TAXONOMIC INVESTIGATIONS OF OKLAHOMA FLORA

By

ADAM KEITH RYBURN

Bachelor of Science

Southwestern Oklahoma State University

Weatherford, Oklahoma

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2003

TAXONOMIC INVESTIGATIONS OF

OKLAHOMA FLORA

Thesis Approved:

Committee Chair

Member

Member

Member

Dean of Graduate College

ACKNOWLEDGEMENTS

This work would not have been possible without the participation of many people. I am deeply indebted to my major advisor, Dr. Ronald J. Tyrl for his guidance, assistance, and friendship throughout my graduate studies at Oklahoma State University. Also, I thank my other dissertation committee members, Dr. Michael W. Palmer, Dr. Becky L. Johnson, and Dr. Mark E. Payton. Their suggestions and comments greatly improved this work. I am grateful to my committee members for their involvement and interest in my future as Biologist.

I thank all my colleagues in the Department of Botany, especially my fellow graduate students in Dr. Tyrl's Lab. Charriss Hayes and Raelene Crandall were both supportive and encouraging. Working with them has been a great pleasure and a valuable learning experience.

I have benefited greatly from funding awarded to me through the McPherson Summer Research Fellowship and the Distinguished Graduate Student Fellowship.

Also, I express gratitude to my family. Thank you Maranda and Addison, Mom, Dad, Aaron, Jennifer, Shannon, Pam, Randy and Marcia for remaining so patient throughout this process. Your helpful comments and enduring love made completion of this project possible.

TABLE OF CONTENTS

Appendixes

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

A TAXONOMIC TREATMENT OF THE GENERA AND SPECIES OF THE SALICACEAE IN OKLAHOMA

INTRODUCTION

Considered by state taxonomists to be one of the more diverse in the United States, the vascular flora of Oklahoma comprises 173 families, 868 genera, and 2,540 species (Tyrl et al., 2003). This diversity of flora is related to the state's ecological and environmental diversity. Across the state, one finds considerable variation in precipitation, temperature, topology, geology, wind, and soils. One finds species representative of the eastern deciduous forest, central grasslands, the Rocky Mountains, the Chihuahuan and Sonoran Deserts, the Gulf Coastal Plain, and the Ozark Mountains.

For three decades, the most commonly used reference for the identification of the vascular plants of Oklahoma was U.T. Waterfall's (1969) *Keys to the Flora of Oklahoma*. As the title implies, it lacks the morphological descriptions of taxa needed to confirm identification of plants. For this information, one had to use descriptions appearing in taxonomic treatments for adjacent areas such as *Gray's Manual of Botany* (Gray, 1950), *Manual of the Vascular Plants of Texas* (Correll and Johnston, 1979), *Flora of the Great Plains* (Great Plains Flora Association, 1986), and *Shinners & Mahler's Illustrated Flora of North Central Texas* (Diggs et al., 1999). In addition, Waterfall's work has become out dated with respect to revisions in classifications and changes in nomenclature.

This prompted a need for a modern, comprehensive treatment for the vascular plants of Oklahoma. As a result, the non-profit corporation Flora Oklahoma Incorporated was formed in 1983 (Tyrl et al., 2003). Foreseeing the immediate need for students, professionals, and other individuals to use the keys and descriptions that will ultimately be published as *Flora of Oklahoma*, the project's editorial committee chose to release parts of the *Flora* as they were written. The key to families, titled *Key to the Vascular Plants of Oklahoma*, appeared in 1994. Three years later the family descriptions appeared in *Key and Descriptions for the Vascular Plant Families of Oklahoma.* Recent work has led to keys to the genera of all families as well as keys to many species. Numerous individuals have been asked by the committee to contribute their taxonomic expertise by writing treatments for the families, genera, and species. The treatment presented here is one such contribution.

The Salicaceae, or willow family, is traditionally recognized as consisting of the two genera *Salix* and *Populus,* although some taxonomists recognize the segregate genera *Chosenia*, and *Toisusu* because they differ greatly in pollen grain morphology from *Salix* and *Populus* (Argus, 1997). Absent from Australasia and New Guinea, the family is nearly cosmopolitan in distribution; with the majority of its members inhabiting the northern temperate and arctic regions (Watson and Dallwitz, 2000). These dioecious, spring-flowering trees and shrubs typically inhabit wet or moist habitats throughout their geographic range. In Oklahoma, *Populus* and *Salix* are represented in every county.

Several species are cultivated for their ornamental value. The somewhat weak wood of many species is used as lumber, plywood, pulpwood, paper pulp, boxes, crates, food containers, matchsticks, matchboxes, and furniture stock (Tesky, 1992; Dirr, 1998; Little, 1998; Taylor, 2001). Members of the family commonly provide critical habitat for many wildlife and livestock species (Uchytil, 1989b; Tesky, 1992; Little, 1998; Taylor, 2001). They provide nesting habitat and cover for some nongame wetland bird species, forage for deer and livestock, and wood and shelter for many game birds and small mammals.

The objective of the work summarized in this chapter was to develop an understanding of the morphological circumscription of each species, clarify the nomenclatural history of each taxon, and write morphological descriptions and keys for identification to be incorporated in the *Flora of Oklahoma*.

METHODOLOGY: PREPARATION OF THE TREATMENT

Preparation of the family treatment began with an extensive literature review. Principal publications examined included those by Stephens (1973), Dorn (1976, 1994, and 1998), Eckenwalder (1977), Correll and Johnston (1979), Argus (1986, 1997), Great Plains Flora Association (1986), Newsholme (1992), Dirr (1998), Little (1998), and Diggs, Lipscomb, and O'Kennon (1999). Loans of specimens from state herbaria were requested (Table 1.1). These specimens were identified using available keys of Gleason and Cronquist (1963), Waterfall (1969), Correll and Johnston (1979), Great Plains Flora Association (1986), and Diggs, Lipscomb, and O'Kennon (1999). All specimens were annotated as a result of this work.

The morphological description of each species was written using DELTA (DEscriptive Language for TAxonomy), a computer program developed by Dallwitz and Paine (1986) that provides a standardized format for taxonomic descriptions. As the borrowed herbarium specimens were examined, a list of characters (each with appropriate states) was compiled in the DELTA format for CHARS (Table 1.2). A corresponding SPECS file was created, in which each character was defined as to type – unordered multistate (UM), ordered multistate (OM), integer numeric (IN), real numeric (RN), and text (TE) (Table 1.3).

The characters and their states of the herbarium specimens of each species were observed or measured, recorded on character data sheets, and subsequently coded in the DELTA format for ITEMS (Table 1.4). As ITEMS was compiled, CHARS was revised as necessary. A TONAT file (Table 1.5) was used to generate natural language descriptions. These descriptions were subsequently edited in Microsoft Word into the format adopted by the editorial committee; they are presented in the following text. Abbreviation of author names is according to Brummitt and Powell (1992). The paragraphs of informational notes are lengthy for the purposes of this dissertation, but will be condensed by the editorial committee.

Table 1.1 Herbaria from which specimens were borrowed and number of sheets of each taxon examined.

	P. alba	*P. deltiodes*	*S. amygdaloides*	*S. caroliniana*	*S. exigua*	*S. humulis*	*S. nigra*	Total
OKLA	0	54	12	40	42	12	97	257
OKL	0	167	26	68	91	6	245	603
CAMU	9	19	0	6	4	0	11	49
NWOSU	0	9	1	0	5	0	7	22
OCLA	2	27	1	7	19	3	47	106
DUR	10	41	1	9	15	4	46	126
NOSU	0	7	1	8	4	0	2	22
CSU	2	13	2	4	3	0	14	38
Total	23	337	44	142	183	25	469	1223

Zero indicates lack of Oklahoma specimens in loan.

Herbaria acronyms follow Holmgren and Holmgren (2003). OKLA = Oklahoma State University Herbarium in Stillwater, OK; OKL = Robert Bebb Herbarium at the University of Oklahoma in Norman, OK; CAMU = Cameron University Herbarium in Lawton, OK; NWOSU = Northwestern State University Herbarium in Alva, OK; OCLA = University of Science and Arts of Oklahoma Herbarium in Chickasha, OK; DUR = Southeastern Oklahoma State University Herbarium in Durant, OK; NOSU = Northeastern State University Herbarium in Tahlequah, OK; CSU = University of Central Oklahoma Herbarium in Edmond, OK.

Table 1.2 CHARS: a list of characters and their states used in DELTA program to generate taxon descriptions.

---

*SHOW: Salicaceae DELTA Character List.

*CHARACTER LIST

#1. plants <habit> <MANDATORY>/
1. trees/
2. small trees/
3. shrubs/

#2. plants <vegetative reproduction>/
1. solitary <implicit>/
2. colonial by rhizomes/
3. colonial by layering/

#3. plants <height of trees>/
m tall/

#4. bark <texture>/
1. smooth/
2. rough/
3. fissured <finely, etc.>/
4. furrowed <deeply, irregularly, etc.>/

#5. bark <specific color or colors that serve to distinguish the taxon>/

#6. stems <habit>/
1. erect/
2. decumbent/

#7. twigs <specific color or colors that serve to distinguish the taxon>/

#8. twigs <presence of indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or

ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#9. terminal buds <presence of terminal buds>/
1. present/
2. absent/

#10. terminal buds <shape> <*Populus* only>/
1. oblong/
2. conical/
3. ovoid/
4. lanceolate/
5. irregular/

#11. terminal buds <bud scales; specific color or colors that distinguish the taxon> <*Populus* only>/

#12. terminal buds <bud scales; presence of indumentum> <*Populus* only>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#13. terminal buds <length> <*Populus* only>/
mm long/

#14. lateral buds <shape>/
1. oblong/
2. conical/
3. triangular/
4. narrowly triangular/
5. ovoid/
6. lancelate/
7. irregular/

#15. lateral buds <bud scales; specific color or color that serves to distinguish the taxon>/

#16. lateral buds <bud scales; presence of indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#17. lateral buds <apices>/
1. apices caudate/
2. apices acuminate/
3. apices acute/
4. apices rounded/

#18. lateral buds <length>/
mm long/

#19. blades <shape>/
1. linear/

2. lanceolate/
3. oblanceolate/
4. ovate/
5. obovate/
6. oblong/
7. deltoid/

#20. blades <width>/
mm wide/

#21. blades <length>/
mm long/

#22. blade <apices>/
1. apices acute/
2. apices acuminate/
3. apices caudate/
4. apices obtuse/

#23. blade <margins>/
1. margins entire/
2. margins serrate/
3. margins dentate/
4. margins revolute/
5. margins sinuate/
6. margins crenate/

#24. blade <margins> <number of teeth per cm> <*Salix* only>/
teeth per cm/

#25. blade <of simple leaves; bases>/
1. bases rounded/
2. bases cuneate/
3. bases acute/
4. bases acuminate/
5. bases cordate/

#26. adaxial surfaces <color or colors that characterize the abaxial surface of blades>/

#27. adaxial surfaces <indumentum of abaxial surfaces of blades>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/

13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#28. abaxial surfaces <color or colors that characterize the adaxial surface of blades>/

#29. abaxial surfaces <indumentum of adaxial surfaces of blades>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#30. basilaminar glands <presence of basilaminar glands>/
1. present <implicit>/
2. absent/

#31. basilaminar glands <number>/

#32. petioles <specific color or colors that serve to distinguish the taxon> <*Salix* only>/

#33. petioles <shape>/

1. terete/
2. flattened perpendicular to blade/

#34. petioles <length>/
mm long/

#35. petioles <presence of indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#36. stipules <if present, distinctive, general descriptors that serve to characterize the taxon>/

#37. stipules <shape>/
1. linear/
2. lanceolate/
3. oblanceolate/
4. ovate/
5. obovate/
6. elliptic/
7. oblong/
8. reniform/
9. oval/

#38. stipules <length>/
mm long/

#39. stipules <indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/

3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. glandular/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#40. stipules <apices>/
1. apices caudate/
2. apices acuminate/
3. apices acute/
4. apices attenuate/
5. apices rounded/
6. apices obtuse/

#41. stipules <margins>/
1. margins entire/
2. margins serrate/
3. margins serrulate/
4. margins dentate/
5. margins glandular/

#42. catkins <flowering time and leaf development relationship>/
1. flowering before leaves/
2. flowering simultaneous with leaves/
3. flowering after leaves/

#43. catkins <density> <*Populus* only>/
1. densely flowered <rachis not or partially visible>/
2. moderately flowered <rachis partially visible>/
3. loosely flowered <rachis clearly visible>/

#44. catkins <length of catkins> <*Populus* only>/
mm long/

#45. catkins <presence of indumentum on rachis>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#46. catkin bracts <specific color or colors that serve to distinguish the taxon> <*Salix* only>/

#47. catkin bracts <shape>/
1. ovate/
2. obovate/
3. oblong/
4. oval/
5. flabellate/

#48. catkin bracts <length>/
mm long/

#49. catkin bracts <indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/

11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#50. catkin bracts <apices> <*Salix* only>/
1. apices caudate/
2. apices acuminate/
3. apices acute/
4. apices attenuate/
5. apices rounded/
6. apices inequalateral/

#51. catkin bracts <margins>/
1. margins entire/
2. margins erose/
3. margins ciliate/
4. margins toothed/
5. margins fimbriate/

#52. catkin bracts <persistence> <*Salix* only>/
1. persistent/
2. deciduous after flowering/

#53. staminate catkins <catkin and stem relationship> <*Salix* only>/
1. sessile on main branches/
2. borne on leafy lateral branchlets/

#54. staminate catkins <catkin and stem relationship> <length of leafy branchlets> <*Salix* only>/
mm long/

#55. staminate catkins <catkin density> <*Salix* only>/
1. densely flowered <rachis not or partially visible>/
2. moderately flowered <rachis partially visible>/
3. loosely flowered <rachis clearly visible>/

#56. staminate catkins <length> <*Salix* only>/
mm long/

#57. pedicels <length of pedicels> <*Populus* only>/
mm long/

#58. floral disks <presence of floral disks>/

1. present <implicit>/
2. absent/

#59. floral disks <width>/
mm wide/

#60. stamens <number>/

#61. anthers <color of anthers>/

#62. anthers <straightness of anthers after dehiscence> <*Salix*>/
1. straight/
2. recurved <slightly or strongly>/

#63. anthers <length of anthers> <*Salix*>/
mm long/

#64. filaments <filament indumentum> <*Salix*>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#65. nectaries on adaxial surfaces <staminate catkins> <whether present on adaxial surface> <*Salix* only>/
1. present/
2. absent/

#66. nectaries on adaxial surfaces < staminate catkins> <number on adaxial surface> <if present in 66> <*Salix* only>/
1. 1/

2. 2/
3. 3/
4. 4/
5. 5/
6. several/

#67. nectaries on abaxial surfaces < staminate catkins> <whether present on abaxial surface> <*Salix* only>/
1. present/
2. absent/

#68. nectaries on abaxial surfaces < staminate catkins> <number on abaxial surface> <if present in 67> <*Salix* only>/
1. 1/
2. 2/
3. 3/
4. 4/
5. 5/
6. several/

#69. pistillate catkins <catkin and stem relationship> <*Salix* only>/
1. sessile on main branches/
2. borne on leafy lateral branchlets/

#70. pistillate catkins <catkin and stem relationship> <length of leafy branchlets if present> <*Salix* only>/
mm long/

#71. pistillate catkins <catkin density> <*Salix* only>/
1. densely flowered <rachis not or partially visible>/
2. moderately flowered <rachis partially visible>/
3. loosely flowered <rachis clearly visible>/

#72. pistillate catkins <length> <*Salix* only>/
mm long/

#73. stipes <length of stipes> <*Salix* only>/
mm long/

#74. stigmas <appearance of stigma> <*Populus* only>/
1. filiform/
2. flabellate with fimbriate margin/

#75. stigmas <lobe number per stigma>/
1. 2-lobed/
2. 3-lobed/
3. 4-lobed/

#76. stigmas <persistence>/
1. persistent after flowering/
2. deciduous after flowering/

#77. styles <length> <*Salix*>/
mm long/

#78. ovaries <shape in longitudinal section>/

1. hemispherical/
2. elliptical/
3. cylindrical/
4. ovoid/
5. obovoid/
6. clavatge/
7. pyriform/

#79. ovaries <ovary indumentum>/
1. glabrous/
2. scabrous <rough to the touch with short, hard, rigid trichomes>/
3. scaberulous <minutely scabrous>/
4. pubescent <soft, short, dense trichomes>/
5. puberulent <minutely pubescent>/
6. velutinous <soft, short, dense, straight trichomes; velvety>/
7. tomentose <soft, short, dense, matted trichomes>/
8. villous <soft, long, dense, curly, ascending trichomes>/
9. sericeous <soft, long, dense or sparse, appressed, straight trichomes; silky>/
10. lanate <soft, long, dense, matted, ascending, curly trichomes; woolly>/
11. pilose <soft, long, sparse, ascending, curly or straight trichomes>/
12. arachnoid <soft, very long, dense, thin, loosely entangled trichomes; cobwebby>/
13. hirsute <stiff, long, dense or sparse, erect or ascending, straight trichomes>/
14. hispid <stiff, long, tapered, dense or sparse, erect or ascending, straight trichomes; bristly>/
15. strigose <stiff, long, sharp, bulbous base, dense or sparse, appressed, straight or curved trichomes>/
16. surfaces with glandular trichomes/
17. stellate <trichomes with branches radiating from base or separate hairs aggregated into star-like clusters>/
18. surfaces with barbed trichomes/
19. strigulose <dimunitive of strigose>/
20. hispidulous <diminutive of hispid>/
21. hirsutellous <diminutive of hirsute>/

#80. capsules <length>/
mm long/

#81. seeds <per carpel> <when applicable>/
1. 1/
2. 2/
3. 3/
4. 4/
5. 5/
6. 6/
7. 7/
8. 8/
9. 9/
10. 10/
11. 11/
12. numerous/

#82. nectaries on adaxial surfaces <pistillate catkins> <whether present on adaxial surface> <*Salix* only>/
1. present/
2. absent/

#83. nectaries on adaxial surfaces <pistillate catkins> <number on adaxial surface> <if present in 83> <*Salix* only>/
1. 1/
2. 2/
3. 3/
4. 4/
5. 5/
6. several/

#84. nectaries on abaxial surfaces <pistillate catkins> <whether present on abaxial surface> <*Salix* only>/

1. present/
2. absent/

#85. nectaries on abaxial surfaces <pistillate catkins> <number on abaxial surface> <if present in 85> <*Salix* only>/
1. 1/
2. 2/
3. 3/
4. 4/
5. 5/
6. several/

#86. Taxonomy and nomenclature/

#87. Distribution/

#88. Ecology/

#89. Economic and wildlife significance/

Table 1.3 SPECS: characters defined as to type and used in DELTA program to generate taxon descriptions. Um=unorder multistate, OM=ordered multistate, IN=integer numeric, RN=real numeric, TE=text.

---

*SHOW: Salicaceae DELTA Specifications.

*NUMBER OF CHARACTERS 89
*MAXIMUM NUMBER OF STATES 21
*MAXIMUM NUMBER OF ITEMS 9

*CHARACTER TYPES 1-2,OM 3,RN 5,TE 7,TE 10,OM 11,TE 13,RN 14,OM 15,TE 17,OM 18,RN 20-21,RN 24,RN 26,TE 27,OM 28,TE 29,OM 31,IN 32,TE 34,RN 36,TE 38,RN 39,OM 44,RN 46,TE 48,RN 49-50,OM 54,RN 55,OM 56-57,RN 59,RN 60,IN 61,TE 63,RN 66,OM 68,OM 70,RN 71,OM 72-73,RN 75,OM 77,RN 80,RN 81,OM 83,OM 85,OM 86-89,TE

*NUMBERS OF STATES 1,3 2,3 4,4 8,21 10,5 12,21 14,7 16,21 17,4 19,7 22,4 23,6 25,5 27,21 29,21 35,21 37,9 39,21 40,6 41,5 42,3 43,3 45,21 47,5 49,21 50,6 51,5 55,3 64,21 66,6 68,6 71,3 75,3 78,7 79,21 81,12 83,6 85,6

*IMPLICIT VALUES 2,1 30,1 58,1

*DEPENDENT CHARACTERS

*MANDATORY CHARACTERS

Table 1.4 ITEMS, measurements and observations of specimens coded using CHARS in DELTA program to generate taxon descriptions.

---

*SHOW: Salicaceae DELTA Items.

*ITEM DESCRIPTIONS

Populus <L. Cottonwood>/
1,1 3,5-20 4,1/4<deeply> 5<grayish-white or gray to light brown> 7<orange-brown to olive-brown> 8,7<densely white>/1 9,1 10,1/4 11<reddish-brown or tan to greenish-brown and resinous> 12,8-7<densely white>/1 13,5-15 14,1/6 15<reddish-brown or tan to greenish-brown and resinous> 16,8-7<densely white>/1 18,6-25 19,4/4-7 20,30-100 21,30-95 22,4-1/2-4<sometimes> 23,5/6 25,1-5 26<green to dark green or grayish-green> 27,1 28<white to silver or green to grayish-green> 29,7<densely white>/1 30,1 31,2<often> 33,1/2 34,15-100 35,7<white>/1 42,1 43,1/2 44,20-150 45,8-7/1 47,1-2/5 48,1-3 49,8/1 51,3&4<shallowly>/5 57,0.5-8 58,1 59,0.5-4 60,6-80 74,1/2 75,2/3 78,4<or narrowly ovoid> 79,7/1 80,1.5-18 81,4-12 86<Typically a northern temperate genus of about 35 species, *Populus* occurs throughout Europe, Asia, tropical Africa, Central America, and North America, of which 11 species are recognized. In Oklahoma, only 2 species are found. *Populus* is the Latin word for "tree of the people." Common names of the genus include Aspen, Poplar, and Cottonwood.> 87<In Oklahoma, *Populus* is represented in every county.> 88<Members of this genus occupy a variety of habitats throughout their distribution in North America. These quick growing trees are often prized for their ornamental value and are easily propagated primarily by using stem cuttings. Many members of the genus are easily susceptible to damage by storms and strong winds, often diminishing their ornamental value. Members of this wind and insect pollinated genus typically flower in early spring.> 89<*Populus* provides critical habitat for many wildlife species that take advantage of cover and herbage. Many species of *Populus* are cultivated as ornamental trees and shrubs. The wood is used as lumber, veneer, plywood, fiberboard, pulpwood, paper pulp, boxes, crates, food containers, cutting boards, interior furniture parts, and agricultural implements.>

Populus alba <L. White or Silver Poplar>/
1,1 2,1-2 3,5-20 4,1<cracking or furrowing basally> 5<grayish-white> 7<orange brown to olive brown> 8,7<dense white> 10,3 11<reddish-brown> 12,8-7<densely white> 13,5-8 14,5 15<reddish-brown> 16,8-7<densely white> 18,6-10 19,4 20,35-75 21,40-80 22,4-1 23,5<often 3-5 palmately lobed> 25,1-5<narrowly> 26<green to dark green> 27,1 28<white to silver> 29,7<dense white> 31,2<often> 33,1 34,15-60 35,7<white> 42,1 43,1 44,20-60 45,7-8 47,1-2 48,1-3 49,8 51,3&4<shallowly> 57,0.5-1 59,0.5-1.5 60,6-14 74,1 75,3 78,4<narrowly> 79,7 80,1.5-4 81,4-6 86<*P. alba* is a morphologically and biologically distinct species whose classification has not been changed since its description by Linnaeus. The common name "abele" is sometimes used in reference to this taxon.> 87<The white poplar is indigenous to central and southern Europe, western Siberia, and central Asia. European immigrants introduced the tree to North America in 1748. The tree has since become naturalized in many parts of the continent including many areas of Oklahoma.> 88<It is an aggressive tree species that can take over portions of natural areas by shading out native vegetation. The species outcompetes many native tree and shrub species because it can grow in a variety of soils, produces large seed crops, and can resprout easily thus forming dense groves that are hard to eradicate. The trees grow best in full sunlight in areas such as fields, forest edges, and the margins of wetlands. Local spread of white poplar is by vegetative means, through root sprouts.> 89<The wood of many Poplars has significant commercial value. It is widely used as a source of pulpwood and for the manufacture of matchsticks, matchboxes, and fruit and flower baskets. Introduced originally as shade and ornamental tree, it now has very little ornamental value due to its susceptibility to a wide variety of pest, insects, and diseases.>

Populus deltoides <Bartr. ex Marsh. Cottonwood>/
1,1 3,20-30 4,4<deeply> 5<tan to yellow green when younger, gray to light brown when older> 7<olive-brown to orange-tan> 8,1 10,4 11<tan to greenish-brown,resionous> 12,1 13,6-15 14,6 15<tan to greenish-brown,resionous> 16,1 18,10-25 19,7 20,30-100 21,30-95 22,2-4 23,6 25,1-5 26<green to grayish green> 27,1 28<green to grayish green> 29,1 31,2<often> 33,2 34,30-100 35,1 42,1 43,3 44,35-150 45,1 47,5 49,1 51,5 57,1-8 59,1-4 60,20-80 61<reddish-brown> 74,2 75,2-3 78,4 80,6-18 81,12 86<: Morphologically variable, the species is divided by some taxonomist into three subspecies on the basis of pedicel length, appearance of leaf apex, and presence of basilaminar glands. Some taxonomic and nomenclatural confusion has been caused due to its ability to hybridize with other members of the genus. The binomial synonym *P. sargentii* is encountered in historic literature. The common name "Cottonwood" relates to the cottony appearance exhibited by the seeds.>
87<: One of eight species of *Populus* native to North America, *P. deltiodes* is distributed throughout the eastern half of the continent with its western most expansion reaching the foothills of the Rocky Mountains. *P. deltiodes* occurs in every county in Oklahoma.> 88<: Germination and seed establishment require barren soils. Such requirements have led to the dominance of floodplains and bottomland hardwood forest. It is not restricted to this habitat and has often been found in upland habitats as well.> 89<: The wood is used as a source for paper pulp, pallets, crates, and food containers. The inner bark has long been used medicinally for treatment of headaches, fevers, and inflammations. The active component is the natural glycoside salicin, a precursor of salicylic acid; which is used in making aspirin. Cottonwoods provide critical habitat for many wildlife and livestock species.>

