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Animal dietary preferences have been studied by feeding-site examinations, animal observation, and stomach and esophageal collections. Analysis of fecal material for undigested plant cuticle is another means for obtaining the same information.

This study was conducted to: (1) prepare a microscope slide series depicting the surface features of leaves and current annual growth of stems of selected plants, (2) evaluate methods for preparing plant and fecal material for cuticle examination, and (3) develop a key for identification of these plant surfaces.

Plants were collected and identified by personnel of the PNW Forest and Range Experiment Station. All other work was conducted at Oregon State University, Corvallis, Oregon.

Reference slides were prepared by three techniques: scraping, maceration and replication. All were appropriate for fresh or

herbarium plant material. Scraping was a tedious method which provided pieces of cuticle representing the surface where other methods were unsuitable. Maceration yielded cuticle fragments strong enough to withstand the acid treatment, but was not adaptable to some plant surfaces. Replication techniques were easiest, but only half of the plant surfaces were acceptably represented in this manner and these caused additional problems during photomicrography. Fecal material was prepared for analysis by a macerating technique.

Photomicrographs of reference slides were prepared for use in subsequent key development. Pictures were taken with a Makam camera mounted on a Wild M20 binocular microscope equipped with photo tube, using Kodak Contrast Process Panchromatic 3-1/4 x 4-1/4 sheet film.

The key was developed using epidermal cell arrangement, trichome characteristics, stomata subsidiary cell arrangement, and silica bodies as dependable characters. Secondary characters included hair lengths, stomata size and cell outline.

Differential digestion attributable to plant and (or) animal differences is a variable in analyzing cuticle fragments in fecal material not tested in this study. However, the key, a first approximation to cuticular identification of these selected plants, appears to handle this variability for most species studied.

Successful use of the key is dependent upon a comprehensive assessment of the vegetation in the study area and the biology of the animal under study. This perspective is necessary to restrict the variables which would otherwise decrease the effectiveness of identification of cuticular fragments in fecal material.

Methodology for Cuticular Identification of
Selected Eastern Oregon Range Plants

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METHODOLOGY FOR CUTICULAR IDENTIFICATION OF SELECTED EASTERN OREGON RANGE PLANTS

INTRODUCTION

Development of methodology for identifying plants by surface characteristics has been stimulated by the desire to assess herbivore dietary preferences by analysis of fecal material. This approach implies the use of microscopic characters of plant surfaces which are represented in feces by the plant cuticle.

Several approaches, other than examination of cuticle fragments in feces, have been used to study dietary preferences: feeding-site examinations, animal observation, and collection of material from stomachs and esophageal fistulas.

Observation of vegetation can reveal animal use. Comparison of data obtained in this manner with data from analysis of deer stomach contents indicates the latter is more exact and positive (Carhart, 1944). Furthermore, data from vegetation observation may confound utilization by domestic livestock and big game animals (Cole, 1956), although this is not a problem if the animals use the vegetation during different seasons of the year (Dasmann, 1949), and sampling is planned accordingly.

Tribe (1950) considered continuous observation of the grazing animal to be the best method for determining botanical composition

of diets. Observations of Cook, Blake and Call (1963), following the animal about and recording species eaten on pastures, was accurate and practical. However, as distance between the point of observation and the animal being observed increases, and as vegetation becomes denser, positive recognition of plants being eaten decreases. This is evident in the reports given by Buechner (1950) and Saunders (1955). Wary animals are difficult to approach even at distances up to 500 yards or more, and delicate feeders such as antelope leave clippings which are difficult to find. Buechner (1950) stated further that clips on grasses are less readily seen than on forbs.

The esophageal fistula technique developed by Torell (1954) and used by Hanker, Torell, and Van Dyne (1964), Heady and Torell (1959) and many others, can provide an accurate determination of diet preferences, but is not applicable to game animals in a free choice situation.

