SEED ANATOMY, MORPHOLOGY, AND FIELD BIOLOGY OF ARGEMONE SPP. (PAPAVERACEAE)

THESIS

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ABSTRACT

Seed ontogeny in Argemone aurantiaca G. B. Ownbey, seed morphology of the eight Texas Argemone species, and the establishment of three selected Argemone species has been investigated.

Ovule primordia are dizonate. The inner integument is initiated before the outer integument. The ovule is anatropous. Just prior to anthesis, the inner integument is composed of three cell layers and the outer integument is composed of two cell layers. The embryo sac follows the Polygonum-type development. Antipodals are enlarged and persistant as described in A. mexicana L. At maturity, the tegmen is composed of one cell layer and the testa is composed of three cell layers. The mature seed is covered by a thick cuticle.

The root systems and soil preferences of A. sanguinea Greene, A. aurantiaca, and A. albiflora Hornem. subsp. texana G. B. Ownbey, were contrasted. Seed and capsule production, capsule dehiscence mechanisms and seedling establishment were investigated in the latter two species. Seed viability and germination rate were determined for A. aurantiaca.

An S.E.M. examination of the seed coats of the eight species revealed differences in seed length, micropyle and chalazal morphology, and density of reticulations, papillae, and stomatal apparati among the eight species. Based on these data, a taxonomic key to the seeds of the Texas Argemone species has been developed.

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Those having torches will pass them on to others.
--Plato, The Republic

CHAPTER I

INTRODUCTION

Argemone L. (prickly poppy) is one of twenty-six genera in the family Papaveraceae. The genus name is derived from the Greek word, argemon, which means "cataract of the eye". Pliny applied the name to a poppy-like plant, the juices of which were used in the treatment of cataracts (Everette, 1977).

Argemone is native to North and South America and to Hawaii. Several species are naturalized in the temperate and subtropic regions of the world (Ownbey, 1958 and1961). Twenty-eight species of Argemone are recognized (Ownbey, 1958; Powell, 1972; Johnston, 1976). The most widespread and documented species is A. mexicana L. The distributional range of A. mexicana and six other species extends into Texas. An eighth species found in Texas, A. aurantiaca G. B. Ownbey, is endemic to the south-central portion of the state (Correll and Johnston, 1970). Argemone typically inhabits disturbed areas such as railroad and highway rights-of-way, drainage ditches, pastures, and fields.

The 1939-1950 outbreak of epidemic dropsy in India was traced to food that was prepared with mustard seed oil which was contaminated with A. mexicana seed oil. The seeds of A. mexicana were harvested with the mustard seeds and the oils extracted from the seeds of both species were mixed during processing (Sanyal, 1950). The oil mixture and pure A. mexicana seed oil are suitable for human ingestion after purification (Shenolikar, 1976).

Sanguinarine, a broad-spectrum antibiotic, is one of several alkaloids which can be extracted from the oil of A. mexicana seed. A 1% emulsion of sanguinarine is suitable for topical application and has a strong antifungal effect on pathogenic fungi (Vichkanova and Adgina, 1971). Tinctures made from A. mexicana seed are used in Viet Nam for the treatment of purulent diseases and fungal skin diseases. The tinctures also reduce inflammation in burns caused by scalding (Bui-Tu-Yu and Sokolov, 1974).

Cook and Collins (1903) reported the use of A. mexicana seeds as a purgative and the use of the latex in the treatment of opthalmia in Puerto Rico. The latex and seed extracts of A. mexicana inhibit the infectivity of the watermelon mosaic and papaya viruses (Tewari and Shukla, 1982; Khurana and Bhargava, 1970).

Oleic and linoleic acids are present in high percentages in A. mexicana seed oil, making the oil suitable for use in paint and varnish manufacture (Garamendi and Leon, 1951). The oil is used as lamp fuel and (on a village scale in Africa) in the manufacture of soap (Vaughan, 1970; Greenwood and Perry, 1972; Bose, 1977).

Although the genus is potentially beneficial, its natural history has not been well-documented for a representative number of species. The present study focuses on selected aspects of the eight Texas Argemone species and has three goals. First, ovule and seed ontogeny in A. aurantiaca will be described and compared to A. mexicana, thus expanding our knowledge of the genus.

Second, seed dispersal and seedling establishment in A. aurantiaca and A. albiflora Hornem. subsp. texana G. B. Ownbey will be investigated. This will continue the documentation of the life cycle of A. aurantiaca and A. albiflora subsp. texana begun by Nichols (1982) and Schneider and Nichols (1984). Information from this study may be useful in landscaping with native species. Argemone seeds are used in very few "wildflower" seed mixes and consitute only 2-3% of the total number of seeds in these mixes (pers. comm. with Paul McCormick, Clyde-Robinson Seed Company, Hayward, California).

Third, the seeds of the eight Texas Argemone species will be described. Prior to this study, Argemone seeds had not been examined at greater than 60X magnification with a dissecting scope and no species-specific descriptions existed for the seed of the genus. A taxonomic key to the seeds of these species may be of use to federal, state, and commercial agencies in quality control of "wildflower" seed mixes and for "weed" seed identification in crop seed and soil samples.

CHAPTER II

HISTORICAL REVIEW

Seed Ontogeny

Bose (1937), Crete (1956), Joshi (1933), Sachar (1953, 1955, 1956), and Soueges (1926) have detailed megasporogensis, megagametogenesis, and embryogenesis in A. mexicana. Studies on seed ontogeny in Argemone are infrequent in the literature.

The ovule primordia of A. mexicana are initiated on parietal placentae. The inner integument develops at about the same time as the hypodermal initial. The outer integument develops later, more proximal to the placenta, and overtops the inner integument as the ovule matures.

In the crassinucellate ovule, megasporogenesis produces a linear tetrad of megaspores. The chalazal megaspore is functional and undergoes the Polygonum-type megagametogenesis.

At maturity, the ovules are bitegmic and anatropous. The inner integument is three cell layers thick and the outer integument is two layers thick. At the micropyle and the chalaza the integuments are multistratose.

The single ovule vascular trace, visible at the megaspore tetrad stage, extends to the chalaza. Xylem and phloem differentiation is complete at the mature embryo sac stage. The nucellus is 3-4 cell layers thick at the sides and the chalaza. Stomatal apparati are located on the raphe.

The pollen tube is usually monosiphonous and porogamous. It grows through a short, hollow, stylar canal which is lined with transmitting tissue.

Endosperm formation begins very soon after fertilization. When coenocytic endosperm lines the embryo sac, the antipodals are prominent and metabolically active.

Except for the persistant epistase and hypostase, the nucellus is absent at seed maturity. At this stage, the tegmen is one cell layer thick and the testa is two cell layers thick (Bandari and Bhargava, 1980; Bose, 1937; Sachar, 1953 and 1955).

The anatomy of the mature seed coat in A. mexicana was described by Vaughan (1970) and Kaul (1970). A description of mature seed anatomy for the genus was given by Corner (1976) and Gunn (1980).

Dispersal and Establishment

Kaul (1970) described seed release and seed dispersal (by wind, water, ants, and rats) in Argemone. Seed dispersal by Zenaida auriculata (eared dove) was documented by Bucher and Nores (1974).

No published data exist on seed viability, germination requirements, or establishment requirements for the genus.

Seedlings initially produce a deep taproot which may mature into an unbranched or a branched root system. Some species develop a perennating rhizome that produces axillary buds. These buds may develop concurrently with the axis or develop after the axis ceases growth. Bud growth may be delayed until the next growing season (Ownbey, 1958).

