12-2011

Bacterial Interactions of Inoculated Price's Potato Bean (*APIOS PRICEANA*): A Biological Study

Rhonda Walker
Western Kentucky University, rhonda.walker@topper.wku.edu

BACTERIAL INTERACTIONS OF INOCULATED PRICE'S POTATO BEAN (*APIOS PRICEANA*): A BIOLOGICAL STUDY

A Thesis
Presented to
The Faculty of the Department of Agriculture
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

By
Rhonda Walker

December 2011

BACTERIAL INTERACTIONS OF INOCULATED PRICE'S POTATO BEAN (*APIOS PRICEANA*): A BIOLOGICAL STUDY

Date Recommended 11/28/2011

Linda Gonzales

Dr. Linda Gonzales, Director of Thesis

Todd Willian

Dr. Todd Willian

S. Sahi

Dr. Shivendra Sahi

Richard C Doerner 11-Jan-2012

Dean, Graduate Studies and Research Date

ACKNOWLEDGEMENTS

To my son – thank you for your support and patience while I pursued my dream.

To my family and friends – thank you for your moral and emotional support, for never allowing me to give up, for believing in me and for reminding me that no mountain is too high.

To Dr. Todd Willian – your input and instruction throughout this project have been invaluable and much appreciated.

To Dr. Linda Gonzales – you have been an inspiration to me as a woman in the industry and have given me faith that anything is possible.

To Dr. Shivendra Sahi – your biology instruction has played an integral part in my research.

To Dr. Jack Rudolph – thank you for your patience and guidance and for listening when I needed you.

To: Dr. Carol Baskin of the University of Kentucky – your donation of collected seeds is much appreciated.

To Dr. Chester of Austin Peay University – your input and referrals have been valuable to this project and are much respected.

To Stephanie Gunn-Zumo – your seed donation and information have been integral to the completion of this project and are much valued.

To Wendy Tennyson – your interest in this project and submission of tubers collected on your farm have been valuable to the project and I thank you for your time.

To the Department of Agriculture – you are the most phenomenal group of professors with whom I could ever have the pleasure of working with.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

BACTERIAL INTERACTIONS OF INOCULATED PRICE'S POTATO BEAN (*APIOS PRICEANA*): A BIOLOGICAL STUDY

Rhonda Walker December 2011 39 Pages

Directed by: Linda Gonzales, Todd Willian, and Shivendra Sahi

Departments of Agriculture and Biology Western Kentucky University

Apios priceana is a native endangered species plant found in the Southeast United States. It is characterized as a leguminous species that bears wisteria like clusters with pea like flowers, a large tuberous root and four to six inch long seed pods. It is believed the Native Americans and early European settlers relied on this species as a source of protein and utilized the seeds for cultivation of the tuberous "potato" which formed. *Apios priceana* contains an average of 13% fiber, 6.9% protein, 71% carbohydrate and 9 of the 11 essential amino acids needed in human diets (Walter et al.,1986). In addition, *A. priceana* tuberous roots contain anti-carcinogenic properties known to be used to treat prostate and breast cancer as well as lowering blood pressure and cholesterol with an added use for diabetes. If removed from the endangered species list it could prove to be a valuable agronomic crop. Its use spans human and animal consumption, bio fuel, medicinal and horticultural purposes. This research was initiated to investigate a biological symbiosis between *A. priceana* and known beneficial soil bacteria which may indicate growth potential of known colonies. Experimental treatments were 1) no inoculation 2*) Azospirillum brasilense* inoculate 3) *Bradyrhizobium japonicum* inoculate and 4) *Rhizobium leguminosarum* biovar *viceae* inoculate. Specimens were evaluated at 30, 60 and 90 day's growth from emergence for taproot length, number of lateral roots and taproot girth. Due to non-germination of seeds, data presented is for treatments 2 and 3. The correlation coefficient for average taproot length, number of lateral roots developed and taproot girth per treatment was as follows: taproot length to number of lateral roots, positive correlation coefficient 0.996; taproot length to taproot girth, positive correlation coefficient 0.999; and number of lateral roots to taproot girth, positive correlation coefficient 0.991. All correlation coefficients are significant at the 0.01 level.

Keywords: Apios priceana, Bradyrhizobium japonicum, Rhizobium leguminosarum, Azospirillum brasilense

CHAPTER 1

INTRODUCTION

Apios priceana is a native plant species in the Southeast region of the United States. It is federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS) and state listed as endangered by the Kentucky and Tennessee Departments of Fish and Wildlife. In the past, this species was used by Native Americans as a food source. Its tuberous root was boiled or fried and its seeds were cooked or used for cultivation purposes. Locally, within the county of Warren in the state of Kentucky, this species possesses historical value, for its original discovery was by a Bowling Green native, Sarah (Sadie) Frances Price in 1896. Price was a native plant artist who, after a miraculous recovery from a bed ridden illness, adventured into the woods of Warren County in pursuit of drawing its native flora. Much of her art was once displayed at Western Kentucky University.

Her happening upon the curious plant left her puzzled as to its origin. She noted, in detail, drawings and collected a specimen which she later sent to B.L. Robinson, a botanist, in Chicago. Robinson classified the plant as belonging to the Fabaceae family with taxonomic likeness to the *Apios* genus. This species was subsequently named after its discoverer, Sarah Price, and given a scientific name of *Apios priceana* and bestowed with a common name of "Price's Potato Bean." Without recovery and reintroduction attempts, a piece of Kentucky, Warren County, Bowling Green and Western Kentucky University history remains elusive.

It is reasonable to assume similarities in a symbiotic relationship with *Apios priceana* and *Bradyrhizobium japonicum* as is seen with *Glycine max* and *B. japonicum*.

These plants are legumes with nodulations that form on the roots in the same manner. Logic assumptions alone are not substantial in determining the theory to be equitable. Prior research performed by Matthew Parker (1999) detailing the positive relationship of *B. japonicum* with *A. americana* serves as the initial reasoning behind this research. In addition to *B. japonicum*, *Rhizobium japonicum* and *Azospirillum brasilense* were used in the experiment due to their nitrogen fixing properties. A positive relationship will rationalize inoculation of field soils of current populations of *A. priceana*.