Analyses of stomach contents have been conducted in several studies (Anderson, Snyder, and Brown, 1965; Chippendale, 1962, 1964; Leach, 1956; Morris and Schwartz, 1957; Norris, 1943). Individuals so collected must be removed from the population being sampled. Bergerud and Russell (1964) and Norris (1943) found that the differential digestion of plants precludes some plants from detection if only larger plant fragments are identified and a substantial

time lapse occurs between eating and collection.

Fecal material provides a number of advantages in diet studies: (1) it can be collected and identified without having to see the animal, (2) the animal need not be removed from the population and consequently a consecutive series of collections can be made from the same population (Dusi, 1949), (3) fecal material is easily collected and stored, and (4) if the material is not fresh, an experienced person can make reasonably good estimates of the time of year when it was voided. Adams (1957) identified plants in fecal material by looking for "recognition items" such as abscission scars on bark and spines on leaves. These items would have to resist digestion, appear commonly in the feces, and be well distributed over edible portions of the plants. This combination of requirements is infrequently met.

These approaches are not mutually exclusive; indeed some of them have been used to complement each other within the same experimental design. Any one of these methods might be the most appropriate, depending upon the demands of the situation which confront the investigator. Features carried by cuticle fragments found in feces not only aid in plant identification but provide a unique advantage in data collection.

Several workers have already applied microscopic techniques to evaluations of contents of crops, rumens and feces. There has

been at least one very thorough attempt to determine the percent each component comprises in the diet, as well as producing qualitative data (Stewart, 1967).

This endeavor has been carried out to achieve three purposes: (1) prepare a series of microscope slides depicting the surface features of the leaves and stem current annual growth of selected eastern Oregon range plants, (2) develop a key to identification of these plant surfaces using slides and photomicrographs, and (3) evaluate methods used in preparing plant and fecal material for cuticle examination.

REVIEW OF LITERATURE

Botanical Basis for Species Identification
by Epidermal Characters

The use of plant epidermal characters in identifying to species is a sound procedure, although sometimes valuable only as confirmatory evidence. Stace (1966) sought to determine the role of epidermal characters in phylogenetic considerations. Mangroves and related plants were studied to determine if those of one family showed greater resemblance of epidermal structure to non-mangroves of the same family or to mangroves of other families. He concluded that the three groups studied, which evidently evolved from entirely different ancestors to eventually tolerate conditions in the mangrove environment, showed a predominance of the interfamilial divergence over the intra-mangrove convergence with regard to epidermal characters. Ontogenetic implications when using epidermal characters for identification also must be considered. Henri Prat (as cited by Davies, 1959) pointed out that grasses tend to show a progressive differentiation of the epidermis in successive leaves of a plant, with leaves nearest the inflorescence differing markedly from the leaves of the vegetative tillers. Davies (1959) found in grasses that silica cells, silica-suberose couples, cork cells, asperites and incipient asperites were frequently absent or confined to the base of the

sheath in vegetative tillers, but occurred over most of the sheath of culm leaves. Silica cells tended to be concentrated in young tillers and silico-suberose couples and cork cells in the sheaths of reproductive tillers. He found the intercostal zone of the lamina to be generally the least affected by growth stage. Metcalfe (1960), after completing the most exhaustive study of leaf anatomy in grasses, concluded that intra-specific differences are minor when compared with inter-specific ones, many of them being quantitative rather than qualitative. The most consistent and useful grass characters were found by Stewart (1965) to be the form of the silica bodies, the presence or absence and form of micro hairs and papillae, the appearance of the base of macro hairs and of the accompanying specialized epidermal cells, the shape and distribution of the stomata and the appearance of the walls of the intercostal long cells.

Paleobotanists have used features of the epidermis and cuticle to help in determining species of plants from fossil prints. J. G. Borneman (cited by Stewart, 1965) discussed in 1856 the use of cuticle features in paleobotanical investigations. Harris (1945) used features of the epidermis and cuticle thickness as measured in folds in his identification and classification of Jurassic plants.

