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About the Institute
The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

COLORADO STATE UNIVERSITY

FORT COLLINS, COLORADO 80521

October 1967

TO: NATIONAL SCIENCE FOUNDATION

TYPE OF SUPPORT REQUESTED:	Research Grant
TITLE OF RESEARCH PROJECT:	Volatile Oils in *Artemisia* as a Taxonomic Tool
PRINCIPAL INVESTIGATOR:	Julius G. Nagy Assistant Professor Department of Wildlife Biology Colorado State University Fort Collins, Colorado 80521 Telephone - (303) 491-5002
AMOUNT REQUESTED:	$23,269
DESIRED GRANT PERIOD:	Three years Starting September 1, 1968
UNIVERSITY OFFICIAL TO BE CONTACTED REGARDING GRANT OR CONTRACT NEGOTIATION:	Mr. B. G. Henrie Contracts and Grants Administrator Colorado State University Fort Collins, Colorado 80521 Telephone - (303) 491-6355

ABSTRACT:

The taxonomy and relationships among the Section Tridentatae will be explored through analyses of the volatile oils as a possible supplement to the traditional characters of morphology and cytology. The identity and composition of the volatile oils in the selected plant populations, as well as their total oil content will be considered in the study. Computer methods have already been developed for the study. Certain portions of the investigations will be conducted on seedlings transplanted to a common nursery to separate the influence of environment from genetic characteristics during the production of the volatile oils.

INTRODUCTION

The species and subspecies of Artemisia covering the western part of the North American continent from southern Canada to northern Mexico are annual or perennial, herbaceous or shrubby plants, usually with aromatic odor. Taxonomically the genus is rather complicated. In the Section Tridentatae, Rydberg (1906) recognized 13 species in 3 sections. Hall and Clements (1923) mention 5 species (A. bigelovii, A. cana, A. pygmaea, A. rigida, and A. tridentata) and divide A. tridentata further into 7 subspecies (arbuscula, bolandery, nova, parishii, rothrockii, trifida, and typica). Beetle (1960) recognized within the Section Tridentatae Rybd. 11 species and 2 forms of Artemisia and mentions that hybridization is also frequent among the species of Section Tridentata; i.e., A. tridentata x A. nova, A. tridentata x A. arbuscula.

Recently Holbo and Mozingo (1965) and Young (1965) characterized several members of the Section Tridentatae by paper chromatography using nucleic proteins and lactones respectively. According to Holbo and Mozingo (1965), A. tridentata can be divided into three subspecies.

Members of the Section Tridentatae contain volatile oils. Total oil content as well as ratios among the individual oils vary. It seems possible that variations in the oil content and composition are of genetic origin. If this hypothesis is true then the oils could be used as an additional tool in taxonomic studies on the genus Artemisia.

The purpose of the proposed research is therefore to investigate the usefulness of the volatile oils in taxonomic studies on the Section Tridentatae, specifically upon A. tridentata and its subpopulations. The methods of Holbo and Mozingo (1965) and Young (1965) will also be used during the investigations.

During the past four years I have worked on various problems connected with the volatile oils of Artemisia such as antibiosis of the volatile oils and seasonal and regional changes of volatile oil content and composition (Nagy et al., 1964; Nagy, 1966). The study covered A. tridentata, (subsp. tridentata and subsp. vaseyana), A. nova and A. frigida. The volatile oil content and composition varied according to species and seasons. In general, the highest oil content was found in A. tridentata leaves and twigs (approximately two to five percent on a dry matter basis), followed by A. nova (one to two percent), and A. frigida (0.5 to one percent). The total oil content was rather constant from samples of A. nova and A. frigida at various locations in Colorado. A. tridentata, however, exhibited considerable variation in this respect at different locations in Colorado.

Each species showed the presence of approximately 14 to 16 different volatile oils. Ratios among the oils from samples of A. nova and A. frigida were remarkably constant at various locations, while the ratios in A. tridentata samples at several locations varied. In spite of this variation the composition and ratios of the oils in A. tridentata, as reflected by gas-chromatograms, were within limits recognizable for the species.

The sum total of environmental factors, such as soil characteristics, altitude, intensity and duration of solar radiation, will undoubtedly influence the oil content and composition. The data, however, obtained so far suggest that the main governing factor determining the oil content and composition is of genetic origin.

