SYSTEMATIC INVESTIGATIONS OF PUTATIVE HYBRIDIZATION BETWEEN RATIBIDA COLUMNARIS (PURSH) RAF. AND R. TAGETES (JAMES) BARNH.

by

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CHAPTER I

INTRODUCTION

An experimental hybridization study of Ratibida columnaris (Pursh) Raf. and R. tagetes (James) Barnh. has suggested the possibility of introgression between these two taxa (Jackson, 1963). In support of this, personal field observations and studies of plantae exsiccatae have revealed morphologically intermediate characteristics.

In addition, considerable variation in ligule coloration occurs in both R. columnaris and R. tagetes as well other taxa in this and closely related genera. The color variants in R. columnaris have been recognized as different species and varieties, and at present they are given the taxonomic rank of forma (Richards, 1968; Sharp, 1935). In contrast, the color variants of R. tagetes have been treated as a single taxonomic unit. Disagreement over the valid specific epithet for R. columnaris has also created taxonomic confusion within this group.

The intent of this study was to determine the extent of introgression in sympatric populations of R. columnaris and R. tagetes and to clarify the taxonomic status of variants within each species by employing chemosystematic, cytological, physiological, and morphological techniques.

CHAPTER II

TAXONOMIC HISTORY

The genus Ratibida was first diagnosed by Rafinesque in his Flora Ludovicina (1817) which contained a very brief description based on Rudbeckia columnaris Pursh (1814). Later, Rafinesque (1819) published a complete description of Ratibida which was followed in the same year with a description of the genus Lepachys based on Rudbeckia pinnata Vent. Lepachys is now considered to be congeneric with Ratibida. Cassini (1825) described the genus Obeliscaria which was also based on Rudbeckia pinnata Vent. and, therefore, also should have been submerged. De Candolle (1836), however, recognized Obeliscaria in his Prodromus and treated Ratibida and Lepachys as sections under Obeliscaria.

Don (1836) was the first worker to recognize the validity of the generic epithet Ratibida after it was originally published by Rafinesque (1817). However, in their Flora of North America, Torrey and Gray (1842) used the generic name Lepachys for their treatment of its three species and one variety. Rudbeckia tagetes James (1823) was transferred to Lepachys tagetes by Gray in "Pacific Railroad Report" in 1836. Watson (1888) in his description of L. mexicana was the last person to use Lepachys.

Barnhart (1897) recognized Ratibida and listed all of the described Lepachys and Obeliscaria taxa as synonyms except Lepachys mexicana, which he did not consider in his treatment. Sharp (1935), in a comparative study of certain of the epappose genera of the Heliantheae, included a complete taxonomic treatment of Ratibida and, at that time, transferred Lepachys mexicana to Ratibida mexicana. The most recent treatment was a monograph of the genus Ratibida by Richards (1968) which considered all of the currently described species.

The name Rudbeckia columnifera first appeared in "A Catalogue of New and Interesting Plants Collected in Upper Louisiana Principally on the Missouri, North America" with the following description:

> Spontaneous varieties of this plant sometimes occur with bright fulvous flowers, coloured like Tagetes patula: the stem is simple, seldom producing more than three flowers, which are of an uncommon length, appearing like a column of flosculi, subtended by 3-8 meutral florets, and a simple calyx (Fraser's Catalogue, 1813).

The catalogue was published by the Fraser Brother's Nursery in August of 1813 (commonly referred to as Fraser's Catalogue). Since the time of its publication, the questionable authorship of the catalogue has caused considerable confusion and debate over the validity of the names therein. Most authors will agree that the plants listed in the catalogue had been collected and brought to England by Thomas Nuttall. Whether he was the sole

author of the catalogue and, if he was, whether he considered the descriptions in the catalogue valid description is the subject of major controversy.

Almost immediately following the publication of "Fraser's Catalogue," Frederick Pursh and John Sims both published "formal" descriptions of Rudbeckia columnaris. The description by Sims was published in Vol. 39 of Curtis Botanical Magazine 1814 (1813 by some authors) and was accompanied with a color plate, No. 1601. In the text following the description, Sims states, "This new species of Rudbeckia was introduced, we believe, by Mr. Nuttall, from the country of the Missouri." Sims also acknowledged that the color plate was taken from material growing in Frasers' Nursery. The description of Rudbeckia columnaris by Pursh was published in his Flora Americae Septentrionalis 1814 (mid-Dec. 1813 according to Graustein, 1955), with "Rudbeckia columnifera Frasers Cat. 1813" cited as a synonym. All three of the preceding descriptions have at one time, by at least one author treating the group, been considered as the valid description.

In Nuttall's The Genera of North American Plants (1818), Rudbeckia columnaris appears without a citation and was not starred as a new species. It should be noted that of the eighteen names which had appeared in "Fraser's Catalogue" with at least a brief description (excluding the brief joint description for the two species

of Sideranthus), only four were acknowledged by Nuttall in The Genera as his, cited "T. N. in Fras. Catal."; two were credited to only "Fras. Catal."; one was credited to Pursh without reference to the catalogue; and Rudbeckia columnifera, as well as nine other taxa found in "Fraser's Catalogue" were not included in The Genera.

In D. Don's treatment of the genus Ratibida (1838), he recognized "Rudbeckia columnaris Pursh fl. amer. stept. 2. p. 575." listing "R. columnifera Fras. Cat. 1813" and "R. columnaris Sims in bot. mag. t. 1601" in synomony. Green (1890) published a reprint of "Fraser's Catalogue" which had "by T. Nuttall" written in Nuttall's handwriting following the title of the catalogue. From this Green concluded that this was proof that Nuttall had been the author of the catalogue. Sharp (1935) credited Rudbeckia columnifera to Nuttall in "Fraser's Catalogue," but stated:

> The description accompanying it is so indefinite that the name R. columnifera may be regarded as nomen subnudum. Nutall himself in The Genera of North American Plants in 1818 abandoned his name R. columnifera and recognized Rudbeckia columnaris Pursh.

Sharp recognized R. columnaris (Sims) D. Don in his treatment considering Sims' description as appearing in 1813. This was apparently an interpretation of the engraver's date appearing on pl. 1601, "Dec. 1, 1813." The title page, however, is dated 1814, which should be considered the date of publication. Fernald (1938) recognized Ratibida columnifera (Nutt.) Woot. & Standl (1915)

as the valid combination. Fernald disagreed with Sharp's (1935) interpretation of the description found in "Fraser's Catalogue," considering it a valid, intelligible description crediting Nuttall as the author. In support of his interpretation of the description, Fernald states:

> Wooten & Standley, Macbride, and Rydberg have all found it distinctive and, even if it seems to some only a "nomen subnudum" in spite of the three distinctive characters given, it should be noted that the name Rudbeckia columnifera was understood by Pursh when in 1814 he cited it without qualification as an exact synonym of his newly proposed R. columnaris.

Fernald also included a discussion of the valid publication dates of both Sim's and Pursh's descriptions of Rudbeckia columnaris, both of which had title page dates of 1814. In order to determine which one of the descriptions had priority, he compared the citations that had appeared in each of the publications and concluded that Pursh's description had pre-dated Sims's. This conclusion was also supported by Graustein's (1954) analysis of the publication date of Pursh's Flora. She contends that Pursh's Flora had actually appeared in December of 1813 based upon the Minutes of the Linnean Society of London which contained a reference to the presentation of The Flora Americae Septentrionalis by Pursh on December 21, 1813.

Shinners (1956) contended that Nuttall had not been the author of "Fraser's Catalogue" and that the names

which had appeared in it should be considered invalid. These conclusions were based upon an analysis of Nuttall's inconsistent treatment of the names that had appeared in "Fraser's Catalogue" in his Genera of North American Plants and the fact that Nuttall's contemporaries, Pursh, Sims, and Ker-Gawler, had treated these names as if they were inconsequential.

Graustein (1956) disagreed with Shinner's interpretation of the authorship of "Fraser's Catalogue," reasserting that Nuttall was the author, stating, "No convincing evidence to the contrary has been furnished by Lloyd H. Shinners in questioning Nuttal's authorship . . . ." Graustein did, however, contend that Nuttall did not consider the description as a valid publication of the species in it. In support of this, she points out that Nuttall had signed an agreement with Professor Benjamin Smith Barton (his patron) prior to his expedition in 1810. This agreement gave Barton exclusive ownership of Nuttall's journals and observations and required that Nuttall not dispose of any of the specimens collected without his consent, lest, "they might otherwise fall into the hands of persons who would use them to my disadvantage." As evidence that Nuttall was honoring this agreement after his arrival in England, she states:

> That he intended to stand by his contract is indicated by the fact that he made no attempt to publish any of his new species in the Botanical Magazine--a device by which he could have anticipated a few items at least of Pursh's Flora--and remained aloof while others became the authors of plants in that publication.

Cronquist, Keck, and Maquire (1956) also disagreed with Shinners's interpretation of the authorship of "Fraser's Catalogue," stating, "It is universally acknowledged that many or all of the new names contained in it were those of Thomas Nuttall." and that the names with descriptions should be considered to be validly published with Nuttall as the author. In Richard's (1968) monograph of the genus, he accepted Nuttal as the author and recognized Ratibida columnifera (Nutt.) Woot. & Standl. as the valid combination.

Reveal (1968) discussed the validity of each species that was listed in "Fraser's Catalogue." His conclusions regarding each of the names appearing in the catalogue were based on the ". . . adequacy of the descriptions and subsequent identifiability of each entity, considering the nomenclatural status and history of each . . ." In his analysis of the description of Rudbeckia columnifera that appeared in "Fraser's Catalogue," he concluded that the description was adequate and that it should be considered as a valid description, rejecting R. columnaris as illegitimate.

I agree with Sharp's (1935) conclusion that the description that accompanied the name Rudbeckia columnifera in "Fraser's Catalogue" (1813) was too vague to constitute a valid description and therefore should be regarded as a nomen subnudum. Fernald's (1938) defense of the R. columnifera description as being a "distincitive" and "intelligible" description which ". . . was understood by Pursh when, in 1814 he cited it without qualification as an exact synonym of his newly proposed R. columnaris" ignores the fact that Pursh, as well as Sims, had the opportunity to visit the Frasers' Nursery and observe representative specimens which corresponded to the names which appeared in the "Catalogue" before he wrote his description of R. columnaris. If subsequent authors, such as Wooten, Standley, Macbride, and Rydberg, had not had the descriptions of Pursh and Sims which refer to R. columnifera as a reference, I doubt that they would have considered the description that appeared in the "Catalogue" distinctive enough to delineate one particular taxon from within the Dracopsis-Rudbeckia-Ratibida complex. I do, however, agree with Fernald's analysis of the publication dates of the descriptions of R. columnaris by Sims and Pursh and therefore consider Ratibida columnaris (Pursh) Rafinesque as the valid combination.

Several subspecific taxa of R. columnaris have been recognized in the past. Obeliscaria pulcherrima was

first described by De Candolle (1836) and was reduced to varietal status by D. Don, appearing as "Ratibida columnaris var. pulcherrima" in Sweet's Brit. Fl. Gard. (1838). Sharp (1935) reduced var. pulcherrima to the rank of forma in his treatment of the epappose genera of Compositae. It was also treated as forma pulcherrima by Richards (1968) in his monograph of the genus Ratibida. This form of R. differs from the typical form in that a portion or all of the ligulate flower is purple to purplish-yellow in color. Since there are several other taxa within the genus which exhibit the same or similar variation in ligule coloration which have not had their color variants afforded taxonomic rank, this form of R. columnaris will not be given taxonomic rank in this treatment. Cockerell (1915, 1916) described four varieties of R. columnaris as follows: var. breviradiata, having short rays 10 mm long; var. incisa, with cleft rays; var. tubularis, with cylindrical or completely quilled rays 25 mm long and 3.5 mm wide; and var. appendiculata, with rays possessing paired long appendages arising from the throat of the ray. Boivin (1960) described forma denudata as individuals with the head devoid of rays. In his monograph, Richards (1968) considered var. breviradiata, var. incisa, var. tubularis, var. appendiculata, and forma denudata as aberrant or

teratological forms and did not afford them with a taxonomic rank. They are considered as such in this treatment.

