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Soil and Leaf Nutrient Analysis of the Endangered Herb, *Baptisia arachnifera*, in Georgia, United States

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Authors' contributions

This work was carried out in collaboration between all authors. This study is part of a master's thesis research carried out by the primary author (RAS) and supervised by the two coauthors (LL, SS). The study was designed by all authors. Author RAS did the literature searches, conducted the experiment, performed the statistical analysis and wrote the thesis. Authors SS and LL served as major adviser and co-adviser, respectively and reformatted the thesis chapter and prepared the manuscript draft for submission. All authors read and approved the final manuscript.

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ABSTRACT

A better understanding of the soil conditions and management practices of an endangered plant may help develop improved restoration and conservation plans. Proper soil and plant nutrient management is critical for the plant's growth and health. To accomplish this goal, it is important to understand the nutrient status of the plant and the soil in which it is growing. *Baptisia arachnifera* (Hairy Rattleweed) is an endangered herbaceous legume for which basic nutrient information is not available. This species occurs only in Wayne and Brantley Counties of Georgia, United States and is found primarily in pine plantations. This study was conducted to investigate the plant and soil nutrient content of *B. arachnifera* populations. Leaf and soil samples were collected from six sites where the species was present and soil samples were collected from six sites where the species was historically absent. Samples were analyzed at the University of Georgia and Georgia Southern University, GA. Results indicated that leaf nutrients including aluminum, boron, copper, iron, manganese, sodium, and zinc ranged from 42.5–96.6, 18–33.1, 3.6–17, 48.8–79.9, 27.8–191.2, 1491.1–5964.1 and 10.6–19.9 ppm, respectively and differed significantly among sites. Differences

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were found in carbon, calcium and nitrogen, concentrations and it varied from 482–12969, 348–870 and <5–99 ppm, respectively. As most of the remaining populations exist in commercial pine plantations, timber management practices such as tillage, soil preparation, fertilizer application and harvesting may affect the nutrient status of soil and plant tissue. This study gives a baseline information about leaf nutrient content of *B. arachnifera* and relevant soil nutrient information, which may have implications to conservation and restoration strategies for this endangered species. Further research should be conducted to understand how soil nutrient availability may influence the leaf nutrient status and population distribution of *B. arachnifera.*

Keywords: Conservation; hairy rattleweed; pine; timber management.

1. INTRODUCTION

Approximately one third of the 17,000 native vascular plant species in the United States are considered endangered or threatened [1]. The southeastern coastal plain region of the United States is an ecoregion that is forested predominantly with species of oak, hickory and pine, especially longleaf pine. The longleaf pine ecosystem is home to approximately 187 species of rare plants, most of which have narrow habitat requirements [2]. One such species is *Baptisia arachnifera* (also known as hairy rattle weed). It is a federally endangered species that is endemic to only Wayne and Brantley Counties of southeast Georgia, USA (Fig. 1). Conservation efforts should focus primarily on species that are rare, as these species have a greater chance of extinction than those that are common [3-5] and *B. arachnifera* is one such species. Most of its populations exist on land that is currently managed for timber [6]. *B. arachnifera* is classified with a rarity rank of G1, S1 (globally and statewide critically imperiled with 5 or fewer occurrences or fewer than 1,000 individuals) [7]. This endangered perennial legume derives its name from the dense tomentose hairs that cover the leaves and stem [8], giving it a "cobwebby" appearance. It prefers the open pinewoods and mixed pine-hardwoods with sandy soil, common in the coastal plain of southeast Georgia [6]. *B. arachnifera* has been listed as endangered since 1978 due to loss of habitat and low numbers of individuals [6]. It has lost more than 80% of individuals in monitoring populations (in sites managed for timber) over the past 23 years [9]. All of the remaining populations are within 16 km of each other. A study [10] showed that the close proximity of the populations and the reduction in populations' sizes suggests that now separate populations may be fragments of a once more continuous gene pool.

