (Butler et al. 2012). This trend has been fueled by the increasing knowledge that native plants can support biodiversity by facilitating improved habitats for other native plants (Butler and Orians 2011; Kramer et al. 2019) and provide food and shelter for a wide range of wildlife, including specialist invertebrates dependent upon certain native plant species (Parsons et al. 2020). In the prairie-based Midwest and Great Plains regions of the United States specifically, previous work has shown that it can be difficult to establish native forbs without additional inputs of water and possibly continuous beds of deeper soil (Monterusso et al. 2005; Armstrong 2009; Sutton et al. 2012; Sutton 2015; Ksiazek-Mikenas et al. 2021). To support more complex urban ecosystems that include native prairie species, new insight into the design and performance of green roofs in the region is needed.

Specific communities may positively impact the survival of individual native species on green roofs and promote stable coexistence (Butler and Orians 2011). Species selection and community composition are known to affect overall ecosystem functioning (Hautier et al. 2018), with direct implications for the survival of individuals (Ksiazek-Mikenas and Köhler 2018). Specifically, previous work has found that greater functional and phylogenetic diversity of plant species on green roofs leads to greater levels of nitrogen and phosphorous in the soil, which results in higher overall plant biomass (Xie et al. 2018). Niche complementarity, or the ability of species with different physiological needs to maximize resource use in a community, may help explain why increased diversity helps promote biodiversity and contribute to greater biomass and carbon sequestration (Cadotte 2013; Barber et al. 2017). Greater diversity of functional traits in an ecosystem can improve defense against foreign invasion (Barber et al. 2017) and therefore support native plant establishment. Increasing diversity beyond the typical array of one or two plant families characteristically included on green roofs may therefore support a wider diversity of both native flora and fauna.

In addition to facilitative support between different plant species, many forbs benefit from below-ground interactions with mutualistic fungi and bacteria (Hoch et al. 2019). In natural areas, soil with robust microbial communities may support increased plant establishment by aiding germination and early survival (Burns and Strauss 2011; Guzman et al. 2021). Plant-microbial interactions have been linked to improved drought tolerance, pathogen protection, nutrient availability, and soil stabilization on green roofs (Fulthorpe et al. 2018). However, green roofs are almost always constructed with sterile, engineered soil that lacks microbial communities; a condition which may have negative consequences for plant health and survival (Fulthorpe et al. 2018). Plant establishment and persistence on green roofs may be enhanced

if beneficial arbuscular mycorrhizal fungi (AMF) are added to the soil early in a plant's life cycle (Al-Yahya'ei et al. 2022). If effective, adding AMF during the planting stage could be an important step to supporting more native plants in urban green roofs. Further research is necessary to support AMF inoculation as a conservation strategy because the ability of AMF to enhance survival and growth of native forbs on green roofs is not yet well established.

Both short-term and long-term survival is important to establish sustainable populations of native plants in urban habitats. Long-term survival of a population depends on the ability of native plants to reproduce (Rondina et al. 2014). Plant reproduction can be low in stressful environments with low nutrient availability and high drought, such as that found on green roofs (Aragón et al. 2008). Reduced reproduction can therefore impact long-term community persistence and resilience to changes in the environment. Previous research has found that reproduction of native plants on green roofs may be affected by pollen limitation due to a difference of the pollinator community between urban roofs and other ground-level habitats (Ksiazek et al. 2012). Reproductive strategies might also be altered given tradeoffs between present and future reproductive abilities in a stressful environment (Aragón et al. 2008).

Previous studies suggest that increasing diversity, both in terms of plant species and below-ground symbionts, will aid in the establishment, growth, and persistence of native plants on green roofs (e.g. Vasal et al. 2017; Ksiazek-Mikenas et al. 2018; Xie et al. 2018; Aguiar et al. 2019; Hoch et al. 2019; Droz et al. 2022). In this experiment, we hypothesized that native forb species grown on green roofs would experience increased rates of survival, growth, and reproduction when (1) they were grown in communities with greater plant diversity and (2) they were grown in soil inoculated with symbiotic AMF.

METHODS

Green Roof Construction

We set up an experimental green roof at Elmhurst University in Elmhurst, Illinois on top of the two-story Frick Center in the center of campus. The green roof was constructed from modular green roof trays (Columbia Green Technologies) measuring 61 cm × 61 cm (2 ft × 2 ft) and 10 cm (4 in) deep. We used 28 experimental trays placed in four rows with room to move around each tray to collect samples and data (Figure 1). The roof was not accessible to the public and was located in full sun. We filled each experimental tray first with a single layer of lightweight lava rock (Vigoro), followed by approximately 6 cm of CM63 Grower's Mix with Pine Fines (Midwest Trading). To test our second hypothesis, we then inoculated half of the trays with *Rhizophagus intraradices* (formerly known as *Globus intraradices*) by mixing 150 ml of Mykos Pure Mycorrhizal Inoculant (Extreme Gardening) with the CM63 Grower's Mix in each tray. We then

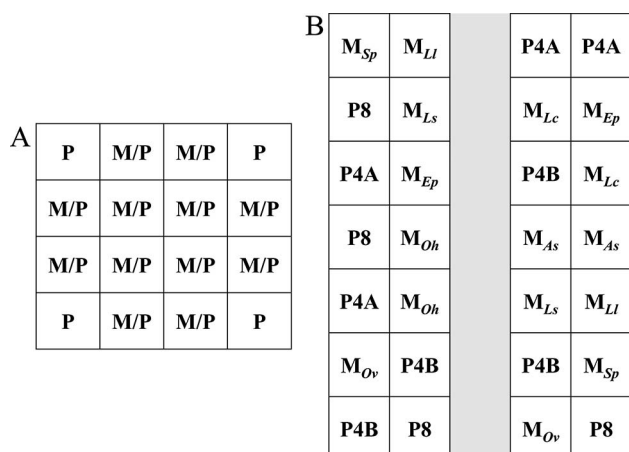


Figure 1. Layout of the planted seedlings in the experimental trays. Part A shows where the seedlings were planted within the tray monoculture treatments (M) and all of the polyculture treatments (P). Part B shows the location of the treatment trays with the top row at the north and bottom row at the south. The species in the monocultures are indicated by the subscripts representing the first letter of the genus and species in the scientific Latin name (e.g., *Ep* = *Echinacea purpurea*). The gray shaded area between columns 2 and 3 indicates a walking path that was kept open for data collection. Placement of the seedlings within the trays as well as the placement of the trays on the roof were determined by a random number generator.

covered the soil in all the trays with another single layer of mixed lava rocks and wood chips (Vigoro) to prevent loss of the soil through wind scouring.

Species Selection, Preparation and Planting

We selected eight species of forbs native to Illinois based on their typical natural habitat, phylogenetic relationships, and commercial availability (Table 1). Key to our selection process was to include a variety of species,

from those that were known to grow successfully on green roofs in the region (i.e., *Allium stellatum* and *Opuntia humifusa* [Armstrong 2009]) to some that, to our knowledge, had not been tried on green roofs before, and even those that came from habitats which would most likely benefit from the water-absorption assistance provided by AMF (i.e., *Lobelia* spp.). Most individuals used in the experiment were purchased as mature seedlings from a regional nursery (Prairie Moon Nursery) while *Sedum pulchellum* and *Liatris ligulistylis* were started from seed in the greenhouse at Elmhurst University. All plants were kept in a greenhouse for at least a week prior to planting on the green roof. Half of the *Lobelia cardinalis* seedlings available from the grower were small seedlings about three months old and the other half were about six months old and slightly larger so both maturity levels were mixed and evenly planted in the experimental treatments.

Each tray was divided into a 16-cell grid and seedlings from each species were planted, one individual per cell, into three diversity treatments: monocultures containing a single species, polycultures containing four species, or polycultures containing eight species. In the monoculture treatments, 12 individuals per species were planted. We created two lower diversity polyculture treatments that included four species each (P4A and P4B) where four individuals per species were planted. In the highest diversity treatment with a polyculture of eight species (P8), two individuals per species were planted (Figure 1). For each of the eight species, this setup resulted in a total of 24 individuals in monoculture, 16 in a four-species polyculture, and 8 in an eight-species polyculture. Placement of species into the grid on the trays as well as placement of the trays on the roof was determined by a random number generator. Placement of each individual into each treatment and placement within a tray was also randomized so that any effects of the tray were minimized. We watered all trays until saturation immediately following the initial planting as well as twice a week for the first two weeks (Figure 2). No supplemental water was provided after that

Table 1: Native Illinois prairie species used in the experiment. All species were included in a monoculture treatment (M; 1 species), polyculture treatment with four species in one of two different combinations (P4A or P4B), and a polyculture treatment with all eight species (P8). All species diversity treatments were also included in both AMF-inoculated and non-inoculated soil treatments. Species names are according to USDA (2024).

Polyculture Group	Family	Species	Common Name
P4A	Asteraceae	<i>Echinacea purpurea</i> (L.) Moench	Eastern purple coneflower
	Asteraceae	<i>Liatris ligulistylis</i> (A. Nelson) K. Schum.	Meadow blazing star
	Crassulaceae	<i>Sedum pulchellum</i> Michx.	Widow's cross
	Oxalidaceae	<i>Oxalis violacea</i> L.	Violet wood-sorrel
P4B	Alliaceae	<i>Allium stellatum</i> Nutt. Ex Ker Gawl.	Prairie onion
	Cactaceae	<i>Opuntia humifusa</i> (Raf.) Raf.	Eastern prickly pear
	Campanulaceae	<i>Lobelia cardinalis</i> L.	Cardinal flower
	Campanulaceae	<i>Lobelia siphilitica</i> L.	Blue cardinal flower



Figure 2. Seedlings of native Illinois forbs were planted in either monocultures or one of two polyculture mixtures (P4A/B and P8) in experimental trays on the green roof. This photograph is from the start of the experiment just after planting in May, 2021.

time, which would be typical of many extensive green roof installations.

Vegetation Assessment

Once per month during the growing season (June – September), we measured survival, height, cover, and reproduction of all individuals. To measure survival, we counted the number of individuals per species in each treatment type that had living above-ground tissues. Any individual that had additional shoots growing from the same root or shoot system was counted as one individual. The percent species survival was simply calculated by dividing the number of individuals that survived to the end of the growing season (September) by the number of individuals planted in May. We measured above-ground growth as the height of the plant from the soil to the highest living tissue for all individuals. We visually estimated the percentage of vegetation cover where the total area of the tray represented 100% cover. All cover measurements were rounded to the nearest 5%. To measure reproduction, we recorded the presence or absence of a reproductive structure (flowers and/or fruits) for each individual during the growing season.

Root Collection, Staining, and Visualization

At the end of the growing season (September), we collected roots from four individuals in each monoculture tray, and two individuals in each of the polyculture trays to measure the rate of AMF colonization for each plant species in the different treatments. We cut primary and lateral roots from the base of the plant and temporarily stored them in Whirl-pak bags for transport to the laboratory. We removed soil and other debris by rinsing the roots in tap water. Following the protocol established by Vierheilig et al. (1998), we cleared the roots by boiling them in 10% KOH and rinsing them with tap water. We then boiled the cleared roots in a solution of 2% acetic acid and black ink (Sheaffer) for three minutes, let them cool, and then rinsed them with tap water. Finally, we placed the stained roots in tap water mixed with a few drops of 2% acetic acid for 20 minutes and then rinsed and stored them in tap water until visualization was performed.

Sedum pulchellum and *Oxalis violacea* did not have above-ground living tissues due to their early senescent phenology so we were unable to collect live roots from these species in September to visualize AMF. Instead, we collected and bulked soil from each tray where the species had been growing in September, filled small pots with the soil, and planted seeds of each species after appropriate pretreatments. The *S. pulchellum* and *O. violacea* plants were then grown in a greenhouse for 16 weeks. We harvested the roots and stained them following the same procedure described above.

For each species in each treatment, we haphazardly selected five stained root sections and visualized them using a light microscope. At 100x total magnification, we haphazardly chose 10 separate viewing fields. In each field, we counted the number of visible AMF structures including arbuscules, spores and vesicles, and recorded the presence or absence of fungal hyphae. Data for all types of AMF structures were pooled and for each species by treatment combination we calculated a total proportion of viewing fields that contained any evidence of AMF.

Statistical Analyses

We used generalized linear mixed-effects models with a binomial response distribution and tray as a random effect to test for effects of inoculation, plant diversity, and their interaction on survival and reproduction for each species. Using “tray” as the random effect in the mixed effects model allowed us to ensure that we were able to consider the effects of the manipulated variables on the plants individually. We used repeated-measures ANOVA of linear models to test for the effects of inoculation, plant diversity, and their interaction on height and the effects of inoculation on cover for each species. We used linear mixed effects models to test for significant relationships between the inoculum treatment and the detected proportion of roots that had been colonized by AMF, using the community diversity as a random effect. All statistical tests were conducted in

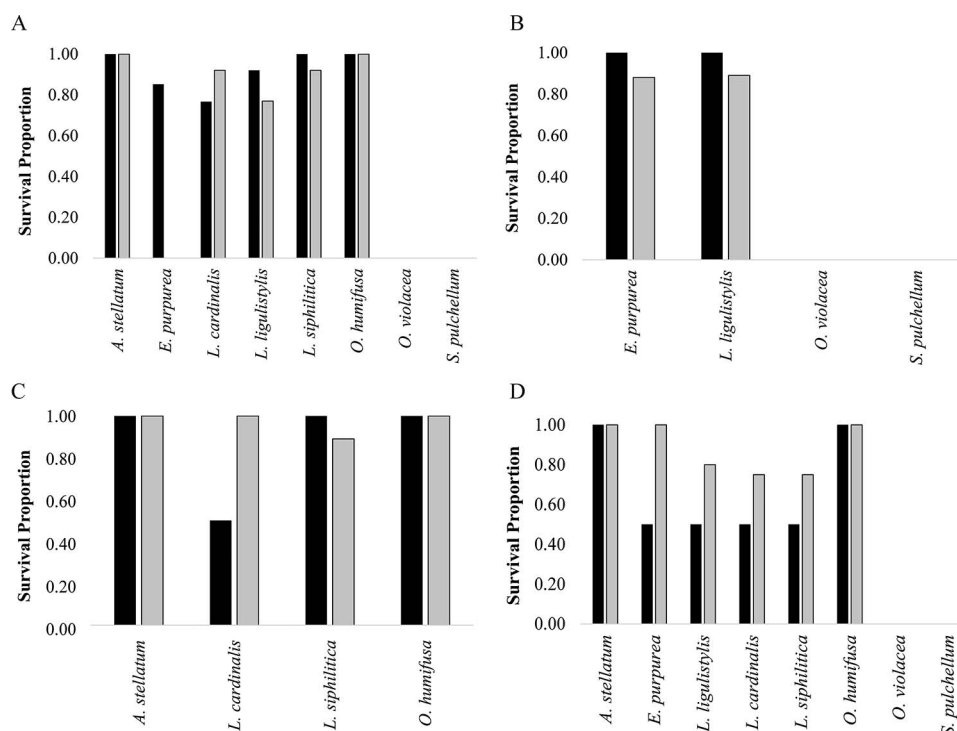


Figure 3. Survival proportion for eight native plant species on a green roof, broken down by both diversity and AMF treatment. Black bars are treatments without AMF, gray bars are treatments with AMF. Species were grown in monocultures (panel A, upper left), polyculture mixes with four species (P4A in panel B, upper right; P4B in panel C, lower left), and a polyculture mix with eight species (panel D, lower right). Lack of data for *O. violacea* and *S. pulchellum* is due to 0% survival of those species.

R, version 4.0.2 (R Core Team 2021), with package lme4 to create the random effects models (Bates et al. 2015).

RESULTS

Survival

There was an observable trend for some species when survival was visualized by community diversity and AMF inoculation treatment (Figure 3). We found no statistically significant effect of the interaction between inoculation and diversity treatment on survival for any species. However, in the monocultures there was a tendency toward slightly greater survival in the AMF treatment for *Lobelia cardinalis* (+15%). *Liatris ligulistylis* (−15%) and *Lobelia siphilitica* (−8%) tended toward slightly lower survival in the AMF treatment. All *Echinacea purpurea* plants died in the AMF treatment but 85% survived in the non-AMF treatment and all *A. stellatum* and *O. humifusa* survived in both the non-AMF and AMF treatments. In the P4A diversity treatments, there was a tendency toward lower survival in the AMF treatment for both *L. ligulistylis* (−12%) and *E. purpurea* (−12%). In P4B, *L. cardinalis* tended toward greater survival (+50%) in the AMF treatment while *L. siphilitica* survived slightly worse in the AMF treatment (−12%). Finally, in the P8 polyculture

treatments, half of the species had a tendency toward greater survival in the AMF treatment (*L. siphilitica* and *L. cardinalis* both at +25%, *L. ligulistylis* +30% and *E. purpurea* +50%). The other four species either had 100% or 0% survival over the study period in both soil treatments.

Because we found no statistical effect of the interaction between community diversity and AMF inoculation on survival, we pooled the data and looked at the effects of diversity and inoculation separately. We found that diversity had a significant effect on survival for three species: *L. ligulistylis* ($p = 0.008$), *L. cardinalis* ($p = 0.018$) and *L. siphilitica* ($p = 0.005$). When looking at the effect of inoculation on survival, we found a significant relationship for half of the species: *E. purpurea* had approximately 18.7% lower survival in the AMF-inoculated treatments ($p = 0.004$), while *L. ligulistylis* (6.4%, $p < 0.001$), *L. cardinalis* (15.7%, $p = 0.002$), and *L. siphilitica* (6.3%, $p < 0.001$) had significantly greater survival in the AMF-inoculated treatments (Figure 4). The other four species either had 100% or 0% survival in both inoculation conditions.

Growth

Overall, we found very few effects of plant diversity or inoculation on plant height. However, we did find a significant interactive effect of diversity and inoculation on

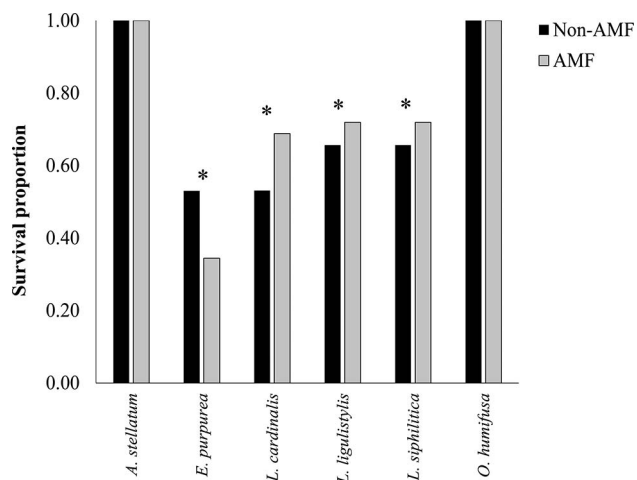


Figure 4. Survival proportion of six species of native plants on a green roof in soil that was either inoculated (AMF) or not inoculated (Non-AMF) with *Rhizophagus intraradices*. An asterisk (*) indicates a statistically significant difference ($p < 0.05$) between the soil treatments. Data for *O. violacea* and *S. pulchellum* are not included because these species had 0% aboveground biomass in both inoculum treatments by the end of the growing season due to their phenologies.

height in *L. siphilitica* ($p = 0.005$, $F = 6.11$). Diversity alone had a significant effect on height for *L. ligulistylis* ($p < 0.001$, $F = 12.82$ on 2 and 45 DF) and inoculation alone had a significant effect on height for *E. purpurea* ($p = 0.009$, $F = 7.020$ on 1 and 190 DF).

