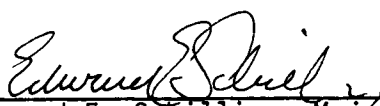
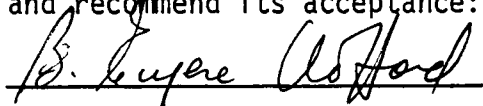


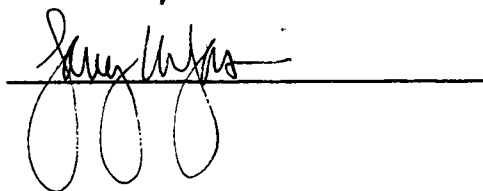
To the Graduate Council:

I am submitting herewith a thesis written by Maureen Cunningham entitled "The Relationship of Helianthus atrorubens and Helianthus silphioides in Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.


Edward E. Schilling, Major Professor

We have read this thesis
and recommend its acceptance:


B. Eugene Cofford


James H. Hays

Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

THE RELATIONSHIP OF HELIANTHUS ATRORUBENS AND
HELIANTHUS SILPHIOIDES IN TENNESSEE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Maureen Cunningham

June 1983

1783006

DEDICATION

This work is dedicated,
with love,
to
Eileen B. and James W. Cunningham

ACKNOWLEDGMENTS

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ABSTRACT

The relationship of Helianthus atrorubens L. and H. silphioides Nutt. in Tennessee was investigated using data from morphology, floral flavonoid constituents, and seed germination.

Analysis of morphology by cluster analysis and principle coordinate analysis indicated two morphological groups with some intermediate specimens. The groups were separated by principle coordinate analysis on the basis of phenotypically stable characters. Half of the intermediate specimens were members of four populations collected in central Tennessee eventually identified as H. silphioides.

Flavonoids forming ultraviolet nectar guides on ray flowers of both species were studied. Anthochlor flavonoids were found to be consistently present in sampled specimens of H. atrorubens but were absent in individuals sampled from most populations of H. silphioides. Three out of four central Tennessee populations comprised of intermediate specimens exhibited absence of anthochlors in sampled individuals.

Seed germination of the species was tested to determine whether there were interspecific differences in dormancy and temperature response after stratification. H. silphioides seed were found to be dormant after 4 weeks dry storage while seed of H. atrorubens germinated slowly to near maximum. After various stratification treatments and incubation at two alternating

temperatures, seed of H. silphioides germinated significantly slower than H. atrorubens seed.

On the basis of these data, the species are recognized as morphologically and, for the most part, chemically distinct in Tennessee. Intermediacy of central Tennessee populations based on morphology was not confirmed by evidence from floral flavonoid profiles. The relationship of these populations to the two species cannot be determined from this study. Differences in seed germination characteristics suggest that the species may have different germination strategies that are related to habitat preference and range limitation.

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CHAPTER I

INTRODUCTION

The genus Helianthus is a large and easily recognized element of the North American flora. Categories within the genus have been problematic, primarily due to morphological variability and natural hybridization. Heiser, Smith, Clevenger, and Martin (1969) proposed an informal division of the genus into three sections based on crossability and persistence of underground parts. Schilling and Heiser (1980) formalized an infrageneric classification in which four sections are described. Section Divaricati, comprised of perennials occurring mostly in the eastern half of North America, has posed particular problems in species delimitation. Virtually all the species in the section at a given ploidy level have the ability to hybridize and produce relatively fertile offspring. Natural backcrosses with parent species may yield progeny exhibiting a morphological continuum between species (Heiser, et al., 1969).

In section Divaricati, one instance of apparent intergradation involves H. atrorubens L. and H. silphioides Nutt., species of the southern United States that are similar enough to have been treated as a single taxon by Cronquist (1952). Currently the taxa are accorded species rank but debate persists over the best taxonomic treatment. Cronquist (1980) suggests that the taxa are perhaps better considered to be varieties of a single species, H. atrorubens.

Heiser, et al. (1969) consider the species to be as distinct as others in the genus.

The two species exhibit a combination of characteristics which attest to their close relationship and distinguish them morphologically from all other sunflowers. The species display oblong and, generally, obtuse involucral bracts, reddish-purple disk corollas, and a scapose appearance at maturity. The two species are distinguished from each other on the basis of leaf shape characteristics, degree of pubescence, number of nodes below the inflorescence, and number of stems per individual (Beatley, 1963; Heiser, et al., 1969; Cronquist, 1980).

The geographical ranges of the species are mostly separate (Figure I-1). H. silphioides center of distribution is in Arkansas and H. atrorubens is extremely abundant from the coast of the Carolinas westward to the Appalachian Mountains in Tennessee. The species ranges overlap in Tennessee and specimens difficult to place in one species or the other have been reported from the central portion of the state (Beatley, 1963; Heiser, et al., 1969). Hybridization of the species in this region is suspected (Cronquist, 1980) but has not been documented.

Reports of morphological blending and suspected hybridization indicate the need for an investigation of the species relationship in Tennessee. In the present study, field collection of specimens was undertaken, and data from multivariate analysis of morphology, floral flavonoid constituents, and seed germination were used to assess the

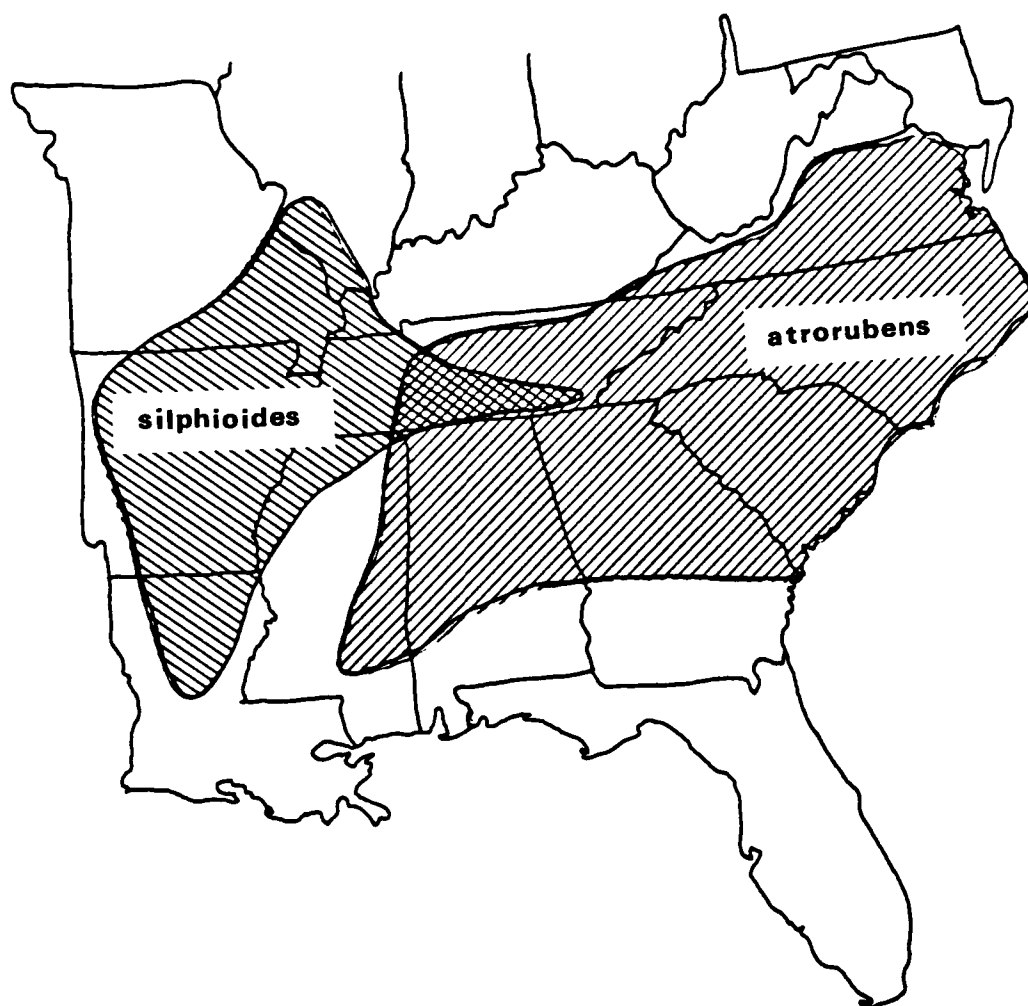


Figure I-1. Generalized Geographical Distribution of *Helianthus silphioides* and *H. atrorubens* (from Heiser, Smith, Clevenger, and Martin, 1969).

relationship of the species in Tennessee. Herbarium specimens were not included in the study since many specimens were incompletely collected or only partially mounted.