Apios fortunei, an Asian species of the *Apios* genus, is currently cultivated in Japan and China as a potato and medicinal crop, respectively. A positive relationship between soil bacteria and *A. priceana* followed by inoculation of known colonies could increase *A. priceana* populations in an effort to remove them from the endangered species listing. Upon this removal from the list, further research could be conducted to determine the cultivation, production and harvest market value of *A. priceana* as a cash crop for the Southeastern United States.

CHAPTER 2

LITERATURE REVIEW

Although the *Apios* genus is cultivated as an economic crop in countries such as Japan and China, its U.S. native species are not currently cultivated as cash crops. Limited scientific research has been performed and/or reported for the *Apios* genus. More specifically, even less research has been performed on *Apios priceana*, resulting in insufficient knowledge and lack of standard inoculation technology that hinders the increased population of *Apios priceana*. A comprehensive inoculation model involving successful bacterial inoculate and known locations is imperative to increased populations of *Apios priceana* before any further research can be performed.

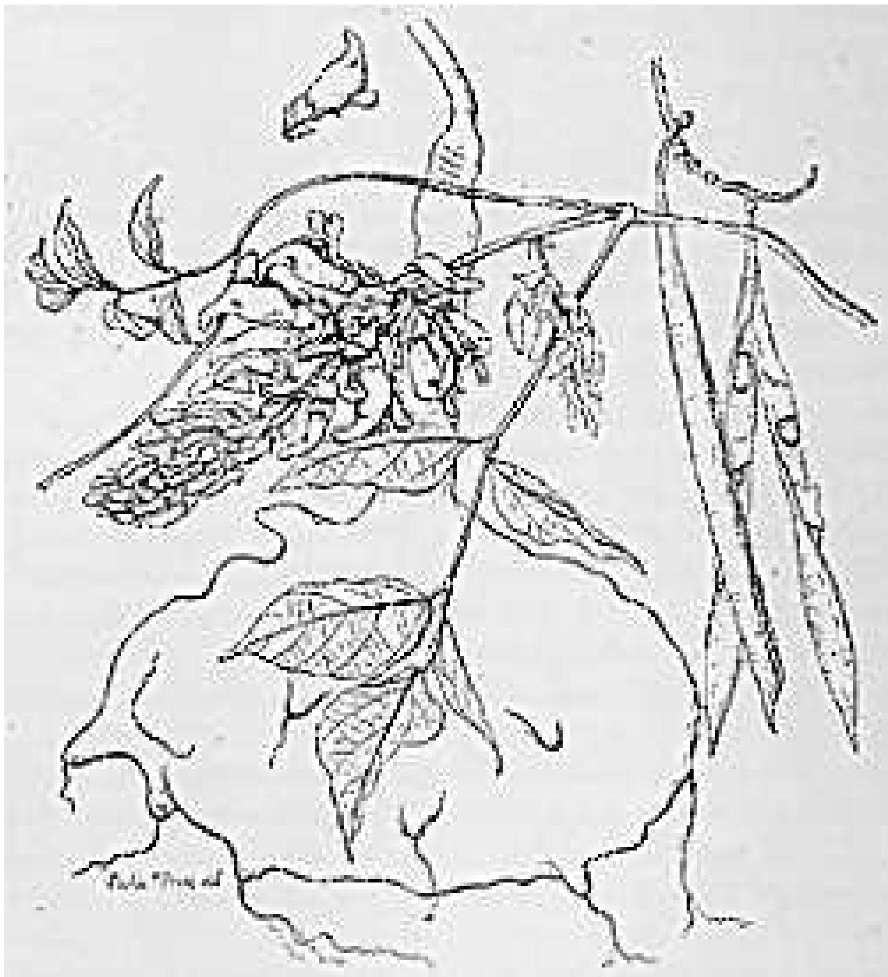
The literature review of this thesis will focus upon the characteristics of *Apios priceana* as well as three beneficial soil bacteria, including *Bradyrhizobium japonicum*, *Rhizobium leguminosarum*, and *Azospirillum brasilense*.

Characteristics of *Apios priceana*

Apios priceana is a native plant species that is currently 1 of 8 Kentucky plants listed as threatened by the United States Fish and Wildlife Service (McKinney. 2006). It is currently known from 40 populations in 15 counties in the states of Kentucky, Tennessee, Mississippi and Alabama. It was first discovered in 1898 by a Bowling Green naturalist, Sarah "Sadie" Frances Price and taxonomically named by B. L. Robinson as belonging to the *Apios* genus, subgenus *Tylosemium* and species *priceana*. In 1913 it was transferred as belonging to the *Glycine* genus and given the name *Glycine priceana* (Britton and Brown 1913). However, Briquet (1906) had already protected the *Apios* genus from classification as belonging to the *Glycine* family and therefore the name was restored to *Apios priceana*. *Apios priceana* is characterized as having a large, thick tuberous root with wisteria like flowers and bean pods 4-6 inches in length (Figure 1). The flowers contain a fleshy apex unlike that of *Apios americana*. To date, no comprehensive reproductive studies of *Apios priceana* have been conducted. According to the USFWS (1993) frequent visitors of the *Apios priceana* include the long tail skipper (*Urbanus proteus* L.), honey bees (*Apis mellifera* L.) and bumble bees (*Bombus sp*.).

Cultivation of *A*. *priceana* would expand land utilization for food production because in the wild it produces a tuber in highly alkaline (pH > 8) and woodland habitats (Walter et al. 1986). It contains 13% fiber, 6.9% protein, 71% carbohydrate and 9 of the 11 essential amino acids needed in human diets (Walter et al. 1986). It is a climbing yellow-green vine that grows from a potato like tuber and contains pink or greenish yellow pea or bean like flowers that bloom from July to August (Alabama Forestry Commission. 2007). It bears trifoliate alternate pinnately compound ovate leaf shapes with wisteria like racemes and clusters of the pea like flowers. It is usually found under mixed hardwoods or clearings therein, usually where ravine slopes or banks break into creek or river bottoms on well drained loamy soils either on old alluvium or over calcareous boulders (Kral, 1983).