Three species of Argemone are long-lived perennials with woody shoots; A. fruiticosa Thurb. ex Gray, A. ownbeyana M. C. Johnst., and A. turnerae A. M. Powell. Other species are annuals, biennials, or short-lived perennials with rhizomatous systems (Ownbey, 1958).

Two species, A. platyceras Link. & Otto. and A. grandiflora Sweet., are commonly cultivated (Everette, 1977). Ownbey (1958) suggested that three other species have potential in cultivation: A. sanguinea Greene, A. polyanthemos (Fedde) G. B. Ownbey, and A. aenea G. B. Ownbey. All three species are found in Texas.

The genus is adapted to sunny, dry climates and provides a succession of flowers throughout the summer (Everette, 1977). A. aurantiaca G. B. Ownbey and A. albiflora Hornem. subsp. texana G. B. Ownbey produce large, showy, white flowers that attract a variety of pollinators, including Diptera, Hymenoptera, and Coleoptera (Nichols, 1982; Schneider and Nichols, 1984).

Seed Systematics

In his monograph on Argemone, Ownbey (1958) described Argemone seed morphology and listed dimensions for the seeds of each species. Shape and dimensions of the seeds of the Texas Argemone were noted by Correll and Johnston (1970). The morphology of Argemone seeds was characterized by Corner (1976) and Gunn (1980) and illustrated by Gunn and Seldin (1976) with the aid of light microscopes at magnifications of 10 to 60X.

The typical Argemone seed is nonarillate, 1-3 mm long, and dull to shiny brown with undertones of red and blue. Characteristic features of the silhoutte of the subspherical seed are the basal nipple (beak) at the micropyle, the ridged raphe extending from one-half to the full length of the seed, and the chalazal umbo.

The crater-like hilum is positioned on the side of the beak or between the beak and the raphe. The testa is scrobiculate and at 10X the reticulations (depressions) are visible as large cells with straight, radial wall sutures. The reticulations are arranged in a more or less regular pattern. The reticulations are most prominent at the maximum distance from the raphe. Stomatal apparati are present on or adjacent to the raphe (Corner, 1976; Gunn and Seldin, 1976; Ownbey, 1958; and Sachar, 1956;).

Taxonomic keys to the seeds of the North American Papaveraceae (Gunn and Seldin, 1976) and to the seeds of the Papaveraceae and Fumariaceae (Gunn, 1980) allow the identification of Argemone seeds only to the genus level. The four seed characters which separate Argemone seeds from the other genera are the subspherical shape, the basal nipple, the absence of an aril, and the smooth surface (except for the reticulations).

CHAPTER III

METHODS AND MATERIALS

Seed Ontogeny

Collections of A. aurantiaca seeds for the ontogenetic study were made in 1984, 1985, and 1986, from open fields in and around San Marcos, Hays County, Texas. Developmental stages of the seed were fixed in 50% ethanol or in 2% glutaraldehyde in 0.05 M sodium cacodylate buffer. Immature seeds were dissected out of the fruit and partially cut open to facilitate processing. Mature seeds were placed directly in 4% ethylene diamine for two weeks to soften the seed coats (Carlquist, 1982). Three other softening agents were used but proved to be less effective: (1) a 30-minute soak in sodium hypochlorite, (2) a 2-week soak in 10% hydrofluoric acid in 70% ethanol (Sachar, 1956), and (3) a 2-week soak in 1% dioctyl sodium sulfosuccinate in 25% methanol (Gunn, 1980).

All material for paraffin sectioning was dehydrated either in a tertiary butyl alcohol series or in 2,2-dimethoxypropane acidified with concentrated hydrochloric acid then embedded in 57° C Paraplast. Transverse and longitudinal sections were cut at 10-12 μm on a conventional rotary microtome. Sections were stained in Harris' hematoxylin, safranin, and fast green according to standard procedures (Johansen, 1940).

Sections were examined with an American Optical Series 10 Microstar compound microscope and photographed with Kodak Pan-X film.

Dispersal and Establishment

Field observations were made in populations in Hays, Wilson, and Guadalupe Counties in 1985 and 1986. Field and laboratory observations of Argemone included the quantity of fruit and seed produced, seed and seedling viability, seed release and dispersal mechanisms, and the morphology of the subterranean system.

A. aurantiaca and A. albiflora subsp. texana plants were selected for seed and fruit counts according to a modified random sample design (Whittaker, 1975). The root systems of A. aurantiaca, A. albiflora subsp. texana, and A. sanguinea plants were exposed by careful excavation of the soil and were painted white for contrast with the soil (Bohm, 1979).

Soil samples were taken from one native population each of A. aurantiaca, A. albiflora subsp. texana, and A. sanguinea. The Soil Testing Laboratory, Texas Agricultural Extension Service, The Texas A. & M. University System, College Station, Texas, and the Caliche Soil Laboratory, Southwest Texas State University, San Marcos, Texas, performed the soil analyses.

The 1984 and 1985 seed crops were stored in similar conditions: in a laboratory under fluorescent lighting, in a 2-liter Nalgene container with the lid ajar, at 25-30° C and 70-99% RH).

Germination of the 1984 A. aurantiaca seed crop was tested in December, 1984. Each lot of 20 seeds was subjected to one of seven treatments (Table 1). The seeds were then dipped in 100% sodium hypochlorite for 30 seconds to inhibit fungal growth. Seeds were germinated at 25° C in petri dishes filled with vermiculite and moistened with deionized water.

Seed viability in the 1985 A. aurantiaca seed crop was tested in two ways:

Tetrazolium method (TZ): 100 seeds were cleaved in a plane perpendicular to the long axis of the embryo. The seed halves, containing the exposed embyo, were placed on filter paper in a petri dish and covered with a 1.0% solution of

TABLE 1

GERMINATION TESTS ON ARGEMONE AURANTIACA

LOT	TREATMENT
1	STRATIFICATION (9° C FOR 4 WEEKS)
2	24-HOUR SOAK IN DEIONIZED WATER
3	15-SECOND DIP IN CONCENTRATED H_2SO_4
4	SCARIFICATION (SEED COAT SANDED)
5	SCARIFICATION (SEED COAT CLIPPED)
6	DARK-GROWN
7	CONTROL

2,3,5-triphenyl tetrazolium chloride (pH 7.0). A 50 seed control was autoclaved.and treated in the same manner as the experimental group (Lakon, 1948).

Excised embryo method: 200 embryos were teased intact from the seeds and placed on filter paper in petri dishes and moistened with deionized water (Heit, 1955).

Seeds collected in 1984 and 1985 were also tested for germination and viability by the tetrazolium method in January, 1985, by the Texas Department of Agriculture Seed Testing Laboratory, Giddings, Texas.

Seeding density experiments were performed with A. aurantiaca seeds. Experiment A was designed to test the effect of soil preparation and seeding density on germination and establishment. Experiment B was designed to test the effect of seeding density and a simple pretreatment on germination and establishment.

Experiment A: Test plots were established in December, 1984, at the National Wildflower Research Center (N.W.R.C.), Austin, Texas, using seed from the 1984 crop. With no pure live seed (PLS) data available on A. aurantiaca, the basic seeding density (n = 16.8 lbs per acre) was estimated based on data for species with seeds of similar sizes. The estimated PLS rating for A. aurantiaca (40) was multiplied by the average of the suggested seeding densities of Iberis umbellata L. and Centaurea cyanus L. (10.5 lbs. per acre; Applewood Seed Company, 1982).Two types of 40' X 160' plots were established at the N.W.R.C. One plot was prepared by flail-mowing, the other plot was prepared by rototilling the soil.Each plot was subdivided and sown with seeds (Fig. 1). The seeds were broadcast by hand. The seeds were raked into the soil in the rototilled plots.

