



Factors affecting utricle fill in fourwing saltbush (*Atriplex canescens* [Pursh] Nutt)  
by Wallace James Johnson

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE  
in Agronomy  
Montana State University  
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Abstract:

Causes of poor utricle fill were studied in fourwing saltbush (*Atriplex canescens* (Pursh) Nutt). Experiments were conducted to determine if differences in chromosome numbers were causing fertility problems in fourwing saltbush accession PI-15585 and Nuttall's saltbush (*Atriplex nuttallii* Watson). The percentage of viable pollen, flowering period for female and male plants and optimum temperature for pollen shed were determined for accession PI-15585 in relation to utricle fill. The fertility of monoecious plants and the morphology of native saltbush plants were also studied. Southwestern accessions of fourwing saltbush were evaluated for seed production in Montana.

The chromosome number of fourwing saltbush accession PI-15585 was  $2n=36$  and Nuttall's saltbush appears to have a chromosome number of  $2n=18$ . If these two species were to cross, the progeny could be semi-sterile. Morphological characteristics of native plants near Circle and Bridger, suggests that these two species could cross and result in the low utricle fill associated with Montana Accession PI-15585. Approximately 95 percent of the pollen shed by fourwing saltbush was viable.

The male and female plants flower for about 24 days and they flower during the same period. Optimum temperature for peak pollen shed was between 16 and 19C. There appears to be no association between pollen factors and poor utricle fill. Irrigation appears to improve utricle fill based on three years of study at Bridger, Montana. The one monoecious plant which produced seed in isolation was self-fertile and had above average utricle fill. Native fourwing saltbush plants in Montana could be hybrids between *A. canescens* and *A. nuttallii*. This may account for the low utricle fill in native populations. According to one year's data, accessions from the southwestern United States did not mature at Bridger, Montana, and would not be suitable for seed production in Montana.

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Date August 8, 1975

FACTORS AFFECTING UTRICLE FILL IN FOURWING SALTBUSH  
(ATRIPLEX CANESCENS (PURSH)NUTT)

by

WALLACE JAMES JOHNSON

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

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Approved

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## ABSTRACT

Causes of poor utricle fill were studied in fourwing saltbush (Atriplex canescens (Pursh) Nutt). Experiments were conducted to determine if differences in chromosome numbers were causing fertility problems in fourwing saltbush accession PI-15585 and Nuttall's saltbush (Atriplex nuttallii Watson). The percentage of viable pollen, flowering period for female and male plants and optimum temperature for pollen shed were determined for accession PI-15585 in relation to utricle fill. The fertility of monoecious plants and the morphology of native saltbush plants were also studied. Southwestern accessions of fourwing saltbush were evaluated for seed production in Montana.

The chromosome number of fourwing saltbush accession PI-15585 was  $2n=36$  and Nuttall's saltbush appears to have a chromosome number of  $2n=18$ . If these two species were to cross, the progeny could be semi-sterile. Morphological characteristics of native plants near Circle and Bridger, suggests that these two species could cross and result in the low utricle fill associated with Montana accession PI-15585. Approximately 95 percent of the pollen shed by fourwing saltbush was viable. The male and female plants flower for about 24 days and they flower during the same period. Optimum temperature for peak pollen shed was between 16 and 19C. There appears to be no association between pollen factors and poor utricle fill. Irrigation appears to improve utricle fill based on three years of study at Bridger, Montana. The one monoecious plant which produced seed in isolation was self-fertile and had above average utricle fill. Native fourwing saltbush plants in Montana could be hybrids between A. canescens and A. nuttallii. This may account for the low utricle fill in native populations. According to one year's data, accessions from the southwestern United States did not mature at Bridger, Montana, and would not be suitable for seed production in Montana.

## INTRODUCTION

Fourwing saltbush (Atriplex canescens (Pursh) Nutt) is a valuable shrub for revegetation of ranges and strip-mined areas in arid and semi-arid climates. It has good drought tolerance and is adapted to a wide spectrum of climatic conditions. Fourwing saltbush is one of the most palatable southwestern shrubs and its leaves, stems, and utricles are readily eaten by livestock and wildlife. Protein percentages have been reported as high as 15 percent in the leaves during the winter.

Many plantings of fourwing saltbush from seed have been unsuccessful because of poor utricule fill, dormancy and low germination. Utricule fill has been improved by removing unfilled utricles by a mechanical process. Utricule fill is probably controlled genetically and may be influenced by climatic conditions.

Utricule fill must be improved if fourwing saltbush is to become easily established from seed on ranges and strip-mined areas. The main objective of this study was to determine causes of low utricule fill and if utricule fill could be improved. The following studies were conducted to accomplish that objective.

1. Determine if infertility was due to incompatible chromosome pairing.
2. Determine the percentage of viable pollen.
3. Determine the requirements for maximum pollen shed.
4. Determine if male and female plants flower at the same time.

and for what length of time.

5. Determine if irrigation would increase utricle fill.
6. Determine if morphological characteristics of native salt-bush populations indicate that crossing between species is a cause of low utricle fill.
7. Determine if accessions from the southwestern United States can produce seed in Montana.

## LITERATURE REVIEW

Fourwing saltbush is one of the most palatable and productive browse plants known to man and is adapted to a wide variety of climatic conditions. Its large range of adaptation extends from the Great Plains on the east, to the shores of the Pacific on the west, and from below sea level on the Mojave desert to the high mountain areas in the western United States (13, 14).

Fourwing saltbush is important in the revegetation of strip-mined areas and ranges throughout the western United States (16). Livestock readily eat the stems, leaves and utricles (1, 3, 4, 5, 6, 11, 21). Protein levels have been reported to remain high throughout the year in the leaves and stems (13). However, Gamrath (8) found that protein levels dropped drastically in Montana in late August.