If this hypothesis proves to be true, then the volatile oil content and composition could possibly be used as an additional tool in taxonomic studies connected with this complicated genus.

Scientific Aims of the Proposed Research

1. To identify the major representative volatile oil compounds in the A. tridentata complex.
2. To investigate the possibility of using the volatile oil content and composition in chemotaxonomic studies.
3. To study the degree to which oil content and composition are genetically determined, and the extent to which they are influenced by environmental conditions.
4. To correlate the findings of the above with the morphological and cytological characters previously established as significant.
5. To test the various previous classifications for their information content against the classification derived from this study.

Brief Description of the Proposed Research

Leaves of various species, subspecies and varieties of Section Tridentatae will be collected during the winter months. The plant collection will include A. tridentata tridentata, A. tridentata vaseyana at various elevations and localities of Colorado and also A. nova and A. bigelovii.

Preliminary results indicate that problems will be encountered with A. tridentata tridentata and A. tridentata vaseyana. Therefore, other species and subspecies will be included in this study only after an intensive research on the above mentioned two subspecies. Most of the samples will be obtained from Colorado, some from Utah and Wyoming. Herbarium specimens will be prepared from each collection site and the specimens deposited in the herbarium of Colorado State University.

Plant species will be tentatively identified in the field (Harrington, 1954), then Dr. Harrington and Dr. Beetle will be consulted for positive identification. The methods of Holbo and Mozingo (1965) and also of Young (1965) will be applied.

Winter has been chosen for collection time because in preliminary experiments we have found that the volatile oil content and composition is subject to less diurnal changes during winter than during the summer months. This variation will be studied, however, at certain selected locations.

The collected leaves will be frozen with dry ice in the field, transported to the laboratory, where dry matter determinations, proximate analyses, volatile oil content and composition determinations will be performed.

Seedlings will be collected from each species or subspecies and transplanted to a common garden. Part of this sample will be analyzed for volatile oil content and composition. The planted seedlings will be grown in the garden and analyzed the following two years for the volatile oil content and composition along with freshly collected seedlings from the same natural locality.

Major compounds of volatile oils will be separated from various collected plants by preparatory gas-chromatography (Nagy, 1966) and identified. Presence or absence of major compounds and ratios of various other compounds present in a sample as reflected by gas-chromatograms will be used to determine taxonomic relationships.

All of the data derived in these tests will be correlated with the morphological and cytological data already available to the investigators, using the computer methods developed in the Taximetrics Laboratory under the direction of David J. Rogers. The methods to be employed include a program entitled "character analysis" and a second entitled "graph theory clustering." These programs are described in: Wirth, Estabrook and Rogers, 1966, Estabrook and Rogers, 1967, and Estabrook, 1967. The first program gives an opportunity to check the data derived from the chromatographic analyses against other types of data used, and gives an indication of the reliability of the character as a piece of taxonomic information. The program also permits an objective analysis of the information content of a particular classification, and will be useful in this study to determine the most useful classification yet published. The second program takes the data (all types) and develops an hierarchical classification with a high predictive ability. The program not only gives the exact levels at which specimens are grouped together into taxa, but also gives accurate indications of the position of hybrids between taxa.

Time Schedule

First year: Establish locations of sampling sites during summer and fall and collect herbarium specimens. Collect plant material in late

December, January and February. Begin with analyses. Transplant seedlings as weather permits. Analyse, separate and identify the volatile oils during summer.

Second year: Select additional sampling sites during fall and adjust methods and techniques. Collect during winter months and collect seedlings as weather permits. Analysis of collected plant material and seedlings from the sagebrush garden.

Third year: Collection of plant material from sites established during the second year to study possible yearly variations. Analyses as described before. Data analysis and preparation of manuscripts.

Facilities Available

A standard research laboratory is available and it is presently used by the grantee. Besides the usual laboratory equipment and instruments, a gas-chromatograph with flame detector (Aerograph Hy Fi 600) and a preparatory gas-chromatograph (Aerograph-Autoprep 700) are routinely used in our present work.

Consulting and Cooperative Possibilities in the Area

The Departments of Botany, Chemistry, and Range Science of Colorado State University have been helpful and cooperative in our previous work along with the Department of Range Management, University of Wyoming.

