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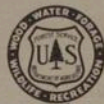
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Shelterbelt Establishment and Management
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STUDY PLAN 1501.20

TAXONOMIC INTERRELATIONSHIPS BETWEEN JUNIPERUS VIRGINIANA L.
AND J. SCOPULORUM SARGENT IN THE MISSOURI RIVER BASIN



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SUMMARY OF STUDY PLAN No. 1501.20

TAXONOMIC INTERRELATIONSHIPS BETWEEN JUNIPERUS VIRGINIANA L. AND
J. SCOPULORUM SARGENT IN THE MISSOURI RIVER BASIN

Natural populations of arborescent Juniperus will be purposively sampled throughout the Missouri River Basin to:

1. determine the extent of variation in certain morphological characters of foliage, cones, and seeds of Juniperus virginiana and J. scopulorum and their putative hybrids,
2. elucidate some of the interrelationships and/or the hybridity between the two species for use in breeding and tree improvement programs.

Fresh and preserved samples of foliage, cones, and seeds will be collected from variable numbers of trees at each of many sampling locations in natural juniper stands scattered throughout the Missouri River Basin. Specimens will be taxonomically evaluated for character differences. Foliage characters to be examined will include: leaf-tip shape, percent overlap of leaf-scales, ratio of leaf-scale gland length to distance to tip of leaf-scale, ratio of leaf-scale gland length to width, width of epidermal cells, and width of leaf-scales. Cone and seed characters will include: number of years to seed maturity, curvature of peduncles, number of seeds per cone, length and width of ovulate cones, ratio of seed length to seed width, and seed markings. Characters of length, spacing, amount and arrangement of branchlets will be analyzed. Field measurements of tree form and size, topographic descriptions, and photographs will be analyzed for their contribution to the analysis of data.

The data will be analyzed graphically, statistically, and photographically.

The results of the study will be presented to the Rocky Mountain Forest and Range Experiment Station for publication in a technical journal or station paper. The manuscript will be presented for technical review on or about June, 1967.

TAXONOMIC INTERRELATIONSHIPS BETWEEN JUNIPERUS VIRGINIANA L. AND
J. SCOPULORUM SARGENT IN THE MISSOURI RIVER BASIN

PROBLEM

Juniper has been used since early pioneer days as a basic component of the shelterbelt plantings which, by the thousands, dot the prairies of the Great Plains from Texas to Canada.

It has frequently been observed that shelterbelts containing conifers have improved in effectiveness over the years, while those not containing conifers have, in general, deteriorated. Engstrom (1940) stated that "conifers, particularly the cedars, are the soundest foundation upon which Plains forestry tree planting can be built. Long-life, adaptability to a wide range of site conditions, year-round protection, regeneration, wildlife value, and comparative freedom from rodent, insect, and livestock damage, as well as disease resistance combine to give the cedars a popular and technical appeal which assure them in my opinion, the most prominent spot in future planting operations".

Concepts of planting and design of field shelterbelts^{1/} have shifted during recent decades from the wide plantings of the 1930's and 1940's containing only 10 to 20 percent coniferous species to narrower belts often consisting entirely of eastern redcedar (Juniperus virginiana L.). These narrower plantings are spaced at 20 to 30-rod intervals across the fields and, along with strip-cropping and "stubble-mulch" tillage, are intended to provide year-round protection to the fields and crops.

^{1/} Terminology in accordance with definition of field plantings given in "Forestry Terminology-- a glossary of technical terms used in forestry." Soc. Amer. For., 1958, p. 76.

Preston and Brandon (1946) have stated that a good all-purpose protective tree planting for northeastern Colorado would consist of two to six rows of ponderosa pine with an exterior row on each side of Rocky Mountain juniper (Juniperus scopulorum Sargent). Thus this native, drought-resistant, and long-lived genus indeed occupies an eminent position in the list of plant materials used in protective tree plantings throughout the Great Plains. This is particularly true in the Central and Northern Great Plains where interest in tree planting - to control soil erosion by winter as well as summer winds - has increased markedly during recent years.

The Central and Northern Great Plains lie within the Missouri River Basin except for a portion of northern North Dakota, southern Kansas and southeastern Colorado. This basin represents a well-defined, natural land unit. Thus the Missouri River Basin, rather than the boundaries of the Great Plains or politically determined state boundary lines will define the boundaries of this study.

Tree improvement and breeding programs designed to develop strains of conifers best suited for withstanding the rigors of Plains environment are planned for the future.^{2/} Read (1956) stated that: "the aim of tree improvement research for Plains planting should be to produce planting stock that will give highest survival and satisfactory growth and longevity under adverse conditions." He further pointed out that research may provide superior planting materials by: (1) selection of seed from

^{2/} Dawson, David H.
1964. Technical problem analysis. U.S.D.A.-- F.S. Lake States
Forest Experiment Station. 65 pp. (refer to pages 10,15,43,51,56 &57).

better stands, (2) selection of seed from better individual trees, (3) controlled breeding of superior individuals, (4) hybridization, and (5) vegetative propagation of superior individuals. To proceed effectively toward these objectives, however, we must first have an adequate understanding of the extent and the nature of the variation of the materials with which we are working.

The importance of seed source is now well established. A study of the comparative taxonomy and morphology of the juniper populations within the Missouri River Basin would provide information on some of the important variable characters of the genus and pinpoint the locations of the variability. If considerable variation exists within the juniper population in the Upper Great Plains a knowledge of that variation and where it is located would be immediately useful to nurserymen who make annual seed collections within the study area. This investigation would also represent a "first-step" leading to extensive provenance testings of juniper seed sources, as suggested in the research programs above. Some information relative to sexual structures and their development may also be gained from the study. This would be a significant contribution of immediate use in tree breeding work.

PAST AND CURRENT WORK

Classification

Chamberlain (1957) classified the genus Juniperus as follows:

Division.....	SPERMATOPHYTA
Sub-division.....	GYMNOSPERMAE
Order.....	CONIFERAE
Family.....	CUPRESSACEAE
Genus.....	JUNIPERUS

Other classifications and descriptions of the genus and its components are given by Sudworth (1915), Gates (1928), Sargent (1933), Van Dersal (1938), Rehder (1940), Fassett (1945c), Fernald (1950), Preston (1947), Pool (1951), Phillips et al (1953), Dallimore and Jackson (1961) and others.