Collection of Specimens

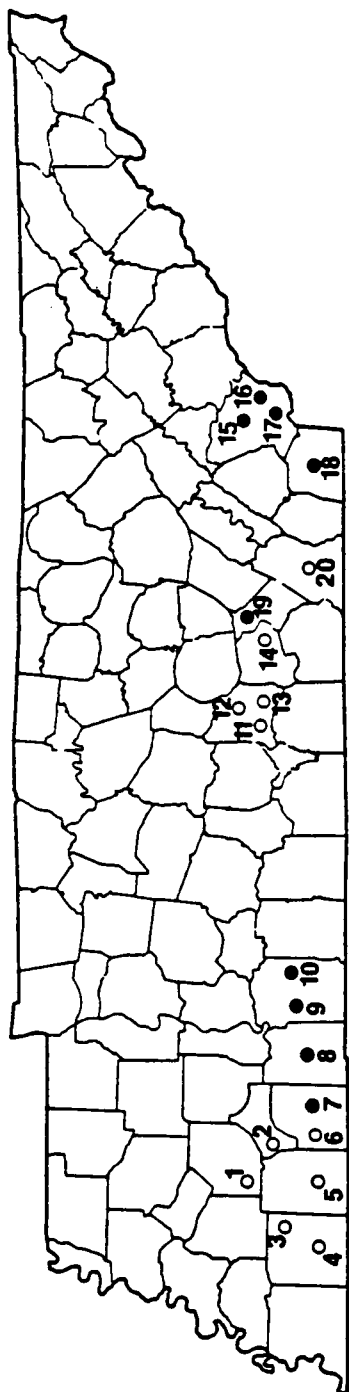
Specimens were collected at anthesis in mid-September 1981 across southern Tennessee in a broad transect defined by latitude lines 35°30' and 35°00'. The transect location was chosen because TENN collections show both species to be present in southern Tennessee. Four populations were collected in west Tennessee to represent H. silphioides in its typical form and four populations from east Tennessee were collected as typical H. atrorubens. Twelve populations were sampled at random, at least 5 miles apart, between the east-west extremes. Voucher specimens of collected populations and their eventual identification by species are listed in Table I-1. Population locations are shown in Figure I-2. The lack of collections from the Central Basin reflects the absence of both species in that region. Five individuals were sampled per population for a total of 100 specimens. Disturbed sites such as roadsides and powerline and railroad rights-of-way, often adjacent to mixed hardwood-pine forests, were the principle collection sites. The species were not found in mixed populations.

Stems were clipped at ground level and a few capitula per plant were clipped below the involucre. At collection, total height measurements were made, and specimens were pressed. Specimens were dried several days later.

Table I-1. List of Collected Populations and Vouchers.

Species	Population Number and Voucher Specimen ^a	
<u>H. silphioides</u>	1. TN Madison Co.:	<u>Cunningham 104</u>
	2. TN Chester Co.:	<u>Cunningham 201</u>
	3. TN Fayette Co.:	<u>Cunningham 302</u>
	4. TN Fayette Co.:	<u>Cunningham 403</u>
	5. TN Hardeman Co.:	<u>Cunningham 504</u>
	6. TN McNairy Co.:	<u>Cunningham 605</u>
	11. TN Coffee Co.:	<u>Cunningham 1102</u>
	12. TN Coffee Co.:	<u>Cunningham 1204</u>
	13. TN Coffee Co.:	<u>Cunningham 1303</u>
	14. TN Grundy Co.:	<u>Cunningham 1401</u>
	20. TN Hamilton Co.:	<u>Cunningham 2005</u>
<u>H. atrorubens</u>	7. TN McNairy Co.:	<u>Cunningham 705</u>
	8. TN Hardin Co.:	<u>Cunningham 802</u>
	9. TN Wayne Co.:	<u>Cunningham 903</u>
	10. TN Wayne Co.:	<u>Cunningham 1001</u>
	15. TN Monroe Co.:	<u>Cunningham 1504</u>
	16. TN Monroe Co.:	<u>Cunningham 1601</u>
	17. TN Monroe Co.:	<u>Cunningham 1705</u>
	18. TN Polk Co.:	<u>Cunningham 1802</u>
	19. TN Sequatchie Co.:	<u>Cunningham 1903</u>

^aAll vouchers on deposit at TENN.



- H. silphioidees
- H. atrorubens

Figure I-2. Location of Collected Populations. Reference numbers of populations as in Table I-1, page 5.

CHAPTER II

NUMERICAL ANALYSIS OF MORPHOLOGY

One approach to the study of plant relationships is to evaluate morphological similarity. Traditionally, taxonomists have based classification on morphological resemblance of specimens with the idea that specimens exhibiting the highest number of shared characteristics constitute natural groups. Overall similarity of specimens has typically been measured in the mind of the investigator. Attempts to render traditional taxonomy more objective and repeatable have led to the use of numerical taxonomic techniques that provide an operational approach to determinations of similarity. Numerical techniques do not provide an absolute measure of similarity or relationship, but the techniques have proved useful in plant systematic studies (Duncan and Baum, 1981). They enable simultaneous evaluation of many characters and improve objectivity of classification schemes.

Assessing the relationship of taxa that exhibit morphological blending in regions of range overlap is a persistent problem in perennial sunflowers. In Tennessee, H. silphioides and H. atrorubens display a blending in some aspects of morphology, primarily cauline leaf characteristics. Apparent intergradation of the species may be explained in several ways. The two species may be hybridizing or a single species may be diverging with two intergrading subspecies. Environmental factors may be having

important influences on morphology, especially since the area of sympatry of H. atrorubens and H. silphioides constitutes the western and eastern range limits, respectively, of each taxon. A study of morphology of specimens collected in Tennessee should provide insights into the species relationship in this region.

In the present study, morphological data has been analyzed by numerical procedures to determine whether specimens fall into distinct morphological groups or exhibit a range of morphology between typical forms of the species.

Methods

The basic procedure in numerical taxonomy is to compute a coefficient of similarity or dissimilarity based on measurement of selected characters for each pair of samples or OTUs (operational taxonomic units, the lowest ranking taxon used in a study, Sneath and Sokal, 1973). Similarity coefficients are analyzed by statistical tests to detect groupings of the OTUs and statements about appropriate classification are made.

Characters selected for measurement are listed in Table II-1. The number and kinds of characters measured are important in a numerical analysis. Ideally, many characters from all stages of the life history should be measured (Sneath and Sokal 1973). In the present study, careful observation of features that varied principally by species led to the selection of characters.

Morphological data from 100 collected specimens were used to compute a measure of resemblance between pairs of OTUs. The

Table II-1. List of Characters Scored in Morphological Analysis.

Characters
1. total height
2. basal leaves present or absent at anthesis
3. basal leaf blade length/width
4. basal leaf petiole length/blade length
* 5. petiole winged more than 1/2 its length or not
* 6. hairs on abaxial midrib of leaf greater than 1 mm or not
* 7. leaf blade length/width
* 8. petiole length/blade length
9. number of nodes below inflorescence
10. number of branches in inflorescence
11. number of flower heads
12. length of second internode from base
13. length of peduncle/total height
14. length of longest ray
15. nectar guide length/ray length

*Largest cauline leaf.

coefficient of similarity used in this study is Gower's coefficient (Gower, 1971), which is capable of combining data of mixed quantitative and qualitative types.

In a taxonomic study the matrix of similarity coefficients is thought to be best analyzed by cluster analysis and ordination (Sneath and Sokal, 1973; Clifford and Stephenson, 1975). Cluster analysis portrays well the relationship of similar OTUs and ordination better depicts the relationship of less similar OTUs. In the current study, unweighted pair group method using arithmetic means (UPGMA) is used for clustering and principle coordinates analysis is used for ordination. Analyses were performed on the DEC System 10 computer at The University of Tennessee, Knoxville, using programs for numerical taxonomy written by Dr. Edward E. Schilling and Statistical Package for the Social Sciences (SPSS) package programs.