Experiment B: Test plots were established on the roof of the Science Building at SW.T.S.U. in December, 1985, with seeds from the 1985 seed

crop. A 2' X 8' wooden frame was filled with soil removed from established populations, subdivided, and sown with seeds (Fig. 2). The n rate was based on the results of the SW.T.S.U. germination trials.

Seed Systematics

The eight Texas Argemone species examined in this study are listed in Table 2. The seeds of A. mexicana and A. polyanthemos were provided by Dr. Charles R. Gunn, Curator of the Seed Collection, Agricultural Research Center, United States Department of Agriculture, Beltsville, Maryland. The remaining six seed vouchers were collected from populations in Texas during 1985. One plant was selected as a typical representative from each species. Seed vouchers are stored in the herbarium of Southwest Texas State University, San Marcos, Texas, 78666.

Twelve seeds from each voucher were fixed 12 hours in 70% ethanol-formalin-acetic acid (Johansen, 1940), dehydrated in an ethanol-acetone series, and critical point dried in a Denton CDP-1 apparatus after standard procedures. The seeds were positioned on holders and sputter-coated with gold. Contrast between the background and the seed surface was increased by painting the hortizontal surfaces around the seeds with colloidal graphite.

Cambridge S90 and JEOL JSM-II scanning electron microscopes were used to examine the seeds at accelerating voltages of 10, 15, and 25 kV. The micrographs were made with Kodak Plus-X 35 mm or Tri-X sheet film.

Reticulation density per 0.5 mm^2 and papillae density per 2000 μm^2 were quantified using grids of the appropriate size on micrographs of the seeds. The stomatal apparati density was determined by counting the apparati on one side of the raphe of each seed.

Seed length was measured on thirty seeds per species with an American Optical-Spencer dissecting stereoscopic microscope equipped with an ocular micrometer. The length was measured in the axis parallel to the raphe.

The validity of the seed characters was tested statistically with three types of tests. The F-max test was employed to determine if homoscedasticity (equal variances) existed

TABLE 2

TEXAS SPECIES OF ARGEMONE EXAMINED

SPECIES	COLLECTION
Argemone aenea G. B. Ownbey	Cresson 85003 (SWT)
A. albiflora Hornem. subsp. texana G. B. Ownbey	Cresson 85001 (SWT)
A. aurantiaca G. B. Ownbey	Cresson 85007 (SWT)
A. chisosensis G. B. Ownbey	Cresson 85006 (SWT)
A. mexicana L.	Gunn 375956 (SWT)
A. polyanthemos (Fedde.) G. B. Ownbey	Wiggins 14986 (SWT)
A. sanguinea Greene	Cresson 85002 (SWT)
A. squarrosa Greene subsp. glabrata G. B. Ownbey	Cresson 85005 (SWT)

between the samples of each species for a given character at P = 0.05. The test compares the highest variance to the lowest variance from a group. The data is homoscedastic if the two extreme variances are equal and heteroscedastic if they are unequal.

Prior to the test, all "counted" data were transformed by the equation $\sqrt{x}$ (or $\sqrt{x + 0.5}$ if any 0 counts were present). The transformation is necessary because this type of data consists of counts of randomly distributed objects which do not fall into a normal distribution. The transformation results in data which falls into a normal distribution. Parametric tests require that the distribution be normal and the variances homoscedastic. Nonparametric tests do not have these requirements.

Since the two variances were heterogenous, parametric tests could not be employed to test the equality of means. The nonparametric, Kruskal-Wallis single factor analysis of variance (ANOVA) was used to test the equality of means among the species for each character at P = 0.05. The means were not equal, but ANOVA's do not indicate where the inequalities are located. A nonparametric, Tukey-type multiple comparsions test was used to determine between which species the inequalities existed at P = 0.05 (Zar, 1984).

CHAPTER IV

OBSERVATIONS

The following observations were made during the course of this investigation.

Seed Ontogeny

Ovule primordia are dizonate. The outer zone divides anticlinally and the inner zone divides both anti- and periclinally. The ovules are initiated by localized, periclinal divisions and subsequent enlargement of the cells in the inner zone. The outer zone continues to divide anticlinally as the ovule primordium expands.

Prior to ovule recurvation, the hypodermal initial forms (Fig. 3). At about the same time, the inner integument is initiated by periclinal divisions in a ring of cells in the outer layer, six to eight cells from the apex.

When the ovule is recurved about 90° towards the base of the gynoecium, the hypodermal initial divides periclinally, producing one megasporocyte and a parietal cell (Fig. 4). The outer integument is initiated by periclinal divisions in a ring of cells three to five cells proximal to the insertion of the inner integument.

Megasporogenesis results in a linear tetrad of megaspores. The crassinucellate ovule follows the Polygonum-type embryo sac development. Procambial tissue, connecting the chalaza with the placental trace, is present at the megaspore tetrad stage.

As the ovule matures, the outer integument overtops the inner integument and fuses cogenitally with the funiculus, forming the raphe of the anatropous ovule. Three to five longitudinal rows of ovules are produced on each longitudinal placenta. Placentation is parietal. The single, placental trace contains annular tracheids.

Just prior to anthesis (Fig. 5), the mature embryo sac is surrounded by the nucellus consisting of a minimum of three layers at the sides of the ovule and up to fourteen layers at the chalaza. Within the embryo sac, the antipodals are conspicious (2.5 μm.).

The outer integument (o.i.) is composed of two cell layers (Fig. 6). The cells of the outer epidermis (o.e.) are large and have cutinized walls. The cells of the inner epidermis (i.e.) are smaller cells with dense cytoplasm and large nuclei.

The inner integument (i.i.) is composed of three cell layers (Fig. 6). The cells of the o.e. and middle layer (m.) are rectangular in outline. The cells of the i.e. are slightly larger and cuboidal. All epidermal cell wall surfaces are cutinized.

Both integuments become multistratose near the micropyle and the chalaza. Transmitting tissue can be found on the placenta adjacent to the micropyle. The transmitting tissue is composed of club-shaped, glandular papillae. Monosiphonous, porogamous pollen tubes were observed.

Two to three days after anthesis (Fig. 7), the o.e. cells of the o.i. enlarge in a tangential plane. The i.e. cells divide periclinally, producing a middle layer of cells (m.) in the o.i.. The i.e. cells accumulate crystals and begin to elongate in the radial plane. The m. cells enlarge in the tangential plane.

The o.e. cells of the i.i. are crushed slightly. The m. layer has patches of periclinal divisions. The i.e. cells are slightly enlarged.

In an immature seed (Fig. 8), the nucellar tissue is reduced as the embryo sac and contents increase in volume. The antipodals are prominent (7.5 μm.) and persist through the coenocytic endosperm stage. A hypostase and epistase are present. The coenocytic endosperm becomes cellular and oil globules accumulate in abundance in the cells.

The o.i. develops characteristic features of the mature testa. Secondary cell walls are formed in the outer tangential walls of the o.e. cells. Pit cavities are found in large numbers in the outer tangential wall. Associated with the pit cavities are papillae on the primary wall. The outer wall is cuticularized. The m. cell layer is flattened, except under the sutures of the

radial walls of the cells of the o.e.. The palisade cells of the i.e. contain rectangular and polyhedral crystals which accumulate along the outer tangential wall in the densely stained cytoplasm.