Characterization of Plant Cuticles

The aerial surfaces of plants are covered with a non-cellular layer of inert substances which are derived from products of cellular metabolism. This structure was studied and reviewed by Crafts and Foy (1962) who cite Brongniart as first to describe the cuticle as early as 1830. Precise definitions of "cuticle" vary according to author, but cuticles themselves also vary both physically and chemically from plant to plant, especially among species. Generally, the cuticle consists of several cuticular layers. The outermost is a pure semi-lipoidal polymer called cutin which is encrusted with wax. Beneath this are impregnations of wax and cementing pectic compounds. The outer walls of the epidermal cells also become involved in the cuticle as cutin impregnates these walls producing alternating layers of cellulose and cutin. Cuticle is found on the surfaces of leaves, current stem growth, floral parts, and nectaries. It has been found present on leaf primordia and meristem and also on cell surfaces lining stomatal chambers and on mesophyll cells exposed to air spaces. Cutin provides the network or matrix for the cuticle. It results from the oxidation and polymerization of various unsaturated lipid compounds deposited on the cell surface. The formation of cutin begins within cells; not only within epidermal cells, but also within cells deeper in the plant tissue. Within a cell

part of some carbohydrate is oxidized to carbon dioxide and the remainder is reduced to fatty acids. Fatty acids represent the initial stage of a continuing process of condensation and reduction which produces long even-numbered chains of carbon atoms, hydrocarbon chains, with a polar group at one end. These molecules migrate via the cell walls to the outer epidermal wall where they are arranged with their polar groups in the water phase of the cellulose wall and their hydrocarbon chains exposed to the air of the atmosphere. In the presence of oxygen these oxidize and gradually condense to form a more or less continuous film over the outer surface of the plant.

Certain atmospheric conditions are a primary requirement for the formation of cuticle, and Lee and Priestley (1924) demonstrated that light and moisture affect cuticular thickness and consistency. They attributed this relationship to the influence of these environmental factors upon the oxidation and condensation of fatty acids.

Ecological interpretations of cuticle are not always consistent. For example, while there is a strong correlation of thick cuticle to xerophytism, there are also some exceptions to this (Daubenmire, 1965).

Skoss (1955) stated that cuticle deposition is continuous until the leaf reaches morphological maturity, at which time the formation ceases. The thickness of the resulting cuticle varies over the

surface; it is thick over the crevices where cells join and thin on the convex surfaces (Craffs and Foy, 1962).

Roberts, Batt and Martin (1959) found that electron micrographs of some leaves showed the probable point of separation between the cell wall and the cuticle. In most of the photographs, however, the juncture between cell wall and cuticle was not well defined, suggesting that the transition is gradual. While using pectic enzymes to isolate cuticles, Orgell (1955) found that isolation occurred most readily with those leaves in which there was a sharp separation, or concentration of pectic materials, between the cuticle and epidermal wall. Isolation occurred less readily with those species which possessed hard, stiff leaves, for in these species the cuticle was not sharply defined but graded into the epidermal and subjacent cell walls. He considered it probable that those species which yielded only very small particles of cuticle during decomposition either had a very thin cuticle which fragmented upon release, or possessed an imbricated cuticle composed of many small platelets cemented together by pectic materials.

Use of Histological Techniques for Identifying Plant Fragments

Baumgartner and Martin (1939) found it necessary to turn to histological methods of plant identification of finely masticated food

fragments in squirrel stomachs. Gross analysis procedures could not be satisfactorily applied. A similar situation was met in analysis of grasshopper crop contents (Mulkern and Anderson, 1959; Brusren and Mulkern, 1960). The application of these techniques to identification of plant fragments in feces was made by Dusi (1949, 1952) in his studies of cottontail rabbits. The fragmentary material in rumen, stomach, and fecal samples from sheep consists of plant cuticle which shows the arrangement of the previously underlying epidermis (Martin, 1955). Croker (1959) developed a key to identification of some grasses and herbs using characters seen on cuticle fragments in feces. She found that digestive juices in sheep darken cellular tissues and suggests that no further staining is necessary for identification purposes. Hercus (1960) isolated cuticle from feces and found no loss of cuticle after being placed in a silk bag in a rumen for two days. Hegg (1961) found microscopic fecal analyses suitable for providing information about the species composition of the diets of red deer, roe deer, and chamois. He suggests that the analyses be supplemented by observations when possible. Specimens from snowshoe rabbits contained identifiable fragments, whereas, those from marmot and ibex showed only cuticula. These differences may be the result of more thorough digestion or a predominantly forb diet of the latter. Meyers and Vaughan (1965) and Vaughan (1967) were able to determine diet composition of plains pocket gophers and