Rudbeckia tagetes was first collected and described by Edwin James while he was a member of the Long Expedition in 1820. The description was not formally published until 1823 when it appeared in the account of the Long Expedition (Phila. Ed. 2:68, 1823). T. Nuttall described Rudbeckia globosa in 1834 which is now considered to be conspecific with the taxon described by James and therefore should be considered as a synonym of R. tagetes. From this date the specific epithet remained unchanged; however, it did appear in several different generic combinations as recognized by different authors. Thus, it appeared as Obeliscaria tagetes in De Candolle's Prodromus (1836), and then was reduced to Lepachys columnaris var. tagetes by Gray in 1852. Gray then elevated it to Lepachys tagetes in 1856. Barnhart (1897) was the first worker to recognize the valid combination Ratibida tagetes (James) Barnh.

Standley (1909) described var. cinera of R. tagetes as differing from the type in being densely strigose-hirsute and cinerous. Richards (1968) found this condition to be the result of bud mite and/or fungal infection on the typical form and there is not afforded taxonomic rank in this treatment.

CHAPTER III

MORPHOLOGICAL STUDIES

Methods and Materials

To determine the extent of morphological variation within each taxon throughout its range, herbarium specimens from four regional herbaria (TEX, OKL, SMU, and TTC) were examined. In addition, specimens were collected from natural populations in Texas and New Mexico for the morphological study as well as for cytogenetic and chemical analyses. In each of the populations examined, an attempt was made to collect at least 10 individuals of each taxon which were representative of the entire population. Sympatric populations were examined carefully for individuals which appeared atypical or intermediate in form. The morphological characteristics used for delinating each taxon were taken from those characteristics which have generally been considered as being diagnostic (Sharp, 1935: Jackson, 1963: Richards, 1968). These characteristics are summarized in Table 1, and Figure 1 illustrates the locations of representative populations examined in this study.

Jackson (1963) was successful in producing a single hybrid between R. columnaris and R. tagetes which was highly sterile (2% pollen fertility). This artificial

TABLE 1

DIAGNOSTIC MORPHOLOGICAL CHARACTERISTICS

Character	R. columnaris	R. tagetes
Head shape	cylindrical	globular
Head length	10-50 mm	6-10 mm
Head width	8-14 mm	8-12 mm
Ligule length	12-40 mm	4-10 mm
Ligule width	8-25 mm	4-8 mm
Papus	2-toothlike projections	no projections
Peduncle length	50-250mm	10-60 mm
Plant height	2.5-12 dm	1-4 dm
Root system	Tap root	Rhizome

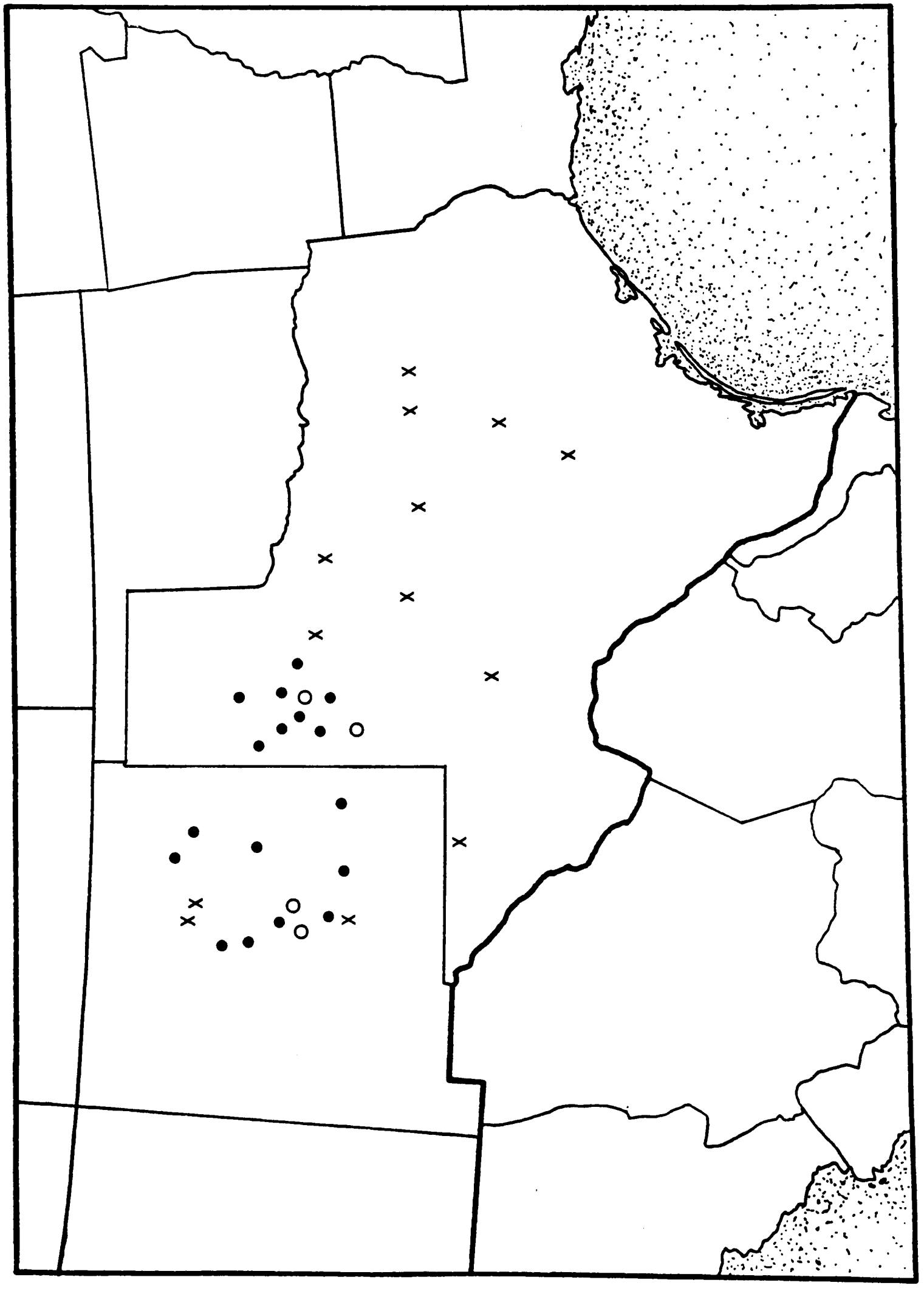
hybrid exhibited a number of characteristics which were intermediate between the two parental taxa including head shape, head length, ligule length, ligule width, peduncle length, and total plant height (Jackson, 1963). In view of this, individuals which were collected from sympatric populations of R. columnaris and R. tagetes that had several intermediate characteristics were examined chemically and cytologically to study the possibility of hybridization between the two taxa.

Results and Discussion

Individuals were found in several sympatric popula-

FIGURE 1. Representative populations of *R. tagetes* and *R. columnaris*.

X - *R. columnaris* population
O - *R. tagetes* population
● - Sympatric populations



tions as well as a few allopatric populations of R. columnaris which exhibited several intermediate characters. Table 2 summarizes the morphological data obtained from 25 individuals from 11 populations which were tentatively classified as putative hybrids. In general, these individuals were less than one half the height of the "normal" R. columnaris individuals in the same population, and most of the heads, ligules, and peduncles produced by these atypical plants were intermediate in length between R. columnaris and R. tagetes (Figures 2 and 3). It should be noted, however, that a few individuals which had several heads of this intermediate form, also had one or more heads that were similar to those of the "typical" R. columnaris plants. In addition, most of the atypical individuals occurred in more xeric habitats.

TABLE 2

MORPHOLOGICAL CHARACTERS OF PUTATIVE NATURAL HYBRIDS

Character	Mean	Range
Head length	18 mm	7-25 mm
Ligule length	13 mm	3-22 mm
Ligule width	11 mm	4-18 mm
Peduncle length	40 mm	26-80 mm
Plant height	3.5 dm	0.6-5.0 dm
Root system	all of the individuals had a tap root	
Pappus	all had 2-toothlike projections	

FIGURE 2. "Normal" floral morphology of *R. columnaris* and *R. tagetes*.

A - *R. columnaris*
B - *R. tagetes*

FIGURE 3. Individuals from sympatric populations of *R. columnaris* and *R. tagetes*.

A - "normal" *R. columnaris*
B - putative hybrid
C - "normal" *R. tagetes*

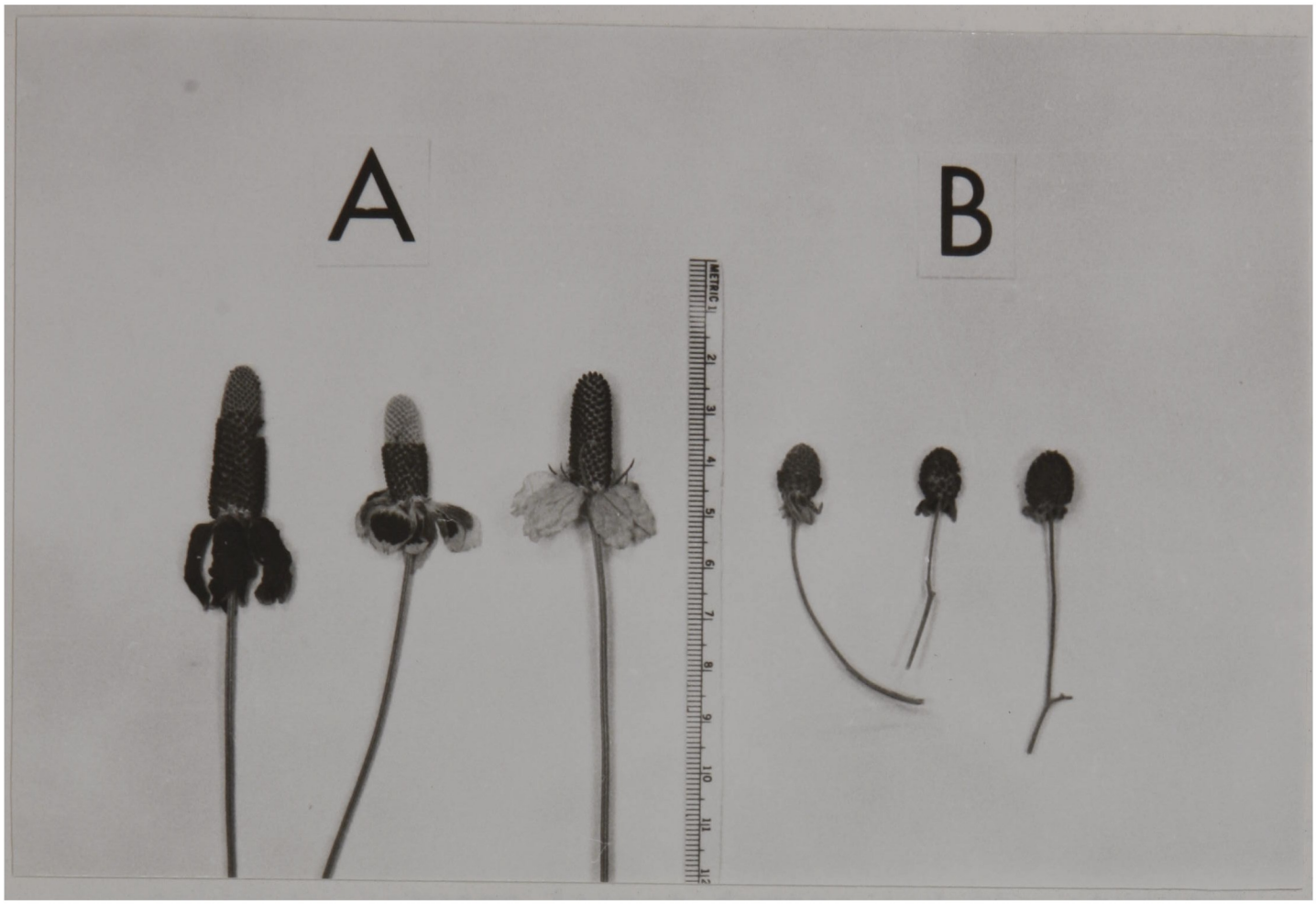


FIGURE 2.