Endlicher (1847) created three sections in the genus Juniperus: Caryocedrus (woody, cupressoid-like cones), Oxycedrus (fleshy berry-cones), and Sabina (acicular-type leaves to reduced scale-type leaves). Of the three sections, Sabina is by far the largest section containing about thirty species. The two species to be investigated in this study are both members of the Sabina section.

Hall (1952) gives a key to the native species of the Sabina section applicable to species found in the Great Plains region. He describes the two species in question as follows:

(Extract from key):

- A. Leaves with entire margins.
 3. Leaves overlapping, tips acute; glands oval or rarely elliptic and shorter than the distance from the gland to the leaf tip.
 4. Upright tree; fruit blue-black, less than 6.5 mm. in diameter.
 5. Leaves 3-4 mm. long; fruit 3.5-6 mm. in diameter, very slightly longer than wide..J. virginiana.
 3. Leaves not overlapping, tips obtuse, glands elliptic or rarely oval and longer than the distance from the gland to the leaf tip.....J. scopulorum.

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History

Eastern redcedar has been recognized since colonial days for its durability, cutting qualities, rich color, and aroma (Williamson, 1957). It is said that Louisiana's capital, Baton Rouge, took its name from this tree - the name being given to the tree by early French colonists (U.S. Forest Service, 1955). Harrar (1957) cites several interesting references to the species by the early English colonists as early as 1632, and in 1749 by Benjamin Franklin. Linnaeus (1753) named the species J. virginiana, apparently from specimens found in the English colony of Virginia.

Rocky Mountain juniper was long considered to be a western form of eastern redcedar, the principle difference being that the former required two years for seed maturity while the latter required only one year. Rocky Mountain juniper, then called mountain redcedar, was first discovered by Lewis and Clark in 1804 (Sudworth, 1915). Sudworth explained that the first technical name given the tree was J. excelsa Pursh in 1814 (preoccupied). Between 1838 and 1897 it was called J. virginiana, J. occidentalis and J. virginiana var. montana. Sargent (1897) distinguished the tree from J. virginiana in 1897 and named it J. scopulorum, although Vasey had given it the common name Rocky Mountain redcedar in 1876 and had pointed out differences between its crown form and that of eastern redcedar. Except for his unfortunate use of a preoccupied name (var. montana), Dr. Vasey's name probably could now be maintained.

Distribution

Juniperus is widely scattered over the northern hemisphere from the arctic zone to the mountains of the tropics. Hall (1947) states that about 40 species are recognized. Harlow and Harrar (1937) state that 13 species are native to the United States.

The distribution of J. virginiana is from southern Maine, southern Ontario, southern Wisconsin, southern Minnesota, most of the eastern half of South Dakota, extending into the southwestern corner of North Dakota; then southward through the eastern half of Nebraska, most of Kansas and Oklahoma (excluding the Panhandle) into northeastern Texas. The extent of the southward extension is not clearly established; but it is in most of Arkansas, the northern portions of Mississippi, Alabama, Georgia, South Carolina to the Atlantic coast and northward again to southern Maine (Williamson, 1957).

Juniperus scopulorum is found throughout the drier, lower mountains and foothills of British Columbia and Alberta, Canada, south through the Great Basin, Rocky Mountain and Northern Plains States to New Mexico and northern Arizona (Sudworth, 1908, 1915). Its distribution in the Northern Plains states, however, is limited to the western portions of North Dakota, South Dakota, Nebraska and northeastern Colorado (Herman, 1958).

Distribution maps for one or both species are illustrated by Williamson (1957), Herman (1958), Hall (1952), Little (1949), Pool (1951), Fassett (1944b and 1945c) and Sudworth (1915).

Taxonomic Studies

Taxonomic varieties or subspecies are described from wild trees observed in their local habitats or from herbarium specimens; the differences are assumed to be due to genetic factors. Genetic ecotypes or races are described from carefully replicated nursery and field tests by which differences can be proved to be due to genetic factors. Wright (1962) has pointed out that the taxonomic approach is not exact because it does not offer a means of separating environmental from genetic effects. He explains, however, that it is inexpensive, rapid, and could be used to supplement genetic studies to the extent to which it produces genetically reliable results.

Wright (1962) states that Jens Clausen, William Hiesey, and David Keck, working in California for the Carnegie Institute of Washington, have played leading roles in advocating combined taxonomic and genetic investigations.

Critchfield (1957), in his taxonomic study of lodgepole pine (Pinus contorta Dougl.), found that his own and previous "genetic" data corresponded very well with the division of the species into four taxonomic subspecies. Wright and Baldwin (1957) used the data from a 17-year-old replicated provenance test of Pinus sylvestris L. in New Hampshire as a standard against which to compare 19th Century taxonomic descriptions of geographic varieties and unreplicated 20th Century "genetic-type" provenance studies. They found that herbarium studies made by leading 19th Century taxonomists led to a better insight into the pattern of variation than did many low-precision provenance tests.

Callaham (1961) strongly advocates an experimental taxonomic approach and concludes that this kind of approach to the analysis of geographic variation should precede seed source studies. He states that physical and biological limitations, limited size, high costs, and the long wait for results all tend to reduce the fact-finding efficiency of the traditional seed source-provenance testing approach. He points out, further, that because of their limited size and high cost, seed source studies can usually accommodate only a few scattered sources and can be represented at a limited number of costly outplantings. This usually precludes keeping track of progenies of individual trees since bulk collections of populations are used and the identity of the individual is lost. At the conclusion of a seed source study one then has the reverse problem of extrapolating from the progeny performances at one plantation site to predict performance at another area a few miles away. He also cites lack of control of the planting site environment as a serious limitation in a seed source study. Extreme fluctuations in one or more environmental factors such as drought, frost, or pests can destroy an investment of many years and of thousands of dollars.

Callaham (1961) lists several advantages of the experimental taxonomic approach over the traditional seed source:

1. the number of populations of a species subjected to analysis can be large.
2. the individual variability within populations is easy to follow,
3. the independent or combined influences of factors of the environment can be tested on each genotype,

4. the genetic responses of progenies to many combinations of environmental factors can be noted, hence the plastic range of the phenotype can be mapped,
5. the pattern of inherent physiological variation can be depicted accurately, for the environmental conditions under which the progenies are grown can be precisely controlled and infinitely varied, and
6. the heritable differences between populations and between individuals within populations can be described.

Ideally, this new approach should encompass the greatest possible number of morphological and physiological characters from many individuals in numerous populations.