We found no significant effect of inoculation on cover for any of the species. In fact, the cover of most of the species decreased over the growing season except for *A. stellatum* and *O. humifusa* and a slight increase in *L. ligulistylis* in the AMF treatments (Figure 5). Notably, the cover of many species decreased in August due to a drought. However, some species (*E. purpurea*, *L. cardinalis*, *L. siphilitica*) experienced a subsequent increase in cover between August and September as basal rosettes regenerated from the roots.

Reproduction

We found that the interaction between inoculation and diversity treatment had a significant effect on the presence of reproductive structures for three species: *A. stellatum* ($p < 0.001$), *E. purpurea* ($p < 0.001$), and *S. pulchellum* ($p = 0.011$). Inoculation alone also had a significant effect on reproduction for two additional species: *L. cardinalis* ($p = 0.004$) and *O. violacea* ($p = 0.002$). The diversity treatment alone had a significant effect on reproduction for *L. siphilitica* ($p = 0.004$).

AMF Infection

We found that all six species remaining at the end of the growing season were colonized by various AM fungal

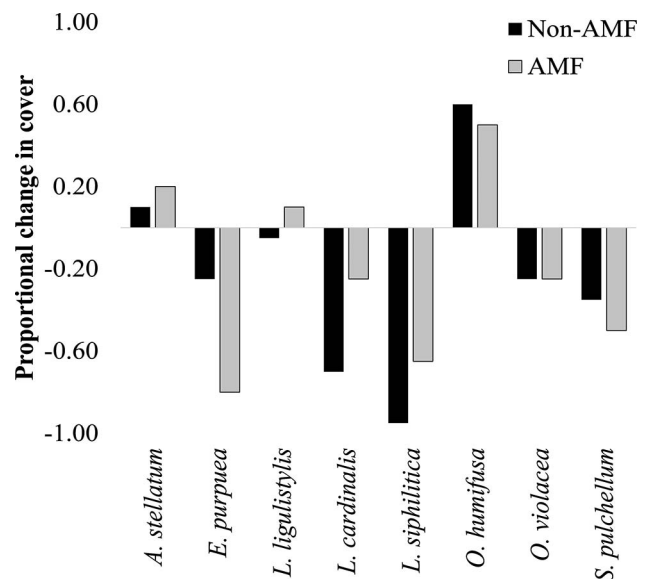


Figure 5. Change in vegetation cover over one growing season across all diversity treatments for eight species on a green roof. Increase in cover is indicated as bars above the zero line while decrease in cover is indicated as bars below the line.

structures (arbuscules, hyphae, and vesicles). The proportions of root sections that were colonized was significantly greater in the non-inoculated condition for all species (Figure 6).

DISCUSSION

During a single growing season, it appears that neither increasing species diversity nor inoculating the soil with the AMF *Rhizophagus intraradices* significantly impacts above-ground growth of native forbs on a green roof. However, using these two methods does impact survival and reproduction for some species and may therefore offer potential strategies for increasing native plant establishment on green roofs in the Midwest USA.

Effects of Plant Diversity

Our study supports the likelihood that the effect of species diversity on growth and survival is highly species-dependent; some species had 100% survival, some had 0% (due to their phenologies) and some saw a significant effect of diversity treatment on survival. There are several reasons for these differences. Each species can vary in its physiological adaptations to competition for space, nutrients, and water, depending on its surrounding conspecifics (Montesinos-Navarro 2017). Within species, a combination of trait plasticity and genetic differences can also lead to individual variation in the degree to which inter-specific and intraspecific competition or facilitation may affect survival (Munzbergova et al. 2020). High species diversity in plant communities can improve plant survival

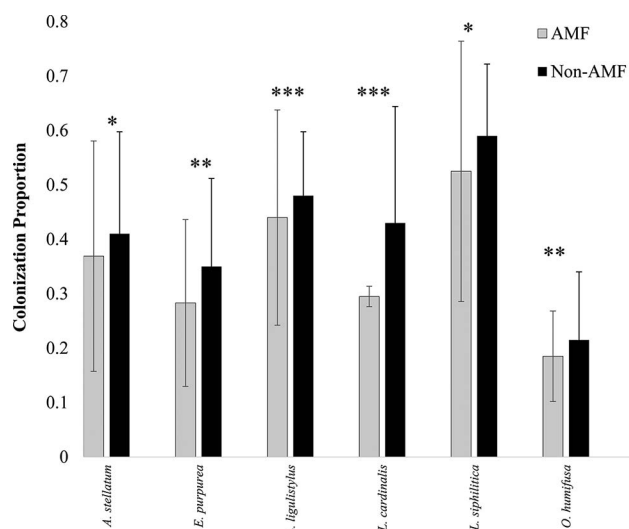


Figure 6. Effect of inoculation with *Rhizophagus intraradicis* on the proportion of root sections colonized by AMF structures, including arbuscules, intraradical hyphae, extraradical hyphae, and vesicles. Asterisks indicate degree of certainty for significant effects: * $p = 0.01 - 0.05$; ** $p = 0.001 - 0.01$; *** $p < 0.001$. Error bars indicate standard deviation.

and coexistence for many species under drought conditions, particularly when the plant species are from a variety of plant families and the phylogenetic diversity is high (Chaves 2021). When increased assemblages of species are grown together in a community under drought conditions, the stress gradient hypothesis suggests that facilitation becomes a more important factor than competition (Chaves 2021). Some species can act as nurse-plants, improving the environment for other species to survive (Navarro-Cano et al. 2016), which has been shown to be beneficial on green roofs (Butler and Oriens 2011). This implies that species having higher survival in our experimental polycultures compared to monocultures may be aided by other species. This finding therefore supports increasing species diversity on green roofs to increase survival although even our most diverse treatment only had eight species; far fewer than the hundreds of native species that make up Illinois' natural prairies (Chadde 2019). Further studies that include long-term monitoring and an increased species pallet could help to further develop this theory.

Some native species may be more likely to survive and reproduce on green roofs if they possess specific advantageous traits or if their traits complement others growing in the community. If the species that are planted have a variety of functional traits, they may be more likely to work together in facultative relationships. It has been shown that increased functional diversity in a community can improve coexistence by reducing competition between species that share similar functional traits (Montesinos-Navarro 2017).

Specifically, functional diversity can govern response to environmental changes by affecting growth, reproduction, and survival (Caruso 2006; Bu et al. 2019). When plant communities are intentionally designed to harbor species with a greater variety of traits, this can increase niche partitioning and promote overall improved resource use by the community (Goberna et al. 2016). For example, Goberna et al. (2016) found that increased plant community diversity resulted in greater uptake of various resources from the soil. This idea is supported in the current experiment by the trend toward an increasing survival rate for *E. purpurea* from the monoculture to the P4 and then to the P8 treatment. While these results should be interpreted with caution due to the low number of replicates in the current investigation (two monocultures per species and four each of the polycultures), they suggest that functional trait complementarity within the community may be especially important for some species. In our experiment, the two species in the P4A treatment that did not survive the entire growing season (*O. violacea* and *S. pulchellum*) are known to be ephemeral species and likely strategized for increased carbon storage below ground rather than production of above-ground biomass after the spring and early summer. This strategy would be supported for species that invest energy in roots, tubers, and other storage structures rather than investing energy in strategies to grow leaves, stems, and flowers under increased abiotic and biotic stress (Bu et al. 2019). *Oxalis violacea* is especially known to use bulbs as a method of underground storage (Nesom 2009). While both *S. pulchellum* and *O. violacea* were absent by September in our study, their expected early arrival in the early spring may support other species in future years, with increased shading and transpiration to cool the soil when the seedlings and new leaves of conspecifics are just starting to emerge. Continued monitoring of species over several generations and with additional research sites is needed for a more complete understanding of how competition and facilitation contribute to community structure on green roofs.

Effects of Below-Ground Diversity

Although supported by studies in natural and agricultural environments, the true impact of AMF on the survival and growth of native forbs on green roofs in general remains undetermined. The interactive effects of inoculation and diversity on survival and reproduction compound the understanding of which facultative relationships plant species may depend on in a green roof habitat. Research has shown that symbiotic AMF can increase nutrient acquisition especially when nutrients are low, in addition to improving protection against microbial pathogens and parasites (Rekret and Maherali 2019). AMF can increase drought tolerance, soil moisture and nutrient uptake during times of stress (Davidson et al. 2016; Qiao 2016), conditions that are typical of green roof habitats in the Midwest U.S.A. Inoculating soil with AMF can also improve plant growth by acting as a biofertilizer, suppressing pathogenic

diseases, and improving water retention in the soil (Samuel and Veeramani 2020), which would show promise for green roofs as well. Adding symbiotic AMF to green roof soil should therefore contribute to growth and survival of plants in these drought-prone, low-nutrient environments. In our study, four species (*E. purpurea*, *L. ligulistylis*, *L. cardinalis* and *L. siphilitica*) out of eight had significantly greater survival when the commercially-available AMF *R. intraradices* was added to the soil. These results suggest that if such species are planted on green roofs, their survival may be enhanced by the ability of *R. intraradices* to increase nutrient uptake and water retention in the soil (Varga 2015).

On the other hand, the surprisingly lower colonization rate of AMF in the roots of all species that we observed in the treatments that had *R. intraradices* added suggests that other factors in addition to inoculation with this one species may also be important. Specifically, these findings suggest that *Rhizophagus intraradices* may not be a symbiotic AMF species for the Illinois natives in our experiment but may instead be competing with other AMF in the environment that more readily colonize the roots of these particular species (Loján et al. 2017). Competition between AMF species is still not well-understood but does occur and can reduce the overall fungal abundance in the soil (Engelmoer et al. 2014) and effect nutrient uptake and growth of plants (Thonar 2014). While beyond the scope of the current study, the countless species of AMF found in soil will no doubt have various species-specific effects on plants in constructed habitats like green roofs. For example, many plant species considered generalists may not benefit much from AMF inoculation, no matter which species are used (Rondina et al. 2014).

Furthermore, some species may not need AMF to tolerate the harsh abiotic conditions of the green roof so the rate of root colonization may be irrelevant. For example, we found that *A. stellatum* and *O. humifusa* did not need AMF to survive, although *A. stellatum* had greater reproduction in the AMF treatment. This is not surprising given the high degree of drought tolerance of these species. At least for *A. stellatum*, it did not appear that the green roof microhabitat was negatively affecting the survival and reproduction of this species. AMF might not improve nutrient and water uptake if it is not needed for certain aspects of the plant's life cycle (Young et al. 2015). For example, previous research in a related cactus species, *Opuntia ficus-indicas*, found no impact of AMF on growth and survival when low but adequate moisture was provided (Neffar and Chenchouni 2015). *Opuntia* and many other species in Cactaceae generally have low AMF colonization (Dhillon and Friese 1994) although *O. humifusa* can be found with high rates of AMF colonization in the wild (Deotare et al. 2014). The *O. humifusa*, as well as the other species used in our experiment, were planted as seedlings that had been growing in greenhouses for a few weeks to several months. Without AMF collection prior to planting, it is difficult to know whether

previous microbial infection occurred within each individual plant.

It is likely that seedlings bought from nurseries are already infected with microbes (Halleen et al. 2003) and that AMF may colonize green roof soils when spores are brought in by wind or animals (Chaudhary et al. 2019; Metzler et al. 2024), although this was not directly measured in this experiment. Late-colonizing AMF species may confer an advantage to plants regardless of previous intentional inoculation. Furthermore, plants can develop specific local adaptations related to the soil microbial community (Rekret and Maherali 2019). Symbiosis with the local microbiota may help explain why we found a dramatically higher survival rate in *E. purpurea* in the non-inoculated monoculture treatment than in the treatment inoculated with *R. intraradices*. Our root staining and visualization confirmed the presence of AMF in both the inoculated and non-inoculated treatments of all six species that were still growing at the conclusion of the first growing season and neither of those grown as fresh seedlings in the greenhouse after the conclusion of the growing season (*O. violaceae* and *S. pulchellum*), suggesting that a single-species inoculum can have a very limited or no effect on plant survival and growth.

It is highly likely that complex and community-wide below-ground dynamics are impacting plants' growth and survival. For example, many AMF species rely on bacteria to colonize plant roots. A recent study in Finland found that inoculation with the AMF species *Rhizophagus irregularis* alone did not significantly increase biomass of forbs on green roofs unless also combined with the bacterium *Bacillus amyloliquefaciens* (Xie G et al. 2018). The plants themselves also contribute to soil biota. For example, vegetation monocultures have been found to have lower AMF diversity in the soil while polycultures support a greater diversity of microbes (Dietrich et al. 2020). In a study conducted by Guzman et al. (2021), *Rhizophagus* was found to dominate plant monocultures, while *Glomus* dominated polycultures, indicating that fungal communities are impacted by plant communities. It is possible, therefore, that the effects of the *R. intraradices* treatment seen on our green roof trays was impacted by both the other plant species and other microspecies present and further investigations would be needed to tease apart these complex ecological interactions.

Additional time will be needed to determine the comprehensive effect that AMF has on herbaceous plants on green roofs. Some AMF species only colonize roots during certain portions of a plant's lifecycle, which can vary considerably by species (Smilauer et al. 2021). Individuals included in the current study were all at the early stages of their life cycles when planted in the inoculated soil. Life cycle differences within a species can further alter AMF symbiosis and nutrient uptake, which may increase intraspecific competition for AMF between plants in different life stages (Merrild et al. 2013). We have yet to examine the effects of AMF on multiple

stages of the plants' life cycles such as seed germination. However, in our experiment, half of the species growing in the inoculated soil (*E. purpurea*, *L. ligulistylis*, *L. cardinalis* and *L. siphilitica*) demonstrated a tendency toward greater vegetation regeneration after a drought in August compared to the non-AMF treatment. In other early successional communities, vegetation regeneration is more likely to occur in soils where AMF communities are present (Neuenkamp et al. 2018), suggesting that AMF inoculation may have contributed to successful regeneration after drought for at least some species in the current study. Long-term monitoring throughout the entire lifecycle of the species would be an ideal next step to developing a more complete understanding of the impacts of AMF.

CONCLUSION

In all habitats, above-ground and below-ground community diversity can be important biotic factors that can alter plant community structure, abundance, growth, and survival. Increasing soil or plant community diversity may contribute to successfully growing native plants in novel urban habitats like green roofs, but more work is needed in this important area of research. Over a single growing season, our results demonstrate that AMF and species diversity should be considered when determining how to best support native plant populations in constructed urban ecosystems, as their effects are complex. Except for one species grown in monoculture (*E. purpurea*), it seems that commercial inoculum containing *R. intraradices* decreases root colonization of native Illinois wildflower species but not to a degree that negatively affects plant growth and reproduction. Community diversity was only found to significantly improve the survival of some species but our experiment was limited by a short time frame. Continued research is needed to determine if the observed trends persist into the future, especially in the case of ephemeral species. Application of these results to support future projects will depend upon the outcome sought by a plant enthusiast, building manager, conservationist, or urban developer in that location. Overall, extensive green roofs remain a very harsh microhabitat for native Illinois forbs, specifically those that lack traits for prolonged drought tolerance.

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LITERATURE CITED

- Aguilar, A.C., S.A. Robinson, and K. French. 2019. Friends with benefits: The effects of vegetative shading on plant survival in a green roof environment. *PLoS ONE*, DOI: 10.1371/journal.pone.0225078.
- Aloisio, J.M., M.I. Palmer, A.R. Tuininga, et al. 2019. Plant colonization of green roofs is affected by composition of established native plant communities. *Frontiers in Ecology and Evolution* 6(238):1-12.
- Al-Yahya'ei, M.N., J. Blaszkowski, H. Al-Hashmi, K. Al-Farsi, I. Al-Rashdi, A. Patzelt, T. Boller, A. Wiemken, and S. Symanczik. 2022. From isolation to application: a case study of arbuscular mycorrhizal fungi of the Arabian Peninsula. *Symbiosis* 86:123-132.
- Aragón, C.F., A. Escudero, and F. Valladares. 2008. Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. *Journal of Ecology* 96:222-229.
- Armstrong, P.K. 2009. Feasibility of using prairie vegetation on a sloped roof with four inches of soil. *Erigenia* 22:3-8.
- Aronson, M.F.J., F.A. La Sorte, C.H. Nilon, M. Katti, M. A. Goddard, C.A. Lepczyk, P.S. Warren, N.G. Williams, S. Cilliers, B. Clarkson, C. Dobbs, R. Dolan, K. Hedblom, S. Klotz, L. Kooijmans, I. Kuhn, I. MacGregor-Fors, M. McDonnell, U. Mortberg, P. Pysek, S. Siebert, J. Sushinsky, P. Werner, and M. Winter. 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proceedings of the Royal Society B* 281:20133330.
- Barber, N.A., H.P. Jones, M.R. Duvall, W.P. Wysocki, M.J. Hansen, and D.J. Gibson. 2017. Phylogenetic diversity is maintained despite richness losses over time in restored tallgrass prairie plant communities. *Journal of Applied Ecology* 54:137-144.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1):1-48.
- Butler, C. and C. Orians. 2011. Sedum cools soil and can improve neighboring plant performance on a green roof. *Ecological Engineering* 37:1796-1803.
- Butler, C., E. Butler, and C. Orians. 2012. Native plant enthusiasm reaches new height: Perceptions, evidence, and the future of green roofs. *Urban Forestry and Urban Greening* 11(1):1-10.
- Burns, J.H. and S.Y. Strauss. 2011. More closely related species are more ecologically similar in an experimental test. *PNAS* 108(13):5302-5307.
- Bu, W., J. Huang, H. Xu, R. Zang, Y. Ding, Y. Li, M. Lin, J. Wang, and C. Zhang. 2019. Plant functional traits are the mediators in regulating effects of abiotic site conditions on aboveground carbon stock-evidence from a 30-ha tropical forest plot. *Frontier Plant Science* 1958(9):1-10.
- Cadotte, M.W. 2013. Experimental evidence that evolutionarily diverse assemblages result in higher productivity.

