THE SOUTHWESTERN NATURALIST 27(3):279-286 AUGUST 20, 1982

THE EFFECTS OF GASES FROM A BURNING COAL SEAM ON MORPHOLOGICAL AND TERPENOID CHARACTERS IN *JUNIPERUS SCOPULORUM* (CUPRESSACEAE)

ROBERT P. ADAMS

ABSTRACT.—Analysis of plants of *Juniperus scopulorum* var. *columnaris* revealed only two siginificant differences in morphology and one difference in terpenoid compostion between columnar trees growing near a burning coal seam and pyramidal trees at a site 3 km away. Canonical variate analysis of the chemical and morphological data using representative specimens of *J. scopulorm*, *J. virginiana*, and *J. horizontalis* indicate that the columnar trees at the type locality near Burning Coal Vein Park, North Dakota, and pyramidal trees from a nearby site cluster with *J. scopulorum*. Putative hybrids between *J. horizontalis* and *J. scopulorum* were confirmed by analysis of both character sets. Species relationships were maintained even when using plants exposed to the gases from the burning coal seam. *Juniperus scopulorum* var. *columnaris* is rejected as being only environmentally induced and, therefore, is conspecific with *J. scopulorum* var. *scopulorm*.

Morphological variation in *Juniperus* has been and continues to be a source of considerable confusion for many scientist. Fassett's grasp of the magnitude of variation is emphasized in his writings as: "*The variation within any colony is often as great as within the species as a whole*: . ."(italics his; Fassett, 1944 *a*. p. 412). This statement was in reference to morphological variation within *Juniperus horizontalis* Moench., *J. scopulorum* Sarg., and *J.virginiana* L. Fassett (1945*a*) recognized two varieties for both *J. scopulorum* and *J. virginiana*. Interestingly, *J. scopulorum* var. *columnaris* Fassett and *J. virginiana* var. *crebra* Fassett are columnar forms, while *J. scopulorum* var. *patens* Fassett and *J. virginiana* var. *ambigens* Fassett are shrubs of likely hybrid origin involving *J. horizontalis* (Fassett, 1945*a*).

The type locality of *J. scopulorum* var. *columnaris* is 7 km NW Amidon, North Dakota, at the Burning Coal Vein Park. This area contains an exposed coal vein that has been burning (underground) since about 1880 (Staudinger, 1966). *Juniperus scopulorum* trees near the burning coal have a columnar shape, whereas *Juniperus scopulorum* 3 km away have the typical pyramidal shape. Examination of the columnar plants showed that few morphological characters separate them from typical *J. scopulorum*, and this apparently led Rehder to reduce the variety to *J. scopulorum* f. *columnaris* (Fassett) Rehder. If only a few genes are involved and the expression is limited to individuals interspersed among typical individuals, the suggestion of Kapadia (1963) for the recognition of a *forma* would seem reasonable, whereas *varietas* would be more appropriate if several to many genes were involved in differentiation which exhibits a regional distribution. Regardless, whether variety or form would be more acceptable, neither variety nor form would be appropriate if the variation, expressed most notably in the columnar shape, is environmentally induced.

Staudinger and Hadley (1965) examined the flavonoids of *J. scopulorum* var. *columnaris* (six plants), *J. scopulorum* (four plants), *J. horizontalis* (three plants), and *J. communis* (two plants). All of these plants were collected in the vicinity of the burning coal vien northwest of Amidon, North Dakota. In additon, one specimen of *J. virginiana* var. *crebra* was analyzed from western Minnesota. Staudinger and Hadley (1965) stated "These columnar junipers have been variously thought to be: merely a variety of *Juniperus scopulorum* Sarg.; an ecological variety of the true *J. scopulorum*; a hybrid between *J. scopulorum* and one of several other juniper species that occur in the area; or a distinct species." even though the flavonoids were not identified and only a few plants were used, the chromatographic data indicated that the columnar junipers were most similiar to *J. scopulorum* (presumedly pyramidal trees) collected from the vicinity, Straudinger and Hadley (1965) concluded that the columnar trees were either a variety of *J. scopulorum* or an "ecological-induced polymorphic type" of *J. scopulorum* and that additional studies were needed to "establish the exact nature of the columnar [trees]." However, they do cite a personal communication with O. A. Stevens who apparently transplanted 40 columnar trees to Fargo, North Dakota, where only one tree maintained its columnar shape. This would seem to greatly favor the ecological-induced polymorphic type hypothesis.