Salix <L. Willow>/
1,1-3 2,1-2-3 3,1.5-20 4,1/3<finely>/4<deeply>/4<irregularly> 5<reddish-brown or gray or light gray or dark brown to blackish> 6,1/2 7<yellowish-brown to dark brown or light yellow to reddish-brown to grayish brown or gray> 8,4<gray>/7-8<becoming glabrous>/1 9,2 14,1/2 15<reddish-brown or yellowish-brown or tan> 16,5/9/1 17,3/4 18,1-4 19,1/2-1<-lanceolate>/4-2<ovate->/3-6-5<narrowly> 20,5-50 21,20-170 22,1/2-3 23,1/2<variously> 24,2-15 25,2/1-3/4 30,2 32<tan or yellow or yellowish to reddish-brown> 26<shiny dark green or yellow-green or green> 27,1/11<along midrib> 28<glaucous or yellowish to pale green or green> 29,4<densely short>/1/4/1<along midrib> 34,1-20 35,7/8/11/1 36<absent or present, in which they are persistent on vigorous twigs or minute or caducous> 37,8/4-2 38,1-12 39,4/16/1 40,3/6 41,2/5 42,1/2 45,8<variously>/1<at time of abscission> 46<yellowish or dark brown to purple> 47,4-3/1/2 48,1-3.5 49,4/8/11 50,5/3-5 51,1/2 52,1/2 53,1/2 54,2-25 55,1/2 56,10-95 60,2/3-7 61<yellow or purple> 62,1/2<slightly or strongly> 63,0.4-0.7 64,8/11/1 66,1-2 68,1-6 69,1/2 70,2-40 71,1/3 72,10-100 73,0.5-2.5 75,1/3 76,1/2 77,0.1-0.8 78,7/4<narrowly> 79,4/1 80,3-8 81,10-12 83,1 84,2 86<*Salix* is the ancient common name of the willows. Comprising some 400 species of cold and temperate areas of the northern hemisphere, with few in the southern hemisphere, the genus is represented by about 90 species in North America and by only six species in Oklahoma.> 87<In Oklahoma, *Salix* is represented in every county.> 88<With the exception of *S. humilis*, members of the genus most commonly occur in bottomland habitats and wet areas in upland sites. *Salix* species require barren soils for germination and seedling establishment. Although willows are difficult to propagate in quantity by seed, rootstocks of young branches sprout prolifically, making this the usual method of artificial regeneration.> 89<A variety of wildlife take advantage of cover and herbage provided by members of the genus. Many species of *Salix* are cultivated as ornamental trees and shrubs. The only North American species of any commercial importance is *S. nigra*, which is used for furniture stock, boxes, crates, doors, and pulp. Used medicinally for millennia as an effective painkiller and treatment for inflammation, the natural glycoside salicin, the precursor of salicylic acid, was isolated in 1829 from the inner bark of *Salix*. Today it is the basic ingredient of aspirin, although the synthesized acetylsalicylic acid is used rather than the natural form.>

Salix amygdaloides <Anderss. Peachleaf Willow>/
1,1 3,4-20 4,4<irregularly> 5<dark brown> 6,1 7<gray to light yellow> 8,1 14,2 15<yellowish-brown> 16,1 17,3 18,2.5-4 19,2-4<-lanceolate> 20,10-50 21,20-100

22,2-3 23,2<finely> 24,6-15 26<yellowish-green> 27,1 28<pale yellow to thickly white glaucous> 29,1 32<yellowish> 25,2-1 34,5-20 35,1-7<sparsely, adaxially> 36<, if present, minute and caducous or sometimes persistent on vigorous twigs> 37,8 38,3-12 41,2 42,2 45,8<densely> 46<pale yellow> 47,2-3 48,1.5-3 49,8 50,6<slightly> 51,1 52,2 53,2 54,10-20 55,2 56,25-60 60,3-7 61<yellow> 62,2<slightly> 63,0.5-0.6 64,11 66,1 67,2 69,2 70,4-40 71,3 72,25-70 73,1-2.5 75,1/3 76,1 77,0.4-0.6 78,7 79,1 80,4-12 81,9 83,1 84,2 86<The synonyms *S. wrightii*, *S. nigra* var. *wrightii*, and *S. nigra* var. *amygdaloides* are encountered in historical literature.> 87<A native species, *S. amygdaloides* is distributed across the continent with the exception of the SE ¼ of the United States. In Oklahoma, populations are restricted to the western panhandle.> 88<A prolific seed producer, *S. amygdaloides* requires barren soils for germination and seed establishment. Germination is rapid, usually within 12 to 24 hours after dispersal if a moist seedbed is reached. Adapted to a variety of soil types, *S. amygdaloides* is characteristically encountered in the moist, fertile sandy or alluvium soils of riparian areas such as banks of streams and rivers. *S. amygdaloides* is characteristic of early stages of succession. The trees are shade intolerant therefore they persist only along a river's edge where repeated flooding prevents other species from being established. Because of the soil-binding properties, the species helps stabilize streambank and protect them from erosion. Flowering occurs in April and May and fruiting follows in late May and early June.> 89<Like many members of the species, *S. amygdaloides* contains the glycoside salicin, a precursor of salicylic acid (aspirin), which has been used medicinally for millennia as an effective painkiller and treatment of inflammation. It belongs to a structurally complex riparian vegetation community that provides an array of habitats and supports many different species of animals.>

Salix caroliniana <Michx. Carolina Willow>/
1,1 3,1.5-10 4,3<finely> 5<light gray> 6,1 7<reddish to grayish-brown> 8,1-4<gray> 14,2 15<reddish-brown> 16,9<sparsely> 17,3 18,1.5-4 19,2 20,7-20 21,50-170 22,2 23,2 24,6-12 25,2-1 26<shiny dark green> 27,1-11<along midrib> 28<thickly, white glaucous> 29,1/4<along veins> 32<yellowish to reddish-brown> 34,3-8 35,7-11<sparsely, adaxially> 36<persistent on vigorous twigs> 37,8 38,2-7 39,16<usually, adaxially> 40,6-3 41,2 42,2 45,8-1<at time of abscission> 46<yellowish> 47,2-1<broadly> 48,1-2 49,8 50,3-5 51,2 52,2 53,2 54,4-25 55,2 56,35-95 60,6<rarely 4 or 5> 61<yellow> 62,1-2<strongly> 63,0.4-0.6 64,11 66,1-2 68,1-2 69,2 70,6-40 71,3 72,45-100 73,1-2 75,1 76,1 77,0.3-0.8 78,7 79,1 80,3-6 81,10-12 83,1 84,2 86<The synonyms *S. longipes*, *S. nigra* var. *longipes*, *S. wardii*, and *S. occidentalis* are encountered in historical literature. Closely related to the black willow, *S. caroliniana* has been known to hybridize with *S. nigra* when ranges intersect> 87<A native species, *S. caroliniana* is found from south Pennsylvania to south Florida, west to central Texas and north to southeast Nebraska. In Oklahoma, populations occur in northeast and southeast as well as isolated populations in south-central and southwest part of the state.> 88<Classified as an obligate wetland species, *S. caroliniana* occurs in wet soils of rocky stream banks and other wet areas. Flowering occurs in late March and April and fruiting typically follows in May.> 89<*S. caroliniana* provides cover for birds and small mammals. The wood of this tree is used in making toys, charcoal, and furniture.>

Salix exigua <Nutt. var. *interior* (Rowlee) Cronq. Sandbar Willow, Coyote Willow>/
1,3 2,2 3,4-6 4,1-3<slightly> 5<gray> 6,1 7<light yellow to reddish-brown> 8,7/8<becoming glabrous> 14,1 15<reddish-brown> 16,9<becoming glabrous> 17,4 18,1-4 19,1 20,3-10 21,45-100 22,1 23,3<remotely or irregularly> 24,2-5 25,4 26<yellowish-green> 27,1-9<sparsely> 28<yellowish to pale-green> 29,1-9<sparsely> 32<yellowish-brown> 34,1-5 35,1 36<absent> 42,2 45,8<sparsely to densely> 46<yellowish> 47,3-2 48,1.5-3.5 49,11 50,3-5 51,2 52,2 53,2 54,2-25<1st catkins, 40-180 mm long on later catkins> 55,1 56,20-50 60,2 61<reddish becoming yellow> 62,2<strongly to slightly> 63,0.4-0.7 64,8 66,2 68,2 71,3 72,35-70 73,0.4-1.5 75,3 76,2 77,0.1-0.2 78,7-4<narrowly> 79,1/9<long silky when mature> 80,5-8 81,12 83,1 84,2 86<*S. exigua* is divided by some taxonomists into a number of varieties on the basis of indumentum type, leaf shape, and the number of teeth on blade margins. One such variety is *S. exigua* var. *interior*. Although some classifications treat *S. exigua* and *S. interior* as separate species, the classification presented here follows that of Cronquist (1964), Argus (1986) and Dorn (1998) in

which *S. interior* is treated as a variety. Variety *interior,* considered the eastern phase of the *Salix exigua* complex by Argus (1986), differs from variety *exigua*, the western phase, in having leaves that are less densely sericeous, more distinctly toothed, and more conspicuously veined. In addition the catkins are more loosely flowered and capsules longer. The specific epithet, *exigua*, is the Latin term meaning small or short, referring to the usually small size of the plant.> 87<A native species, *S. exigua* is distributed across the western two thirds of the continent. In Oklahoma, populations occur across the state.> 88<*S. exigua* requires barren soils for germination and seed establishment. Unlike the other members of the genus in Oklahoma, *S. exigua* can reproduce vegetatively by sprouting from underground shoot buds in a process called suckering. Characteristic of early seral communities, *S. exigua* is commonly found in association with other members of the family, namely *Populus deltoides* and *Salix nigra*. *S. exigua* is characteristically encountered in the moist, fertile sandy or alluvium soils of riparian areas such as banks of streams and rivers but can occasionally be found in periodically wet areas in upland sites. Flowering can typically occur twice during a growing season, the first in April and May, then again during mid to later times during the summer.> 89<Like many members of the species, *S. exigua* contains the glycoside salicin, a precursor of salicylic acid (aspirin), which has been used medicinally for millennia as an effective pain killer and treatment of inflammation. *S. exigua* is used for erosion control along streambanks, lakeshore, and riparian area development and restoration. A common forage species for deer, *S. exigua* also provides wood and shelter for many game birds.>

Salix humilis <Marsh. Prairie Willow>/
1,3 2,3 3,1-3 5<reddish-brown> 6,2 7<yellowish brown to dark brown> 8,4<gray> 14,1 15<reddish-brown> 16,5 17,4 18,2-5 19,3-5-6<narrowly> 20,5-20 21,30-85 22,1 23,1-4-2<remotely or irregularly> 24,7-13 25,2 26<shiny dark green> 27,1 28<glaucous> 29,4<densely short> 32<tan> 34,2-8 35,7/11/8 36<foliaceous on vigorous twigs, minute rudiments, or absent on older twigs> 37,2-4 38,3-7 39,4 40,3 41,2<sparsely> 42,1 45,8<sparsely> 46<dark brown to purple> 47,3-4 48,1-2 49,8 50,5 51,1 52,1 53,1<with 2 or 3 leafy bracts> 55,1 56,10-40 60,2 61<purple> 62,1 63,0.4-0.6 64,1-11<sparsely> 66,1 68,2 71,1 72,10-60 73,0.5-2 75,1/3 76,1 77,0.2-0.4 78,7-4<narrowly> 79,4 80,4-7 81,12 83,1 84,2 86<Some taxonomists recognize two varieties of *S. humilis*, based on leaf shape and abaxial leaf appearance.> 87<A native species, *S. humilis* occurs in southeastern Canada and distributed through the eastern part of the Great Plains, south to Texas. In Oklahoma, populations occur in the southeastern part of the state.> 88<As its common name, "prairie willow" suggests, *S. humilis* is found in upland prairies and savannas, especially in sandy soils.> 89<The species provides cover for small and medium sized mammals and deer and livestock occasionally eat the herbage.>

Salix nigra <Marsh. Black Willow>/
1,1 3,2-20 4,4<deeply> 5<dark brown to blackish> 6,1 7<light reddish-brown to darker grayish-brown> 8,1-4 14,2 15<tan> 16,1 17,3 18,1.5-2.5 19,2-1<-lanceolate> 20,7-20 21,40-150 22,2 23,2 24,7-13 25,3-2-1 26<green> 27,1-11<sparsely along midrib> 28<green> 29,1-11<sparsely along midrib> 32<yellowish-brown> 34,3-10 35,11<sparsely adaxially> 36<caducous or sometimes persistent on vigorous twigs> 37,4-2 38,1-8 40,3 41,5 42,2 45,8-1<at time of abscission> 46<yellowish> 47,2 48,1-3 49,4 50,3-5 51,1 52,2 53,2 54,4-15 55,2 56,15-70 60,6<rarely 4 or 5> 61<yellow> 62,1-2<strongly> 63,0.4-0.6 64,11 66,1 68,2-3 69,2 70,5-35 71,1 72,30-100 73,0.5-2 75,3 76,1 77,0.2-0.4 78,7 79,1 80,3-5 81,10-12 83,1 84,2 86<Some taxonomists recognize numerous varieties of *S. nigra*, based on petiole length, blade shape, and blade width. *S. nigra* has been known to hybridize with *S. amygdaloides* (*Salix* x *glatfferteri* Schnedider) when ranges intersect in other states, although this has not been encountered in collections of Oklahoma.> 87<A native species, *S. nigra* is distributed across the eastern half of the continent. In Oklahoma, populations occur across the state, except for the panhandle.> 88<Along with *Populus deltoides*, *S. nigra* occurs as a codominant in many early seral floodplain communities where it often forms gallery forest with distinct cohorts of different heights. *S. nigra* requires barren soils for germination and seed establishment. Due to the distance that can be traveled by the largely wind dispersed cottony diaspore, it is not restricted to bottomland habitat and has often been found in upland habitats, such as ditches, drainages, and other period wet

areas, where conditions for germination and seedling establishment are favorable. *Salix nigra* is very intolerant of shade. Very susceptible to fire damage, *S. nigra* has the ability to sprout from the base following fire. Flowering occurs in late March and April and fruiting typically follows in May.> 89<Like many members of the species, *S. nigra* contains the glycoside salicin, a precursor of salicylic acid (aspirin), which has been used medicinally for millennia as an effective pain killer and treatment of inflammation. *S. nigra* is the largest and only commercially important willow of about 90 species native to North America. Once used extensively for artificial limbs because of its light weight, the wood holds its shape well and does not splinter. The most common uses of the wood today is for furniture stock, boxes, crates, doors, and pulp. Rated fair in nutritional value, *S. nigra* is a food source for birds, deer, small mammals, and some livestock. *S. nigra*, along with associated species *Populus deltoides*, are commonly used as nesting habitat and cover by some non-game wetland bird species. Honeybees are common spring visitors, obtaining substantial amounts of pollen from flowers.>

Table 1.5 TONAT: instructions to generate word descriptions of taxa from CHARS in DELTA program.

```
*SHOW: Translate into natural language
*HEADING: Salicaceae DELTA
*LISTING FILE TONAT.LST *PRINT FILE TONAT.PRT
*DATA BUFFER SIZE 6000
*INPUT FILE  SPECS.TXT
*SPECIAL STORAGE
*TRANSLATE INTO NATURAL LANGAUGE
*OMIT TYPSETTING MARKS
*REPLACE ANGLE BRACKETS
*OMIT CHARACTER NUMBERS
*OMIT INAPPLICABLES


*ITEM SUBHEADINGS

*LINK CHARACTERS 1-3 4-5 7-8 9-13 14-18
19-21 22-25 26-27 28-29 30-31 32-35 36-41
42-45 46-52 53-56 58-59 61-63 65-66 67-68
69-72 74-76 78-79 82-83 84-85

*INPUT FILE CHARS.TXT

*PRINT HEADING
*INPUT FILE ITEMS.TXT
```

TAXONOMIC TREATMENT OF THE SALICACEAE

SALICACEAE C.F.B. de Mirabel Willow Family

Plants trees or shrubs; dioecious. **Leaves** simple; alternate; venation pinnate; stipules present, persistent or caducous. **Inflorescences** catkins; axillary; bracts present, small or scale like, often deciduous. **Flowers** produced before or simultaneously with leaves; imperfect, staminate and pistillate similar; perianths absent or in 1-series. **Sepals** absent or modified into cup-like disk or 1 or 2 glands. **Petals** absent. **Androecia** bilaterally symmetrical. **Stamens** 1 or 2 to numerous; free or fused by filaments. **Pistils** 1; compound, carpels 2 or 4; sessile or short stipitate; stigmas 2 or 4, 2-lobed or not lobed; styles short or absent; ovaries superior; locules 1; placentation parietal or rarely basal. **Nectaries** absent or present. **Fruits** capsules. **Seeds** numerous; comose.

The family is represented in Oklahoma by 2 genera and 7 species. Its distribution is almost cosmopolitan with greatest diversity in north temperate and arctic regions. Ours are typically found in wet or moist habitats. The inner bark of both genera contains the precursor of aspirin and acetametaphin which has been used medicinally for headaches, fevers, and as an anti-inflammatory for thousands of years (Tyrl et al., 2003).

1. Leaves deltoid or ovate. Terminal buds present. Axillary bud scales 3-7. Bract margins fimbriate or laciniate. .... ***Populus***

1. Leaves lanceolate or falcate or elliptic or linear or oblanceolate. Terminal buds absent. Axillary bud scales 1. Bract margins entire. .... ***Salix***

Populus C. Linnaeus Cottonwood

Plants trees; 5-20 m tall. **Bark** smooth or deeply furrowed; grayish-white or gray to light brown. **Twigs** orange-brown to olive-brown; densely white tomentose or glabrous. **Terminal buds** present; oblong or lanceolate; reddish-brown or tan to greenish-brown and resinous; villous to densely white tomentose or glabrous; 5-15 mm long. **Lateral buds** oblong or lanceolate; reddish-brown or tan to greenish-brown and resinous; villous to densely white tomentose or glabrous; 6-25 mm long. **Blades** ovate or ovate to deltoid; 30-100 mm wide; 30-95 mm long; apices obtuse to acute or acuminate; margins sinuate or crenate; bases rounded to cordate; adaxial surfaces green to dark green or grayish-green; glabrous; abaxial surfaces white to silver or green to grayish-green; densely white tomentose or glabrous. **Basilaminar glands** present or absent. **Petioles** terete or flattened perpendicular to blades; 15-100 mm long; white tomentose or glabrous. **Catkins** flowering before leaves; densely or moderately flowered; 20-150 mm long; villous to tomentose or glabrous; bracts ovate to obovate or flabellate; 1-3 mm long; villous or glabrous; margins ciliate and shallowly toothed or fimbriate. **Pedicels** 0.5-8 mm long. **Floral disks** present; 0.5-4 mm wide. **Stamens** 6-80. **Stigmas** filiform or flabellate with fimbriate margins; 3-lobed or 4-lobed. **Ovaries** ovoid or narrowly ovoid; tomentose or glabrous. **Capsules** 1.5-18 mm long. **Seeds** 4 to numerous.

Populus is a Latin word meaning "of the people." Other common names of the genus are aspen and poplar. Principally a northern temperate genus of about 35 species, *Populus* occurs throughout Europe, Asia, tropical Africa, Central America, and North America (Dirr, 1998), where 11 species are recognized (Schreiner, 1974; USDA, 2003). In Oklahoma, only two species occur, but the genus is present in every county (Figure 1.1a-b). Rapid growing, trees are often prized for their ornamental value and are easily propagated, primarily via stem cuttings. They are, however, susceptible to damage by storms and strong winds, which diminishes their ornamental value (Taylor, 2001). Their wood is used for lumber, veneer, plywood, fiberboard, pulpwood, paper pulp, boxes, crates, food containers, cutting boards, interior furniture parts, and agricultural implements (Young and Young, 1992; Dirr, 1998; Taylor, 2001). *Populus* provides critical habitat for many wildlife species that take advantage of cover provided or consume the herbage (Little, 1998; Taylor, 2001; Tyrl et al., 2002). Members of this wind pollinated genus typically flower in early spring with fruiting and seed dispersal soon following.

1. Leaves ovate; abaxial surfaces white tomentose. Petioles terete. Catkin rachises tomentose. Capsules 3-5 mm long.............................. ***P. alba***

1. Leaves deltoid; abaxial surfaces glabrous. Petioles flattened. Catkin rachises glabrous. Capsules 15-20 mm long..................... ***P. deltiodes***

Populus alba **L.** White Poplar, Silver Poplar. **Plants** trees; solitary to colonial by rhizomes; 5-20 m tall. **Bark** smooth cracking or furrowing basally; grayish-white. **Twigs** orange brown to olive brown; densely white tomentose. **Terminal buds** ovoid; reddish-brown; villous to densely white tomentose; 5-8 mm long. **Lateral buds** ovoid; reddish-brown; villous to densely white tomentose; 6-10 mm long. **Blades** ovate; 35-75 mm wide; 40-80 mm long; apices obtuse to acute;

margins sinuate often 3-5 palmately lobed; bases rounded to narrowly cordate; adaxial surfaces green to dark green; glabrous; abaxial surfaces white to silver; densely white tomentose. **Basilaminar glands** 2. **Petioles** terete; 15-60 mm long; white tomentose. **Catkins** flowering before leaves; densely flowered; 20-60 mm long; tomentose to villous; bracts ovate to obovate; 1-3 mm long; villous; margins ciliate and shallowly toothed. **Pedicels** 0.5-1 mm long. **Floral disks** 0.5-1.5 mm wide. **Stamens** 6-14. **Stigmas** filiform; 4-lobed. **Ovaries** narrowly ovoid; tomentose. **Capsules** 1.5-4 mm long. **Seeds** 4 to 6.

P. alba is a morphologically and biologically distinct species whose classification has not been changed since its description by Linnaeus. The common name "abele" is sometimes used in reference to this taxon. Indigenous to central and southern Europe, western Siberia, and central Asia, *P. alba* was introduced in North America in 1748 by European immigrants (Dirr, 1998). The taxon has since naturalized in many regions of the continent, including areas of Oklahoma (Figure 1.1a). Because of its ability to grow in a variety of soils, produce large seed crops, and form dense groves via root sprouts, it is an aggressive species that can take over portions of natural areas by outcompeting many native tree and shrub species. When shoots are cut or damaged it resprouts easily, thus making established colonies difficult to eradicate (Glass, 1990; Little, 1998). The trees grow best in full sunlight in fields, at forest edges, and at the margins of wetlands. The wood of *P. alba* has significant commercial value. It is widely used as a source of pulpwood and for the manufacturing of matchsticks, matchboxes, and fruit and flower baskets. Introduced originally as a shade and ornamental tree, it now has little ornamental value due to its susceptibility to a wide variety of pest, insects, and diseases (Dirr, 1998). Flowering occurs in March and April, with fruiting and seed dispersal following in late April and May.

***Populus deltoides* Bartr. ex Marsh.** Cottonwood, Eastern Cottonwood. **Plants** trees; 20-30 m tall. **Bark** deeply furrowed; tan to yellow green when younger gray to light brown when older. **Twigs** olive-brown to orange-tan; glabrous. **Terminal buds** lanceolate; tan to greenish-brown resinous; glabrous; 6-15 mm long. **Lateral buds** lanceolate; tan to greenish-brown resinous; glabrous; 10-25 mm long. **Blades** deltoid; 30-100 mm wide; 30-95 mm long; apices acuminate to obtuse; margins crenate; bases rounded to cordate; adaxial surfaces green to grayish green; glabrous; abaxial surfaces green to grayish green; glabrous. **Basilaminar glands** 0-2. **Petioles** flattened perpendicular to blade; 30-100 mm long; glabrous. **Catkins** flowering before leaves; loosely flowered; 35-150 mm long; glabrous; bracts flabellate; glabrous; margins fimbriate. **Pedicels** 1-8 mm long. **Floral disks** 1-4 mm wide. **Stamens** 20-80; anthers reddish-brown. **Stigmas** flabellate with fimbriate margins; 3 to 4-lobed. **Ovaries** ovoid. **Capsules** 6-18 mm long. **Seeds** numerous.

Morphologically variable, the species is divided by some taxonomists into three subspecies on the basis of pedicel length, appearance of leaf apex, and presence of basilaminar glands (Eckenwalder, 1977; Cooper and Van Haverbeke, 1990). Some taxonomic and nomenclatural confusion exists due to its ability to hybridize with other members of the genus (Great Plains Flora

Association, 1986; Dirr, 1998). The synonym *P. sargentii* is encountered in the older literature (Gleason and Cronguist, 1963). The common name "cottonwood" reflects the cottony appearance exhibited by the masses of comose seeds. One of eight species of *Populus* native to North America, *P. deltiodes* is distributed primarily throughout the eastern half of the continent, but extends into the southwestern part of the United States (Eckenwalder, 1977; Little, 1979). *Populus deltoides* occurs in every county in Oklahoma (Figure 1.1b). Often forming gallery forests of distinct cohorts of differing heights, *P. deltiodes* requires barren soils for germination and seed establishment (Schreiner, 1974). Such requirements result in its codominance with *Salix nigra* in floodplains and bottomland hardwood forests (Cooper and Van Haverbeke, 1990; Tyrl et al, 2002). Due to the distance that the wind dispersed seeds can travel, it is not restricted to bottomland habitats and is often found in upland habitats, such as ditches, drainages, and other periodically wet areas, where conditions for germination and seedling establishment are favorable (Taylor, 2001). Although difficult to propagate in quantity by seed, rootstocks of young branches sprout prolifically, making this the usual method of artificial regeneration (Dirr, 1998). Cottonwoods provide important habitat for many wildlife and livestock species. The wood is used for pallets, crates, food containers, and paper pulp (Taylor, 2001). The pulp produces a very high-grade gloss paper (Cooper and Van Haverbeke, 1990). The inner bark has long been used medicinally for treatment of headaches, fevers, and inflammations. The active component is the natural glycoside salicin, a precursor of salicylic acid, which is used in making aspirin

(2002; Duke 1983). An important component of windbreak plantings in the Great Plains, *P. deltiodes* is frequently used as an ornamental to provide quick, yet rather temporary, esthetic and protective effects (Cooper and Van Haverbeke, 1990). Flowering occurs from early March through April, with fruiting and seed dispersal soon following in mid May through July.