Figure 1. *Apios priceana* illustration by Sadie Price
1898 Botanical Gazette 25 (6): 451



Bradyrhizobium japonicum

Members of the Fabaceae (Legume) family share a symbiotic nitrogen fixing relationship with many soil bacteria. In some cases, when several different legumes exist in the same location, the beneficial soil bacteria may prefer one legume as a host over another. Matthew Parker (1999) conducted a study in which isolates of soil bacteria *B. japonicum* from *Apios americana* and *Desmodium glutinosum* were collected and tested along with *Amphicarpaea bracteata* for a symbiotic relationship. According to his findings there is support for a historical relationship between *A. bracteata* and *A. americana* with *Bradyrhizobium*. Furthermore, Parker states, "The simultaneous presence of divergent Bradyrhizobial lineages with affinity *to B. japonicum* and *B. elkanii* emphasizes the high diversity of root nodule bacteria that may be present within even a single local population."

Apios priceana has historically been known to cohabitate with *Amphicarpaea bracteata* which is commonly called the Hog Nut or Hog Peanut (USFWS, 1993). With the combination of historical information of cohabitation and the positive relationship found from Parker's research, testing *A. priceana* for a positive symbiotic relationship with *B. japonicum* was performed. The decision not to test *B. elkanii* was based on the limited number of seeds which could be acquired for this study. In addition, *Apios americana* showed a 30% greater plant growth and yield when inoculated with *B. japonicum* (Putnam et.al., 1991).

Rhizobium leguminosarum

The Asian species, *Apios fortunei*, is a cultivated crop in the Asian market where the seeds (beans) are cooked like lentils. *Apios priceana* has a pea like flowering habit. In addition, soil inoculation (*Rhizobium leguminosarum* biovar *viceae*)of the pulse crop Mungbean (*Vigna radiate* L.) resulted in the number of pods per plant and the number of seeds per pod increasing from 15.3 to 16.8 and 95.7 to 109.2 when comparing control to inoculated specimens respectively (Anjum et al., 2006). The developed fruit of *Apios priceana* closely resembles that of the green bean.

Furthermore, inoculation of the peanut (*Arachis hypogaea* L.) using TAL309, an isolated cowpea rhizobial strain, resulted in a beneficial relationship between the peanut and the TAL309 inoculate. When compared to the indigenous rhizobial strain, the TAL309 successfully competed for nodulation (Hadad et al., 1986). *Rhizobium leguminosarum* biovar *viacieae* was utilized as this is a traditional inoculate for pulse crops such as mungbean, cowpea and groundnut.

Azospirillum brasilense

Azospirillum brasilense are plant growth promoting bacteria (PGPB). Plant growth promoting hormones play an integral role in plant health under stress conditions (Walker et al., 2010. The Southeast region of the United States rainfall pH levels range from 4.3 – 4.9 which are indicative of acid rain conditions (NADP, 2007). Plants exposed to prolonged periods of acid rain, can exhibit leaf drop and loss of nutrients. Plant growth promoters such as gibberellins, auxins and cytokinin can be produced by plant growth promoting bacteria present in the rhizosphere and thus aid in stress reduction (Walker et al., 2010). *Azospirillum brasilense* produces abscisic acid (ABA) (Cohen et al., 2008). ABA is a natural plant hormone that plays an integral role in how the plant survives stresses due to weather. PGPB such as *A. brasilense* which produces ABA may be used to alleviate plant stresses (Cohen et al., 2008).

According to Bakanchikova et al. (1993), *A. brasilense* has antimicrobial activity against *A. tumefaciens* in which *A. brasilense* inhibits the growth of crown galls in dicotyledonous plant species. In addition, a recent study by Somers et al. (2005) revealed that *A. brasilense* produces phenylacetic acid (PAA) which showed auxin-like activity and has an antimicrobial effect on other gram negative bacterial species including *Erwinia carotovora*, *Pseudomonas syringae* pv. *glycinea* and *Escherichia* *coli*. Moreover, *A. brasilense* contains antifungal activity against *Alternaria brassici-cola*, *Fusarium oxysporum*, and *Neurospora crassa* (Somers et al., 2005).

Phaseolus vulgaris inoculated with *A. brasilense* resulted in root lengths that were 431 mm and 194 mm for inoculated and non-inoculated specimens respectively (German et al. 2000). *Azospirillum brasilense* is a nitrogen fixing bacteria that, due to its plant hormone synthesis capacity, has the potential to increase root elongation and nodule formation. When co-inoculated with *B. japonicum* on soybeans (*Glycine max*) increased nodulation, root length and dry matter (Molla et al., 2001). This bacterium was chosen for this study due in large part to its capacity to simultaneously promote cell elongation and nitrogen fixation.

CHAPTER 3

MATERIALS AND METHODOLOGY

Inoculates NDure (*B. japonicum*), NDure (*R. leguminosarum* biovar *viceae*) and Accolade (*A. brasilense*) from INTX Microbials, LLC., Kentland, Indiana were mixed in individual sterile petri dishes at a concentration of 5g of inoculate to 10 mL deionized H_2O. *Apios priceana* seeds were presoaked for two hours and scarred just above the hilum (USFWS. 1993) and then submerged into inoculate and allowed to sit for 15 minutes to ensure inoculate adhesion onto the seed. Seeds were then planted in a random complete block design in sterile BM1 potting media containing 80% Canadian sphagnum peat moss, 14% Perlite and 5% Vermiculite in individual pots at a depth of 0.6 cm and incubated at 15°C for two weeks and then 20°C for two weeks. As the ambient air temperature rose in nature rose, the incubation temperature was adjusted to reflect natural conditions. Information on specific inoculates is presented in Table 1.