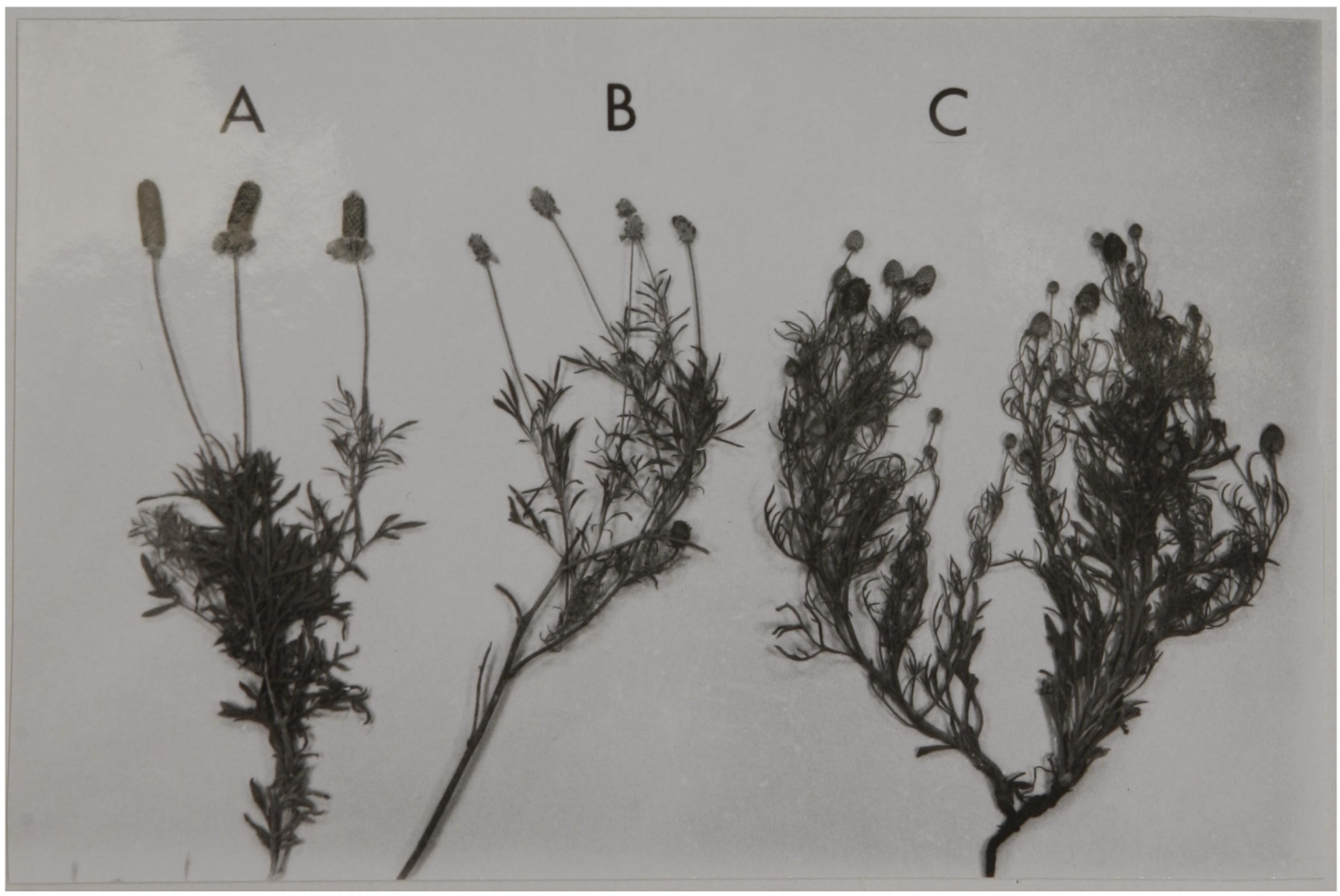


FIGURE 3.

The fact that some of the putative hybrid plants occurred in allopatric populations of R. columnaris led me to suspect that some genetic or environmental factor other than hybridization was capable of producing an intermediate morphology in at least some of the populations. Careful examination of the atypical individuals revealed that many were infested with boring insect larvae which had done considerable damage to the stem, while others were infested with root aphids and/or mealy bugs. There were, however, some individuals which appeared to be insect-free, which discounted the direct correlation between insect damage and the atypical forms. This led to the investigation of other environmental factors which could have affected morphological development, including water stress, which is discussed in detail in Chapter VI.

CHAPTER IV

CYTOLOGICAL STUDIES

Methods and Materials

Mitotic material was obtained from recently germinated achenes collected from natural populations and from individuals used in a greenhouse crossing study. In most cases, achenes from individuals within a population were combined to give a populational sample. However, if the parental plants appeared to represent a putative hybrid or an aberrant form, the achenes from each individual were treated separately. The achenes were germinated in flasks containing one-half strength Hoagland's solution. Prior to being placed in the Hoagland's solution, the achenes were washed by placing them in distilled water which was changed three times a day for three days in order to prevent the chemical inhibition of germination. The achenes were then placed in the Hoagland's solution which was changed once a day until the achenes germinated. The flasks were also aerated continually to prevent the solutions from becoming anaerobic. After germination, the seedlings were grown under artificial light on a long day cycle (14 hr light, 10 hr dark). Six to eight days after germination the seedlings either were karyotypically analyzed or transferred to peat pots and allowed to grow in

the greenhouse until they were needed for chemical or cytological analysis. The seedlings used for karyotypic analysis were pretreated in either an aqueous solution of 8-hydroxyquinoline (0.3 g of 8-hydroxyquinoline per liter of water) or in a saturated aqueous solution of para-dicholorobenzene to inhibit spindle fiber formation. The effectiveness of the two chemicals was different for each of the two species and thus the duration, the chemical inhibitor used, and the temperature of the pretreatment varied with the species being examined. The R. tagetes seedlings were harvested three to four hr after the beginning of the light period and were placed in a saturated aqueous solution of paradichlorobenzene for 2½ hr at 25° C. The R. columnaris seedlings were harvested 10 hr after the beginning of the light period and were placed in 8-hydroxyquinoline solution for 8 to 16 hr at 4° C. After their respective pretreatments, all of the seedlings were fixed in a modified Carnoy's solution (four parts ethanol to one part glacial acetic acid) for at least two hr before examination. The seedlings were hydrolyzed in 6 N hydrochloric acid for 15 min and returned to the fixative for 20 min before examination. The root tips were then excised, placed in a drop of FLP orecin stain (Jackson, 1973), squashed and examined.

Meiotic chromosome observations were made from bud material collected in the field as well as from the greenhouse. Best results were obtained when the bud material was collected from two to four pm and fixed in a modified Carnoy's solution of chloroform, absolute ethanol, and glacial acetic acid (4:3:1 v/v). After fixation, which required a minimum of six hours, florets of a suitable size were dissected out of the head, placed on a slide in a drop of the FLP orecin stain, squashed and examined. The stained material was examined at diakinesis and anaphases I and II to detect abnormal meiotic configurations. Some chromosome counts for populations were also obtained from meiotic material. Difficulty in obtaining suitable material in R. columnaris due to the tendency of the chromosome material to stick together has to date limited detailed analysis of the meiotic configurations.

In an attempt to produce artificial hybrids between R. columnaris and R. tagetes for comparison with the putative field hybrids, specimens of both taxa from sympatric and allopatric populations were crossed in the greenhouse. Although both taxa have been reported as being self-sterile (Jackson, 1963), all of the individuals involved in the crossing study were checked for self-sterility.

Just prior to anthesis of the first disc flower, the heads were bagged with a commercial paper laboratory

tissue (Kimwipes) to prevent possible pollen contamination. The crosses were made by removing the tissue bags from the heads of the two individuals being crossed, rubbing the heads together, and then rebagging the heads twice daily (10 am and 4 pm) until at least one of the heads had terminated flowering. The heads remained bagged until the achenes reached maturity (5-7 weeks). In the individuals being checked for self-sterility, heads from the same plant were rubbed together. The archenes produced were germinated by the techniques described for the mitotic material.

To determine pollen fertility of individuals being examined, pollen grains were stained with Buffalo Black B dissolved in FLP solvent (Jackson, 1973). Pollen grains which were uniformly stained were considered to be fertile. In excess of 300 pollen grains were counted in each pollen fertility determination.

Results and Discussion

All previously reported chromosome counts for R. tagetes and R. columnaris (Jackson, 1959; Perdue, 1959; Jackson, 1963) have been verified in this study. Perdue's mitotic counts of 2n=26 and 2n=27 as well as Jackson's (1963) counts of 2n=28 for R. columnaris were observed. However, 2n=26 was found to be the most prevalent. The R. columnaris karyotype is characterized by one large

metacentric, nine relatively large acrocentrics and three (2n=26) four (2n=28) small metacentric pairs of chromosomes as illustrated in Figure 4. The 2n=27 karyotype idffers from the 2n=26 only in that it has one additional small metacentric chromosome. The additional metacentrics in the 2n=27 and 2n=28 are possibly supernumary in nature. Jackson's (1963) reported 2n=32 for R. tagetes was also observed and the karyotype is characterized by nine relatively large acrocentric and seven small metacentric pairs of chromosomes as illustrated in Figure 5. Vouchers from which counts were obtained are deposited in the Texas Tech Herbarium (TTC) and are listed in Table 3.

Over 1000 achenes were collected from the experimental crosses, however, only 21 germinated and of those only one survived long enough to produce a single true leaf before it died. All of the achenes that germinated were from R. tagetes X R. columnaris crosses, none of the R. columnaris X R. tagetes achenes were viable. All of individuals checked for self sterility were found to be completely self-sterile. R. columnaris X R. columnaris and R. tagetes X R. tagetes crosses yielded achenes with 70-85% germination.

Although only a few of the putative natural hybrids collected had either mature achenes or bud material suitable for cytogenetic analysis, of those that did,

FIGURE 4. Karyotype of R. columnaris.

FIGURE 5. Karyotype of R. tagetes.

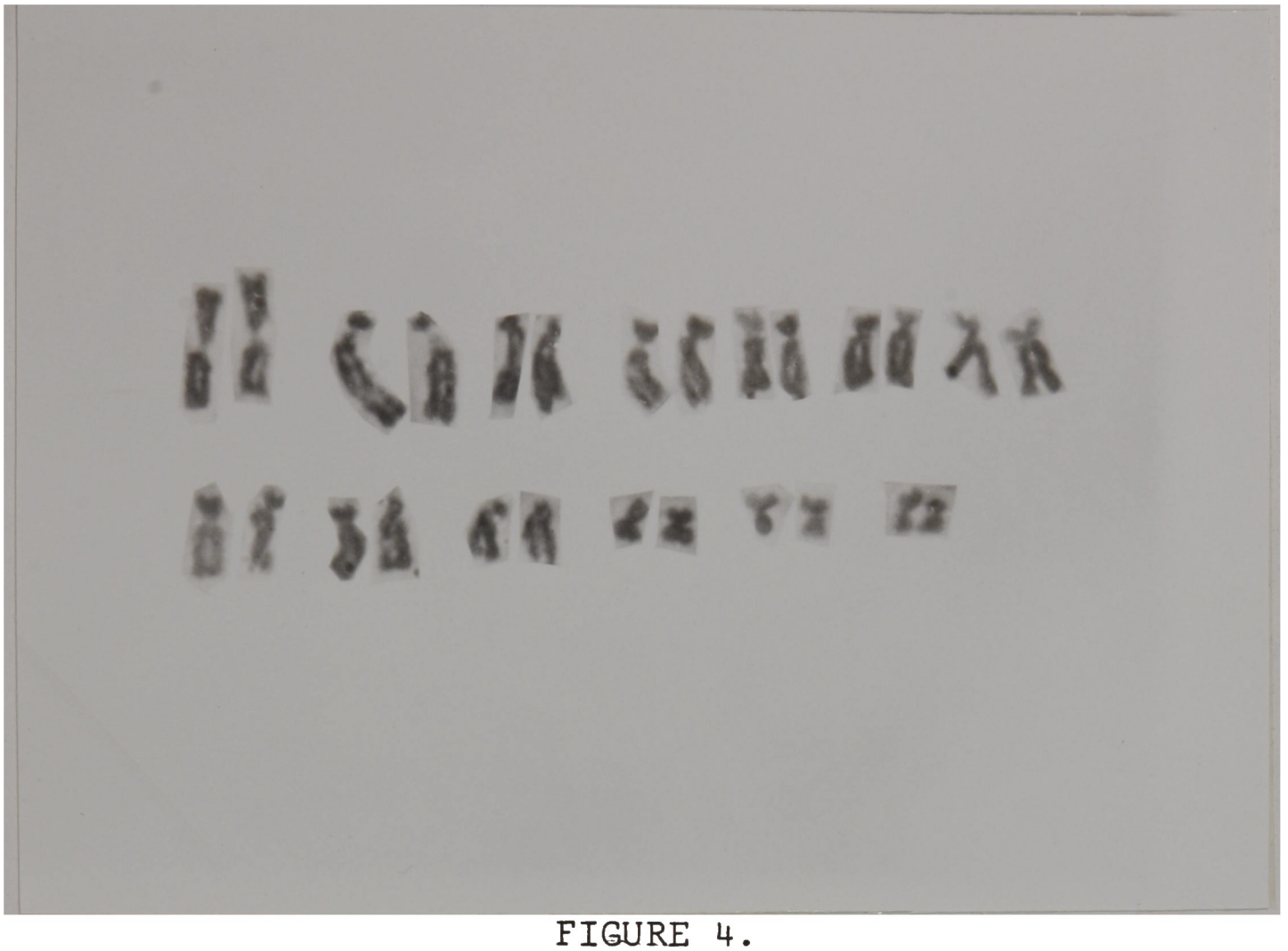


FIGURE 4.