- Proceedings of the National Academy of Science* 110(22):8996-9000.
- Cao, Y. and Y. Natuhara. 2020. Effect of anthropogenic disturbance on floristic homogenization in the floodplain landscape: Insights from the taxonomic and functional perspectives. *Forests* 11(10):1-22.
- Caruso, C.M. 2006. Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. *American Journal of Botany* 93(4): 531-538.
- Chadde, S.W. 2019. *Prairie Plants of Illinois: A Field Guide to the Wildflowers and Prairie Grasses of Illinois and the Midwest*. An Orchard Innovations Book.
- Chaudhary, V.B., E.L. Sandall, and M.V. Lazarski. 2019. Urban mycorrhizas: predicting arbuscular mycorrhizal abundance in green roofs. *Fungal Ecology* 40:12-19.
- Chaves, R., P. Ferrandis, A. Escudero, and A.L. Lazuriaga. 2021. Diverse phylogenetic neighborhoods enhance community resistance to drought in experimental assemblages. *Scientific Reports* 11(1):2249.
- Cook-Patton, C. and T.L. Bauerele. 2012. Potential benefits of plant diversity on vegetated roofs: a literature review. *Journal of Environmental Management* 106: 85-92.
- Davidson, B.E., S.J. Novak, and M.D. Serpe. 2016. Consequences of inoculation with native arbuscular fungi for root colonization and survival of *Artemisia tridentata* ssp. *wyomingensis* seedlings after transplanting. *Mycorrhiza* 26(6):595-608.
- Deotare, P.W., S.S. Metkar, R.C. Maggirwar, and S.P. Khodke. 2014. AMF status of naturally growing *Opuntia humifusa*. *International Journal of Life Sciences* 2(1):63-66.
- Dietrich, P., C. Roscher, A.T. Clark, N. Eisenhauer, B. Schmid, and C. Wagg. 2020. Diverse plant mixtures sustain a greater arbuscular mycorrhizal fungi spore viability than monocultures after 12 years. *Journal of Plant Ecology* 13(4):478-488.
- Dhillon, S.S. and C.F. Friese. 1994. The occurrence of mycorrhizas in prairies: Application to ecological restoration. Pages 103-114 in R.G. Wickett, P.D. Lewis, A. Woodliffe and P. Pratt, eds. *Proceeding of the 13th North American prairie conference*, University of Windsor, Canada.
- Droz, A.G., R.R. Coffman, A.C. Eager, and C.B. Blackwood. 2022. Drivers of fungal diversity and community biogeography differ between green roofs and adjacent ground-level green space. *Environmental Microbiology* 24(12):5809-5824.
- Dunnett, N. and N. Kingsbury. 2004. *Planting Green Roofs and Living Walls*. Timber Press, Portland.
- Engelmoer, D.J.P., J.E. Behm, and E.T. Kiers. 2014. Intense competition between arbuscular mycorrhizal mutualists in an in vitro microbiome negatively affects total fungal abundance. *Molecular Ecology* 23(6): 1584-1593.
- Francis, R.A. and J. Lorimer. 2011. Urban reconciliation ecology: The potential of living roofs and walls. *Journal of Environmental Management* 92:1429-1437.
- Fulthorpe, R. 2018. The green roof microbiome: improving plant survival for ecosystem service delivery. *Frontiers in Ecology and Evolution* 6(5):1-10.
- Goberna, M., J.A. Navarro-Cano, and M. Verdu. 2016. Opposing phylogenetic diversity gradients of plant and soil bacterial communities. *Proceedings of The Royal B* 283(1825):1-6.
- Guzman, A., M. Montes, L. Hutchins, G. DeLaCorda, P. Yang, A. Kakouridis, R.M. Dahlquist-Williard, M.K. Firestone, T. Bowles, and C. Kremen. 2021. Crop diversity enriches arbuscular mycorrhizal fungal communities in an intensive agricultural landscape. *New Phytologist* 231(1):447-459.
- Halleen, F., R.W. Crous, and O. Petrin. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special references to pathogens involved in the decline of young vines. *Australasian Plant Pathology* 34:47-52.
- Hallfors, M., S. Lehvavirta, T. Aandahl, L.-M. Lehtimäki, L.O. Nilsson, A. Ruotsalainen, L.E. Schulman, and M. T. Hyvarinen. 2020. Translocation of an arctic seashore plant reveals signs of maladaptation to altered climatic conditions. *Peer J*. 2020 Nov 20; 8:e10357. doi: 10.7717/peerj.10357.eCollection 2020.
- Hautier, Y., F. Isbell, E.T. Borer, E.W. Seabloom, W.S. Harpole, E.W. Lind, A.S. MacDougall, C.J. Stevens, P.B. Adler, J. Alberti, J.D. Bakker, L.A. Brudvig, Y.M. Buckley, M. Cadotte, M.C. Caldeira, E.J. Chaneton, C. Chu, P. Daleo, C.R. Dickman, J.M. Dwyer, A. Eskelinen, P.A. Fay, J. Firn, N. Hagenah, H. Hillebrand, O. Iribarne, K.P. Kirkman, J.M.H. Knops, K.J. La Pierre, R.L. McCulley, J.W. Morgan, M. Partel, J. Pascual, J.N. Price, S.M. Prober, A.C. Risch, M. Sankaran, M. Schuetz, R.J. Standish, R. Virtanen, H.M. Wardle, L. Yahdjian, and A. Hector. 2018. Local loss an spatial homogenization of plant diversity reduce ecosystem multifunctionality. *Nature Ecology & Evolution* 2:50-56.
- Hobbs, R.J., E. Higgs, and J.A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. *Trends in Ecology and Evolution* 24(11):599-605.
- Hoch, J.M.K., M.E. Rhodes, K.L. Shek, D. Dinwiddie, T. C. Hiebert, A.S. Gill, A.E.S. Estrada, K.L. Griffin, M. I. Palmer, and K.L. McGuire. 2019. Soil microbial assemblages are linked to plant community composition and contribute to ecosystem services on urban green roofs. *Frontiers in Ecology and Evolution* 7(198):1-14.
- Kowarik, I. 2011. Novel urban ecosystems, biodiversity, and conservation. *Environmental Pollution* 159: 1974-1983.
- Kramer, A.T., B. Crane, J. Downing, J.L. Hamrick, K. Havens, A. Highland, S.K. Jacobi, T.N. Kaye, E.V. Lonsdorf, J.R. Neale, A. Novy, P.E. Smouse, D.W. Tallamy, A. White, and J. Zeldin. 2019. Sourcing

- native plants to support ecosystem function in different planting contexts. *Restoration Ecology* 27(3): 470-476.
- Ksiazek, K., J. Fant, and K. Skogen. 2012. An assessment of pollen limitation on Chicago green roofs. *Landscape and Urban Planning* 107(4):401-408.
- Ksiazek-Mikenas, K., J. Herrmann, S.B. Menke, and M. Köhler. 2018. If you build it, will they come? Plant and arthropod diversity on urban green roofs over time. *Urban Naturalist* 1:52-72.
- Ksiazek-Mikenas, K. and M. Köhler. 2018. Traits for stress-tolerance are associated with long-term plant survival on green roofs. *Journal of Urban Ecology* 4(1):juy016.
- Ksiazek-Mikenas, K., V.B. Chaudhary, D.J. Larkin, and K.A. Skogen. 2021. A habitat analog approach established native plant communities on green roofs. *Ecosphere* 12(9):e03754.
- Loján, P., C. Senés-Guerrero, J.P. Suárez, et al. 2017. Potato field-inoculation in Ecuador with *Rhizophagus irregularis*: no impact on growth performance and associated arbuscular mycorrhizal fungal communities. *Symbioses* 73:45-56.
- Merrild, M.P., P. Ambus, S. Rosendahl, and I. Jakobsen. 2013. Common arbuscular mycorrhizal networks amplify competition for phosphorus between seedlings and established plants. *New Phytologist Foundation* 200(1):229-240.
- Metzler, P., K. Ksiazek-Mikenas, and V.B. Chaudhary. 2024. Tracking arbuscular mycorrhizal fungi to their source: active inoculation and passive dispersal differentially affect community assembly in urban soils. *New Phytologist*. DOI: 10.1111/nph.19526.
- Monterusso, M.A., D.B. Rowe, and C.L. Rugh. 2005. Establishment and persistence of *Sedum* spp. and native taxa for green roof applications. *HortScience: a publication of the American Society for Horticultural Science* 40(20):391-396.
- Montesinos-Navarro, A., M. Verdu, J.I. Querejeta, and A. Valiente-Banuet. 2017. Nurse plants transfer more nitrogen to distantly related species. *Ecology* 98(5): 1300-1310.
- Munzbergova, Z., V. Kosova, R. Schnablova, M. Rokaya, H. Synkova, D. Haisel, N. Wilhelmova, and T. Dostalek. 2020. Plant origin, but not phylogeny, drive species ecophysiological response to projected climate. *Frontier Plant Science* 400(11):1-28.
- Navarro-Cano, J.A., P.P. Ferrer-Gallego, E. Laguna, I. Ferrando, M. Goberna, A. Valiente-Banuet, and M. Verdu. 2016. Restoring phylogenetic diversity through facilitation. *Restoration Ecology* 24(4):449-455.
- Neffar, S. and B.H. Chenchouni. 2015. Effects of soil chemical properties and seasonality on mycorrhizal status of Prickly Pear (*Opuntia ficus-indica*) planted in hot arid steppe rangelands. *Sains Malaysiana* 44(5): 671-680.
- Nesom, G.L. 2009. Taxonomic notes on acaulescent *Oxalis* (Oxalidaceae) in the United States. *Phytologia* 91(3):501-526.
- Neuenkamp, L., M. Moora, M. Opik, J. Davison, M. Gerz, M. Mannisto, T. Jairus, M. Vasar, and M. Zobel. 2018. The role of plant mycorrhizal type and status in modulating the relationship between plant and arbuscular mycorrhizal fungal communities. *New Phytologist Foundation* 220(4):1236-1247.
- Orbendorfer, E., J. Lundholm, B. Bass, R.R. Coffman, H. Doshi, N. Dunnett, S. Gaffin, M. Köhler, K.K.Y. Liu, and D.B. Rowe. 2007. Green roofs as urban ecosystems: Ecological structures, functions, and services. *BioScience* 57(10):823-834.
- Parsons, S.E., L.M. Kerner, and S.D. Frank. 2020. Effects of native and exotic congeners on diversity of invertebrate natural enemies, available spider biomass, and pest control services in residential landscapes. *Biodiversity and Conservation* 29:1241-1262.
- Pouso, S., A. Borja, L.E. Fleming, E. Gomez-Baggethun, M.P. White, and M.C. Uyarra. 2020. Contact with blue-green spaces during the COVID-19 pandemic lockdown beneficial for mental health. *Science of the Total Environment* 756:1-12.
- Qiao, X., S. Bei, H. Li, P. Christie, F. Zhang, and J. Zhang. 2016. Arbuscular mycorrhizal fungi contribute to overyielding by enhancing crop biomass while suppressing weed biomass in intercropping systems. *Plant and Soil* 406:173-185.
- R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rekret, P. and H. Maherali. 2019. Local adaptation to mycorrhizal fungi in geographically close *Lobelia siphilitica* populations. *Oecologia* 190:127-138.
- Rondina, A.B.L., L.E.A.M. Lescano, R.D.A. Alves, E.M. Matura, M. Nogueira, and W. Zangaro. 2014. Arbuscular mycorrhizas increase survival, precocity and flowering of herbaceous and shrubby species of early stages of tropical succession in pot cultivation. *Journal of Tropical Ecology* 30(06):599-614.
- Samuel, S.S. and A. Veeramani. 2020. Advantages of arbuscular mycorrhizal fungi (AMF) production for the profitability of agriculture and biofertilizer industry. Mycorrhizal Fungi, Utilization in Agriculture and Forestry. *IntechOpen*. DOI: 10.5772/intechopen.95458.
- Smilauer, P., J. Kosnar, M. Kotilinek, S. Pechackova, and M. Smilauerova. 2021. Host age and surrounding vegetation affect the community and colonization rates of arbuscular mycorrhizal fungi in a temperate grassland. *New Phytologist* 232(1):290-302.
- Sutton, R.K., J.A. Harrington, L. Skabelund, L.P. MacDonagh, R. Coffman, and G. Koch. 2012. Prairie-based green roofs: Literature, templates, and analogs. *Journal of Green Building* 7:143-172.
- Sutton, R.K. 2015. Green roof plant traits for the central Great Plains. *Journal of Living Architecture* 2(2):1-10.

- Swanston, C., L.A. Brandt, M.K. Janowiak, S.D. Handler, P. Butler-Leopold, L. Iverson, F.R. Thompson III, T.A. Ontl, and P.D. Shannon. 2018. Vulnerability of forests of the Midwest and Northeast United States to climate change. *Climatic Change* 146:103-116.
- Thonar, C.C., E. Frossard, P.Š. Milauer, and J. Jansa. 2014. Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. *Molecular Ecology* 23:733-746.
- USDA, NRCS. 2024. The PLANTS Database (<http://plants.usda.gov>, 4/01/2024). National Plant Data Team, Greensboro, NC USA.
- Varga, S. 2015. Effects of arbuscular mycorrhizal fungi and maternal plant sex on seed germination and early plant establishment. *American Journal of Botany* 102(3):358-366.
- Vasal, A., H. Shalom, G.J. Kadas, and L. Balustein. 2017. *Sedum*-annual plant interactions on green roofs: Facilitation, competition and exclusion. *Ecological Engineering* 108:318-329.
- Vierheilig, H., A.P. Coughlan, U. Wyss, and Y. Piche. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied Environmental Microbiology* 64(12):5004-5007.
- Williams, N.S.G., J. Lundholm, and J.S. MacIvor. 2014. Do green roofs help urban biodiversity conservation? *Journal of Applied Ecology* 51:1643-1649.
- Xie, G., J.T. Lundholm, and J.S. MacIvor. 2018. Phylogenetic diversity and plant trait composition predict multiple ecosystem functions in green roofs. *Science of the Total Environment* 628-629:1017-1026.
- Young, T., D.D. Cameron, and G.K. Phoenix. 2015. Using AMF inoculum to improve the nutritional status of *Prunella vulgaris* plants in green roof substrate during establishment. *Urban Forestry & Urban Greening* 14(4):959-967.
- Zettlemoyer, M.A., D.D. McKenna, and J.A. Lau. 2019. Species characteristics affect local extinctions. *American Journal of Botany* 106(4):547-559.

AUXIN-PRODUCING BACTERIA ISOLATED FROM THE ROOTS OF ILLINOIS *SPIRANTHES* ORCHIDS

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ABSTRACT: Orchid conservation efforts often focus on utilizing fungal symbionts to increase germination rates. Less well known is the role specific endophytic root bacterial associates play in promoting orchid growth. Notably, auxin-producing endophytic bacteria in orchid roots have been shown to induce orchid germination in the absence of a fungal symbiont. To determine if bacteria that produce auxin were present in *Spiranthes* orchids, bacteria were isolated from three native Illinois species and colorimetric analysis was used to quantify auxin production. Using 16s rRNA sequencing, a total of 15 isolates were found to produce measurable concentrations of auxin. *Spiranthes* seeds plated with media supernatant, which contained auxin from four isolates, did not significantly increase germination percent compared to seeds plated with a known fungal symbiont. To quickly determine if these isolates impacted root development in plants, the model plant *Arabidopsis* was utilized. *Arabidopsis* seeds did show decreased primary root formation when plated on supernatant media containing auxin from the four isolates, suggesting the auxin has biological activity in plants. Two of the isolates were labelled with green fluorescent protein and found to colonize root structures of *Spiranthes magnicamporum* Sheviak. Overall, these findings suggest that root-associated bacteria that produce auxin should be examined more thoroughly for their impact on orchid growth for conservation purposes.

INTRODUCTION

The genus *Spiranthes* Rich. (Orchidaceae) contains terrestrial orchids typically found throughout temperate zones in North America and are commonly known as Ladies' Tresses (NatureServe 2022). These orchids have white flowers (occasionally pink) that are arranged helically along a terminal inflorescence (Dueck et al. 2014). Currently, there are 35 species recognized within the genus *Spiranthes* with nine being native to Illinois (Pace and Cameron 2017). Three of these Illinois native species, *Spiranthes cernua* (L.) L.C. Rich, *Spiranthes magnicamporum* Sheviak, and *Spiranthes vernalis* Engelm. & Gray, are in danger of population loss (NatureServe 2022) (Fig. 1). Notably, *S. magnicamporum* is listed as globally vulnerable, while *S. cernua* and *S. vernalis* are globally secure but listed, respectively, as vulnerable and critically imperiled in Illinois (NatureServe 2022). Both *S. cernua* and *S. magnicamporum* are autumn-flowering species with a widespread range that

extends further north into Canada and are agamospermic depending on geographical location (Catling 1982; Pace and Cameron 2017; NatureServe 2022). Conversely, *S. vernalis* is a summer-flowering species in Illinois and is not known to be agamospermic, possibly contributing to its critically imperiled status (Catling 1982). To combat further population decline due to overcollection from wild populations and habitat loss from urbanization, current conservation efforts for *Spiranthes* orchids involve the use of mycorrhizal fungi to improve germination and promote later stages of development such as flowering (Zettler and McInnis 1993).

Mycorrhizal fungi are required for orchid development because nearly all species start off as tiny, dust-like seeds that lack resource-containing endosperm (Barthlott et al. 2014). Because orchid seeds lack endosperm, they must obtain energy from another source to fuel growth and development prior to establishing photosynthesis. This energy requirement is fulfilled by mycorrhizal fungi which forms a beneficial relationship with the seeds to provide carbohydrates and nitrogen necessary for development (McCormick et al. 2006; Mohammadi et al. 2011). This union results in development of a protocorm which houses the fungal symbiont and produces a shoot apical meristem (Yeung et al. 2017). Thus, most orchid conservation efforts employ *in vitro* symbiotic germination (Fig. 2) with the seeds and mycorrhizal fungi collected from natural orchid populations (McCormick et al. 2006).

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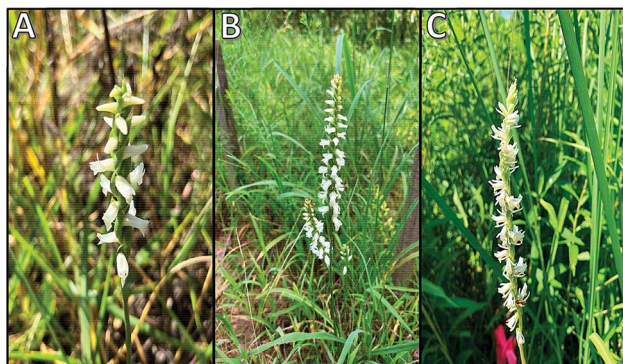


Figure 1. Photos of *Spiranthes* orchids: A) *Spiranthes cernua* collected in Pocahontas, IL; B) *Spiranthes magnicamporum* collected in Edwardsville, IL; C) *Spiranthes vernalis* collected in Edwardsville, IL.