Kriebel (1957) studied the sugar maple complex and showed that in the southern part of the range the break in various growth characters corresponded with the difference between Acer saccharum Marsh. and A. barbatum Michx. He did find, however, that in the northern part of the range the growth-character variation pattern did not correspond to the distribution of A. saccharum Marsh. and A. nigrum Michx.

Wright (1944) found that the presence of pubescence in a population of white ash (Fraxinus americana L.) was a good indicator that the population belonged to either the southern or intermediate ecotypes.

Several workers have reported on taxonomic investigations of the genus Juniperus. The number working specifically with the species and in the area to be investigated in this particular study is limited, however. These and some of the others will be discussed here, especially as they pertain to J. virginiana and J. scopulorum.

Hell (1947) did a taxonomic study of Juniperus in Oklahoma. He reported that eastern redcedar is generally distributed throughout Oklahoma, except in the extreme western portion, including the Panhandle. He concluded that Rocky Mountain juniper was not to be found in Oklahoma, although he reported specimens from northwestern Oklahoma which showed intermediate characteristics tending toward both species. His variants were similar to those described by Fassett (1944b) as hybrid swarms in the zone of species overlap between the two species.

Hall (1961a) states that in the Northern Great Plains there are a great many hybrids of redcedar and Rocky Mountain juniper and that many horticulturally valuable variants have been found in this area by such men as D. Hill and others. He relates that the percentages of many of these variants cannot be identified positively because the local populations from which they were obtained have not been properly studied.

Hall (1961b) lists three major processes distinct in both operation and result which set on a population either simultaneously or successively to cause variation. They are inbreeding, differential selection, and hybridization. Effects of inbreeding are not apparent in Juniperus. Differential selection produces divergence consequent to environmental adaptation. Where genotypes persist in the face of the production of abundant hybrid progeny, it is by the selective advantage of the adapted genotypes and the elimination of the hybrids. He states that hybridization may result in swamping partial discontinuities between divergent populations through the resumption of gene interchange. After crossings between adapted races or species, there is further elimination of hybrid genotypes poorly adapted to habitat conditions and increase for those hybrids suited to the disturbed habitat.

Hall (1947) acknowledges a taxonomic relationship between eastern red cedar and Rocky Mountain juniper but states that the species are sometimes difficult to distinguish. He states that in the regions where the two species meet it is necessary to turn to quantitative characters, since there apparently are no qualitative characters which separate them.

Fassett (1944a) found three factors which makes the differentiation of the two species difficult. First, the variation within either one of the species is often more conspicuous but never as constant as the differences between them. He found that the variation within any colony of either species is often as great as within the species as a whole. This is probably unlikely since it suggests, in effect, that a "part" is greater than the "whole" Second, the specific characters are highly variable and necessitate the use of statistical analysis in differentiating the species. Third, the two species may occur together in a colony and hybrid forms may or may not be found.

Fassett (1944b) reported on presumed hybrid swarms of J. virginiana and J. scopulorum. He based his findings on 16 sampling sites (six in Kansas; four in Nebraska; four in South Dakota; and two in North Dakota). He concluded that: (1) where each species grows by itself, each retains pure specific characters; (2) in the western part of the J. virginiana range certain tendencies toward J. scopulorum suggest an ancient incursion of that species; and (3) where the ranges of the two species meet, all recombinations of the characters of each occur in individuals of one colony.

Van Haverbeke (1965) made a preliminary study of the genus in Nebraska. This study verified the presence of two arborescent species: J. virginiana L., in the eastern two-thirds and J. scopulorum Sargent, in the western portion of Nebraska. Interpretation of hybrid indices in this study suggested that: (1) the western population more closely resembles its pure parental type; than the eastern population resembles its pure parental type; (2) specimens in the western part of the J. virginiana range express tendencies toward

J. scopulorum and , (3) J. scopulorum may influence the juniper populations throughout Nebraska, along easterly flowing river drainagess, as well as in the zone of species overlap or abutment.

Other taxonomic and cytological studies within the genus include (Fassett, 1943, 1945a, 1945b, 1945c; Mathews, 1939; and Ross and Duncan, 1949).

Taxonomic Characters

Fassett (1944a) reports that J. virginiana and J. scopulorum differ in ten sets of characters as follows:

1. percent of peduncles curved
2. shape of leaf tips
3. relation of gland length to distance to tip of leaf
4. overlap of leaves
5. width of epidermal cells
6. branching character of stems
7. number of years to fruit maturity
8. size of fruits
9. number of seeds per cone
10. seed markings

Hall (1952, 1961b and 1964) used the following characters to distinguish various species of juniper:

1. length of terminal whip leaves
2. gland length-width ratio for whip leaves
3. distance from tip of gland to tip of leaf
4. length of lateral whips
5. berry-cone diameters
6. percent of decussate spur shoots on secondary shoots
7. leaf margins (scored as smooth, denticulate, or intermediate).

Cytological Studies

Mathews (1939) studied the morphology and cytological development of sporophylls and seed of eastern redcedar in North Carolina. He also included a review of past cytological work in the genus. He studied the morphological and cytological development of the staminate strobilis and microsporophyll, pollen mother cells, male gametophyte, ovulate strobilus, megasporangium, female gametophyte, pollination, fertilization processes and embryo formation.

Ross and Duncan (1949) reported on some cytological evidences of hybridization between J. virginiana and J. horizontalis in the "Driftless area" of Wisconsin. Their results were based primarily on a study of meiosis in pollen mother-cells of presumed hybrids and parental types. They found an imbalance in the somatic chromosome complements as evidenced by the presence of heterobrachial chromosomes without counterparts in pollen mother-cells during meiosis.

Analysis

Taxonomic data normally lend themselves well to graphical analysis. Such an analysis, is in most instances, equally as effective and meaningful as a purely statistical analysis. The essence of taxonomy is the accumulation of supporting evidences from examinations of specimen characters from populations made up of variable individuals. As in most other biological areas of study, one can rarely say with absolute certainty that any given character always expresses itself in a given manner. Therefore, it is through the process of amassing evidence from various fields of investigations that one is able to establish reliable trends within or between variable populations which are so clear that statistical verification is oftentimes of little significance.

Methods of graphic treatment of quantitative data have been used by Thorbjornsen (1961), Mergen (1958), Fowler and Heimburger (1958), Mergen and Furnival (1960), Clifford (1955), Fisher (1936, 1938), Hall (1952, 1961b, 1964) Critchfield (1967), Davidson (1947) and Ross and Duncan (1949).

