ILLINOIS
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HISTORY
SURVEY

February 15, 2005

Kathy Barker
Grants Administrator
Illinois Department of Natural Resources
Office of Resource Conservation – Operations
One Natural Resources Way
Springfield, IL 62702-1271

Dear Ms. Barker:

Enclosed you will find the following report: An investigation of hybridization between *Baptisia tinctoria* (yellow wild indigo) and *B. leucantha* (white wild indigo). This report is being submitted to fulfill the requirements of the Illinois Wildlife Preservation Fund Grant Agreement for Grant #04-038W. As I have previously discussed with you by phone, the results of the portion of this study dealing with the Random Amplified Polymorphic DNA (RAPD) data have not been included in this report. These results will be presented in an addendum to this report that we will submit no later than June 2005.

Sincerely,

Mary Ann Feist
Associate Research Scientist

607 East Peabody Drive, Champaign, Illinois 61820-6970 USA
(217) 333-6880 Fax (217) 333-4949
http://www.inhs.uiuc.edu

An investigation of hybridization between *Baptisia tinctoria* (yellow wild indigo) and *B. leucantha* (white wild indigo).

Mary Ann Feist
Associate Research Scientist

&

Jason A. Koontz, Ph.D.
Adjunct Assistant Professional Scientist

Illinois Natural History Survey
Center for Wildlife and Plant Ecology
607 East Peabody Drive
Champaign, IL 61820

15 February 2005

Prepared for:

Illinois Department of Natural Resources
Illinois Wildlife Preservation Fund
13608 Fox Rd.
Yorkville, IL 60560

Introduction

During the summer of 2002, *Baptisia tinctoria* (L.) R. Brown (yellow wild indigo) was rediscovered in Illinois at Leesville East in Kankakee County (Carroll and Feist 2004, Phillippe et al. 2003). This species was thought to be extirpated from Illinois for as long as 70 years. Since its rediscovery, it has been given endangered status in Illinois (Nyboer & Ebinger 2004). *Baptisia tinctoria* occurs in 25 states in the eastern and southeastern United States as well as Ontario, Canada (Gleason and Cronquist 1991, USDA, NRCS 2002). It prefers dry, sterile, and sandy soils, often in dry, open woodlands, savannas, or clearings (Larisey 1940, Fernald 1950, Gleason and Cronquist 1991, Isely 1998). Considered common throughout most of its range, *B. tinctoria* is less common near the limits of its distribution and is listed as endangered in Maine and now Illinois, threatened in Kentucky (KSNPC 2001, USDA, NRCS 2002), on the watch list in Indiana (Yatskievych 2000, Homoya pers. comm. 2003), and of special concern in Iowa (IAC 1999) as well as in Wisconsin (WDNR 2002) where its presence was documented for the first time in 1969 (Cochrane 1976). Minnesota holds one vouchered specimen from the late 1800's. Due to declines in populations as a result of commercial demands for its medicinal properties, *B. tinctoria* has been included on a list of U.S. medicinal plant species that have been designated as 'priorities for further study' by The Nature Conservancy and TRAFFIC (a joint wildlife trade monitoring program of WWF-World Wide Fund For Nature and IUCN-The World Conservation Union; Robbins 1999).

Potential hybrid individuals of *B. tinctoria* and another *Baptisia* species were observed at the Leesville East site in 2002. These appear to be morphological intermediates between *B. tinctoria* and *B. leucantha* (Raf.) Thieret (white wild indigo) however, *B. bracteata* Nuttall (cream wild indigo) also occurs at the site. Morphological and phenological evidence (Larisey

1940, Gleason and Cronquist 1991), however, seem to support the hypothesis that *B. leucantha* is the other parent. Flower color and flower, fruit, and leaf size of the hybrid are all intermediate between *B. tinctoria* and *B. leucantha* (Appendix A, Photos 1-5) In addition, the phenology of *B. tinctoria* and *B. leucantha* overlap (July-August), whereas *B. bracteata* blooms earlier in the season (May-June) (Larisey 1940, personal observation).

The role of hybridization in conservation biology is a controversial issue (e.g., Arnold 1997) and continues to be debated. It appears that hybridization can have both positive and negative consequences for rare species. Hybridization with a congener may cause the rare species to be genetically 'assimilated' into the widespread species (reviewed in Levin et al. 1996; Rhymer and Simberloff 1996; Soltis and Gitzendanner 1999), however, the introduction of 'new' genetic material could bolster the rare species' genetic diversity and increase its chance of survival. To understand and effectively manage the newly discovered populations of yellow wild indigo, we need to determine the nature of hybridization. For this study we used genetic markers to attempt to determine if hybridization is indeed occurring and if so, to what extent and between which species. These markers will be the basis for future conservation of yellow wild indigo at this site.