Evidence has been presented by Murphy and Holden (1979) that ethylene is produced from the burning of coal and they postulate that the columnar habit is chiefly a response to ethylene. Of course other gases are produced from burning coal, such as SO_2, NO and CO, and these also might affect plant growth. Murphy and Holden (1947) also propagated 25 columnar junipers by cuttings, none of which maintained their coulumnar form. They concluded that the columnar form was induced by ethylene in the vicinity of the burning coal.

Columnar forms of *J. scopulorum* have been reported (Cringen and Dix, 1975) north of Sheridan, Wyoming, and I have obsereved columnar forms near Kalispell and Butte, Montana, and Soda Springs, Idaho. Each of these sites is near a smeltering operation.

The small microhabitat affected by the burning coal offers a unique, naturally occurring experiment on environmentally induced characters available for the study of character stability, classification, and the detection of hybridization from plants that are obviously affected by the environment. The questions to be addressed are: Do the columnar trees in the vicinity of the burning coal differ in morphological and terpenoid characters from typical *J. scopulorum* in the area and/or from *J. scopulorum* plants from a more distant area? Can the reported hybridization in the region between *J. horizontalis*, *J. scopulorum*, and *J. virginiana* (Fassett, 1944*b*, 1945*b*, 1945*c*; Van Haverbeke, 1968; Schurtz, 1971; Flake et al., 1978; Von Rudloff, 1975) be detected using field collected samples or must the trees be grown in a uniform garden?

Materials and Methods.—Fresh foliage was taken from the following: 15 plants of *Juniperus virginiana*, Quartz Mounain State Park, Altus, Oklahoma; 15 *J. scopulorum*, 2 km W of Soda Spings, Idaho; 10 plants of *J. horizontalis*, along the Saskatchewan River, Saskatoon, Saskatchewan; 5 plants of *J. horizontalis*, 3 km S Grass Range, Montana; 8 columnar plants from Burning Coal Vein Campground, 7 km NW of Amidon, North Dakota; 7 pyramidal plants of *J. scopulorum*, approximately 3 km S of the aforementioned columnar plants. The foliage was frozen until voucher specimens and material for steam distillation were processed. The volatile terpenoids were removed by steam distillation and analyzed by capillary gas/liquid chromatography (Adams, 1975, 1977). Peak identification was based on the mass spectral computer search report for these taxa (Adams et al., 1981). Forty-four morphological characters were scored (Table 1) and subjected to analysis of variance (ANOVA) between the eight columnar and seven pyramidal trees from the western North Dakota Burning Coal Vein area. ANOVA

TABLE 1.—*Analysis of variance between columnar (eight trees) from the Burning Coal Vein and pyramidal (seven trees) from 3 km away for 44 morphological characters. Characters noted with an asterisk (*) were subsequently used in canonical variate analysis.*

Character	*F* ratio	Probability
whip gland length	0.404	0.542
whip gland width	0.634	0.555
whip gland area	0.093	0.762
whip gland length/width	0.966	0.654
whip blade length	3.114	0.098
whip sheath length	3.961	0.065
*whip blade/sheath length	1.878	0.191
whip gland length/sheath length	3.42	0.084
whip glands sunken-smooth-protrudes	no variation seen	
whip glands ruptured no-some-all	0.039	0.841
*whip leaves recurved no-some-all	no variation seen	
*whip leaves glaucous beneath	0.867	0.628
whip leaf margins (at 40X)	no variation seen	
*scale leaf length	0.169	0.690
*scale leaf branch width	0.662	0.564
*scale leaf gland length	0.652	0.561
*distance from scale leaf gland to leaf tip	0.107	0.747
*scale leaf overlap	0.079	0.779
*scale leaf overlap/leaf length	0.541	0.519
*scale leaf length/branch width	0.001	0.978
*scale leaf gland length/distance from gland to leaf tip	0.705	0.579
scale leaf tip shape obtuse-acute-acuminate	2.400	0.143
scale leaf width at 0.66 mm from tip	0.001	0.972
scale leaves persistent	no variation seen	
*branching angle of ultimate twigs	1.647	0.220
*female cone diameter	8.317	0.017
*female cone area (length × width)	9.575	0.013
*female cone ratio (length/width)	1.121	0.318
*female cone color (pink-violet to blue-violet to black)	1.062	0.331
bloom on female cones none-some-all	0.112	0.744
female cones; red component (0.0-1.0)	0.213	0.658
female cones: green component (0.0-1.0)	0.474	0.514
female cones; blue component (0.0-1.0)	0.222	0.652
*female cones mature in: 1 yr.-2 yrs.	no variation seen	
female cones lobed; percent with 2 versus one	2.217	0.169
*seeds per female cone	0.696	0.570
seed length × width	2.204	0.167
*seed length/width	0.704	0.572
*hilum seed scar/seed length	0.010	0.920
*grooves or pits in the seed (no. on one side)	0.009	0.925
flaccid foliage erect-some flaccid-flaccid	0.389	0.550
*crown height/width	9.216	0.010
*crown tip shape; flat-obtuse-acute-acuminate	1.181	0.297
*plant habit; scored for columnar through pyramidal, round to prostrate	0.235	0.641