Initially, analyses were run using population averages for OTUs. Data for individual specimens (100 OTUs) were analyzed secondly to investigate the effects of within population variability on the relationships depicted by analysis of population averages.

Results and Discussion

The phenogram derived from cluster analysis of population averages is shown in Figure II-1. OTUs are labeled by population number and a letter to designate species as eventually defined. Two clusters are evident. All OTUs of H. silphioides clustered

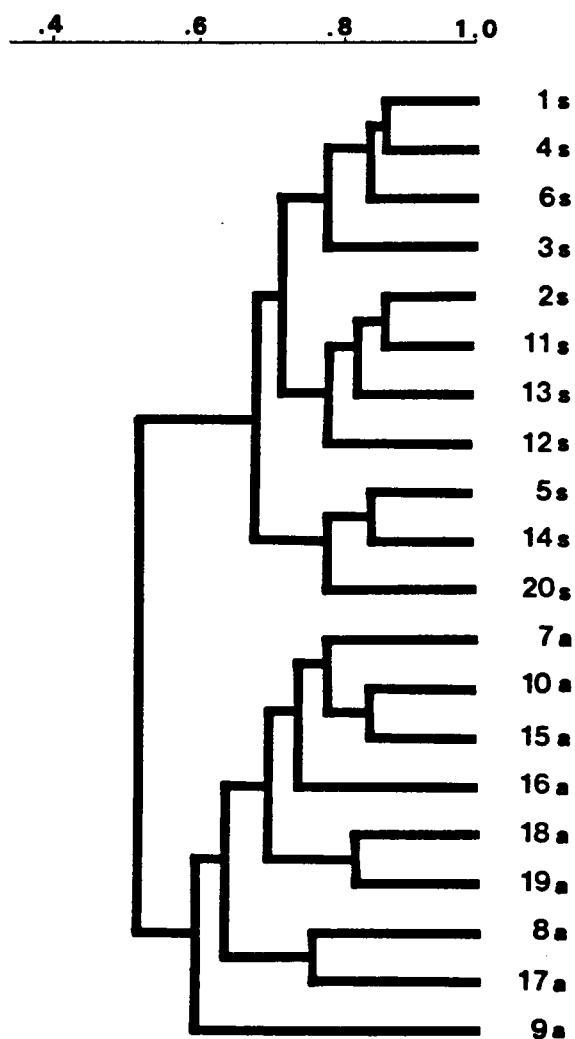


Figure II-1. Phenogram Produced by UPGMA Cluster Analysis of 20 Populations. OTUs are population averages for 15 morphological characters (Table II-1, page 9). Gower's coefficient of similarity is scale at top. Reference numbers of OTUs are population numbers as in Table I-1, page 5.

together and all OTUs of H. atrorubens grouped together. Principle coordinate analysis of population averages results in a separation of populations along Axis 1, the horizontal axis, as shown in Figure II-2. OTUs are labelled by population number. The results of cluster analysis and principle coordinate analysis indicate that when intrapopulation variation is averaged, the OTUs fall into two clearly distinct groups.

Cluster analysis of 100 OTUs produced a phenogram showing two large clusters (Figure II-3). OTUs are labelled by population number and a letter to designate individuals sampled from each population. All OTUs included in one cluster are from populations eventually identified as H. silphioides; all OTUs in the other group are from populations of H. atrorubens. The results show that cluster analysis of all collected individuals does not alter the relationship depicted by analysis of population averages. All specimens of H. silphioides exhibit greater similarity to other H. silphioides specimens than to samples of H. atrorubens and vice versa.

Principle coordinate analysis of 100 OTUs results in most specimens falling into two relatively discrete groups separated along Axis 1, the horizontal axis (Figure II-4). All OTUs included in the group to the left are from populations eventually identified as H. silphioides; those in the group to the right are H. atrorubens. A dotted line separates specimens of the species. Although most OTUs are included in two well defined groups, some

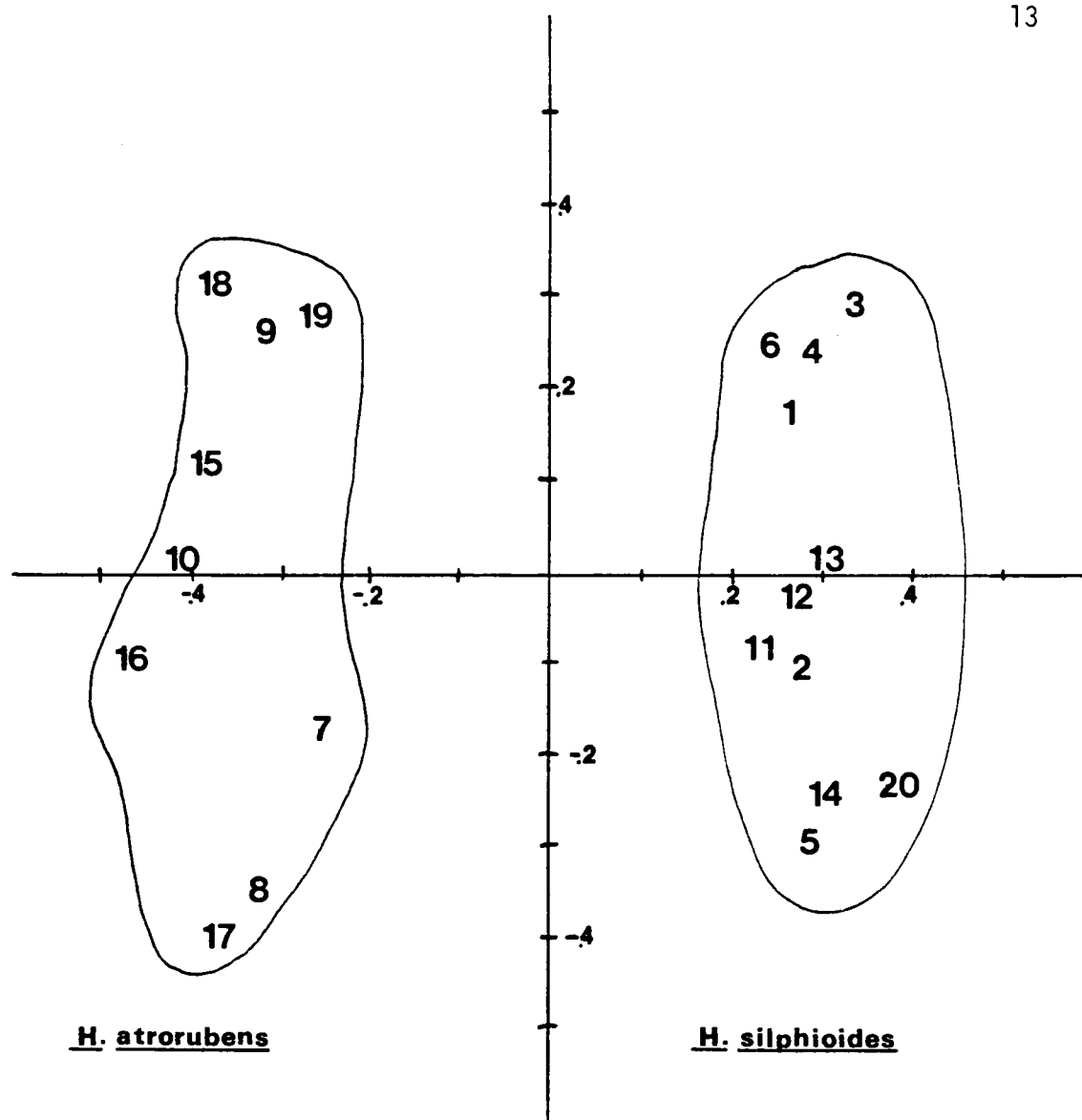


Figure II-2. Graph of 20 OTUs on the First Two Axes Determined by Principle Coordinate Analysis. OTUs are population averages for 15 morphological characters (Table II-1, page 9). Reference numbers of OTUs are population numbers as in Table I-1, page 5. Axis 1 (horizontal) accounts for 30.7% of total variance. Axis 2 (vertical) accounts for 14.9% of total variance.

Figure II-3. Phenogram Derived from UPGMA Cluster Analysis of 100 OTUs for 15 Morphological Characters (Table II-1, Page 9).

Gower's coefficient of similarity is scale at top.