northern pocket gophers, respectively, by preparing slides of stomach materials and comparing fragments to reference slides. Storr (1961) investigated diets of quokkas and found that the epidermis from all perennial and a few annual plants passed undigested through the quokka, and experienced little difficulty in identifying to species the epidermis from plants growing in a restricted area. He determined that the digestibility of the epidermis is all or nothing and that there is no differential digestibility of epidermis among perennial species. Maceration treatments disintegrated the epidermis of most annual species leaving only the cuticle. Similar pieces of cuticle found in feces were identified only as "annual herb". The epidermis of perennials survived maceration. This differential behavior of annuals and perennials was further investigated and attributed to the fact that in the perennials examined, cutin was deposited in and (or) on all the walls of the epidermal cells, whereas in the case of the annuals only the outer walls are cuticularized. Kiley (1966) investigated the diets of waterbuck by fecal analysis and concluded that the differential digestion of some species was not a particularly important factor in qualitative fecal analysis because young species eaten, which she inferred would have possessed a more delicate cuticle, were represented in the feces.

After working with penned deer which were fed known diets, Urness and Zyznar (1968) reported that identification of some

species is simplified by the presence of unique diagnostic structures while other plants, in mixed diets, were not as easily separated.

Because only a low percentage of material in each microscope field is identifiable, they suggested that identification begin at the lowest possible level of magnification and be increased only as necessary.

Permanent mounts of positively identified fecal remnants were preferred in comparison with mounts prepared directly from plants.

Stewart (1965, 1967) constructed a comprehensive key based on cuticular characteristics of grass species from the East African plains. He relied most heavily upon those characteristics visible on the abaxial epidermis of the lamina. He used this key in subsequent work with fecal material from the following species: wildebeest, hartebeest, gazelles, buffalo, and common zebra. His work was primarily an attempt to quantify the analysis of fecal material.

Each of the investigators reviewed above prepared a reference collection of microscope slides to be used for comparison purposes. These slides were prepared directly from the plants by various procedures or from fecal material resulting from a known diet. Many of the investigators also prepared species lists of plants available in the specific locale used by the animals.

MATERIALS AND EVALUATION OF METHODS

Plants

The plants selected for this work were collected and identified by personnel of the Pacific Northwest Forest and Range Experiment Station. The plants were collected in the Fremont National Forest of southcentral Oregon and in the Wallowa and Blue Mountains of northeastern Oregon. The plant species are listed in Appendix A.

Reference Slide Preparation

A reference slide collection of plant surfaces or replicas is one of the essential first steps for identification of plants from fecal material. There are three types of methods most frequently employed for slide preparation. Each method can be used on both fresh plant material and dry herbarium mounts. Plant material from the latter need only be soaked in water to restore flexibility to the tissues. The three types of methods are scraping, maceration, and replication. Other approaches available are more specialized in their application and these will be reviewed first.

Reference slides can be made from fecal material when the plant fragments can be positively identified. When the animal of interest can be fed specific plants in pure diets, then it can be determined whether or not the plant cuticle can be recovered in the feces

and also its size and appearance. This approach was not practical for a reference collection of a large number of plants required in this study. Urness and Zyznar (1968) preferred reference slides obtained from pure diets and were able to feed specific plant species to penned deer being maintained on a stock diet of rolled barley and pelleted alfalfa. The microscopic appearance of the remnants of the stock diet was easily recognized in feces.

Brusven and Mulkern (1960), and Mulkern and Anderson (1959) used material from crops of grasshoppers feeding on known materials for reference. They also ground plant material into sizes approximating that to be found in the crops and placed this on microscope slides. The same approach was used by Myers and Vaughan (1965) for their work with pocket gophers.