Because low germination rates and mycorrhizal fungi incompatibility significantly limit current conservation efforts, the development of new techniques should be explored (Zettler and McInnis 1993). Notably, bacteria have been discovered on the rhizoplane and endophytically within several terrestrial orchid roots (Li et al. 2017; Alibrandi et al. 2020; Kaur and Sharma 2021). Promising research has shown that endophytic Root Associated Bacteria (RAB) can protect against pathogens by priming plants for systematic resistance through ethylene, jasmonic acid, and salicylic acid defense signaling pathways (Brock et al. 2013; Glaeser et al. 2016). Root associated bacteria can also aid in plant nutrient uptake by solubilizing phosphorous, fixing atmospheric nitrogen, and processing insoluble iron present in soil (Rajkumar et al. 2009; Gupta et al. 2013; Afzal et al. 2019). More pertinent to orchid conservation is the ability of RAB to promote seed germination possibly through the production of plant hormones (Tsavkelova et al. 2007a; Kaur and Sharma 2021).

One of the most common plant hormones produced by RAB in orchids is auxin in the form of Indole-3-acetic acid (IAA) (Júnior et al. 2011; Tsavkelova et al. 2016; Yang et al. 2014). Auxin is involved in almost every aspect of plant growth and development including root formation, germination, post embryonic development, vascular tissue development, and tropisms (Evans et al. 1994; Liu et al. 2013; Teale et al. 2006). Past research has indicated that exogenous application of auxins such as IAA and 1-Naphthaleneacetic acid (NAA) positively promote orchid germination by increasing protocorm diameters, trichomes, and DNA contents through endoreduplication (Lim and Loh 2003; Novak and Whitehouse 2013).

One conservation-focused advantage of RAB that produce auxin may be their ability to promote vegetative growth as well as germination in multiple orchid species (Tsavkelova et al. 2007b; Tsavkelova et al. 2016; Kaur

and Sharma 2021). Germinated seeds from epiphytic orchid species outperformed asymbiotic germination controls, while established plantlets displayed increased root numbers and foliar area when inoculated with auxin producing RAB such as *Bacillus*, *Sphingomonas*, and *Enterobacter* (Tsavkelova et al. 2007b; Júnior et al. 2011).

Auxin is naturally transported cell-to-cell in a unidirectional manner known as polar auxin transport (PAT). Polar auxin transport inhibitors, such as monensin, obstruct the unidirectional flow of auxin and impede the allocation of auxin within certain plant regions thereby impacting organ development. When orchid seedlings were placed on media containing such PAT inhibitors, first leaf formation was diminished (Novak and Whitehouse 2013). In *Arabidopsis*, PAT occurs from an area known as the auxin organizing center to the site of cotyledon initiation. This could suggest that protocorms have an auxin organizing center and that auxin plays an integral role in orchid germination by facilitating shoot development through PAT post-embryogenesis (Novak et al. 2014). Overall, the role of auxins produced by RAB and their potential to induce growth benefits are still unclear.

The purpose of this study is to: 1) isolate and compare the endophytic auxin-producing bacteria in three different *Spiranthes* species, 2) determine the genus of auxin-positive bacteria using 16s rRNA sequencing, 3) track bacterial localization within root structures using fluorescently labelled cells, and 4) perform *in vitro* symbiotic germination experiments with *Spiranthes* and *Arabidopsis* seeds to uncover potential benefits for growing orchids *in vitro* with auxin-producing bacteria.

METHODS

Root Collection

Roots from three *S. cernua*, *S. magnicamporum*, and *S. vernalis* orchids were collected from populations in Illinois while the plants were flowering. Each species was collected from populations within 30 miles of each other (Table 1). Single roots were collected in sterile falcon tubes, stored at 4°C and processed within 48 hours of collection.

Root Surface Sterilization for Bacterial Collection

Roots were initially rinsed with DI water to remove residual soil. Roots were then surface sterilized with a 5:5:90 sterilization solution of alcohol (95% ethanol), bleach (7.5% sodium hypochlorite), and DI autoclaved water for two minutes with shaking. After sterilization, the roots were washed three consecutive times with DI water for one minute with shaking.

Isolation of Root Associated Bacteria

Surface-sterilized root samples were crushed using a sterile mortar and pestle with 5 mL of sterile DI water. A series of tenfold dilutions were carried out six times with each root sample. After this, 50 µL of each dilution was

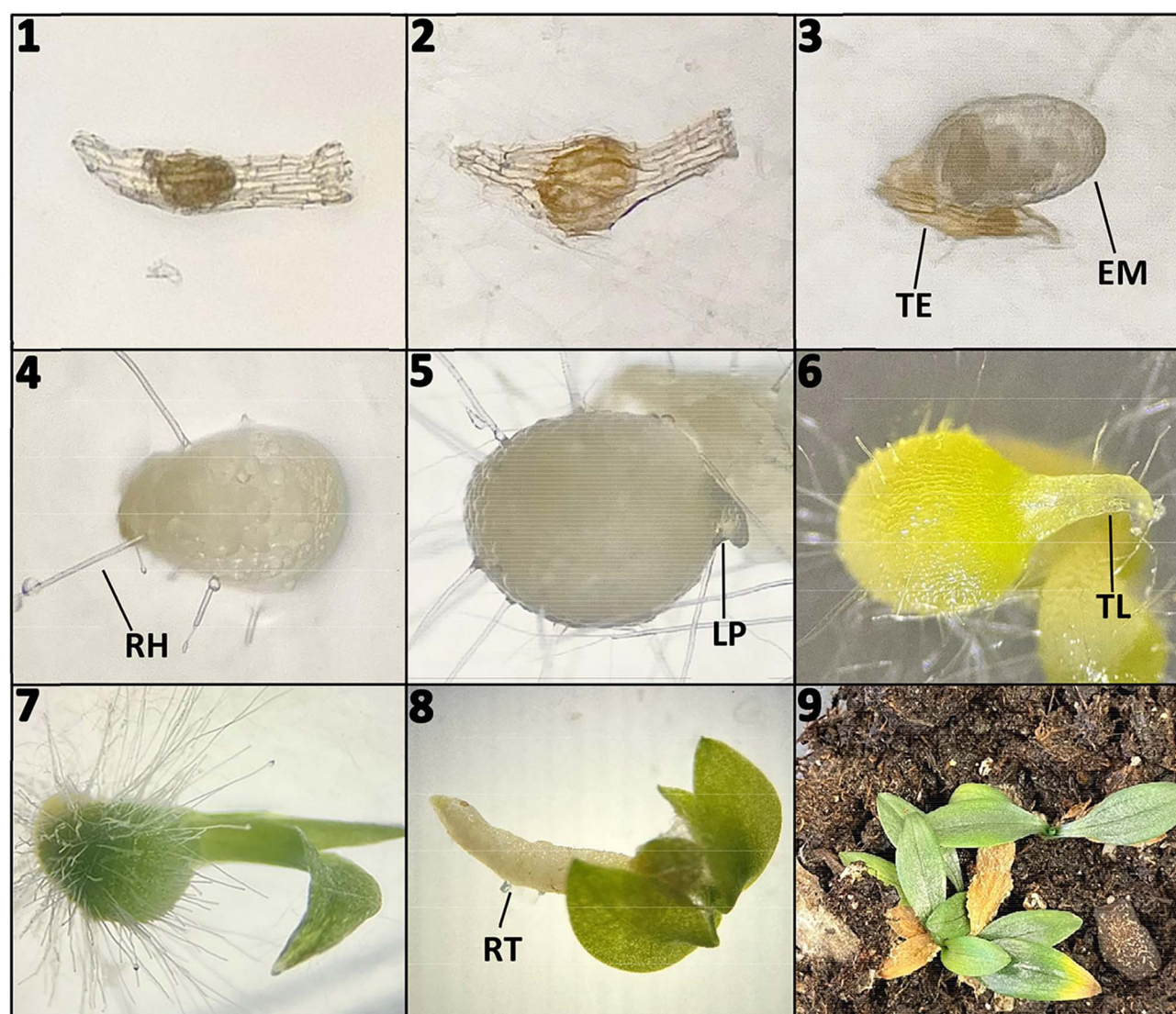


Figure 2. *Spiranthes* germination stages: 1. No germination; 2. Embryo swells; 3. Germination- swollen embryo (EM) ruptures testa (TE); 4. Protocorm produces rhizoids (RH); 5. Emergence of leaf primordium (LP); 6. Development of first true leaf (TL); 7. Elongation of leaves; 8. Emergence of roots (RT); 9. Orchids established in soil.

plated on BD™ Bacto™ Tryptic Soy Agar (TSA) plates and left to incubate at ambient temperature (21°C-23°C). Newly formed bacterial colonies were streaked on new TSA plates every 24 hours. Isolated colonies were grown in BD™ Bacto™ Tryptic Soy Broth (TSB) for 12 hours at 115 rpm at ambient temperature (21°C-23°C). Colonies cultured in TSB were mixed with glycerin and frozen at –80°C for storage.

Auxin Quantification

Bacteria were screened for auxin production using a colorimetric analysis assay. Colorimetric analysis is utilized to quantify an unknown concentration of a particular compound in a sample with the aid of a color reagent. In

this case, colorimetric analysis is being used to determine the concentration of auxin in RAB supernatant samples and Salkowski reagent is the color reagent. Salkowski reagent reacts with auxin to produce a pink color change which can be measured and compared to a set of standards with known auxin concentrations to determine the amount of auxin in a sample.

Individual bacterial colonies were grown in TSB for 12 hours at 30°C with shaking at 115 rpm. Samples were diluted to an OD₆₀₀ of 1.0 with sterile TSB. Following this, 100 µL of each diluted sample was used to inoculate 100 mL of TSB cultures in triplicate. These were incubated at 30°C at 115 rpm for 48 hours. One mL was centrifuged at 1000 xg for 5 minutes to remove cell bodies and 100 µL of supernatant were transferred to a 96 well

Table 1: Sample collection information. Information regarding the location identity and collection dates for root samples.

Orchid Species	Location	Collection Dates
<i>S. cernua</i>	Pocahontas, IL	September 24, 2022
<i>S. magnicamporum</i>	Edwardsville, IL	September 20, 2022
<i>S. vernalis</i>	Edwardsville, IL	July 14, 2022

plate. A standard curve was made by adding 3-Indoleacetic acid (IAA) (Sigma Life Science, St. Louis, MO) to TSB to create the following concentrations: 3.75, 7.5, 15, 30, 60 µg/mL. Salkowski reagent (50 ml of 35% HClO₄ and 1 ml of 0.5 M FeCl₃) was added to each well in a 2:1 (v/v) ratio. The plate was incubated at ambient temperature for 60 minutes, followed by Abs 530 nm readings. The linear trendline constructed from the colorimetric curve was used to quantify the amount of auxin concentration using absorbance values.

Sequencing of Root Associated Bacteria

DNA was extracted from auxin-positive colonies using the DNeasy® PowerSoil Pro® Kit (Qiagen). After extraction, the Extract-N-Amp™ Tissue PCR Kit (Sigma-Aldrich) was used to prep samples for PCR Amplification. The following primers were used to amplify the 16s rRNA gene: 16F27 Forward= 5'- AGAGTTTGATCMTGGCT-CAG -3' and 16R1492 Reverse=5'- TACGGYTACCTTG TTACGACTT -3' (Heuer et al., 1997). The parameters of the thermocycler were set to run one cycle at 94°C for 5 minutes, 35 cycles at 90°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute, and one cycle at 72°C for 4 minutes. The PCR products were cleaned with the Zymo Research DNA Clean & Concentrator™ kit and sent to the Core DNA Sequencing Facility at the University of Illinois, Urbana-Champaign for sequencing. The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) was used to identify the bacterial isolates that produced auxin (Altschul et al., 1990).

Creating Green Fluorescent Protein Labelled Bacteria Using the Triparental Mating Method

Green Fluorescent Protein (GFP) is a protein that was originally isolated from jellyfish and is often used to label microorganisms as it produces a detectable phenotype (green fluorescence) when the protein is exposed to light in the blue to ultraviolet range. Therefore, bacteria were created using a triparental conjugation system to insert a mini-Tn7 transposon containing P_{lac}:GFP into auxin producing RAB. Donor strains of *Escherichia coli* WM3064 containing the plasmid pURR25 (mini-Tn7-KSGFP; Teal et al., 2006) or pUX-BF13 (Tn7 transposase; Bao et al. 1991) were grown in TSB supplemented with 100 µg/mL Ampicillin (Amp) and 300 µg/mL 2,6-Diaminopimelic acid (DAP). The recipient auxin producing bacteria were

cultured in regular TSB with no supplements. All samples were grown for 12 hours at 30°C. *E. coli* pURR25 and *E. coli* pUX-BF13 were subsequently diluted to 0.1 A in TSB supplemented with 300 µg/mL DAP and recipient bacteria were diluted to 0.1 A in TSB. Samples were incubated at 30°C for three hours with shaking at 115 rpm to reach log phase. 1 mL of each culture was harvested and mixed as follows: Recipient only (negative control); *E. coli* pURR25 only (negative control); *E. coli* pUX-BF13 only (negative control); Recipient + *E. coli* pURR25 + *E. coli* pUX-BF13 (positive control) and centrifuged at 10,000 rpm for 1 min to pellet. The supernatant was removed, leaving about 20 µL of residual media which was used to resuspend and spot the pellet onto TSA plates supplemented with 200 µg/mL DAP for a 12-hour incubation at ambient temperature (21°C-23°C). Bacterial spots were then collected in 1 mL of TSB and 200 µL of each were spread onto TSA plates supplemented with 20 µg/mL kanamycin (Kan). Plates were incubated at ambient temperature (21°C-23°C) for three days until colonies developed. GFP positive colonies were identified using a transilluminator, restreaked on new TSA plates, cultured in TSB and frozen with 20% glycerol at -80°C for storage. To confirm the recipient identity of GFP positive colonies, sequencing was performed as described above.

Inoculation of Orchids with GFP Labelled Bacteria

Roots of soil-established two-year-old *S. magnicamporum* orchids were sterilized as described above. Only *S. magnicamporum* orchids were used in this particular experiment due to their availability. GFP-labelled *Enterobacter* spp. were grown in TSB and incubated at 30°C at 115 rpm for 48 hours. Each bacterial culture was placed in a centrifuge tube and pelleted at 1000xg for five minutes. The supernatant was decanted, and the bacterial pellet was resuspended in fresh TSB and sterile water in a 1:1 (v/v) ratio. The *S. magnicamporum* roots were then submerged in the diluted TSB media for 10 minutes at 115 rpm. Plants were transplanted into closed plastic containers with sterilized soil. After the initial transplant, 1 mL of bacterial broth was pipetted into the soil near the rhizosphere of each transplant. Root cross sections were prepared after 48-96 hours and fluorescently imaged using a Leica DM50000 B microscope. Brightfield and fluorescent images were merged using ImageJ.

Seed Collection and Processing

Spiranthes magnicamporum and *S. cernua* inflorescences were collected from the same root collection sites described above when flowers were wilted and seed capsules had begun to turn slightly brown. The inflorescences were placed in a 15 mL falcon tube without a lid. Tubes were placed in a mason jar containing approximately 6 cm of WiseSorbent Technology Indicating Silica Gel beads. Inflorescences were stored in a closed jar for a minimum of 10 days to remove moisture. Under a dissecting microscope, 2-3 capsules were placed on a piece of paper. Sterile forceps and scalpels were used to open capsules followed by manual shaking to dispense all seeds. Seeds were placed in a glass tube with a lid and parafilm. Individual jars were then placed in a falcon tube filled a fourth of the way with silica beads. Tubes were stored in -20°C freezer.

Preparation of OMA Plates Supplemented with Bacterial Auxin

Tryptic soy broth was inoculated with frozen bacterial isolates 3,4,5, and 8 and grown at 30°C with shaking at 115 rpm for 12 hours. Isolate concentration was then measured at 600 nm and normalized to 1 A by diluting in sterile TSB. Following this, 100 μL of diluted bacterial sample was added to 100 mL of Oatmeal Broth (OMB) media with 200 $\mu\text{g/mL}$ L(-)-Tryptophan, 99% (Acros Organics). Isolate cultures were collected after 48 hours of growth with shaking at 115 rpm and 30°C . Cultures were then placed in 50 mL tubes centrifuged at 1000 G for 5 minutes to collect supernatant. The pH of the supernatants was adjusted to 6.0 using HCl and NaOH and BactoTM Agar was added to a final concentration of 1%. Supernatants were then autoclaved for 20 minutes and poured into petri dishes to make plates for germination studies. These plates were wrapped in aluminum foil and stored at 4°C to avoid deterioration of auxin by light and temperature.

Colorimetric Analysis of Bacterial Auxin Before and After Autoclaving

To determine if autoclaving would degrade bacterial auxin, each isolate was grown as described above. Supernatant from the culture was then tested for auxin concentration before and after a 20-minute autoclave exposure. To make blanks and standards for colorimetric analysis, OMB (pH 6) media supplemented with 200 $\mu\text{g/mL}$ L(-)-Tryptophan, 99% (Acros Organics) was used.

Orchid Seed Sterilization and Sowing

About 200 *S. magnicamporum* seeds collected from Edwardsville, Illinois and *S. cernua* seeds collected from Pochontas, Illinois were placed on filter paper that was folded and stapled. Packets were then placed in a clean container with 1 drop Tween/100 mL DI water. Seeds were agitated at 112 rpm using an orbital shaker for 10 minutes while soaking to eliminate surface tension.

Packets were then removed and placed in a vessel with 0.5% NADCC (Sodium Dichloroisocyanurate) and 1 drop of Tween/100 mL. The seeds were agitated for 30 minutes in the bleach solution and subsequent processing was carried out in a biological safety cabinet to ensure sterility. Packets were removed and put into sterile DI water with manual shaking for three minutes to dilute remaining NADCC. Packets were then cut open with sterile scalpels using forceps to stabilize them. Seeds were sowed on Oatmeal agar (OMA) plates supplemented with bacterial supernatant, OMA plates with 1 cm. fungus (positive control), and OMA plates without fungi. The fungus used in the positive control plates was *Ceratobasidium* collected from Stoddard County, MO from *Spiranthes praecox* in 2018 by Caleb Dvorak. Plates were then wrapped in aluminum foil and stored at ambient temperature (21°C - 23°C). Germination scores were assigned after 90 days using a dissection microscope to examine seeds.