Once seedlings had emerged, the pots were removed from the incubator and placed under a grow light providing 14/10 light/darkness. When seedlings reached 15cm in height and exhibited a developing root system they were transplanted into BM1 mix in 3.8 liter pots in the greenhouse for observation and evaluation. A trellis support system was constructed for each pot using bamboo sticks and floral wire.

Roots were evaluated and measured for taproot length, number of lateral roots, taproot girth and nodulation formation at 30 days, 60 days, and 90 days after emergence. Planting, emergence, and evaluation dates for each treatment are presented in Table 2. Statistical analyses were performed using Microsoft Excel and XLStat macros.

Table 1. Bacterial inoculates used for each treatment.

Treatment	Inoculate
1	Control
2	Accolade™ *Azospirillum brasilense*
3	N-Dure™ *Bradyrhizobium japonicum*
4	N-Dure™ *Rhizobium leguminosarum* biovar *viceae*

Inoculates acquired from INTX Microbials, LLC, Kentland, Indiana.

Table 2. Date of planting, emergence and evaluation periods for inoculated *A. priceana* across the four treatments in 2011.

Treatment	Replicate	Planting Date	Emergence Date	30 Days	60 Days	90 Days
Control No Inoculate	1	March 22	None	N/A	N/A	N/A
	2	March 22	None	N/A	N/A	N/A
	3	March 22	None	N/A	N/A	N/A
Azospirillum brasilense	1	March 22	March 26	April 25	May 25	June 24
	2	March 22	March 27	April 26	May 26	June 25
	3	March 22	None	N/A	N/A	N/A
Bradyrhizobium japonicum	1	March 22	March 31	April 30	May 30	June 29
	2	March 22	None	N/A	N/A	N/A
	3	March 22	None	N/A	N/A	N/A
Rhizobium leguminosarum	1	March 22	None	N/A	N/A	N/A
	2	March 22	None	N/A	N/A	N/A
	3	March 22	None	N/A	N/A	N/A

Results are expressed for level of significance and correlation between inoculation and taproot length, inoculation and lateral root development, and inoculation and taproot girth. Further comparison among treatments was conducted by LSD (Least Significant Difference) test in order to identify the difference between treatments.

CHAPTER 4

RESULTS AND ANALYSIS

<u>Taproot development</u>

To study the effects of inoculates on taproot development specimens were examined at 30, 60 and 90 days. Raw data is shown in Appendix A. Analysis of variance (ANOVA) is presented in Appendix B for each of the three evaluation periods. There was no significant difference in taproot length development between the treatments where $P=0.13$ and 0.07 at 30 and 60 days respectively. However, at 90 days there was a significant difference between treatments where $P=< 0.05$. Further comparison among treatments was conducted by LSD (Least Significant Difference) test in order to identify the difference among each specific treatment. Results indicated at 90 days the taproot development from the *B. japonicum* inoculated specimen was significantly lower ($P=<0.05$) than the *A. brasilense* inoculated specimen (Table 3). Although there was no statistical significant difference between treatments in taproot length development at 30 and 60 days; algebraically average *A. brasilense* taproot development was 207%, 274% and 279% greater than *B. japonicum* at 30, 60 and 90 days respectively (Figure 2).

Results signify the presence of *A. brasilense* induces rapid onset of taproot development in contrast to the slow taproot development of *B. japonicum*. This is indicative of *A. brasilense* and its plant hormone synthesis capacity which has the potential to simultaneously increase root elongation and nodule formation.

<u>Lateral root development</u>

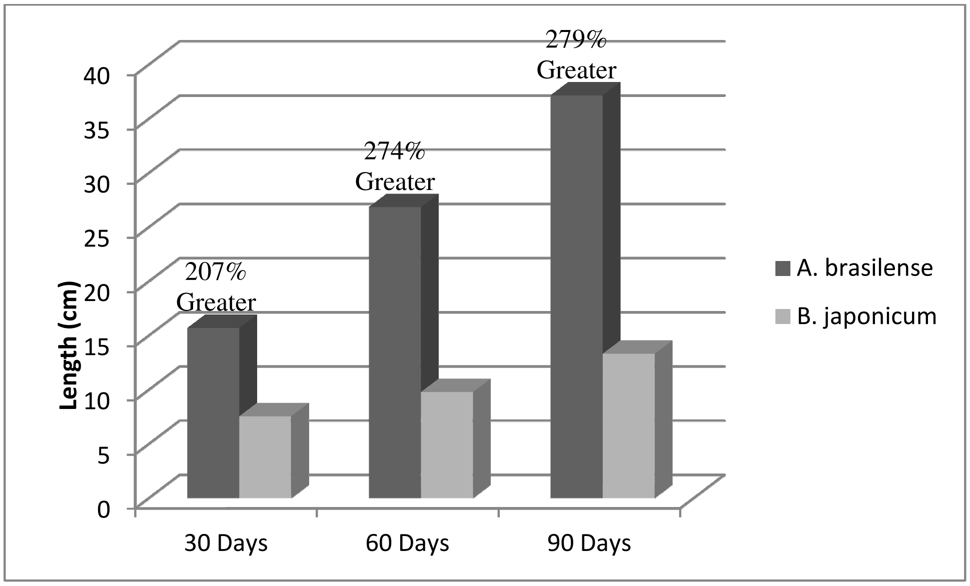
To study the effects of inoculates on taproot development specimens were examined at 30, 60 and 90 days. Raw data is shown in Appendix A. Analysis of variance

Table 3. Mean taproot lengths as influenced by inoculate.

Taproot Length LSD			
Evaluation Period	Inoculate		Mean Taproot Length (cm)
30 Days	*A. brasilense*		15.7
	B. japonicum		7.6
		LSD	18.0
60 Days	*A. brasilense*		26.9
	B. japonicum		9.8
		LSD	18.9
90 Days	*A. brasilense*		37.2
	B. japonicum		13.3 *
		LSD	17.1

* Means significantly different at $P = 0.05$.