Reference numbers of OTUs are population numbers as in Table I-1, page 5, and captions to designate individuals sampled from each population.

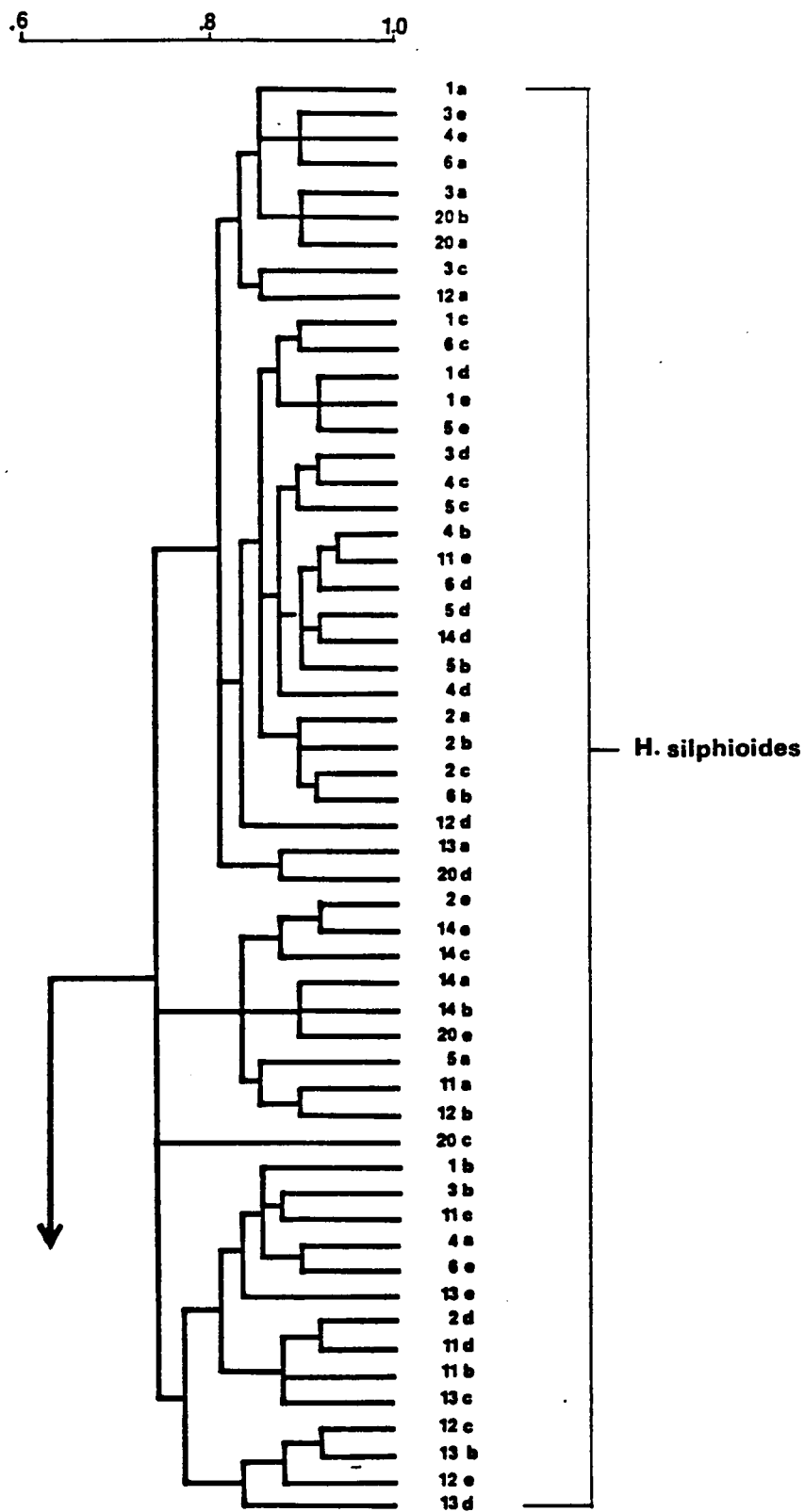


Figure II-3

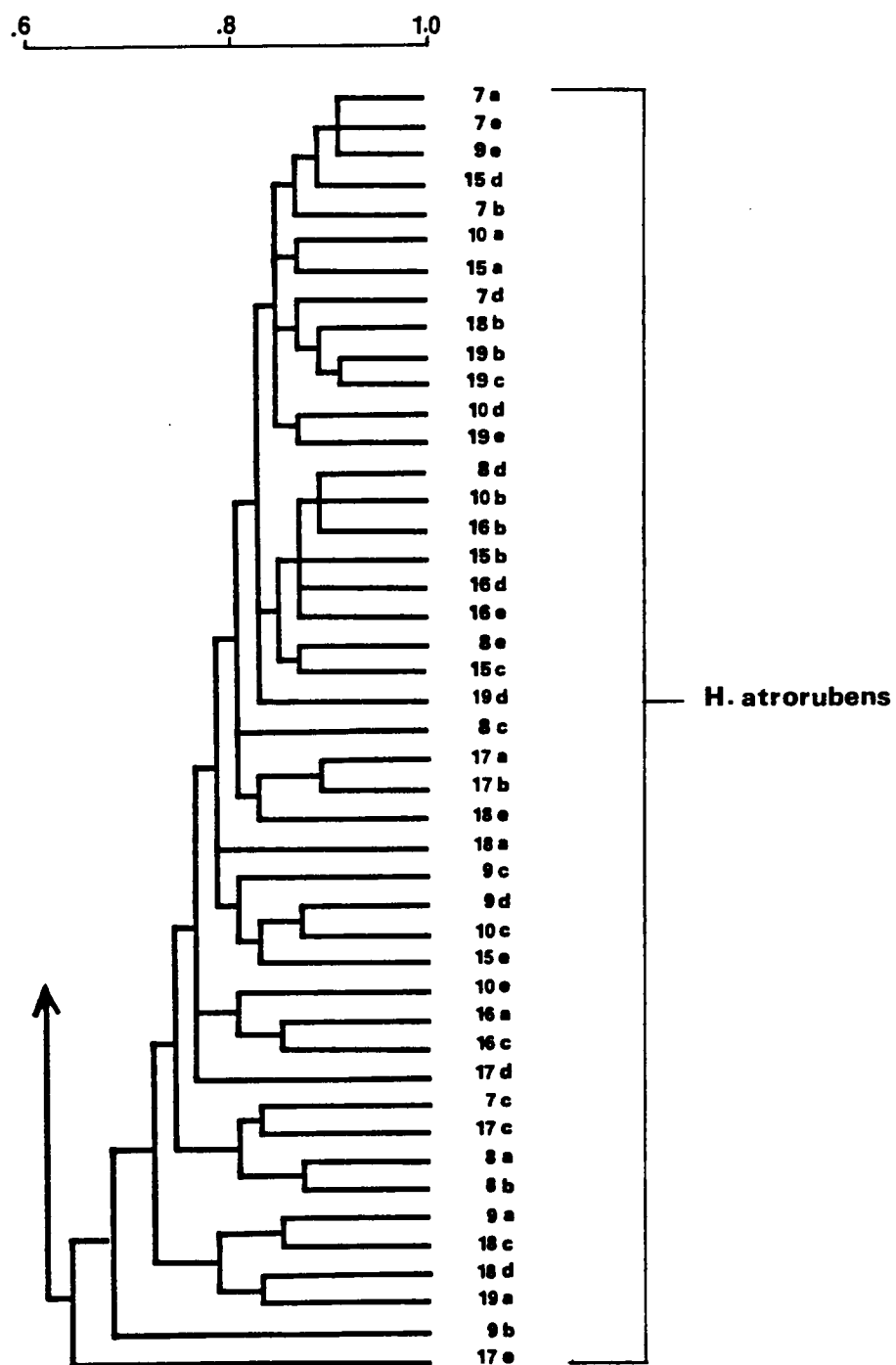


Figure II-3 (Continued)