also was run on a total of 36 terpenoids that were present in amounts of greater than 0.1% of the total oil in either the columnar or pyramidal population. The morphological characters then were subjected to ANOVA among *J. scopulorum* (Soda Springs), *J. virginiana* (Altus), and *J. horizontalis* (Saskatoon and Grass Range). From that analysis, 24 characters were selected (based primarily of *F* ratios from ANOVA) for subsequent use (Table 1) in canonical variate analysis (CVA). A similar ANOVA was run on the terpenoids of the aforementioned taxa and 30 compounds with the largest *F* ratios were then used for CVA. Canonical variate analysis follows the programs of Blackrith and Reyment (1971), Cooley and

Lohnes (1971), and Pimentel (1979). CVA was run with morphological and then with terpenoid characters with five *a priori* groups: *J. scopulorum* (Soda Springs), *J. horizontalis* (Saskatoon and Grass Range), *J. virginiana* (Altus), eight columnar trees from Burning Coal Vein, and seven pyramidal *J. scopulorum* trees approximately 3 km S Burning Coal Vein. Two of the columnar samples and two of the pyramidal plant samples were omitted from the CVA of the morphology due to missing data for female cones(no female cones). Three additional plants collected at the Burning Coal Vein also were added as exemplars. These were: *1910*—a putative *J. horizontalis* × *J. scopulorum* hybrid, 1 m tall and 3 m wide; *1911*—an unusual juniper (perhaps a backcross from *J. horizontalis* to *J. scopulorum*) with foliage in tight balls on the limbs similar to a bonsai tree; and *1912*—a typical *J. horizontalis* plant 8 cm tall and 3 m wide.

Female cone colors were scored subjectively and also measured for the percent reflected red, green-yellow, and blue with a Sarasota Instruments colorimeter.

The choices of morphological characters were based on previous studies of these species (Fassett, 1944*a*; Hall, 1952; Schurtz, 1971; Van Haverbeke, 1968). Although several of the characters are ratios of other characters and thus exhibit strong correlations, this analysis should provide a useful illustration of the environmental effects on linear characters and ratios. Some of the highly correlated characters were included in CVA which is ideally suited to resolve such correlation.

RESULTS AND DISCUSSION.—Differences in morphology between columnar and pyramidal trees are slight. Only three of the morphological characters had significant ($P = 0.05$) F ratios in ANOVA (Table 1). These were female cone diameter (FDI), female cone area (FRA), and crown height/width (CHW). Due to the statistical correlation and the logical redundancy of FDI and FRA, these should be considered only one character and henceforth are so treated. Most of the taxonomic characters used in *Juniperus* were not affected by the burning coal gases. Two of the whip leaf length characters (BLL, SHL) and the highly correlated G/S character were nearly significantly different. The scale leaves (SLL) and branching angle (BAN), two characters thought to be important in determining overall tree crown shape, showed no significant differences.

Gas chromatographic analysis of the eight columnar and seven pyramidal trees gave a total of 100 components, of which 36 were present in quantities of at least 0.1% of the total oil in one of the groups. ANOVA of these 36 terpenoids revealed only one with a significant difference between columnar and pyramidal trees (compound 53, $F = 10.086$, $P = 0.0072$). That compound is a minor component (0.37% in columnar trees; 0.52% in pyramidal trees) and appears to be a sesquiterpene (MW204, base peak 105) whose complete structure has not been determined. One other component (peak 76, unknown) showed some signs of differentiation ($F = 3.559$, $P = 0.079$) but it is a minor component (0.15% and 0.04% total oil in columnar and pyramidal).