Figure 2. Comparison of mean taproot development of *B. japonicum* treatment to *A. brasilense* treatment at 30, 60 and 90 day evaluation periods.



(ANOVA) is presented in Appendix B for each of the three evaluation periods. The analysis of variance (ANOVA) demonstrated that lateral root development was not statistically significant between the treatments where *P=0.15*, *0.08* and *0.14* at 30, 60 and 90 days respectively. Further comparison among treatments was conducted by LSD (Least Significant Difference) test in order to verify if a difference among each specific treatment exists. Results indicated there is no significant difference between treatments at 30, 60 and 90 days where (*P=<0.05*) (Table 4). Although there was no statistical significant difference between treatments; algebraically average *A. brasilense* lateral root development was 275%, 172% and 167% higher than *B. japonicum* at 30, 60 and 90 days respectively (Figure 3). Results signify the presence of *A. brasilense* or *B. japonicum* inoculates is not statistically integral in lateral root development for *A. priceana*.

<u>Taproot girth development</u>

To study the effects of inoculates on taproot girth development specimens were examined at 30, 60 and 90 days. Raw data is shown in Appendix A. Analysis of variance (ANOVA) is presented in Appendix B for each of the three evaluation periods. There was no significant difference in taproot length development between the treatments where *P=0.10* at 60 days. However, at 30 and 90 days there was a significant difference between treatments where *P=0.03 and <0.01*respectively. Further comparison among treatments was conducted by LSD (Least Significant Difference) test in order to identify the difference among each specific treatment. Results indicated at 30 and 90 days the taproot girth development from the *B. japonicum* inoculated specimen was significantly lower (*P=<0.05*) than the *A. brasilense* inoculated specimen (Table 5). Although there was no statistical significant difference between treatments in taproot girth development

Table 4. Mean lateral roots as influenced by inoculate.

Lateral Root Development LSD			
Evaluation Period	Inoculate		Mean Number of Lateral Roots
30 Days	*A. brasilense*		11.0
	B. japonicum		4.0
		LSD	18.0
60 Days	*A. brasilense*		15.5
	B. japonicum		9.0
		LSD	9.0
90 Days	*A. brasilense*		20.0
	B. japonicum		12.0
		LSD	18.0

Figure 3. Comparison of mean lateral root development of *B. japonicum* treatment to *A. brasilense* treatment at 30, 60 and 90 day evaluation periods.

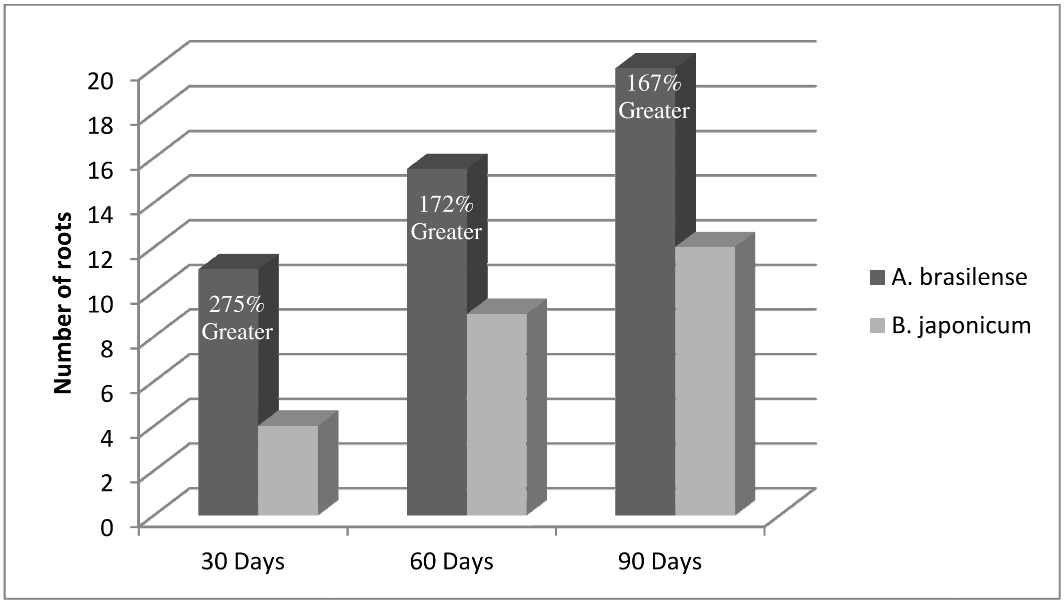


Table 5. Mean taproot girth as influenced by inoculate.

Taproot Girth LSD

Evaluation Period	Inoculate		Mean Taproot Girth (cm)
30 Days	*A. brasilense*		1.1
	B. japonicum		0.4 *
		LSD	0.3
60 Days	*A. brasilense*		2.0
	B. japonicum		0.6
		LSD	2.3
90 Days	*A. brasilense*		3.8
	B. japonicum		1.3 *
		LSD	0.4

* Means significantly different at $P = 0.05$.

at 60 days; algebraically average *A. brasilense* taproot development was 294%, 320% and 302% greater than *B. japonicum* at 30, 60 and 90 days respectively (Figure 4). Results signify the presence of *A. brasilense* bacteria is fundamental in taproot girth development for *A. priceana* in comparison to *B. japonicum*.

Nodule development

There were no results for nodule formation from the two specimens, *A. brasilense* or *B. japonicum* when evaluated at the 90 day interval. However, Figure 5 shows the possible beginning formation of nodules on the *A. brasilense* 2 specimen.

Combination of taproot, lateral root and taproot girth development

Comparison of mean taproot length, number of lateral roots and taproot girth for each treatment are presented in Figure 6. As taproot length increased, both the number of lateral roots and taproot girth increased. Pearson correlation analysis showed taproot length was significant and positively correlated ($P=<0.01$) to both number of lateral roots and taproot girth (Table 6).