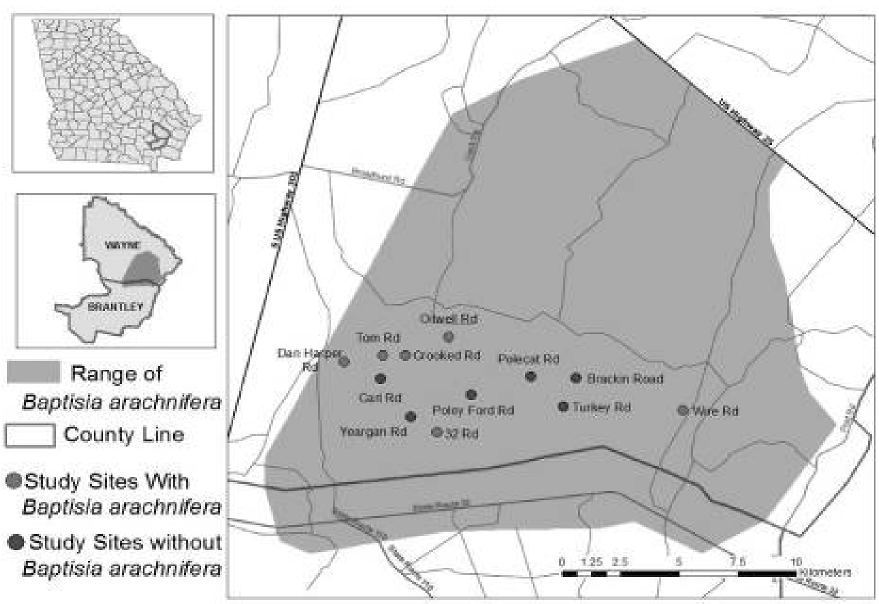

Fig. 1. Field sites with and without *Baptisia arachnifera*

To develop the recovery efforts of endangered species, it is important to study and understand the patterns of the decline of the species [11]. Discovering what causes these patterns is equally as important to the conservation efforts of this endangered species. Nutrients are required by plants for both developmental and physiological processes and deficiency of a nutrient may result in disorder, sickness and even death of plants. It is very important to understand the nutrient dynamics in the plant and soil to develop better conservation and restoration strategies. One or more nutrient(s) may play critical role(s) in growth and survival of the species. On the other hand, soils are the reservoirs and sources of the nutrients and that may determine the presence and distribution of the plant species. However, no study has measured the plant nutrient status of *B. arachnifera* and the soil in which it grows.

Timber management practices and their consequences have been shown to affect the availability of nutrients [12]. Application of fertilizer in forest stands may significantly affect nutrient levels available to plants. Five common fertilizer types are used in pine stands of the southeastern US with varying N-P-K values [13]. These include triple superphosphate (TSP, 0-46-0), diammonium phosphate (DAP, 18-46-0) for phosphorous, ammonium nitrate (34-0-0) and urea (46-0-0) for nitrogen, and muriate of potash (MOP, 0-0-60) for potassium fertilization. The amount of fertilizer applied in pine plantations varies and is usually based on a previous foliar analysis [14]. Management practices such as soil preparation and harvesting can affect nutrient availability. Soil nutrients can be lost from timber sites through the removal of biomass as well as through the increased nutrient mobilization and leaching that can occur during soil disturbance [15,16]. Both harvesting and mechanical site preparation have the potential to accelerate the mineralization of nutrients [15]. Additionally, it has been found that the clear-cutting of trees on sites may lead to increased erosion rates and/or percolation losses of nutrients in the soil [17]. Another study [18] reported that short cutting rotations and clear cutting of sites leads to increased loss of nutrients and reduction of site quality. *B. arachnifera* is a legume and in legume crop farming it has become common practice to add nutrients such as zinc, boron, copper, molybdenum and nickel to increase crop yield and increase drought tolerance [19]. Iron has been recognized as an important nutrient for nitrogen-fixing plants [20] such as *Baptisia*.

The objectives of this study were to, i) measure the leaf nutrient contents of *B. arachnifera*, ii) compare soil nutrients between the sites with and without *B. arachnifera* and iii) determine the differences in *B. arachnifera* leaf nutrients among the sites. The findings of this study may help better understand the nutrient dynamics of *B. arachnifera*, which in turn may develop better conservation and restoration strategies of this endangered species.