### General Description of Fourwing Saltbush

Hall and Clements (9) divide Atriplex canescens (Pursh) Nutt into five sub-species on the basis of morphological characteristics. A. canescens typica is a shrub 4 to 15 dm high and is located throughout North America. It is the most common sub-species and is distinguished by lanceolate shaped leaves, definite fourwinged appendages on the fruiting bracts, and fruiting bracts which are extended on pedicels.

A. canescens ssp. aptera Nelson is a wood shrub 2 to 4 dm high. This type is commonly found in Montana, Washington, and Wyoming. Hall and Clements (9) indicate it may be a cross between Nuttall's saltbush and fourwing saltbush because of its low growth form, fruiting bracts which lack fourwinged appendages, and sessile fruiting bracts.

The other sub-species are difficult to distinguish morphologically and are only located in isolated areas. The main difference among sub-species is in plant heights.

Fourwing saltbush is primarily dioecious, with the distribution of the sexes about equal (9, 13, 14). Gamrath (8), however, reports about 20 percent of the plants being monoecious in accession number PI-15585 grown at the Bridger Plant Materials Center, Bridger, Montana. Within the dioecious group male and female plants were distributed equally.

Male plants produce small yellowish staminate flowers on the end of stems on the current year's growth (8). Female plants produce conspicuous clusters of fourwinged, one-celled pods known as utricles, which contain the seeds (5).

Seed and leaf size vary greatly from area to area, plant to plant, and within a plant (16). The number of utricles per pound averages 55,000 and the seed itself is about one-half the size of alfalfa seed (13). Utricle fill in Montana is somewhat lower than that found in Utah by Plummer (13).

Variation in growth form has been reported by a number of people (8, 13, 16). The fourwing saltbush found in Montana has many different leaf shapes and sizes, plant height varies from 3 to 12 dm, and utricle shape and size show great variation (8). Stutz (20) reported that *Gigas A. canescens* may reach a height of 30 dm. This species is located on the sand dunes in Utah.

Fourwing saltbush has high drought tolerance. Its root system penetrates to great depths for water, and it has the ability to store water in its woody stalks and leaves (22).

Fourwing saltbush will hybridize with a number of other saltbush species and will produce fertile progeny. Female fourwing saltbush plants were bagged and hand pollinated with pollen from the following species; A. garrettii, A. gardneri, A. confertifolia, A. cuneata, and A. falcata. Limited fertility resulted from these crosses (7). No exact statements were made as to percentages of fertile crosses. Fourwing saltbush will also hybridize with greasewood (Sarcobatus vermiculatus) (11).

#### Causes of Poor Establishment

Fourwing saltbush has been a difficult plant to establish from seed (17). Poor utricle fill, seed dormancy in newly harvested seed (10 to 12 months after harvest) and slow germination are all causes of poor establishment (16). The utricle or outer covering surrounding the seed is extremely hard and slows germination (16). Gamrath (8) showed that dewinging of the utricles increased speed of germination (8). After dewinging, the utricles were placed on a gravity table to separate light from heavy utricles. Heavy utricles had 95 percent seed fill and light utricles had 23 percent seed fill. Smaller but not the lightest utricles had the highest percent of fill (8). Fourwing saltbush can be germinated at 15, 20 or 15-25C (2, 8).

Date of harvest affects utricle fill and germination percentages

(8). Utricle fill was highest when utricles were harvested on August 17 and declined after that date (8). Highest germination however, was obtained when utricles were harvested on October 1 (8). Springfield (16) reports that utricule fill is possibly under genetic control. He showed that the largest utricles on a plant had the highest percent fill. Soaking seeds for eight hours in water did not affect germination. He concluded that germination inhibitors were not removed by soaking in water. He was unable to correlate utricule fill with elevation, annual precipitation, or latitude of collection site. Storage of seed near 12C will improve speed of germination as will dewinging of utricule; however, total germination was not increased (16). Other factors which may affect the germination of fourwing saltbush are embryo dormancy, temperature, moisture, and age of seed (16).

Morphology of Nutall's Saltbush (*A. nutallii*) and Fourwing Saltbush (*A. canescens*)

Hall and Clements (9) describe fourwing saltbush as an erect shrub, woody throughout, with numerous scurfy leaves alternate or sessile, linear to spatulate or oblong in shape. Flowers are generally one-nerved and dioecious (rarely monoecious), staminate flowers are found in dense spikes on long terminal panicles. Female flowers are non-showy, dense leafy-bracted spikes and panicles; perianth 4 or 5 cleft in staminate flowers. Utricles are sessile or stalked and free tips project as wings. The utricule face is smooth or with small projections between wings.

Nuttall's saltbush is described as an erect or slightly spreading sub-shrub, woody at the base, with ascending stems mainly herbaceous. Leaves are scurfy in appearance and spatulate or ovate in shape. Flowers mostly dioecious (rarely monoecious), staminate flowers appear in terminal spikes. Pistillate flowers on long, compact terminal spikes and spike-like panicles. Utricles are generally sessile but one sub-species has been known to have stalked utricles (9).

The Genetics of *Atriplex* sp.

Fourwing saltbush apparently has a broad genetic base which makes it adaptable to many climatic conditions. Cytological studies are being completed on many *Atriplex* species. The basic chromosome number for *Atriplex* is 9. Stutz (19) found that Gigas *A. canescens* has a chromosome number of  $2n=18$  and that the typical *A. canescens* has a chromosome number of  $2n=36$  (10, 11).