Arabidopsis Seed Sterilization, Sowing, and Root Analysis

Wildtype (Col-0) *Arabidopsis* seeds were submerged in 1 mL of a 30% bleach (7.5% sodium hypochlorite) and 0.1% Tween20 solution and mixed for 15 minutes horizontally on a vortex. Seeds were then pelleted by centrifuging at 6,000 G for 10 seconds and the supernatant was replaced with sterile dd H₂O water. This wash procedure was repeated three times for a total of four rinses. For the last step, the water was not decanted and kept in the tube. The tube was covered with aluminum foil and placed at 4°C for 72 hours before sowing on $\frac{1}{2}$ Murashige and Skoog (MS) plates (Caisson Labs) (0.8% BactoTM Agar, pH = 5.8, and 1% sucrose), OMA plates (pH = 6), and OMA plates containing supernatant from isolates 3, 4, 5, and 8. For each plate, 10 seeds were sowed in a single row 0.7 cm apart. Plates were incubated at ambient temperature (21°C - 23°C) for 12 hours in the dark, uncovered, and then placed vertically in a growth chamber with a 18:6 L/D cycle. Photos of the plates were captured every three days using a Canon EOS Rebel T3i Digital SLR Camera with EF-S 18-55mm f/3.5-5.6 IS lens. Photos were then analyzed using ImageJ to measure primary root length.

RESULTS

Isolation of RAB and Quantification of Auxin Production in *Spiranthes* Orchids

To determine if any *Spiranthes*-associated endophytic bacteria were capable of auxin production, RAB were isolated from three species of *Spiranthes* orchids and tested with Salkowski reagent to identify auxin production. A total of 15 isolates were found to produce auxin. Of these, six belonged to the *Enterobacter* genus, six belonged to the *Agrobacteria* genus, two belonged to the *Pantoea* genus, and one belonged to the *Bacillus* genus as determined from 16S rRNA sequence analysis (Table 2). *Enterobacter* isolated from *S. cernua* produced the most auxin with an average

Table 2: Auxin-producing bacteria isolated from *Spiranthes* roots. Top NCBI nucleotide BLAST scores for each bacterial isolate that produced auxin. Isolate 1 served as a negative control and did not produce auxin.

Isolate Number	Species Isolated From	BLAST Description	Bit Score	E Value	Percent Identity	Query Cover	Accession
1	<i>S. magnicamporum</i>	<i>Bacillus cereus</i> strain yasmun8	1,443.35	0	96.9	100	OK632087
2	<i>S. magnicamporum</i>	<i>Enterobacter huaxiensis</i> strain WCHdHu090008	1,238.38	0	100.0	100	OP818075
3	<i>S. magnicamporum</i>	<i>Pantoea agglomerans</i> strain DAPP-PG734	1,264.23	0	100.0	100	OW970315
4	<i>S. magnicamporum</i>	<i>Enterobacter huaxiensis</i> strain WCHdHu090008	1,284.54	0	99.9	100	OP818075
5	<i>S. magnicamporum</i>	<i>Pantoea agglomerans</i> strain DBM 3797	1,275.31	0	99.3	100	CP086133
6	<i>S. magnicamporum</i>	<i>Enterobacter huaxiensis</i> strain WCHdHu090008	1,415.65	0	99.6	100	OP818075
7	<i>S. cernua</i>	<i>Enterobacter Cloacae</i> isolate 96	1,389.80	0	100.0	100	OW969784
8	<i>S. cernua</i>	<i>Enterobacter Cloacae</i> isolate 732	1,308.55	0	100.0	100	OW969624
9	<i>S. cernua</i>	<i>Enterobacter Cloacae</i> isolate 96	1,424.89	0	100.0	100	OW969784
10	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> P8-19	1,788.68	0	100.0	100	MN181152
11	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> strain RB-076	1,781.29	0	100.0	100	MT453929
12	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> strain GU-RP21	1,714.81	0	100.0	100	OQ421733
13	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> strain GU-RP21	1,554.15	0	100.0	100	OQ421733
14	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> strain P8-19	1,899.48	0	100.0	100	MN181152
15	<i>S. vernalis</i>	<i>Bacillus manihonensis</i> strain 12L-2	1,790.52	0	100.0	100	KT720103
16	<i>S. vernalis</i>	<i>Agrobacterium tumefaciens</i> strain GU-RP21	1,779.44	0	99.9	100	OQ421733

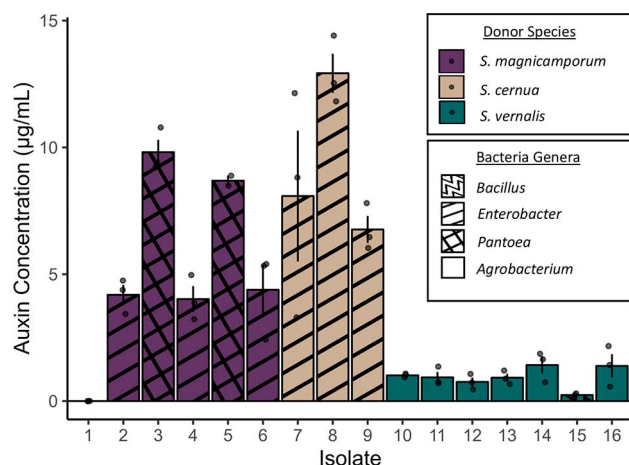


Figure 3. Auxin quantification in RAB supernatant. Amount of auxin produced by each bacterial isolate grown in tryptic soy broth for 48 hours at 30°C. Black dots represent the average of three technical replicates. Each bar represents an average of three biological replicates. Bar color corresponds to *Spiranthes* species from which the isolates were identified. Isolate 1 served as a negative control and did not produce auxin. Bars represent \pm standard error of the three biological replicates.

concentration of 12.92 µg/mL. In comparison, the lowest auxin-producing RAB belonged to the *Bacillus* genus isolated from *S. vernalis*. Supernatant from this strain showed an average auxin concentration of 0.24 µg/mL (Fig. 3). Notably in *S. cernua*, all auxin-producing isolates belonged to the *Enterobacter* genus, while *Agrobacteria* were commonly found in *S. vernalis* specimens. *Spiranthes magnicamporum* orchids housed a mixture of *Enterobacter* and *Pantoea* bacteria (Table 2).

Localization of Auxin Producing Root-Associated Bacteria in Roots

To determine the location of auxin-producing isolates in *S. magnicamporum* roots, isolates two and four were transformed with a constitutively expressed GFP gene. Both isolates were *Enterobacter* sp. with a 99.85% 16S rRNA sequence identity. After 48 hours, fluorescent microscopy revealed that these bacteria colonized root hairs, epidermal layers, the cortex, and steles (Fig. 4). Notably, a high concentration of bacteria was observed colonizing root hairs, while a low concentration of bacteria was observed colonizing root steles.

Impact of Auxin by *Spiranthes* RAB on Orchid Germination

To determine if the auxin produced by the bacterial isolates was biologically active and could impact germination or root growth, *S. magnicamporum* and *S. cernua* were grown on agar plates supplemented with bacterial culture supernatant. The auxin concentrations of supernatants from individual isolates grown in oatmeal broth

was determined using colorimetric analysis before and after being autoclaved. These analyses indicated that the supernatant from all isolates still appeared to contain comparable levels of auxin before and after sterilization, suggesting that the auxin is stable during this process. Specifically, supernatant from isolate 4 contained the most auxin with an average of around 33 µg/mL (Fig. 5a). Slightly higher concentrations post-autoclaving may be due to evaporation. Increased concentrations of auxin in autoclaved supernatant compared to initial quantification of auxin in samples is most likely contributed to the addition of tryptophan in culture broth.

To examine if auxin produced by the isolates could improve orchid germination in the absence of a fungal symbiont, *S. magnicamporum* and *S. cernua* seeds were sown on supernatant-supplemented plates. For *S. magnicamporum* germination, the OMA bacterial supernatant plates containing auxin were comparable to the negative control which had seeds placed on plain OMA plates with no fungal symbiont (Fig. 5b). The positive control plates containing a known fungal symbiont had the highest rate of germinated seeds with 21.7% of seeds reaching the germination stage. Seeds grown on plates made with supernatant from isolate 8 contained the most germinated seeds (6.7%) among isolate plates that germinated; however, rhizoid production was not observed. *Spiranthes cernua* germination plates performed similarly with isolate 8 plates showing the most seeds in stage three (2.7%). However, *S. cernua* seeds grown on plates with a fungal symbiont only resulted in 2 out of 197 seeds successfully germinating, suggesting that this overall germination approach may not be suitable for this species. All isolate plates were also comparable to the negative control that did not contain a fungal symbiont, suggesting that in the absence of mycorrhizal fungi, the auxin produced by these bacteria do not impact germination (Fig. 5c).

Impact of RAB Auxin on *Arabidopsis* Germination and Root Growth

To determine if the auxin produced by the RAB was biologically active, *Arabidopsis* germination and root growth were quantified on agar plates supplemented with RAB supernatant. *Arabidopsis* was utilized because of its high germination reliability, quick root development compared to *Spiranthes* orchids, and the ample amount of auxin-related literature on its growth. *Arabidopsis* seeds plated on OMA bacterial supernatant plates containing RAB supernatant had comparable germination results to the control plates (OMA and 1/2MS) (Fig. 5d). Notably, 1/2 MS plates displayed 100% seed germination while OMA and all other isolate plates displayed a 90% germination rate. Seeds grown on isolate 8 plates displayed the lowest germination rates at 84.2%. Nine days after sowing seeds on auxin plates, the primary root lengths of *Arabidopsis* seedlings were an average of 2.5 cm when grown on 0.5x MS media. Plants grown on OMA media resulted in root lengths that averaged 0.5 cm. All plants grown on isolate plates (containing bacterial supernatant grown in OMA

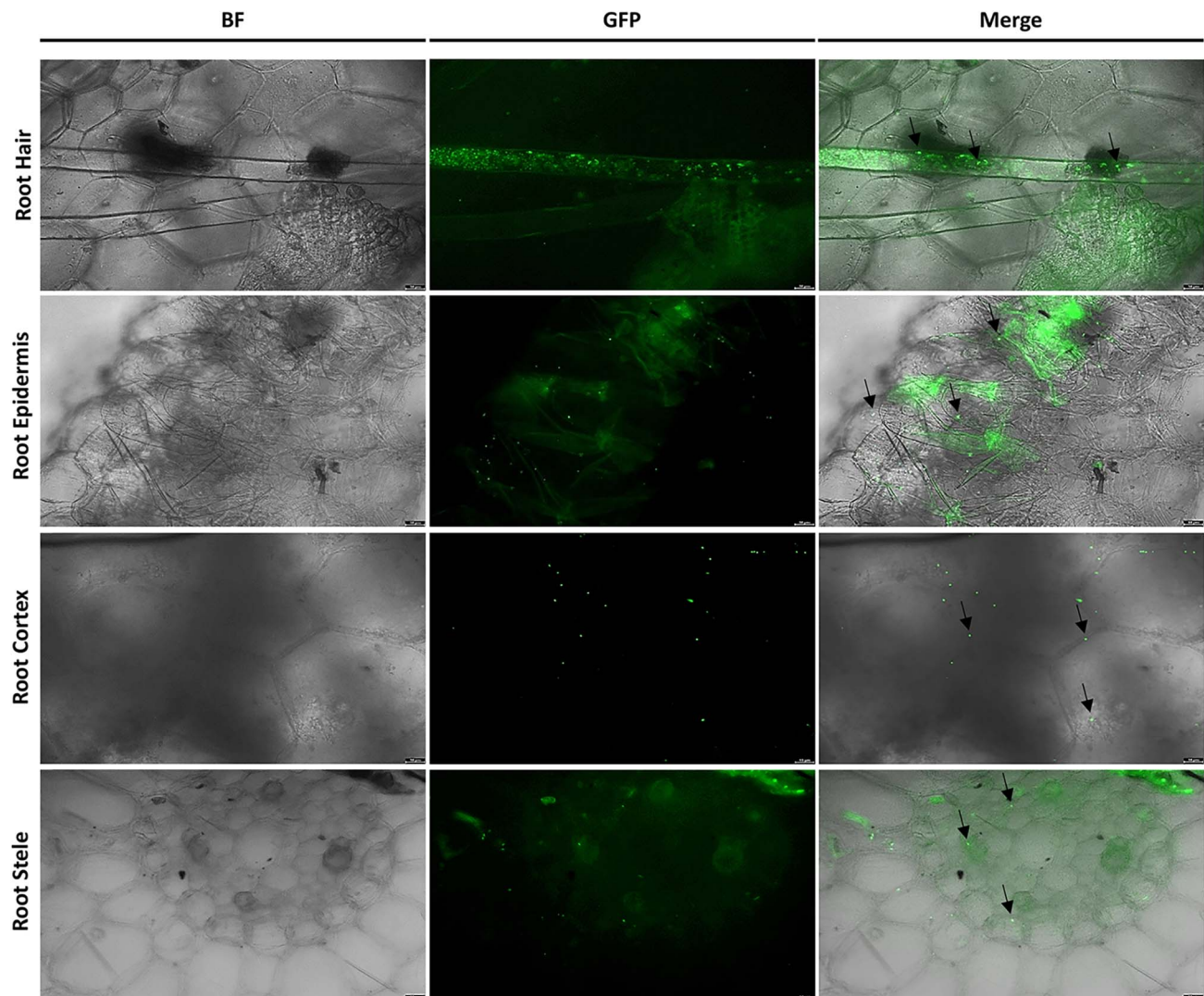


Figure 4. Infection of *S. magnicamporum* plants with GFP-labelled auxin producing RAB. *Enterobacter* sp. expressing GFP was introduced into *S. magnicamporum* plants and analyzed using fluorescent microscopy. Black arrows represent *Enterobacter* sp. that successfully infiltrated the roots. Scale bars present in each image are 15 µm. The image columns are labelled BF (images generated using bright-field microscopy), GFP (images generated using fluorescent microscopy which detects GFP labelled bacteria), and Merge (BF and GFP images are combined).

media) resulted in root lengths that were less than 0.2 cm in length and were significantly different than the root lengths of plants grown on OMA media as determined by a one-way ANOVA test ($p < 0.05$) (Fig. 5e), suggesting that the auxin produced by the RAB is active and able to influence *Arabidopsis* growth.

DISCUSSION

Colorimetric Analysis of RAB for Auxin Quantification

Auxin assays involving colorimetric quantification revealed endophytic microbes that were auxin-positive in *Spiranthes* roots. *Pantoea agglomerans* and *Enterobacter*

cloacae were high-auxin producers while *Agrobacterium tumefaciens* and *Bacillus manliponensis* were low-auxin producers. It should be noted that while auxin compounds were produced by isolates from *Spiranthes* orchids, the specific types of auxins were not deduced. Various research shows that common auxin compounds produced by RAB from orchids are indole-3-lactic acid (ILA), indole-3-acetaldehyde (IAAld), indole-3-propionic acid (IPA), and IAA (Tsavkelova et al. 2007b; Júnior et al. 2011). Previous research using height performance liquid chromatography (HPLC) suggests that *Enterobacter* isolates are primarily producing IAA and IPA, while *Bacillus* isolates are primarily producing ILA (Júnior et al. 2011).

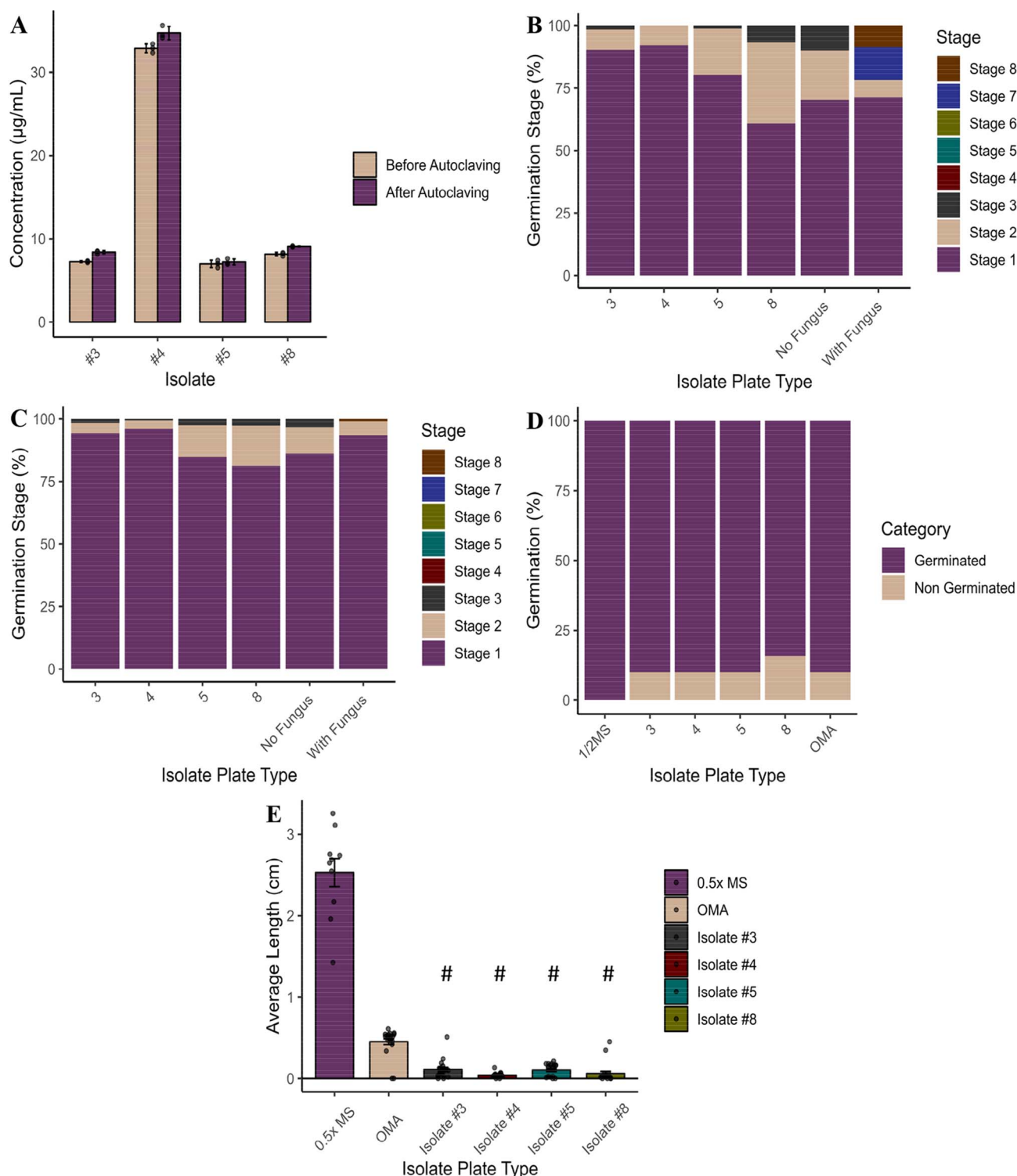


Figure 5. Impact of auxin-producing RAB on *Spiranthes* and *Arabidopsis* germination and growth: A) Concentration of auxin present before and after supernatant from isolates was autoclaved for 20 minutes. For each isolate tested, $n = 3$; B) Percent germination of *S. mangicamporum* seeds grown on plates supplemented with RAB culture supernatant. Stages correspond to the stages represented in Figure 2. Therefore, true seed germination occurs at stage three. For isolate plate type 3 ($n = 135$), 4 ($n = 102$), 5 ($n = 258$), 8 ($n = 253$), No Fungus ($n = 91$), With Fungus ($n = 129$); C) Percent germination of *S. cernua* seeds grown on plates supplemented with RAB culture supernatant. For isolate plate

A limitation of using colorimetric analysis with Salkowski reagent to quantify auxin production is that IAA concentrations have been observed to be as much as 19 times greater than HPLC detection methods. This discrepancy could be attributed to the addition of tryptophan, an IAA precursor, often added in other studies to increase IAA production by RAB or by the accumulation of other indole compounds which are sensitive to Salkowski reagent (Tsavkelova et al. 2007b). Therefore, IAA levels determined using Salkowski reagent are likely inflated, with the functional auxin produced at levels lower than reported. In future studies, HPLC quantification could improve IAA concentration accuracy. (Sachdev et al., 2009; Júnior et al., 2011).