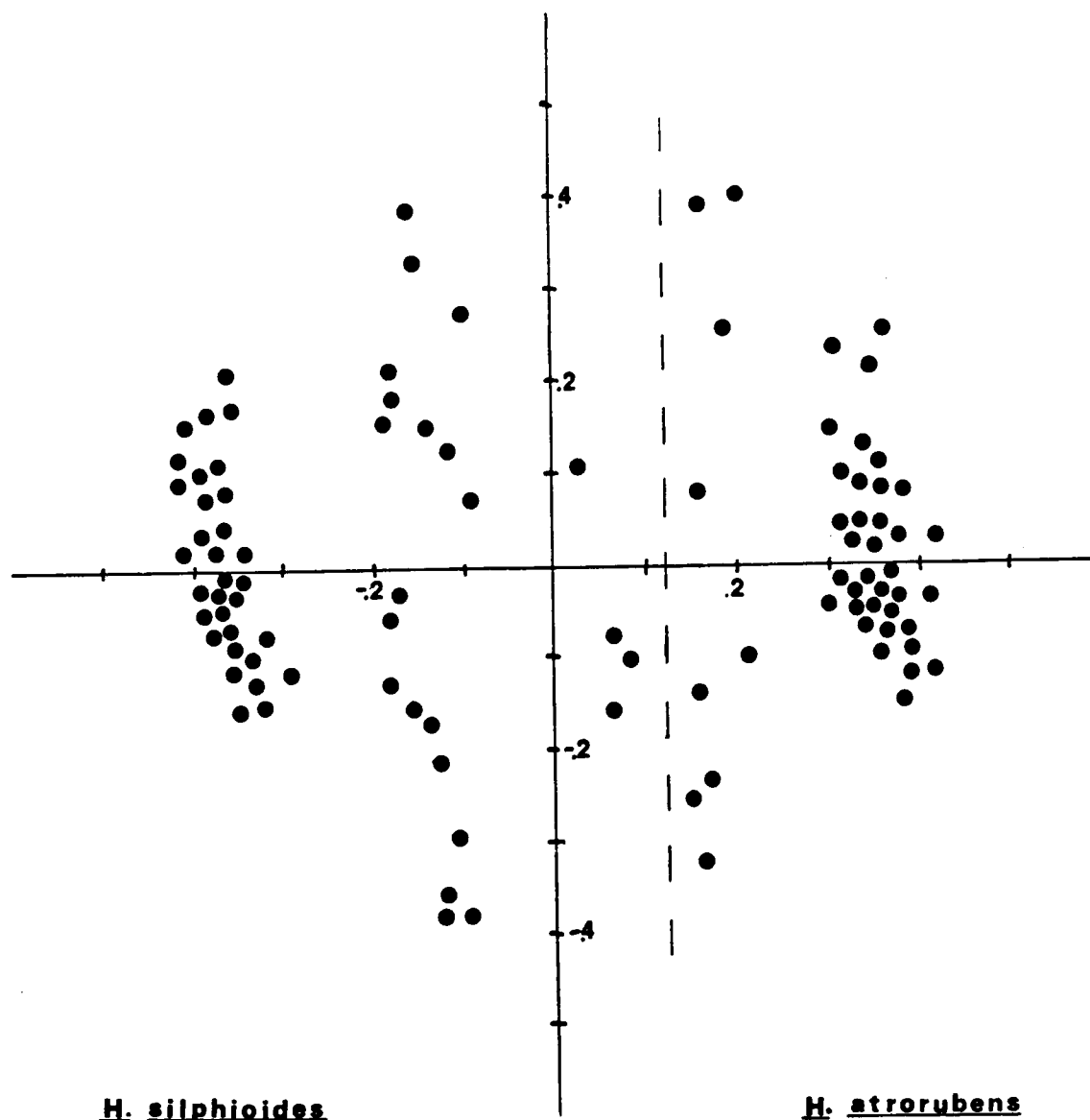


Figure II-4. Graph of 100 OTUs on the First Two Axes Determined by Principle Coordinate Analysis. Axis 1 (horizontal) accounts for 35.2% of total variance. Axis 2 (vertical) accounts for 9.0% of total variance.

individuals of both species exhibit intermediacy. Half of the specimens in the intermediate region are members of four populations collected in central Tennessee. The other intermediate specimens are made up of one or two variants from most other populations.

Knowledge of the characters most important in the separation of the OTUs along Axis 1 of principle coordinates analysis aids interpretation of the results. Factor analysis showed the following characters to be responsible for separation of groups: length of hairs on leaf midrib, presence or absence of basal leaves at anthesis, petiole of cauline leaves winged more than 1/2 its length or not, and number of nodes below the inflorescence. The expected stability of these features indicates that the groups are based on consistent differences between taxa. Axis 1 may thus be considered the taxonomically meaningful axis in the analysis. The specimens in the intermediate region require evaluation, particularly those from central Tennessee populations noted above. This region is the area where difficult specimens have been reported. Inspection of original data indicates that winged condition of the petiole and presence or absence of basal leaves at maturity are the characters responsible for the intermediate position of central Tennessee populations. These populations exhibit features typical of H. silphioides except for the two characters mentioned. Unfortunately, numerical techniques cannot assess the source of the variability pattern noted.

In conclusion, the numerical analyses of morphology indicate that although some individuals and some populations show a degree

of phenotypic intermediacy, most collected specimens fall into one of two morphological groups. The groups may be considered species groups since both cluster analysis and principle coordinate analysis result in the same group composition and stable morphological features are the characters useful in separation of the groups. Extensive hybridization of the species in Tennessee is not indicated by this study.

CHAPTER III

ANALYSIS OF FLORAL FLAVONOIDS

The usefulness of flavonoid compounds as taxonomic markers has been abundantly discussed and demonstrated (Harborne, Mabry and Mabry, 1975). Flavonoids occur throughout the plant kingdom, are chemically stable, and are easily and inexpensively separated by paper chromatography. Techniques for the extraction, separation and identification of flavonoids have been standardized (Markham, 1982) and the major biosynthetic pathways have been elucidated. In many cases, species have been found to have specific flavonoid complements, and in cases where this occurs, hybrids between species can be detected (Alston and Turner, 1963; Smith and Levin, 1963; Carter and Brehm, 1969). Thus flavonoid compounds are useful in assessing plant relationships and constructing classifications.

In previous studies of flavonoids present in Helianthus, three classes of compounds have been found: flavone aglycones that are localized in glandular trichomes, and flavonol glycosides and anthchlors that are present in leaf and floral material (Harborne and Smith, 1978; Schilling and Mabry, 1981; Schilling, in press). Flavone aglycones and floral flavonoids are the most useful compounds to study since they can be extracted without large amounts of other phenolic compounds that obscure their presence. Glandular trichomes are absent in H. atrorubens and

H. silphioides so floral flavonoids are the useful compounds to study in these species.

Floral flavonoids have a possible biological significance in addition to their potential taxonomic importance. In Helianthus, floral flavonoids are localized in the basal region of ligules (Harborne and Smith, 1978; Schilling, in press), where their ultra-violet absorption produces a pattern of absorbance that has been called a UV nectar guide. Abrahamson and McCrea (1977) report that Daumer (1956, 1958) showed UV nectar guides to be visible to most pollinating insects. Basal regions of UV absorbance may indicate location of fertile floral parts and provide a pattern that pollinators recognize. Currently, the role of UV nectar guides in pollination biology is not fully understood.

In the present study, the objective was to determine whether UV nectar guides are present in populations of H. atrorubens and H. silphioides collected in Tennessee and, if so, whether they differ in relative size and flavonoid constituents.

Methods

Dried pressed flower heads of collected specimens were viewed under a long wave UV lamp (366nm) to detect UV absorbing regions on the rays. Several rays from two individuals per population were extracted in methanol. Compounds were separated using thin layer and two dimensional paper chromatography with standard solvent systems (15% acetic acid, 40% acetic acid, and 3:1:1 tertiary butyl alcohol:acetic acid:water). Ray flowers were sectioned and

extracted separately to detect localization of flavonoids within the rays. Compounds were identified by color characteristics, R_f values, and cochromatography with an authentic standard, sulfurein. All procedures follow those outlined in Mabry, Markham, and Thomas (1970).

Results and Discussion

UV nectar guides were found to be present in all populations. Figure III-1 shows flower heads of the species as viewed under a UV lamp. On the average, the lower third of the ligules of sampled specimens was UV absorbing. Nectar guide length in relation to ray length was entered as a character in the numerical analysis of morphology, but was not found to be important in separation of species groups (page 18).