Salix C. Linnaeus Willow

Plants shrubs to trees; solitary to colonial by rhizomes or by layering; 1.5-20 m tall. **Bark** smooth or finely deeply or irregularly furrowed; reddish-brown or gray or light gray or dark brown to blackish. **Stems** erect or decumbent. **Twigs** yellowish-brown to dark brown or light yellow to reddish-brown to grayish-brown or gray; gray pubescent or tomentose to villous becoming glabrous or glabrous. **Terminal buds** absent. **Lateral buds** oblong or conical; reddish-brown or yellowish-brown or tan; puberulent or sericeous or glabrous; apices acute or rounded; 1-4 mm long. **Blades** linear or lanceolate to linear-lanceolate or ovate to ovate-lanceolate or oblanceolate to oblong to narrowly obovate; 5-50 mm wide; 20-170 mm long; apices acute or acuminate to caudate; margins entire or variously serrate; 2-15 teeth per cm; bases cuneate or rounded to acute or acuminate; adaxial surfaces shiny dark green or yellow-green or green; glabrous or pilose along midribs; abaxial surfaces glaucous or yellowish to pale green or green; densely short pubescent or glabrous or pubescent or glabrous along midribs. **Basilaminar glands** absent. **Petioles** tan or yellow or yellowish to reddish-brown; 1-20 mm long; tomentose or villous or pilose or glabrous. **Stipules** absent or present, when present persistent on vigorous twigs or minute or caducous; reniform or ovate to lanceolate; 1-12 mm long; pubescent or glandular or glabrous; apices acute or obtuse; margins serrate or glandular. **Catkins** flowering before or simultaneous with leaves; variously villous or glabrous at time of abscission; bracts yellowish or dark brown to purple; oval to oblong or ovate or obovate; 1-3.5 mm long; pubescent or villous or pilose; apices rounded or acute to rounded; margins entire or erose; persistent or deciduous after flowering. **Staminate catkins** sessile on main branches or borne on leafy lateral branchlets 2-25 mm long; densely or moderately flowered; 10-95 mm long. **Stamens** 2 or 3-7; anthers yellow or purple; straight or slightly or strongly recurved; 0.4-0.7 mm long; filaments villous or pilose or glabrous. **Nectaries** on adaxial surfaces 1 to 2; on abaxial surfaces 1 to several. **Pistillate catkins** sessile on main branches or borne on leafy lateral branchlets 2-40 mm long; densely or loosely flowered; 10-100 mm long. **Stipes** 0.5-2.5 mm long. **Stigmas** 2 or 4-lobed; persistent or deciduous after flowering. **Styles** 0.1-0.8 mm long. **Ovaries** pyriform or narrowly ovoid; pubescent or glabrous. **Capsules** 3-8 mm long. **Seeds** 10 to numerous. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

Salix is derived from the Celtic word *sallis*, the ancient common name for willows, which is derived from *'sal'* meaning 'near', and *'lis'* meaning 'water' (Warren-Wren, 1973). A taxonomically confusing genus due to a high degree of morphological variability, *Salix* comprises some 400 species in cold and temperate areas of the northern hemisphere, with only few species in the southern hemisphere (Dorn, 1976; Burnsfeld et al., 1992; Argus, 1997). The genus is represented in North America by about 90 species (USDA, 2003) and in Oklahoma by six species, where it is present in every county (Figure 1.1c-g). With the exception of *S. humilis*, members of the genus most commonly occur in bottomland habitats or wet areas in upland sites (Stephens, 1973). Plants require barren soils for germination and seedling establishment (Brinkman, 1974; Pitcher and McKnight, 1990). Although willows are difficult to propagate in quantity by seed, rootstocks of young branches sprout prolifically, making this the usual method of artificial regeneration (Tesky, 1992). A variety of wildlife species take advantage of cover and herbage provided by members of the genus (Uchytil, 1989b; Tesky, 1992; Little, 1998). Many species of *Salix* are cultivated as ornamental trees and shrubs. The only North American species of any commercial importance is *S. nigra*, which is used for furniture stock, boxes, crates, doors, and pulp (Pitcher and McKnight, 1990; Tesky, 1992). Used medicinally for millennia as an effective painkiller and treatment for inflammation, the natural glycoside salicin, the precursor of salicylic acid, was isolated in 1829 from the inner bark of *Salix*. Today it is the basic ingredient of aspirin, although synthesized acetylsalicylic acid is used rather than the natural form (Pitcher and McKnight, 1990; Newsholme, 1992). Members of this wind and insect pollinated genus typically flower in early spring.

1. Plants producing catkins before leaves. Stems decumbent. Staminate and pistillate catkins borne on main stems. Leaves obovate or oblanceolate. .... ***S. humilis***

1. Plants producing catkins simultaneously with leaves or after leaves are formed. Stems erect. Staminate and pistillate catkins borne on leafy lateral twigs. Leaves linear or lanceolate or ovate.

2. Leaf surfaces similar, both green; abaxial surfaces not glaucous.

   3. Plants trees; not rhizomatous. Leaves lanceolate to linear-lanceolate; margins with 7-13 teeth per cm. Stamens 4-6 .... ***S. nigra***

   3. Plants shrubs; rhizomatous. Leaves linear; margins with 2-6 teeth per cm. Stamens 2......... ***S. exigua* var. *interior***

2. Leaf surfaces different; adaxial surfaces green or yellowish green; abaxial surfaces white or whitish green glaucous.

   4. Adaxial leaf surfaces dark green. Margins of catkin bracts erose. Plants of eastern 3/4 of state..................... ***S. caroliniana***

   4. Adaxial leaf surfaces yellow green. Margins of catkin bracts entire. Plants of western Panhandle............... ***S. amygdaloides***

***Salix amygdaloides* Anderss.** Peachleaf Willow. **Plants** trees; 4-20 m tall. **Bark** irregularly furrowed; dark brown. **Stems** erect. **Twigs** gray to light yellow; glabrous. **Lateral buds** conical; yellowish-brown; glabrous; apices acute; 2.5-4 mm long. **Blades** lanceolate to ovate-lanceolate; 10-50 mm wide; 20-100 mm long; apices acuminate to caudate; margins finely serrate; 6-15 teeth per cm; bases cuneate to rounded; adaxial surfaces yellowish-green; glabrous; abaxial surfaces pale yellow to thickly white glaucous; glabrous. **Petioles** yellowish; 5-20 mm long; glabrous to sparsely tomentose adaxially. **Stipules** if present minute and caducous or sometimes persistent on vigorous twigs; reniform; 3-12 mm long; margins serrate. **Catkins** flowering simultaneous with leaves; densely villous; bracts pale yellow; obovate to oblong; 1.5-3 mm long; villous; apices slightly inequalateral; margins entire; deciduous after flowering. **Staminate catkins** borne on leafy lateral branchlets 10-20 mm long; moderately flowered; 25-60 mm long. **Stamens** 3-7; anthers yellow; slightly recurved; 0.5-0.6 mm long;

filaments pilose. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent. **Pistillate catkins** borne on leafy lateral branchlets 4-40 mm long; loosely flowered; 25-70 mm long. **Stipes** 1-2.5 mm long. **Stigmas** 2 or 4-lobed; persistent after flowering. **Styles** 0.4-0.6 mm long. **Ovaries** pyriform; glabrous. **Capsules** 4-12 mm long. **Seeds** 9. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

S. amygdaloides is sometimes referred to as the almondleaf willow, which more appropriately recognizes the origin of the specific epithet which stems from *Amygdalus*, the genus for almond (Warren-Wren, 1973). The synonyms *S. wrightii*, *S. nigra* var. *wrightii*, and *S. nigra* var. *amygdaloides* are encountered in the older literature. *S. amygdaloides* hybridizes with *S. nigra* (*Salix* x *glatferteri* Schnedider) where their distributions overlap (Stephens, 1973; Tesky, 1992); this phenomenon, however, has not been reported in Oklahoma populations. A native species, *S. amygdaloides* is distributed across the continent with the exception of the southeast quarter of the United States (Little, 1971; USDA, 2003). In Oklahoma, populations are restricted to the western end of the Panhandle (Figure 1.1c). A prolific seed producer, germination is rapid, usually 12 to 24 hours after dispersal if a moist seedbed is reached (Brinkman, 1974). Adapted to a variety of soil types, *S. amygdaloides* is characteristically encountered in the moist, fertile sandy or alluvial soils of riparian areas (Froiland, 1962; Dorn, 1977). The tree willow of the Panhandle, it is characteristic of early stages of succession and is usually associated with *Populus deltoides*. Trees are shade intolerant, persisting only along a river's edge where repeated flooding prevents other species from being established. Because of its soil-binding properties, the species helps stabilize streambanks and protect from erosion (Uchytil, 1998a). It contributes to the structural complexity of riparian communities and thus provides an array of habitats that support many different species of animals (Stevens and Dozier, 2001). Flowering occurs in April and May and fruiting follows in late May and early June.

***Salix caroliniana* Michx.** Carolina Willow. **Plants** trees; 1.5-10 m tall. **Bark** finely fissured; light gray. **Stems** erect. **Twigs** reddish to grayish-brown; glabrous to gray pubescent. **Lateral buds** conical; reddish-brown; sparsely sericeous; apices acute; 1.5-4 mm long. **Blades** lanceolate; 7-20 mm wide; 50-170 mm long; apices acuminate; margins serrate; 6-12 teeth per cm; bases cuneate to rounded; adaxial surfaces shiny dark green; glabrous to pilose along midribs; abaxial surfaces thickly white glaucous; glabrous or pubescent along veins. **Petioles** yellowish to reddish-brown; 3-8 mm long; tomentose to sparsely pilose adaxially. **Stipules** persistent on vigorous twigs; reniform; 2-7 mm long; usually glandular adaxially; apices obtuse to acute; margins serrate. **Catkins** flowering simultaneous with leaves; villous to glabrous at time of abscission; bracts yellowish; obovate to broadly ovate; 1-2 mm long; villous; apices acute to rounded; margins erose; deciduous after flowering. **Staminate catkins** borne on leafy lateral branchlets 4-25 mm long; moderately flowered; 35-95 mm long. **Stamens** 6 rarely 4 or 5; anthers yellow; straight to strongly recurved; 0.4-0.6 mm long; filaments pilose. **Nectaries** on adaxial surfaces 1 to 2; on abaxial surfaces 1 to 2. **Pistillate catkins** borne on leafy lateral branchlets 6-40 mm long; loosely flowered; 45-100 mm long. **Stipes** 1-2 mm long. **Stigmas** 2-lobed; persistent after flowering. **Styles** 0.3-0.8 mm long. **Ovaries** pyriform; glabrous. **Capsules** 3-6 mm long. **Seeds** 10 to numerous. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

The synonyms *S. longipes*, *S. nigra* var. *longipes*, *S. wardii*, and *S. occidentalis* are encountered in the older literature (Argus, 1986; USDA, 2003). Closely related to *S. nigra, S. caroliniana* has been known to hybridize with it where their ranges intersect in the Gulf Coastal Plains region (Diggs et al., 1999). This phenomenon, however, apparently is not seen in populations of the Ozarks (Argus, 1986). A native species, *S. caroliniana* is found from southern Pennsylvania to southern Florida, west to central Texas, and north to southeastern Nebraska (Little, 1971; Argus, 1986). Populations occur primarily in eastern Oklahoma, with isolated populations in the Arbuckle and Wichita mountains (Figure 1.1d). Classified as an facultative wetland species (Reed, 1988), *S. caroliniana* occurs in wet soils of rocky stream banks and other wet areas. Flowering occurs in late March and April and fruiting typically follows in May.

Salix exigua **Nutt. var.** ***interior*** **(Rowlee) Cronq.** Sandbar Willow, Coyote Willow. **Plants** shrubs; colonial by rhizomes; 4-6 m tall. **Bark** smooth to slightly fissured; gray. **Stems** erect. **Twigs** light yellow to reddish-brown; tomentose or villous becoming glabrous. **Lateral buds** oblong; reddish-brown; sericeous becoming glabrous; apices rounded; 1-4 mm long. **Blades** linear; 3-10 mm wide; 45-100 mm long; apices acute; margins remotely or irregularly dentate; 2-6 teeth per cm; bases acuminate; adaxial surfaces yellowish-green; sparsely sericeous to glabrous; abaxial surfaces yellowish to pale-green; sparsely sericeous to glabrous. **Petioles** yellowish-brown; 1-5 mm long; glabrous. **Stipules** absent. **Catkins** flowering simultaneous with leaves; sparsely to densely villous; bracts yellowish; oblong to obovate; 1.5-3.5 mm long; pilose; apices acute to rounded; margins erose; deciduous after flowering. **Staminate catkins** borne on leafy lateral branchlets 2-25 mm long on 1st catkins and 40-180 mm long on later catkins; densely flowered; 20-50 mm long. **Stamens** 2; anthers reddish becoming yellow; strongly to slightly recurved; 0.4-0.7 mm long; filaments villous. **Nectaries** on adaxial surfaces 2; on abaxial surfaces 2. **Pistillate catkins** loosely flowered; 35-70 mm long. **Stipes** 0.4-1.5 mm long. **Stigmas** 4-lobed; deciduous after flowering. **Styles** 0.1-0.2 mm long. **Ovaries** pyriform to narrowly ovoid; glabrous or long silky sericeous when mature. **Capsules** 5-8 mm long. **Seeds** numerous. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

S. exigua is divided by some taxonomists into a number of varieties on the basis of indumentum type, leaf shape, and the number of teeth on blade margins. One such variety is *S. exigua* var. *interior*. Although some classifications treat *S. exigua* and *S. interior* as separate species (Burnseld et al., 1992; USDA, 2003), the classification presented here follows that of Cronquist (1964), Argus (1986) and Dorn (1998) in which *S. interior* is treated as a variety. Variety *interior,* considered the eastern phase of the *Salix exigua* complex by Argus (1986), differs from variety *exigua*, the western phase, in having leaves that are less densely sericeous, more distinctly toothed, and more conspicuously veined. In addition the catkins are more loosely flowered and capsules longer.

The specific epithet *exigua* means small or short, referring to the usually small size of the plants. A native species, *S. exigua* is distributed across the western two-thirds of the continent (Brinkman, 1974; Little, 1971). In Oklahoma, populations occur across the western two-thirds of the state, as well as isolated populations in eastern Oklahoma (Figure 1.1e). Unlike other members of the genus in Oklahoma, the species can reproduce vegetatively by sprouting from underground shoot buds in a process called suckering (Uchytil, 1989b). Characteristic of early seral communities, *S. exigua* is commonly associated with other members of the family, namely *Populus deltoides* and *Salix nigra*. The species is characteristically encountered in the moist, fertile, sandy or alluvial soils of riparian areas such as banks of streams and rivers, but can occasionally be found in periodically wet areas in upland sites. It is used for erosion control along streambanks, lakeshores, and for development and restoration of riparian areas. A common forage species for deer, *S. exigua* also provides shelter for many game birds (Stevens et al., 2000). Flowering typically occurs twice during the growing season, first in April and May and again mid to late summer.

***Salix humilis* Marsh.** Prairie Willow. **Plants** shrubs; colonial by layering; 1-3 m tall. **Bark** reddish-brown. **Stems** decumbent. **Twigs** yellowish-brown to dark brown; gray pubescent. **Lateral buds** oblong; reddish-brown; puberulent; apices rounded; 2-5 mm long. **Blades** oblanceolate to obovate to narrowly oblong; 5-20 mm wide; 30-85 mm long; apices acute; margins entire to revolute to remotely or irregularly serrate; 7-13 teeth per cm; bases cuneate; adaxial surfaces shiny dark green; glabrous; abaxial surfaces glaucous; densely short pubescent. **Petioles** tan; 2-8 mm long; tomentose or pilose or villous. **Stipules** foliaceous on vigorous twigs minute rudiments or absent on older twigs; lanceolate to ovate; 3-7 mm long; pubescent; apices acute; margins sparsely serrate. **Catkins** flowering before leaves; sparsely villous; bracts dark brown to purple; oblong to oval; 1-2 mm long; villous; apices rounded; margins entire; persistent. **Staminate catkins** sessile on main branches with 2 or 3 leafy bracts; densely flowered; 10-40 mm long. **Stamens** 2; anthers purple; straight; 0.4-0.6 mm long; filaments glabrous to sparsely pilose. **Nectaries** on adaxial surfaces 1; on abaxial surfaces 2. **Pistillate catkins** densely flowered; 10-60 mm long. **Stipes** 0.5-2 mm long. **Stigmas** 2 or 4-lobed; persistent after flowering. **Styles** 0.2-0.4 mm long. **Ovaries** pyriform to narrowly ovoid; pubescent. **Capsules** 4-7 mm long. **Seeds** numerous. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

Some taxonomists recognize two varieties of *S. humilis*, based on leaf shape and abaxial leaf appearance. A native species, it occurs throughout the eastern part of the Great Plains, from southeastern Canada south to Texas (Stevens and Dozier, 2000). In Oklahoma, populations occur in the southeastern part of the state (Figure 1.1f). As its common name "prairie willow" suggests, *S. humilis* is found in upland prairies and savannas, especially in sandy soils (Stephens, 1973). The species provides cover for birds and small and medium-sized mammals and deer and livestock occasionally eat the herbage (Stevens and Dozier, 2000). Flowering occurs in April and May.

***Salix nigra* Marsh.** Black Willow. **Plants** trees; 2-20 m tall. **Bark** deeply furrowed; dark brown to blackish. **Stems** erect. **Twigs** light reddish-brown to darker grayish-brown; glabrous to pubescent. **Lateral buds** conical; tan; glabrous; apices acute; 1.5-2.5 mm long. **Blades** lanceolate to linear-lanceolate; 7-20 mm wide; 40-150 mm long; apices acuminate; margins serrate; 7-13 teeth per cm; bases acute to cuneate to rounded; adaxial surfaces green; glabrous to sparsely pilose along midribs; abaxial surfaces green; glabrous to sparsely pilose along midribs. **Petioles** yellowish-brown; 3-10 mm long; sparsely pilose adaxially. **Stipules** caducous or sometimes persistent on vigorous twigs; ovate to lanceolate; 1-8 mm long; apices acute; margins glandular. **Catkins** flowering simultaneous with leaves; villous to glabrous at time of abscission; bracts yellowish; obovate; 1-3 mm long; pubescent; apices acute to rounded; margins entire; deciduous after flowering. **Staminate catkins** borne on leafy lateral branchlets 4-15 mm long; moderately flowered; 15-70 mm long. **Stamens** 6 rarely 4 or 5; anthers yellow; straight to strongly recurved; 0.4-0.6 mm long; filaments pilose. **Nectaries** on adaxial surfaces 1; on abaxial surfaces 2 to 3. **Pistillate catkins** borne on leafy lateral branchlets 5-35 mm long; densely flowered; 30-100 mm long. **Stipes** 0.5-2 mm long. **Stigmas** 4-lobed; persistent after flowering. **Styles** 0.2-0.4 mm long. **Ovaries** pyriform; glabrous. **Capsules** 3-5 mm long. **Seeds** 10 to numerous. **Nectaries** on adaxial surfaces 1; on abaxial surfaces absent.

Some taxonomists recognize numerous varieties of *S. nigra*, based on petiole length, blade shape, and blade width. *S. nigra* hybridizes both with the closely related *S. amygdaloides* (*Salix* x *glatfelteri* Schnedider) and *S. caroliniana* where their ranges intersect (Argus, 1986). *S. nigra* is the largest and most widespread tree species of the genus. A native species, it is distributed across the eastern half of the continent (Little, 1971; Duncan and Duncan, 1988). In Oklahoma, populations occur across the state, except for the western Panhandle (Figure 1.1g). *Salix nigra* occurs with *Populus deltoides* as a codominant in many early seral floodplain communities where it often forms gallery forests with distinct cohorts of differing heights. Due to the distances that the wind dispersed comose seeds travel, it is not restricted to bottomland habitats and is often found in upland sites such as ditches, drainages, and other periodically wet areas, where conditions for germination and seedling establishment are favorable (Argus, 1986). Very susceptible to fire damage, *S. nigra* has the ability to sprout from the shoot base following fire (Adams et al., 1982). It is the only commercially important willow of the 90 species native to North America (Pitcher and McKnight, 1990). Once used extensively for artificial limbs because of its light weight, the wood holds its shape well and does not splinter. The most common uses of the wood today are for furniture stock, boxes, crates, doors, and pulp (Pitcher and McKnight, 1990; Tesky, 1992). Rated fair in nutritional value, *S. nigra* is a food source for birds, deer, small mammals, and some livestock. Plants are commonly used as nesting habitat and cover by some nongame wetland bird species. Honeybees are common spring visitors and obtain substantial amounts of pollen from the staminate flowers (Tesky, 1992). Flowering occurs in late March and April with fruiting and seed dispersal following in May.

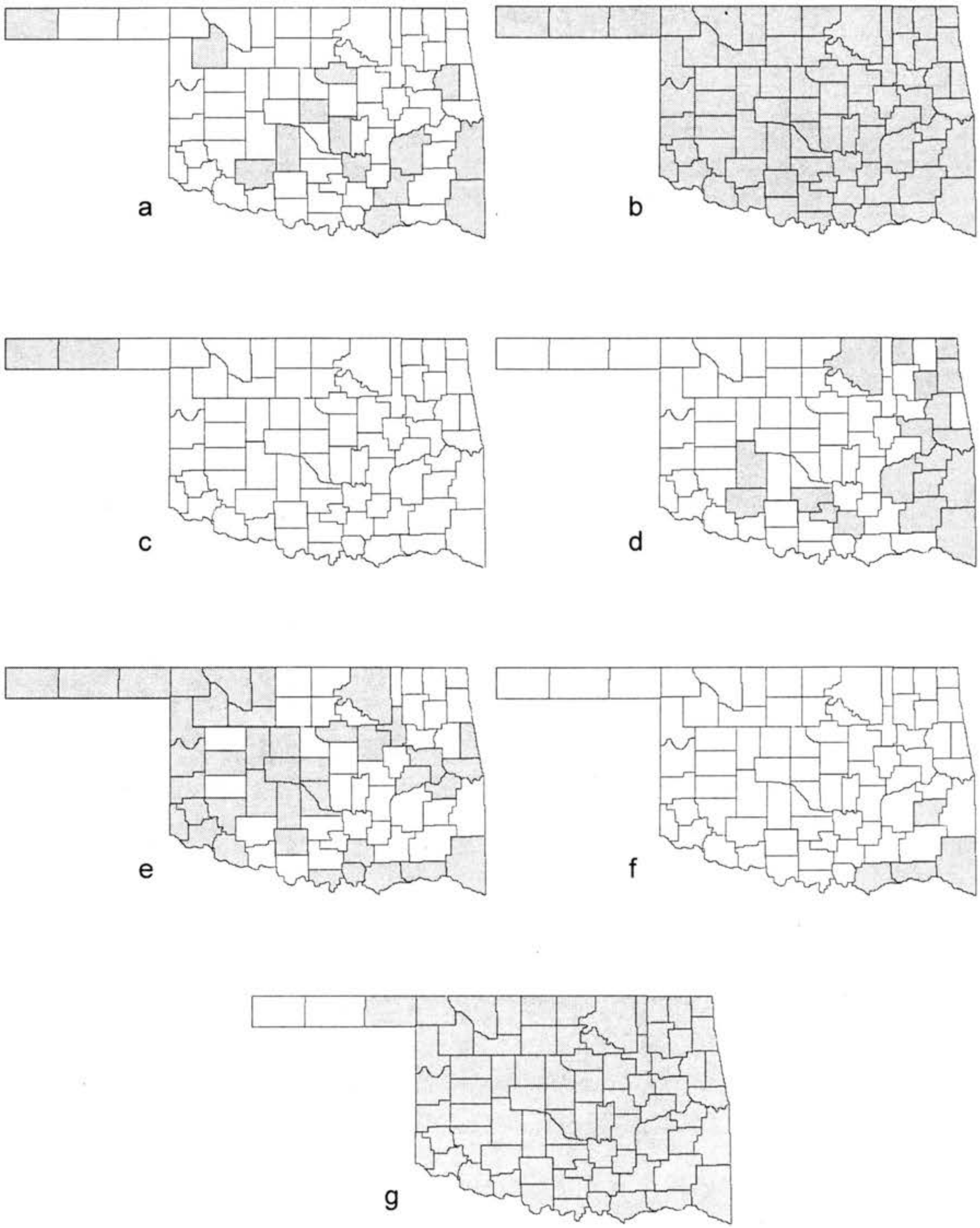


Figure 1.1 County distribution maps of Salicaceae in Oklahoma based on herbaria collections. a) *Populus alba*, b) *P.* deltiodes, c) *Salix amygdaloides*, d) *S. caroliniana*, e) *S. exigua*, f) *S. humilis*, g) *S. nigra*.