The study will be a cooperative effort between the principal investigator and Dr. David J. Rogers. A graduate student majoring in wildlife nutrition but with background and interest in plant systematics will work on the project toward his Ph.D. degree. Part of his work will be supervised by Dr. Rogers. It is intended that the principal investigator and Dr. Rogers will serve as co-major professors on the committee of the selected student.

Consultants Whose Fields Are Related to the Proposed Research

A. A. Beetle, Ph.D., Department of Range Management, University of Wyoming.

Robert E. Dils, Ph.D., Leader, C. S. U. Coop. Watershed Management Unit.

H. D. Harrington, Ph. D., Department of Botany, Colorado State University.

Harold W. Steinhoff, Ph.D., Department of Fishery and Wildlife Biology, Colorado State University.

Biographies

Principal Investigator: Julius G. Nagy,
Assistant Professor of Wildlife Biology

Born: 7 August 1925, Balatonbolgar, Hungary, United States Citizen

Education:			
	B. S.	Wayne State University, Bacteriology	1960
	M. S.	Colorado State University, Wildlife Nutrition	1963
	Ph.D.	Colorado State University	1966

Professional Experience:

1956-61 C. F. Burger Creamery Co., Detroit, Michigan. Bacteriologist in charge of laboratory, quality control and sanitation bacteriology. Active research in the bacteriological aspect of products development.

1961-63 Colorado State University. Research Assistant. Problems connected with *in vitro* and *in vivo* rumen metabolism studies on cattle, sheep, deer, and antelope.

1963-65 Colorado State University, Jr. Animal Scientist. Co-investigator NSF Project No. GS 1507.

1965-67 Colorado State University, Instructor

1967- Colorado State University, Assistant Professor

Special Training:

1963 Rumen microbiology techniques with Marvin P. Bryant, U.S.D.A., Beltsville, Maryland.

1964 Gas-chromatography course. University of Colorado, Boulder, Colorado.

Fields of Present Major Scientific Interest:

Nutrition of wild and domestic ruminants.

Membership in Scientific Societies:

Member of Phi Kappa Phi, Sigma Xi

Publications Related to the Proposed Study by the Above-Mentioned Personnel:

Nagy, J. G., Gy. Vidacs and G. M. Ward. 1963. Effects of essential oils of sagebrush on bacteria (Abstr.). Proc. Rocky Mt. Soc. of Microb.

Nagy, J. G. 1963. Effects of essential oils of sagebrush (Artemisia tridentata) on deer rumen microorganisms. M.S. Thesis, Colorado State University.

Nagy, J. G., Gy. Vidacs and G. M. Ward. 1963. Effects of sagebrush and its essential oils on bovine appetite and metabolism of rumen microflora (Abstr.). J. Animal Sci. 22:841.

Nagy, J. G., Gy. Vidacs and G. M. Ward. 1964. Separation of the essential oils of Artemisia app. by gas-chromatography and the effects of the oils on bacteria (Abstr.). Proc. 35th Annual Meeting Colorado-Wyoming Academy of Science.

Nagy, J. G., H. W. Steinhoff, and G. M. Ward. 1964. Effects of essential oils of sagebrush on deer rumen microbial function. J. Wildlife Management 28:785-790.

Nagy, J. G. and R. P. Tengerdy. 1967. Antibacterial effect of the volatile oils of sagebrush on bacteria: I. Effect of the oils on aerobic bacteria. J. Appl. Bact. 15:819-821.

Manuscripts Prepared for Publication

Nagy, J. G. and G. M. Ward. Volatile oils of some Artemisia species.

Nagy, J. G. and G. M. Ward. Antibacterial effect of the volatile oils of sagebrush on bacteria. II. Effect of the oils on deer rumen bacteria.

LITERATURE CITED

Beetle, A. A. 1960. A study of sagebrush: the section Tridentatae of Artemisia. Univ. of Wyo. Agr. Exper. Sta. Bul. 368, 83 p.

Estabrook, George F. and David J. Rogers. 1966. A general method of taxonomic description for a computer similarity measure. Bioscience 16:789-793.

Estabrook, George F. 1967. An information theory model for character analysis. Taxon. 16:86-97.

Hall, H. M. and F. E. Clements. 1923. The phylogenetic method in taxonomy. Carnegie Inst. Wash. Publ. No. 326:1-156.

Holbo, H. R. and H. N. Mozingo. 1965. The chromatographic characterization of Artemisia, Section Tridentatae. Am. J. Bot. 52:970-978.