Flavonoids were found to be localized in the nectar guide region of the rays and were responsible for UV absorbance. The presence of anthochlor flavonoids in the rays of collected populations is indicated in Table III-1. The only anthochlor flavonoids reported to be present in Helianthus are sulfurein and its chalcone isomer, coreopsin (Figure III-2). These compounds occurred in all sampled individuals of H. atrorubens but were consistently absent in sampled individuals of 7 out of 11 collected populations of H. silphioides. Anthochlors were present in at least one sampled individual of four H. silphioides populations. All specimens of both species that lacked anthochlors in the ligules exhibited the presence of another compound, probably quercetin-7-glucoside based

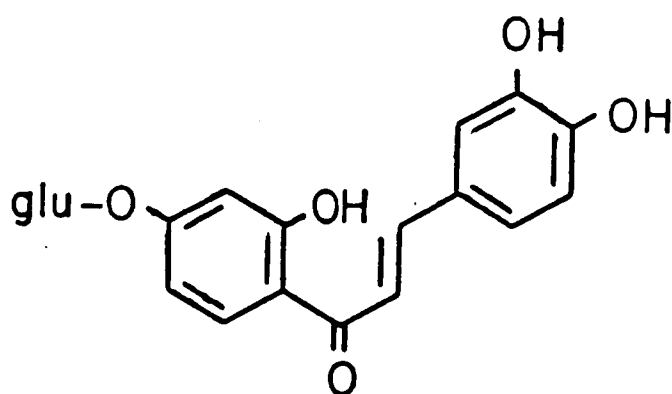


Figure III-1. Ultraviolet Nectar Guides of H. silphoides (Bottom) and H. atrorubens (Top) as Seen under a Long Wave (366nm) UV Lamp.

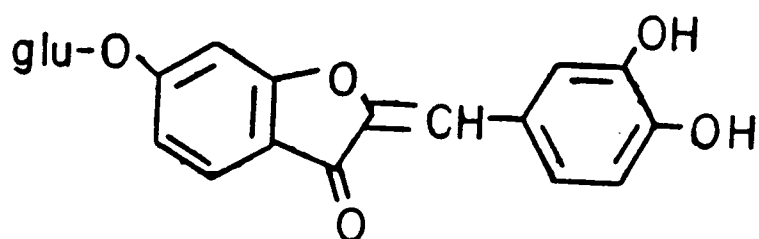
Table III-1. Presence of the Anthochlors, Coreopsin and Sulfurein, in Ray Flowers of Two Individuals from Each Population.*

Species	Population Number	Anthochlors Present
<u>H. silphioides</u>	1	*/-
	2	-
	3	-
	4	-
	5	*
	6	*/-
	11	*
	12	-
	13	-
	14	-
	20	-
<u>H. atrorubens</u>	7	*
	8	*
	9	*
	10	*
	15	*
	16	*
	17	*
	18	*
	19	*

*Population numbers as in Table I-1, page 5.



coreopsin



sulfurein

Figure III-2. Chemical Structure of Anthochlors Present in H. silphioides and H. atrorubens.

on Rf values. This compound was absent in rays containing coreopsin and sulfurein. The presence of quercetin-7-glucoside in ligules of Helianthus species that lack ray flower anthochlors and the absence of quercetin-7-glucoside in ray flowers containing anthochlors has been previously demonstrated (Harborne and Smith, 1978; Schilling, in press). Harborne and Smith (1978) suggest the possibility that insects may be able to perceive differences between UV nectar guides formed by yellow anthochlors and those formed by colorless quercetin glycosides, and that nectar guide color and pattern may play a role in the reproductive biology of Helianthus species. The present study was not designed to investigate nectar guide recognition by insects.

Since, on the whole, the species show different ray flower flavonoid profiles, the compounds present in populations with individuals indicated as morphologically intermediate by principle coordinate analysis (numbers 11, 12, 13, and 14) should be closely inspected. No novel compounds or new combination of compounds suggestive of hybrid origin are present in these populations. Harborne and Smith (1978) have suggested that the anthochlor character is inherited as a dominant or codominant in Helianthus species of hybrid origin. If this is true, one would expect populations of F₁ hybrids of H. atrorubens and H. silphioides to exhibit the presence of anthochlors. Of the four populations comprised mostly of intermediate individuals, only population number 11 showed the presence of anthochlors. Therefore, if

populations 12, 13, and 14 are of hybrid origin, they are probably not F₁ hybrids. These populations might be of later generation or backcross origin, but if so, they seem notably isolated from their parental taxa. More intensive sampling in the central Tennessee region should help elucidate the relationship of intermediate specimens to the two species.

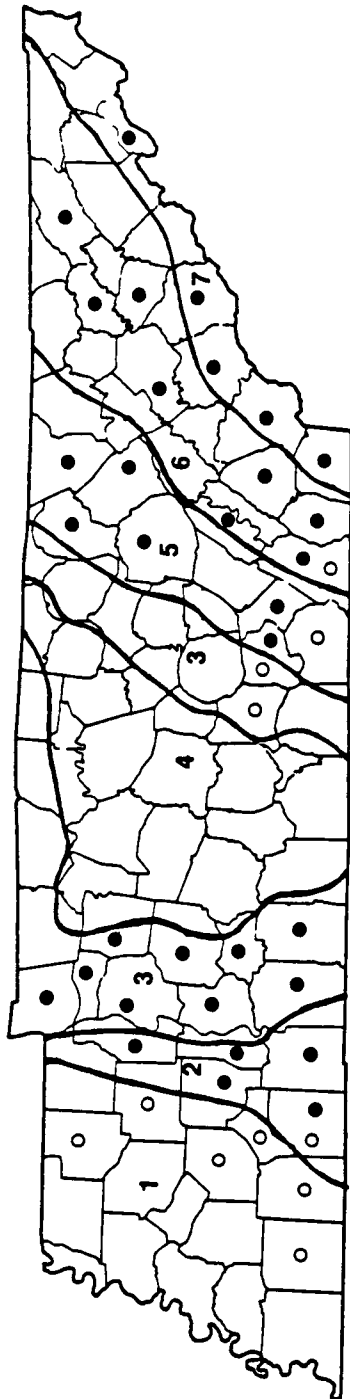
In conclusion, floral flavonoids support the species groupings based on morphology. Anthochlors were consistently present in all specimens of H. atrorubens sampled but were generally absent in specimens of H. silphioides. The significance of variability in anthochlor presence in H. silphioides is unknown. The lack of anthochlors in most populations made up of intermediate individuals suggests that these populations are probably not of F₁ hybrid origin. Flavonoid data is useful in this study because of its correlation with morphological groups and its indication that central Tennessee populations are not chemically intermediate.

CHAPTER IV

SEED GERMINATION CHARACTERISTICS

Helianthus atrorubens and H. silphioides are mostly allopatric, but populations of each species occur in southern Tennessee. As shown in Figure IV-1, the species are typically not found in the same physiographic provinces in the state. H. silphioides is found mainly in relatively low elevation flatlands: the Mississippi Embayment, the Barrens of the southeastern Highland Rim, and the southern Tennessee Valley. H. atrorubens is typically found at higher elevations: on the Coastal Plain Upland, the Highland Rim, the Cumberland Plateau, and in the Ridge and Valley. This distribution suggests that temperature and moisture may be important in the localized distribution of the species within the state. Seed germination characteristics of the species may play an important role in range limitation and habitat preference. Presently no information is available on germination characteristics of perennial sunflowers other than general comments that germination is better after an exposure to cold temperature (Heiser, et al., 1969).

Germination and establishment of the seedling are crucial times in the life of a plant. Emergence of the embryo from the protective seed coat is an irreversible event; the young plant must find adequate moisture and tolerable temperatures in order to survive. It is not surprising that the timing of germination is known to be far from haphazard, especially for species in areas



○ H. silphiooides
● H. atrorubens

- 1 Mississippi Alluvial Plain and Embayment
- 2 Coastal Plain Upland
- 3 Undissected Highland Rim
- 4 Central Basin and Dissected Highland Rim
- 5 Cumberland Mountains and Plateau
- 6 Ridge and Valley
- 7 Unakas

Figure IV-1. Distribution of H. silphiooides and H. atrorubens by Counties in Tennessee. Physiographic provinces after Shanks (1958) and Fenneman (1938).

with seasons unfavorable for growth and survival in the delicate seedling stage (Went, 1949; Mayer and Poljakoff-Mayber, 1963; Thompson, 1973).