## MATERIALS AND METHODS

### Chromosome Number Determination of *Atriplex* sp.

Attempts were made to determine the chromosome number for the following types: Accession PI-15585 from Bridger, Montana; Nuttall's saltbush, a selection from a native stand near Bridger; and from plants having morphological characteristics of both species which were collected near Circle, Montana. Seeds were germinated at 15-25°C. Root tips were cut to 1 cm in length and placed in oxyquinoline (21) for 4 to 6 hours, and then placed in 5 percent aceto-carmine stain for 24 to 36 hours (15). After staining, root tips were placed in Farmer's solution for one hour to set the stain and to preserve them for later examination. Root tips were placed in 10 percent HCL for eight minutes to break up the outside layer of cells. They were macerated using a razor blade and dissecting needles. The material was then transferred onto a microscopic slide and a cover slip placed over the material. Aceto-carmine was diffused under the cover slip to keep the macerated tips moist and the cover slips were sealed in place with clear fingernail polish.

Cells containing chromosomes were located using 10 x magnification and then viewed more carefully under high power and phase contrast.

### Pollen Study

The first study was to determine if sunlight and high temperatures would affect pollen viability. Microscope slides, with glycerine used as an adhesive substance to catch pollen, were placed at four random areas in a seed production field at Bridger, Montana, at 10:00 A.M. and left until 4:00 P.M. Slides used as checks were set out and collected

every hour to determine the percentage of viable pollen. Each slide was divided into three sections with a grease pencil for counting purposes. A small drop of aceto-carmin stain was then applied to each slide and one minute allowed before counting viable pollen grains with the aid of 10 x magnification. Three areas were counted on each slide. Pollen grains were considered viable if they stained deep pink (15).

The second part of this study was to determine peak pollen shed during the day. Three glycerine coated slides were placed in the field at random and collected at two-hour intervals. Slides were set out in two hour intervals from 8-10, 10-12 AM, 12-2 and 2-4 PM. Temperatures were recorded at the beginning and end of each time period. Slides were examined and stained as previously explained.

The third study was to determine the period of pollen shed and if period of pollen shed coincided with the bloom of the female flower. Glycerine coated slides were placed in the field from 11 AM to 5 PM at four randomly selected areas. The slides were collected and stained as previously described. Female flowers were also selected at random in the field during this time period and examined under the microscope. If female flowers were extruding feathery stigmas without signs of deterioration, they were considered able to accept pollen.

The pollen experiments were analyzed as: 1) a factorial analysis, 2) a regression analysis where the number of pollen grains dispersed were regressed on temperature, and 3) analysis of variance using a Duncan's Multiple range test for mean separation.

### Irrigation Study

This study was designed to determine the effect of irrigation practices upon utricle fill and was conducted at the Bridger Plant Materials Center. Irrigations were applied as follows: none in 1972; twice in 1973, once on June 15, just before flowering took place, and again on July 20 after flowering was completed; twice in 1974, during flowering on July 1 and after flowering on July 24. Environmental conditions did not vary greatly during the three years. Three groups of 75 utricles from each years seed harvest were cut and evaluated for seed fill by cutting.

### Bisexual Plant Study

Self-fertility of bisexual plants was studied. In the fall of 1972, a bisexual plant was selected from the seed production field at Bridger, Montana, for its ability to produce a large number of utricles and for its leafy appearance. Male and female dioecious plants were also selected from the same field, using the same selection criteria. The plants were carefully removed from the field by digging, and top growth was pruned back and the roots packed in moist vermiculite to keep them moist. The plants were then taken to the Montana State University greenhouse where the crown area of each plant was split into four equal parts. These parts were put in a mist bench to allow the root system to develop. After three months, they were transplanted to pots and watered sparingly until spring. On May 15, 1973, the clones were transplanted in the Cottonwood Creek drainage, located approximately 15 miles southeast of Bridger.

This area was chosen because of the absence of other saltbush plants. The plants were spaced one-fourth mile apart and randomly arranged. Plants were caged to protect them from livestock and rodents, and the plants were watered once a week until established. Seeds were collected in the fall of 1974 and percent utricle fill determined.

#### Plant Morphology Study

This study was conducted on native plants at Circle, Montana, and near Bridger, Montana. Both areas have plants with morphological characteristics of A. nutallii and A. canescens.

Seeds were collected and percent utricle fill was determined for plants from both areas. Morphological characteristics studied to describe each plant included plant height, utricle shape and size, fruit pedicelled or sessile, leaf shape, woody or herbaceous stems. The plants in this study were classified into three groups:

1. Type I which closely resembled A. canescens.
2. Type II which showed characteristics of both species.
3. Type III which closely resembled A. nutallii.

#### Evaluation of Southwest Accessions of Fourwing Saltbush for Seed Production in Montana

The main objective of this study was to determine if accessions from other areas could be grown for seed production in Montana and to determine if they could be combine harvested. Seeds of nine accessions were obtained from plant material centers in the Southwest. Accessions evaluated included, Utah 621, Texas 1041, New Mexico 155, Utah 8871,

Arizona 17279, California 32461, California 17364, and Arizona 15644.

Accession PI-15585 was used as the check.

A completely randomized block design with three replications was used. Plots were 1.6 x 3.3 meters. The accessions were planted in the spring of 1973 and harvested in the fall of 1974.

## RESULTS

### Chromosome Study

The chromosome number for fourwing saltbush accession PI-15585 was determined to be  $2n=36$  (Plates 1 and 2). Chromosome numbers of native collections at Circle, Montana, were not obtained.

The Nuttall's saltbush seed collected near Bridger, Montana, appears to have a chromosome number of  $2n=18$ . Several counts were made showing  $2n=16$  and 17. However, a photograph of those chromosomes was not obtained.

### Pollen Study

Exposure to sunlight for one to five hours did not affect pollen viability. The average viable pollen grain percentage was 96.0% for the hourly checks and 95.5% for those slides left in the field for five hours. Plate 3 shows a viable and a non-viable pollen grain.