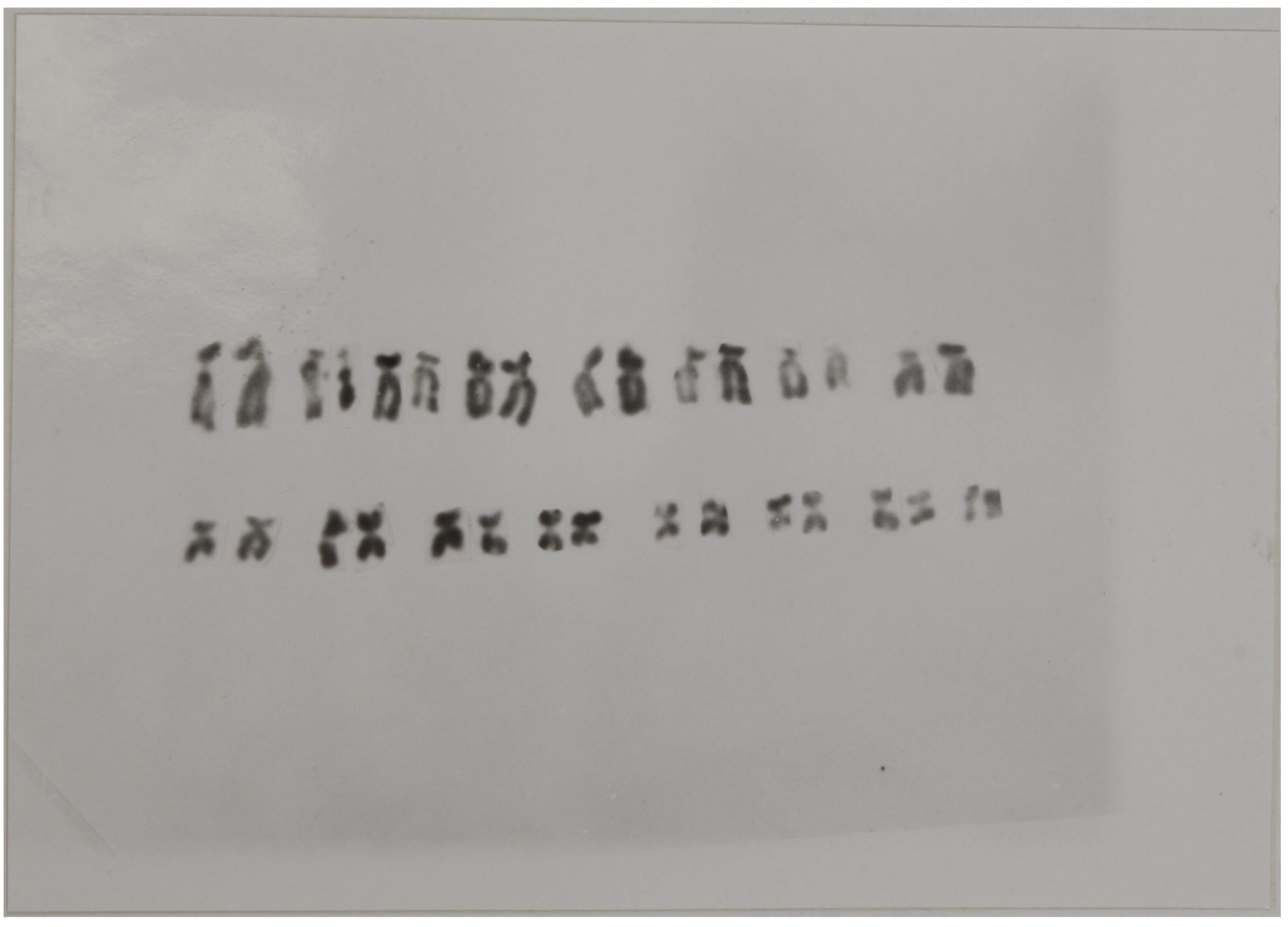


FIGURE 5.

TABLE 3

POPULATIONS OF *R. columnaris* AND *R. tagetes* EXAMINED FOR CHROMOSOME NUMBER

Species	Chromosome Number	Voucher
R. columnaris	n=14	New Mex. Lincoln Co.: Jahns 68.
	n=13	New Mex. Lincoln Co.: Jahns, Northington, & La Duke 80.
	n=13	New Mex. Lincoln Co.: Jahns, Northington, & La Duke 86.
	2n=26	New Mex. Lincoln Co.: Jahns & Goldthwaite 132.
	2n=26	Texas Archer Co.: Jahns 56.
	2n=26&27*	Texas Deaf Smith Co.; Jahns & Goldthwaite 150.
	n=16	Texas Floyd Co.: Jahns 100.
	2n=26	Texas Floyd Co.: Jahns 118.
	n=13	Texas Crosby Co.: Jahns 107.
	2n=28	Texas Hockley Co.: Jahns & La Duke 77.
	2n=26	Texas Lubbock Co.: Jahns 128.
	n=26	Texas Parker Co.: Jahns 90.
R. tagetes	n=16	New Mex. Lea Co.: Jahns & Goldthwaite 130.
	2n=32	New Mex. San Miguel Co.: Jahns & Goldthwaite 145.
	2n=32	New Mex. Santa Fe Co.: Jahns 155.

TABLE 3--CONTINUED

Species	Chromosome Number	Voucher
	2n=32	Texas Deaf Smith Co.: Jahns & Goldthwaite 151.
	2n=32	Texas Floyd Co.: Jahns 117.
	n=16	Texas Hale Co.: Jahns 116.
	n=16	Texas Oldham Co.: Jahns & Goldthwaite 149.
	n=16	Texas Lamb Co.: Jahns 100.

NOTE: n=meiotic count; 2n=mitotic count

*A single individual grown from seed collected from the population had a 2n=27 karyotype; the remainder had a 2n=26 karyotype.

all had a chromosome number of 2n=26 (8 individuals) and a pollen fertility ranging from 82-96% (24 individuals). The single hybrid that Jackson (1963) was able to produce in his crossing study had a pollen fertility of less than 3% and was also female sterile. Based upon the high pollen fertility and the chromosome numbers of the individuals examined cytologically, I concluded that they were aberrant forms of R. columnaris. Due to the observed incompatibility between R. tagetes and R. columnaris in this experimental crossing study, it became apparent that the probability of producing a natural hybrid was very remote, and if such a hybrid did survive that it would be effectively sterile, thus greatly reducing the possibility of introgression between these two taxa.

CHAPTER V

CHEMICAL STUDIES

Methods and Materials

Natural populations of both taxa were sampled and examined chromatographically to determine if any consistent chemical differences exist between the two taxa, and to determine the extent of any inter- and intra-populational variation in flavonoid content within each. The techniques used in this chromatographic survey closely follow those described in Mabry, Markham, and Thomas (1970).

Approximately .05 g of dried mid-stem leaf material was extracted with 85% aqueous methanol for three days. The extract was then spotted directly on Whatman 3MM chromatographic paper. The resulting chromatogram was first developed in a solvent system of t-butanol, glacial acetic acid, and water (3:1:1: v/v). Subsequently, 15% glacial acetic acid was used for development in the other dimension. After air drying, the chromatograms were observed over ultraviolet light alone and in the presence of ammonia vapors in order to determine the color characteristics of the flavonoid compounds present on the chromatogram. Rf values were also calculated. Vouchers of the populations examined in this survey are listed in Table 4 and are deposited in the

Texas Tech Herbarium (TTC). Duplicate specimens are to be distributed.

TABLE 4

POPULATIONS OF *Ratibida* EXAMINED FOR FLAVONOIDS BY PAPER CHROMATOGRAPHY

Species	Voucher
R. columnaris	New Mex. Guadalupe Co.: *Jahns*, *Finley* & *La Duke* 152.
	New Mex. Lea Co.: *Jahns* & *Goldthwaite* 129.
	New Mex. Lincoln Co.: *Jahns* 65.
	New Mex. Lincoln Co.: *Jahns* 67.
	New Mex. Lincoln Co.: *Jahns* 68.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 80.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 81.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 84.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 85.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 86.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 87.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 88.
	New Mex. Lincoln Co.: *Jahns*, *Northington* & *La Duke* 89.

TABLE 4--CONTINUED

Species	Voucher
	New Mex. Lincoln Co.: Jahns & Goldthwaite 131.
	New Mex. Lincoln Co.: Jahns & Goldthwaite 132.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 143.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 143.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 146.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 148.
	New Mex. Santa Fe. Co. : Jahns & Goldthwaite 142.
	New Mex. Santa Fe Co.: Jahns, Finley, & La Duke 154.
	New Mex. Torrance Co.: Jahns & Goldthwaite 137.
	New Mex. Torrance Co.: Jahns & Goldthwaite 139.
	New Mex. Torrance Co.: Jahns & Goldthwaite 141.
	Texas Archer Co.: Jahns 56.
	Texas Crosby Co.: Jahns 105.
	Texas Crosby Co.: Jahns 107.
	Texas Crosby Co.: Jahns 126.
	Texas Deaf Smith Co.: Jahns & Goldthwaite 150.
	Texas Dickens Co.: Jahns 95.

TABLE 4--CONTINUED

Species	Voucher
	Texas Floyd Co.: Jahns 100.
	Texas Floyd Co.: Jahns 112.
	Texas Floyd Co.: Jahns 118.
	Texas Floyd Co.: Jahns 121.
	Texas Hale Co.: Jahns 115.
	Texas Hockley Co.: Jahns 61.
	Texas Hockley Co.: Jahns & La Duke 77.
	Texas Hockley Co.: Jahns 96.
	Texas Hockley Co.: Jahns 103.
	Texas Jack Co.: Jahns 93.
	Texas Knox Co.: Jahns 57.
	Texas Lamb Co.: Jahns 99.
	Texas Lamb Co.: Jahns 102.
	Texas Lubbock Co.: Jahns 60.
	Texas Lubbock Co.: Jahns 63.
	Texas Lubbock Co.: Jahns & La Duke 75.
	Texas Lubbock Co.: Jahns 128.
	Texas Lynn Co.: Jahns 64.
	Texas Parker Co.: Jahns 90.
	Texas Young Co.: Jahns 55.
	Texas Young Co.: Jahns 94.

TABLE 4--CONTINUED

Species	Voucher
R. tagetes	New Mex. Lea Co.: Jahns & Goldthwaite 130.
	New Mex. Lincoln Co.: Jahns, Northington, & La Duke 82.
	New Mex. Lincoln Co.: Jahns, Northington, & La Duke 83.
	New Mex. Lincoln Co.: Jahns & Goldthwaite 133.
	New Mex. Lincoln Co.: Jahns & Goldthwaite 134.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 145.
	New Mex. San Miguel Co.: Jahns & Goldthwaite 147.
	New Mex. Santa Fe Co.: Jahns, Finley, & La Duke 153.
	New Mex. Santa Fe Co.: Jahns 155.
	New Mex. Torrance Co.: Jahns & Goldthwaite 135.
	New Mex. Torrance Co.: Jahns & Goldthwaite 136.
	New Mex. Torrance Co.: Jahns & Goldthwaite 138.
	New Mex. Torrance Co.: Jahns & Goldthwaite 140.
	Texas Deaf Smith Co.: Jahns & Goldthwaite 151.
	Texas Floyd Co.: Jahns 109.
	Texas Floyd Co.: Jahns 117.