Germination timing results from an interplay by regulating mechanisms within the seed and the ambient environment. Two important internal factors controlling germination are temperature response characteristics and dormancy (Thompson, 1973). Dormancy is the endogenous inhibition of further embryo growth. Embryo dormancy is typically broken by exposure to cool, moist conditions, i.e., stratification, that simulates natural overwintering. Mechanisms controlling response to temperature enable the embryo to respond to appropriate germination temperatures once dormancy has been broken.

Mechanisms affecting the timing of germination may collectively be labelled "strategies." Although the term implies volition, it is appropriate to consider germination timing as a crucial maneuver in a species ability to successfully compete in given areas. It may be possible to relate a species geographical range or habitat preference to germination strategies. Definition of germination requirements related to dormancy and temperature response is necessary before range and germination patterns can be correlated.

Another reason for investigating germination in a taxonomic study is the potential use of germination "behavior" as a taxonomic character. Although germination characteristics may vary widely between populations of species noted for their adaptive capacity in

a broad array of habitats (Thompson, 1973; Farmer and Cunningham, 1981), in populations of species restricted to particular phyto-geographical regions, germination responses remain notably stable (Thompson 1973). Dormancy has been shown to be at least partially under genetic control in species and hybrids of Papaver (Harper and McNaughton, 1960) and in some agronomic species (Whittington, 1973). Thus dormancy and temperature response characteristics may be of systematic importance in some plant groups. The distribution of H. atrorubens and H. silphioides suggests that germination patterns may be species specific.

Germination of H. atrorubens and H. silphioides seed was investigated in the present study to determine whether there were interspecific differences in features such as seed dormancy and temperature response. Germination at alternating incubation temperatures after stratification treatments was tested. The results are discussed in relation to environmental parameters operative in nature.

Methods

Fully ripened seed were collected at dispersal time in a mixed lot from three populations per species. Seed source for H. atrorubens was the Cherokee National Forest near Reliance, TN. H. silphioides seed were collected near Tullahoma, TN. Voucher specimens with Cunningham collection numbers are on deposit at TENN: H. atrorubens Polk Co.; 1802, H. silphioides Coffee Co.; 1102. Flower heads were clipped off parent plants and the seed

were removed by rubbing the capitula. Seed were separated from dried corollas with an air column separator, which also removed many empty seed. A filled seed count was made by cutting open 40 seed per species. H. atrorubens seed were 100% filled and H. silphioides were 88% filled.

Seed were dried on a shelf at room temperature for 4 weeks before assignment to the treatment combinations outlined in Table IV-1. The species were tested at two alternating incubation temperatures, 5-15°C and 10-20°, and four stratification periods.

Unstratified (0 weeks) seed were used for controls. The 4, 8, and 12 weeks treatments were placed in a 3-4°C dark environment (subject to infrequent exposures to light) for the specified time prior to incubation. Each treatment consisted of three 100-seed replicates. Germination and stratification were carried out in 9 cm plastic petri dishes lined with moistened qualitative grade filter paper (4 ml distilled water) and wrapped in clear plastic. Water lost by evaporation was added during incubation.

Seed were incubated in controlled environment chambers with 13 hours light concurrent with the higher alternating temperature. Light was provided by 20 W cool white fluorescent bulbs. Intensity was between 240 and 290 foot candles.

Each treatment was incubated at least 4 weeks to initiate germination. Germinated seed were counted and removed from petri dishes every 2 days until no further germination occurred for 5 days. Remaining seed were examined and classified as germinated, filled

but ungerminated, or empty/decayed. $\sqrt{X+1}$ transformations of germination data were subjected to analysis of variance.

Results and Discussion

Final germination percentages are listed in Table IV-1. All germination percentages of H. silphioides are adjusted for 88% filled seed basis in order to compare differences between species in physiological response of filled seed to certain treatments.

Unstratified seed of H. silphioides failed to germinate at 5-15°C and reached only 1% at 10-20°C. Unstratified H. atrorubens seed germinated slowly at 10-20°C over 12 weeks to about 90% of total, while at 5-15°C essentially no germination occurred. Under natural conditions, seed of both species probably germinate in spring; H. silphioides seed must overwinter to break dormancy and H. atrorubens seed germinate so slowly without stratification that winter temperatures would prevail before a substantial proportion of the seed lot germinates.

All stratified H. atrorubens seed germinated to very high percentages (94% or higher) at 5-15°C and 10-20°C. Seed of H. silphioides stratified for 4, 8, and 12 weeks germinated to relatively high levels (over 80%) at both alternating temperature regimes. Germination was slightly higher (over 90%) with 4 weeks stratification.

Decrease in final germination percentage with longer stratification periods and higher incubation temperatures can be

Table IV-1. Final Germination Percentages of H. atrorubens and H. silphioides Seed.

Species	Incubation Temperature*	Replicate**	Stratification Time (Weeks)			
			0	4	8	12
<u>H. atrorubens</u>	5-15°C	I	0	86	86	79
		II	0	90	88	83
		III	0	98	90	86
		$\overline{m}$	0	91	88	83
	10-20°C	I	1	90	72	83
		II	1	88	88	83
		III	1	99	88	78
		$\overline{m}$	1	92	83	81
	5-15°C	I	3	95	97	96
		II	1	94	97	96
		III	2	100	95	95
		$\overline{m}$	2	96	96	96
<u>H. silphioides</u> ***	10-20°C	I	92	92	95	95
		II	90	96	99	98
		III	92	95	97	98
		$\overline{m}$	91	94	97	97

*13 hours light concurrent with higher temperature.

**100 seed per replicate.

***Based on 88% filled seed.

attributed to a greater tendency for H. silphioides seed to decay under those conditions (Table IV-2).

In addition to final percentages, an important consideration in germination studies is the time period over which germination takes place. Rapidity of germination response to incubation after 4 weeks and 12 weeks stratification is illustrated in Figure IV-2.

After 4 weeks chilling, seed of both species germinated faster at 10-20°C than at 5-15°C. H. silphioides seed exhibited slower germination than H. atrorubens seed at both temperatures, particularly at 5-15°C. Stratification for 12 weeks promoted rapid germination of both species at both incubation temperatures. This indicates that longer chilling treatments not only break embryo dormancy, but also substantially lower the temperature at which rapid germination will occur. A similar pattern of response has been demonstrated for other east Tennessee plants and is probably typical of many species native to the southeastern United States (Farmer and Cunningham, 1980, 1981). Seed that received 8 weeks stratification were intermediate to the 4 and 12 weeks treatments in germination speed. Analysis of variance of the time (number of days) to half maximum germination revealed stratification, species, and temperature are significant factors at the .05 level of probability (Table IV-3).

The results indicate that physiological differences exist between species in relation to temperature response and dormancy. Sampling seed populations of the species throughout their ranges is

Table IV-2. Number of Seed Per Replicate Classified as Decayed or Empty after Incubation.

Species	Incubation Temperature	Replicate	Stratification Time (Weeks)		
			4	8	12
<u>H. silphioides</u>	5-15°C	I	23	24	31
		II	18	19	27
		III	14	20	25
		$\overline{m}$	<u>18</u>	<u>21</u>	<u>28</u>
	10-20°C	I	18	35	27
		II	22	20	27
		III	11	20	32
		$\overline{m}$	<u>17</u>	<u>25</u>	<u>29</u>
<u>H. atrorubens</u>	5-15°C	I	3	2	4
		II	4	1	2
		III	0	5	4
		$\overline{m}$	<u>2</u>	<u>3</u>	<u>3</u>
	10-20°C	I	7	5	4
		II	4	1	1
		III	5	3	2
		$\overline{m}$	<u>5</u>	<u>3</u>	<u>2</u>