Orgell (1954, 1955) used pectic enzymes to isolate sheets of cuticle from leaves. The cuticle from some leaves was isolated quickly, within a few hours, but others took up to 48 hours. This approach was tried using the enzymes Pectinol R-10 and Cellulase 36 supplied by Special Products Department, Rohm and Haas Company, Independence Mall West, Philadelphia, Pennsylvania. Sheets of cuticle were obtained but epidermal cell arrangement and outline could not be seen due to the thorough action of the enzymes. The length of time required also made this method less desirable.

The scraping method involves placing the plant leaf or stem

on a smooth surface such as glass or tile with the desired surface facing down. Using needles, fine forceps, and scalpel the tissue is removed starting with the opposite epidermis and ending with the cuticle and epidermis desired. Davies (1959), Metcalfe (1960) and Prat (1948) used this method exclusively for their work. Others, (Brusven and Mulkern, 1960; Stewart, 1965) turned to it only in necessity. Stewart (1965) preferred to use one of the macerating techniques. Clarke (1960) modified the scraping technique by placing the plant material in 88 percent lactic acid and heating this in a boiling water bath for 7 to 15 minutes for fresh material and 20 to 25 minutes for herbarium material. The leaf or stem was then transferred to cold lactic acid for 5 to 10 minutes and scraped while continuing to flood with the cold acid. He then stained the epidermis in water soluble aniline blue.

Scraping was used extensively in the present study, along with a replicating method. It was found that herbarium mounts required only a few minutes soaking in water before scraping. Scraping was conducted with the aid of a Bausch & Lomb dissecting microscope with variable magnification from 7 to 30X. The procedure was used with satisfaction. The size of cuticle obtained varied from quite small (about one square mm) to several times that size. The smaller pieces were obtained from plant surfaces which possessed thin cuticles and whose spongy parenchyma could not be scraped

away without tearing the cuticle (Appendix D, Figures 64, 66, 72). Occasional squirts of water were added from a medicine dropper to keep the tissues wet and to wash away the freed cellular materials. Pieces of cuticle obtained in this manner were mounted unstained as in Appendix D, Figures 31, 33, 34, 36, 51, 52, 61, 64; or they were stained in one percent safranin. The staining procedure was conducted in a ceramic spot dish having three rows of depressions. The cuticle was held in the stain for 15 seconds then dehydrated quickly through 50, 70, 95, and 100 percent ethyl alcohol and placed in xylene for a couple of minutes. While bringing the material through the alcohol series and xylene, it was continuously flattened to keep the edges from curling under. The material was then transferred with forceps to a drop of xylene on a microscope slide. While viewing through the binocular microscope the material was orientated with the original outer side facing up. A drop of Permount¹ was added and a cover slip placed over the material by first setting it down on edge and then easing it down over the piece of plant material. This avoided the formation of bubbles under the cover slip. An ordinary clothes pin (metal spring type) was used to apply pressure on the mount from top and bottom to flatten the specimen while

¹Permount is a mounting medium available from Van Waters and Rodgers, Scientific Supply Company Division, Portland, Oregon.

drying. After drying, excess mounting medium was removed with a razor blade and xylene. For many, the procedure of scraping would seem very tedious, for some it would undoubtedly be impossible. However, practice helps in learning how the materials are likely to behave during scraping, and even a very small piece can often show all the features necessary.

Macerating techniques involve variations of the use of acids to disintegrate the mesophyll of leaves and sub-epidermal tissues of stems to leave only the cuticle and attached cutinized cell walls of the epidermis. Nitric acid and a mixture of chromic acid and nitric acid are used for this purpose. Martin (1955) used 50 percent nitric acid over a water bath, which reduced the convection currents set up by direct heating and thereby allowed recovery of delicate cuticles that tended to break up. He removed the margins of the grass leaves in order to facilitate separation of the two surfaces. He found that some thin cuticles were destroyed by maceration, but he states that the same situation was found to occur in the rumen and fecal samples of sheep. The same method was used by Croker (1959).