The objectives of this study were to (1) identify/locate populations of *B. tinctoria*, *B. lactea*, *B. leucophaea* and the putative hybrid to study and (2) conduct allozyme and DNA analyses. We will use allozyme and DNA analyses to identify species-specific markers that can be used to test the hypothesis of hybridization. At the end of the project we hope to have a set of markers that are unique to *B. tinctoria*, *B. leucantha*, *B. bracteata,* and the hybrid to study. If hybridization is occurring, then the putative hybrids should share a combination of the unique markers found in the parental species. The data can also be used to infer genetic variation and

gene flow of the *B. tinctoria* populations. This information is also valuable for conservation and management of the species.

Materials and Methods

Population sampling

All collections were made from the Leesville East site located in the southeast corner of Kankakee County, Illinois. This site is owned by the Illinois Department of Natural Resources and is part of an area known as the Pembroke Savannas. We searched the area for all individuals of *Baptisia tinctoria*, *B. leucantha*, *B. bracteata*, and the putative hybrid. We sampled 65 indidividuals of *B. tinctoria*, 40 of *B. leucantha*, 3 of *B. bracteata*, and 13 of the putative hybrid. We sampled two leaves per individual. One was kept fresh by placing it in a moistened paper towel in a plastic bag and kept on ice until arrival at the laboratory at the Illinois Natural History Survey. We ground and then froze the one leaf for allozyme analyses. The second leaf sample provided material for DNA extraction. We dried these leaves by placing them in a paper coin envelope containing Drierite desiccant (anhydrous calcium sulfate, W. A. Hammond Drierite Co., Xenia, OH).

Molecular Markers

Allozyme procedures followed Koontz et al. (2001). Buffer system 1 of Soltis et al. (1983) was used to resolve glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and fructose-1,6-diphosphatase (F1,6DP). We used buffer system 6 to resolve aspartate amino transferase (AAT) and leucine amino peptidase (LAP). We used buffer system 8, as modified by Haufler, 1985 to resolve phosphoglucoisomerase (PGI), malic enzyme (ME), and triosephosphate

isomerase (TPI); and system 9 to resolve 6-phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), aldolase (ALD), and shikimate dehydrogenase (SKDH). Phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH), and menadione reductase (MNR) were resolved using the morpholine system (Odrzykoski and Gottlieb, 1984) at pH 6.1. We used agarose overlay stain recipes for TPI and PGI. We numbered allozymes sequentially starting with the most anodal as 1, and we designated alleles alphabetically, beginning with the most anodal as *a*. We used BIOSYS-1 (Swofford and Selander, 1989) to compute allele frequencies and genetic identities, and to perform UPGMA to assess the allozymic similarity of the populations.

We also decided to pursue DNA-based markers because they are more variable and are better able to distinguish differences between species. Random Amplified Polymorphic DNA (RAPD) marker protocols generally followed those of Koontz et al. (2001), but are briefly discussed here. DNA was extracted from 10 mg of dried leaf tissue using the Wizard DNA extraction Kit (Promega), following the manufacturers instructions; however, the ground leaf material in the nuclei lysis buffer was incubated at 65° C for at least 1 hr. DNAs were quantified with a SmartSpec 3000 spectrophotometer (Bio-Rad) and diluted to a standard 10 ng/µl with TE pH 8.0. Primers from Operon's (Qiagen) A and B RAPD kits were surveyed using a subset of the *Baptisia* samples collected. RAPD reactions consisted of a 25 µl reaction containing 17 µl of sterile distilled H_2O, 0.1 µl of *Taq* polymerase (Promega, buffer B), 1 µl of primer, 1 µl of BSA (Amresco, at 2.5 mg/ml), 5 µl of a mastermix [500 µl 10x reaction buffer supplied with the *Taq*, 290 µl sterile distilled H_2O, 10 µl 1M $MgCl_2$ (Amresco), 200 µl dNTPs (USB) at 10mM], and 1 µl of diluted DNA. Reactions were run out on 1.5% agarose gels (Type 1, Amresco) in 0.5x TBE buffer until a bromophenol blue dye maker migrated 8 cm. Gels were stained with

ethidium bromide for 20 min and destained in distilled H_20 for 30 min. Gels were visualized and photographed in UV light with a Kodak EDAS 290 gel imaging system. After screening approximately 40 RAPD primers, ten were selected because they exhibited the most stable banding patterns.