Characterization of RAB that Produce Auxin in *Spiranthes* Orchids

The breadth of auxin-producing RAB reported here is consistent with orchids collected from Vietnam and Brazil (Tsavkelova et al. 2007a; Júnior et al. 2011). However, these results are not directly comparable as many of these RAB were found in orchid species that were epiphytic, while our research identified *Enterobacter*, *Agrobacterium*, and *Pantoea* in terrestrial species. Thus, this could suggest that various orchid species, despite type and location, share a common auxin-producing endophytic profile. These findings further implicate the need for RAB to be characterized for conservation purposes as their use could be implemented across several orchid species. This would offer great advantages compared to conservation efforts that use fungal symbionts, which can be species dependent.

Location of Auxin-Producing RAB in *S. magnicamporum* Plants

Isolated auxin-producing RAB, specifically *Enterobacter* sp., was confirmed to be present endophytically within *Spiranthes* roots. Large concentrations of bacteria colonizing root hairs suggest this route as a common source of entry. *Enterobacter* was also discovered to colonize cortical cells but appeared to be in greater abundance in cortical cell walls. These results support previous research in which GFP labelled *Pseudomonas*, another RAB commonly found in terrestrial orchids, greatly colonized root hairs and cortical intercellular spaces of olive roots (Prieto et al. 2011; Alibrandi et al. 2020). Our results also found that *Enterobacter*

was present in small abundances within the root stele supporting prior research detailing *Enterobacter* sp. colonization in *Brassica oleracea* (Tanaka et al. 2006).

Impact of Auxin produced by RAB on Plant Growth

Plants are known to produce a wide variety of auxins, not all of which show the same levels of activity and modes of regulation (Zhao 2010). While the likely function of bacterial-produced auxin is to somehow regulate plant growth and development in a way that benefits the bacteria, compounds reacting with the Salkowski reagent are not necessarily biologically active in plants. As a quick test to determine if this auxin is biologically active, we chose an *Arabidopsis* root-growth assay because of the well-established root-growth-inhibition phenotype caused by excess auxin in growth media (Evans et al. 1994). Indeed, addition of the RAB supernatant to growth media suppressed almost all root growth, suggesting that these compounds are active in plants. In spite of this, the RAB supernatant failed to have an impact on germination in either *Spiranthes* or *Arabidopsis*. This was not surprising for *Arabidopsis*, as auxin has not been shown to have much impact on its germination (Liu et al. 2013). While previous work in other orchid species has suggested that auxin-producing RAB can have a positive impact on germination, our results did not support this (Tsavkelova et al. 2007b; Tsavkelova et al. 2016). Conversely, our results were loosely supported by conflicting research which has shown that exogenous application of auxin upregulates production of Absciscic Acid (ABA), a plant hormone that delays germination (Shuai et al. 2017; Liu et al. 2013). The concentration of IAA present in the supernatant plates may have also played a role in deterring germination in *Spiranthes* orchids. Evident by past research on orchids, IAA concentrations that are too high or too low can have a negative impact on protocorm diameter size and first leaf emergence (Novak and Whitehouse 2013). Past studies on orchids with increased germination rates due to RAB utilized living bacteria, while in this study bacteria were killed by autoclaving and only the supernatant was collected and used for germination experiments. These bacteria could therefore be producing other metabolites that could benefit germination other than auxin (Tsavkelova et al. 2007b). When considering that complicated germination requirements present a barrier to efficient orchid conservation efforts, improvements

Figure 5. Continued. type 3 (n = 125), 4 (n = 153), 5 (n = 197), 8 (n = 75), No Fungus (n = 122), With Fungus (n = 197); D) Percent germination of *Arabidopsis* seeds grown on plates supplemented with the supernatant of auxin producing bacteria. For each treatment, n = 20, except for plants grown on ½ MS media (n=10); E) Average primary root lengths of *Arabidopsis* plants grown on plates containing the supernatant of auxin producing bacteria. For each treatment, n = 20, except for plants grown on ½ MS media (n = 10). Error bars represent +/- standard error. The # represents a significant decrease in primary root length compared to the OMA control as determined by one-way ANOVA test (p < 0.05).

promoted by auxin-producing RAB would be welcomed in the field. Unfortunately, the isolates reported here do not appear to have a beneficial impact on *Spiranthes* germination, although this does not preclude eventual discovery of beneficial impacts of this microbe-plant association in other species of orchids.

CONCLUSIONS

Root associated bacteria that produced auxin were found endophytically within the roots of *Spiranthes* orchids. GFP-labelled RAB confirmed that bacteria could colonize root structures and suggested root hairs as a common source of entry. While culture supernatant from these bacteria did not appear to promote germination in *S. magnicamporum*, *S. cernua*, or *Arabidopsis*, they did significantly decrease the length of primary roots in *Arabidopsis*, suggesting biological activity in plants. While more tests would need to be conducted to confirm that the auxin produced by the RAB was directly impacting root growth, these results are consistent with prior research on exogenous application of auxin in plants. Overall, these results outline the need to examine root microbes in concurrence with mycorrhizal fungi for conservation purposes.

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LITERATURE CITED

Afzal, I., Z.K. Shinwari, S. Sikandar, and S. Shahzad. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research* 221:36–49.

Alibrandi, P., S. Schnell, S. Perotto, and M. Cardinale. 2020. Diversity and structure of the endophytic bacterial communities associated with three terrestrial orchid species as revealed by 16S rRNA gene metabarcoding. *Frontiers in Microbiology* 11:604964.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410.

Bao, Y., D.P. Lies, H. Fu, and G.P. Roberts. 1991. An improved Tn7-based system for the single-copy insertion of cloned genes into chromosomes of gram-negative bacteria. *Gene* 109:167–168.

Barthlott, W., B. Grosse-Veldmann, and N. Korotkova. 2014. Orchid Seed Diversity: A Scanning Electron Microscopy Survey. Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin.

Brock, A.K., B. Berger, I. Mewis, and S. Ruppel. 2013. Impact of the PGPB *Enterobacter radicincitans* DSM 16656 on growth, glucosinolate profile, and immune

responses of *Arabidopsis thaliana*. *Microbial Ecology* 65:661–670.

Catling, P.M. 1982. Breeding systems of northeastern North American *Spiranthes* (Orchidaceae). *Canadian Journal of Botany* 60:3017–3039.

Dueck, L.A., D. Aygoren, and K.M. Cameron. 2014. A molecular framework for understanding the phylogeny of *Spiranthes* (Orchidaceae), a cosmopolitan genus with a North American center of diversity. *American Journal of Botany* 101:1551–1571.

Evans, M.L., H. Ishikawa, and M.A. Estelle. 1994. Responses of *Arabidopsis* roots to auxin studied with high temporal resolution: Comparison of wild type and auxin-response mutants. *Planta* 194:215–222.

Glaeser, S.P., J. Imani, I. Alabid, H. Guo, N. Kumar, P. Kämpfer, M. Hardt, J. Blom, A. Goesmann, M. Rothballer, A. Hartmann, and K. Kogel. 2016. Non-pathogenic *Rhizobium radiobacter* F4 deploys plant beneficial activity independent of its host *Pisiformospora indica*. *The ISME Journal* 10:871–884.

Gupta, G., J. Panwar, and P.N. Jha. 2013. Natural occurrence of *Pseudomonas aeruginosa*, a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br. *Applied Soil Ecology* 64:252–261.

Heuer, H., M. Krsek, P. Baker, K. Smalla, and E.M. Wellington. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology* 63:3233–3241.

Júnior, R.F., E.A.N. Pedrinho, T.C.L. Castellane, and E.G.M. Lemos. 2011. Auxin-producing bacteria isolated from the roots of *Cattleya walkeriana*, an endangered Brazilian orchid, and their role in root acclimatization. *Brazilian Journal of Soil Science* 35: 729–737.

Kaur, J. and J. Sharma. 2021. Orchid root associated bacteria: linchpins or accessories? *Frontiers in Plant Science* 12:1–13.

Li, O., R. Xiao, L. Sun, C. Guan, D. Kong, and X. Hu. 2017. Bacterial and diazotrophic diversities of endophytes in *Dendrobium catenatum* determined through barcoded pyrosequencing. *PLOS ONE* 12:1–21.

Lim, W.L. and C.S. Loh. 2003. Endopolyploidy in *Vanda* Miss Joaquim (Orchidaceae). *New Phytologist* 159: 279–287.

Liu, X., H. Zhang, Y. Zhao, Z. Feng, Q. Li, H. Yang, S. Luan, J.Li, and Z. He. 2013. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in *Arabidopsis*. *Proceedings of the National Academy of Sciences* 110:15485–15490.

McCormick, M.K., D.F. Whigham, D. Sloan, K. O'Malley, and B. Hodkinson. 2006. Orchid–fungus fidelity: A Marriage Meant to last? *Ecology* 87:903–911.

Mohammadi, K., S. Kholesro, Y. Sohrabi, and G. Heidari. 2011. A Review: Beneficial Effects of the Mycorrhizal

- Fungi for Plant Growth. *Journal of Applied Environmental and Biological Sciences* 9:310–319.
- NatureServe. 2022. *NatureServe Network Biodiversity Location Data*. <https://explorer.natureserve.org/>. Accessed 09 August 2022.
- Novak, S.D. and G.A. Whitehouse. 2013. Auxin regulates first leaf development and promotes the formation of protocorm trichomes and rhizome-like structures in developing seedlings of *Spathoglottis plicata* (Orchidaceae). *AoB Plants* 5:1–12.
- Novak, S.D., L.J. Luna, and R.N. Gamage. 2014. Role of auxin in orchid development. *Plant Signaling & Behavior* 9:1–8.
- Pace, M.C. and K.M. Cameron. 2017. The Systematics of the *Spiranthes cernua* Species Complex (Orchidaceae): Untangling the Gordian Knot. *Systematic Botany* 42:1–30.
- Prieto, P., E. Schilirò, M.M. Maldonado-González, R. Valderrama, J.B. Barroso-Albarracín, and J. Mercado-Blanco. 2011. Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. *Microbial Ecology* 62: 435–445.
- Rajkumar, M., N. Ae, and H. Freitas. 2009. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. *Chemosphere* 77:153–160.
- Sachdev, D.P., H.G. Chaudhari, V.M. Kasture, D.D. Dhavale, and B.A. Chopade. 2009. Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. *Indian Journal of Experimental Biology* 47:993–1000.
- Shuai, H., Y. Meng, X. Luo, F. Chen, W. Zhou, Y. Dai, Y. Qi, J. Du, F. Yang, J. Liu, W. Yang, and K. Shu. 2017. Exogenous auxin represses soybean seed germination through decreasing the gibberellin/abscisic acid (GA/ABA) ratio. *Scientific Reports* 7:12620.
- Tanaka, K., T. Shimizu, M. Zakria, J. Njoloma, Y. Saeki, M. Sakai, T. Yamakawa, K. Minamisawa, and S. Akao. 2006. Incorporation of a DNA Sequence Encoding Green Fluorescent Protein (GFP) into Endophytic Diazotroph from Sugarcane and Sweet Potato and the Colonizing Ability of these Bacteria in *Brassica oleracea*. *Microbes and Environments* 21:122–128.
- Teal, T.K., D.P. Lies, B.J. Wold, and D.K. Newman. 2006. Spatiometabolic Stratification of *Shewanella oneidensis* Biofilms. *Applied and Environmental Microbiology* 72:7324–7330.
- Teale, W., I. Paponov, and K. Palme. 2006. Auxin in action: Signaling, transport and the control of plant growth and development. *Nature Reviews. Molecular Cell Biology* 7:847–859.
- Tsavkelova, E.A., T.A. Cherdynseva, S.G. Botina, and A.I. Netrusov. 2007a. Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research* 162:69–76.
- Tsavkelova, E.A., T.A. Cherdynseva, S.Yu. Klimova, A.I. Shestakov, S.G. Botina, and A.I. Netrusov. 2007b. Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. *Archives of Microbiology* 188:655–664.
- Tsavkelova, E.A., M.A. Egorova, M.R. Leontieva, S.G. Malakho, G.L. Kolomeitseva, and A.I. Netrusov. 2016. *Dendrobium nobile* Lindl. seed germination in co-cultures with diverse associated bacteria. *Plant Growth Regulation* 80:79–91.
- Yang, S., X. Zhang, Z. Cao, K. Zhao, S. Wang, M. Chen, and X. Hu. 2014. Growth-promoting *Sphingomonas paucimobilis* ZJSH1 associated with *Dendrobium officinale* through phytohormone production and nitrogen fixation. *Microbial Biotechnology* 7:611–620.
- Yeung, E.C. 2017. A perspective on orchid seed and protocorm development. *Botanical Studies* 58:33.
- Zettler, L. and T. McInnis. 1993. Symbiotic seed germination and development of *Spiranthes cernua* and *Goodyera pubescens* (Orchidaceae: Spiranthoideae). *Lindleyana* 8:155–162.
- Zhao, Y. 2010. Auxin biosynthesis and its role in plant development. *Annual Review of Plant Biology* 61:49.

RELATIONSHIPS BETWEEN FOREST COVER AND CANEBRAKES IN SOUTHERN ILLINOIS

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ABSTRACT: Giant cane (*Arundinaria gigantea* [Walt.] Muhl.) is a woody perennial bamboo species native to the southeastern United States. Giant cane forms monodominant ecosystems, known as “canebrakes”, that primarily occur in riparian areas and bottomland hardwood forests. Due to land conversion and alteration of disturbance regimes, canebrake ecosystems have drastically declined, and remnant stands now mainly persist as forest understory and edge vegetation. Although canebrake restoration efforts are increasing, information on the current conditions of canebrake remnants remains sparse. We assessed the relationships between forest characteristics and canebrake growth (i.e., culm density, culm height, and canebrake area) using generalized linear mixed effect models. Data were collected at 47 sites during June–August, 2023 in southern Illinois, USA. Canebrake area was negatively associated with overstory basal area, while midstory height and percentage herbaceous cover was positively associated with increased canebrake area. Cane culm density was negatively associated with leaf litter depth and percentage canopy cover, while culm density was positively associated with understory stem density. Culm height was negatively associated with midstory height, midstory density, understory density, and overstory density, while culm height was positively related to overstory basal area and percentage herbaceous cover. Understanding the relationship between canebrake conditions and forest characteristics can aid in determining which forest components should be managed to increase canebrake growth and vigor. Our findings corroborate previous studies indicating that overtopping forest tree competition is associated with limited giant cane growth, which emphasizes the importance of overstory management for canebrake conservation and restoration.

INTRODUCTION

Giant cane (*Arundinaria gigantea* [Walt.] Muhl.) is a woody perennial bamboo species native to the United States (Tucker 1988; Ward 2009; Triplett et al. 2010). Its distribution extends across 22 southeastern states from Maryland to eastern Oklahoma, southward to east Texas, and eastward to Florida (Marsh 1977). Giant cane occurs in almost any landcover type from dense forest to open lands mostly along hydric riverbanks and streams (Marsh 1977), but is also found on mesic, xeric, and sub-xeric upland sites (Platt 1999). It can form vigorous monodominant communities known as “canebreaks”, characterized by dense aboveground culms and mats of spreading underground rhizomes in more open areas with few associated

trees. Giant cane also occurs in less dense stands of reduced health and vigor in the understory of closed-canopy forests. Canebrakes vary in area and extent; they may occur in discrete patches or in continuous patches that extend over a large area (Gagnon and Platt 2008). However, the literature does not suggest a universally-accepted definition of the area, density, and ecosystem functionality of a cane patch that qualifies it as a canebrake. For this study, we referred to giant cane patches that were approximately 100 m² or larger as canebrakes.

Historically, canebrakes occupied a vast area throughout the southeastern United States; however, conversion of wetlands to agriculture and suppression of disturbances reduced suitability of habitats for giant cane growth (LMVJV 2007; Shoemaker 2018). Currently, less than 2% of canebrake ecosystems remains from their pre-settlement distribution and typically persist as forest understory and edge vegetation in Bottomland Hardwood Forests (BLH) or along fencelines (Marsh 1977; Noss et al. 1995; Brantley and Platt 2001).

Giant cane provides many ecological and cultural benefits. Historically, giant cane was an important part of the Native American culture and livelihood (Platt et al. 2009). Ecologically, canebrakes serve as a potential buffer

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species by improving soil and water quality, increasing soil infiltration rate, and enhancing soil organic matter content (Schoonover et al. 2005, 2010; Blattel et al. 2009; Singh et al. 2018). Moreover, canebrakes support a diverse wildlife community (Platt et al. 2001, 2013; Geise 2011). Due to the cultural importance and ecological services provided by giant cane, interest in the restoration of canebrake habitat has increased, particularly for mitigating erosion along streams and riverbanks, improving water quality, and providing habitat for terrestrial and aquatic organisms (NRCS 2021).

Although the effective area and quality of a canebrake to support various wildlife species is not well understood, historical canebrake conditions (i.e., dense and extensive) provided refuge for many wildlife species (Brantley and Platt 2001). In addition, dense understory vegetation including canebrakes are known to increase prey abundance and provide refuge for breeding, nesting, and foraging of many bird species (Wilson and Twedt 2003; Horn et al. 2005; Moorman et al. 2012). Understanding how giant cane growth and vigor is influenced by surrounding habitat is necessary to enhance canebrake characteristics to meet specific management goals.