TABLE 4--CONTINUED

Species	Voucher
	Texas Hale Co.: Jahns 116.
	Texas Hockley Co.: Jahns & La Duke 78.
	Texas Hockley Co.: Jahns 97.
	Texas Hockley Co.: Jahns 104.
	Texas Lamb Co.: Jahns 100.
	Texas Lamb Co.: Jahns 101.
	Texas Lubbock Co.: Jahns 62.
	Texas Lubbock Co.: Jahns 59.
	Texas Lubbock Co.: Jahns 58.
	Texas Lubbock Co.: Jahns 127.
	Texas Oldham Co.: Jahns & Goldthwaite 149.

After completing the preliminary survey, a large populational sample was collected in order to obtain approximately 1 kg (dry weight) of leaf material for each taxon. The dried leaf material was then ground into a coarse powder. This was extracted with three volumes of chloroform over a period of three days in order to remove most of the chlorophyll. The chloroform fractions were then combined, reduced in volume, and analyzed for flavonoid content. Following the chloroform extraction, the plant material was air dried to remove any residual chloroform, and was then extracted with two volumes 85% aqueous methanol. The methanol extract was then taken to dryness on a flash evaporator and the residue was redissolved in a mixture of chloroform and water, allowing the remaining chlorophyll to be separated from the flavonol-rich water layer as the two immiscible solvents separated. The resulting water layer was partitioned with ethyl acetate in order to remove aglycones and mono-glycosides from the water layer. All of the layers were then checked for flavonoid content using paper chromatography, and flavonoids present in each fraction were isolated. The flavonoid skeletons of the isolated compounds were analyzed using ultraviolet and, when necessary, nuclear magnetic resonance (NMR) spectroscopy. The

techniques used in isolation and spectral analysis follow those described by Mabry, Markham, and Thomas (1970) with the exception that fused sodium acetate was substituted for sodium acetate.

Acid hydrolyses were employed to remove the sugar moiety from suspected flavonoid O-glycosides for characterization of the sugar to obtain the aglycone for positive identification. The samples were refluxed for 45-60 min in 2 N HCl on a steam cone, neutralized with NaOH, and taken to dryness on a flash evaporator. The residue was dissolved in a mixture of ethyl acetate and water. This mixture was allowed to stand for one hr to allow for complete separation of the ethyl acetate and water phases. The ethyl acetate phase was analyzed for flavonoid content while the aqueous phase was analyzed for the sugar-moiety using gas chromatography. Before injection into the chromatograph, trimethysilyl (TMS) ethers of the sugars were prepared by techniques outlines by Mabry, Markham, and Thomas (1970). A Beckman model GC-5 with a flame ionization detector equipped with a 6-ft x 0.25 inch coiled aluminum column packed with 2% SE-33 on Chromosorb W (HP AW DMCS 100/120, Analabs lot no. 011-3) maintained at 165^{o} C, with a carrier gas flow rate of 40 ml/min, was used for the analysis. The identification of each sugar was based upon a

comparison of retention times of known sugars which had been prepared in an identical manner to the unknowns obtained from hydrolysis. It should be noted that during acid hydrolysis both α and β cofigurations are produced, resulting in two peaks of different retention times for a single sugar. Retention times of known sugars are presented in Appendix A.

Results and Discussion

The preliminary chromatographic survey revealed that R. columnaris and R. tagetes had consistently different flavonoid spot patterns. These are depicted in Figures 6 and 7. The various compounds have been numbered to facilitate their discussion, and chromatographic spots of both taxa which are thought to be chemically identical are numbered the same. The identities and paper chromatographic characteristics of these compounds are presented in Table 5. Table 6 presents spectral data, and substitutions to the flavonoid skeleton are shown in Table 7.

It appeared originally that each taxon had some flavonoid compounds which were unique to that taxon. However, in the process of isolating the flavonoid constituents from large amounts of plant material, compounds 8, 9, and 11 which originally had been thought to be unique to R. tagetes were found in low

FIGURE 6. Flavonoid spot profile of R. columnaris.

FIGURE 7. Flavonoid spot profile of R. tagetes.

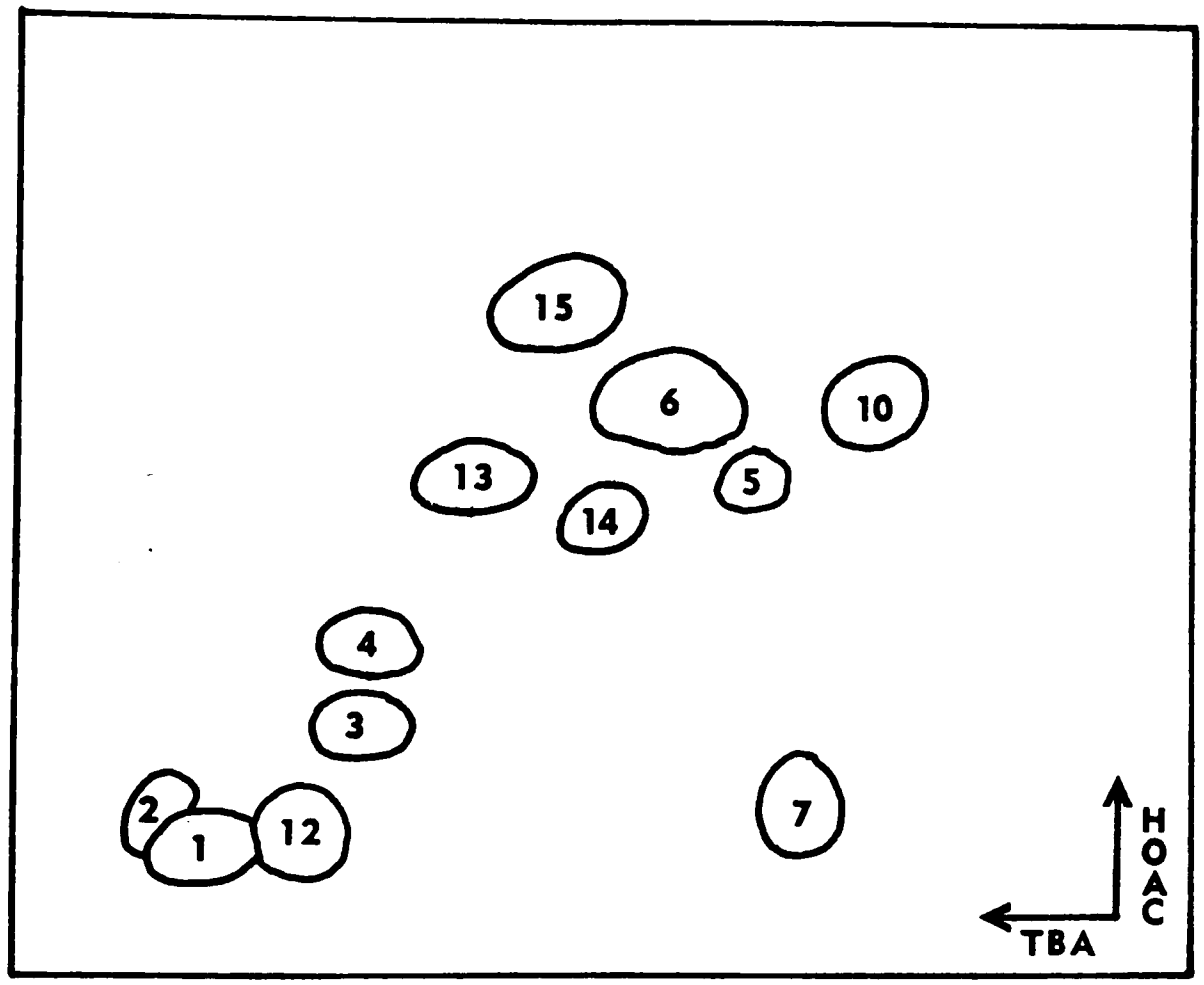


FIGURE 6.

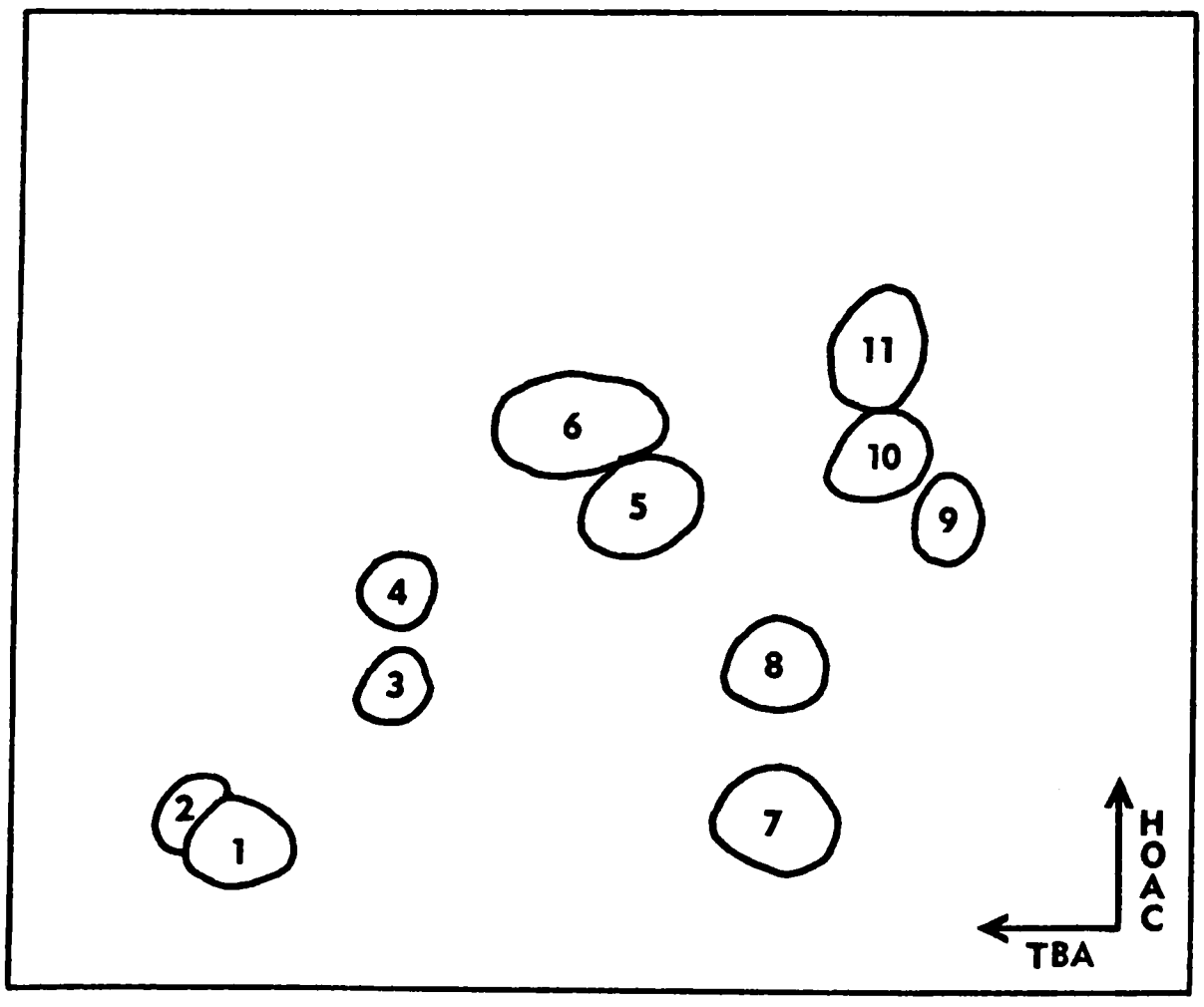


FIGURE 7.