LITERATURE CITED

Adams, D. E., R. C. Anderson, and S. L. Collins. 1982. Differential response of woody and herbaceous species to summer and winter burning in an Oklahoma grassland. Southwestern Naturalist. 27:55–61.

Argus, G. W. 1986. The genus *Salix* (Salicaceae) in the Southeastern United States. Systematic Botany Monographs 9:1–70.

Argus, G. W. 1997. Infrageneric classification of *Salix* (Salicaceae) in the New World. Systematic Botany Monographs 52:1–121.

Brinkman, K. A. 1974. *Salix* L. Willow. *in* C. S. Schopmeyer, ed. Seeds of Woody Plants in the United States. Agriculture Handbook, 450. U.S. Department of Agriculture, Forest Service, Washington, DC. Pages 746–750.

Brummitt, R. K. and C.E. Powell. 1992. Authors of Plant Names. Royal Botanical Gardens, Kew, Surrey. United Kingdom.

Burnsfeld, S. J., D. E. Soltis, and P. S. Soltis. 1992. Evolutionary patterns and process in *Salix* sect. *Longifoliae*: Evidence from chloroplast DNA. Systematic Botany 17:239–256.

Cooper, D. T. and D. F. Van Haverbeke. 1990. *Populus deltoides*. *in* R. M. Burns and B. H. Honkala, eds. Silvics of North America: Volume 2. Hardwoods.

Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC.

Correll, D. S. and M.C. Johnston. 1979. Manual of the Vascular Plants of Texas. The University of Texas at Dallas, Richland, Texas.

Cronquist, A. 1964. *Salix in* C. L. Hitchcock, A. Cronquist, M. Ownbey, and J.W. Thompson. Vascular Plants of the Pacific Northwest. Part 2. University of Washington Press, Seattle, WA. Pages 37–71.

Dallwitz, M. J. and T.A. Paine. 1986. User's Guide to DELTA System: A General System for Processing Taxonomic Descriptions. CSIRO Division of Entomology, Canberra, Australia.

Diggs, G. M., B. L. Lipscomb, and R. J. O'Kennon. 1999. Shinners & Mahler's Illustrated Flora of North Central Texas. Botanical Research Institute of Texas., Fort Worth, TX.

Dirr, M. A. 1998. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Fifth Edition. Stipes Publishing L.L.C., Champaign, IL.

Dorn, R. D. 1976. A synopsis of American *Salix*. Canadian Journal of Botany 54:2769–2789.

Dorn, R. D. 1977. Willows of the Rocky Mountain states. Rhodora 79:390–429.

Dorn, R. D. 1994. North American *Salix* (Salicaceae): typifications and notes.

Phytologia 77:89–95.

Dorn, R. D. 1998. A taxonomic study of *Salix* section *Longifoliae* (Salicaceae). Brittonia 50:193–210.

Duke, J. A. 1983. *Populus deltoides* Bartr. ex. Marsh. *in* J. A. Duke, ed. Handbook of Energy Crops. Center for New Crops and Plants Products, Purdue University. West Lafayette, IN. www.hort.purdue.edu/newcrop/duke_energy/*Populus*_deltoides.html.

Duncan, W. H. and M. B. Duncan. 1988. Trees of the Southeastern United States. The University of Georgia Press, Athens, GA.

Eckenwalder, J. E. 1977. North American cottonwoods (*Populus*, Salicaceae) of sections *Abaso* and *Aigeiros*. Journal of the Arnold Arboretum 58:193–208.

Froiland, S. G. 1962. The genus *Salix* (Willows) in the Black Hills of South Dakota. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO.

Glass, W. 1990. White Poplar; Vegetation Management Guideline Vol.1, No. 25. Illinois Nature Preserves Commission.

Gleason, H. A. and A. Cronquist. 1963. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. D. Van Nostrand Company, Inc., Princeton, NJ.

Gray, A. 1950. Gray's Manual of Botany, Eighth edition. American Book Company, New York, New York.

Great Plains Flora Association. 1986. Atlas of the Flora of the Great Plains. University of Kansas Press., Lawrence, Kansas.

Holmgren, P. K. and N. H. Holmgren. 2003. Index Herbariorum, Part I: The Herbaria of the World, Eighth Edition. NYBG Press, The New York Botanical Garden, Bronx, New York. http://www.nybg.org/bsci/ih/ih.html.

Little, E. L. Jr. 1971. Atlas of United States Trees. Vol. 1. Conifers and Important Hardwoods. Misc. Publ. 1146. U.S. Department of Agriculture, Forest Service, Washington, D.C.

Little, E. L. Jr. 1979. Checklist of United States Trees (Native and Naturalized). Agriculture. Handbook. 541. U.S. Department of Agriculture, Forest Service, Washington, DC.

Little, E. L. Jr. 1998. Forest Trees of Oklahoma. Oklahoma Forestry Services, State Department of Agriculture, Oklahoma City, OK.

Newsholme, C. 1992. Willows: The Genus *Salix*. B.T. Batsford Ltd, London, UK.

Pitcher, J. A. and J.S. McKnight. 1990. *Salix nigra*. *in* R. M. Burns and B. H. Honkala, eds. Silvics of North America: Volume 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC.

Reed, Jr. P. B. 1988. National List of Plant Species That Occur in Wetlands: National Summary. U.S. Fish & Wildlife Service Biological Report 88:1–244.

Schreiner, E. J. 1974. *Populus* L. Poplar. Pages 645-655 *in* C. S. Schopmeyer, ed. Seeds of Woody Plants in the United States. Agriculture Handbook, 450. U.S. Department of Agriculture, Forest Service, Washington, DC.

Stephens, H. A. 1973. Woody Plants of the North Central Plains. The University Press of Kansas, Lawrence, KS.

Stevens, M., G. Fenchel, and C. Hoag. 2000. Coyote Willow, *Salix exigua* Nutt., Plant Guide. Natural Resource Conservation Service, United States Department of Agriculture, Washington D.C.

Stevens, M. and I. Dozier. 2000. Prairie Willow, *Salix humulis* Marsh., Plant Guide. Natural Resource Conservation Service, United States Department of Agriculture, Washington D.C.

Stevens, M. and I. Dozier. 2001. Peachleaf Willow, *Salix amygdaloides* Anderrs., Plant Guide. Natural Resource Conservation Service, United States Department of Agriculture, Washington D.C.

Taylor, J. L. 2001. *Populus deltiodes*. Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. http://www.fs.fed.us/database/feis.

Tesky, J. L. 1992. *Salix nigra*. Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. http://www.fs.fed.us/database/feis.

Tyrl, R. J., R. E. Masters, and T. G. Bidwell. 2002. Field Guide to Oklahoma Plants. Oklahoma State University, Stillwater, OK.

Tyrl, R. J., S. C. Barber, P. Buck, J. R. Estes, P. Folley, L. K. Magrath, C.E.S. Taylor, and R. A. Thompson. 2003. Keys and Descriptions for the Vascular Plants of Oklahoma. Flora Oklahoma Incorporated, Noble, OK.

Uchytil, R. J. 1989a. *Salix amygdaloides*. Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. http://www.fs.fed.us/database/feis.

Uchytil, R. J. 1989b. *Salix exigua*. Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. http://www.fs.fed.us/database/feis.

USDA, NRCS. 2003. The PLANTS Database, Version 3.5. National Plant Data Center, Baton Rouge, LA. http://plants.usda.gov.

Warren-Wren, S. C. 1973. The Complete Book of Willows. A.S. Barnes and Co., Inc., Cranbury, NJ.

Waterfall, U. T. 1969. Keys to the Flora of Oklahoma, 4th ed. Published by the author for sale by Student Union Bookstore., Oklahoma State University.

Stillwater, OK.

Watson, L. and M. J. Dallwitz. 2000. The Families of Flowering Plants: Descriptions, Illustrations, Identification, and Information Retrieval. http://biodiversity.uno.edu/delta/.

Young, J. A. and C. G. Young. 1992. Seeds of Woody Plants in North America. Dioscorides Press, Portland, OR.

CHAPTER 2

MOLECULAR SYSTEMATIC INVESTIGATIONS OF *ECHINACEA* (ASTERACEAE: HELIANTHEAE) BASED ON NUCLEAR RIBOSOMAL ITS AND ETS SEQUENCES

INTRODUCTION

Described by Conrad Moench in 1794 *Echinacea* is a North American genus comprising 4–9 species. It is distributed primarily in the Midwest (Figure 2.1). Taxonomists differ in their opinions as to its position in the Asteraceae (Snyder, 1991; Baskin et al., 1993). Using only morphological characters, Cox and Urbatsch (1990) and Bremer (1994) classify the genus in the subtribe Rudbeckiinae. Related genera of the tribe include *Rudbeckia*, *Ratibida*, and *Dracopsis*. In contrast, Urbatsch and coworkers (2000) using chloroplast DNA restriction site data, suggested *Echinacea* to belong in the Zinniinae. Related genera of this tribe are *Heliopsis*, *Sanvitalia*, and *Zinnia*.

Likewise, there are differences of opinion as to the number of species. In the last 40 years, taxonomists have followed the classification scheme of Ronald L. McGregor (1968) or Arthur Cronquist (1955, 1980); both of whom based their systems on morphological characters. McGregor recognized nine species and four varieties, whereas Cronquist circumscribed only four species and four varieties (Table 2.1). Binns and coworkers (2002a) recently proposed a revision of the genus based on a morphometric analysis. The results of their work agree with the scheme of Cronquist except for their recognition of *E. pallida* var.

tennesseensis as a distinct variety rather than an eastern outlier of *E. pallida* var. *angustifolia* as proposed by Cronquist (Table 2.1).

Advances in molecular biology, especially in the techniques of sequencing DNA, now permit from a molecular perspective an examination of the taxonomic position of *Echinacea* in the family and the relationship of its species. Thus the objective of this study was to construct a phylogeny of the genus using nucleotide sequence data from the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions of nuclear ribosomal DNA (nrDNA) to examine the monophyly and circumscription of *Echinacea* and its species, and possibly provide evidence in support of the classification proposed by McGregor (1968), Cronquist (1955, 1980), or Binns and coworkers (2002a). I present here an analysis of DNA sequences from the nine species recognized by McGregor (1968) and from six related genera in the Asteraceae.

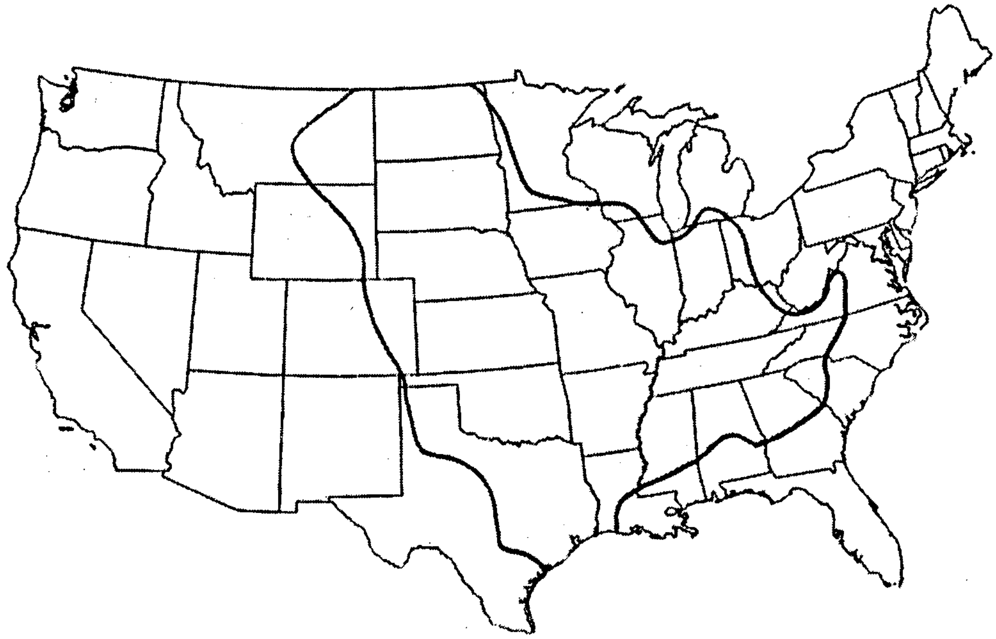


Figure 2.1. Distribution of *Echinacea* Moench in North America (McGregor, 1968; Binns et al., 2002)

Table 2.1. Taxonomic treatments of *Echinacea* Moench. Synonyms are indented and placed in brackets.

R.L. McGregor (1968)	A. Cronquist (1955, 1980)	S.E. Binns, B.R. Baum, and J.T. Arnason (2002a)
E. pallida (Nutt.) Nutt.	*E. pallida* (Nutt.) Nutt. var. *pallida*	*E. pallida* (Nutt.) Nutt. var. *pallida*
E. simulata McGregor	[*E. simulata* McGregor]	*E. pallida* (Nutt.) Nutt. var. *simulata* (McGregor) Binns, B.R. Baum & Arnanson
E. sanguinea Nutt.	[*E. sanguinea* Nutt.][a]	*E. pallida* var. *sanguinea* (Nutt.) K.N.Gandhi & R.D.Thomas
E. angustifolia DC. var. *angustifolia*	*E. pallida* (Nutt.) Nutt. var. *angustifolia* (DC.) Cronquist	*E. pallida* (Nutt.) Nutt. var. *angustifolia* (DC.) Cronquist
E. angustifolia DC. var. strigosa R.L. McGregor	[*E. angustifolia* DC. var. *strigosa* R.L. McGregor]	[*E. angustifolia* DC. var. *strigosa* R.L. McGregor]
E. tennesseensis (Beadle) *Small*	[*E. tennesseensis* (Beadle) *Small*][b]	*E. pallida* (Nutt.) Nutt. var. *tennesseensis* (McGregor) Binns, B.R. Baum & Arnanson
E. atrorubens Nutt.	*E. atrobubens* Nutt. var. *atrorubens*	*E. atrobubens* Nutt. var. *atrorubens*
E. paradoxa (J.B.S. Norton) Britt. var. *neglecta* R.L. McGregor	*E. atrobubens* Nutt. var. *atrorubens*	*E. atrobubens* Nutt. var. *neglecta* (McGregor) Binns, B.R. Baum & Arnanson
E. paradoxa (J.B.S. Norton) Britt. var. *paradoxa*	*Echinacea atrorubens* var. *paradoxa* (Norton) Cronquist	*Echinacea atrorubens* var. *paradoxa* (Norton) Cronquist
E. laevigata S.F. Blake	*E. laevigata* S.F. Blake	*E. laevigata* S.F. Blake
E. purpurea (L.) Moench	*E. purpurea* (L.) Moench	*E. purpurea* (L.) Moench

a Suggested that plants might be formally recognized as a variety, but was not doing so at this time.
b Suggested that plants formerly recognized as *E. tennesseensis* are merely eastern outliers.

METHODS AND MATERIALS

The ITS and ETS regions are part of the 18S-26S nrDNA multigene family which includes the 18S, 5.8S, and 26S subunits. This multigene family is highly repeated in the plant nuclear genome, allowing for a high level of detection, amplification, cloning, and sequencing of nrDNA (Baldwin et al., 1995).

ITS is one of the most widely used sources of characters for phylogenetic studies of closely related plant taxa. Many of the present-day phylogenies, at least at the family and genus level, use nuclear sequences based on ITS (Savard et al., 1993; Sang et al., 1995a; Bayer et al., 1996; Bena et al., 1998; Prather and Jansen, 1998). Illustrative of this are recent publications presenting data attempting to resolve the phylogeny of many members of the Asteraceae (Baldwin et al., 1995; Sang et al., 1995b; Urbatsch and Jansen, 1995; Urbatsch et al., 2000).

The popularity of the ITS and ETS regions can be attributed to the relatively high rate of nucleotide substitution resulting in rapid sequence evolution. This allows for systematic comparison of relatively recently diverged taxa (Liston et al., 1996; Moritz and Hillis, 1996). Using these gene regions offers increased phylogenetic precision over that of restriction site mapping techniques typical of studies incorporating chloroplast DNA (Baldwin et al., 1995).

The ITS comprises two regions, ITS-1 and ITS-2, located on either side of the 5.8S subunit, which exhibits very low levels of sequence variation (Figure

2.2). An appealing aspect of using ITS is that this region can be readily polymerase chain reaction (PCR) amplified and sequenced using universal primers. A limiting factor in using ITS to examine angiosperm phylogeny is that it provides a small amount of sequence data. Baldwin and coworkers (1995) report ITS region as varying from 565-700 base pairs (bp) in length, although I have encountered sequences as long as 900 bp. Excluding the 5.8S nrDNA region (163-165 bp), about 400-700 bp of ITS-1 and ITS-2 are phylogenetically informative.

Until recently, most of the nrDNA data used in phylogenetic studies involved only ITS, and the phylogenetic utility of ETS was relatively unexplored, with the exception of few plant groups (Andreasen and Baldwin, 2001). ETS is part of the larger intergenic spacer (IGS), which also includes the nontranscribed spacer (NTS) (Figure 2.2). ETS is longer than ITS-1 and ITS-2 combined, with products varying between 800 and 1,900 bp in length for members of the Asteraceae (Linder et al., 2000). Its also appears to have the similar rapid evolution characteristic of the ITS region. Comparisons of ITS and ETS sequence data in the *Calycadenia/Osmadenia* complex by Baldwin and Markos (1998) suggested the evolutionary rate in ETS is 1.3 to 2.4 times faster than that of ITS. They concluded that ETS fulfills the need for additional nucleotide characters to augment the small number of sites present in ITS and predicted that ETS will prove useful in increasing resolution and support for nrDNA-based phylogenies. This has recently been the case in a number of phylogenetic studies involving members of the Asteraceae that incorporate sequence data from the two gene regions, e.g., Clevinger and Panero (2000); Linder and coworkers (2000); Chan and coworkers (2002); Lee and coworkers (2002); and Markos and Baldwin (2002).

The major disadvantage of using ETS sequence data involves the amplification process. Exhaustive efforts to construct universal primers for ETS amplification by Baldwin and Markos (1998) and Linder and coworkers (2000) have yielded substantial primer possibilities for amplification of the ETS region over a wide range of taxa. Finding the best fitting primer for the taxa in question is a tedious process; one which this investigator was not spared.

Taxa Sampled. Seventeen taxa were examined in this study—the 11 taxa of *Echinacea* recognized by McGregor (1968), and 6 outgroup species (Table 2.2). Only one specimen of each taxon was used. The outgroup species are representative of the Rudbeckiinae and Zinniinae, the two subtribes in which *Echinacea* has been positioned (Cox and Urbatsch, 1990; Bremer, 1994; and Urbatsch et al., 2000). With the exception of *Sanvitalia fruticosa*, all of the outgroup taxa are native to North America north of Mexico and are typically found associated with species of *Echinacea*. *Sanvitalia fruticosa* is a Mexican and Central American genus of about seven species (Bremer, 1994).

Genomic DNA Extraction. Established molecular techniques were used to extract total genomic DNAs from the taxa (Table 2.2). Quality DNA was extracted from leaves of herbarium material (20–40 mg), silica gel dried leaf material (20–40 mg), and fresh material (approximately 100 mg). Many of the ITS regions of taxa were isolated using the 2X CTAB protocol of Doyle and Doyle

(1987), whereas the ETS region of all taxa were isolated using plant DNA extraction kits–DNeasy Plant Mini Kit (Qiagen, Clarita, CA) and REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, MO).

Sequences of ITS-1 and ITS-2 for *Zinnia grandiflora* (ZGU74397 and ZGU74446), *Heliopsis helianthoides* (HHU73154 and HHU74424), and *Sanvitalia fruticosa* (SFU74394 and SFU74443 were obtained from GenBank®.

Polymerase Chain Reaction. Total genomic DNA (template DNA), primers, and the ready-to-use solutions—*Taq* PCR Master Mix (Qiagen, Clarita, CA) and REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, MO)—were used in the amplification of the ITS and ETS regions by the polymerase chain reaction (PCR). Appropriate primers were obtained from Integrated DNA Technologies (IDT, Coralville, IA). Primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') designed by White and coworkers (1990), were used in the amplification of the ITS region. PCR was conducted in a 9600 Perkin-Elmer thermal cycler using a modified protocol of Clevinger and Panero (2000). This protocol involved an initial denaturation of 95°C for 4 minutes followed by 33 cycles of 95°C for 45 seconds (denaturation), 48°C for 45 seconds (annealing of primers), and 72°C for 1 minute. The reaction was terminated with an 8-minute primer extension at 72°C and then held at 4°C.

Primers 18S-ETS (5'-ACTTACACATGCATGGCTTAATCT-3') and ETS-Hel-1 (5'-GCTCTTTGCTTGCGCAACAACT-3') (Baldwin and Markos, 1998) were used for amplification of the ETS region. PCR was conducted in a PTC-0150 Mini Cycler (MJ Research Inc., Waltham, MA) using the protocol of Baldwin and

Markos, (1998). This protocol involved an initial denaturation of 97°C for 1 minute followed by 40 cycles of 97°C for 10 seconds (denaturation), 55°C for 30 seconds (annealing of primers), and 72°C for 20 seconds, and concluding with 72°C for 7 minutes (for completion of primer extension). The final product was held at 4°C.

Sequencing and Sequence Alignment. ITS nucleotide sequences of PCR products were determined using automated cycle-sequencing and an ABI Prism 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA) at the University of Oklahoma. The sequences were assembled and edited using Sequencer™ 4.0 (Gene Codes Corp., Ann Arbor, MI).

All sequences were aligned using the program Clustal X (Thompson et al., 1997) with default settings. Afterwards it was necessary to make some manual adjustments to the alignment.

Sequences from this study that are new to science were submitted to GenBank® using the protocols of the National Center for Biotechnology Information.

Phylogenetic Analyses. Sequences were imported into PAUP* 4.0 (Swofford, 2002), from which phylogenies were generated. Characters were equally weighted and their states were unordered. Parsimony analyses were carried out with gaps treated as missing data, and heuristic tree search options included RANDOM sequence addition for 500 replicates—each of which held 10 trees—, following tree bisection-reconstruction (TBR) branch swapping, MulTrees on, and steepest descent off.

Bootstrap analyses of 500 replicates were conducted to measure relative support for clades (Felsenstein, 1985); heuristic tree search options were simple sequence addition, TBR branch swapping, MulTrees on, and steepest decent off. In bootstrap analysis, multiple randomized matrices are constructed from the data by a random sampling of characters with replacement of the characters. From these matrices, most-parsimonious trees are constructed and used to form a consensus tree. The bootstrap values given indicate a 50% or higher representation of a branch in the parsimonious trees produced. Only those branches that receive a 50% or higher value are retained in the consensus tree, all others are collapsed (Judd et al., 2002).

To further evaluate the relative robustness of clades found in the most parsimonious trees, decay index values were determined. The decay value indicates how many extra steps are required to find a tree without a particular branch or clade. So the higher the decay value, the more robust a particular clade (Donoghue et al., 1992).

Robustness and topology of the trees were evaluated using the consistency index (CI), the retention index (RI), and the rescaled consistency index (RC). The consistency index measures homoplasy by dividing the minimum amount of evolutionary steps by the actual tree length. CI falls between 0 and 1. The lower the CI value, the more characters contradict the evolutionary tree (Wiley et al., 1991). In other words, the closer to one the better the topology. The retention index differs in that it accounts for multiple independent origination events for derived characters. This index equals the maximum tree length minus the actual tree length, divided by the maximum length minus the minimum length (Judd et al., 2002). Not all characters may be contributing to the tree topology (e.g. autapomorphies). In cases like this the CI may be an overestimate. This may be overcome by calculating a rescaled consistency index (RC), sometimes referred to in the literature as "CI excluding uninformative characters." The RC is simply calculated by multiplying the CI and RI. All three of these indices are indicators of the validity of the tree produced.

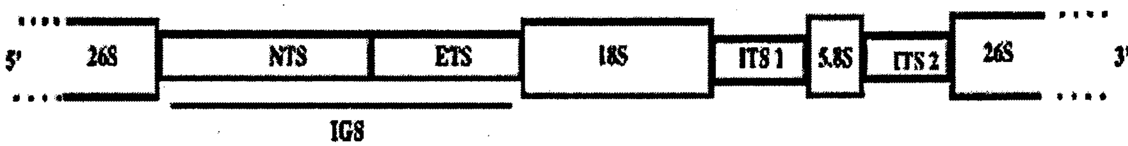


Figure 2.2. Structure of 18S-26S nrDNA multigene family (not drawn to scale). Taken from Markos and Baldwin (2002).

Table 2.2. Taxa examined, sources of leaf material, preparation methods, and DNA extraction methods. Classification follows McGregor (1968). Herbaria acronyms follow Holmgren and Holmgren (2003)

Taxa	ITS	ETS
	Leaf Sourse / DNA Extraction Method	
Echinacea angustifolia DC. var. *angustifolia*	OKL / CTAB[a]	Fresh / DNeasy
Echinacea angustifolia DC. var. *strigosa* R.L. McGregor		Fresh / XNAPS
Echinacea atrorubens Nutt.	SiGel / CTAB	Fresh / DNeasy
Echinacea laevigata S.F. Blake		KANU / DNeasy
Echinacea pallida (Nutt.) Nutt.	OKLA / CTAB	Fresh / XNAPS
Echinacea paradoxa (J.B.S. Norton) Britt. var. *neglecta* R.L. McGregor	OKL / CTAB	KANU / DNeasy
Echinacea paradoxa (J.B.S. Norton) *Britt.* var. *paradoxa*	OKL / CTAB	KANU / DNeasy
Echinacea purpurea (L.) Moench	OKL / CTAB	Fresh / DNeasy
Echinacea sanguinea Nutt.	OKL / CTAB	KANU / DNeasy
Echinacea simulata McGregor	MO / CTAB	KANU / DNeasy
Echinacea tennesseensis (Beadle) Small	MO / CTAB	KANU / DNeasy
Dracopis amplexicaulis (Vahl) Cass.	SiGel / CTAB	Fresh / XNAPS
Heliopsis helianthoides Sweet	GenBank®	KANU / DNeasy
Ratibida columnifera (Nutt.) Wooton & Standl.	SiGel / CTAB	Fresh / XNAPS
Rudbeckia hirta L.	SiGel / CTAB	Fresh / XNAPS
Sanvitalia fruticosa Hemsl.	GenBank®	KANU / DNeasy
Zinnia grandiflora Nutt.	GenBank®	KANU / DNeasy

OKL: Robert Bebb Herbarium, University of Oklahoma, Norman
OKLA: Oklahoma State University, Stillwater
MO: Missouri Botanical Garden, St. Louis
KANU: R.L. McGregor Herbarium, University of Kansas, Lawrence

[a] DNA extracted without regard for variety represented.