2. MATERIALS AND METHODS

Baptisia arachnifera leaves were collected in May 2014 from six different sites in Wayne and Brantley counties, Georgia that has a history and presence of this endangered plant (Fig. 1). Five of those sites: Tom Road (site 1), Dan Harper Road (site 2), Oilwell Road (site 3), Crooked Road (site 4), and Wire Road (site 5) are owned by a pulp and paper company, and a sixth site (Lewis Tract) is owned by the Nature Conservancy. The sites on pulp and paper Company property received management practices such as bedding, thinning, fertilizer application, spraying of herbicide, and tree harvesting and no prescribed burning. The Nature Conservancy (site 6), received treatments including sporadic burning and thinning, with no bedding, fertilizer or herbicide application. Ten mature *B. arachnifera* plants were randomly selected from each site (total 60 plants) and 10–15 recently mature leaves were collected from each plant, totaling 60 sets of 10-15 leaf samples.

Soil samples were collected from the same six sites and from six additional sites in which *B. arachnifera* was absent. These additional six sites were owned by the same pulp and paper company and had no historical record of any presence of *B. arachnifera*. These sites include Yeargan Road (site 7), Poley Ford Road (site 8), Carl Road (site 9), Polecat Road (site 10), Brackin Road (site 11) and Turkey Road (site 12) (Fig. 1).

The 12 sites exhibited a variety of soil profiles (Table 1) including mostly loamy sand with several of fine sand [21]. At each of the 12 sites, soil samples were collected from two sampling points and then mixed to make one composite sample as representative of each site. In sites with *B. arachnifera*, two plants were chosen randomly and one set of soil was sampled from a spot near to each plant.

Table 1. Location and soil types of field sites (*Baptisia arachnifera* was present in sites 1–6, and was historically absent in sites 7–12)

Site	GPS coordinates	Soil type
1	31°20'36.46" N, 81°54'00.89" W	Leefield loamy sand, Albany-Leefield complex, Mascotte find sand
2	31°20'36.21" N, 81°54'01.71" W	Albany-Leefield complex, Leefield loamy sand, Rigdon-Olustee complex
3	31°20'45.29" N, 81°53'48.43" W	Surrency mucky fine sand, Mascotte find sand
4	31°20'36.55" N, 81°53'56.60" W	Surrency mucky fine sand, Mascotte find sand
5	31°20'49.78" N, 81°46'57.96" W	Rigdon-Olustee complex, Mandarin fine sand, Leon find sand
6	31°20'19.70" N, 81°54'20.27" W	Bonifay loamy sand, Fuquay loamy sand, Leefield loamy sand, Olustee loamy fine sand, Mascotte find sand
7	31°20'33.28" N, 81°53'52.83" W	Surrency mucky fine sand, Mascotte find sand
8	31°20'31.92" N, 81°53'40.66" W	Mascotte find sand, Rigdon-Olustee complex
9	31°20'32.54" N, 81°54'02.27" W	Olustee loamy fine sand, Mascotte find sand
10	31°21'09.77" N, 81°47'18.07" W	Rigdon-Olustee complex, Mandarin fine sand
11	31°21'12.11" N, 81°47'29.07" W	Rigdon-Olustee complex, Mascotte find sand
12	31°21'12.89" N, 81°47'24.98" W	Rigdon-Olustee complex, Mandarin fine sand

In sites without *B. arachnifera*, two sampling points were chosen randomly. Soils were collected at a depth of 0–10 cm using metal augers. The average rooting depth of *B. arachnifera*, as we observed, was 10 cm and that makes the top 0–10 cm soil layer most critical source for nutrients.