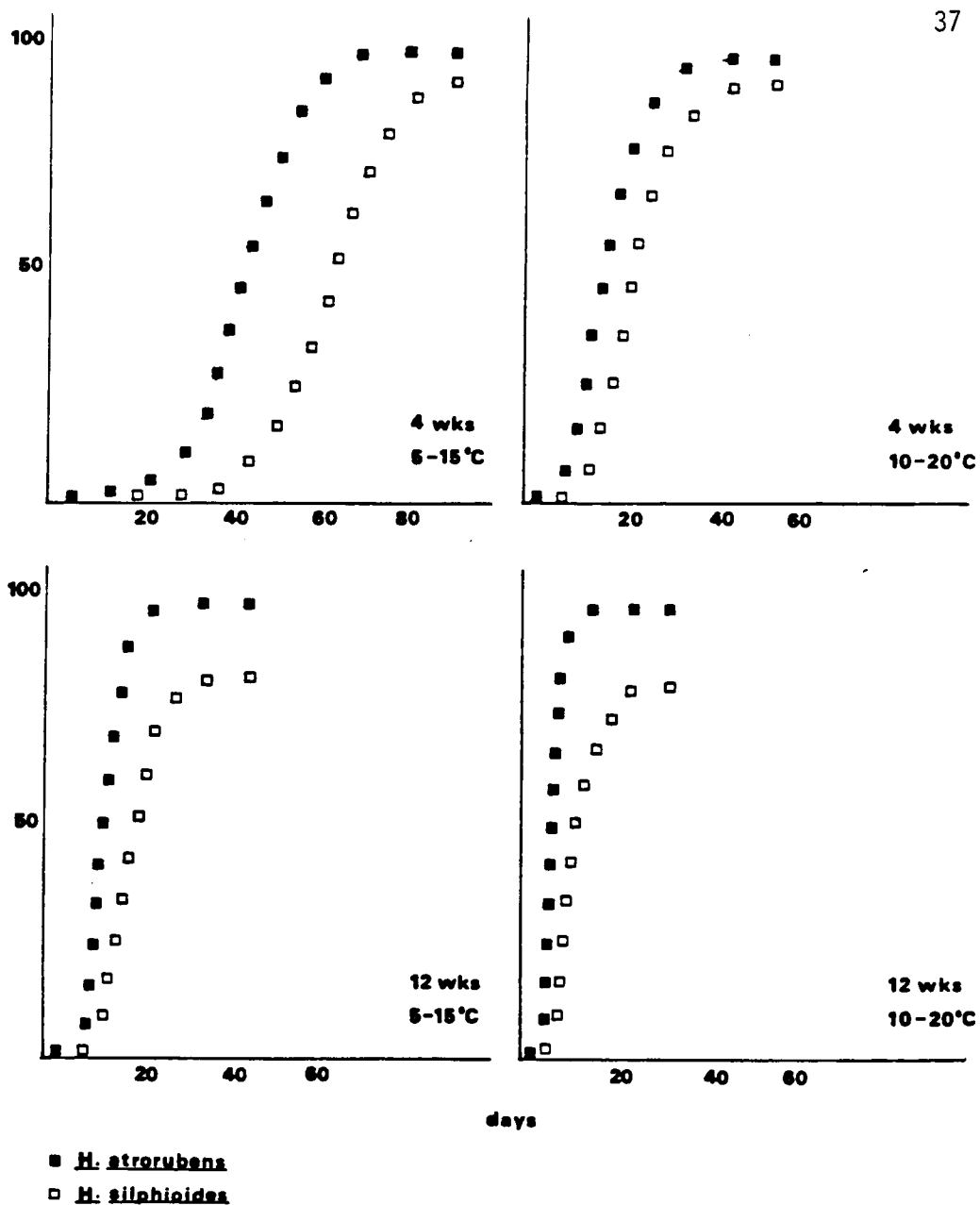


Figure IV-2. Germination Percentage of *H. atrorubens* and *H. silphioides* over Time at Two Alternating Incubation Temperatures after 4 Weeks and 12 Weeks Stratification.

Table IV-3. Analysis of Variance of Time (Days) to Half Maximum Germination of H. atrorubens and H. silphioides.

Source	df	SS	MS	F
A (stratification)	2	35.97	17.98	59.93*
B (species)	1	8.31	8.31	27.70*
C (temperature)	1	19.80	19.80	66.00*
AB	2	0.12	0.06	0.20
AC	2	4.42	2.21	7.37
BC	1	0.07	0.07	0.23
ABC	2	0.33	0.16	0.53
error	2	0.60	0.30	

*Significant at .05 level.

necessary before a complete analysis of germination strategies can be performed and related to range limitations. However, the data generated in this study can be discussed as possible adaptations to environmental factors. Dormancy is a major point of difference between the species but, for reasons previously mentioned, probably does not have an important effect on season of germination. The tendency for seed of H. silphioides to decay following 12 weeks stratification may indicate a reduced capability for seed survival in areas with poor water drainage that experience cold, wet winters. The slower response of H. silphioides seed compared to H. atrorubens seed serves to spread germination of the seed lot over a greater time period. This may indicate that uncertain conditions related to temperature fluctuations and moisture availability during embryo emergence prevail in the habitats to which it is adapted. An extended time period over which germination occurs is one strategy to better insure that at least a portion of each season's seed population will produce offspring. In addition to correspondence with climatic factors, germination timing is related to competition between neighboring seedlings for light and space, particularly in species like the two under study that invade disturbed sites. Thus small differences in time of germination may have a drastic affect on competitive ability.

In conclusion, seed from a sampled portion of the range of each species displayed differences in dormancy and response to alternating incubation temperature. H. silphioides seed were

dormant after 4 weeks dry storage while seed of H. atrorubens germinated slowly without stratification. H. silphioides seed germinated slower than seed of H. atrorubens after various stratification and incubation treatments. These differences in germination characteristics may be an indication that the species have different germination strategies that better insure establishment of seedlings in certain habitats.

CHAPTER V

CONCLUSIONS

The objective of the present study was to investigate the biological relationship of H. silphioides and H. atrorubens in Tennessee using data from morphology, ray flower flavonoid constituents and seed germination. The results indicate that the species are distinct.

Analysis of morphology using numerical techniques showed that when intrapopulational variation was averaged, both cluster analysis and principle coordinate analysis revealed two distinct morphological groups. Cluster analysis of all collected individuals also resulted in two distinct groups. Principle coordinate analysis of all specimens showed two discreet morphological groups with some specimens intermediate to the groups. The groups may be considered species groups since both cluster analysis and principle coordinate analysis showed the same group composition and factor analysis indicated that stable morphological features are important in their separation. Morphology of specimens collected in Tennessee bears no evidence of extensive hybridization in this region.

Combinations of morphological characteristics shown by this study to be useful in discriminating between the species are:
H. atrorubens--hairs on abaxial leaf midvein longer than 1 mm, basal leaves usually present at anthesis, petioles of basal leaves over

1/2 length of blade and winged more than 1/2 petiole length, 8 or fewer nodes below the inflorescence, and not colonial (if more than 1 stem is present, all stems flower), H. silphioides--hairs on abaxial leaf midvein not longer than 1 mm, basal leaves generally absent at maturity, petioles of basal leaves not over 1/2 length of blade and only rarely winged more than 1/2 petiole length, more than 8 nodes below the inflorescence, and colonial (nonflowering stems present).

Study of flavonoids forming UV nectar guides on ray flowers supports the species groupings based on morphology. Anthochlor flavonoids, coreopsin and sulfurein, were consistently present in specimens of H. atrorubens but were generally absent in H. silphioides. Further studies on presence of floral anthochlors in H. silphioides throughout its range are necessary to assess the significance of variability in the anthochlor character. Only one of four central Tennessee populations indicated as morphologically intermediate by principle coordinate analysis exhibited the presence of anthochlors. On the basis of chemical evidence these populations are probably not F₁ hybrids.

Seed germination characteristics of the species varied in temperature response and dormancy. After 4 weeks dry storage H. silphioides seed were dormant while seed of H. atrorubens germinated slowly at moderate temperatures to a high percentage of total. After various stratification treatments, seed of H. silphioides germinated slower than H. atrorubens seed at several

alternating temperature regimes. The extended time period over which germination of H. silphioides occurs relative to H. atrorubens may indicate a strategy of "caution" (Mayer and Poljakoff-Mayber, 1975) related to greater uncertainties in environmental conditions in its habitat. Seed lots throughout the range of the species must be tested before germination patterns can be correlated with habitat and range.

In conclusion, specimens of the two species in Tennessee fall into two distinct groups based on morphology and floral flavonoids. Four populations collected on the eastern Highland Rim generally exhibit morphological and chemical features typical of H. silphioides but display some morphological characteristics intermediate between the species. The source of this intermediacy was not determined. Seed germination characteristics of the species suggest that germination strategies may be important to survival in their ecologically distinct habitats.

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