The i.i. begins to degenerate into the final form of the tegmen as the o.e. cells are radially flattened. The m. cells have enlarged in all directions and the i.e. cells are of a moderate size.

In a mature seed (Fig. 8), the testa is composed of the three layers of cells. As the seed dries, the outer tangential wall of the o.e. becomes concave. The m. and i.e. are present as described in the immature seed. The o.e. and the m. layers of the tegmen are crushed. The radial walls of the i.e. become plicated. The nucellus is one layer thick.

The embryo (Fig. 9) is about 1.0 mm long and produces two linear cotyledons. The cotyledons are frequently slightly unequal in length. Each cotyledon contains three veins which arise form a single trace. No evidence of polyembryogeny was observed in A. aurantiaca.

In the mature seed, a thick cuticle lies close to the surface of the seed (Fig. 10). The cuticle may be separated from the seed mechanically with forceps or chemically by soaking the seeds in sodium hypochlorite (Fig. 10).

Dispersal and Establishment

The seed and capsule production data for A. aurantiaca and A. albiflora subsp. texana are provided in Table 3.

In A. aurantiaca and A. albiflora subsp. texana, fruit dehiscence occurs 30-35 days after corolla and staminal abscision. As the capsules dry, the valves in A. aurantiaca open downward one-fourth the length of the ventral sutures, while those in A. albiflora subsp. texana open one-third the length. The globular stigma remains supported by a cage of vertical fibrous strands.

In A. aurantiaca, many of the seeds remain in the basal portion of the capsule, held in by one of two mechanisms. The first mechanism is a result of one of the various capsule

shapes found in the species. In capsules with lanceolate outlines, the fibrous strands may be too close together to permit the large seeds from falling out (Fig. 12). In capsules with ellipsoidal outlines, the fibrous strands are spaced further apart and seeds can fall free of the capsule (Fig 13). In the second mechanism, the seeds may be held in a mass by the hyphae of a fungus (Fusarium ? sp.). The fungus is not present in the unopened capsule. Open capsules with either of these two conditions were artificially shaken in an attempt to release the seeds. A few seeds were released, but the majority of the seeds remained in the capsule.

The small seed size and the clavate outline of the capsule allowed the seeds of A. albiflora subsp. texana to fall freely from the capsule (Fig. 14). Capsules of both species were observed with holes in them near the bases. These holes were apparently chewed or bored by insects. Seeds in these capsules were not disturbed. The openings of some A. aurantiaca capsules were occluded by spider (Thomisidae) webs. These capsules also contained undisturbed seeds.

Natural decomposition released the seeds "trapped" in the base of the A. aurantiaca capsules. The decomposing capsules were found on upright stems, on prostrate stems that fell naturally, or on stems trampled by cattle. In A. albiflora subsp. texana, the capsules decomposed while attached to the upright or reclined stem.

Capsule dehiscence was not observed in A. sanguinea. No seed dispersal mechanisms were observed in any species.

Results of the germination and viability tests on the seed of A. aurantiaca are provided in Table 4. The 1984 crop was retested in 1985 by the Seed Testing Lab (Table 4). No embryo growth was observed in the excised embryo test.

No A. aurantiaca seedlings were observed in the established populations or in the N.W.R.C. plots. Two seedlings were produced in Experiment B: one in P-DS and one in NP-B (Fig. 15). Neither seedling lived more than two weeks. A. albiflora subsp. texana seedlings were present in great numbers in the native populations. Establishment of A. sanguinea was not observed. Seedling morphology was not studied in any of the species.

TABLE 3

SEED AND CAPSULE PRODUCTION OF ARGEMONE AURANTIACA AND ARGEMONE ALBIFLORA SUBSP. TEXANA

	AUR		ALB	
CROP	MEAN ±SD	N	MEAN ±SD	N
SEEDS PER CAPSULE				
1985	80 ± 44	32	--	
1986	73 ± 56	25	112 ± 38	25
CAPSULES PER PLANT				
1985	23 ± 11	25	--	
1986	25 ± 13	25	18 ± 11	25

-- NOT OBSERVED
AUR = A. AURANTIACA
ALB = A. ALBIFLORA SUBSP. TEXANA

TABLE 4

GERMINATION AND VIABILITY TEST RESULTS

GIDDINGS SEED TESTING LABORATORY

CROP	% GERMINATED	% NONVIABLE (TZ)
1984	0.25	99.75
1985	16.00	84.00

AUTHORS TESTS

% GERMINATION								
	LOT	1	2	3	4	5	6	7
1984		0	20	10	5	5	5	10
% NONVIABLE								
	TZ				EXCISED EMBRYO			
1984	0.0				100			

In native populations of A. aurantiaca, rosettes arose from axillary buds located on a perennating rhizome (Fig. 16). Rosettes of A. albiflora subsp. texana developed from the seedlings (Fig. 17). Each rhizome produced three to five buds, one to three of which emerged above ground. Remnants of aerial stems from the previous growing season were found attached to the same rhizome as the rosette (Fig. 18). The annual species (A. albiflora subsp. texana and A. sanguinea) produce taproots (Figs. 19-20).

A. albiflora subsp. texana and A. aurantiaca may grow to 15 dm. A. sanguinea is slightly less tall (12 dm).

The data from the soil analyses are provided in Table 5.

Seed Systematics

Hilum position (with respect to the beak and raphe), raphe morphology, chalazal umbo configuration, and the density of the reticulations per 0.5 mm^2 of seed surface were the gross characteristics examined on S.E.M. micrographs of the seeds of the eight species Argemone (Fig. 21). Fine morphological characters examined included the presence and number of stomatal apparati on the raphe (Figs. 22 and 23), and the morphology and density of papillae per 2000 μm^2 on the surface of the reticulations (Fig. 24).

Micrographs of typical seeds of the eight species are provided in Figs. 25-32. The hilum may be positioned either on the side of an elongate beak (Fig. 32) or between a short beak and the raphe (Fig. 25). The raphe may be normal (Fig. 29) or enlarged into a flap in the area immediately adjacent to the hilum (Fig. 31). The chalazal umbo may be inconspicuous (Fig. 27) or prominent. If prominent, the edge of the umbo may (Fig. 26) or may not (Fig. 30) extend beyond the edge of the seed body.

Micrographs of the papillae present on the reticulation surface are provided in Figs. 33-40. Papillae were either constricted (Fig. 35) or simple (without constrictions, Fig. 38).

A summary of the characters for each species is provided in Table 6. The results of statistical tests are provided in Fig. 41.