It appears that the normal suite of morphological taxonomic characters, aside from crown height/width and female cone size, are not affected by the gases from burning coal. The terpenoids also were not affected by the burning coal gases except for one compound (which could be due to chance, considering the number of compunds checked). In an effort to quantify the overall effect of the burning coal gases, another population of *J. scopulorum* was selected to compare with both the columnar and pyramidal plants treated as separate populations.

Canonical variate analysis (CVA) using 24 morphological characters with the highest F ratios in ANOVA among the five groups (Table 1) yielded four eigenroots which accounted for 70.95, 19.49, 8.56, and 0.83% of the variance amoung groups. The first three eigenroots were highly significant by Bartlett's

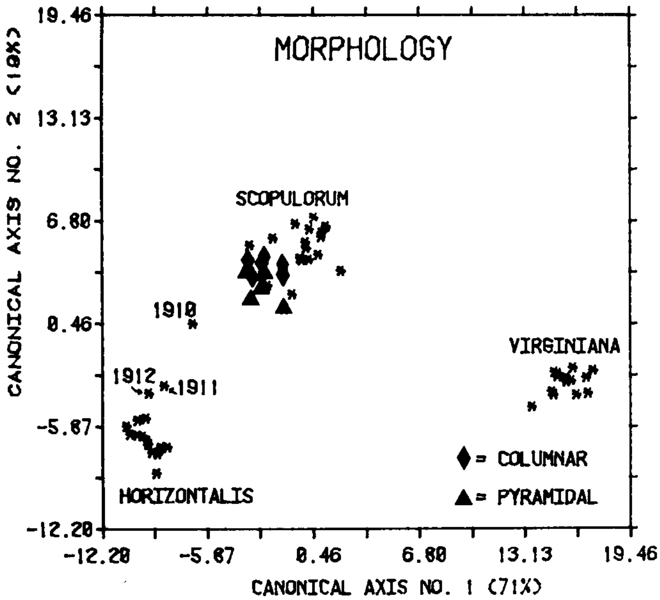

FIG. 1.—Plot of canonical axes 1 and 2, accounting for approximately 71 and 19% of the variance in the morphology among the five groups, respectively. Columnar and pyramidal plants from western North Dakota cluster with *Juniperus scopulorum* with no subclustering. Note the intermediate positon of individual *1910*.

test of sphericity (Blackrith and Reyment, 1971; Pimentel, 1979). The plot of the first two axes (Fig. 1) accounts for 90.44% of the among-group variance and shows three well-defined clusters for *J. horizontalis, J. scopulorum,* and *J. virginiana.* The columnar and pyramidal trees (and groups) cluster with *J. scopulorum* with no sub-clustering of the two growth forms. The putative *J. horizontalis* × *J. scopulorum* hybrid (*1910*) is depicted as intermediate between *J. horizontalis* and *J. scopulorum.* The ball-foliaged individual (*1911*) clusters towards *J. horizontalis,* as does *1912,* a *J. horizontalis* plant from the Burning Coal Vein site. Plotting the third canonical axis (8.5% variance) against the first axis (Fig. 2) shows an interesting separation of all the columnar and pyramidal plants from *J. scopulorum.* Again the columnar and pyramidal plants are not readily divisible into sub-clusters. The three individuals, *1910, 1911,* and *1912,* are ordinated about as before. This differentiation of the western North Dakota plants from *J. scopulorum* is not in the direction of the other taxa and is undoubtedly part of the intraspecific variation in *J. scopulorum.*

Canonical variate analysis of the terpenoids gave four eigenroots that accounted for 81.30, 17.46, 0.79, and 0.34% of the variance amoung the five groups. The first two eigenroots were highly significant by Bartlett's test; the third root was significant and the fourth root was not significant. Plotting the first two axes for the terpenoids accounts for 98.76% of the intergroup variance (Fig. 3). All of the pyramidal and columnar trees cluster closely with *J. scopulorum* with no sub-clusterings of the two growth forms. The putative hybrid (*1910*) again falls intermediate between *J. scopulorum* and *J. horizontalis.* Plant