Dice and Leraas (1936), Hubbs and Perlmutter (1942) and Simpson and Roe (1939) presented methods of illustrating statistical data graphically. Standard statistical methods, applicable to analysis of quantitative taxonomic data are also available in Snedecor (1956) and Fresse (1956).

OBJECTIVES

The objectives of this study are:

1. to taxonomically determine the extent of variation in morphological characters of foliage, cones, and seed of Juniperus virginiana and Juniperus scopulorum in the Missouri River Basin with special attention to major river drainages and to the reported areas of species overlap and/or abutment in the Great Plains; and,
2. using the information obtained in objective 1, to elucidate the interrelationships and/or hybridity between the two species within the above geographic region.

METHODS

Sampling

Natural populations of Juniperus will be purposively sampled throughout the Missouri River Basin (Appendix A). Several major east-flowing rivers: Milk, Yellowstone, Missouri, Cheyenne, Niobrara, Loups, Platte, Republican, Solomon and Kansas and their tributaries are contained within this Basin. Most of these rivers have their origin in the range of J. scopulorum and their ending in the range of J. virginiana. It is possible that these drainages could serve as avenues of species intrusion, thus enabling one population to exert an influence upon the other. This could be a major factor contributing to the variation in Juniperus throughout the Plains. Areas of species overlap or abutment are also considered prime areas in which variation and presumed hybridization between the two species could be found (Appendix A).

Approximately fifty to seventy-five sampling sites will be purposively selected throughout this major drainage system. Strongest emphasis will be in sampling along major river drainages and their tributaries and in the areas of reported or suspected species overlap or abutment (Appendix A).

The initial collection will consist of two trips. The first, in September, 1965, will sample juniper populations north and westward up the Missouri River from Nebraska through the Dakotas and into Montana; then southward and eastward down the Yellowstone River, through the Black Hills into central Nebraska (Appendix A). The second trip in October, 1965, will sample southward down the Missouri River into Kansas and Missouri; then westward through Kansas along the Kansas and Solomon Rivers into northeastern Colorado, Wyoming, and then eastward into Nebraska along the Platte River Valley (Appendix A).

Precise routes will be determined after consulting various state botanical publications describing the flora and its distribution in each state to be sampled. Supplemental sampling will be done later that winter or in the fall of 1966 if necessary.

It is anticipated that a minimum of ten individuals will be sampled from each local population (five male and five female). However, there may be instances where less than ten trees will be available. More important, however, the sample taken should reflect the variation present within the population. Thus at some collection sites, considerably more than ten individuals may be sampled.

Each local population will be visually inspected for the amount of variation present. Trees representing extremes of variation will be sampled first; then additional individuals lying between these extremes will be sampled. Thus, fewer individuals from less variable populations will be required, and greater numbers of individuals will be needed from highly variable populations. The aim of this scheme will be to collect enough material to insure adequate sampling between the extremes represented in each population.

Identification of "pure" parental types of both species will be necessary. Therefore, specimens from more distant parts of their respective ranges will also be examined. These will be obtained either by requesting in-service agencies for supplemental collections or the loan of herbarium specimens from appropriate institutions. Sampling of an east-west transect across the respective ranges should accomplish this.

Foliage and Cone Collections

Four branch tips each approximately 12-inches long, or long enough to include all current year's growth, possessing staminate or ovulate strobili, and scale-like leaves, will be cut from each sample tree. Branches will be cut from that part of the crown fully exposed to light (south side) to avoid possible morphological differences associated with exposure. Branch tips will be selected at a height equal to one-half the height of the crown whenever possible. The cut branch tips from each tree will be placed in conventional botanical presses for drying and storage. Material from each sample tree will be labeled with an identifying number (Example, VM-8-10; Van Haverbeke, Montana, collection site no. 8, tree no.10).

Four (two ternate and two tertiary) branchlet segments, approximately two-inches long, and five staminate or five ovulate cones will also be collected from each sample tree. These will be placed immediately in properly marked glass vials (3-inches tall) containing a standard killing and fixing solution (FPA) as in Sess (1958). Reproductive structures will be sectioned in half with a razor blade prior to its immersion in the fixative solution to insure penetration of the fixative. This material will be kept as reserve material for possible future investigation of anatomical characters of foliage, staminate and ovulate reproductive structures.

In addition, seed will be collected from each female tree sampled. The seed from each tree will be handled as a single lot and will be kept moist and later placed in cold storage for possible use in future tree improvement studies of Juniperus seed sources.

Field Observations

Field observations of location, elevation, soil type, and a brief topographic description of each collection site will be recorded. Also, for each individual tree sampled, the diameter (d.b.h.), height, age (increment boring), crown spread, bark thickness, branching habit, foliage color and cone persistence will be noted for consideration in the analysis of data (Appendix B). Although it is not planned to revisit the sample areas, the location of each tree will be mapped and marked by spray painting a number on the stem at breast height. This will insure relocation of sample trees if it is necessary to revisit them later for additional material or measurements. Photographs will be taken of representative specimens and/or of general areas within certain sampling sites if needed to describe conditions of topography and tree forms.

Measurement of Characters

A preliminary taxonomic study of the genus Juniperus in Nebraska by the author (1965) and other investigations within the genus by Hall (1947, 1952), Hall et al (1961b) and Fassett (1944a, b, 1945 a,b,c)) have identified a series of valid morphological foliage, cone, seed and branch characters.

The following characters will be analyzed in this study:

Foliage

1. Leaf-scale tip shape - qualitatively determined by comparing leaf-scale tips with standard samples of acuminate, acute, sub-acute or blunt.
2. Percent overlap of leaf-scale - quantitatively measured in mm. as the distance from the upper leaf-scale tip to the next lower leaf-scale tip and from the tip of the upper leaf-scale to the point of intersection of the lower extremities of the upper leaf-scale with the edge of the lower leaf-scale.
3. Ratio of length of leaf-scale gland to distance from its upper end to the tip of the leaf- mm. (converted to a number).
4. Ratio of leaf-scale gland length to leaf-scale width (mm. converted to a number).
5. Width of epidermal cells (microns). Measured with compound microscope).
6. Width of leaf scales (mm.)

The above values will be determined by randomly selecting and measuring six leaf-scales, three from each of two fresh branch tips.

Cones and Seed

1. Number of years to seed maturity. (1 year vs. two or more years)
2. Length of cone in mm.
3. Width of cone in mm.
4. Number of seeds per cone.
5. Shape of pits or grooves on seed coat(qualitatively by scores).
6. Ratio of seed length to seed width.