TABLE 5

SOIL CHARACTERISTICS FOR NATIVE POPULATIONS OF THREE ARGEMONE SPECIES

		SAN	AUR	ALB
	TEXTURE	S C L	C L	SL
	pH	7.4	7.7	5.8
ppm	N	1.0	13.0	1.0
available	P	1.0	42.0	5.0
form	K	168	240	100
	Ca	140	3521	191
	Mg	64	426	32
	Zn	0.56	2.00	2.00
	Fe	4.6	5.8	12.0
	Mn	2.7	10.0	5.5
	Na	70	105	175
µmole/l	salinity	71	403	72

SAN = A. sanguinea
AUR = A. aurantiaca
ALB = A. albiflora subsp. texana

SCL = SANDY CLAY LOAM
CL = CLAY LOAM
SL = SANDY LOAM

TABLE 6

SUMMARY OF SEED CHARACTERS FOR EIGHT ARGEMONE SPECIES

SPECIES / CHARACTER	SQA	AUR	POL	CHI	MEX	AEN	ALB	SAN
BULKY HILUM	0	0	0	0	0	0	+	0
CU PROMINENT	+	+	0	+	+	+	+	0
CU EXTENDS	0	+	--	+	0	+	+	+
HILUM BET. RAPHE & BEAK	+	+	+	0	+	0	+	0
HILUM ON BEAK	0	0	0	+	0	+	0	+

+ = YES 0 = NO -- = N/A

SQA = A. squarrosa
AUR = A. aurantiaca
POL = A. polyanthemos
CHI = A. chisosensis
MEX = A. mexicana
AEN = A. aenea
ALB = A. albiflora
SAN = A. sanguinea

CHAPTER V

DISCUSSION

One feature which A. aurantiaca exhibits during ontogeny but A. mexicana does not is the presence of a middle layer of cells in the mature outer integument and in the testa. In all other respects, ovule and seed ontogeny in A. aurantiaca are similar to that described in A. mexicana. The ovule primordia are dizonate. In the crassinucellate ovule, the megasporocyte divides into a linear tetrad. The Polygonum-type megagametogenesis is followed. The antipodals are prominent and persistant. The nuclear endosperm becomes cellular and accumulates numerous oil droplets. The i.i. has three layers at anthesis, but only the inner layer remains in the tegmen at seed maturity.

A. aurantiaca is a perennial species that produces a perennating rhizome. It would be suitable for use in long-term displays using native species. No seedlings were observed in the native populations, possibly due to an allelopathic effect from the parent plants or other species in the area. The fact that seedlings were not observed in the artificial plots at the N.W.R.C. soil indicates that the soil may not be suitable for germination and establishment of this species. The germination plots at SW.T.S.U. yielded two seedlings, but the hot, dry environment on the roof may have affected establishment of the seedlings.

Excavations of subterranean systems in native populations revealed no organic connections between individuals in close proximity. However, this does not exclude the possiblity of vegetative reproduction. The connecting organ could decompose rapidly after the establishment of a new individual.

A. aurantiaca was observed growing almost twice as high as previously reported (Correll and Johnston, 1970).

In contrast, A. albiflora subsp. texana and A. sanguinea are annual species which produce short taproots. A. albiflora subsp. texana apparently reproduces readily by seed, making this species desirable for annual displays. Seed viability and germination rates for A. albiflora subsp. texana and A. sanguinea and the pollination syndrome for A. sanguinea need to be investigated.

All three Argemone species are suitable for use in landscaping with native species. Drought-hardy, these plants would require little watering. They produce a colorful floral display throughout the growing season and provide height to wildflower plantings. Insects attracted by Argemone may help pollinate other species in the vicinity. The soil samples provided information about proper habitat when these species are to be used in native species landscaping.

Seed length, beak, raphe, and chalazal morphology, reticulation density, papillae density, and stomatal apparati density were the major seed characters used in describing the seed of the eight Texas Argemone species. Although no one character could be used to separate all eight species, each species has a unique suite of the characters which allows it to be distinguished form the rest.

In contrast with Ownbey (1958), the number of reticulations in a given area (and hence the size of the reticulations) was not found to be proportional to seed size. The seed lengths measured in this study were not identical to earlier descriptions, although the ranges of the seed lengths and relative seed sizes among the species were consistant with earlier descriptions.

A taxonomic key to the seeds of the eight Texas Argemone species, based on the characters described in this study is provided in Fig. 42.

EXPLANATION OF FIGURES

PLATE I

Figures 1-2. Figure 1--Plot diagram for N.W.R.C. seeding experiment. Figure 2--Plot diagram for SW.T.S.U. seeding experiment.

PLATE II

Figures 3-9. Bar represents 0.1mm (1.0 mm in Fig. 9). Figure 3--Ovule with hypodermal initial and periclinal divisions which initiate the inner integument in the protoderm. Figure 4--Immature ovules with megasporocyte and two layers of parietal derivatives. The periclinal divisions in the protoderm which initiate the outer integument are visible below the insertion of the inner integument. Note the degree of curvature of the ovule. Figure 5--Pre-anthesis ovule with mature Polygonum-type embryo sac. Figure 6--Detail of the seed coat and nucellus of a pre-anthesis ovule. Figure 7--Detail of the seedcoat and nucellus of an ovule 2-3 days after anthesis. Figure 8--Detail of the seed coat of a mature seed. Figure 9--A mature embryo.

PLATE III

Figures 10-17. Figure 10--A. aurantiaca seed without chemical treatment to raise cuticle. Bar represents 50 µm. Figure 11--A. aurantiaca seed after chemical treatment to raise cuticle. Bar represents 200 µm. Figure 12--Dehisced capsule of A. aurantiaca (lanceolate). Bar represents 1 cm. Figure13--Dehisced capsule of A. aurantiaca (ellipsoidal). Bar represents 1 cm. Figure 14--Dehisced capsules of A. albiflora subsp. texana (clavate). Bar represents 1

cm. Figure 15--Seedling of A. aurantiaca. Bar represents 1 cm. Figure 16--Rosette of A. aurantiaca. Note the branch from the previous growing season still attached. Bar represents 5 cm. Figure 17--Rosette of A. albiflora subsp. texana. Note the small stage of rosettes. Bar represents 5 cm.

PLATE IV

Figures 18-24. Figure 18--Excavated root system of A. aurantiaca. Segments of rod represent 1 dm. Figure 19--Excavated root system of A. albiflora subsp. texana. Segments of rod represent 1 dm. Figure 20--Excavated root system of A. sanguinea. Segments of rod represent 1 dm. Figure 21--Seed of A. mexicana: B, beak; H, hilum; R, raphe; CU, chalazal umbo. Bar represents 1 mm. Figure 22--Stomatal apparatus on raphe of A. aurantiaca. Bar represents 200 μm. Figure 23--Detail of the stomatal apparatus. Bar represents 50 μm. Figure 24--Reticulation covered with papillae. Bar represents 200 μm.

PLATE V

Figures 25-32. S.E.M. micrographs of seed of the eight Texas Argemone species. Bar represents 1.0 mm. Figure 25-- A. squarrosa subsp. glabrata. Figure 26--A. aurantiaca. Figure 27--A. polyanthemos. Figure 28--A. chisosensis. Figure 29--A. aenea. Figure 30--A. mexicana. Figure 31-- A. albiflora subsp. texana. Figure 32--A. sanguinea.

PLATE VI

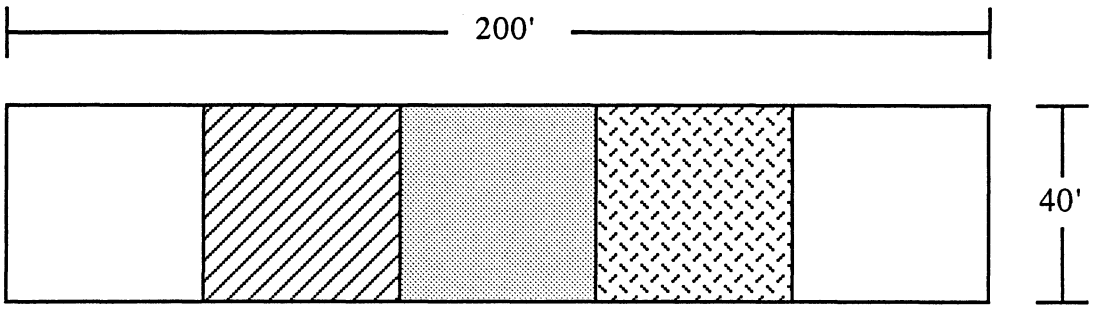
Figures 33-40. S.E.M. micrographs of papillae. Bar represents 10 μm. Figure 33-- A. squarrosa subsp. glabrata. Figure 34-- A. aurantiaca. Figure 35--A. polyanthemos. Figure 36--A. chisosensis. Figure 37--A. aenea. Figure 38--A. mexicana. Figure 39-- A. albiflora subsp. texana. Figure 40--A. sanguinea.