Nagy, J. G., H. W. Steinhoff, and G. M. Ward. 1964. Effects of essential oils of sagebrush on deer rumen microbial function. J. Wildl. Mgmt. 28:785-790.

Nagy, J. G. 1966. Volatile oils and antibiosis of Artemisia. Ph.D. Dissertation, Colo. State Univ. 73 p.

Rogers. 1966. A graph theory model for systematic biology, with an example for the Onidiinae (Orchidaceae). Systematic Zoology. 15:59-69.

Rydberg, P. A. 1916. Artemisia (and) Artemisiastrum. North Am. Flora. 34:244-285.

Young, A. L. 1965. Chromatographic technique in the identification of sagebrush taxa. J. Range Mgmt. 18(1):35.

Budget	1st year NSF	1st year CSU	2nd year NSF	2nd year CSU	3rd year NSF	3rd year CSU	Totals NSF	Totals CSU
Salaries - Faculty								
Julius G. Nagy, Ph.D., Prin. Investigator, 2 mos. summer salary 9 mo. appointee	1,840	- 0 -	1,940	- 0 -	2,040	- 0 -	5,820	- 0 -
PERA-Retirement Contribution - 6%	110	- 0 -	116	- 0 -	122	- 0 -	348	- 0 -
Salaries - Non-Faculty								
Grad. Student ½ time	2,800	- 0 -	2,800	- 0 -	3,000	- 0 -	8,600	- 0 -
Student Labor	800	- 0 -	800	- 0 -	800	- 0 -	2,400	- 0 -
Consumable Supplies								
Chemicals, gas-chromatography materials, glassware, dry ice	800	- 0 -	800	- 0 -	800	- 0 -	2,400	- 0 -
Travel								
Travel to collection areas	500	- 0 -	400	- 0 -	400	- 0 -	1,300	- 0 -
Other Expenses								
Statistical analysis & computer time	- 0 -	- 0 -	250	- 0 -	250	- 0 -	500	- 0 -
Photographic supplies	40	- 0 -	40	- 0 -	70	- 0 -	150	- 0 -
Reprints, publication costs	- 0 -	- 0 -	50	- 0 -	150	- 0 -	200	- 0 -
Indirect Costs - 45% of salaries, wages and fringe benefits*	489	389	519	406	543	430	1,551	1,225
TOTAL COSTS	$7,379	$ 389	$7,715	$ 406	$8,175	$ 430	$23,269	$1,225

*The overhead rate for Colorado State University is 45% of salaries, wages, annuities, sick leave, holidays and vacation. Audit was performed by the Denver Branch of the Defense Contract Audit Agency (3800 York Street, Denver, Colorado, 80205) over the accounting period 7-1-64 to 6-30-65. The foregoing rates are to be used as provisional rates.

INFORMATION CONCERNING INSTITUTION SUBMITTING PROPOSAL

A. This proposal is submitted by Colorado State University. Contracts and grants that result from this proposal should be made in the name of COLORADO STATE UNIVERSITY.

B. The officials authorized to sign and to submit proposals are: (1) Rue Jensen, Vice-President for Research, or (2) Merle H. Schmohl, Administrative Assistant to Vice-President for Research.

C. The official authorized to negotiate contracts is the Contracts and Grants Administrator or his assistant.

D. Correspondence should be addressed to: Colorado State University, Fort Collins, Colorado; Attention: Contracts and Grants Administrator; Telephone 491-6355. Area Code 303.

E. The VICE-PRESIDENT FOR RESEARCH OF COLORADO STATE UNIVERSITY is authorized to sign contracts or to acknowledge grants.

F. Colorado State University is an agency of the STATE OF COLORADO and enjoys exemption from tort liability.

STATEMENT ON SOLICITING

We hereby certify that we have not employed or retained a company or person (other than a full-time employee) to solicit or secure this contract (grant) and agree to furnish information relating thereto as requested by the Sponsor's cognizant officer.

We certify that the distribution of costs between the direct and indirect categories as shown in the proposal conforms to the usual accounting practices of the institution and to the distribution used by the cognizant Federal audit agency.

Date

Julius G. Nagy
Principal Investigator

G. A. Swanson, Head
Department of Fishery and Wildlife Biology

Merle H. Schmohl
Administrative Assistant to
Vice-President for Research
Colorado State University