Optimum temperature for pollen shed ranged from 16 to 19C (Figure 1) and with an increase in temperature above this range, pollen shed decreased. Pollen shed was associated with temperature but not with time of day. A significant negative correlation coefficient for pollen shed and temperature was found to be a  $-.77^*$ . The correlation coefficient for pollen shed and time of day was .34.

Few pollen grains were collected the first day because of low pollen shed. On the second and third day pollen production peaked at the optimum temperature of 19C and decreased as temperature increased. On the fourth day, the temperature was close to optimum for pollen shed during the entire day, however; a rain shower stopped pollen shed.



Plate 1. A phase contrast photomicrograph of mitotic metaphase in a root tip cell of fourwing saltbush. The chromosome number is  $2n = 36$  (magnification 3111 x).

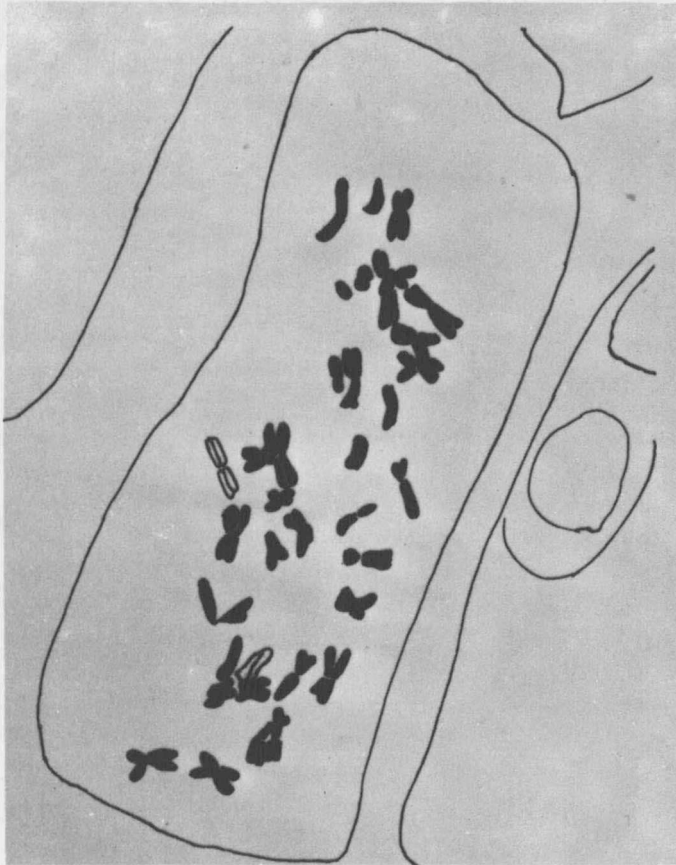


Plate 2. A schematic drawing of the cell in Plate 1. The two white chromosomes cannot be seen on the photomicrograph because they were on a different plane of focus.

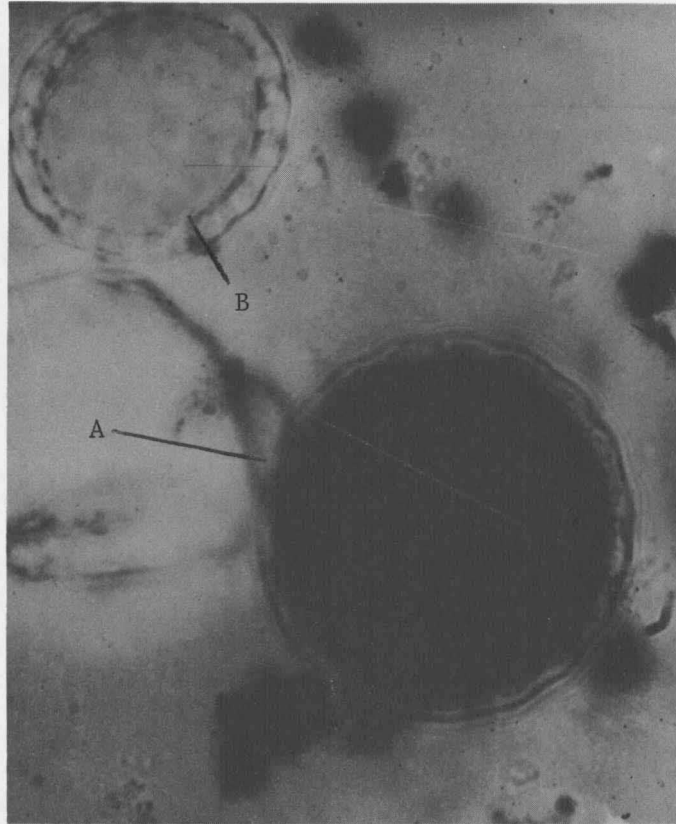


Plate 3. Fourwing pollen grains. A) Viable pollen grain and B) Non-viable pollen grain.

Pollen was shed from June 26 through July 16 (Table 1) and pollen production was about the same for all dates except on July 16 when pollen production was reduced. Pollen grains were not detected after July 16.

Female flowers selected at random throughout the field had receptive stigmas from June 26 through July 16. Flowering distribution was similar for both male and female plants. Plate 4 shows a stigma which is receptive to pollen.

#### Irrigation Study

Timely irrigation of fourwing saltbush was necessary for utricle fill. In 1972, when plants were not irrigated, only 3% of the utricles were filled. In 1973, when water was applied on June 15, before flowering occurred, and July 20, when flowering was completed, 59% of the utricles were filled (Table 2). With a somewhat similar irrigation regime in 1974, 50% of the utricles were filled. Climatic conditions during the three years of this study at Bridger did not vary greatly.