With the exception of no. 1, values will be determined by selecting six cones, three from each of two pressed branches for analysis.

Branching Habits

It is not known at present whether or not reliable comparisons of branching characteristics will be possible. Characters listed below are based on those used by Hall (1952, 1961b, 1964). They will be considered during the course of this investigation and will be utilized if deemed desirable.

1. Length of tertiary branches
2. Relative spacing of tertiary branches
3. Amount of long shoot growth on secondary branches
4. Amount of long shoot on tertiary branches
5. Arrangements of leaves (decussate vs. ternate) on secondary and tertiary branches.

Cones and Embryos

No specific characters have been reported for microsporophylls, microsporangia or microspores. Some useful characters may be recognized following examination of preserved staminate strobili and their contents.

It is also possible, although not included within the scope of the present study, that preserved ovulate cones can be utilized for a study of developing embryos. Prepared sections of immature embryos of J. scopulorum could be compared with comparable sections (in time) of J. virginiana as illustrated for J. virginiana by Mathews (1939), to gain some knowledge as to why seed matures in one year in J. virginiana and two years in J. scopulorum.

It seems likely that the difference in length of time required for seed maturity between the two species probably involves a delay or an "arrest" in an early developmental stage of either the male or female gametophyte following pollination since both species apparently hybridize with ease. For example, the development of the [♀]archegonium, containing the megaspore mother cell, may be slowed or delayed resulting in the postponement of fertilization until the following spring. If this were

true, then one might expect to find the pollen tube of the germinated pollen grain (microspore), containing the undivided body cell and pollen tube nucleus, either still growing or its development arrested in the nucellus tissue of the female gametophyte, rather than inside the archegonial chamber.

Other Characters

Data collected and listed under the section "Field Observations" (diameter, height, branching habit, geographic and topographic features) will be analyzed for possible correlation either among themselves or with characters considered under the above categories.

Quantitative values for tree characters will be obtained by measuring sample material with dissecting or compound microscopes equipped with ocular micrometers. Qualitative data will be derived by comparison of characters in question with a set of standard ratings.

It cannot be stated with absolute certainty which of the above characters, or others, will be utilized to analyze the specimens in the present study. They will, nevertheless, form the basis for evaluation of the material collected for analysis.

Further, in a study of this type, one cannot always foresee all possibilities for investigation. It is entirely possible that one will be led into an unconsidered but important area of investigation. If this happens and the results promise to add significant meaning to the study, it will be pursued. It is also reasonable to assume that some of the characters listed above may prove unsatisfactory. If so, they will be eliminated from the analysis.

ANALYSIS

Analyses of the data will be mainly graphic, and wherever needed, mathematical. The intent will be to compare the different populations, character by character, to determine any continuous or discontinuous variations-- using the two "pure" types as the extremes of the base.

Graphic analyses will be in the form of character frequency distributions, correlations, character-correlation associations, character-geographic area associations, and hybrid indices. Examples are shown in Appendix C.

Hybrid indices will be determined for each presumed or putative hybrid population. These will be compared with each other and with the values of the pure species in the standard manner. Also, an attempt to determine if a hybrid habitat exists, and to gain some knowledge of clinal variation; phenotypic variations in the populations will be correlated with climatic conditions where possible.

Maximum, minimum and mean values, standard error of means, and significant differences will be presented graphically.

Since the data will be multivariate, any mathematical analyses will be patterned after methods outlined by Sokal and Sneath (1963) for processing numerical taxonomic data.

PRESENTATION OF EXPECTED RESULTS

The results of this study will be presented to the Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. The work will be prepared for publication in a technical paper (Forest Science, Amer. Jour. Botany, or Silvae Genetica) or, as a station paper. Figures, tables, analyses and photographs will be included.

MISCELLANEOUS

Personnel Assignments

Van Haverbake will be responsible for all phases of the study. Some cooperators will be contacted for collections outside the Missouri River Basin. Hovlond will assist in the field collections and laboratory work.

Time Schedule

<u>Job</u>	<u>Date Initiated</u>	<u>Completed</u>	<u>Man-days</u>
Field Collection	September, 1965	November, 1965	30
Laboratory Analysis	December 1965	July, 1966	100
Field Collection	September, 1966	September, 1966	10 (if needed)
Laboratory Analysis and Write-up	October, 1966	June 1967	240

Costs

(other than salaries)

Per Diem (2 men, 30 days @ 12.00/day).....	\$720.00
Vehicle....(5000 mi.@ 9¢ mi.).....	450.00
Equipment.....	200.00
	<u>\$1370.00</u>

Equipment Needs

Field

vehicle	camera and film	collection vials
maps	record forms	preservative fluids
diameter tape	field notebook	india ink and pens
bark gauge	ID tags	specimen labels
abney level	press and blotters	hand clippers
pocket knife	altimeter	50' tape

Laboratory

dissecting or binocular scope	rotary or freezing microtome
compound microscope	dehydration, infiltration and
ocular micrometer	staining and mounting materials.
paper envelopes	

The above equipment is now available or can be purchased at minimal cost.

APPENDIX A

Juniperus distribution map and
route of sampling anticipated.