TABLE 5

IDENTITY AND CHROMATOGRAPHIC PROPERTIES OF THE FLAVONOIDS OF *R. columnaris* AND *R. tagetes*

	Identity	Color		Rf Values	
		U.V.	$+NH_3$	TBA	HOAC
1.	Apigenin 6-0 methyl	P	DYG	.80	.12
2.	Unknown	B	B-B	.86	.14
3.	Unknown	B	FLB	.61	.25
4.	Unknown	B	FLB	.65	.38
5.	Isoorientin	P	YG	.39	.41
6.	Isoorientin 7-0-glycosly	P	YG	.45	.51
7.	Orientin	P	YG	.30	.20
8.	Orientin [6-0 methyl/ether]?	P	FY	.28	.32
9.	Vincenin-1	P	Y	.16	.49
10.	Violanthin-1	P	Y	.26	.55
11.	Violanthin-2	P	Y	.25	.63
12.	Luteolin 6-0 methyl ether	P	DGY	.68	.06
13.	Isoorientin [7-0 glucosyl-2]?	P	Y	.68	.58
14.	Isoorientin [7-0 glucosyl-3]?	P	Y	.55	.40
15.	Unknown	P	Y	.56	.68

P=purple; DYG=dark yellow-green; B=blue; B-B=bright blue; FLB=fluorescent light blue; YG=yellow-green; FY=floures-cent yellow; Y=yellow.

TABLE 6

ABSORPTION MAXIMA OF ISOLATED FLAVONOIDS

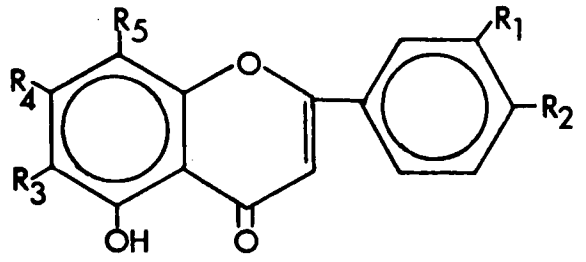
	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAC	NaOAC/H_3BO_3
1.	272, 331	272, 305, 325, 389	255, 275sh, 295sh, 355	255, 375sh, 295sh, 350	272, 290sh, 378	274, 331
5.	250, 265, 345	265, 275sh, 330, 405	272, 295sh, 330, 425	255sh, 275, 290sh, 355, 385	265, 320, 388	264, 300sh, 375, 415sh
6.	240, 254, 265, 345	265, 290sh, 400	274, 290sh, 325, 422	265sh, 275 290sh, 370	260, 380	260, 372, 420sh
7.	255, 265 345	265, 405	270, 295sh, 410	260, 272, 295sh, 355, 385	268, 410	255, 375
8.	250, 265, 342	265, 390	268, 290sh, 420	255, 270, 290sh, 350 385	255, 260 375	255, 365
9.	257, 272, 349	268, 280, 340sh, 405	280, 300sh, 334, 428	265sh, 278 297sh, 359, 384sh	282, 326, 400	265, 285sh, 382, 430
10.	270, 329	282, 334, 400	275, 301, 345, 385	275, 301, 345, 385	280, 295sh, 387	275, 282sh, 320, 340sh, 380sh

TABLE 6--CONTINUED

	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAC	NaOAC/H_3BO_3
11.	270, 329	282, 334, 400	275, 301, 345, 385	275, 301, 345, 385	280, 295sh, 387	275, 282sh, 320, 340sh, 380sh
12.	250sh, 270, 340	260, 270sh, 332, 405	275, 302sh, 320, 428	259, 280, 290sh, 368	269, 320, 402	262, 375, 410sh
13.	254, 290, 355	269, 320, 408	272, 320, 435	265, 295sh, 359, 400	265, 301sh, 320, 384	259, 375
14.	252, 265sh, 290, 355	269, 322, 405	274, 304sh, 330, 435	265, 295sh, 362, 404	268, 310, 405	260, 375

TABLE 7

SUBSTITUTIONS ON FLAVONOID SKELETON

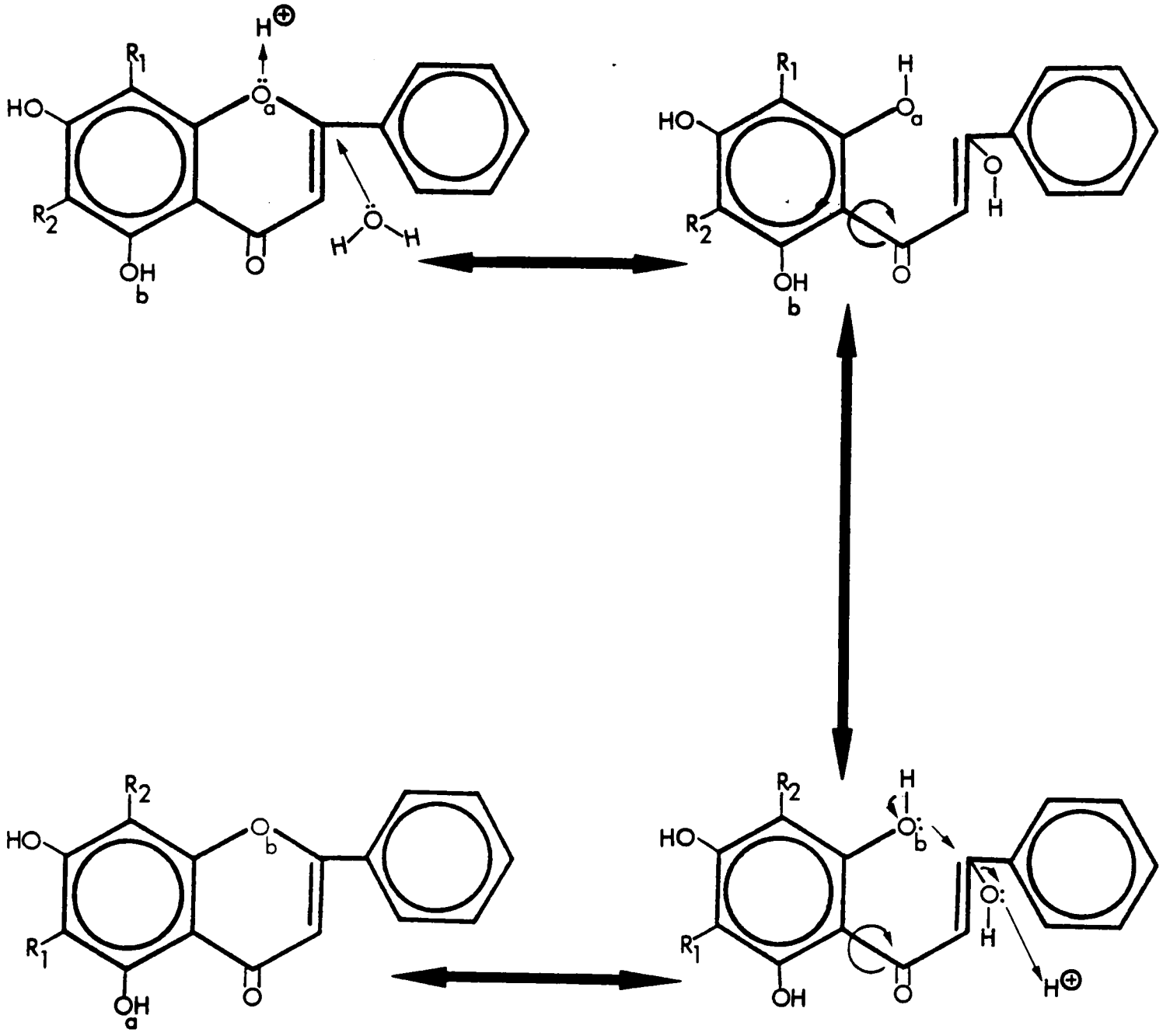


Spot No.	R_1	R_2	R_3	R_4	R_5
1.	H	OH	OCH_3	OH	H
5.	OH	OH	C-glycosyl	OH	H
6.	OH	OH	C-glycosyl	O-glucosyl	H
7.	OH	OH	H	OH	C-glycosyl
8.	OH	OH	OCH_3	OH	C-glycosyl
9.	OH	OH	C-glycosyl	OH	C-glycosyl
10.	H	OH	C-glycosyl	OH	C-glycosyl
11.	H	OH	C-glycosyl	OH	C-glycosyl
12.	OH	OH	OCH_3	OH	H
13.	OH	OH	C-glycosyl	O-glucosyl	H
14.	OH	OH	C-glycosyl	O-glucosyl	H

concentrations in R. columnaris. In addition, several other compounds were isolated which had not appeared in either taxon in the preliminary chromatographic survey. Due to the low concentration of flavonoids in the plant material, the identification of these additional compounds has not been completed and they have been omitted from the following discussion. Their chromatographic characteristics, however, are listed in Appendix B.

The acid hydrolyses of compounds that were suspected of being glycosides revealed that most of the compounds present had at least one C-glycosyl attached to the flavonoid skeleton. Since this type of hydrolysis is ineffective in cleaving the carbon-bound sugar moieties from the flavonoid skeleton, the C-glycosides have not had their sugar moieties identified. However, the acid hydrolyses were useful in the determination of the position of the substitutions on the A-ring of the flavonoid. During the acid hydrolysis of a compound, there is a rearrangement of the 6 and 8 positions (i.e., Wessely-Moser rearrangement), as illustrated in Figure 8. As a result of this, compounds with a C-glycosyl at the 6 or 8 position will produce two compounds. For example, the acid hydrolysis of compound 7 yielded both compounds 7 and 5 (orientin and isoorientin).

FIGURE 8. Reversal of C_6 and C_8 positions during acid hydrolysis.


H⊕
R1
HO
Oa
R2
OH
b
O
H
O
H
R1
H
HO
Oa
R2
O
H
OH
b
O
R2
H
HO
Ob
R1
OH
a
O
R2
H
HO
Ob
R1
OH
O
O
H
H⊕

All of the putative hybrids examined had chromatographic spot profiles identical to those of the typical R. columnaris. Since the detection of a hybrid with flavonoid analysis depends upon both parents having distinct flavonoid spot profiles of which neither is an additive pattern of the other, the discovery of the compounds 8, 9, and 11 in low concentrations in R. columnaris led me to question the ability to detect a hybrid between these two taxa using flavonoid spot profiles. The relative concentrations of these compounds in the two taxa are, however, very different, and a hybrid might produce these compounds in an intermediate concentration. This has not been substantiated, however, since I was unable to produce an artificial hybrid that lived long enough to produce sufficient leaf material for flavonoid analysis.

The evolutionary significance of compounds 8, 9, and 11 being found in low concentration in R. columnaris is unclear at this point. However, one might speculate that the relatively simple chromatographic profile of R. tagetes represents primitive or ancestral flavonoid constituents. Accepting this, the compounds present in very low concentration in R. columnaris could represent the end products of metabolic pathways which are being suppressed or bypassed in favor of new pathways. This

could result in a more complex chromatographic profile, as seen in R. columnaris. This hypothesis can be substantiated only after other taxa in this and closely related genera have been examined and all of the metabolic pathways in these taxa elucidated.

CHAPTER VI

PHYSIOLOGICAL STRESS STUDIES

Methods and Materials

In a preliminary study to examine the effects of water stress on floral morphology, seedlings of R. columnaris representing five natural populations from different habitats were selected for analysis. These plants, four per population, were grown in the greenhouse on a long day light cycle, in clay pots, with identical soil mixtures. The control group was watered daily, while the other group was subjected to a water stress by allowing the plants to reach their wilting point before watering. Observations of the heads produced by the plants grown under water stress revealed that approximately 20% of the heads produced exhibited abnormal floral development, as indicated by malformed ray and disc flowers, abnormally short receptacles and peduncles, and, in a few cases, reduced pollen fertility. The responses of the individuals representing different populations to the water stress were not equal, with some of the populations showing a proportionately greater number of abnormal heads per plant.