Results and Discussion

The results of the allozyme analysis were inconclusive. No fixed differences of alleles were found among *Baptisia tinctoria B. leucantha,* and *B. bracteata* (Appendix B). All species shared the same alleles. As a result, no strict additivity could be demonstrated in the putative hybrid. Although these findings do not refute the hypothesis of hybridization, they do not allow any of the three species to be eliminated from the pool of potential parents of the hybrid. The lack of unique alleles indicates that the species are all closely related and that more variable genetic markers are need to distinguish between them.

A total of 40 RAPD primers (A1-20, B1-20) were surveyed for markers that distinguish the three *Baptisia* species from one another. RAPD markers are dominant (can not differentiate a homozygous dominant individual from a heterozygous individual) and each band is treated as a different locus, with two alleles (present or absent). To date, 12 loci from one primer have been scored. It appears that there are at least three loci unique to *B. tinctoria* and no unique loci in *B. leucantha* or B. bracteata. The hybrid individuals do not contain unquie RAPD markers and those unique to B. tinctoria are present in the hybrids. More loci will be scored during April and May 2005. An addendum will be submitted on or by June 2005 with the complete RAPD data set scored and analyzed. If hybridization is indeed occurring, we expect unique bands found in two

of the three taxa (*B. tinctoria*, *B. leucantha*, and *B. bracteata*) to be found in combination in hybrid individuals.

Summary

During the summer of 2002, *Baptisia tinctoria* (L.) R. Brown (yellow wild indigo) was rediscovered in Kankakee County, Illinois. It had been thought to be extirpated from Illinois for as long as 70 years. Since its rediscovery, it has been listed as endangered in Illinois (Nyboer & Ebinger 2004). In addition to *B. tinctoria*, potential hybrid individuals of yellow wild indigo and *B. leucantha* (Raf.) Thieret (white wild indigo) were observed. These appear to be morphological intermediates between *B. tinctoria* and *B. leucantha*; however, *B. bracteata* Nuttall (cream wild indigo) also occurs at the site. To understand and effectively manage the newly discovered populations of yellow wild indigo, we conducted a study using genetic markers to determine the nature of hybridization. Allozyme results were inconclusive. Because there were no fixed allelic differences for any of the taxa in our study, we could not exclude any of the three *Baptisia* species (*B. tinctoria,* and *B. leucantha*, and *B. bracteata*) as parent species of the hybrid. The most we can say at this point, is that the three species are closely related.

The results of preliminary RAPD analyses show some unique markers for yellow wild indigo that appear in the hybrid individuals. We have several more markers to score and these results will be discussed in an addendum to this report to be submitted in June 2005.

Acknowledgments

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APPENDIX A

Baptisia Photographs

Photograph 1. Connie Carroll-Cunningham, INHS, with *Baptisia tinctoria* at Leeseville East in Kankakee County, Illinois. Photo by Mary Ann Feist

Photograph 2. *Baptisia tinctoria* in flower at Leeseville East in Kankakee County, Illinois. Photo by Mary Ann Feist

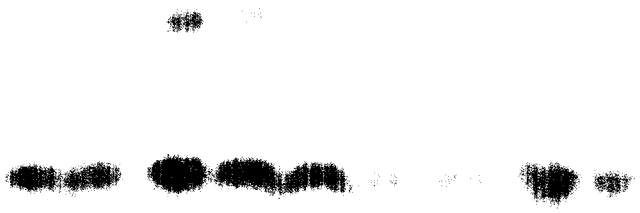
Photograph 3. *Baptisia tinctoria* in fruit at Leeseville East in Kankakee County, Illinois. Photo by Mary Ann Feist

Photograph 4. Mary Ann Feist and the *Baptisia* hybrid at Leeseville East in Kankakee County, Illinois. Photo by Jason Koontz.

Photograph 5. *Baptisia leucantha* at Leeseville East in Kankakee County, Illinois. Photo by Mary Ann Feist

APPENDIX B

Gel Photograph



Photograph 1. 6pgd Lanes 1, 5-13, 16-19 are all homozygous for the fast allele ("AA"); lanes 2-4, 15 are heterozygous,("AB") and lane 14 is homozygous for the slow allele ("BB"). Lanes 1-4 are the hybrid, lanes 5-11 are *B. leucantha*, lanes 12-14 are *B. bracteata*, lanes 15-19 are *B. tinctoria*.