GenBank®: sequences acquired from GenBank
SiGel: silica gel dried leaf material
Fresh: fresh leaf material
CTAB: 2X CTAB protocol of Doyle and Doyle (1987)
DNeasy: DNeasy Plant Mini Kit (Qiagen, Clarita, CA)
XNAPS: REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, MO)

RESULTS

ITS—Genomic DNA Extraction. High-quality DNA was extracted from the ITS region of all ingroup taxa and three of the six outgroup taxa (*Dracopis amplexicaulis, Ratibida columnifera,* and *Rudbeckia hirta.*, in the spring of 2001. It should be noted that not all taxa recognized by McGregor (1968) were included as part of the ingroup taxa. DNA was extracted from *Echinacea angustifolia* without regard for the variety represented (i.e. var. *angustifolia* and *strigosa*). Also, DNA from *E. laevigata* was not obtained because of its limited availability due to its federally threatened status (U.S. Fish & Wildlife Service, 1999). Herbarium material of *E. laevigata* has since been acquired but difficulty in extracting quality DNA for PCR amplification has hindered the efforts of including the species in this molecular systematic study up to now.

ITS—PCR, Sequencing, and Sequence Alignment. Primers ITS4 and ITS5 provided quality PCR amplification of the ITS region. Amplification of the 18S-26S nrDNA ITS region (ITS-1, 5.8S, and ITS-2 combined) and subsequent alignment resulted in an average fragment length of 867 base pairs (ranging from 804 bp in *Ratibida columnifera* to 918 bp in *E. tennesseensis*) for all taxa studied (Table 3). This included 165 bp for the 5.8S subunit. Proper alignment of ITS-1 and ITS-2 sequences required the introduction of minimal gaps for most taxa, resulting in the total of 743 sites (414 for ITS-1, 329 for ITS-2).

ITS—Phylogenetic Analyses. Of the 918 characters, 647 were constant. The number of parsimony uninformative characters (autapomorphies) was 136, and the number of parsimony informative characters (synapomorphies) was 135.

The heuristic search of ITS sequences resulted in two most parsimonious trees requiring 416 evolutionary steps (a.k.a. tree length). The consensus tree, with a consistency index (CI) of 0.803, a retention index (RI) of 0.733, and a rescaled consistency index (RC) of 0.588, is presented in Figure 2.3. Support for the *Echinacea* clade—using bootstrap values—was very strong (100%) while infrageneric branches were very weakly supported (56, 61, 52%).

ETS—Extraction. Numerous unsuccessful attempts were made to amplify the ETS region of the 17 taxa. PCR product was obtained from only six: *E. atrorubens*, *E. paradoxa* var. *paradoxa* and var. *neglecta*, *S. fruticosa*, *Rudbeckia hirta*, and *Ratibida columnifera*. Because PCR product from the remaining taxa was not obtained, these six were not sequenced and included in the phylogenetic analysis. Inability to obtain amplified ETS product is most likely attributed to the existence of phenolics and polysaccharides in the 11 taxa. These compounds are known inhibitors in extraction techniques (Michael Berg, Gnanambal Naidoo, personal communication; Couch and Fritz, 1990; Rether et al., 1993; Savolainen, 1995, and Buldewo and Jaufeerally-Fakim, 2002.)

DISCUSSION

Phylogenetic analysis of the ITS region for *Echinacea* has generated a well-supported cladistic hypothesis for the placement of the genus in the Zinniinae as suggested by Urbatsch and coworkers (2000) (Figure 2.3). *Echinacea* is monophyletic with 100% bootstrap support. This is supported by studies of Urbatsch and Jansen (1995) and Urbatsch and coworkers (2000) who looked at the phylogenetic affinities among the coneflower genera of the Asteraceae based on chloroplast DNA and ITS sequences.

The relationships among species of the genus, however, are not well defined. In this thesis, I had hoped to elucidate the relationships and provide evidence in support of one of the previously published treatments. ITS data by themselves do not provide overwhelming support for any of the three classification schemes. Low levels of intrageneric ITS variation illustrated by very small branch lengths—some as low as zero—as well as decay values of one, preclude detailed phylogenetic inferences and any consideration of classification would be premature until better resolution can be provided by complementary data (such as ETS). A number of observations can be drawn from the topology produced using only ITS data. They are the following with the caveat that they lack the traditional strong support associated with such analyses.

1. Representing the most basal taxon in the *Echinacea* clade and noticeably distant from the other species of the genus, the federally threatened

(U.S. Fish & Wildlife Service, 1999) *Echinacea tennesseensis* appears to be a distinct taxon as recognized by McGregor (1968) (Figure 2.3). It does not appear to be an eastern outlier of *E. angustifolia* as suggested by Cronquist (1980) or a variety of *E. pallida* as proposed by Binns and coworkers (2002a). Work by Baskauf and coworkers (1994) on the relative genetic variability of *E. tennesseensis* and *E. angustifolia* revealed that *E. tennesseensis* has substantially less genetic variability than its widespread prairie relative, at both the species and population levels. My results agree with their observations.

2. The taxon recognized as *E. paradoxa* var. *paradoxa* by McGregor and *E. atrorubens* var. *paradoxa* by Binns and coworkers (2002a) appears to be a distinct taxon not related to other members of either species (Figure 2.3). Baldwin (1993) noted that useful phylogenetic information can be derived at the intraspecific level using ITS sequences. If this is indeed the case, one would expect these two varieties to be in the same clade. My ITS data also are supported by ecological and morphological differences. Restricted to the prairies and open wooded hillsides of the Arbuckle Mountains of southern Oklahoma, *Echinacea paradoxa* var. *neglecta* has the rose-colored ray florets typical of other members of *Echinacea.* In contrast, var. *paradoxa* is endemic to the west-central and southern portions of the Ozark Plateau in Missouri and Arkansas is the only member of the genus with yellow ray florets. Work done by Urbatsch and Jansen (1995) has provided chloroplast DNA data that suggests that the yellow pigmented rays unique to var. paradoxa are autapomorphic rather than pleisomorphic.

3. Cronquist's and Binns and coworkers' recognition of *E. pallida* with four varieties is not supported by the topology generated in this study (Figure 2.3). Only var. *pallida* and var. *angustifolia* show a close relationship. Interestingly, they are sister taxa to the morphologically quite different *E. purpurea*.

4. Likewise, Cronquist's and Binns and coworkers' recognition of *E. atrorubens* with three varieties is not supported by the topology (Figure 2.3). As noted above variety *paradoxa* pairs with E. *pallida* var. *simulata* and is quite different from both *E. paradoxa* and *E. atrorubens.* Variety *atrorubens* pairs with *E. pallida* var. *sanguinea*; and var. *neglecta* pairs with *E. purpurea,* and *E. pallida* var. *pallida* and *var. angustifolia.*

5. My ITS data provide support for the hypothesis of Binns (2001) that the parents of tetraploid (2n = 44) *E. pallida* included one or more taxa from a complex that comprises *E. atrorubens*, *E. paradoxa* var. *paradoxa*, and var. *neglecta*. Sharp (1935), McGregor (1968) Binns (2001) believed *Echinacea pallida* to be the most recently derived taxon of the genus, albeit they differed in their opinions as to its origin. McGregor (1968) hypothesized that its origin was the result of stabilized introgression of an allopolyploid hybrid between *E. sanguinea* and *E. simulata*. Binns combined phytochemical, morphological, and geographical data in her hypothesis.

Future work will be an examination of the relationship among *E. purpurea, E. angustifolia*, and *E. pallida,* which have been touted for their putative medicinal benefits (Li, 1998; Sari et al., 1999). My ITS results suggest these three species are sister taxa (Figure 2.3). An interesting question is whether genetic material from the 18S-26S nrDNA multigene family might code for the properties that have led the medicinal popularity of the genus. Numerous publications (Bauer and Wagner, 1985; Bauer et al., 1988; Bailey et al., 1999; Letchamo et al., 1999; Binns et al., 2000a; Binns et al., 2000b; Binns et al., 2000c) have cited phytochemicals such as phenolics, polysaccharides, and caffeic acid derivatives as the sources of *Echinacea*'s medicinal properties. The use of the germplasm of *Echinacea* species to predict quantitative phytochemical markers is being evaluated by Benard Baum and his colleagues at the Eastern Cereal & Oilseed Research Center in Ottawa, Ontario (Baum and Binns; 1999; Baum et al.; 2001). Future collaborations with this group may prove to be beneficial in achieving my goals.

I intend to continue my efforts to extract quality DNA and PCR product from the 11 taxa from which ETS sequences were not attained. I plan to employ the techniques of Couch and Fritz (1990), Rether and coworkers (1993), Savolainen (1995), and Buldewo and Jaufeerally-Fakim (2002). Once all ETS sequences are obtained and aligned, another phylogenetic analysis using both ETS and ITS data will be conducted. Results will hopefully elucidate relationships among the species and provide information on the relationship of *Echinacea* to other members of the family.

Table 2.3. Aligned matrix of DNA sequences (5' to 3') of ITS region of ingroup and outgroup taxa. ITS1, 1–414; 5.8S, 415–579; and ITS2, 580–918

	10 20 30 40 50
Echinacea angustifolia	-----------------------------GCGACGTGGGCGGTTCGCTGC
E. atrorubens	---------------------------GGGCG-CGTGGGCGGTTCGCTGC
E. pallida	---------------------CCCTTGGGGCGACGTGGGCGGTTCGCTGC
E. paradoxa var. *neglecta*	----GGCCCGGTAAAGTGTTCGCATCGTGGCGACGTGGGCGGTTCGCTGC
E. paradoxa var. *paradoxa*	-------------------------------GACGTGGGCGGTTCGCTGC
E. purpurea	---------------------------GGGCG-CGTGGGCGGTTCGCTGC
E. sanguinea	-------------------------------GACGTGGGCGGTTCGCTGC
E. simulata	---------------------------------CGTGGGCGGTTCGCTGC
E. tennesseensis	TTAAGGCCCGGTAAAGTGT-CGGATCGTGGCGACGTGGGCGGTTCGCTGC
Dracopis amplexicaulis	---------------------CCCTTTTGGCGACGTGGGCGGTTCGCTGC
Heliopsis helianthoides	--
Ratibida columnifera	--------------------------------------GCGGTTCGCTGC
Rudbeckia hirta	-------------------------------------GCCGGTTCGCTGC
Sanvitalia fruticosa	--
Zinnia grandiflora	--

	60 70 80 90 100
E. angustifolia	CCGCGACGTCGCGAGAA-TTCCACTGAACCTTATCATTTAGAGGAAGGAG
E. atrorubens	C-GCGACGTCGCGAGAA-TTCC-CTGAACCTTATCATTTAGAGGAAGGAG
E. pallida	CCGCGACGTCGCGAGAAATTCCACTGAACCTTATCATTTAGAGGAAGGAG
E. paradoxa var. *neglecta*	CCGCGACGTCGCGAGAAATTCCACTGAACCTTATCATTTAGAGGAAGGAG
E. paradoxa var. *paradoxa*	CCGCGACGTCGCGAGAA-TTCCACTGAACCTTATCATTTAGAGGAAGGAG
E. purpurea	C-GCGACGTCGCGAGAA-TTCC-CTGAACCTTATCATTTAGAGGAAGGAG
E. sanguinea	CCGCGACGTCGCGAGAAATTCCACTGAACCTTATCATTTAGAGGAAGGAG
E. simulata	CCGCGACGTCGCGAGAA-TTCC-CTGAACCTTATCATTTAGAGGAAGGAG
E. tennesseensis	CCGC-ACGTCCCGAGAA-TTCCACTGAACCTTATCATTTAGAGGAAGGAG
Dracopis amplexicaulis	CGGCGACGTCGCGAGAAATTCCACTGAACCTTATCATTTAGAGGAAGGAG
Heliopsis helianthoides	--
Ratibida columnifera	CGGCGACGTCGCGAGAA-TTCCACTGAACCTTATCATTTAGAGGAAGGAG
Rudbeckia hirta	TGGCGACGTCGCGAGAA-TTCCACTGAACCTTATCATTTAGAGGAAGGAG
Sanvitalia fruticosa	--
Zinnia grandiflora	--

	110 120 130 140 150
E. angustifolia	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. atrorubens	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. pallida	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. paradoxa var. *neglecta*	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. paradoxa var. *paradoxa*	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. purpurea	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. sanguinea	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. simulata	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
E. tennesseensis	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
Dracopis amplexicaulis	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
Heliopsis helianthoides	--
Ratibida columnifera	AAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
Rudbeckia hirta	AAGTTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGA
Sanvitalia fruticosa	--TCGA
Zinnia grandiflora	--TCGA

```
                              160       170       180       190       200
E. angustifolia               ATCCTGCATAGCAGAACGACCCGTGAACATGTAAAAACTACTGGCC-TTT
E. atrorubens                 ATCCTGCATAGCAGAACGACCCGTGAACATGTATAAACTACTGGCC-TTT
E. pallida                    ATCCTGCATAGCAGAACGACCCGTGAACATGTAAAAACTACTGGCC-TTT
E. paradoxa var. neglecta     ATCCTGCATAGCAGAACGACCCGTGAACATGTAAAAACTACTGGCC-TTT
E. paradoxa var. paradoxa     ATCCTGCATAGCAGAACMACCCGTGAACATGTAWAAACTACTGGCC-TTT
E. purpurea                   ATCCTGCATAGCAGAACGACCCGTGAACATGTAAAAACTACTGGCC-TTT
E. sanguinea                  ATCCTGCATAGCAGAACGACCCGTGAACATGTATAAACTACTGGCC-TTT
E. simulata                   ATCCTGCATAGCAGAACGACCCGTGAACATGTATAAACTACTGGCC-TTT
E. tennesseenis               ATCCTGCATAGCAGAACGACCCGTGAACATGTAAAAACTATTGGCC-TTT
Dracopis amplexicaulis        ACCCTGCAAAGCAGAACGACTTGTGAACAAGTAAAAACAACTGGTC-TTT
Heliopsis helianthoides       ---------------ACAACCCGTGAACATGTAAAA-CTATTGGCC-TTG
Ratibida columnifera          ACCCTGCATGGCAGAACGACCCGTGAACATGTTAAAACAGGCGGCC-TCC
Rudbeckia hirta               AACCTGCCTAGCAGAACGACCCGTGAACAAGTTAAAACAGCTGGTC-TTT
Sanvitalia fruticosa          ATCCTGCATAGCAAA-CAACCTGTGGACACNTAAAAAATACT-GCC-TTG
Zinnia grandiflora            ATCCTGCATAGCAAAACAACCCGTGAACATGTAAATTCTACCGTCCATTG

                              210       220       230       240       250
E. angustifolia               CAGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
E. atrorubens                 CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACCA
E. pallida                    CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
E. paradoxa var. neglecta     CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACAA
E. paradoxa var. paradoxa     CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
E. purpurea                   CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
E. sanguinea                  CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACCA
E. simulata                   CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
E. tennesseensis              CGGGGACCGAAGCA--TTTGTTTCGAGCCTTGTGAGGCCTTGTT-GACGA
Dracopis amplexicaulis        TGTGGTTTGAAGCACATTTGTTTTGAGCCTCATGAGTCCTTGTT-GACGG
Heliopsis helianthoides       TTGTGATCAAAGCA--TTTGTTTTGAGAATCATGTGGCCTT-TTCGGCAT
Ratibida columnifera          TGGGTCTTGAAGCA--TATGCTTTGAGCCTTGTGAGTCCTTGTT-GACGT
Rudbeckia hirta               TAGGGCTTGAAACA--TTTGTTTTGAGCCTTATGAGGCCTTGTC-GACGT
Sanvitalia fruticosa          CAAGGGTCAAANCG--TTTGTTTTGAGCCTCCTGAGGCCTTGTT-GACG-
Zinnia grandiflora            CAGGGACCAAAAC---TTTGTTTTGAGCCCTGTGGTGACTTGTT-GGCGT

                              260       270       280       290       300
E. angustifolia               GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. atrorubens                 GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. pallida                    GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. paradoxa var. neglecta     GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. paradoxa var. paradoxa     GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. purpurea                   GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. sanguinea                  GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. simulata                   GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
E. tennesseensis              GCATTCATGCTTGCCTCT-ACGGGGCATCATGGTTGTCTGGTTGACACAC
Dracopis amplexicaulis        GTGTTCATGTTTGCCCCT-T-GGAGCATCATGGATGTCAAGTTGACAAAC
Heliopsis helianthoides       GCGTTCATGCTTGTCCCT-ATGGG-CATCATGGATGC-ATGTCGATGCAC
Ratibida columnifera          GTGTCCATGCTTTCC-CC-ACGGGGCATCATGGATGCAATGTTGACACAC
Rudbeckia hirta               GTGTTCATGGTTGCC-C--ATAGGGCATCATGGATGCAA-GTTGACACAC
Sanvitalia fruticosa          GCCTTCATGCTTGCCCCT-ATGGGGCACCACGGATGTCAAGTTGACGCAC
Zinnia grandiflora            GCATTCATGG-----CCT-CACGGGGATCATGGATGTCAGGTTAACGCAC
```

	310 320 330 340 350
E. angustifolia	T-AACAACCCCC-GGCACAAAATGTGCCAAGGAAAACAAAACTTAAAGGG
E. atrorubens	T-AACAACCCCC-GGCACAACATGTGCCAAGGAAAACAAAACTTAAAGGG
E. pallida	T-AACAACCCCC-GGCACAAAATGTGCCAAGGAAAACAAAACTTAAAGGG
E. paradoxa var. *neglecta*	T-AACAACCCCC-GGCACAAAATGTGCCAAGGAAAACAAAACTTAAAGGG
E. paradoxa var. *paradoxa*	T-AACAACCCCC-GGCACAACATGTGCCAAGGAAAACAAAACTTAAAGGG
E. purpurea	T-AACAACCCCC-GGCACAAAATGTGCCAAGGAAAACAAAACTTAAAGGG
E. sanguinea	T-AACAACCCCC-GGCACAACATGTGCCAAGGAAAACAAAACTTAAAGGG
E. simulata	T-AACAACCCCC-GGCACAACATGTGCCAAGGAAAACAAAACTTAAAGGG
E. tennesseensis	T-AACAACCCCC-GGCACAACATGTGCCAAGGAAAACAAAACTTAAAGGG
Dracopis amplexicaulis	TTAACAACCCCC-GGCACGGAATGTGCCAAGGATANCATAACTTGAAGTG
Heliopsis helianthoides	T-AACAACCCCC-GGCACAACACGTGCCAAGGAAAACAAAACATAAAGGG
Ratibida columnifera	T-AACAACCCCC-GGCACGGAATGTGCCAAGGAAAAGTAAACATGAAGGG
Rudbeckia hirta	T-AACAACCCCCCGGCACGGCATGTGCCAAGGAAAACTAAAATTGAAGTA
Sanvitalia fruticosa	T-AACAACCCCC-GGCAGAACACGTGCCAAGGAAAACATAACTTAAAGGG
Zinnia grandiflora	T-AACAACCCCC-GGCACAACACGTGCCAAGGAAAACTAAACTAAAAGGG

	360 370 380 390 400
E. angustifolia	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. atrorubens	-CTTGTGCTGTTATGCCCCATCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. pallida	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. paradoxa var. *neglecta*	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGGGCATACTGTGCGTTGCTTC
E. paradoxa var. *paradoxa*	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. purpurea	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. sanguinea	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. simulata	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
E. tennesseensis	-CTTGTGCTGTTATGCCCCGTCA-TTGGTGTGCATACTGTGCGTTGCTTC
Dracopis amplexicaulis	-CCCGTGCTATTACGCCCCGTTT-GCGGTGTGCGCATTGTGTGTGGCTC-
Heliopsis helianthoides	-CCTGTGCCATTACGCCCCGCTT-GCGGTTTGTGCAATGCA-GTGGCTTC
Ratibida columnifera	-CATGTGCTATTGCGCCCCGCTG-GCGGTGTGCGCATTGTACCTTGCTTC
Rudbeckia hirta	-CACGTACTGTTATGACCCGTTT-GCGGTGTGATTATGGTTGTGT-CTTC
Sanvitalia fruticosa	-CCCGTGCTATTATGCCC-GTCA-CCGGTGTGCGTGTTGTGCGTGGCTTC
Zinnia grandiflora	-CCCGTGCTCCTGCGCCCCGTTT-ACGGTGTGCGTATTGTTCGTCGGTTC

	410 420 430 440 450
E. angustifolia	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. atrorubens	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. pallida	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. paradoxa var. *neglecta*	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. paradoxa var. *paradoxa*	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. purpurea	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. sanguinea	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. simulata	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
E. tennesseensis	TTTTGTAAACTTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
Dracopis amplexicaulis	-TTTATAAA-TTATAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
Heliopsis helianthoides	-TTTG-AAACTT--AACGACTCTCGGCAACGGATATCTTGGCTCACGCAT
Ratibida columnifera	TTT-GTAAACATATAACGACTCTCGGCAACGGATATCTCGGCTCACGCAT
Rudbeckia hirta	TTC-A-AAACTAATAACGACTCTCGGCAACGGATATCTTGGCTCACGCAT
Sanvitalia fruticosa	TTTTGTAAACTTA-AACGACTCTCGGCAACGGATATCTTGGCTCACGCAT
Zinnia grandiflora	CTTTGTGAACTT--AACGACTCTCGGCAACGGATATCTTGGCTCACGCAT

```
                                   460        470        480        490        500
E. angustifolia                CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. atrorubens                  CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. pallida                     CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. paradoxa var. neglecta      CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. paradoxa var. paradoxa      CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. purpurea                    CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. sanguinea                   CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. simulata                    CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
E. tennesseensis               CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Dracopis amplexicaulis         CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Heliopsis helianthoides        CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Ratibida columnifera           CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Rudbeckia hirta                CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Sanvitalia fruticosa           CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC
Zinnia grandiflora             CGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCC

                                   510        520        530        540        550
E. angustifolia                GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. atrorubens                  GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. pallida                     GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. paradoxa var. neglecta      GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. paradoxa var. paradoxa      GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCATCCGGTTG
E. purpurea                    GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. sanguinea                   GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. simulata                    GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
E. tennesseensis               GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG
Dracopis amplexicaulis         GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCTGGTTG
Heliopsis helianthoides        GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCTGGTTG
Ratibida columnifera           GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAGGCCATCTGGTTG
Rudbeckia hirta                GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCTGGTTG
Sanvitalia fruticosa           GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCTGGTTG
Zinnia grandiflora             GTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCTGGTTG

                                   560        570        580        590        600
E. angustifolia                AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. atrorubens                  AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. pallida                     AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. paradoxa var. neglecta      AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. paradoxa var. paradoxa      AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. purpurea                    AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. sanguinea                   AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. simulata                    AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCCA-----
E. tennesseensis               AGGGCACGTCTGCCTGGGCGTCACGCATCAC---GTTGCCCCC------
Dracopis amplexicaulis         AGGGCACGTCTGCCTGGGCGTCACGCATCAA---ATCGCCCTCAMCAA-G
Heliopsis helianthoides        AGGGCACGTCTGCCTGGGCGTCACGCATC------TTGCCCCAACC----
Ratibida columnifera           AGGGCACGTCTGCCTGGGCGTCACGCATCTC---ATCGCCCCCCAC---C
Rudbeckia hirta                AGGGCACGTCTGCCTGGGCGTCACGCATCAT---GTCGCTTCTA-----C
Sanvitalia fruticosa           AGGGCACGTCTGCCTGGGCGTCACGCATC-----GTTGCCACACA-----
Zinnia grandiflora             AGGGCACGTCTGCCTGGGCGTCACGCATC-----ATCGCCCCACC----A
```

	610 620 630 640 650
E. angustifolia	AA-CATCT---ATTTAGATGTT-CTG-GTT----GGGSCGGAGATTGGTC
E. atrorubens	AA-CATCT---ATTTAGATGTT-CTG-GTT----GGGGCGGAGATTGGTC
E. pallida	AA-CATCT---ATTTAGATGGT-CTG-GTT----GGGGCGGAGATTGGTC
E. paradoxa var. *neglecta*	AA-CATCT---ATTTAGATGKT-CTG-GTT----GGGGCGGAGATTGGTC
E. paradoxa var. *paradoxa*	AA-CATCT---ATTTARATGTT-CTG-GTT----GGGGCGGAGATTGGTC
E. purpurea	AA-CATCT---ATTTAGATGTT-CTG-GTT----GGGGCGGAGATTGGTC
E. sanguinea	AA-CATCT---ATTTAGATGTT-CTG-GTT----GGGGCGGAGATTGGTC
E. simulata	-AGCATCT---ATTTAGATGTT-CTG-GTT----GGGGCGGAGATTGGTC
E. tennesseensis	AAGCATCT---ATTTAAT-GTT-CTG-GTT----GGGGCGGAGATTGGTC
Dracopis amplexicaulis	AA-TATCAA--TATGSGGTGTT-TTT-GTT----GTGGCGGATATTGGTC
Heliopsis helianthoides	AAGCATCCCTTTCAGTGATGCTTAT--GTT----GGGGCGAAGATTGGTC
Ratibida columnifera	AACCAGCCC--ATCTTGG-GTTGCTT-TTTTTTGGGGGCGGATGTTGGTC
Rudbeckia hirta	TGTCAACCC--ATCTTGG-GTTGTTT-TGT----GGGGCGGATATTGGTC
Sanvitalia fruticosa	AA-AAAT-A-TCATTAGATGTTTTT--GTT----GCGGCGGAGATTGGTC
Zinnia grandiflora	AACTTCT-ATTTCAAAGATGTGTTG--GTC----GGGGCGGAGATTGGTC