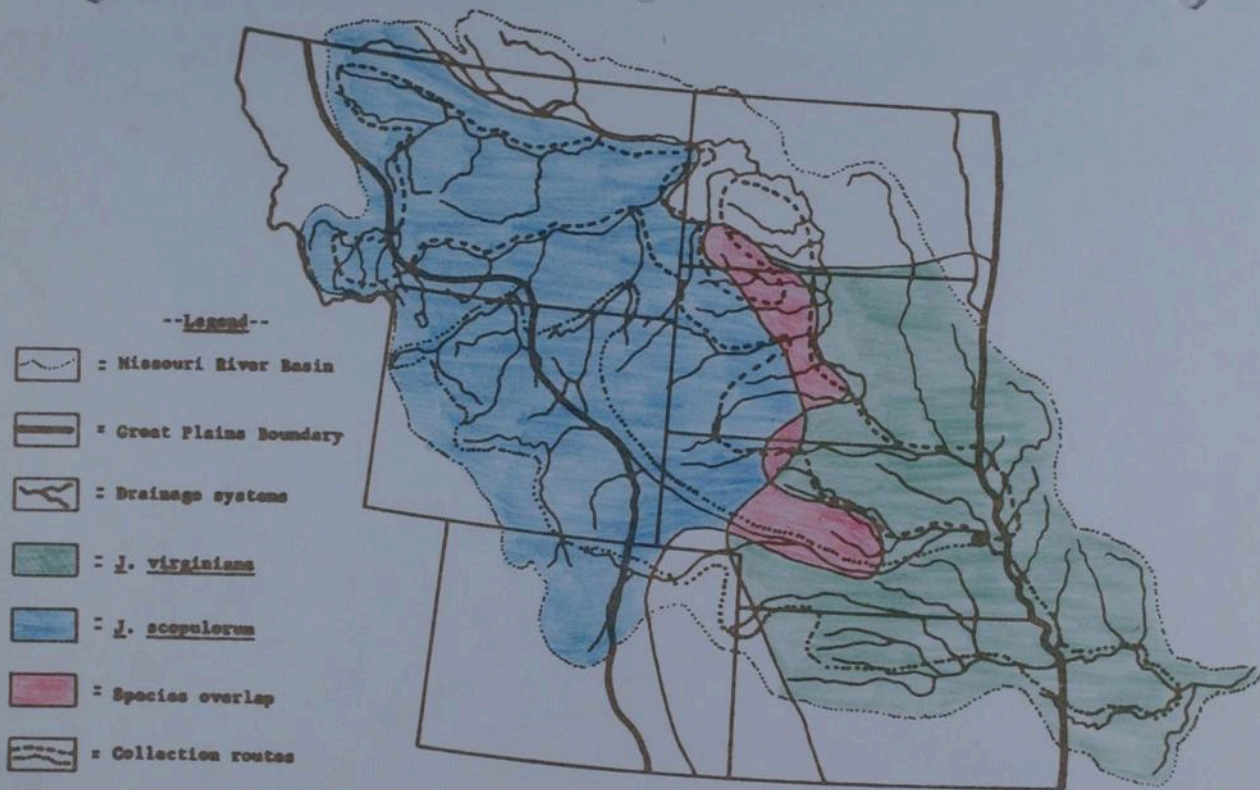


Fig. 1.-- Missouri River Basin showing boundaries of Great Plains, drainage systems, ranges of *J. virginiana* and *J. scopulorum*, areas of species overlap and collection routes.

APPENDIX B

Field Record Form

FIELD RECORD FORM

Study No. 1501.20

Taxonomic interrelationships between J. virginiana L. and J. scopulorum
Sargent in the Missouri River Basin.

Date _____

Collector _____ State _____

Collection Site No. _____ Elevation _____

Soil Type _____ Tag Description _____

Topographic Description and remarks _____

Tree No.	d.b.h. (in.)	ht. (ft.)	age (yrs.)	crown spread (ft.)	back thickness (in.)	sex	br. habit	cone persist. (yrs.)

Fig. 2.-- Sample field record form for collections of Juniperus foliage.

APPENDIX C

Examples of data analysis and presentation
of results, (after Van Haverbeke, 1965)

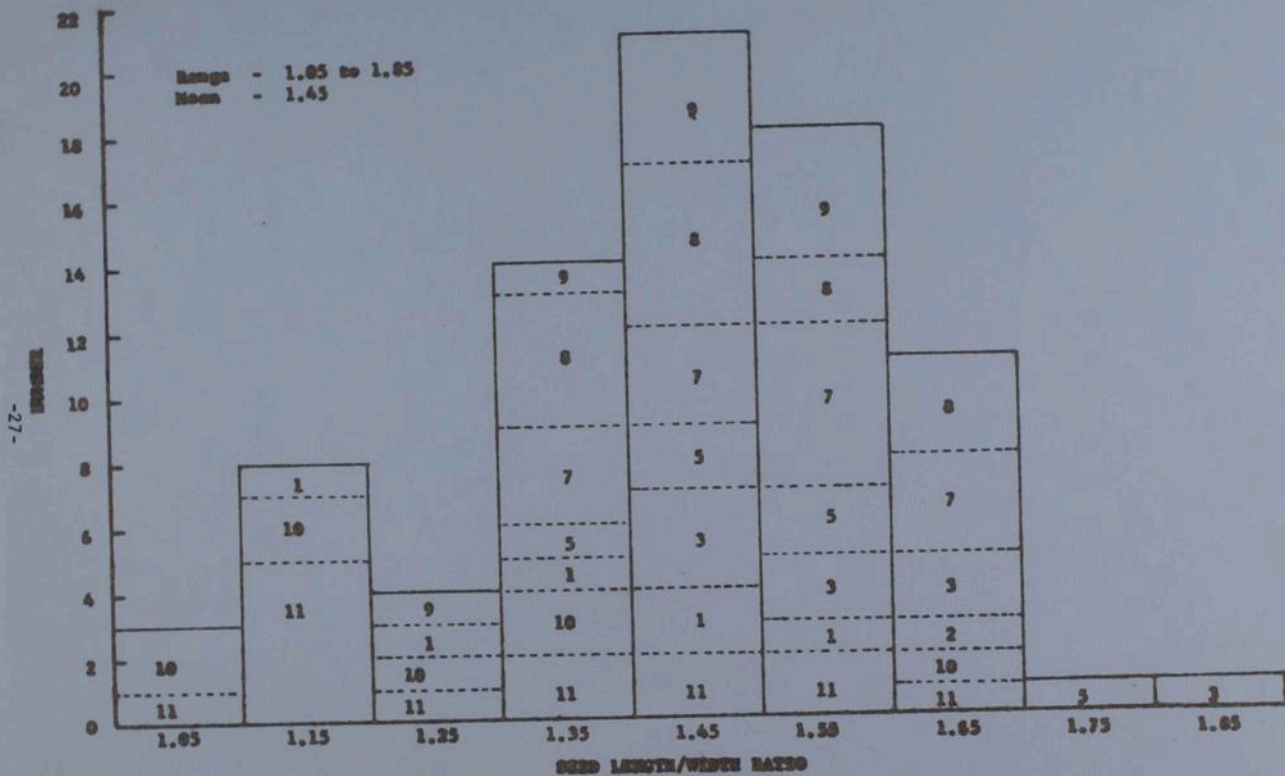


Fig. 4. -- Frequency distribution of seed length/width ratios in *Juniperus* in Nebraska.
(Numbers refer to collection sites).