PLATE VII

Figure 41. Results of statistical tests on interspecific comparisons. Means are reported untransformed ± standard deviation. Lines under subgroups of species represent no significant difference detected in the subgroup at P = 0.05.

PLATE VIII

Figure 42. Taxonomic key to the seeds of the eight Texas Argemone species.



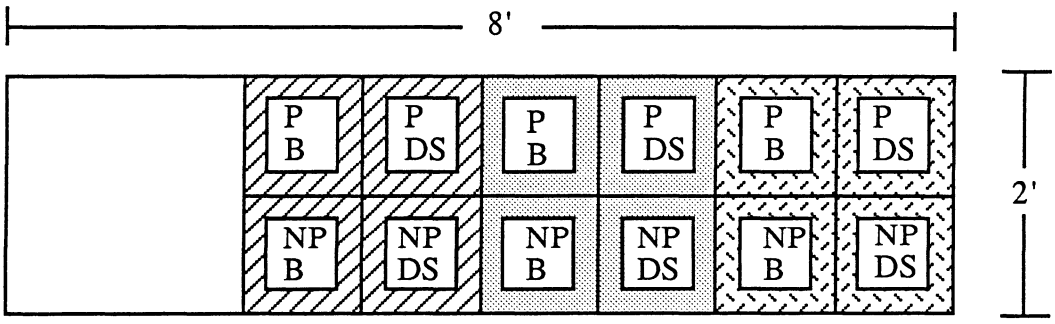
CONTROL (NO SEEDS SOWN)

2n (76 SEEDS PER FT ^{2})

n (38 SEEDS PER FT ^{2})

1/2n (19 SEEDS PER FT ^{2})

(1)



CONTROL (NO SEEDS SOWN)

2n (100 SEEDS PER FT ^{2})

n (50 SEEDS PER FT^2)

1/2n (25 SEEDS PER FT ^{2})

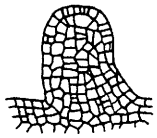
P 24-HOUR DI RINSE

NP NO PRETREATMENT

B SEEDS BROADCAST

DS SEEDS 6MM UNDER SOIL

(2)



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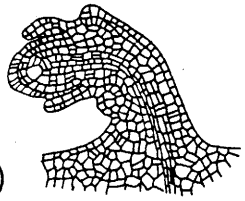

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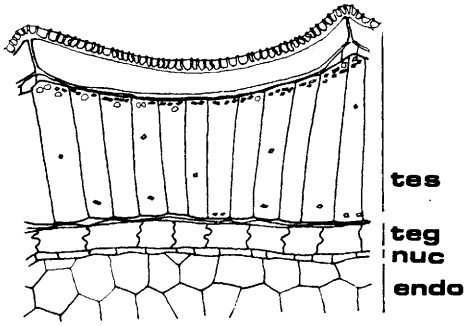

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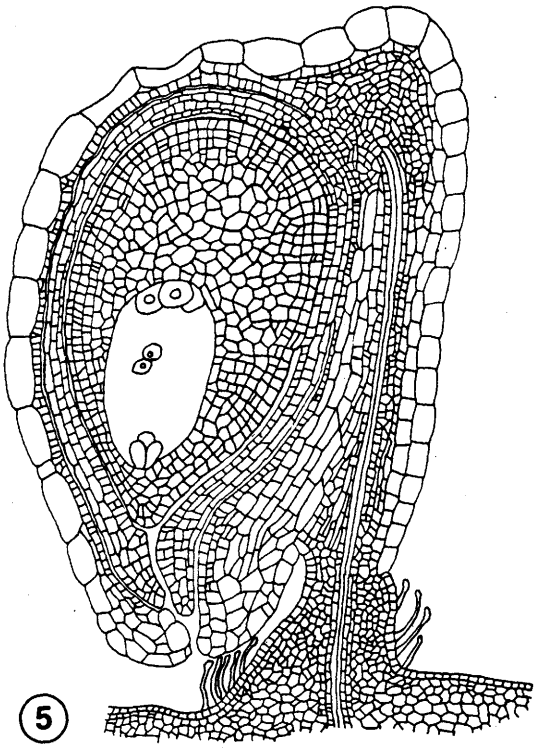

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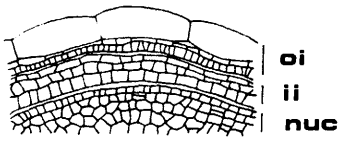

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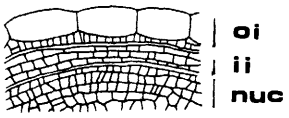