In Illinois, much research has been done on propagation techniques for the reestablishment and expansion of existing canebrake stands (e.g., Zaczek et al. 2004; Brendecke and Zaczek 2008; Schoonover et al. 2011); however, information on the status of remnant canebrakes, which are fragmented and usually occur as less dense understory vegetation, remains sparse. In addition, giant cane is listed as a conservation target as a natural community in the Illinois Comprehensive Wildlife Conservation Plan (IDNR 2005) and The Joint Venture Partnership's Cache River Wetlands Site Conservation Plan (Bouska et al. 2012), emphasizing a need for appropriate and effective management actions to restore and to enhance remaining canebrake habitat.

Given the loss and fragmentation of BLH forests and canebrake habitats, obtaining information on the current distribution and characteristics of the remaining canebrakes, such as area and vigor, could aid in determining its ecological value and resiliency as sparse understory vegetation. Cane growth and survivorship within associated forest cover is positively affected by disturbances such as fire and windstorm forest blowdown which leads to an increase in light intensity from canopy gaps and reduction in woody competition (Gagnon and Platt 2008). Growth and vigor of giant cane respond positively to increased light intensity and nutrient availability such as nitrogen (Cirtain et al. 2009). Understanding canebrake status in relationship with associated forest cover characteristics could guide canebrake management practices to meet restoration and rehabilitation goals in promoting growth of remnant canebrakes by alteration of forest overstory conditions.

We aimed to understand the relationships between forest cover and canebrakes in southern Illinois. Specifically, we compared characteristics of canebrakes (i.e., density, height, and area) to forest characteristics that influence light intensity and nutrient availability (e.g., potential

competitions and indicators of site quality) in southern Illinois. Forest characteristics associated with low light intensity such as high percentage canopy cover, leaf litter, and tree density were hypothesized to negatively influence canebrake density, height, and area (Gagnon et al. 2007; Gagnon and Platt 2008). In addition, we hypothesized that the presence of other herbaceous and woody shrub species could compete for nutrient and light by overtopping cane (Brantley and Platt 2001; Shoemaker 2018), and therefore, negatively affect cane growth.

MATERIAL AND METHODS

Study Area

Forty-seven study sites with established canebrakes of at least 100 m² in area were chosen in southwestern Illinois, USA, across Jackson, Union, Alexander, and Pulaski counties (Figure 1). In addition, canebrakes were required to be on public land due to permits and were not planted. Twenty-five, 7, and 15 study sites were located within the Cypress Creek National Wildlife Refuge, the Shawnee National Forest, and lands managed by the Illinois Department of Natural Resources, respectively. Forty-one sites were located within the non-glaciated area, while the other six study sites were located within the glaciated area and included a coal mine reclamation site, where mining operation concluded in 1992. Prescribed fire was included as a management action during the early stage of land restoration at the coal mine reclamation site. Although we did not observe any signs of burning at our study sites, prescribed burning was observed regionally within the large public land units. Dominant vegetation communities were oak-hickory forest associations. Haymond-Petrolia-Karnak soils, which form clay-sandy alluvium in floodplains drainages under deciduous forests (Fehrenbacher et al. 1984), were characteristic of study sites. Mean annual temperature of the study area is 15 °C, reaching 27 °C during the hottest month and 2 °C during the coldest month and the mean annual precipitation is 120 cm, with the growing season during April–October (Fehrenbacher et al. 1984; Robeson 2002).

Data Collection

Surveys were conducted during June–August 2023 at 47 sites. At the start of data collection in June, flooding from heavy rains in May had subsided and the emergence of new culms occurred across study sites. At each site, 10 m × 10 m sample plots were established in the center and at the edge of canebrake; sample plots were 30 m apart, measuring from the edge of the plots. If the canebrake was too small to fit multiple sample plots (ranging from 1–3 plots/site), only one sample plot was established at the center of the canebrake. Thirty-two sites contained only 1 sample plot, 10 sites contained 2 sample plots, and 5 sites contained 3 sample plots. We surveyed a total of 62 sample plots across all sites.

At each sample plot, we measured 11 site-specific variables that were hypothesized to affect cane growth (i.e., nutrient availability and light intensity [Brantley and Platt

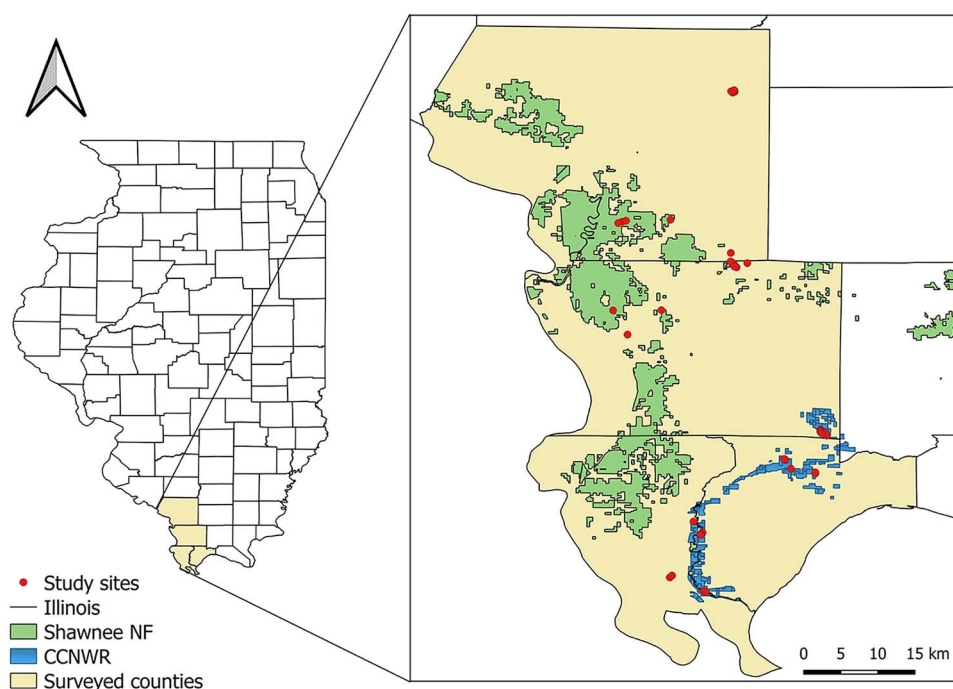


Figure 1. Map of 47 study sites (red dots) surveyed during June–August 2023 across Jackson, Union, Alexander, and Pulaski Counties in southern Illinois, USA. Study sites were located within Cypress Creek National Wildlife Refuge (CCNWR), Shawnee National Forest (Shawnee NF), and areas managed by the Illinois Department of Natural Resources (IDNR).

2001; Gagnon and Platt 2008; Cirtain et al. 2009; Shoemaker 2018]) including tree canopy cover (%), tree density (stems/ha, i.e., overstory, midstory, and understory), overstory tree basal area (m^2/ha), tree height in the overstory, midstory, and understory strata (m), percentage cover of shrub species (woody vegetation with < 1 m height), percentage cover of herbaceous plants, and leaf litter depth (mm). Trees were grouped into three categories: understory (< 3 m height), midstory (3–9 m height), and overstory (> 9 m height). Measurements were collected for trees that were within the boundary of sample plots; trees along the border of a sample plot were included if more than half of the base was within the sample plot. For each tree category, stem density (stems/ha) was calculated by multiplying the number of total stems counted within a sample plot ($10 \text{ m} \times 10 \text{ m}$) by 100. Tree height to the nearest m was calculated by averaging the height of trees for each category within the plot using a laser range finder (Pariyar and Mandal 2019). Tree diameter at breast height (DBH) was measured 1.37 m above ground to the nearest cm using a diameter tape for trees in plots. Basal area (m^2/ha) was calculated for each tree using the formula for the area of a circle, $\pi \cdot (\text{DBH}/2)^2$ and summed by groups of overstory trees. Percentage tree canopy cover (overstory and midstory trees) was measured to the nearest 1.0% using a convex spherical densiometer at four cardinal directions around the center of the sample plot, and the mean was taken among all four directions (Lemmon 1956).

Within each sample plot, we established 5 subplots and followed the Daubenmire method (Daubenmire 1959) within a 1 m^2 -quadrat to estimate percentage of shrub and herbaceous cover at the center of the sample plot and at 5-m distances from the center in the four cardinal directions. Six cover classes were used: 0–5%, 5–25%, 25–50%, 50–75%, 75–95%, and 95–100% (Daubenmire 1959). Leaf litter depth was measured using a ruler to the nearest mm at the center of the sample plot and at 5-m distances in the four cardinal directions from the center and averaged for each plot. Giant cane culm density (stems/ m^2) was measured using a 1 m^2 -quadrat at the center of the sampling plot and at 5-m distances from the center in four cardinal directions. In addition, culm height (cm) was measured using a meter stick to the nearest cm and averaged for all older (> 1 year) live culms among 5 quadrats. Density (culms/ m^2) of first-year culms, newly emerged culms with the presence of culm sheath, older culms, and dead culms were determined within each quadrat. The area of each canebrake was also measured (to the nearest m^2) by walking the perimeter of the site with a GPS-unit to delineate a polygon.

Statistical Analyses

To assess the relationships between forest variables and cane growth, we used generalized linear mixed-effect models with gamma distribution and log-link function in R packages ‘lme4’ (Bates et al. 2009). We tested 3 models that we determined to be measures of cane growth: 1)

Table 1: Summary forest and canebrake measurements taken during June–August 2023 across 62 sample plots in southern Illinois, USA.

Forest variables	min	max	mean (standard deviation)
Percentage tree canopy cover (%)	66.8	99.8	95.1 (8.2)
Overstory density (stems/ha)	0	800	114.5 (199.9)
Overstory basal area (m ² /ha)	0	147.2	40.3 (37.9)
Overstory height (m)	9.1	38.0	15.3 (5.5)
Midstory density (stems/ha)	0	1,000	350 (232.4)
Midstory basal area (m ² /ha)	0	53.0	4.8 (8.2)
Midstory height (m)	3.7	9.0	6.3 (1.6)
Understory density (stems/ha)	0	1,000	396 (271.3)
Understory height (m)	2.0	3.0	2.7 (0.5)
Percentage cover of shrub (%)	0	17.0	0.6 (2.5)
Percentage cover of herbaceous (%)	0	49.5	13.4 (11.7)
Leaf litter depth (mm)	0	39.6	8.9 (9.3)
Canebrake variables			
Live culm density (culms/m ²)	1.2	24	6.0 (4.1)
First-year culm density (culms/m ²)	0	5.6	0.7 (1.2)
Old culm density (culms/m ²)	0.8	20.8	5.3 (3.5)
Dead culm density (culms/m ²)	0	12.6	2.05 (2.0)
Culm height (cm)	46.5	372.5	116.9 (57.1)
Canebrake area (m ²)	104.3	24,726.3	1,602.0 (4,100.2)

culm density (live culms, both first-year and old culms), 2) culm height (old live culms only as new culms may have not fully expanded in height and diameter for plots measured in early summer vs those in late summer), and 3) canebrake area. We treated site as a random effect to account for variations between sites which could affect giant cane growth pattern. We used a generalized linear model with the gamma distribution and log-link function to fit a model to assess the relationship between forest variables and the area of canebrake at each site. The forest variable measurements were averaged among the sample plots for each site in the canebrake area model.

Prior to running the analyses, highly correlated variables ($|r| \geq 0.7$) were eliminated from further consideration based on a Pearson's correlation test. We found a strong correlation between understory tree height and overstory stem density ($r = 0.7$); therefore, we removed understory tree height from the predictor variables due to its minimal biological effect on light availability for giant cane. Additionally, due to a low average percentage shrub cover ($< 0.6\%$) across our sample plots where shrubs were present, we removed percentage shrub cover as a predictor variable. All variables were standardized to a mean of 0 and standard deviation of 1 and outliers (i.e., data points that fell below or above the upper and lower bounds based on the length of 1.5 times the interquartile range) were removed prior to analysis (Tukey 1977). For the canebrake area analysis, the sample size was 37 sites after outliers were removed. For the culm density and culm height analyses, the sample size was 60 sample plots

after outliers were removed. We used backward elimination approach for model selection as described in Zuur et al. (2009). A variable with the highest p-value was eliminated, then the process was repeated until all remaining variables were significant ($p < 0.05$). Significant variables were then included in the final model. In addition, for each final model, we calculated r-squared values to assess model explanatory performance. Relative importance of predictors were calculated for each model using the package 'glmm.hp' (Lai et al. 2022). All statistical analyses were performed in R version 4.2.3 (R Core Team 2024).

RESULTS

Canebrakes were found in small discrete patches and in larger areas on continuous tracts of land (Table 1). They varied in size and morphology across our study sites, ranging from an area of 104 m² to 24,726 m² ($\bar{x} = 1,602.1$ m²), a live culm density range of 1.2–24.0 culm/m² ($\bar{x} = 6.0$ culm/m²) and culm height that ranged from 46.5 cm to 372.5 cm ($\bar{x} = 116.9$ cm; Table 1). The differences in numbers of live culms and dead culms varied across sites; three sites had greater numbers of dead versus live culms. Canebrakes also differed in regeneration rate, by which the percentages of new culms in comparison to the total culms (dead and live culms) ranged from 0.0 to 42.9% ($\bar{x} = 8.2\%$). Canebrakes were found in locations that were forested or forest-adjacent with a wide range of overstory tree densities, from no trees to 800 stems/ha (Table 1).

Table 2: Significant forest variables based on the final models for: 1) canebrake area, 2) culm density, and 3) culm height using backward-elimination approach for model selection. Marginal (R^2_m ; variances explained only by the fixed effects) and conditional r-squared (R^2_c ; variances explained by the fixed effects and random effects) values are reported for each model. Relative importance of each variable is represented by the marginal R^2 .

Variables	Coefficient	Standard error	P-value	R^2
Canebrake area ($R^2_m = 0.37$)				
Intercept	5.94	0.08		
Percentage herbaceous cover	0.16	0.08	0.060	0.07
Understory density	0.22	0.08	<0.001	0.10
Overstory basal area	-0.27	0.07	<0.001	0.20
Culm density ($R^2_m = 0.33$, $R^2_c = 0.86$)				
Intercept	1.43	0.14		
Percentage canopy cover	-0.33	0.09	<0.001	0.10
Understory density	0.25	0.06	<0.001	0.10
Leaf litter depth	-0.37	0.10	<0.001	0.13
Culm height ($R^2_m = 0.26$, $R^2_c = 0.88$)				
Intercept	4.61	0.08		
Percentage herbaceous cover	0.08	0.03	<0.001	0.03
Midstory height	-0.06	0.03	<0.001	0.02
Overstory basal area	0.09	0.02	<0.001	0.03
Midstory density	-0.06	0.02	<0.001	0.03
Understory density	-0.07	0.03	<0.001	0.04
Overstory density	-0.15	0.05	0.020	0.11

Other forest-based measurements relating to understory, midstory, and overstory characteristics also varied across sample plots (Table 1).

For the canebrake area analysis, three variables were among the top model including overstory basal area, percentage herbaceous cover, and understory density, of which two were statistically significant predictors (p -value < 0.05; Table 2). Of these variables, overstory

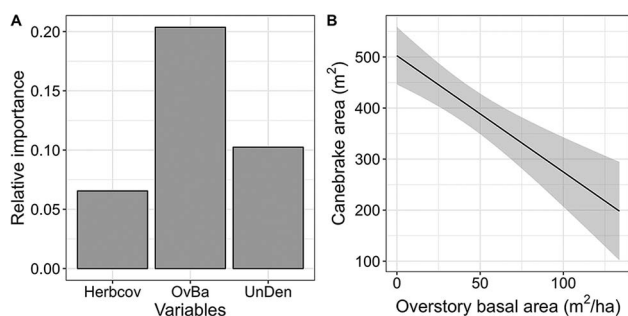


Figure 2. A) Relative importance of individual predictors within the top models for predicting canebrake area (m^2) including percentage herbaceous cover (HerbCov), understory density (UnDen), and overstory basal area (OvBa). B) Predicted plot with standard errors showing the effect of overstory basal area, which was a predictor with the highest relative importance value (R^2) on canebrake area (m^2), using the 'add_ci' function in R.

basal area was negatively associated with canebrake area and accounted for the most variation ($R^2 = 20\%$; Figure 2). Understory density and percentage herbaceous cover were positively associated with increased canebrake area, together accounting for $\sim 17\%$ of the variation (Figure 2a).

For culm density analysis, three explanatory variables were statistically significant (p -value < 0.05; Table 2), including percentage canopy cover, understory density, and leaf litter depth. Cane culm density was negatively associated with leaf litter depth and percentage canopy cover accounting for a total $\sim 23\%$ of the culm density variation ($R^2 = 13\%$ and 10% , respectively; Figure 3). Understory density was positively associated with culm density accounting for $\sim 10\%$ of the variation (Figure 3a).

For culm height analysis, six variables were statistically significant (p -value < 0.05; Table 2). Height predictors including percentage herbaceous cover, midstory height, overstory basal area, midstory density, understory density, and overstory density together accounted for 26% of the variation in culm height (Figure 4). Culm height was negatively associated with most variables, accounting for 20% of the variation (Table 2). Only overstory basal area and percentage herbaceous cover were positively related to culm height.

DISCUSSION

Historically, canebrakes occupied hundreds of thousands of hectares throughout the southeastern United States

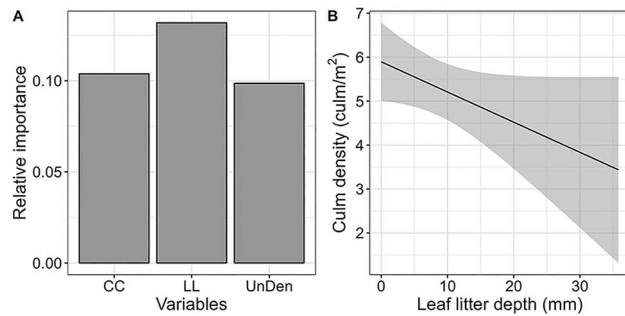


Figure 3. A) Relative importance of individual predictors within the top models for predicting culm density (culm/m²) including percentage canopy cover (CC), leaf litter depth (LL), and understory density (UnDen). B) Predicted plot with standard errors showing the effect of leaf litter depth (mm), which was a predictor with the highest relative importance value (R^2) on culm density (culm/m²), using the 'add_ci' function in R.

including southern Illinois (Brantley and Platt 2001). Roosevelt (1908) described canebrakes in Louisiana as going on for “miles”. However, the southern Illinois cane patches observed in this study were relatively small with a mean of 0.16 ha. Canebrake area reduction is thought to be a result of overgrazing from livestock, agricultural land conversion, and lack of the historical disturbance regime which included fire and flooding (Marsh 1977; Platt and Brantley 1997; Brantley and Platt 2001). Though it is not well understood how large a cane patch must be to be ecologically functional, most giant cane in southern Illinois has been relegated to scattered patches adjacent to agricultural fields along stream edges, fence lines, roadsides, ditches, and under and adjacent to forest stands. Giant cane stands growing in riparian zones can function to reduce excess nutrients such as nitrate in groundwater by 90% in the first 3.3 m

from an agricultural field edge (Schoonover and Williard 2003; Schoonover et al. 2005). Geise (2011) found a diverse community of invertebrates inhabiting canebrake of less than 0.1 ha. However, more research is needed to evaluate how large a patch or stand of cane needs to qualify as an ecologically functional canebrake.