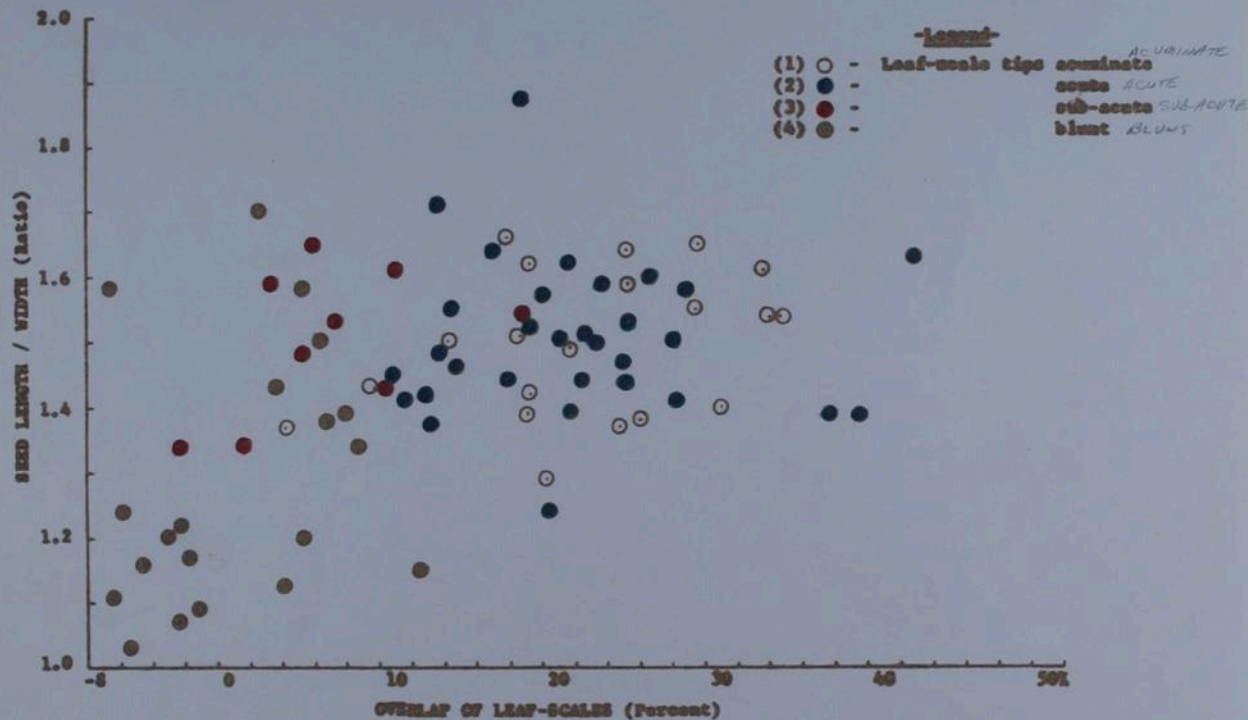


Fig. 15. -- Relationship between seed length / width ratio and percent overlap of leaf-scales, with shape of leaf tips superimposed for *Juniperus* in Nebraska.

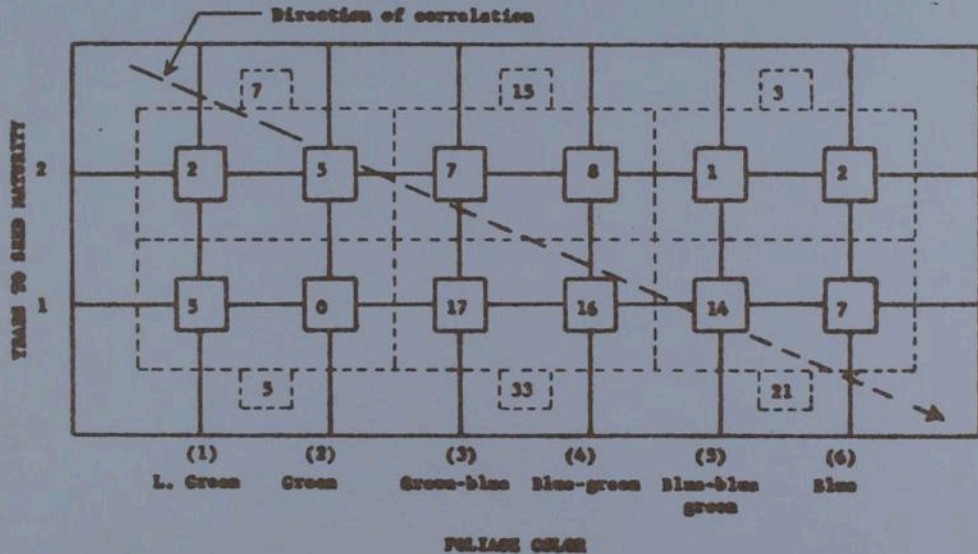


Fig. 9. -- Relationship between foliage color and years required for seed maturity in *Mimulus* in Nebraska.

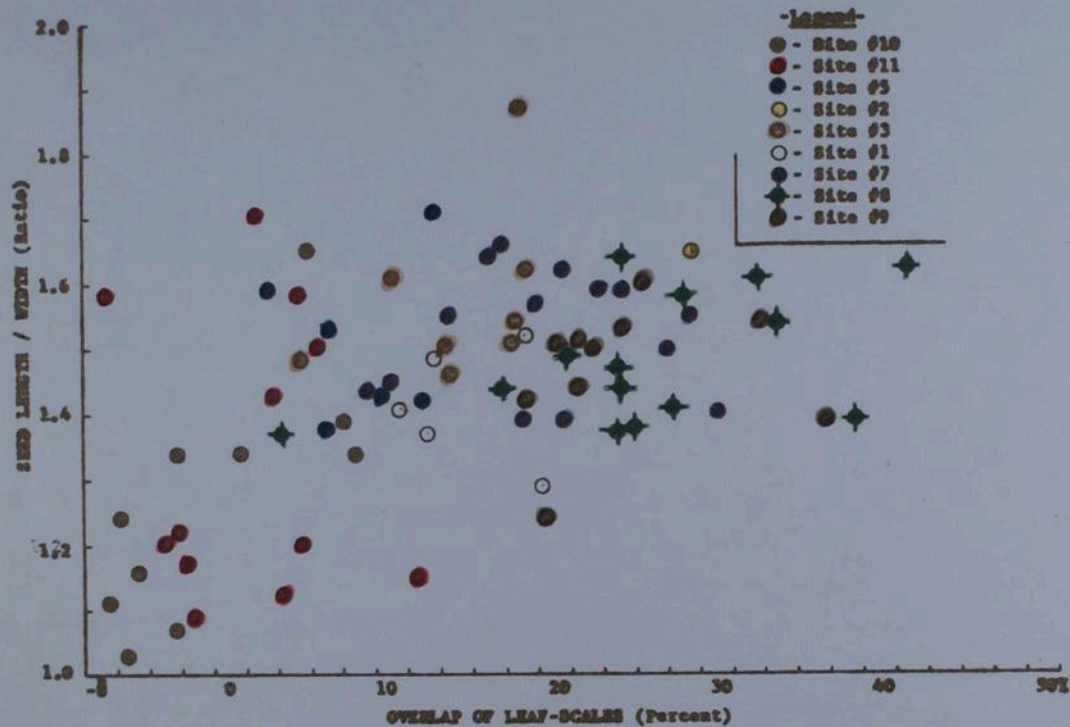


Fig. 14. -- Relationship between seed length/width ratio and percent overlap of leaf-scales with distribution of collection sites superimposed for Juniperus in Nebraska.