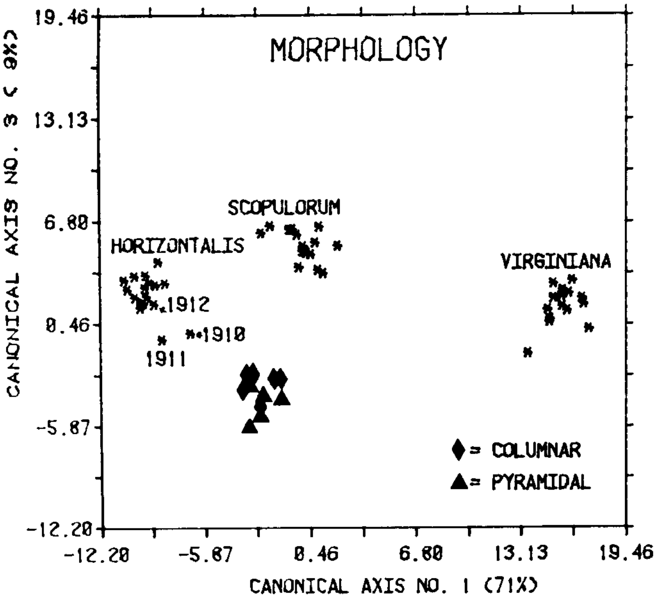

FIG. 2.—Plot of canonical axes 1 and 3 based on the morphology of the five groups. Axis 3 accounts for almost 9% of the intergroup variance and shows the differentiation of both the columnar and pyramidal plants from western North Dakota from *J. scopulorum*.

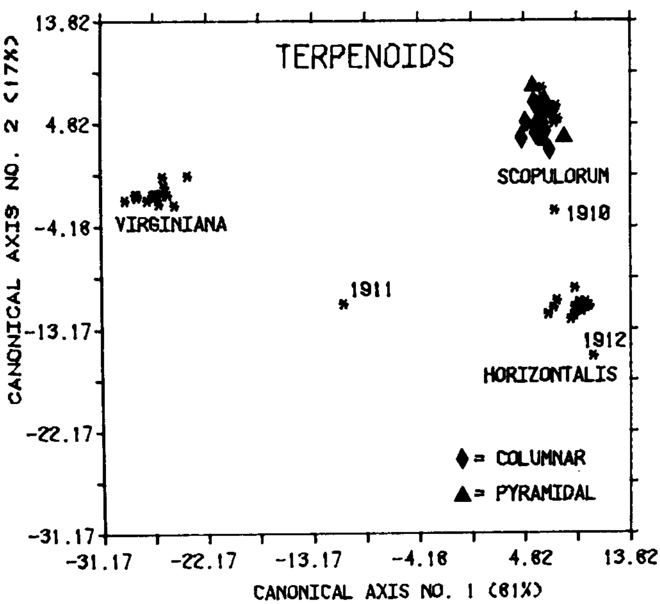

FIG. 3.—Plot of canonical axes 1 and 2 based on terpenoid characters in the five groups. These first 2 axes account for about 98% of the variance among groups. The western North Dakota plants, both columnar anf pyramidal, cluster well with *J. scopulorum*. Individual *1910* shows intermediacy between *J. scopulorum* and *J. horizontalis*. Individual *1911* might be intermediate to the taxa but is most likely a "sport"; see text for discussion.

1911 is ordinated rather midway among the three taxa. The oil of this plant showed major shifts in the concentration for many components and it is not surprising that it fails to cluster. This plant may be a mutant or reflect the result of genetic incompatibility between *J. horizontalis* and *J. scopulorum*. The *J. horizontalis* plant form the Burning Coal Vein (*1912*) clusters loosely with other *J. horizontalis* and not in the direction of *J. scopulorum*.

Examination of the third canonical axis showed only a slight differentiation among the plants from western North Dakota and is not presented.

From the data presented above, it is clear that the columnar junipers at the Burning Coal Vein differ primarily in their growth habit from typical pyramidal *J. scopulorum* collected 3 km away. In addition, these columnar junipers differ little from *J. scopulorum* at Soda Springs.

In conclusion, the effects of the burning coal gases on both the morphology and terpenoids did not interfere with taxonomic analysis involving the classification of columnar plants and the ability to detect putative hybridization. Although the gases from the burning coal have a major effect on the plant crown shape, the vast majority of taxonomic characters are unaffected and thus present no major problem in the analyses of regional differentiation and/or hybridization in *J. scopulorum*. The columnar type *J. scopulorum* var. *columnaris* Fassett (or f. *columnaris* [Fassett] Rehder) does not represent genetic differences from typical *J. scopulorum* Sarg, and its taxonomic segregation is therefore rejected.