	660 670 680 690 700
E. angustifolia	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. atrorubens	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. pallida	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. paradoxa var. *neglecta*	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. paradoxa var. *paradoxa*	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. purpurea	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. sanguinea	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. simulata	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
E. tennesseensis	TCCCGTGCCACTT-GC-ATGGTTGACCTAAATATGAGTCTC-CTCA-C-G
Dracopis amplexicaulis	TCCTGTGCCCATG-GT-GTGGTTGGCCTAAATAGGAGTCGCGCTCT-C-G
Heliopsis helianthoides	TCCCATG-TGCATTCT-ATGGTTGCCCTAAATTTGAGTATC-CTCTTCAG
Ratibida columnifera	TTCCGTGCCCATG-GC-GTGGTTGGCCTAAATAGGAGTCGC-CTCT-T-G
Rudbeckia hirta	TCCTGTGCTATTG-GT-GCGGTTGGCCTAAATAGGAGCTGC-ATCT-T-G
Sanvitalia fruticosa	TCCCGTGC-ATTTGC-GTGGTTGACCTAAATGTGAGTCTC-CTCA-C-G
Zinnia grandiflora	TCCCGCGC-CCGC-GC-GTGGTTGGCCTAAATAGGAGTCTC-CTCA-C-G

	710 720 730 740 750
E. angustifolia	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. atrorubens	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. pallida	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. paradoxa var. *neglecta*	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. paradoxa var. *paradoxa*	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. purpurea	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. sanguinea	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. simulata	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
E. tennesseensis	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGCCGTAC
Dracopis amplexicaulis	AGTGACGCATGACTAGTGGTGGTTGATATGACAGTCGTCTCGTGTCGTGT
Heliopsis helianthoides	AG-AACGCACGGCTAGTGGTGGTTGATAACATAGTCATCTTGTGATGTGC
Ratibida columnifera	AGTGACGCACGACTAGTGGTGGTTGATAAGACAGTCGTCTCGTGTCGCGT
Rudbeckia hirta	AATGACGCAATACTAGTGGTGGTTGATAATACAGTCGTCTCGTGTCTTGT
Sanvitalia fruticosa	AGAGACGCACGGCTAGTGGTGGTTGATAACACAGTCGTCTCGTGTTGTGC
Zinnia grandiflora	AGAGTCGCACGACTAGCGGTGGTTGATAACACAGTCGTCTCGTGTCCTGT

	760 770 780 790 800
E. angustifolia	GGTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. atrorubens	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. pallida	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. paradoxa var. *neglecta*	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAMCC-TGACGCGTCGTCT
E. paradoxa var. *paradoxa*	GTTTATGATTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. purpurea	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. sanguinea	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. simulata	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
E. tennesseensis	GTTTATGTTTGTGAGTGTCTAGACTTGTGAAAAACC-TGACGCGTCGTCT
Dracopis amplexicaulis	GTTTTCATTCYTGAGTCAA-ATTCTCTTAACCTACCAAGATGTGTTGTCT
Heliopsis helianthoides	GTTTTCATCCGTGTGTGGCTTTACTTTTAAAGAACCCA-ATGCGTTGTCT
Ratibida columnifera	GTTTTCATTCTTRAGT-CAGACGCTCTTAACATACCAAGATGCGTTGTCT
Rudbeckia hirta	GTTTTCATTCTCGAGT-TAGATGCTCTTAACCTACAATGATGTGTTGTCT
Sanvitalia fruticosa	GTTTT-G-CCGTGAGTGTTTAGACTCGTAAAAAACC-CGACGCGTTGTCC
Zinnia grandiflora	GCTTTCATTCGTGAGGGAAGATACTCGTATACAAACCCGACGCGCTGTCT

	810 820 830 840 850
E. angustifolia	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. atrorubens	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. pallida	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. paradoxa var. *neglecta*	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. paradoxa var. *paradoxa*	TCAGATCATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. purpurea	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. sanguinea	TCAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. simulata	TSAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
E. tennesseensis	TGAGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
Dracopis amplexicaulis	TATGATGACGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
Heliopsis helianthoides	CGTGACATAGCTTGGAT---------------------------------
Ratibida columnifera	TGTGACGACGCTTCGATCGCGACCCCAGGTCAGGCGGG-T----------
Rudbeckia hirta	TGTGATGATGCTTCGATCGCGACCCCAGGTCAGGCGGGACTACCCGCTGA
Sanvitalia fruticosa	TTGGATGATGCTTCGAT---------------------------------
Zinnia grandiflora	TGTGACGATGCTTCGAT---------------------------------

	860 870 880 890 .900
E. angustifolia	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTAC------------
E. atrorubens	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGA-------
E. pallida	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAG---------
E. paradoxa var. *neglecta*	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACA-G---------
E. paradoxa var. *paradoxa*	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACT-GCAAGGAT------
E. purpurea	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATTCC---
E. sanguinea	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATTCCCTT
E. simulata	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACT-GC------------
E. tennesseensis	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATTCC-TT
Dracopis amplexicaulis	GTTTAAGCATATCAATAAGCGGAGGAAAAGAAACKTACTAGGATA-----
Heliopsis helianthoides	--
Ratibida columnifera	--
Rudbeckia hirta	GTTTAAGCATATCAATAAGCGGAGGAAA-G-AACTTTCAGGGTTCCC-TT
Sanvitalia fruticosa	--
Zinnia grandiflora	--

	910 920	
E. angustifolia	------------------	[860]
E. atrorubens	------------------	[866]
E. pallida	------------------	[871]
E. paradoxa var. *neglecta*	------------------	[888]
E. paradoxa var. *paradoxa*	------------------	[858]
E. purpurea	------------------	[870]
E. sanguinea	A-----------------	[871]
E. simulata	------------------	[857]
E. tennesseensis	AATAACGGGGAACCGAAC	[918]
Dracopis amplexicaulis	------------------	[875]
Heliopsis helianthoides	------------------	[653]
Ratibida columnifera	------------------	[804]
Rudbeckia hirta	ATACG-------------	[869]
Sanvitalia fruticosa	------------------	[672]
Zinnia grandiflora	------------------	[673]

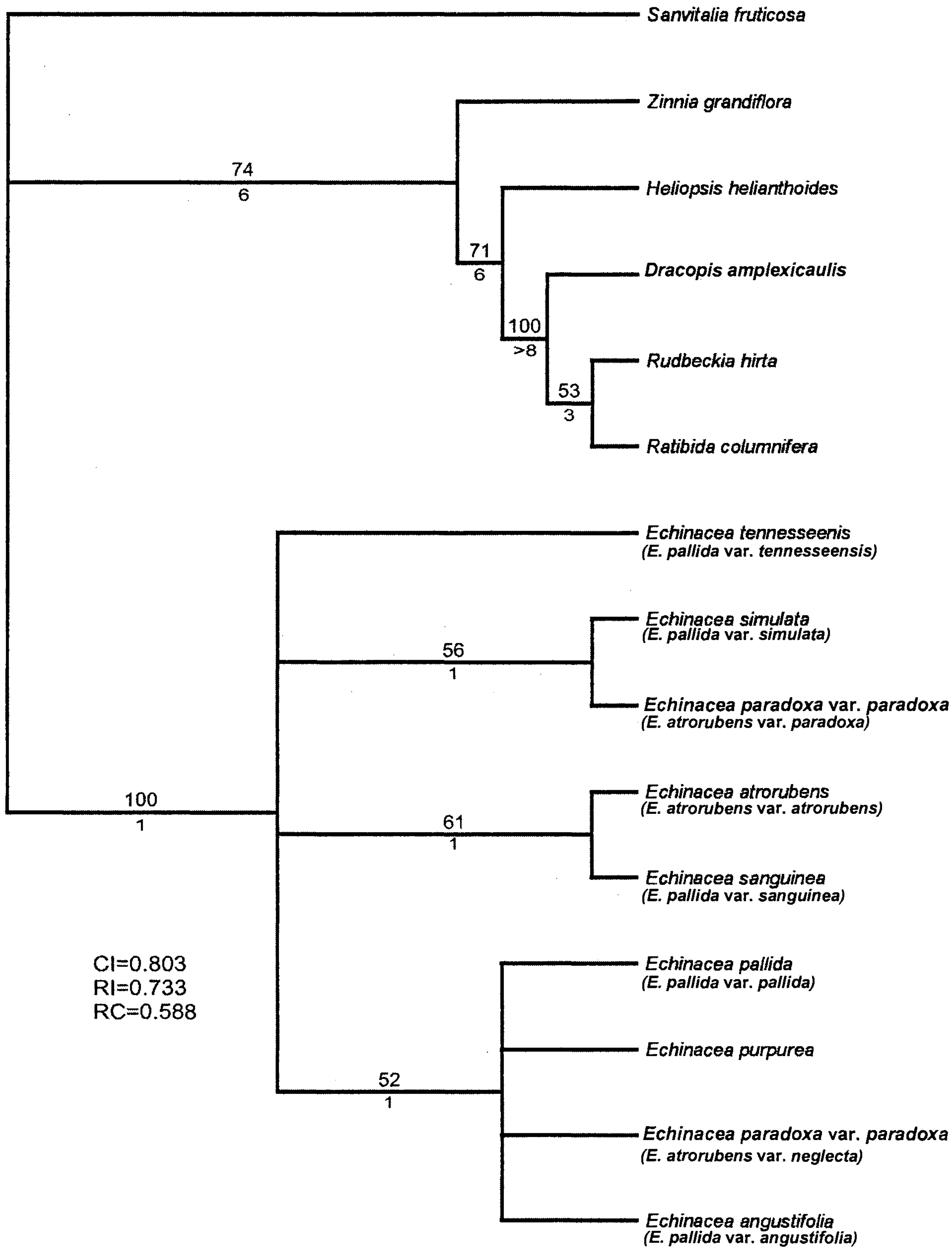


Figure 2.3. Strict consensus tree of the *Echinacea* and closely related taxa based on ITS data. Bootstrap support (%) is shown above branches and decay index below. Upper name of pairs is that of McGregor (1968), lower name of pairs in parentheses is that of Cronquist (1955, 1980) and Binns and coworkers (2002a).

LITERATURE CITED

Andreasen, K. and B. G. Baldwin. 2001. Unequal evolutionary rates between annual and perennial lineages of checker mallows (*Sidalcea*, Malvaceae): evidence from 18S–26S rDNA Internal and External Transcribed Spacers. Molecular Biology and Evolution 18:936–944.

Bailey, D. T., S.L. Richheimer, Z. Liu, G. Jayatilake, and C. Mannila. 1999. HPLC Anaylsis of *Echinacea* Phenols and Amides. 1999 AHPA International Echinacea Symposium, June 3–5, 1999. Kansas City, MO.

Baldwin, B. G. 1993. Molecular systematics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. American Journal of Botany 80:222–238.

Baldwin, B. G., M.J. Sanderson, J.M. Porter, M.F. Wojciechowski, C.S. Campbell, and M.J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of Missouri Botanical Garden 82:247–277.

Baldwin, B. G. and S. Markos. 1998. Phylogenetic utility of the external transcriber spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). Molecular Phylogenetics and Evolution 10:449–463.

Baskauf, C. J., D. E. Mccauley, and W. G. Eickmeier. 1994. Genetic analysis of a rare and a widespread species of *Echinacea* (Asteraceae). Evolution 48:180–188.

Baskin, J. M., K.M. Snyder, and C.C. Baskin. 1993. Nomenclatural history and taxonomic status of *Echinacea angustifolia, E. pallida, E. tennesseensis* (Asteraceae). SIDA 15:597–604.

Bauer, R. and H. Wagner. 1985. Echinacea species as potential immunostimulatory drugs. Pages 253–321 *in* H. Wagner, Hiroshi Hikino, and Norman R. Farnsworth, eds. Economic and Medicinal Plant Research, Vol. 5. Academic Press, Inc., Orlando, FL.

Bauer, R., I.A. Khan, and H. Wagner. 1988. TLC and HPLC analysis of *Echinacea pallida* and *E. angustifolia* roots. Planta Medica 54:426–430.

Baum, B. R., J. F. L. S. Mechandra, S.E. Binns, and J.T. Arnason. 2001. Predicting quantitative phytochemical markers in single *Echinacea* plants or clones from their DNA fingerprints. Phytochemistry 56:543–549.

Baum, B. R. and Shannon E. Binns. 1999. The *Echinacea* germplasm enhancement project, DNA fingerprinting, and intellectual property protection. 1999 AHPA International Echinacea Symposium, June 3–5, 1999. Kansas City, MO.

Bayer, R., L. Hufford, and D. E. Soltis. 1996. Phylogenetic relationships in Sarraceniaceae based on rbcL and ITS sequences. Systematic Botany 21:121–134.

Bena, G., M. F. Jubier, I. Olivieri, and B. Lejeune. 1998. Ribosomal External and Internal Transcribed Spacers: combined use in the phylogenetic analysis of *Medicago* (Leguminosae). Journal of Molecular Evolution 46:299–306.

Binns, S. E., B. R. Baum, and J. T. Arnason. 2002a. A Taxonomic Revision of *Echinacea* (Asteraceae: Heliantheae). Systematic Botany 27:610–632.

Binns, S. E., J. Hudson, S. Merali, and J. T. Arnason. 2002b. Antiviral activity of characterized extracts from *Echinacea* spp. (Heliantheae: Asteraceae) against herpes simplex virus (HSV-I). Planta Medica 68:780–3.

Binns, S. E., J. F. Livesey, J. T. Arnason, and B. R. Baum. 2002c. Phytochemical variation in *Echinacea* from roots and flowerheads of wild and cultivated populations. Journal of Agricultural and Food Chemistry 50:3673–3687.

Binns, S. E. 2001. The Taxonomy and Phytochemistry of the Genus *Echinacea*. Ph.D. dissertation. Unversity of Ottawa, Ottawa, Ontario.

Bremer, K. 1994. Asteraceae: Cladistics and Classification. Timber Press, Inc., Portland, OR.

Buldewo, S. and Y.F. Jaufeerally-Fakim. 2002. Isolation of clean and PCR-amplifiable DNA from *Anthurium andreanum*. Plant Molecular Biology Reporter 20:71a–71g.

Chan, R., B. G. Baldwin, and R. Ornduff. 2002. Cryptic Goldfields: A molecular phylogenetic reinvestigation of *Lasthenia californica sensu lato* and close relatives (Compositae: Heliantheae *sensu lato*). American Journal of Botany 89:1103–1112.

Clevinger, J. A. and J. L. Panero. 2000. Phylogenetic analysis of *Silphium* and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. American Journal of Botany 87:565–572.

Couch, J. A. and P.J. Fritz. 1990. Isolation of DNA from plants high in polyphenolics. Plant Molecular Biology Reporter 8:8–12.

Cox, P. B. and Lowell E. Urbatsch. 1990. A phylogenetic analysis of the coneflower genera. Systematic Botany 15:394–402.

Cronquist, A. 1955. Vascular Plants of the Pacific Northwest, Part 5: Compositae. University of Washington Press, Seattle.

Cronquist, A. 1980. Vascular Flora of the Southeastern United States, Volume I. The University of North Carolina Press, Chapel Hill, NC.

Donoghue, M. J., R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of *Dipsacales* based on rbcL sequences. Annals of the Missouri Botanical Garden 79:333–345.

Doyle, J. J. and J.L. Doyle. 1987. A rapid DNA isolation prodecure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Holmgren, P. K. and N. H. Holmgren. 2003. Index Herbariorum, Part I: The Herbaria of the World, Eighth Edition. NYBG Press, The New York Botanical Garden, Bronx, New York. http://www.nybg.org/bsci/ih/ih.html.

Judd, W.S., C.S. Campbell, E.A. Kellogg, P.F. Stevens. 2002. Plant Systematics: A Phylogenetic Approach. 2nd Edition. Sinauer Associates, Sunderland, Mass.

Lee, J., B. G. Baldwin, and L. D. Gottlieb. 2002. Phylogeny of *Stephanomeria* and related genera (Compositae: Lactuceae) based on analysis of 18S–26S nuclear rDNA ITS and ETS sequences. American Journal of Botany 89:160–168.

Letchamo, W., J. Livesey, T.J. Arnason, C. Bergeron, and V.S. Krutilina. 1999. Cichoric acid and isobutylamide content in *Echinacea purpurea* as influenced by flower developmental stages. Page 494–498 *in* J. Janick, ed. Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA.

Li, T. S. C. 1998. *Echinacea*: cultivation and medicinal value. HortTechnology 8:122–129.

Linder, C. R., L. R. Goertzen, B. V. Heuvel, J. Francisco-Ortega, and R. K. Jansen. 2000. The complete external transcribed spacer of 18S–26S rDNA: amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. Molecular Phylogenetics and

Evolution 14:285–303.

Liston, A., W. A. Robinson, and J. M. Oliphant. 1996. Length variation in the nuclear ribosomal DNA Internal Transcribed Spacer region of non-flowering seed plants. Systematic Botany 21:109–120.

Markos, S. and B. G. Baldwin. 2002. Structure, molecular evolution, and phylogenetic utility of the 5 ' region of the External Transcribed Spacer of 18S-26S rDNA in *Lessingia* (Compositae: Asteraceae). Molecular Phylogenetics and Evolution 23:214–228.

McGregor, R. L. 1968. The taxonomy of the genus *Echinacea* (Compositae). University of Kansas Science Bulletin 48:113–142.

Moench, C. 1794. Methodas plantas. 780 pages.

Moritz, C. and D.M. Hillis. 1996. Molecular Systematics: Contexts and controversies. Pages 1–16 *in* D. M. Hillis, C. Moritz, and B.K. Mable, eds. Molecular Systematics. 2nd Edition. Sinauer Associates, Sunderland, Mass.

Prather, L. A. and Robert K. Jansen. 1998. Phylogeny of *Cobaea* (Polemoniaceae) based on sequence DNA data from the ITS region of nuclear ribosomal DNA. Systematic Botany 23:57–72.

Rether, B., G. Delmas, and A. Laouedj. 1993. Isolation of polysaccharide-free DNA from plants. Plant Molecular Biology Reporter 11:333–337.

Ryburn, A. K. 2003. Molecular Systematic Investigations of *Echinacea* (Asteraceae: Heliantheae) Based on Nuclear Ribosomal ITS and ETS Sequences. *in* A. K. Ryburn, ed. Taxonomic Investigations of Oklahoma Flora. Ph.D. dissertation. Oklahoma State University, Stillwater, OK.

Sang, T., D. J. Crawford, and T. F. Stuessy . 1995a. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proceedings of the National Academy of Sciences 92:6813–6817.

Sang, T., Daniel J. Crawford, Tod F. Stuessy, and Mario Silva O. 1995b. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). Systematic Botany 20:55–64.

Sari, A. O., M.R. Morales, and J.E. Simon. 1999. *Echinacea angustifolia*: An Emerging Medicinal. Page 490–493 *in* J. Janick, ed. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Savard, L., M. Michaud, and J. Bousquet. 1993. Genetic diversity and phylogenetic relationships between birches and alders using ITS, 18S rRNA, and rbcL gene sequences. Molecular Phylogenetics and Evolution 2:112–118.

Savolainen, V., P. Cuenoud, R. Spichiger, M.D.P. Martinez, M. Crevecoeur, and J-F Manen. 1995. The use of herbarium specimens in DNA phylogenetics:

evaluation and improvement. Plant Systematics and Evolution 197:87–98.

Sharp, W. M. 1935. A critical study of certain epappose genera of the Heliantheae-Verbesininae of the natural family Compositae. Annals of the Missouri Botanical Garden 22:51–152.

Snyder, K. M. 1991. The comparative ecology of the narrow endemic *Echinacea tennesseensis* (Asteraceae: Heliantheae) and two geographically widespread congeners: a greenhouse study and literature review. M.S. thesis. University of Kentucky, Lexington, KY.

Swofford, D. L. 2002. Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0 Beta. Sinauer Associates, Inc., Sunderland, Massachusetts.

Thompson, J. D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.

U.S. Fish & Wildlife Service. 1999. Threatened and Endangered Species System (TESS). Division of Endangered Species, http://endangered.fws.gov.

Urbatsch, L. E., B. G. Baldwin, and M. J. Donoghue. 2000. Phylogeny of the coneflowers and relatives (Asteraceae: Heliantheae) based on nuclear rDNA Internal Transcribed Spacer (ITS) sequences and chloroplast DNA restriction site data. Systematic Botany 25:539–565.

Urbatsch, L. E. and R. K. Jansen. 1995. Phylogenetic affinities among and within the coneflower genera (Asteraceae: Heliantheae), a chloroplast DNA analysis. Systematic Botany 20:28–39.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 *in* M. Innis, D. Gelfand, J. Sninsky, and T. White, eds. PCR Protocols: A Guide to Methods and Application. Academic Press, San Diego, CA.

Wiley, E. O., D. Siegel-Causey, D.R. Brooks, and V.A. Funk. 1991. The Complete Cladist. A Primer of Phylogenetic Procedures. Museum of Natural History, University of Kansas Special Publication No. 19, Lawrence, KS.

CHAPTER 3

POPULATION DYNAMICS OF *ECHINACEA PALLIDA* (ASTERACEAE) AT THE TALLGRASS PRAIRIE PRESERVE (OKLAHOMA)

INTRODUCTION

Commonly known as pale purple coneflower, *Echinacea pallida* is one of the more widespread species of the New World genus (Figure 3.1). It occurs from the Prairie Peninsula of Iowa and Illinois to the eastern one-third of the lower Great Plains, as well as the Ozark and Ouachita Mountains of Oklahoma, Missouri, and Arkansas and reaches its southern most distribution in southwestern Louisiana (McGregor, 1968; McKeown, 1999; Binns et al., 2002). Although small populations have been recorded in a number of eastern states—some as disjunct as northeastern Maine—these populations are probably adventive; their occurrence no doubt a result of cultivation because of the medicinal value of the taxon (McGregor, 1968).

The geographic distribution of *E. pallida* reflects its specific habitat requirements. Populations are restricted to rocky prairies, open wooded hillsides, and cedar glades, all of which must have calcareous, well-drained soils (McGregor, 1968). Many such sites exist throughout the Great Plains, most commonly in the area known as the tallgrass prairie.

Echinacea pallida differs from the other members of the genus in that it typically has light purple to pink or white, highly reflexed ray florets and dark brown disk florets that are subtended by brown or darkly colored receptacular bracts. Stems are typically 30–90 cm tall, and the distinctly 3-nerved, hirsute leaves are 10–30 cm long and 1–4 cm wide. The most important distinguishing character for this species is pollen grain color. It is the only member of the genus with white pollen, and the specific epithet actually refers to the color of these pollen grains rather than the light colored ray florets.

Containing a variety of compounds such as alkaloids, polysaccharides, glycoproteins, caffeic acid derivates, flavonoids, and essential oils, *Echinacea* is an extremely popular medicinal taxon (Bauer et al. 1988; Bauer and Wagner, 1985, Small and Catlig, 1999). *Echinacea pallida* is one of three species in the genus being studied as a medicinal plant. Its economic and medicinal value is well documented (Bauer et al., 1988; Schulthess et al., 1991; Li, 1998; Small and Catlig, 1999).

The popularity of this medicinal genus has led to an increase in cultivation as well as a rapid decrease in the wild populations of its species because of indiscriminant extirpation of plants by collectors (Ladd and Oberle, 1995). Devastation of natural populations of *E. pallida* increases the need for a better understanding of its biology. Previous work on the genus has focused on *E. tennesseensis* and *E. laevigata*, designated as endangered by the U.S. Fish and Wildlife Service (1999). *Echinacea angustifolia* and *E. purpurea* also have been investigated in field studies by Leuszler (1996) and Hurlburt (1999). *Echinacea pallida* has not been studied, especially with respect to it's population biology. Thus the purpose of this study was to provide information about the population dynamics of *Echinacea pallida*. Using methods employed by population dynamics, the following objectives were addressed:

1) To determine whether the population of *Echinacea pallida* at the Tallgrass Prairie Preserve (TGPP) is stable, increasing, or decreasing.
2) To determine the variables most likely contributing to the transformation of vegetative individuals into reproductive ones and vice-versa.
3) To determine the variables most likely contributing to the mortality of individuals at the TGPP.