HYBRID INDEX VALUES FOR SIX SEED AND FOLIAGE CHARACTERS
OF JUNIPERUS SPECIES IN NEBRASKA

<u>CHARACTER</u>	<u>HYBRID INDEX VALUE</u>				
	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1. Years to seed maturity	2	0	0	0	1
2. Leaf-scale tip shape	4	3	0	2	1
3. Foliage color	1	2	3-4	5	6
4. Seed length/width ratio	1.03-1.19	1.20-1.36	1.37-1.52	1.53-1.69	1.70-1.87
5. No. seeds/cone	1.0 -1.3	1.4 -1.7	1.8 -2.2	2.3 -2.6	2.7 -3.0
6. % overlap of leaf-scales	-8.0 -1.9	2.0 -11.9	12.0-21.9	22.0-31.9	32.0-41.9

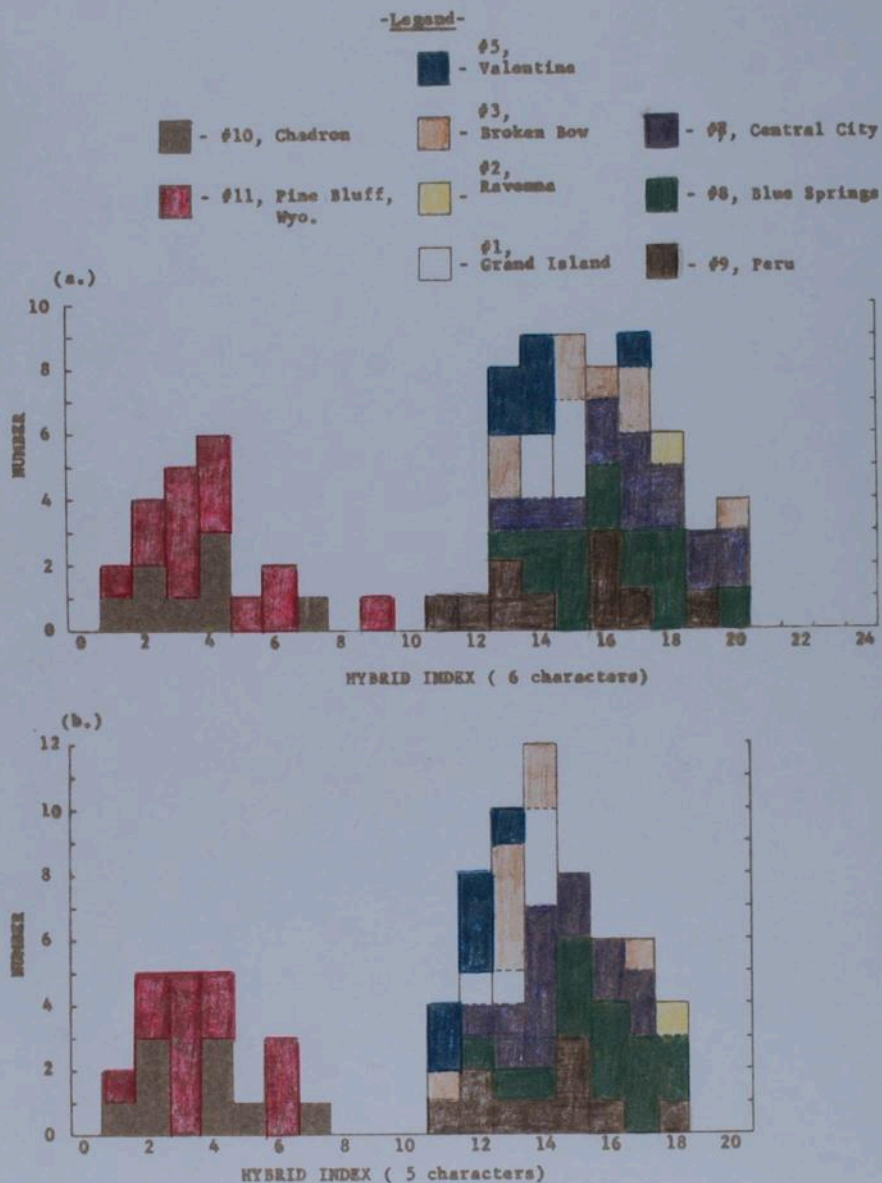


Fig. 19. -- Frequency distribution of hybrid index values for characters of *Tuminius* species in Nebraska.

HYBRID INDEX CHECK FOR SIX CHARACTERS OF JUNIPERUS IN NEBRASKA

HYBRID INDEX CHECK

CHARACTER	1	2	3	4	5	6	7	9	11	T L Σ	12	13	14	15	16	17	18	19	20	T R Σ
	Yrs. to Sd. Maturity	0	0	0	0	0	0	0	0	8	8	8	8	8	8	8	8	8	8	8
-33- Leaf Tip Shape	0	0	0	1	0	0	0	2	6	9	6	7	7	7	6	7	7	8	8	63
Foliage Color	1	3	4	2	2	5	6	4	0	27	4	4	4	4	4	6	5	5	7	43
Seed L/W Ratio	0	1	0	3	6	6	4	6	4	30	2	2	4	4	6	4	6	6	6	40
No. Seeds per Cone	0	0	1	1	0	0	2	4	0	8	0	1	0	3	3	3	4	4	5	23
% Foliage Overlap	1	0	1	1	2	1	2	2	4	14	4	2	5	4	5	6	6	7	6	45

$\frac{1}{\Sigma}$ Total Left Side; Total Right Side

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