This research was supported by NSF grants DEB-7722331 and DEB-7921757.

Literature Cited

Adams, R. P. 1975. Gene flow versus selection pressure and ancestral differentiation in the composition of species; analysis of population variation of *Juniperus ashei* Buch, using terpenoid data. J. Molec. Evol., 5:177-185.

———. 1977. Chemosystematics-analyses of populational differentiation and variability of ancestral and recent populations of *Juniperus ashei*. Ann. Missouri Bot. Gard., 64:184-209.

Adams, R. P., E. Von Rudloff, T. A. Zanoni, and L. Hogge. The volatile terpenoids of *Juniperus blancoi* and its affinities with other entire leaf margin junipers of North America. J. Nat. Prod., 42:21-26.

Blackrith, R. E., and R. A. Reyment. 1971. Multivariate morphometrics. Academic Press, London.

Cooley, W. W., and R. P. Lohnes. 1971. Multivariate data analysis. John Wiley & Sons, New York.

Cringan, A. T., and R. L. Dix. 1975. Ecological theme analysis and inventory of potential landmarks of the Great Plains natural region. Rep. Nat. Park Serv. USDI, contr. no 14-10-9-900-248.

Fassett, N. C. 1944*a*. *Juniperus virginiana, J. horizontalis* and *J. scopulorum* I. The specific characters. Bull. Torrey Bot. Club, 71:410-418.

———. 1944*b*. *Juniperus virginiana, J. horizontalis* and *J. scopulorm* II. Hybrid swams of *J. virginiana* and *J. scopulorum*. Bull. Torrey Bot. Club, 71:475-483.

———. 1945*a*. *Juniperus virginiana, J. horizontalis,* and *J. scopulorum*—V. Taxonomic treatment. Bull. Torrey Bot. Club, 72:480-482.

———. 1945*b*. *Juniperus virginiana, J. horizontalis,* and *J. scopulorum*—III. Possible hybridization of *J. horizontalis* and *J. scopulorum*. Bull. Torrey Bot. Club., 72:42-46.

———. *Juniperus virginiana, J. horizontalis,* and *J. scopulorum*—IV. Hybrid swarms of *J.virginiana*, and *J. horizontalis*. Bull. Torrey Bot. Club., 72:379-384.

Flake, R. H., L. Urbatsch, and B. L. Turner. 1978. Chemical documentation of allopatric introgession in *Juniperus*. Syst. Bot., 3:129-144.

HALL, M. T. 1952. Variation and hybridization in *Juniperus*. Ann. Missouri Bot. Gard., 39:1-64.
KAPADIA, Z. J. 1963. Varietas and subspecies, a suggestion towards greater uniformity. Taxon, 12;257-258.
MURPHY, J. M., AND D. J. HOLDEN. 1979. The ethylene from burning lignite as a probable cause of columnarity in North Dakota Junipers. Prairie Nat., 11:11-22.
PIMENTEL, R. A. 1979. *Morphometrics*. Kendall/Hunt Publ. Co., Dubuque.
RUDLOFF, E. VON. 1975. Chemosystematic studies of the volatile oils of *Juniperus horizontalis, J. scopulorum* and *J. virginiana*. Phytochemistry, 14:1319-1329.
SCHURTZ, R. H. 1971. A taxonomic analysis of a triparental hybrid swarm in *Juniperus* L. Ph.D. thesis., Univ. Nebraska, Lincoln.
STAUDINGER, T. D. 1966. A chromatographic and ecological study of the columnar juniper of western North Dakota. Masters thesis, Univ. North Dakota, Grand Forks.
STAUDINGER, J. D., AND E. B. HADLEY. 1965. A chromatographic study of the junipers of western North Dakota. North Dakota Acad. Sci., 19:178-185.
VAN HAVERBEKE, D. F. 1968. A population analysis of *Juniperus* in the Missouri River Basin, Univ. Nebraska Studies, 38., Lincoln.

Address of author: *Science Research Center at Salt Lake, Hardin-Simmons Univ., Plant Resources Institute, 360 Wakara Way, Salt Lake City, UT 84108.*