#### Bisexual Plant Study

The one bisexual plant which produced seed in the Cottonwood Creek area had a utricle fill of 50.6% compared to 32.0% for unprocessed utricles for accession PI-15585 from the seed production field at Bridger, and 53.6% for the same utricles which were not processed (Table 3). Plates 5, 6, 7, and 8 show a sequence of flower morphology from bisexual plants.

The bisexual plant appears to be fertile. The Cottonwood area is extremely dry with an average annual precipitation of 15 cm. Other

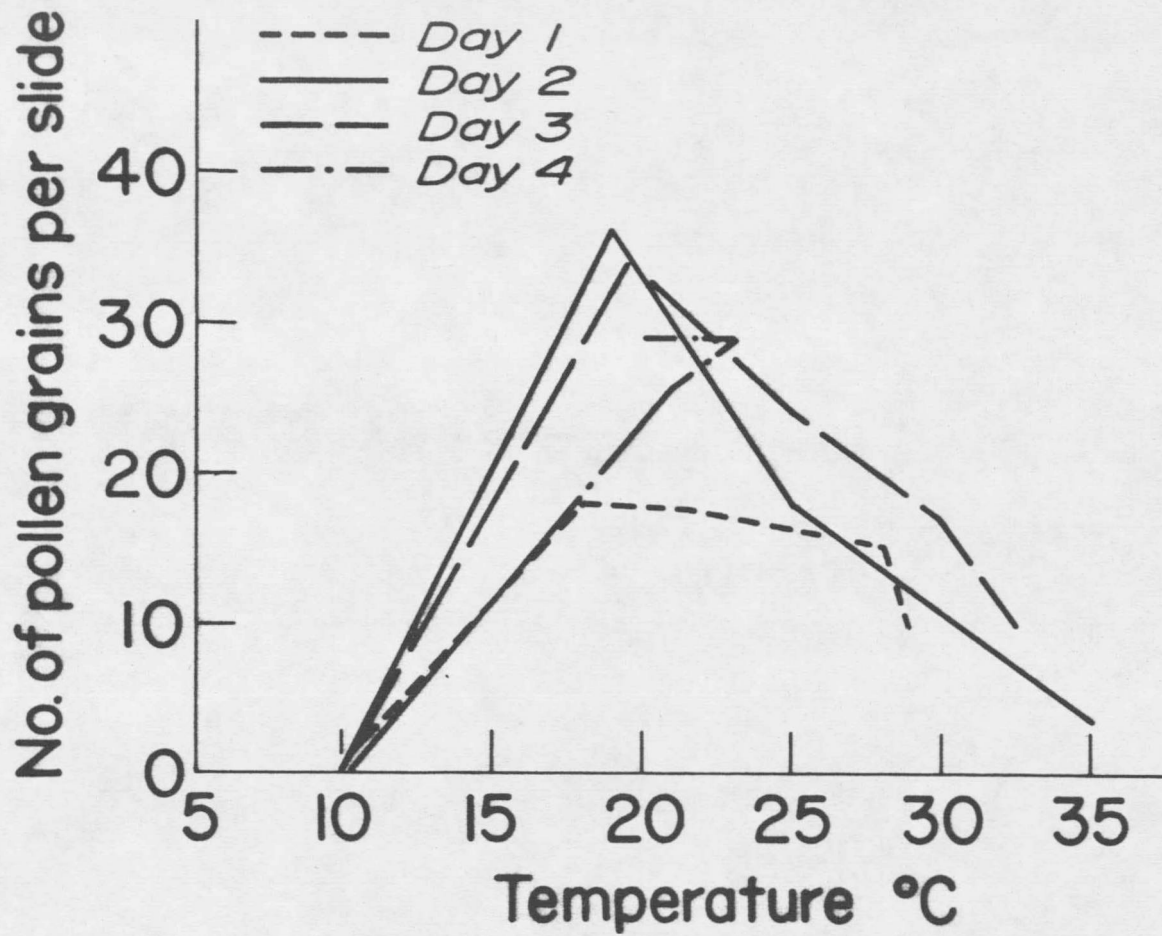


Figure 1. Effect of temperature on the number of pollen grains collected over a four day period at Bridger, Montana.

Table 1. Seasonal amount of fourwing saltbush pollen shed at three random areas on six dates in the seed production field at Bridger, Montana in 1973.

Sampling date	Area showing number of pollen grains/slide			
	I	II	III	Means
6/26	7.2	6.3	5.0	5.8a*
6/28	4.3	5.6	8.3	5.4a
7/16	1.6	1.3	1.6	1.5 b
7/18	0.0	0.0	0.0	0 c
7/23	0.0	0.0	0.0	0 c
8/1	0.0	0.0	0.0	0 c
Means	4.4a	4.4a	4.9a	

\* Means with a letter in common are not significantly different at  $p=0.05$ , Duncan's Multiple range test. (Means for columns can not be compared in those for rows).

Table 2. Effect of irrigation on percent utricle fill of fourwing saltbush being grown at the Bridger Plant Materials Center, Bridger, Montana.

Year	Irrigation dates	% utricle fill
1972	None	3.3
1973	June 15, July 20	59.3
1974	July 1, July 18	50.6

Table 3. Percent utricle fill of bisexual and dioecious plants selected from PI-15585.