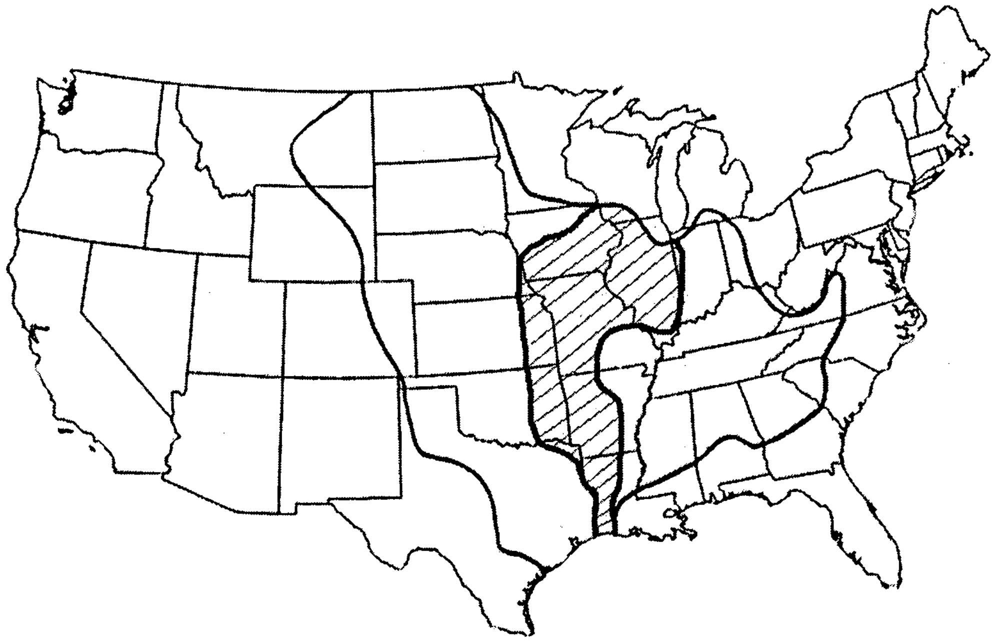


Figure 3.1 Distribution map of *Echinacea*. The shaded portion indicates the distribution of the genus. Diagonal lines designate the distribution of *Echinacea pallida*.

METHODS AND MATERIALS

Field Site Description

This study was conducted in the bison (*Bos bison*) grazing area of the Tallgrass Prairie Preserve in Osage County, Oklahoma (36.8481°N, 96.4219°W). This native tallgrass prairie of approximately 15,800 ha has been owned and managed by The Nature Conservancy since 1989. Approximately 90% of the site is grassland which contains a diverse assemblage of plant species (Palmer, 2003). Approximately 80% of the prairie vegetation is dominated by C_4 warm-season grasses including *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), *Sorghastrum nutans* (Indian grass), *Sporobolus compositus* (composite dropseed), and *Panicum virgatum* (switch grass). Other common species include members of the Asteraceae—*Erigeron philadelphicus* (Philadelphia fleabane), *Coreopsis grandiflora* (largeflower tickseed), *Rudbeckia hirta* (Black-eyed Susan), and *Ratibida columnifera* (Mexican hat)—, Fabaceae—*Mimosa quadrivalvis* (sensitive briar), *Amorpha canescens* (lead plant), *Dalea candida* (white prairie clover), and *Dalea purpurea* (purple prairie clover)—, as well as other species such as *Eryngium yuccifolium* (rattlesnake master), *Linum sulcatum* (grooved flax), and *Asclepias tuberosa* (butterfly milkweed). Much of the remaining area of the TGPP is forested predominantly by *Quercus stellata* (post oak) and *Quercus marilandica* (blackjack oak) (Arévalo, 2002).

The TGPP is managed to restore a remnant of the tallgrass prairie landscape in Oklahoma (Hamilton, 1996). Many studies on the tallgrass prairie stress the importance of fire and bison grazing in determining species composition and structure (Collins, 1989; Collins, 1990; Fahnestock and Knapp, 1994). Prescribed burns are conducted throughout the year—dormant spring burns (March-April), late growing season burns (July-September), and dormant fall burns (October-November). Burn locations are randomly selected according to the fuel load of the vegetation (Hamilton, 1996).

We selected one population of *Echinacea pallida* located just north of the preserve's headquarters, in an area where bison grazing and fire have been restored (Figure 3.2). A 100 X 100 m area was established. Within this area, seven 10 x 10 m sites were randomly selected and permanently marked at their corners with rebar and aluminum caps. These sites were identified by a four digit number which symbolized the Cartesian coordinates with respect to the 100 X 100 m study area. The first two digits signified placement along the X axis and the last two along the Y axis (e.g., XXYY). Within each site, one hundred 1 x 1 m quadrats were established and used to map the location of each plant observed.

Data Collection

The population was studied each year from 1997 to 2000 from about the first of June to mid July, the peak flowering period for this prairie species. We monitored the population at the individual plant level. The location of each plant was marked by an aluminum tag driven into the ground beside it by a galvanized nail. The number of the plant and its Cartesian coordinates with respect to the 10 X 10 m sites were recorded and placed on each tag. The following characters were measured and recorded: number of leaves, length of each leaf, number of flowering heads per plant (if present), and height of each flowering head (if present). These measurements were recorded using a handheld microcassette recorder, transcribed later onto field data sheets, and then entered into a Microsoft® Excel spreadsheet (Appendix A). Incidence of pathogens, fungal infection, and herbivory was not recorded for individuals of the population.

Data Analysis

Assuming that the population was closed, i.e., no immigration or emigration occurring, the following equation was used to determine its current status (N_t) with respect to its status one year earlier (N_{t-1}):

$$N_t = N_{t-1} + \mathrm{B} - \mathrm{D} \qquad (3.1)$$

B is the number of births—or new individuals—and D, the number of deaths (Akcakaya et al., 1999).

We summarized the dynamics of the population by calculating the finite rate of increase or λ (lambda). Lambda was calculated by the equation:

$$\lambda = N_t / N_{t-1} \qquad (3.2)$$

A population is considered stable when $\lambda=1$, increasing when $\lambda>1$; and decreasing when $\lambda<1$ (Silvertown and Doust, 1993).

The following variables were calculated for each plant: sum of leaf lengths, maximum leaf length, number of flowering heads (if present), sum of stem heights (if present), maximum stem heights (if present). The status of each plant was recorded each year as either vegetative (basal rosette of leaves), reproductive (having one or more flowering heads), or missing (dead or not found). From this information it was possible to construct a database (Appendix B) that allowed us to describe the status of each plant from year to year.

In order to examine the variables most likely contributing to the transformation of vegetative individuals into reproductive ones and vice-versa, as well as those variables most likely contributing to the mortality of individuals at the site, we employed backward stepwise logistic regression using the LOGISTIC procedure in SAS 8.2 (SAS Institute, Cary, NC). Logistic regression, like most regression analyses, is a valuable tool in that it allows us to find the best fitting, yet biologically most reasonable model to describe the relationship between a response or dependent variable and a set of independent variables (Lemeshow and Hosmer, 1998). Unlike other regression models, such as linear regression, logistic regression requires that the response or dependent variables be recorded in a binary fashion. In a backward stepwise logistic regression, the analysis begins with a large model consisting of all independent variables and then eliminates the least significant ones until only the significant (α=.05) remains (Menard, 1995).

The following equation is the model used to illustrate the relationship between a single dependent variable and the independent variables:

$$P(Y = 1) = \frac{e^{\alpha + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_3 \chi_3 \cdots\cdots \beta_K \chi_K}}{1 + e^{\alpha + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_3 \chi_3 \cdots\cdots \beta_K \chi_K}} \tag{3.3}$$

where $P(Y = 1)$ is the probability that the dependent variable is equal to 1, e is the natural log base (2.718), α is the intercept or constant, β is the value of independent variable, and X is the estimate of the independent variable (given in a SAS output).

The dependent variables used in the analyses were those of the stage of the life cycle (vegetative, reproductive, and missing) from one year to the next. The independent variables were the size characteristics cited above (number of leaves, sum of leaf lengths, maximum leaf length, number of flowering heads, sum of stem heights, maximum stem heights). The squared values of the size characteristics also were included in the analyses to allow for nonlinearity. Appendix C illustrates the input file used for the LOGISTIC procedure.

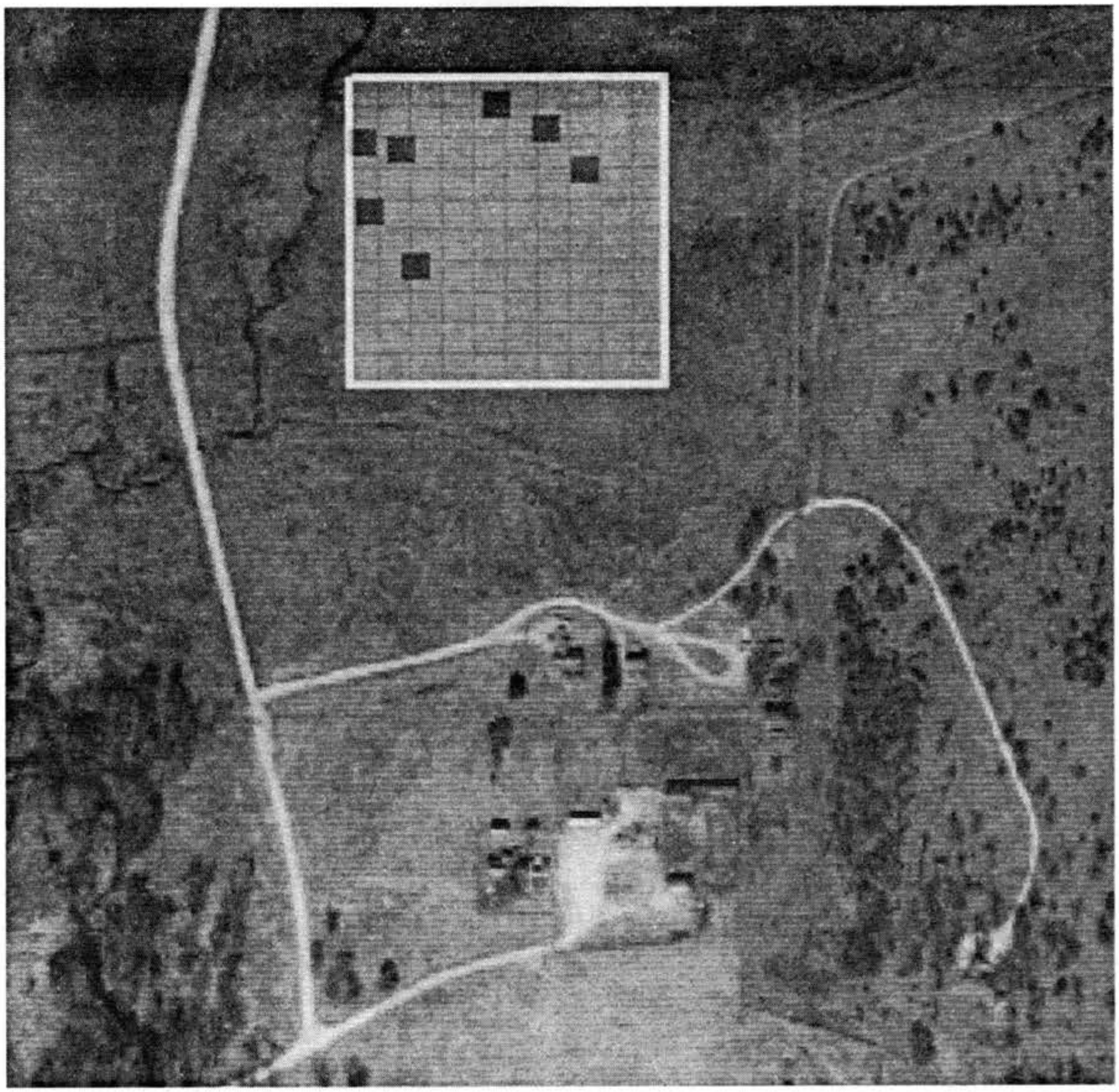


Figure 3.2 Aerial photograph of the Tallgrass Prairie Preserve headquarters and adjacent area. Inset is an outline of the study area containing the seven randomly selected sites.

RESULTS AND DISCUSSION

Population Status

During the four-year period, 8,038 recordings were made during the data collection process. A majority of the individuals studied (73%) were located at two sites—0351 (44%) and 0074 (29%). There were 2,813 new plants encountered at the seven randomly selected sites. Of these, 1,299 were mapped and tagged in 1997; 313 in 1998; 679 in 1999; and 504 in 2000. Appendix D illustrates the distribution of individuals present at each site over the four-year period.

The number of new individuals in the population was considerably higher than those that died in nearly all sites during all years (Table 3.1). An overall increase in the population size of *E. pallida* was seen—662 individuals ($N_{2000} - N_{1997}$)—from the time the study began until it ended, resulting in a λ value of 1.53. An increase in individuals at every site was seen in every growing season (1997–1998, 1998–1999, and 1999–2000) except at 0351 in 1999–2000 (λ = 0.74). At this site, a large number of individuals died. Because it encompasses the highest percentage (44%) of individuals in the entire population, it caused a slight decrease in the population during 1999–2000 (λ = 0.97).

These results agree with those of Arévalo and coworkers (2003a) who concluded the population is still in a state of change and has not yet reached a steady state.

Transition Analyses Using Logistic Regression

Although the study sites showed differences in numbers of individuals, all possessed similar size structure and flowering patterns in each study period. Considerable differences are seen in the ratio of vegetative to reproductive individuals observed during 1998 and 2000 versus 1997 and 1999 (Table 3.2). Throughout the four-year period, the total number of vegetative (V) individuals recorded were 5,559, reproductive (R) individuals 1,647, and those that died or were missing (M), 832. Logistic regression involving stage classes as dependent variables and plant size characters as independent variables provided the following results (Table 3.3).

The transition from a vegetative plant to a reproductive one the following year was best explained by maximum leaf lengths ($p<0.0001$), maximum leaf lengths squared ($p=0.0020$), and number of leaves ($p=0.0021$). As illustrated in Figure 3.3, the smaller the maximum leaf lengths, the less likely a vegetative plant was to be recruited to a reproductive stage the next year. As maximum leaf lengths increase, so too did the likelihood the plant to make the transition from a vegetative stage of its life cycle to a reproductive one. The same can be said for number of leaves. Individuals with fewer leaves were less likely than those with more leaves to make a transition to a reproductive stage the following year.

The transition from a reproductive plant to a vegetative one the following year was best explained by maximum leaf lengths ($p<0.0001$) and maximum leaf lengths squared ($p=0.0004$). The likelihood of a reversion is illustrated in Figure 3.4, a plot of the probability of individuals remaining reproductive as a function of maximum leaf lengths. The data clearly suggest that the greater the maximum leaf lengths of a reproductive individual, the higher the probability of that individual to remain in a reproductive stage and not revert to a vegetative stage the following year.

Mortality is as an important part of the dynamics of a population as the establishment of viable offspring. Because of this, it is important to know what variables might suggest when individuals will reach the end of their life cycle. The transition of an individual from a reproductive plant to a dead (missing) plant the following year was best explained by maximum leaf lengths ($p=0.0097$). The probability of individuals remaining reproductive as a function of maximum leaf lengths is shown in Figure 3.5. The results suggest that as maximum leaf lengths of reproductive individuals increase, so too does the likelihood of these individuals to remain reproductive and not succumb to mortality before the next growing season.

The transition of an individual from a vegetative stage to a dead (missing) plant the following year was best explained by maximum leaf lengths ($p<0.0001$) and maximum leaf lengths squared ($p<0.0001$). The results suggest that vegetative plants with smaller maximum leaf lengths will most likely not return the next year. This result agrees with the trend found in the raw data. It appears that once a vegetative individual has reached a maximum leaf length of about 10 cm, its chances of remaining a part of the population increases.

Possible environmental variables affecting the population dynamics of E. pallida at the TGPP

One might hypothesize that a variety of environmental conditions could be affecting the population of *E. pallida* at the TGPP and contributing to the results of this study. Some of these environmental variables were examined by Arévalo and coworkers (2003a&b). They did not find a strong relationship between soil composition and chemistry and population features. Likewise, climatic parameters (mean monthly average temperature, total precipitation, and mean monthly soil temperature) also were considered. These parameters, acquired from a Mesonet station (Oklahoma Climatological Survey, 2002) located less than 500 m from the study site, were not clearly related with the differences among the transition probabilities during the study (Arévalo et al., 2003b).

Arévalo and coworkers (2003b) suggested that the difference in numbers of vegetative and reproductive plants in 1998 and 2000 from those in 1997 and 1999 are a result of the prescribed fire regime instituted at the TGPP. Their results suggest that *E. pallida* exhibits a dynamic response to fire, i.e., producing a large number of reproductive individuals in the growing season after fall fires (1997 and 1999) and smaller numbers of reproductive individuals when fire is absent (1998 and 2000). In years without fire, there was more competition for adequate solar radiation due to accumulation of litter and grass biomass from the previous growing season. This resulted in an increase in leaf biomass per plant, both in terms of surface area and number of leaves, as well as a decrease in the number of flowering heads. Although the effect of fire in reproductive dynamics of prairie plants has been extensively reported (Dudley and Lajha, 1993; Hamilton, 1996; Hurlburt, 1999; Vickery, 2002), especially with respect to survivorship in different species (Maret and Wilson, 2000), reversion of the size during those years has not been documented as a common response. Arévalo and coworkers (2003b) considered a regular rate of fire an intrinsic factor of the population dynamic of *E. pallida* that facilitates the maintenance of the population.

It should clearly be determined what effect fire has on the population with demographic parameters. Altering the current method of fire management may result in a non sustainable population of *Echinacea pallida*. If current management practices on the preserve are maintained, populations of *E. pallida* will persist.

Table 3.1 Finite rate of increase λ and summed values of individuals new to the population (B), those that died (D), and total number of individuals for each site in a given year (N_t).

Site	1997	1998			1997-1998	1999			1998-1999	2000			1999-2000	Overall population status (1997-2000) λ
	N_t	B	D	N_t	λ	B	D	N_t	λ	B	D	N_t	λ	
0074	373	81	5	449	1.20	229	64	614	1.37	168	131	651	1.06	1.75
0351	691	82	10	763	1.10	245	114	894	1.17	78	306	666	0.74	0.96
1371	2	0	0	2	1.00	1	0	3	1.50	2	0	5	1.67	2.50
1834	0	1	0	1		0	0	1	1.00	2	0	3	3.00	
4387	26	10	0	36	1.38	32	3	65	1.81	21	9	77	1.18	2.96
5979	127	87	0	214	1.69	133	32	315	1.47	123	80	358	1.14	2.82
7065	80	70	3	147	1.84	39	32	154	1.05	110	43	221	1.44	2.76
Totals	1299	331	18	1612	1.24	679	245	2046	1.27	504	569	1981	0.97	1.53

Table 3.2 Numbers of individuals in vegetative (V), reproductive (R), and dead/missing (M) stage classes for each site during each year.

	1997		1998			1999			2000		
Site	V	R	V	R	M	V	R	M	V	R	M
0074	181	192	441	8	5	398	218	64	702	17	131
0351	349	342	737	26	10	498	406	114	725	59	306
1371	1	1	2	0	0	1	2	0	5	0	0
1834	0	0	1	0	0	0	1	0	3	0	0
4387	7	19	35	1	0	38	27	3	79	1	9
5979	37	90	210	4	0	218	97	32	385	5	80
7065	26	54	144	3	3	83	72	32	253	2	43
	601	698	1570	42	18	1236	823	245	2152	84	569

Table 3.3. Summary of backward stepwise logistic regression results as explained in text. Estimates and intercepts generated using SAS 8.2 (SAS Institute, Cary, NC).

Transition from vegetative to reproductive individual	Significant independent variables (a=0.05)	Estimate used for equation 3.3
maximum leaf lengths	p<0.0001	0.3926
maximum leaf lengths squared	p=0.0020	-0.00462
number of leaves	p=0.0021	0.0334
	intercept	-5.9648
Transition from reproductive to vegetative individual	**Significant independent variables (a=0.05)**	**Estimate used for equation 3.3**
maximum leaf lengths	p<0.0001	0.7476
maximum leaf lengths squared	p=0.0004	-0.0146
	intercept	-10.6814
Transition from reproductive to dead individual	**Significant independent variables (a=0.05)**	**Estimate used for equation 3.3**
maximum leaf lengths	P=0.0097	-0.0844
	intercept	2.1684
Transition from reproductive to dead individual	**Significant independent variables (α=0.05)**	**Estimate used for equation 3.3**
maximum leaf lengths	p<0.0001	-0.2245
maximum leaf lengths squared	p<0.0001	0.00828
	intercept	-0.1642

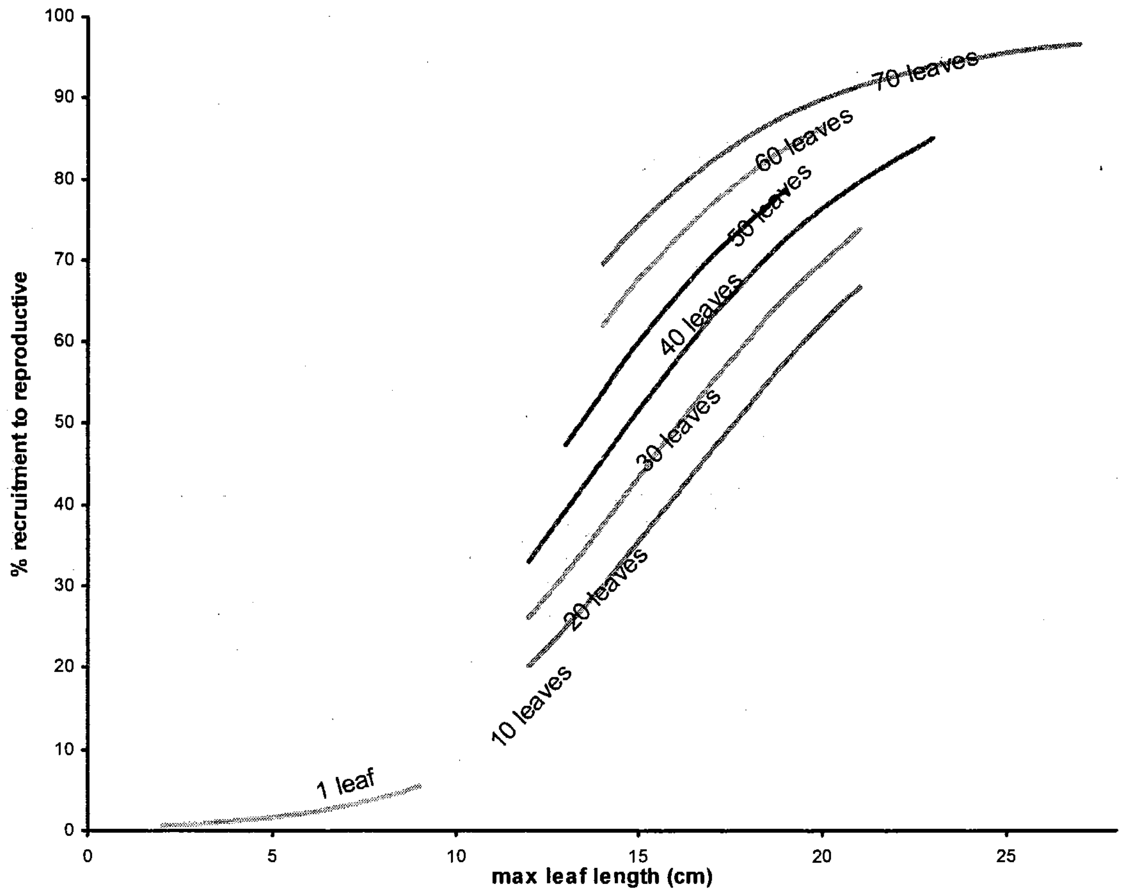


Figure 3.3 Transition from vegetative stage previous year to reproductive stage the following year. Constructed from logistic model (Equation 3.3).

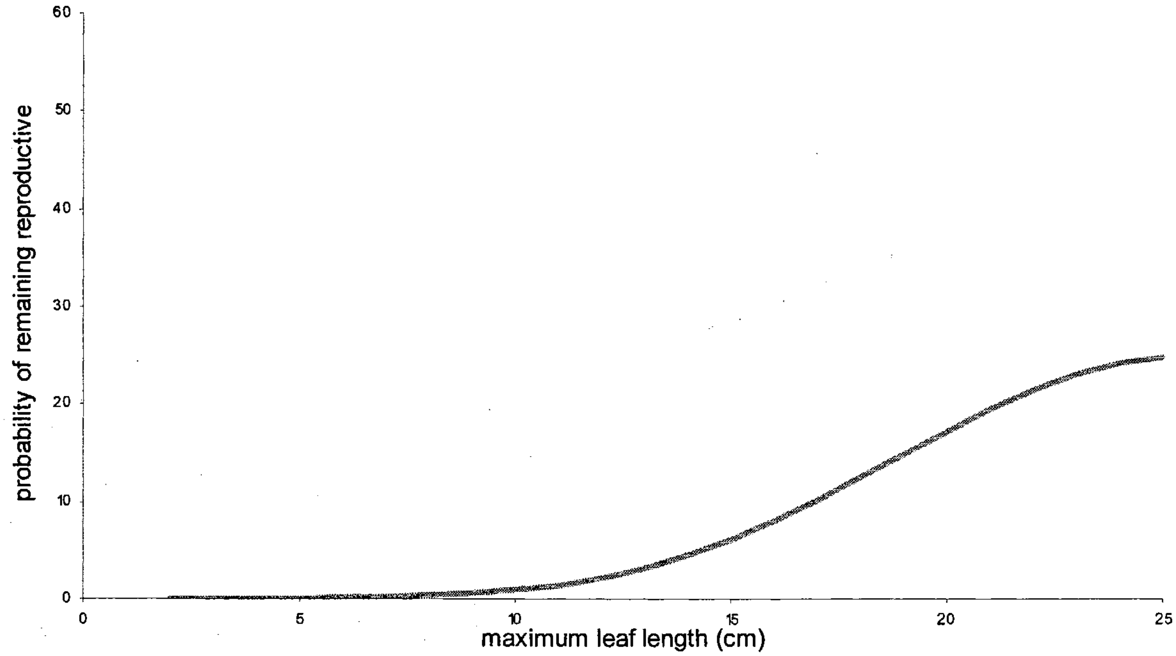


Figure 3.4 Transition from reproductive stage previous year to vegetative stage the following year. Constructed from logistic model (Equation 3.3).