Basic leaf and soil preparations were done at the Agroecology Lab at Georgia Southern University, Statesboro, GA, USA. Then further analyses were done at the Agricultural and Environmental Services Laboratories (AESL) of University of Georgia, Athens, GA, USA. Both leaf and soil samples were analyzed for the following nutrients: Aluminum (Al), Boron (B), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Lead (Pb), and Zinc (Zn). In addition soils were also tested for Calcium (Ca), Magnesium (Mg), Sulfur (S), Phosphorus (P), and Potassium (K) at the AESL. At the Agroecology Lab at Georgia Southern, leaf and soil samples were dried in an Isotemp drying oven (Fischer Scientific) at 60°C for 72 hours. Dried soil samples were then sieved through the 2 mm sifter. The dried leaf and soil samples were then ground in Ball Mill grinder (8000 M Mixer /Mill, SPEX Sample Prep) using 20 ml plastic vials. Ground samples were used for further analysis at AESL. The analytical methodology involved HNO_3 Microwave Digestion (EPA 3051) and Inductively Coupled Plasma Spectrography (ICP) (CEM Mars5 microwave digestion).

Soil carbon (C) and nitrogen (N) were analyzed at the Agroecology Lab. The same process was used for drying, sifting and grinding. 100 milligrams of soil was measured from each sample using an XSE Analytical Balance (105 DU model, Mettler, Toledo) and carefully packed inside an aluminum cup that went through a Flash 2000 Combustion NC Soil Analyzer (CE Elantech Inc.) and C and N contents were determined.

Statistical analysis was done to calculate differences in soil and leaf tissue nutrients among sites. Nonparametric Wilcoxon test and Mann-Whitney U tests were performed to compare both leaf and soil nutrient data among 6 and 12 sites. Means were compared using a Steel-Dwass test. All statistical analyses were done using JMP Pro 10 ® 2012.

3. RESULTS AND DISCUSSION

Leaf nutrient content varied among sites. Leaf Al, B, Cu, Fe, Mn, Na and Zn content differed significantly among sites and ranged from 42.5 – 96.6, 18 – 33.1, 3.6 – 17, 48.8 – 79.9, 27.8 – 191.2, 1491.1 – 5964.1, and 10.6 – 19.9 ppm, respectively (Table 2). Average leaf Cd, Cr, Mo, Ni and Pb were, <0.8, <1, <1, 1.1 – 1.4, <2.0 – 5.3 ppm, respectively. Aluminum concentrations for sites 1, 3, 4 and 6 was 50% lower than in sites 2 and 5 ($P<0.0001$). Boron concentrations were 50% higher in sites 1, 2 and 4 than sites 3, 5 and 6 ($P<0.0001$). For copper, concentrations in sites 1, 2, 3 and 4 were 66% lower than in sites 5 and 6 ($P<0.0001$). Iron was 50% higher in site 2 than all of the other sites ($P = 0.0005$) and manganese was 81% higher in site 6 than site 5.

Sites 1, 2, 3 and 4 were 55% lower in manganese concentration than site 5 (P = 0.0001). Sodium was 44% lower in sites 6 and 5 than sites 2 and 1, which were 13% higher than sites 3 and 4 (P = 0.0001). Finally, zinc was 88% higher in site 6 than the sites with the lowest concentration of zinc, sites 1 and 3 (P = 0.0015) (Table 2).

Results of soil nutrient content analysis indicate that Ca, C and N were significantly different among sites with and without *B. arachnifera* (Tables 3,4). Soil Ca, C and N concentrations were 84% (P = 0.0222), 77%, and 45% (P = 0.0039) lower, respectively on sites without *B. arachnifera*. Other nutrients, Al, B, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, and Zn did not differ significantly between site types (Table 3).

Types and intensity of timber management practices such as tillage and fertilizer application may have influenced the nutrients in soil and plant leaves. Timber management has the potential to change the nutrient content of the soil through practices like tilling and draining, as well [22,23].

Table 2. *Baptisia arachnifera* leaf nutrient content averages by site (ppm)