Plant type	% utricle fill
PI-15585	
Bisexual	50.6
Unprocessed dioecious	32.0
Processed dioecious (empty utricles removed)	53.6

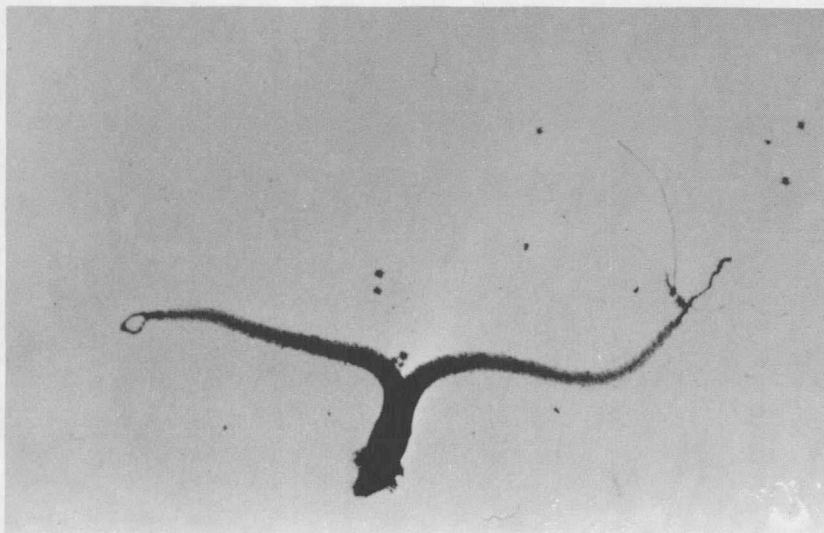


Plate 4. A stigma from a female fourwing saltbush flower which has a feathery appearance indicating that it is receptive to pollen.

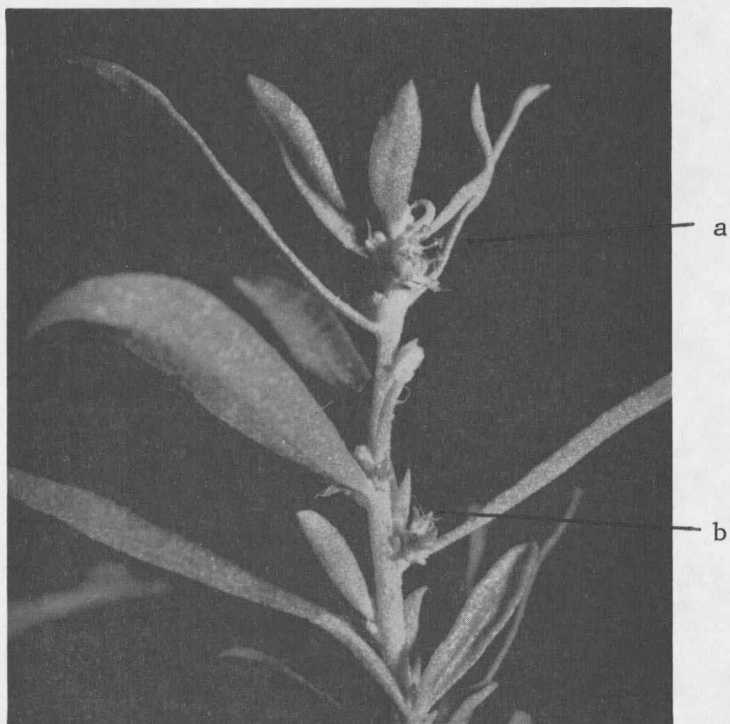


Plate 5. A monocious fourwing saltbush plant showing (a) anthers of a male flower and (b) exposed stigmas of a female flower.

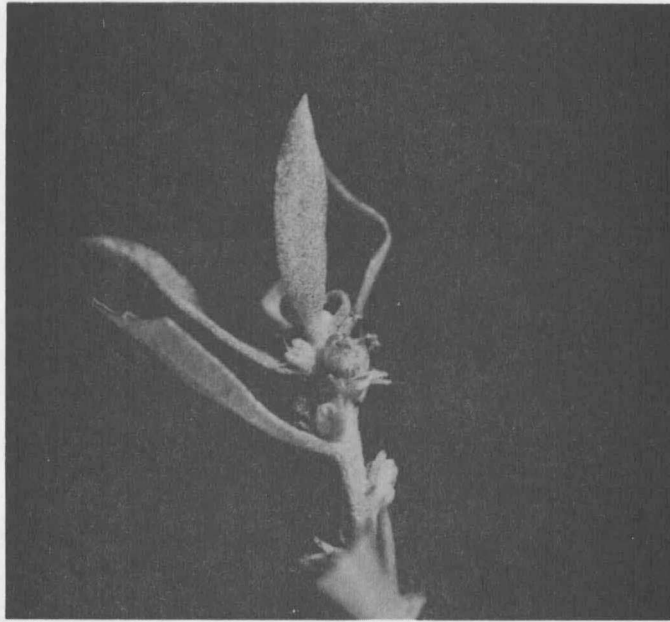


Plate 6. Close-up view of a stigma from a female flower of a monoecious fourwing saltbush plant.

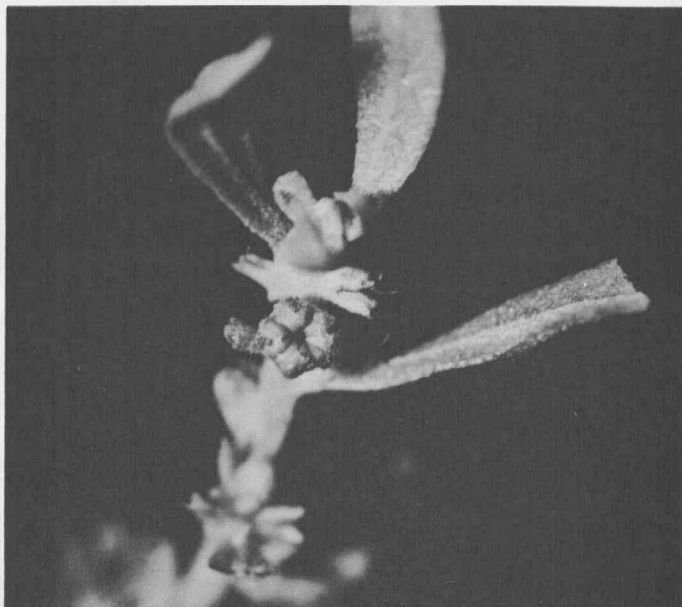


Plate 7. Close-up view of the anthers of an unopened male flower of a monoecious fourwing saltbush plant.