We documented several forest characteristics explaining 37%, 34% and 26% of the variation in canebrake area, cane culm density, and culm height, respectively. However, no single forest characteristic affected all cane growth parameters consistently. The relationships between giant cane and individual forest factors, though significantly related, did not alone account for more than 20% of the variation which was the case for overstory basal area and canebrake area. Canebrake area decreased as forest basal area increased, indicating that more intact and less disturbed forests limits canebrake area. Forests with high levels of basal area are likely to be old and more structurally developed, have high levels of canopy cover, and have not recently received substantial canopy-level disturbance to open large gaps in the overstory. However, it is important to note that the basal area values reported in our study were extrapolated from a small subplot size; therefore, they were sensitive to the presence of any large trees (>20 DBH) and might not represent the characteristics of the forest stands. Although interpretation of the forest structure should be done with caution, the relationship between canebrake characteristics and relative basal area among sites still represented the relationship between large overtopping trees and small canebrake habitat. Disturbances such as periodic fires and windstorms that reduce forest canopy cover and thus increase understory light levels and reduce below-ground competition are important for canebrake growth and vigor given giant cane is a disturbance-dependent species (Marsh 1977; Gagnon and Platt 2008; Certain et al. 2009).

Although some sample plots did not contain any overstory trees, thus zero basal area, all plots had canopy cover recorded which resulted from nearby overstory and midstory trees as indicated by a minimum sample plot

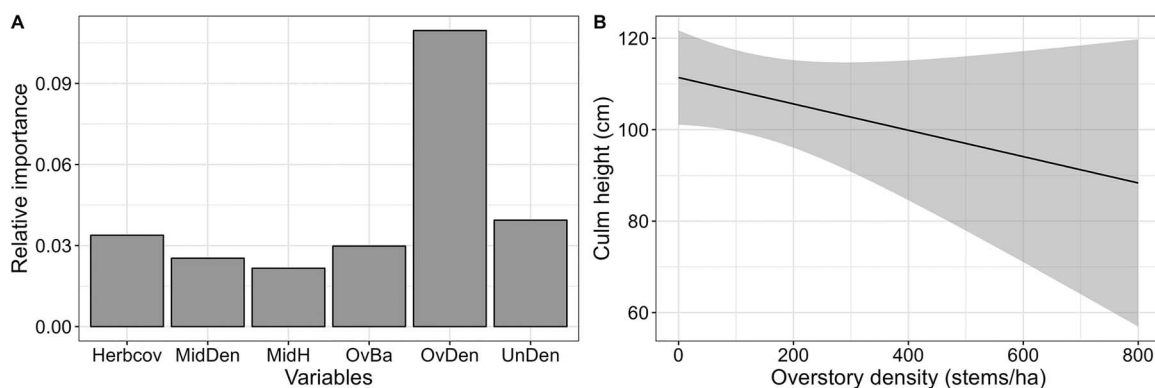


Figure 4. A) Relative importance of individual predictors within the top models for predicting culm height (cm) including percentage herbaceous cover (Herbcov), midstory density (MidDen), midstory height (MidH), overstory basal area (OvBa), overstory density (OvDen), and understory density (UnDen). B) Predicted plot with standard errors showing the effect of overstory density (stems/ha), which was a predictor with the highest relative importance value (R^2) on culm height (cm), using the 'add_ci' function in R.

canopy cover of 66.8%. Culm density was negatively associated with canopy cover. Closed canopy limits light availability, which could inhibit cane growth (Cirtain et al. 2003; Gagnon et al. 2007). Additionally, culm density was negatively related to leaf litter depth. The result was not unexpected as leaf litter depth would have inputs from overstory, midstory, and understory trees, which could also reflect the amount of light availability. Cane culm density was low with a mean of 6.04 culms/m² compared to 16.5 culms/m² for 11 other remnant cane patches of unknown ages growing in riparian zones with scattered tree cover adjacent to agricultural fields in southern Illinois (Anderson 2014). Additionally, in the region within a single location, a mean culm density of 21.8 \pm 3.0 culms/m² was recorded across 60 plots distributed within an 11-year-old 0.2 ha giant cane nursery with little to no canopy cover at Southern Illinois University (Ganden, unpublished data). Moreover, we found that only 10% of the variation in culm density was associated with percentage canopy cover, so other unmeasured factors influence culm density. Cane height was negatively associated by increased overstory, understory, and midstory density and midstory height accounting for 20% of the variation. In our study, mean cane height was 116.9 cm which is less than 241.4 cm at the open-grown cane nursery at Southern Illinois University (Ganden, unpublished data) and 242.7 cm in Anderson's (2014) study.

There were mixed but weak correlations between cane growth and understory factors. Canebrake area was positively associated with increased understory density and percentage herbaceous cover. Also, cane density was positively related to understory density suggesting that conditions favorable for cane density also benefit other woody understory plants. This was expected because areas with increased light level promote cane culm production (Gagnon et al. 2007) as well as understory vegetation growth, which could explain the positive trends between understory density, herbaceous cover, and culm density observed in our study.

Regeneration of new culms is important in determining persistence and vigor of canebrakes. Although we did not directly study the regeneration rates of cane culms, we observed 3 sites with more dead culms than live culms. In addition, we also observed 8 sites with no new culms at the time of our surveys. Large numbers of dead culms and relatively low number of new culms observed (8.18%) could indicate nutrient limitation at our sites (Zaczek et al. 2010). Increasing nutrient and light availability and reducing competition through prescribed burning can promote new culm growth and emergence (Cirtain et al. 2003; Dattilo and Rhoades 2005; Zaczek et al. 2010). In addition, large numbers of dead culms could be a result of flowering events. Although sexual reproductive ecology of giant cane is not well-studied, it is inconsistent and unpredictable characterized by long intervals of vegetative growth with monocarpic flowering events that may occur every 20 to 25 years or more (Hughes 1951; Marsh 1977; Gagnon and Platt 2008). Habitat fragmentation also

creates an additional barrier to cane regeneration; culms within a stand often belonged to a single clone with low seed viability and low rate of germination, perhaps due to self-incompatibility (Mathews et al. 2009).

In this study, the area of cane patches and the density and height of giant cane culms were mainly limited by overtopping overstory forest tree competition. Because we only measured these stands once, we cannot definitively state that forest competition limited the area or spread of cane patches. However, it is likely that expansion of cane patches was limited by associated forest cover since cane vigor (height and density) was considerably lower compared to other measured open-grown cane patches in the region.

Summary and Management Recommendations

Based on our findings, management of overstory trees can improve growth of existing canebrakes. To rehabilitate remnant cane stands under dense forest cover, land managers should consider reducing forest overstory basal area and percentage canopy cover through thinning alone, prescribed fire alone or thinning and prescribed fire in combination. Timing, intensity, and frequency of fire should be considered for maintaining canebrakes (Hughes 1966; Gagnon and Platt 2008; Zaczek et al. 2010) as it is a disturbance-dependent species. For example, burning at an interval of 10 years was optimal for cane (Hughes 1966). Although prescribed fire applied to a developing 6-year-old cane restoration planting decreased culm height and diameter, it also increased culm density within the planting and spread into adjacent areas one year after treatment (Zaczek et al. 2010), thus benefitting the persistence and expansion of giant cane stands. Reduction of overstory vegetation would increase understory light levels and soil resources, improving the vigor of cane and other understory plants. Moreover, to prevent a mass die-off following flowering, propagating cane using rhizomes from multiple stands and clones can promote genetic diversity and seed viability (Mathews et al. 2009).

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LITERATURE CITED

- Anderson, M.M. 2014. Fire and fertilization effects on the growth and expansion of existing native canebrakes [*Arundinaria gigantea* (Walt) Muhl] in southern Illinois. Master thesis. Southern Illinois University, Carbondale, IL.
- Bates, D., M. Maechler, B. Bolker, S. Walker, R.H.B. Christensen, H. Singmann, B. Dai, F. Scheipl, G. Grothendieck, and P. Green. 2009. Package 'lme4'.
- Blattel, C.R., K.W. Williard, S.G. Baer, J.E. Schoonover, and J.J. Zaczek. 2009. Ground water nitrogen dynamics in giant cane and forest riparian buffers. *Castanea* 74:259–270.
- Brantley, C.G. and S.G. Platt. 2001. Canebrake conservation in the southeastern United States. *Wildlife Society Bulletin* 1:1175–1181.
- Bouska, K., K. Erndt-Pitcher, A. Lloyd, A. Nelson, and T. Stoeber. 2012. Challenges to integrating human and natural systems: An assessment of the Cache River Watershed. Reports. Southern Illinois University, Carbondale, Illinois.
- Brendecke, W.W. and J.J. Zaczek. 2008. Greenhouse and field performance of giant cane propagules from natural and planted stands. Pages 8–19 in D.F. Jacobs and C.H. Michler, technical coordinators, Proceedings of the 16th Central Hardwood Forest Conference. US Department of Agriculture, Forest Service, Northern Research Station NRS-P-24, Newtown Square, PA.
- Cirtain, M.C., J.R. Franklin, and S.B. Franklin. 2003. Effects of nutrients and shading on *Arundinaria gigantea* (Walt.) Walt. Ex. Muhl. seedling growth. Pages 49–56 in L.I. Lyle, E.W. Chester, and A.F. Scott, eds. Proceedings of the Tenth Annual Symposium on the Natural History of the Lower Tennessee and Cumberland River Valleys. The Center of Excellence for Field Biology, Austin Peay State University, Clarksville, TN.
- Cirtain, M.C., S.B. Franklin, and S.R. Pezeshki. 2009. Effect of light intensity on *Arundinaria gigantea* growth and physiology. *Castanea* 74:236–246.
- Dattilo, A.J. and C.C. Rhoades. 2005. Establishment of the woody grass *Arundinaria gigantea* for riparian restoration. *Restoration Ecology* 13:616–622.
- Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. *Northwest Science* 33:39–64.
- Fehrenbacher, J.B., K.D. Alexander, I.J. Jansen, R.G. Darmody, R.A. Pope, and M.A. Flock. 1984. Soils of Illinois. Bulletin 778, University of Illinois at Urbana-Champaign, Agricultural Experiment Station, Urbana-Champaign, IL.
- Gagnon, P.R., W.J. Platt, and E.B. Moser. 2007. Response of a native bamboo [*Arundinaria gigantea* (Walt.) Muhl.] in a wind-disturbed forest. *Forest Ecology and Management* 241:288–294.
- Gagnon, P.R. and W.J. Platt. 2008. Multiple disturbances accelerate clonal growth in a potentially monodominant bamboo. *Ecology* 89:612–618.
- Geise, J.J. 2011. The effects of habitat fragmentation on arthropod biodiversity in native canebrakes. Master thesis. University of Memphis, Memphis, TN.
- Horn, S., J.L. Hanula, M.D. Ulyshen, and J.C. Kilgo. 2005. Abundance of green tree frogs and insects in artificial canopy gaps in a bottomland hardwood forest. *The American Midland Naturalist* 153:321–326.
- Hughes, R.H. 1951. Observations of cane (*Arundinaria*) flowers, seed, and seedlings in the North Carolina coastal plain. *Bulletin of the Torrey Botanical Club* 18: 113–121.
- Hughes, R.H. 1966. Fire ecology of canebrakes. Pages 149–158 in R. Komarek, ed. Proceedings of the 5th Annual Tall Timbers Fire Ecology Conference. No. 5. Tall Timbers Research Stations, Tallahassee, FL.
- Illinois Department of Natural Resources [IDNR]. 2005. The Illinois comprehensive wildlife conservation plan & strategy: as prescribed by the wildlife conservation & restoration program and state wildlife grants program. Illinois Department of Natural Resources, Springfield, IL.
- Lai J., Y. Zou, S. Zhang, X. Zhang, and L. Mao. 2022. glmm. hp: an R package for computing individual effect of predictors in generalized linear mixed models. *Journal of Plant Ecology* 15:1302–1307.
- Lemmon, P.E. 1956. A spherical densiometer for estimating forest overstory density. *Forest Science* 2:314–320.
- Lower Mississippi Valley Joint Venture Forest Resource Conservation Working Group [LMJVJ]. 2007. Restoration, Management, and Monitoring of Forest Resources in the Mississippi Alluvial Valley: Recommendations for Enhancing Wildlife Habitat. Lower Mississippi Joint Venture, Vicksburg, MS.
- Marsh, D.L. 1977. The distribution of cane, *Arundinaria gigantea* (Poaceae: Bambusoideae). *Journal of the Arkansas Academy of Science* 31:72–74.
- Mathews, K.G., J. Huguelet, M. Lanning, T. Wilson, and R.S. Young. 2009. Clonal diversity of *Arundinaria gigantea* (Poaceae: Bambusoideae) in western North Carolina and its relationship to sexual reproduction: an assessment using AFLP fingerprints. *Castanea* 74: 213–223.
- Moorman, C.E., L.T. Bowen, J.C. Kilgo, J.L. Hanula, S. Horn, and M.D. Ulyshen. 2012. Arthropod abundance and seasonal bird use of bottomland forest harvest gaps. *The Wilson Journal of Ornithology* 124: 31–39.
- Natural Resources Conservation Service [NRCS]. 2021. Plant Materials Technical Note. No.4. Giant cane and other native bamboos: establishment and use for conservation of natural resources in the southeast. United States Department of Agriculture, Greensboro, NC.
- Noss, R.F., E.T. Laroe, III, and J.M. Scott. 1995. Biological Report 28. Endangered ecosystems of the United States: A preliminary assessment of loss and degradation. U.S. Department of Interior, National Biological Service, Washington, DC.

- Pariyar, S. and R.A. Mandal. 2019. Comparative tree height measurement using different instrument. *International Journal of Ecology and Environmental Sciences* 1:12–17.
- Platt, S.G. and C.G. Brantley. 1997. Canebrakes: an ecological and historical perspective. *Castanea* 62:8–21.
- Platt, W.J. 1999. Southeastern pine savannas. Pages 23–51 in R.C. Anderson, J.S. Fralish, and J.M. Baskin, eds. *Savannas, Barrens, and Rock Outcrop Plant Communities of North America*. Cambridge University Press, N.Y.
- Platt, S.G., C.G. Brantley, and T.R. Rainwater. 2001. Canebrake fauna: wildlife diversity in a critically endangered ecosystem. *Journal of the Elisha Mitchell Scientific Society* 1:1–9.
- Platt, S.G., C.G. Brantley, and T.R. Rainwater. 2009. Native American ethnobotany of cane (*Arundinaria* spp.) in the Southeastern United States: A Review. *Castanea* 74:271–285.
- Platt, S.G., T.R. Rainwater, R.M. Elsey, and C.G. Brantley. 2013. Canebrake fauna revisited: additional records of species diversity in a critically endangered ecosystem. *The Journal of the American Bamboo Society* 26:1–2.
- R Core Team. 2024. R: A language and environment for statistical computing R Foundation for Statistical Computing. Vienna, Austria.
- Robeson, S.M. 2002. Increasing growing-season length in Illinois during the 20th century. *Climatic Change* 52: 219–238.
- Roosevelt, T. 1908. In the Louisiana canebrakes. *Scribner's Magazine* 43:47–66.
- Schoonover, J.E., K.W. Williard, J.J. Zaczek, J.C. Mangun, and A.D. Carver. 2005. Nutrient attenuation in agricultural surface runoff by riparian buffer zones in southern Illinois, USA. *Agroforestry Systems* 64: 169–180.
- Schoonover, J.E., K.W.J. Williard, C. Blattel, and C. Yocum. 2010. The utility of giant cane as a riparian buffer species in southern Illinois agricultural landscapes. *Agroforest Systems* 80:97–107.
- Schoonover, J.E., J.L. Hartleb, J.J. Zaczek, and J.W. Groninger. 2011. Growing giant cane (*Arundinaria gigantea*) for canebrake restoration: greenhouse propagation and field trials. *Ecological Restoration* 29:234–242.
- Schoonover, J.E. and K.W.J. Williard. 2003. Ground water nitrate reduction in giant cane and forest riparian buffer zones. *Journal of the American Water Resources Association* 39:347–354.
- Shoemaker, C.M. 2018. Environmental and landscape factors affecting the continued suppression of canebrakes (*Arundinaria gigantea*, Poaceae) within restorations of bottomland hardwood forests. *The Journal of the Torrey Botanical Society* 145:156–162.
- Singh, G., J.E. Schoonover, K.W. Williard, A.L. Sweet, and J. Stewart. 2018. Giant cane vegetative buffer for improving soil and surface water quality. *Journal of environmental quality* 48:330–339.
- Triplett, J.K., K.A. Oltrogge, and L.G. Clark. 2010. Phylogenetic relationships and natural hybridization among the North American woody bamboos (Poaceae: Bambusoideae: *Arundinaria*). *American Journal of Botany* 97:471–492.
- Tucker, G.C. 1988. The genera of Bambusoideae (Gramineae) in the southeastern United States. *Journal of the Arnold Arboretum* 69:239–273.
- Tukey, J.W. 1977. *Exploratory Data Analysis*. Addison-Wesley Publisher, Reading.
- Ward, D.B. 2009. Scientific Note: *Arundinaria gigantea* and *Arundinaria macrosperma*, the Correct Names Respectively for the Switch Cane and the Giant Cane. *Castanea* 74:189–195.
- Wilson, R.R. and D.J. Twedt. 2003. Spring bird migration in Mississippi alluvial valley forests. *The American midland naturalist* 149:163–175.
- Zaczek, J.J., R.L. Sexton, K.W.J. Williard, and J.W. Groninger. 2004. Propagation of giant cane (*Arundinaria gigantea*) for riparian habitat restoration. Pages 103–106 in L.E. Riley, R.K. Dumroese, and T.D., technical coordinators. *National proceedings: Forest and Conservation Nursery Associations*. U.S. Department of Agriculture Forest Service Proceeding RMRS-P-33, Fort Collins, CO.
- Zaczek, J.J., S.G. Baer, and D.J. Dalzotto. 2010. Fire and fertilization effects on the growth and spread of rhizome-transplanted giant cane (*Arundinaria gigantea*). *Restoration Ecology* 18:462–468.
- Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G. M. Smith. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Vol. 574. Springer, New York.