Figure 4. Comparison of mean taproot girth development of *B. japonicum* treatment to *A. brasilense* treatment at 30, 60 and 90 day evaluation periods.

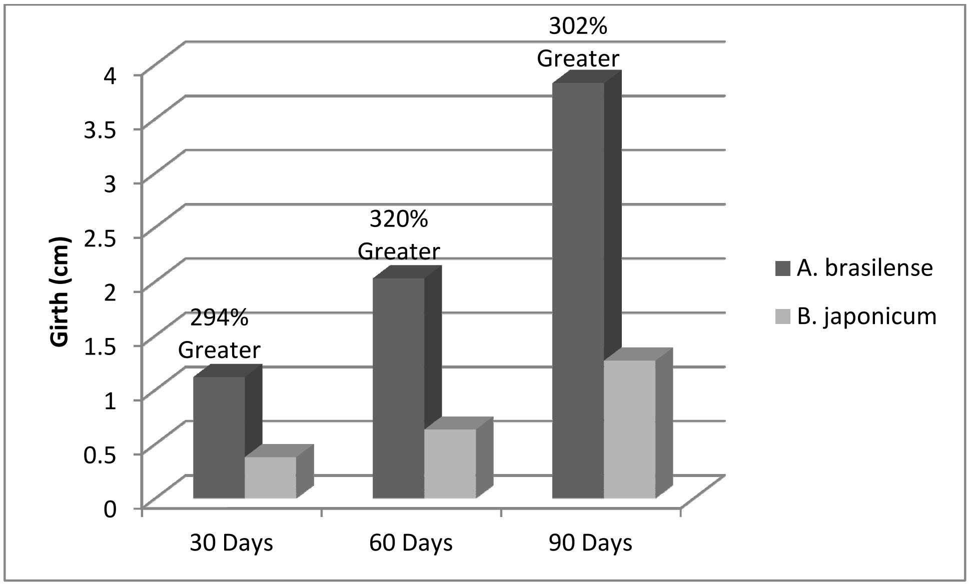


Figure 5. Beginning white nodule formation on *A. brasilense* 2 specimen as seen at 90 day evaluation.

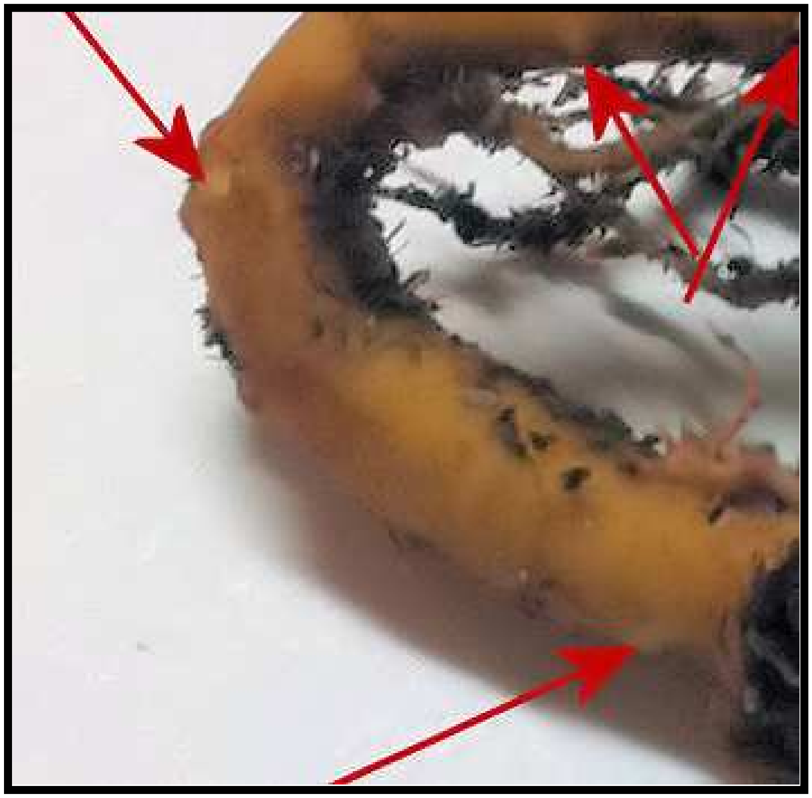


Figure 6. Mean comparison of taproot length, number of lateral roots and taproot girth development.