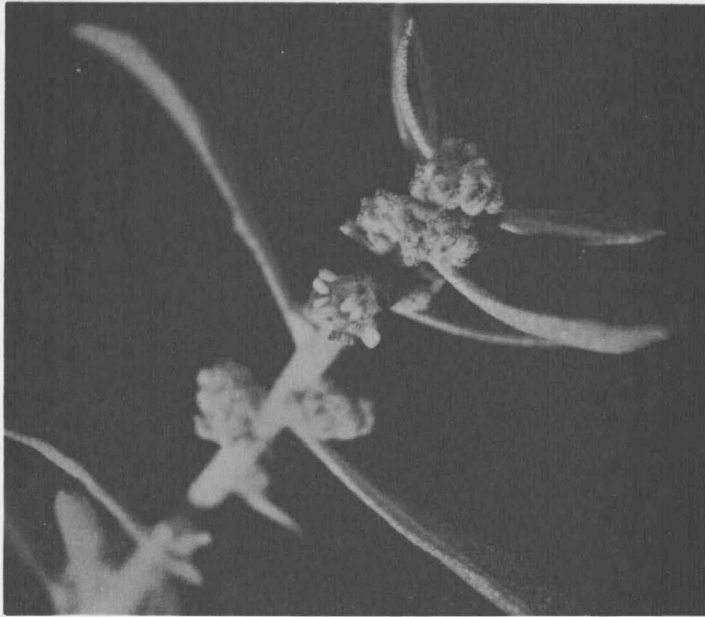


Plate 8. Male flowers of a monoecious fourwing saltbush plant with extruded anthers.

plants transplanted to the Cottonwood Canyon did not set seed, were injured by rodents or livestock, or died. Seed was not obtained from any of the other bisexual or dioecious plants.

#### Plant Morphology Study

Three categories of plant types were identified at Bridger and Circle, Montana. These plant types had similar morphological characteristics regardless of the area in which they were grown. Plate 9 shows the site where the three plant types were discovered at Circle. The first type closely resembled Nuttall's saltbush (Plate 10). This type had small to medium sessile utricles (Plates 11 and 12). Utricle fill ranged from 17 to 24 percent (Table 4). Wing formation was not distinct and all plants had an elongated type of inflorescence. Upright stems were herbaceous with leaves ovate in shape.

The second group of plant types exhibited morphological characteristics similar to those of both fourwing and Nuttall's saltbush (Plate 13). Utricles were small or medium with wings present which varied from distinct to minute (Plates 14 and 15). Utricle fill ranged from 0 to 40 percent (Table 4). Some plants had many pedicelled utricles but others had sessile or minutely pedicelled utricles similar to type 1. All plants had an elongated raceme type of inflorescence with herbaceous stems. Leaves were ovate to spatulate.

The third type closely resembled the fourwing accession PI-15585, grown at Bridger, Montana (Plate 16). Utricles were large with definite wings with finger-like projections and were usually pedicillate. Utricles