Nutrients	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Significance
Al*	42.5^{b}	93.3^{a}	43.3^{b}	56.1^{b}	96.6^{a}	47.2^{b}	P=<0.0001
B*	32.2^{a}	31.9^{a}	25.5^{b}	33.1^{a}	18^{b}	21.2^{b}	P=<0.0001
Cd	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	-
Cr	<1	<1	<1	<1	<1	<1	-
Cu*	3.6^{b}	5.6^{b}	4.4^{b}	5.6^{b}	17^{a}	11.6^{a}	P=<0.0001
Fe*	51.9^{b}	79.9^{a}	48.8^{b}	56.4^{b}	55.7^{b}	52.1^{b}	P=<0.0005
Mn*	39.3^{c}	38.2^{c}	27.8^{c}	44^{c}	105.5^{b}	191.2^{a}	P=<0.0001
Mo	<1	<1	<1	<1	<1	<1	-
Na*	5515.9^{a}	5964.1^{a}	5076.6^{b}	5076.9^{b}	1491.1^{c}	2663.1^{c}	P=<0.0001
Ni	1.3	1.2	1.4	1.4	1.4	1.1	-
Pb	<2.0	<2.0	<2.0	<2.0	5.3	<2.0	-
Zn*	10.9^{b}	12.2^{ab}	10.6^{b}	11.6^{ab}	11.6^{ab}	19.9^{a}	P=<0.0115

**Indicates that there were significant differences of this nutrient among sites; Lower case letters indicate differences in leaf nutrient concentration (ppm) among sites*

Table 3. Soil nutrient (ppm) in different sites (*Baptisia arachnifera* was present in sites 1–6 and was historically absent in sites 7–12)

Site	1	2	3	4	5	6	7	8	9	10	11	12
Al	2320	2329	2121	926	3423	1287	3116	869	1137	563	2108	989
B	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
C*	12969	9820	10493	11779	12932	4441	482	2906	2720	1959	3193	3230
Ca*	<5	<5	98.93	11.51	39.83	25.73	<5	<5	<5	<5	<5	<5
Cd	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cr	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cu	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Fe	891	1387	633	235	1157	578	880	347	394	197	880	166
K	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Mg	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Mn	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Mo	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
N*	870.3	737.7	674.3	693.3	795.6	503.7	348.4	378.3	411.5	378.6	447.5	379.3
Na	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
Ni	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
P	<6	<6	<6	<6	<6	<6	<6	<6	<6	<6	<6	<6
Pb	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
S	10.77	19.87	73.58	<8	31.86	<8	<8	<8	<8	<8	<8	<8
Zn	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

**Indicates that there were significant differences of this nutrient among sites*

Table 4. Soil carbon and nitrogen analysis results (ppm) from sites with and without *Baptisia arachnifera*

Elements	*Baptisia arachnifera*	Mean	Standard deviation	P-value
Carbon	Present	10405.94	3186.466	0.0039
	Absent	2415	1053.4	0.0039
Nitrogen	Present	712.4902	124.7673	0.0039
	Absent	390.6093	34.29749	0.0039

The average leaf nutrient levels of *B. arachnifera* were different from soybean (*Glycine max*), a well-studied legume. *B. arachnifera* had 92% less zinc, 24% less boron and 71% less iron but 62% more copper and 51% more manganese than soybean levels [24,25]. A congener (same genus) species *B. lanceolata* occurred on soil with higher levels of manganese than *B. arachnifera* [26]. It was assumed by the researchers that *B. arachnifera* has a lower tolerance for manganese than *B. lanceolata* [26]. Sites 5 and 6 had almost three-fold manganese in the leaves compared to other sites. High levels of aluminum were found on sites 5 and 6. Aluminum has been found to be toxic to plants that grow in soils with a pH of 5.5 or lower even in very small amounts [27] with soybean being a prime example of a plant that is stressed by aluminum [28,29], but has also developed some tolerance to it. It is possible that *B. arachnifera* is tolerant to high aluminum, but further research is required to confirm it. Calcium was 6.2 times higher in the soil in sites with *B. arachnifera* than sites that did not have the plant. A study [30] reported that calcium in the soil helped reduce damage caused by the toxic effects of aluminum on root growth and this raises the question if calcium is having the same effect on aluminum concentrations in *B. arachnifera*.

4. CONCLUSION

It is speculated that timber management practices may have effects on the soil and leaf nutrient dynamics of *Baptisia arachnifera*. The results of this study may give baseline information about the nutrient content of the *B. arachnifera* plants and relevant soil nutrient information, which may help understand the nutrient dynamics of this species and develop better conservation and management. This study can be used as stepping stone for future nutrients studies of *B. arachnifera*. Further research is required to understand the correlation between soil nutrient availability and population distribution and leaf nutrient concentration of *B. arachnifera*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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