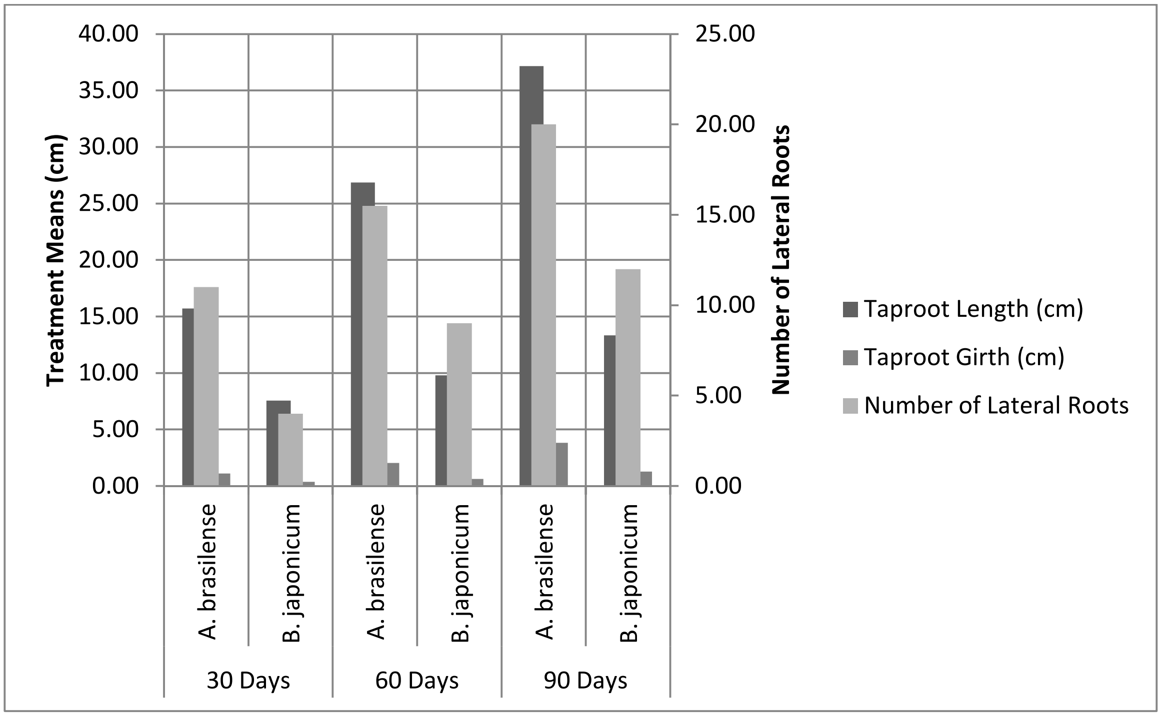


Table 6. Linear correlation coefficients for taproot length, number of lateral roots and taproot girth for two treatments.

		TL	LR	TG
TL	Pearson Correlation	1	0.95*	0.98*
LR	Pearson Correlation		1	0.94*
TG	Pearson Correlation			1

* Correlation is significant at the 0.01 level.

TL = Taproot length

LR = Number of lateral roots

TG = Taproot girth

CHAPTER 5

DISCUSSION

Interval of root evaluation

Inoculated *Apios priceana* roots were evaluated at 30, 60 and 90 day intervals. The *Azospirillum brasilense* inoculate produced greater results when compared to *Bradyrhizobium japonicum* inoculate. Data collection for the control and *Rhizobium leguminosarum* biovar *viceae* was not able to be performed due to non-germination of specimen seeds. Overall the germination rate was 25%. Although this seems to be a low rate a tele-conference with the USDA Forestry Service Land Between the Lakes (LBL) biologist, Elizabeth Raikes, would provide their current germination rate in the lab as being 32%. The decision to conclude data collection at the 90 day interval was based on the principle that symbiosis and nodulation would be seen in soybeans (dicotyledonous) species within that time period (Molla et al. 2001). Figure 6 shows the possible beginning formation of nodules on the *A. brasilense* 2 specimen. Even though there is small data collection, *A. brasilense* showed a positive attribute to plant root growth and development in a rapid manner in comparison to the slow growing *B. japonicum* inoculates in the short amount of time the inoculations were evaluated.

Azospirilum brasilense contribution to improved growth

Given that *Azospirillum brasilense* are plant growth promoting bacteria (PGPB) it is not unexpected to discover the rapid growth capacity from using this bacteria as an inoculate for *Apios priceana*. However, the phenomenal amount of increased production over *Bradyrhizobium japonicum* was surprising. Use of the bacteria as a soil inoculates for known *Apios priceana* populations might demonstrate an increase in production for the species. Acid rain conditions of the Southeast region of the United States, combined with the karst environment of known populations, may cause stress to the species that could be overcome by use of this particular bacteria inoculate. Most definitely the increased taproot elongation could attribute to the species' ability to absorb soil water at greater depths, thus giving it improved sustainability capacities. In addition, the Southeast region of the United States is susceptible to drought which is another plant stress that affects known *Apios priceana* populations. This increase in root elongation could prove valuable to the species' survival under drought conditions.

Although some *A. brasilense* strains are known to cause inhibition of nodulation in dicotyledonous specimens (Bakanchikova et al. 1993), it worthy to note the beginning formation of galls as seen in Figure 6 on the *A. brasilense* inoculated specimen and the possible induction of nodulation for this particular dicotyledonous species. It would be cause worthy to continue studying the affect this particular bacterial strain has on *A. priceana* for nodulation capacity through symbiosis. Symbiosis could provide an increase in nitrogen needed by the species especially during high nitrogen required growth stages such as flowering. Moreover, this study was conducted for root effects the bacteria strains would have on *A. priceana*. However, Figure 7 shows the elongation capacity that *A. brasilense* has on the immediate elongation of the stem of the species as well. And although there was no data collected for this particular point in the study, it would be worthwhile to further study the effects *A. brasilense* may have on *A. priceana* stem, leaf and flowering morphology. An improved upward and outward growth of the stems might increase the species' ability to absorb light in less favorable light conditions. Figure 8 displays the disparity between *A. brasilense* 2 and *B. japonicum* 1 with respect to taproot length and girth.

<u>Decision making for different treatments</u>

The decision to use the particular bacterial inoculates was made based upon previous studies. *Azospirillum brasilense* was chosen due to it being a nitrogen fixing bacteria that, due to its plant hormone synthesis capacity, has the potential to increase root elongation and nodule formation. When co-inoculated with *B. japonicum* on soybeans (*Glycine max*) increased nodulation, root length and dry matter has been observed (Molla et al. 2001). *Apios priceana* has historically been known to cohabitate *with Amphicarpaea bracteata* which is commonly called the Hog Nut or Hog Peanut (USFWS, 1993). With the historical information of cohabitation and the positive relationship found from Parker's research testing *A. priceana* for a positive symbiotic relationship with *B. japonicum* was performed. In addition, *Apios americana* showed a 30% greater plant growth and yield when inoculated with *B. japonicum* (Putnam et.al., 1991).The Asian species, *Apios fortunei*, is a cultivated crop in the Asian market where the seeds (beans) are cooked like lentils. The decision was made to utilize *Rhizobium leguminosarum* biovar *viacieae* as this is a traditional inoculate for pulse crops such as mungbean, cowpea and groundnut.