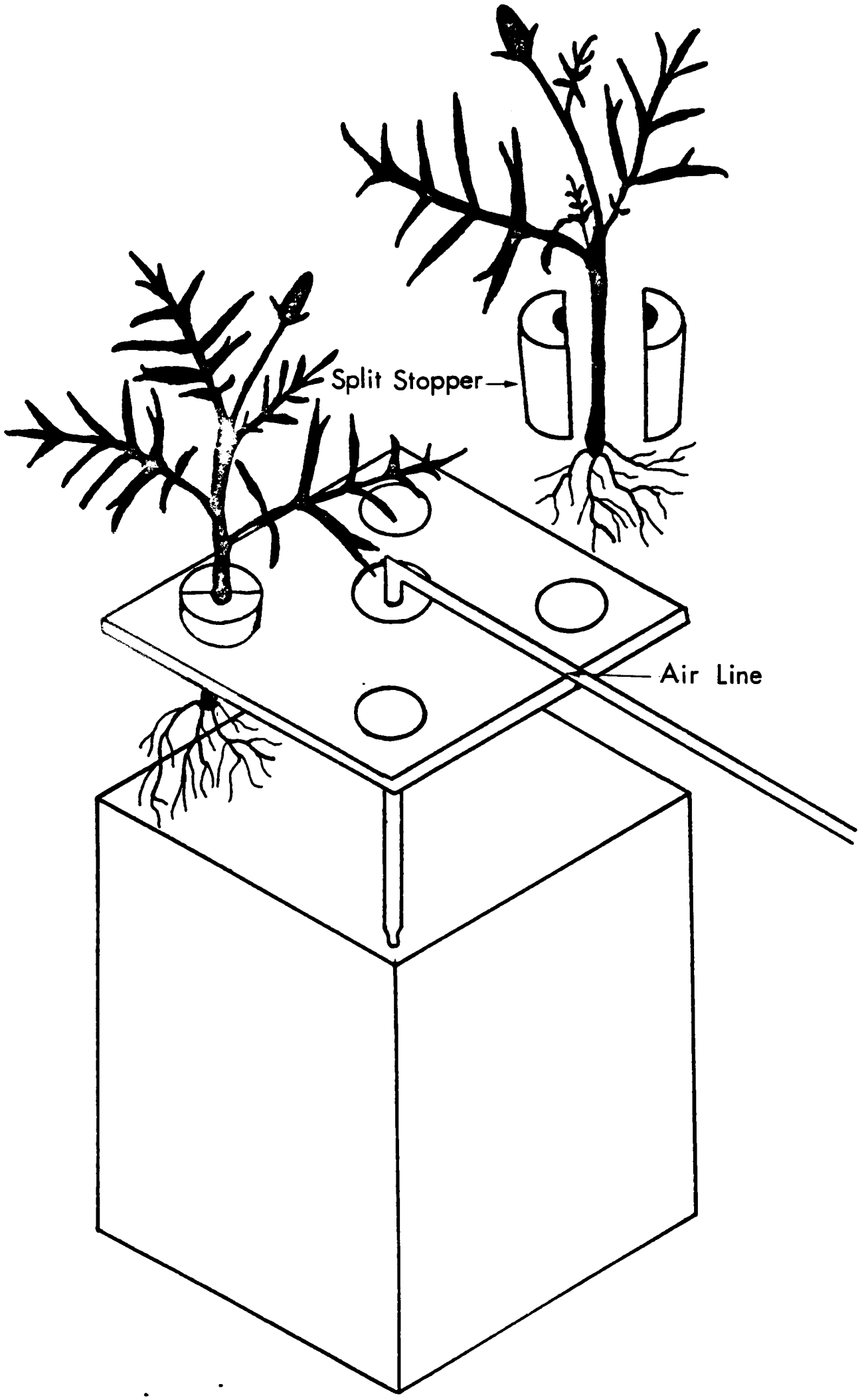
In order to eliminate some of the variables which could have affected the results in the preliminary study,

a second series of plants were subjected to water stress under controlled conditions. To eliminate the genetic variability between individuals, all of the plants used were members of a clone produced from a single individual. In the preliminary study, fluctuations in the degree of water stress existed and ranged from a relative abundance of soil moisture which was available to the plant immediately after watering, to severe water stress as the plants approached their observed wilting point 2-4 days after watering. In the controlled study, the plants were grown hydroponically and the water stress was imposed osmotically by the addition of sodium chloride to the culture solution, which allowed the degree of water stress to be maintained at a reasonably constant level.

Initially, one large seedling (GCJ 118a) was used to produce six stem cuttings. These cuttings were allowed to grow until there was sufficient stem material to produce 40 stem cuttings. Each cutting was treated with a rooting hormone (Rootone) and placed in moist vermiculite. Rooting required approximately five weeks, and at the end of six weeks, 20 cuttings of approximately the same size were transferred to a modified Hoagland's solution (Machlis & Torrey, 1956) for hydroponic culture. The cuttings were grown in five plastic containers,

four plants per container (Figure 9). Each container held approximately one liter of solution which was continuously aerated to prevent the solutions from becoming anaerobic. At the time of transfer, each cutting had 4-6 mature leaves, and all of the visible floral initials were removed. The cuttings were allowed to grow in the modified Hoagland's solution for a period of one week before being subjected to any water stress. At the end of this period, the solutions were sequentially increased in NaCl concentration in order to obtain four treatment solutions of 0.1 M, 0.2 M, 0.3 M, and 0.4 M NaCl (solutions were made with modified Hoagland's solution) with four plants per treatment plus a control left in the modified Hoagland's solution. The levels of each solution were checked twice daily and were maintained at the appropriate level by adding distilled water when necessary. The solutions were completely changed every other day to insure that the solutions were not deficient in nutrients. The cuttings were maintained in hydroponic culture for a period of 10 weeks before they were harvested for analysis (cuttings in the 0.4 M treatment died four weeks after being placed in that solution). After being harvested, the plants were examined to determine the effects of water stress on general growth and floral morphology.

FIGURE 9. Hydroponic culture container.


Split Stopper
Air Line

Results and Discussion

Analysis of the plant material harvested from the osmotically-induced water stress revealed that as the degree of stress increased there was a decrease in total biomass, plant height, number of heads produced, peduncle length, ligule length, and receptacle length. These effects of water stress are summarized in Table 8. Figure 10 illustrates the effect of stress on floral morphology, and Figure 11 compares the 0.2 M NaCl and the 0.3 M NaCl treatment with greenhouse-grown R. tagetes (as a reference, Figure 2 illustrates "normal" R. columnaris and R. tagetes heads). The heads produced by the plants in the 0.2 M and the 0.3 M NaCl treatments were very similar in general appearance to the atypical individuals which had originally been collected as putative hybrids and fell within the range of measurements recorded for the diagnostic characters of these individuals (see Table 2).

The cytological and chemical analyses have indicated that the atypical individuals which had been collected as putative hybrids were not of hybrid origin. This, in conjunction with the similarity of the water stressed R. columnaris plants to these atypical individuals, convinced me that these putative hybrids were simply products of natural water stress. The apparent disparity between "normal" plants and the atypical forms in the

TABLE 8

EFFECTS OF IMPOSED WATER STRESS ON *R. columnaris*

Treatment	Dry Weight[1]	Plant Height[2]	No. of Heads[3]	Average Length[4] Ped.	Lig.	Recept.
Control	21.45 g	28 cm	32	16 cm	22 mm	24 mm
0.1 M NaCl	11.93 g	20 cm	24	14 cm	20 mm	22 mm
0.2 M NaCl	4.96 g	16 cm	17	9 cm	8 mm	9 mm
0.3 M NaCl	2.37 g	12 cm	8	5 cm	7 cm	8 mm
0.4 M NaCl*	------	------	------	------	------	------

[1]Total dry matter accumulated from all four plants in each treatment

[2]Average height of the four plants in each treatment

[3]Number of heads with some disc florets open

[4]Ped.=peduncle; Lig.=ligule; Recept.=receptacle

*All plants died four weeks after stress was initiated

FIGURE 10. Effects of water stress on R. columnaris floral morphology.

FIGURE 11. Comparison of floral morphology of water stressed R. columnaris and R. tagetes.

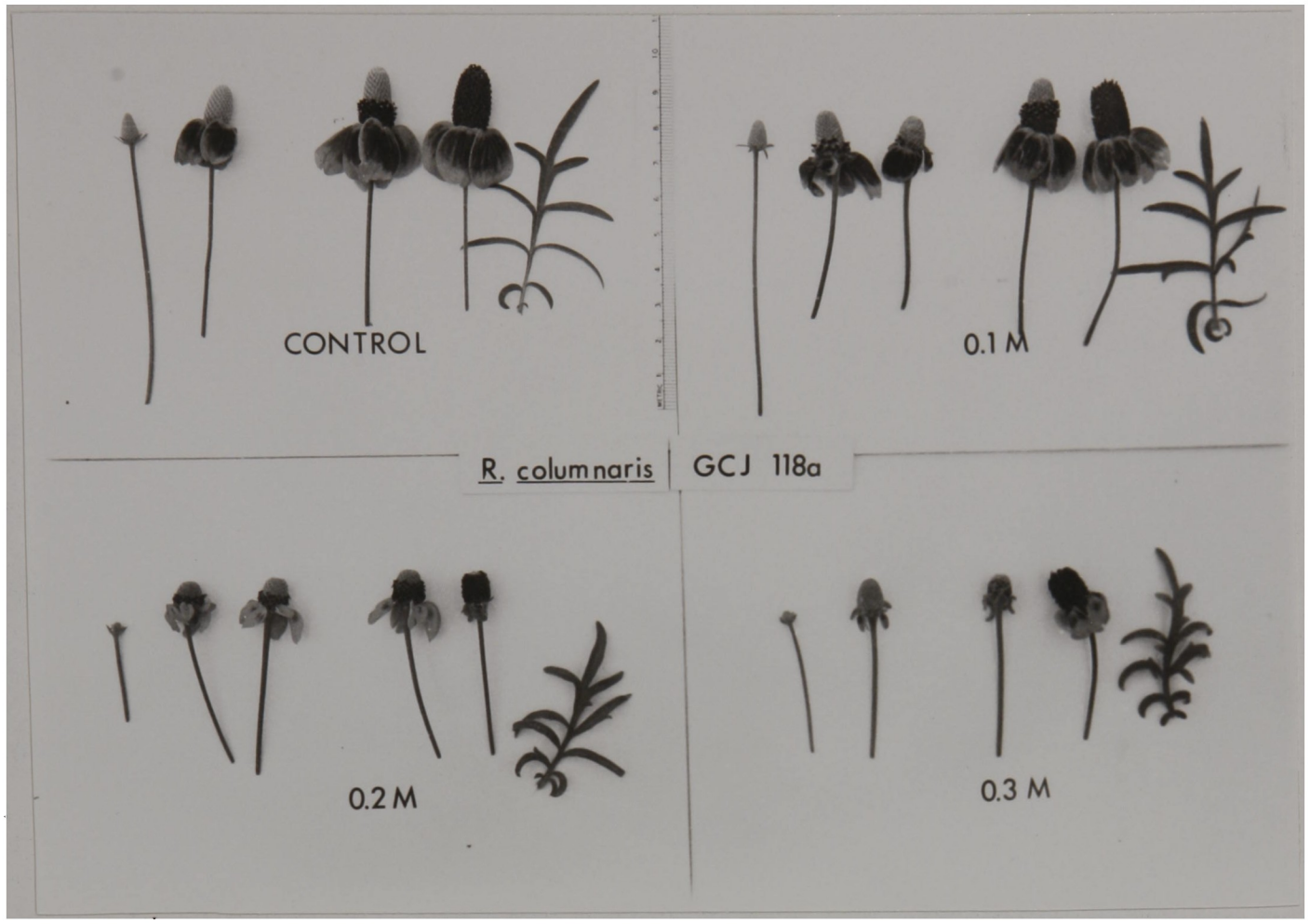


FIGURE 10.