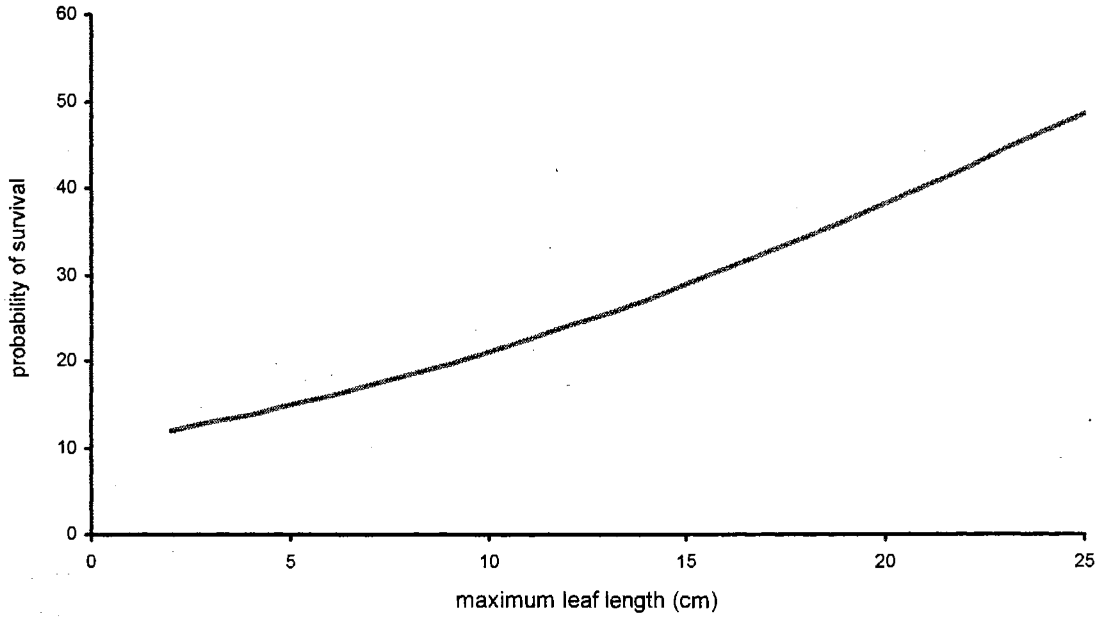


Figure 3.5 Transition from reproductive stage previous year to dead (missing) plant the following year. Constructed from logistic model (Equation 3.3).

LITERATURE CITED

Akcakaya, H. R., Mark A. Burgman, and Lev R. Ginzburg. 1999. *Applied Population Ecology using RAMAS EcoLab*. Sinauer Associates, Inc., Sunderland, MA.

Arévalo, J. R. 2002. Distribution of trees and saplings at the edge of cross timbers forests, Oklahoma, USA. Natural Areas Journal 22:99–107.

Arévalo, J. R., A.K. Ryburn, and S.D. Jaiswal. 2003a. Temporal changes in the spatial structure of a population of *Echinacea pallida* at the Tallgrass Prairie Preserve, Oklahoma. Southwestern Naturalist. Manuscript in review.

Arévalo, J. R., A.K. Ryburn, and S.D. Jaiswal. 2003b. Temporal changes in the structure of a population of *Echinacea pallida.* Tallgrass Prairie Preserve, Oklahoma. Journal of Vegetation Science. Manuscript in review.

Bauer, R. and H. Wagner. 1985. Echinacea Species as Potential Immunostimulatory Drugs. Pages 253–321 *in* H. Wagner, Hiroshi Hikino, and Norman R. Farnsworth, eds. Economic and Medicinal Plant Research, Vol. 5. Academic Press, Inc., Orlando, FL.

Bauer, R., I.A. Khan, and H. Wagner. 1988. TLC and HPLC analysis of *Echinacea pallida* and *E. angustifolia* roots. Planta Medica 54:426–430.

Binns, S. E., B. R. Baum, and J. T. Arnason. 2002. A taxonomic revision of *Echinacea* (Asteraceae: Heliantheae). Systematic Botany 27:610–632.

Collins, S. L. 1989. Experimental analysis of patch dynamics and community heterogeneity in tallgrass prairie. Vegetatio 85:57–66.

Collins, S. L. 1990. Patterns of community structure during succession in tallgrass prairie. Bulletin of the Torrey Botanical Club 117:397–408.

Dudley, J. L. and K. Lajtha. 1993. Effects of prescribed burning on nutrient availability and primary production in sandplain grasslands. American Midland Naturalist 130:286–298.

Fahnestock, J. T. and A. K. Knapp. 1994. Plant responses to selective grazing by bison: Interactions between light, herbivory and water stress. Vegetatio 72:175–185.

Hamilton, R. G. 1996. Using fire and bison to restore a functional tallgrass prairie landscape. Pages 208–214 *in* Transcript of the 61st North American Wildlife and Natural Resource Conference.

Hurlburt, D. P. 1999. Population Ecology and Economic Botany of *Echinacea angustifolia*, A Native Prairie Medicinal Plant. Doctoral Dissertation. Univeristy of Kansas , Lawrence, KS.

Ladd, D. and Frank Oberle. 1995. Tallgrass Prairie Wildflowers. Falcon Publishing Inc., Helena, Montana.

Lemeshow, S. and David W. Hosmer Jr. 1998. Logistic Regression. Pages 2316–2327 *in* P. Armitage and Theodore Colton, eds. Encyclopedia of

Biostatistics. John Wiley & Sons, Ltd., Chichester, UK:

Leuszler, H. K., Vincent J. Tepedino, and Diane G. Alston. 1996. Reproductive biology of purple coneflower in southwestern North Dakota. The Prairie Naturalist 28:91–102.

Li, T. S. C. 1998. *Echinacea*: Cultivation and Medicinal Value. HortTechnology 8:122–129.

Maret, M. P. and M. V. Wilson. 2000. Fire and seedling population dynamics in western Oregon prairies. Journal of Vegetation Science 11:307–314.

McGregor, R. L. 1968. The taxonomy of the genus *Echinacea* (Compositae). University of Kansas Science Bulletin 48:113–142.

McKeown, K. A. 1999. A Review of the Taxonomy of the Genus Echinacea. Pages 482–489 *in* J. Janick, ed. Perspectives on new crops and new uses. American Society for Horticultural Science Press, Alexandria, VA.

Menard, S.W. 1995. Applied Logistic Regression Analysis. Sage University Paper series on Quantitative Applications in Social Sciences. No. 107. Sage University Publications, Inc. Thousand Oaks, CA.

Oklahoma Climatological Survey. 2002. Mesonet Climatological Data Summary (Foraker) . Oklahoma Climatological Survey, College of Geological Sciences, University of Oklahoma, Norman, Oklahoma.

Palmer, M. W. 2003. The Vascular Flora of the Tallgrass Prairie Preserve, Osage County, Oklahoma. Unpublished manuscript.

Schulthess, B. H., E. Giger, and T. W. Baumann. 1991. *Echinacea* - Anatomy, Phytochemical Pattern, and Germination of the Achene. Planta Medica 57:384–388.

Silvertown, J. W. and J.L. Doust. 1993. Introduction to Plant Population Biology. Blackwell Science Ltd., Malden, MA.

Small, E. and Paul M. Catling. 1999. Canadian Medicinal Crops. National Research Council of Canada, Ottawa.

U.S. Fish & Wildlife Service. 1999. Threatened and Endangered Species System (TESS). Division of Endangered Species, http://endangered.fws.gov.

Vickery, P. 2002. Effects of prescribe fire on the reproductive ecology of northern blazing star *Liatris scariosa* var. *novae-angliae*. American Midland Naturalist 148:20–27.

Appendix A. Representative database sheet used in organizing field data of *Echinacea pallida*. site = 0074, 0351, 1371, 1834, 4387, 5979, and 7065; year = 1997-2000; plant ID = 1-2236; x and y = Cartesian coordinates with respect to 10 X 10 m site; quad # = 1 X 1 m quadrat within each site (1-100); stage (life cycle stage at time of data collection) = R (reproductive/flowering), V (vegetative), M (missing/dead); fh # = number of heads; L1-L200 = leaf lengths; FH1-FH15 = stem heights.

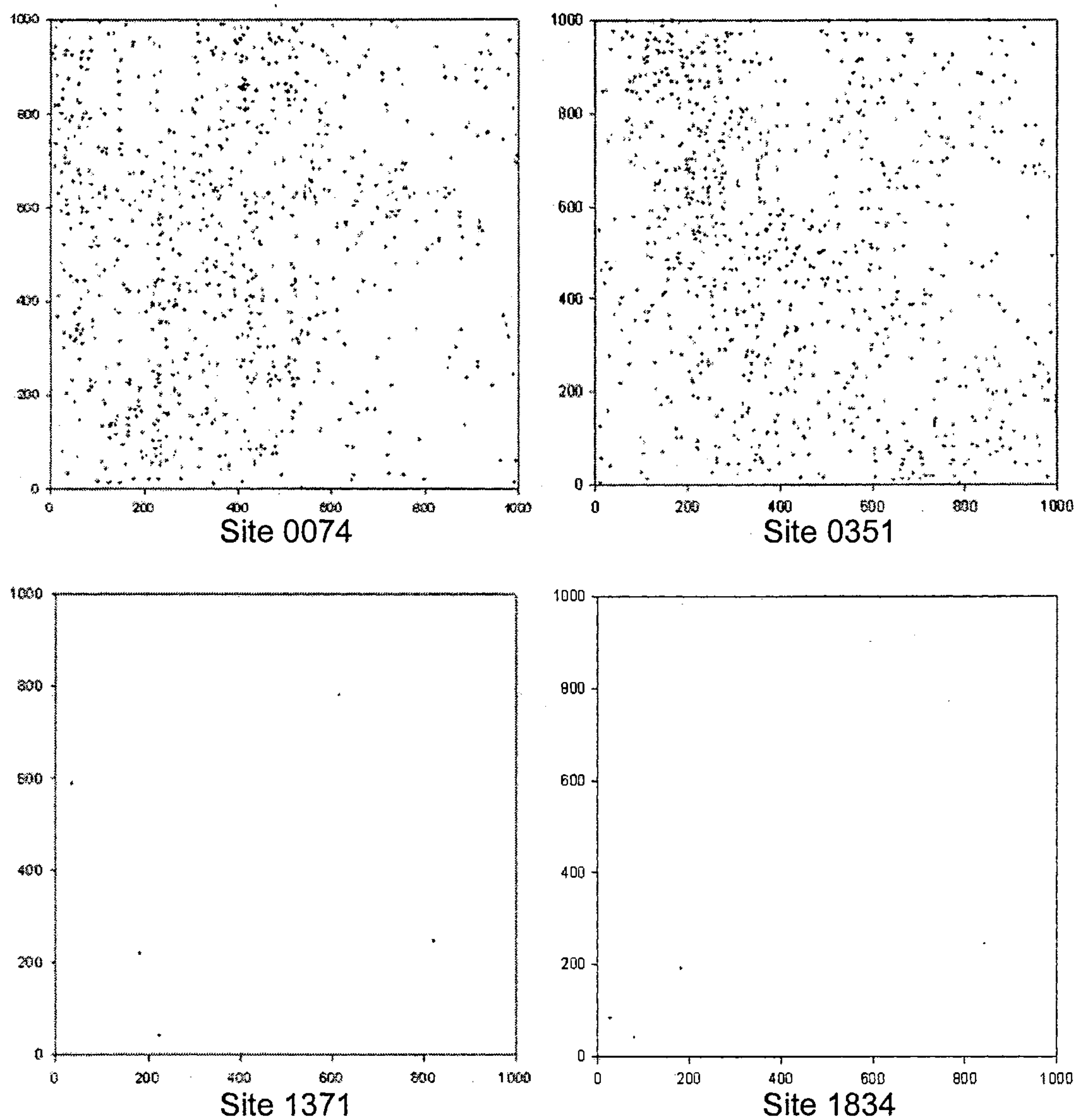
site	year	plant ID	x	y	quad#	stage	fh#	L1	L2	L3	L4	L5	L6	L7	FH1	FH2
1371	1997	1	36	588	6	R	1	9	7	13	5	15	2	3	28	
1371	1998	1	36	588	6	V	0	16	12	4	3	25	12	12		
1371	1999	1	36	588	6	R	4	14	13	14	12	10	11	12	64	58
1371	2000	1	36	588	6	V		16	17	15	13	14	13	13		
1371	1997	2	182	219	13	R	1	8	7	6	9	10	11	12	49	
1371	1998	2	182	219	13	V	0	6	6	12	7	6	8	13		
1371	1999	2	182	219	13	V		30	29	18	18	15	19	23		
1371	2000	2	182	219	13	V		16	25	27	36	33	35	35		
7065	1997	3	41	511	6	R	3	6	9	11	11	9	11	8	51	53
7065	1998	3	41	511	6	V	0	7	8	10	17	18	11	15		
7065	1999	3	41	511	6	R	2	17	16	17	14	14	13	12	61	72
7065	2000	3	41	511	6	V		6	21	13	26	21	21	24		
7065	1997	4	41	311	6	R	1	4	3	5	5	8	5	3	41	
7065	1998	4	41	311	6	V	0	10	10	4						
7065	1999	4	41	311	6	R	1	14	13	12	11	11	10	8	46	
7065	2000	4	41	311	6	V		10	14	5	5	2	15	8		
7065	1997	5	9	590	7	V	0	12	9	11	12	12	12	12		
7065	1998	5	9	590	7	V	0	7	12	10	8	12	12	6		
7065	1999	5	9	590	7	R	1	13	12	13	14	13	11	11	69	
7065	2000	5	9	590	7	V		9	6							
7065	1997	6	6	662	7	V	0	12	12	11						
7065	1998	6	6	662	7	V	0	5	13	12						
7065	1999	6	6	662	7	V		21	22	19	17	11				
7065	2000	6	6	662	7	M										
7065	1997	7	15	756	8	R	2	10	9	11	12	10	7	9	50	59
7065	1998	7	15	756	8	V	0	4	3	8	8	6	6	7		
7065	1999	7	15	756	8	R	1	14	13	14	13	11	11	10	61	25
7065	2000	7	15	756	8	V		8	12	26	8	16	23	29		
7065	1997	8	38	770	8	R	2	9	9	12	11	8	9	3	45	38
7065	1998	8	38	770	8	V	0	11	17	6	16	8	5			
7065	1999	8	38	770	8	R	2	15	15	16	15	16	16	17	59	47
7065	2000	8	38	770	8	M										
7065	1997	9	45	752	8	R	4	10	8	10	11	14	10	11	55	61
7065	1998	9	45	752	8	V	0	11	18	18	18	9	11	16		
7065	1999	9	45	752	8	R	3	16	15	14	13	12	10	11	65	68
7065	2000	9	45	752	8	V		8	13	25	15	12	32	27		
7065	1997	10	67	799	8	R	2	7	7	7	8	9	9	6	45	43
7065	1998	10	67	799	8	V	0	5	7							
7065	1999	10	67	799	8	M										

Appendix B. Representative database sheet summarizing recorded variables. site = 0074, 0351, 1371, 1834, 4387, 5979, and 7065; year = 1997-2000; plant ID = 1-2236; stage this year (life cycle stage at time of data collection) = R (reproductive/flowering), V (vegetative), M (missing/dead); stage previous year (life cycle stage year prior to data collection if applicable) = R (reproductive/flowering), V (vegetative), M (missing/dead).

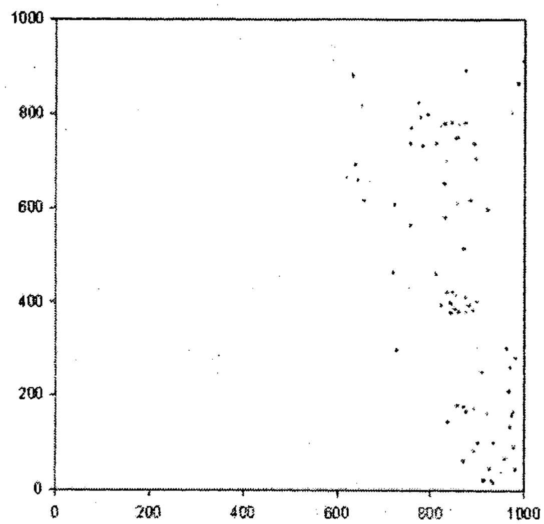
site	year	plant ID	stage this year	stage following year	sum leaf length (cm)	max leaf length (cm)	number of leaves	sum stem heights (cm)	max stem height (cm)	number of stems
4387	1999	1713	V	V	23	9	3	0	0	0
4387	1999	1714	V	V	69	15	5	0	0	0
4387	1999	1715	V	V	21	11	2	0	0	0
4387	1999	1716	V	V	14	10	2	0	0	0
4387	1999	1717	V	V	13	9	2	0	0	0
4387	1999	1718	V	V	59	14	5	0	0	0
4387	1999	1719	V	V	35	10	4	0	0	0
4387	1999	1720	V	D	34	14	4	0	0	0
4387	1999	1721	V	V	63	15	7	0	0	0
4387	1999	1722	V	V	37	15	3	0	0	0
4387	1999	1723	R	V	181	17	15	67	67	1
4387	1999	1724	V	V	16	10	2	0	0	0
4387	1999	1725	V	V	18	11	2	0	0	0
4387	1999	1726	V	V	18	9	2	0	0	0
4387	1999	1727	V	V	55	11	6	0	0	0
4387	1999	1728	V	V	47	12	5	0	0	0
4387	1999	1729	R	V	110	14	12	54	54	1
4387	1999	1730	V	V	44	16	4	0	0	0
5979	1997	83	R	V	109	13	15	60	60	1
5979	1998	83	V	V	11	6	2	0	0	0
5979	1999	83	V	V	147	24	8	0	0	0
5979	1997	84	V	V	38	10	5	0	0	0
5979	1998	84	V	V	53	22	3	0	0	0
5979	1999	84	V	V	90	18	6	0	0	0
5979	1997	85	R	V	204	13	26	47	47	1
5979	1998	85	V	R	13	6	3	0	0	0
5979	1999	85	R	V	119	14	11	96	50	2
5979	1997	86	R	V	70	9	15	47	47	1
5979	1998	86	V	D	8	4	2	0	0	0
5979	1997	87	R	V	172	17	16	58	58	1
5979	1998	87	V	R	90	25	5	0	0	0
5979	1999	87	R	V	511	21	44	215	76	3
5979	1997	88	V	V	57	15	5	0	0	0
5979	1998	88	V	D	25	15	2	0	0	0
5979	1997	89	V	V	21	8	3	0	0	0
5979	1998	89	V	D	12	5	3	0	0	0
5979	1997	90	V	V	30	12	3	0	0	0
5979	1998	90	V	R	32	13	4	0	0	0
5979	1999	90	R	V	116	12	14	46	46	1
5979	1997	91	R	V	79	14	10	47	47	1
5979	1998	91	V	V	98	20	10	0	0	0
5979	1999	91	V	V	73	10	8	0	0	0
5979	1997	92	R	V	82	10	12	61	61	1

Appendix C. SAS input for logistic regression (LOGISTIC procedure).

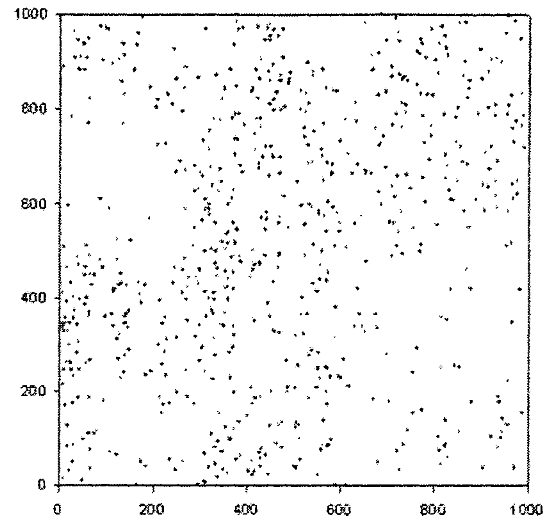
```
DATA ONE;

INFILE 'C:\DOCUMENTS AND SETTINGS\ADMINISTRATOR\MY DOCUMENTS\ECHINACEA STUFF\RYBURN2.PRN';
INPUT PLANTID YEAR SITE STAGETHIS$ STAGEFOLLOW$ SUMLEAFLEN MAXLEAFLEN NOLEAVES
SUMSTEMHT MAXSTEMHT NOSTEMS;

SUMLEAFLEN2 = SUMLEAFLEN**2;
MAXLEAFLEN2 = MAXLEAFLEN**2;
NOLEAVES2 = NOLEAVES**2;
SUMSTEMHT2 = SUMSTEMHT**2;
MAXSTEMHT2 = MAXSTEMHT**2;
NOSTEMS2 = NOSTEMS**2;

DATA TWO;
SET ONE;

IF STAGETHIS = 'V';

DATA TWOVR;
SET TWO;

IF STAGEFOLLOW = 'V' OR STAGEFOLLOW = 'R';

PROC LOGISTIC;
TITLE 'PREVIOUS STAGE = V';
MODEL STAGEFOLLOW = SUMLEAFLEN SUMLEAFLEN2 MAXLEAFLEN MAXLEAFLEN2 NOLEAVES NOLEAVES2
SUMSTEMHT SUMSTEMHT2 MAXSTEMHT MAXSTEMHT2 NOSTEMS NOSTEMS2 /SELECTION = BACKWARD;

DATA TWOVD;
SET TWO;

IF STAGEFOLLOW = 'V' OR STAGEFOLLOW = 'D';

PROC LOGISTIC;
TITLE 'PREVIOUS STAGE = V';
MODEL STAGEFOLLOW = SUMLEAFLEN SUMLEAFLEN2 MAXLEAFLEN MAXLEAFLEN2 NOLEAVES NOLEAVES2
SUMSTEMHT SUMSTEMHT2 MAXSTEMHT MAXSTEMHT2 NOSTEMS NOSTEMS2 /SELECTION = BACKWARD;

DATA THREE;
SET ONE;

IF STAGETHIS = 'R';

DATA THREERV;
SET THREE;

IF STAGEFOLLOW = 'R' OR STAGEFOLLOW = 'V';

PROC LOGISTIC;
TITLE 'PREVIOUS STAGE = R';
MODEL STAGEFOLLOW = SUMLEAFLEN SUMLEAFLEN2 MAXLEAFLEN MAXLEAFLEN2 NOLEAVES NOLEAVES2
SUMSTEMHT SUMSTEMHT2 MAXSTEMHT MAXSTEMHT2 NOSTEMS NOSTEMS2 /SELECTION = BACKWARD;

DATA THREERD;
SET THREE;

IF STAGEFOLLOW = 'R' OR STAGEFOLLOW = 'D';

PROC LOGISTIC;
TITLE 'PREVIOUS STAGE = R';
MODEL STAGEFOLLOW = SUMLEAFLEN SUMLEAFLEN2 MAXLEAFLEN MAXLEAFLEN2 NOLEAVES NOLEAVES2
SUMSTEMHT SUMSTEMHT2 MAXSTEMHT MAXSTEMHT2 NOSTEMS NOSTEMS2 /SELECTION = BACKWARD;

RUN;
```



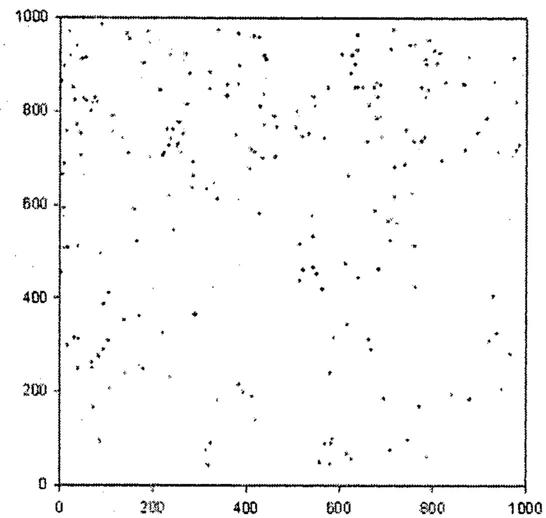
Appendix D. Distribution of individual plants in each 10 X 10 m site studied. Mapping is based on Cartesian coordinate system.



Site 4387



Site 5979



Site 7065

Appendix D Continued.

CURRICULUM VITAE

ADAM K. RYBURN

Candidate for the Degree of

Doctor of Philosophy

Thesis: Taxonomic Investigations of Oklahoma Flora

Major Field: Plant Sciences/Botany

EDUCATION

1999 - Present Oklahoma State University, Stillwater, OK Pursuing Doctoral Degree in Plant Sciences/Botany, to be conferred December, 2003

1993 - 1998 Southwestern Oklahoma State University, Weatherford, OK Bachelor of Science/Biological Science

1990-1993 Anadarko High School, Anadarko, OK

PROFESSIONAL EXPERIENCE

Visiting Assistant Professor
Presently Department of Botany / Oklahoma State University

Teaching Associate
1999 - Present Department of Botany / Oklahoma State University

Teaching Assistant
1999 - 2003 University of Oklahoma Biological Station, Lake Texoma, OK

Research Assistant
2002 Department of Botany / Oklahoma State University

Research Assistant
1999 - 2000 Department of Botany / Oklahoma State University

Teaching Assistant
1998 - 1999 Department of Biological Sciences / Southwestern Oklahoma State University (SWOSU)

AWARDS RECEIVED

OSU Phoenix Award Finalist for Outstanding PhD Candidate, 2003

OSU Distinguished Graduate Student Fellowship, 2001-present

OSU Leadership Legacy Recipient, 2000

Outstanding Teaching Assistant, College of Arts and Sciences, 2000 (OSU)

Southwestern Oklahoma State University Outstanding Biology Student of the Year, 1997, 1998

Who's Who Among American College Students, 1997 (SWOSU)

Mary Miller Award for Outstanding Field Research Student, 1996 (SWOSU)