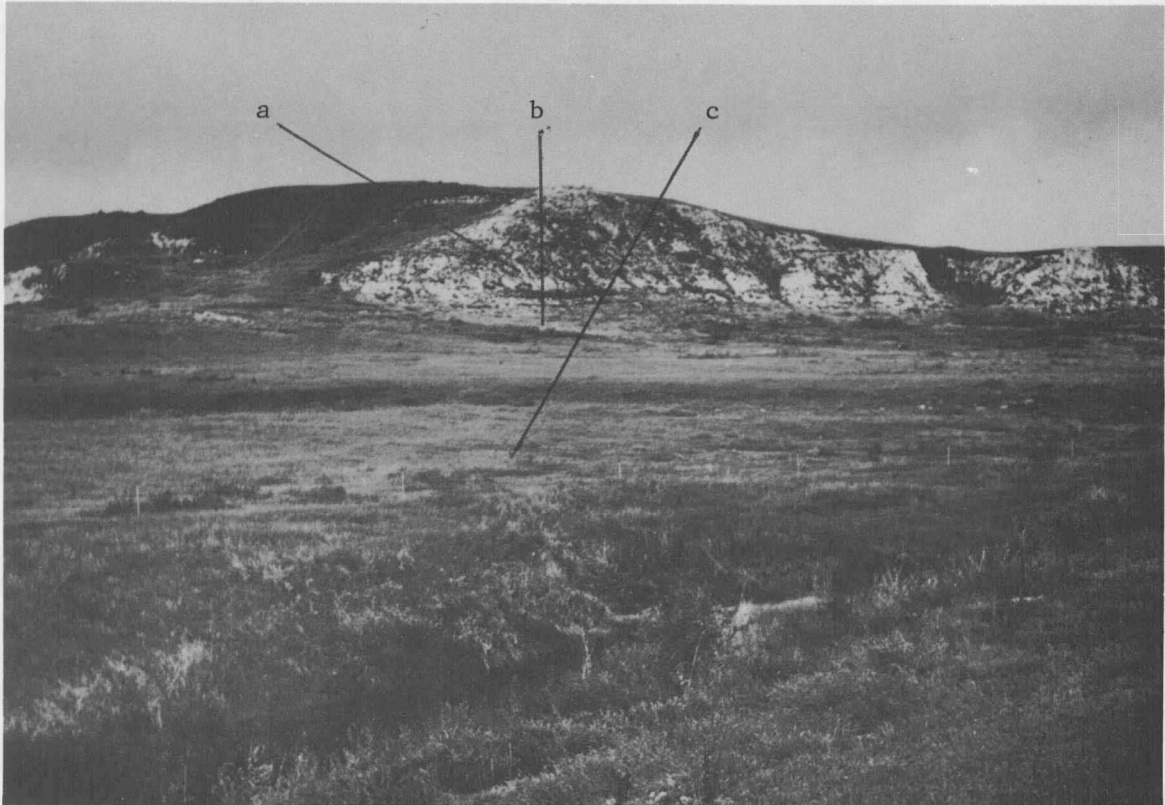


Plate 9.. The collection area near Circle, Montana, where the three types of fourwing saltbush were located; (a) Type III, (b) Type II, and (c) Type I.



Plate 10. A Type I plant from the Circle collection which resembles Nuttall's saltbush. It is low growing and herbaceous.

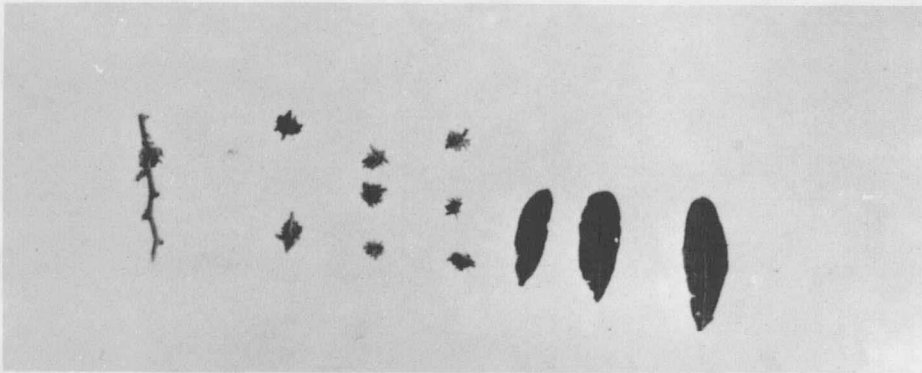


Plate 11. The leaves and utricles from a Type I plant from a native collection of fourwing saltbush at Circle, Montana.

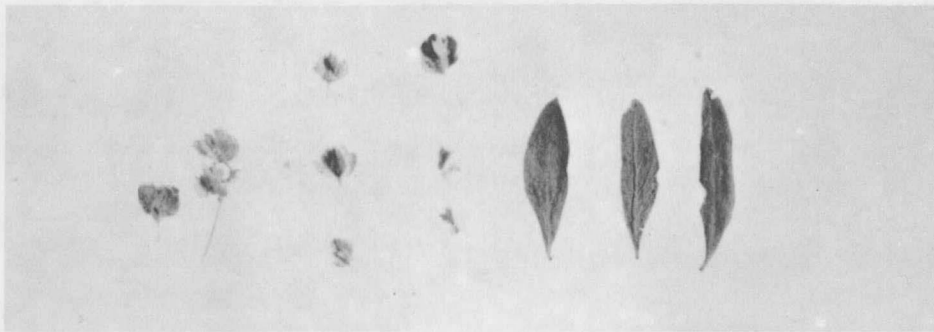


Plate 12. The leaves and utricles from a Type I plant from a native collection of fourwing saltbush at Bridger, Montana.

Table 4. Morphological characteristics of three types of Atriplex species growing near Circle and Bridger, Montana.

Plant characteristics	Plant Types					
	I		II		III	
	Bridger	Circle	Bridger	Circle	Bridger	Circle
Plants observed	2	4	3	3	2	4
Stems	2	4	3	3	2	4
Woody					2	4
Herbaceous	2	4	3	3		
Inflorescence						
Raceme	2	4	3	3	2	4
Elongate	2	4	2	3		
Cluster			1		2	4
Utricle						
Size						
Large					2	4
Medium	2	2		3		
Small		2	3			
Wings						
Lacking		4				
Present	2		3	3	2	4
Large				3	2	4
Medium	2	4		3		
Small			3			
Serrated	2	4	3	3		4
Smooth					2	
Utricle Attachment						
Pedicled		1	2	3	2	4
Non-pedicled	2	3	1			
Utricle Fill %	17	24	40	0	48	0



Plate 13. A Type II plant from the Circle collection which resembles both A. canescens and A. nutallii. It is a more robust plant with semi-woody stems.

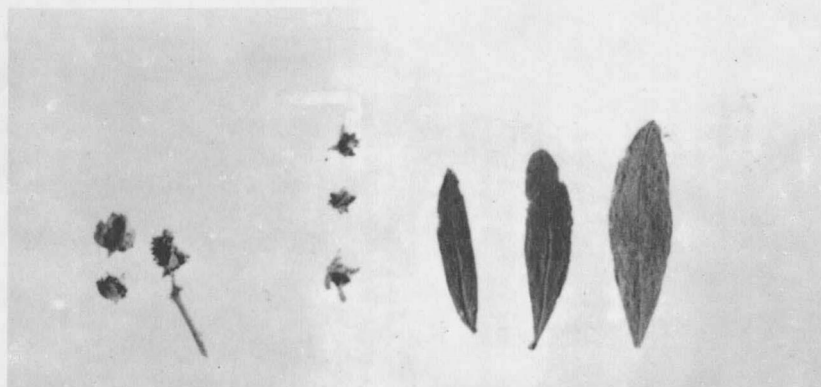


Plate 14. The leaves and utricles from a Type II plant from a native collection of fourwing saltbush at Circle, Montana.





