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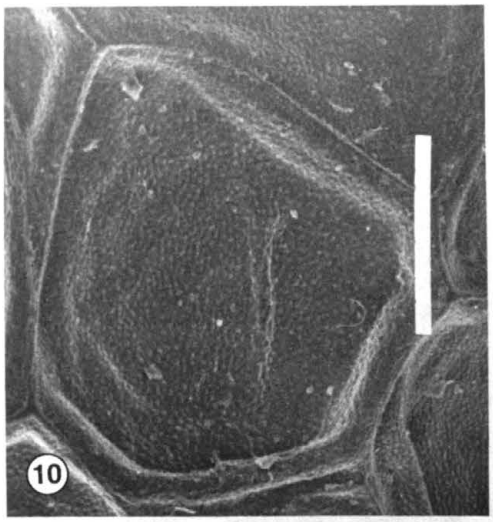
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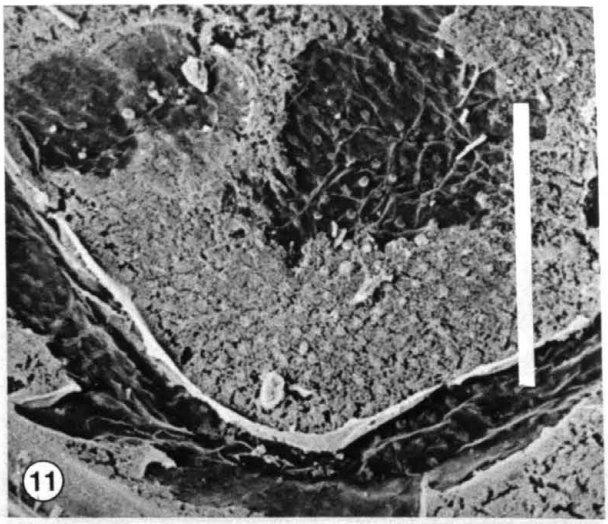
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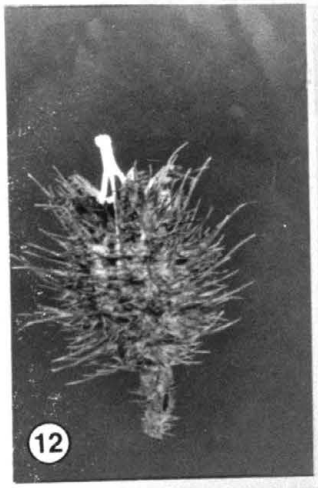
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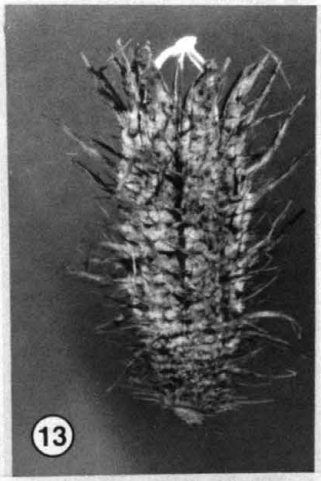
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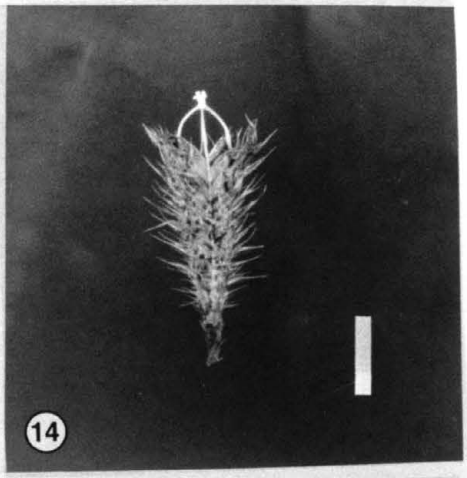
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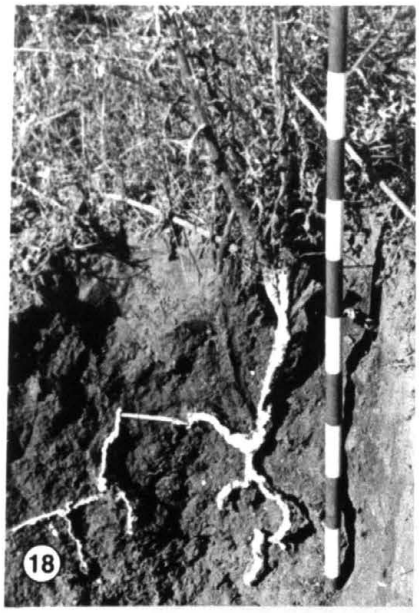
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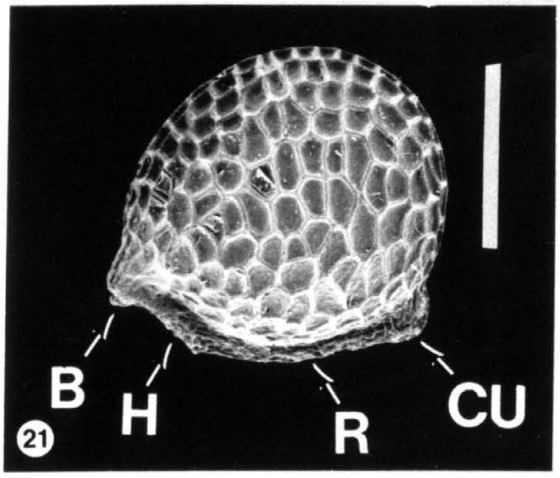
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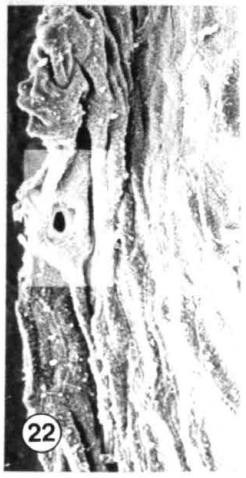
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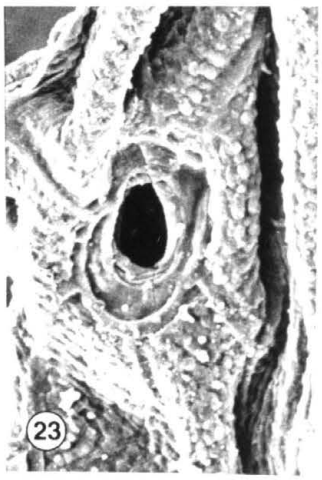
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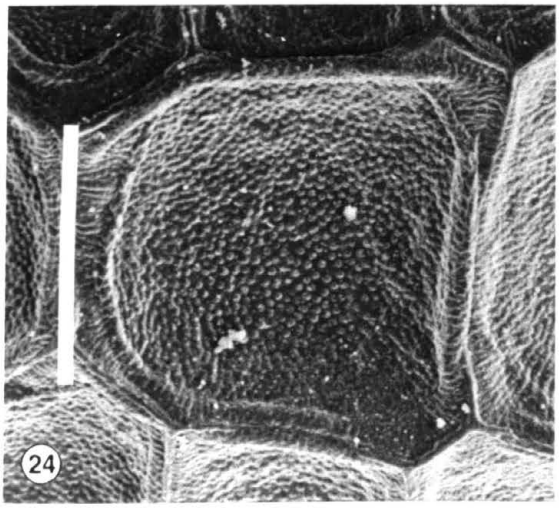
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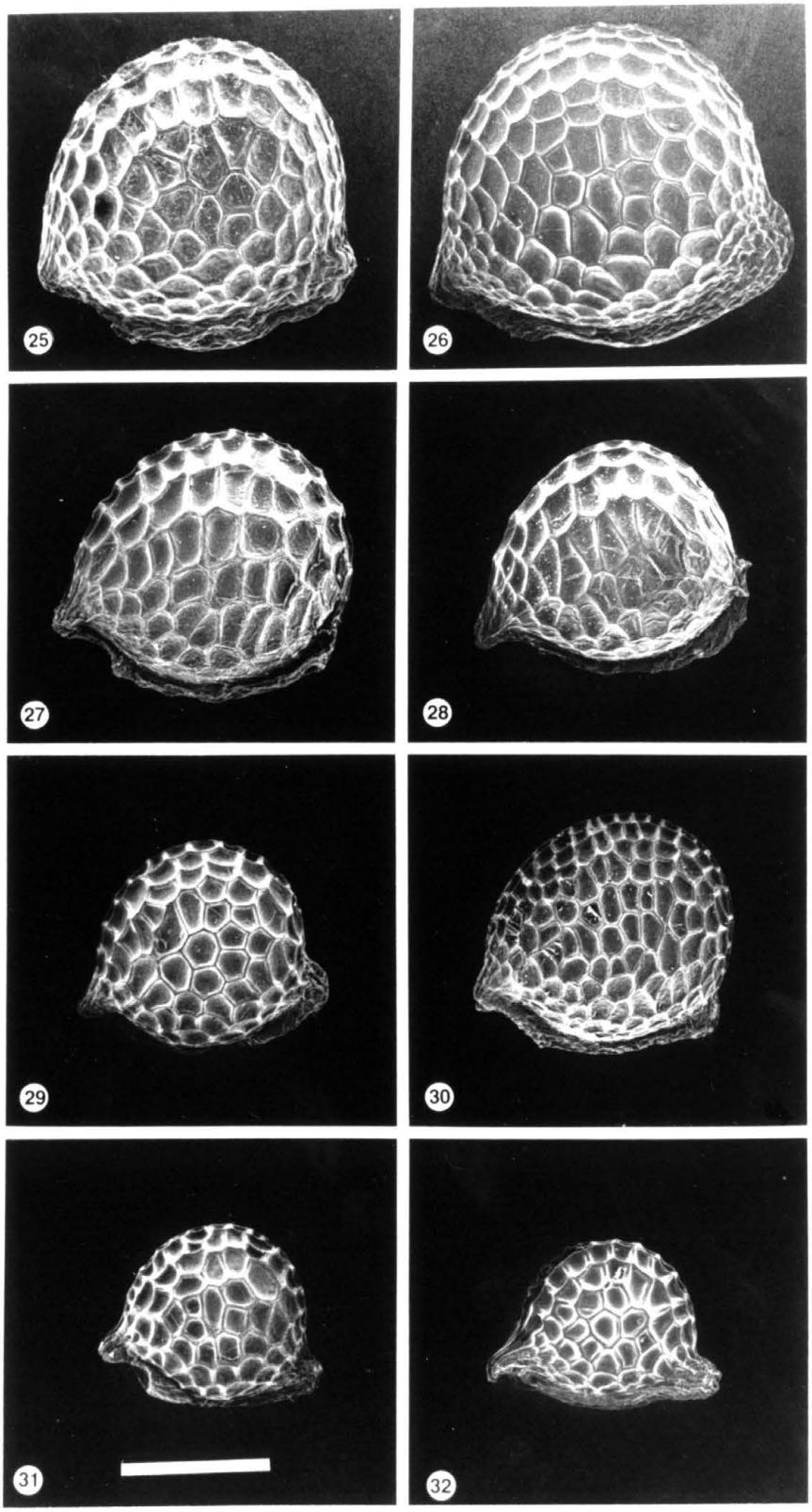
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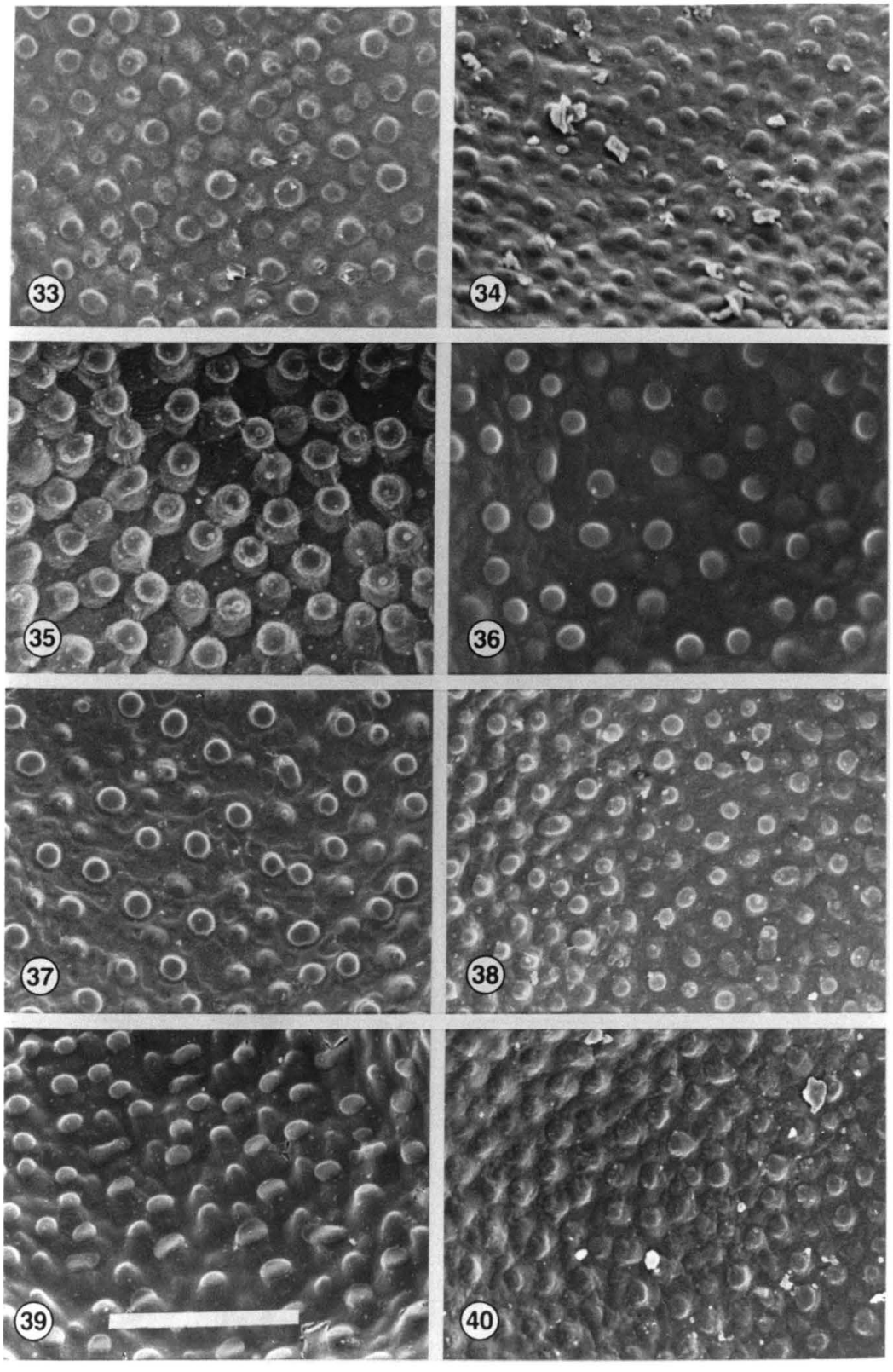
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MEAN SEED LENGTH (MM ± SD)