<u>Limitations of the study</u>

Apios priceana is a native endangered species for the Southeast region of the United States. It is for this reason acquisition of adequate seeds was limited. The initial seeds the study began with were more than 12 years old and not viable. A second set of seeds harvested in 2009 was acquired through a private source and had been stratified since the seed harvest. Fresh seeds with a stratification period no greater than 60 days may improve germination percentage and provide more data collection capacity for the study. Initially this study was to contain the affects each bacteria would have on flowering capacity. However, due to non-viable initially acquired seeds, the study had to be extended by 6 months until a second batch of seeds could be obtained and flowering for this particular species, *A. priceana*, occurs in the second year. In addition, obtaining soil from known locations is nearly impossible due to heavy regulations on endangered species. Using native soil conditions/samples for *A. priceana* populations in lieu of a peat based soil may provide different results when fresh seeds are inoculated with the bacteria.

Although the data collected provided insight to the effect *A. brasilense* and *B. japonicum* have on *A. priceana* root morphology, additional study is needed. It may be worthwhile to further the study with inclusion of the stem, leaf and flowering growth capacity of *A. priceana* using these particular bacterial strains.

Figure 7. Comparison of stem elongation between *A. brasilense* 2 and *B. japonicum* 1 at 3 days of seedling growth.

Figure 8. Disparity between A. brasilense 2 and B. japonicum 1 with respect to taproot length and girth.

CHAPTER 6

SUMMARY

Apios priceana is a native plant species in the Southeast region of the United States. It is federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS) and state listed as endangered by the Kentucky and Tennessee Departments of Fish and Wildlife. In the past, this species was used by Native Americans as a food source. Its tuberous root was boiled or fried and its seeds were cooked or used for cultivation purposes. Limited scientific research has been performed and/or reported for the *Apios* genus. More specifically, even less research has been performed on *Apios priceana*, resulting in insufficient knowledge and lack of standard inoculation technology that hinders the increased population of *Apios priceana*. Three bacterial strains were used to study their effect on root morphology of *A. priceana*. Due to inadequate seed germination, *R. leguminosarum* data was not collected. Overall, *A. brasilense* showed greater growth capacity effect in comparison to *B. japonicum* on *A. priceana* when evaluating taproot length, lateral root formation and taproot girth providing results which were greater than *B. japonicum* by 269%, 196% and 313% respectively.

Fresh seeds with a stratification period no greater than 60 days may improve germination percentage and provide more data collection capacity for the study. In addition, using native soil conditions/samples for *A. priceana* populations in lieu of a peat based media may provide different results when fresh seeds are inoculated with the bacteria. Although the data collected provided insight to the effect *A. brasilense* and *B. japonicum* have on *A. priceana* root morphology, additional study is needed. It may be worthwhile to further the study with inclusion of the stem, leaf and flowering growth capacity of *A. priceana* using these particular bacterial strains.

APPENDIX A – RAW DATA

Taproot Length Evaluation (cm)

30 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	14.7	7.6	0
2	0	16.7	0	0
3	0	0	0	0
Σ		31.4	7.6	
Mean		15.7	7.6	
% Difference		207%		

60 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	25.8	9.8	0
2	0	27.9	0	0
3	0	0	0	0
Σ		53.7	9.8	
Mean		26.85	9.8	
% Difference		274%		

90 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	36.2	13.3	0
2	0	38.1	0	0
3	0	0	0	0
Σ		74.3	13.3	
Mean		37.15	13.3	
% Difference		279%		

Number of Lateral Roots Evaluation

30 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	10	4	0
2	0	12	0	0
3	0	0	0	0
Σ		22	4	
Mean		11	4	
% Difference		275%		

60 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	15	9	0
2	0	16	0	0
3	0	0	0	0
Σ		31	9	
Mean		15.5	9	
% Difference		172%		

90 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	19	12	0
2	0	21	0	0
3	0	0	0	0
Σ		40	12	
Mean		20	12	
% Difference		167%		

Taproot Girth Evaluation (cm)

30 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	1.106	0.381	0
2	0	1.143	0	0
3	0	0	0	0
Σ		2.249	0.381	
Mean		1.1245	0.381	
% Difference		295%		

60 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	1.905	0.635	0
2	0	2.159	0	0
3	0	0	0	0
Σ		4.064	0.635	
Mean		2.032	0.635	
% Difference		320%		

90 Days Replicates	Control	*Azospirillum brasilense*	*Bradyrhizobium japonicum*	*Rhizobium leguminosarum*
1	0	3.81	1.27	0
2	0	3.85	0	0
3	0	0	0	0
Σ		7.66	1.27	
Mean		3.83	1.27	
% Difference		302%		

APPENDIX B – ANOVAS

Taproot Length Analyses

30 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	43.74	1	43.74	21.87	0.134111	161.4476
Within Treatments	2	1	2			
Total	45.74	2				

60 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	193.8017	1	193.8017	87.89191	0.06765	161.4476
Within Treatments	2.205	1	2.205			
Total	196.0067	2				

90 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	379.215	1	379.215	210.0914	0.043852	161.4476
Within Treatments	1.805	1	1.805			
Total	381.02	2				

Lateral Root Analyses

30 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	32.6666667	1	32.66667	16.33333	0.154421	161.4476
Within Treatments	2	1	2			
Total	34.6666667	2				

60 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	28.1666667	1	28.16667	56.33333	0.084323	161.4476
Within Treatments	0.5	1	0.5			
Total	28.6666667	2				

90 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	42.6666667	1	42.66667	21.33333	0.135737	161.4476
Within Treatments	2	1	2			
Total	44.6666667	2				

Taproot Girth Analyses

30 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	0.3685282	1	0.368528	538.3903	0.02742	161.4476
Within Treatments	0.0006845	1	0.000684			
Total	0.3692127	2				

60 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	1.3010727	1	1.301073	40.33333	0.099425	161.4476
Within Treatments	0.032258	1	0.032258			
Total	1.3333307	2				

90 Days
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Treatments	4.3690667	1	4.369067	5461.333	0.008614	161.4476
Within Treatments	0.0008	1	0.0008			
Total	4.3698667	2				

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