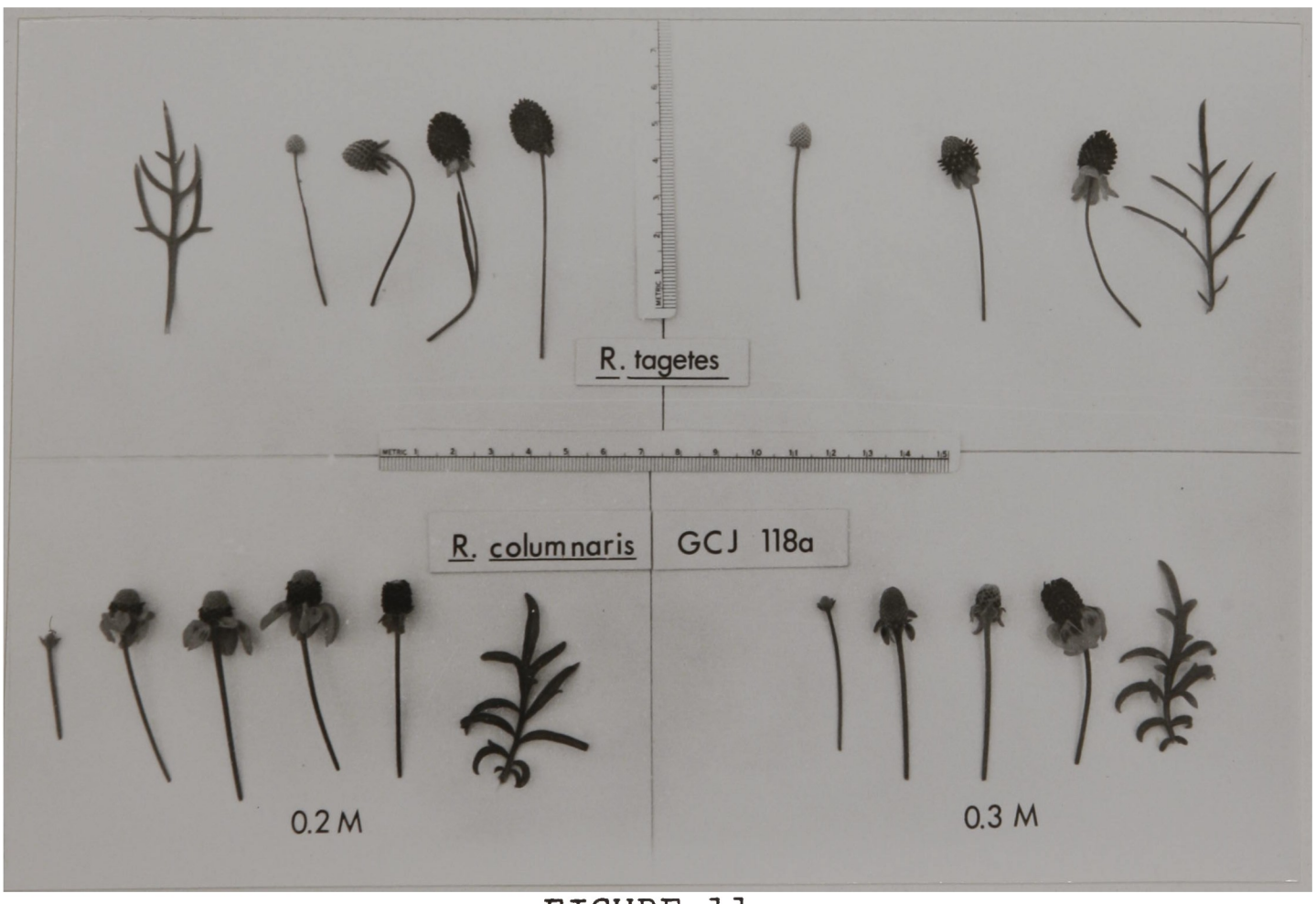


FIGURE 11.

same population can be explained by the fact that R. columnaris is weakly perennial which results in a mixed population of plants of different ages. The "normal" forms represent individuals of one to several years of age which have a well-established taproot system, whereas the atypical forms represent recently-germinated seedlings which are more sensitive to water deficits in the uppor soil profiles. In more mesic habitats, insect damage to the stem or root system is thought to have a similar effect.

CHAPTER VII

TAXONOMIC TREATMENT

As explained in the Taxonomic History (Chapter II), I am of the opinion that the name Rudbeckia columnifera should be regarded as a nomen subnudum and that Ratibida columnaris (Pursh) Rafinesque is therefore the valid combination. Furthermore, I am also of the opinion that the currently recognized (Richards, 1968) forma pulcherrima should be submerged for the following reasons: Several other taxa in this genus exhibit the same color variants and they are not recognized as subspecific taxa. If forma pulcherrima is recognized, then, to be consistent, the other color forms should also be recognized. This would result in considerable taxonomic confusion. In addition, there appear to be several subforms of forma pulcherrima, each having differing amounts of purple ligule coloration. Although it is possible that after the genetics of the various forms has been examined more thoroughly, they may yet warrant taxonomic rank. I currently do not recognize any infraspecific taxa. The synonymy for R. columnaris, then is as follows:

Ratibida columnaris (Pursh) Raf. Florula Ludovicia. p. 73. 1817.

Rudbeckia columnifera, Fraser's catal. 1813.
Rudbeckia columnaris Pursh, Flora Am. Sept. 2: 575 1814.

Rudbeckia columnaris Sims, Curt. Bot. Mag. 39 pl 1601 1814.

Ratibida sulcata Raf. J. Phys. 80:100 1819.

Obeliscaria columnaris DeCandolle, Prodr. 5:559. 1836.

Obeliscaria pulcherrima DeCandolle, Prodr. 5: 559. 1836.

Ratibida columnaris var. pulcherrima (DC) D. Don, Sweets Brit. Fl. Gard. Ser. 2, 4:361. 1838.

Lepachys columnaris Torr, & Gray, Flora N. Am. 2:315. 1842.

Lepachys columnaris Torr, & Gray var. pulcherrima (DC) Torr. & Gray, Flora N. Am. 2:315. 1842.

Ratibida columnifera (Nutt.) Woot. & Standl., Contr. U. S. Nat. Herb. 19:706. 1915.

Ratibida columnifera (Nutt.) Woot. & Standl. var. pulcherimma (DC) Woot. & Standl., Contr. U. S. Nat. Herb. 19:706. 1915.

Ratibida columnifera (Nutt.) Woot. & Standl. var. breviradiata Cockerel, Am. Natur. 49(586): 620. 1915.

Ratibida columnifera (Nutt.) Woot. & Standl. var. incisa Cockerel, Am. Natur. 49(586):620. 1915.

Ratibida columnifera (Nutt.) Woot. & Standl. var. tubularis Cockerel, Am. Natur. 49(586):620. 1915.

Ratibida columnifera (Nutt.) Woot. & Standl. var. appendiculata Cockerel, J. Hered. 7:428. 1916.

Lepachys columnifera (nutt.) MacBride, Contrib. Gray Herb. New Ser. 3(65):45. 1922.

Lepachys columnifera (Nutt.) Rydb. var. pulcherrima (DC) Rydb. Flora Prairies and Plains of Central North America 838. 1932.

Ratibida columnaris (Sims) D. Don forma pulcherrima (DC) Sharp, Ann. Mo. Bot Gard. 22:70-71. 1935.

Ratibida columnifera (Nutt.) Woot. & Standl. forma pulcherrima (DC) Fernald, Rhodora 40:353. 1938.

Ratibida columnaris (Pursh) Raf. forma denudata Boivin Natur. Can. 87:49. 1960.

Type Locality ---- No known type exists, however, evidence indicates that T. Nuttall collected the plants in "the country of Missouri" in 1813.

Distribution ---- From southern Canada to northern Mexico primarily in the prairies and foothills.

Selected specimens examined:

ARIZONA. Coconino Co., Clark 12142 (OKL).
COLORADO. Baca Co., Taylor 34 (TEX).
IOWA. Polk Co., Hayden 10546 (TEX).
KANSAS. Ottawa Co., Barker 2556 (SMU), Ellsworth Co., Raven 19494 (TEX).
LOUISANA. Ouachita Parish, Kral 20349 (SMU).
MONTANA. Mineral Co., Cronquist 6739 (TEX), Fergus Co., Olmstead G-24 (TEX).
NEBRASKA. Kearney Co., Stephens 6720 (SMU).
NEW MEXICO. Curry Co., Shinners 33245 (SMU).
OKLAHOMA. Mc Curtain Co., Herron 20 (TEX), Murray Co., Merrill 639 (OKL), Harmon Co., Stevens 1079 (OKL).
SOUTH CAROLINA. Berkeley Co., Ahles 53370 (SMU).
TEXAS. Presidio Co. Warnock 14604 (TEX), Grayson Co., Gentry 51-418 (TEX), Hale Co., Whitehouse 9927 (SMU), Earth Co., Gould 5615 (SMU), Ector Co., Shinners 33178 (SMU).
WYOMING. Albany Co., Goodman 776 (OKL).
MEXICO. Coahuila. F. Chiang 9208 (TEX)

I accept the taxonomic treatment of Ratibida tagetes of Richards (1968) and therefore the synonymy for this taxon as follows:

Ratibida tagetes (James) Barnh., Bull. Torr. Bot. Club. 24:410. 1897.

Rudbeckia tagetes James, Long's Exped. 2:68. 1823.
Rudbeckia globosa Nutt., J. Acad. Natur. Sci. Phila. 7:79. 1834.
Obeliscaria tagetes DC., Prodr. 5:559. 1836.
Lepachys columnaris Torr. & Gray var. tagetes Gray Smiths. Contrib. Knowl. (Pl. Wright.) 3:106. 1852.
Ratibida tagetes (James) Barnh. var. cinera Standley Muhlenb. 5:30. 1909.

Type Locality ---- Otero County, New Mexico "south of La Junta in prairie near stagnant pool" July 24, 1820, James (NY!).

Distribution ---- On prairies and high plains of Colorado, Kansas, Oklahoma, Texas, New Mexico, Arizona, and Chihuahua, Mexico.

Selected specimens examined.

ARIZONA. Apache Co., Damaree 48748 (OKL).
Colorado. Las Animas Co., Weber 4399 (TEX), Otero Co., McGregor 13323 (SMU).
KANSAS. Ellis Co., Runyon 14744 (SMU), Wallace Co., McGregor 18582 (SMU).
OKLAHOMA. Cimarron Co., Goodman 5393 (TEX), Cimarron Co., Goodman 3137.
NEW MEXICO. Colfax Co., Turner 4817 (TEX), Santa Fe Co., Rusby 78 (TEX).
TEXAS. Brewster Co., Warnock 4600 (TEX), Culberson Co., Tharp 49-1002 (SMU), Deaf Smith Co., Shinners 8334 (TEX), El Paso Co., Warnock 5808 (TEX), Parmer Co., Shinners 8330 (SMU), Pecos Co., Tharp 43-939 (SMU), Ochiltree Co., Cory 32228 (TEX), Sherman Co., Shinners 8225 (SMU).

CHAPTER VIII

SUMMARY AND CONCLUSIONS

In a preliminary examination of sympatric populations of R. tagetes and R. columnaris to determine the extent of introgression, some individuals were found which exhibited intermediate floral morphology. The cytological and, to a limited extent, chemical analyses of the individuals revealed that they were not of hybrid origin but were "atypical" forms of R. columnaris. In an effort to determine the environmental factors which caused the "atypical" forms, individuals from a clone of R. columnaris were subjected to varying degrees of water stress. The individuals grown under the higher water stress had a floral morphology almost identical to the "atypical" forms found in the field. From this, I concluded that natural water deficits were a primary factor in producing these "atypical" individuals.

In addition, Jackson (1963) was successful in producing only a single artificial hybrid which was highly sterile, and I was unable to produce a hybrid which lived beyond one week. Therefore, I am of the opinion that the probability of a natural hybrid being produced is very remote and that introgression between the two taxa at this time is nonexistent. There does, however, seem to be considerable

variation within each taxon especially within R. columnaris. More extensive studies of the color forms and chromosomal races of 2n=26 and 2n=28 are needed before the full significance of these variation can be determined.

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APPENDIX

APPENDIX A

RETENTION TIMES OF KNOWN SUGARS

Sugar	Retention Time Peak min	Peak sec	Peak min	Peak sec
Arabinose	4	35	5	00
Galactose	11	35	13	45
Glucose	12	53	18	20
Phamnose	4	40	5	50
Xylose	6	10	7	25

NOTE: A Beckman model GC-5 with a flame ionization detector equipped with a 6 ft x 0.25 inch coiled aluminum column packed with 2% SE-33 on Chromosorb W (HP AW DMCS 100/120, Analabs lot no. 011-3) maintained at 165° C, with a carrier gas flow rate of 40 ml/min was used for the analysis.

APPENDIX B

CHROMATOGRAPHIC CHARACTERISTICS OF ADDITIONAL COMPOUNDS ISOLATED

Compound no.	Rf Values		Color	
	TBA	HOAC	U.V.	$+NH_3$
16	.10	.22	B	B
17	.50	.88	P	Y
18	.72	.71	P	Y
19	.37	.74	P	Y
20	.45	.78	P	Y
21	.45	.65	P	Y
22	.51	.21	P	Y
23	.50	.31	P	Y

NOTE: B=blue; P=purple; Y=yellow