2.1	2.1	2.1	2.0	1.7	1.4	1.3	1.2
± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1
SQA	AUR	CHI	POL	MEX	AEN	ALB	SAN

MEAN STOMATAL APPARATI DENSITY (± SD)

8 ± 4	5 ± 1	6 ± 2	4 ± 2	4 ± 2	3 ± 2	2 ± 1	1 ± 1
SQA	POL	AUR	ALB	AEN	SAN	MEX	CHI

MEAN PAPILLAE DENSITY PER 2000 μM^2 (± SD)

57 ± 13	39 ± 8	38 ± 14	36 ± 10	22 ± 1	27 ± 8	28 ± 6	21 ± 3
CHI	SQA	AUR	MEX	SAN	ALB	AEN	POL

MEAN RETICULATIONS PER O.5 MM^2 (± SD)

10 ± 1	9 ± 2	8 ± 1	6 ± 1	5 ± 1	4 ± 1	4 ± 1	4 ±1
MEX	ALB	SAN	AEN	SQA	CHI	AUR	POL

KEY TO THE SEEDS OF THE TEXAS ARGEMONE SPECIES

1 Chalazal umbo prominent and extends beyond the seed body (2)

1 Chalazal umbo inconspicuous or within the limits of the seed body (6)

2(1) Seed length 1.7 mm or longer (3)

2 Seed length less than 1.7 mm (4)

3(2) Micropyle located on prominent nipple ..... 1. A. chisosensis

3 Micropyle located on small nipple ..... 2. A. aurantiaca

4(2) Raphe around the hilum enlarged ..... 3. A. albiflora subsp.texana

4 Raphe around the hilum not enlarged (5)

5(4) Micropyle located on prominent nipple, seed length 1.1-1.3 mm, 7-10 reticulations per 0.5 mm^2..... 4. A. sanguinea

5 Micropyle located on small nipple, seed length 1.4-1.5 mm, 4-8 reticulations per .... 0.5 mm^2 ..... 5. A. aenea

6(1) Umbo inconspicuous ..... 6. A. polyanthemos

6 Umbo conspicuous (7)

7(6) Seed length 2.0-2.3 mm, 3-6 reticulations per 0.5 mm^2..... ..... 7. A. squarrosa subsp. glabrata

7 Seed length 1.5-1.8 mm, 8-11 reticulations per 0.5 mm^2 ..... 8. A. mexicana

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