Pohl (1967) further developed this method by using several different concentrations of nitric acid in the range of 40 to 80 percent in order to obtain acceptable results with each species handled.

A mixture of equal parts of chromic and nitric acids, each at ten percent concentration (Jeffrey's solution) was first applied by

Storr (1961) for the purpose of obtaining plant cuticles. The macerating procedure is described and evaluated by Stewart (1965). He used the method extensively with grasses and his comments are relative here because the method was used to a limited extent in this work. A one centimeter length from the midpoint of the lamina was chosen as a standard in order to observe surfaces which had attained a comparable degree of differentiation. The material was boiled in 10 ml of the chromic-nitric acid mixture under a reflux condenser usually for about three minutes. Longer times were required for more fibrous leaves. The materials and solution were then transferred to a 250 ml beaker, filled with water, the fragments allowed to settle, decanted and washed again. A few drops of ammonia were added to the second washing. Cuticles obtained in this manner can be observed without further preparation other than mounting or they may be stained to produce greater contrast of characters. This treatment was not found to distort any epidermal features and both surfaces were obtained simultaneously provided one did not disintegrate. The surfaces could usually be easily identified as adaxial or abaxial by referring back to the original leaf with a dissecting microscope, and the preparations were quite clear so that it made no difference which side was mounted up. Stewart (1965) turned to scraping methods only when the cuticle was too thin to be recovered by macerating processes. The cuticles can

be stained by transferring the fragments left, after the final wash is decanted, to a small test tube. Safranin stain, dehydrating series and xylene as described earlier can be added to the test tube and removed with a very fine-tipped pipette, the final products being transferred in xylene to the microscope slide before Permount and cover slip are added.

Replication by some type of plastic peel method is a simple and quick technique for duplicating a plant surface. There are, however, some inherent difficulties that can accompany this technique. Fresh material need only be cleaned if necessary with a brush and water. Herbarium material must be soaked in water for several hours to restore the wrinkled surfaces of the epidermal cells to their original smooth form (Sinclair and Dunn, 1961).

The simplest and most easily obtained material for replication is cellulose acetate, available as clear fingernail polish. After the leaf surface has been blotted dry, the polish can be applied with the brush that accompanies this product (Stoddard, 1965). One application is sufficient, and when the polish has dried, it can be peeled off. Care must be taken not to stretch the cellulose acetate peel as it is being pulled from the leaf.

Sinclair and Dunn (1961) suggested the use of Archer Adhesive, a clear herbarium plastic, for making replicas. They had to compose the plastic from its basic ingredients, but it is now

available in prepared form from Carolina Biological Supply Company, Gladston, Oregon. Silicone rubber products have also been used for replicating surface features (Sampson, 1961; Shutak and Dayawon, 1966; Zelitch, 1961). These silicone rubber products are opaque and require that a second step be carried out to make a replica of the rubber mold in a clear medium such as cellulose acetate. The resulting clear mount is a positive replica of the original whereas the other three replicas mentioned above produce only negative replicas. This, however, is insignificant in the transparent replicas. Each of these replicating materials can produce outstanding mounts showing intimate details of the relief across the surface.

Of the three media described above, Archer Adhesive was used most extensively throughout this study. In addition to the unique pros and cons mentioned above regarding the cellulose acetate and silicone rubber methods, the following evaluation given for Archer Adhesive method holds true for all three of the techniques. Two dilutions were prepared: 9:6:1 and 6:4:1 of toluene, methanol and Archer Adhesive, respectively. These were kept in air tight jars to prevent undue evaporation of solvents. Leaves were soaked, blotted dry and placed on a grass plate. Grass leaves that tended to curl were held flat with a razor blade pinned to the top of a rubber cork. This provided enough weight

concentrated on the razor's edge which held the leaves flat. These grasses usually possessed deeply furrowed adaxial surfaces that could not be replicated.

Three to four applications of plastic with four minute drying intervals were flowed over the leaf surface, the less viscous solution first, followed by the other. After drying for ten to fifteen minutes, the leaf was scraped from the glass, soaked in water and torn from the plastic. If cellular material and trichomes remained adhered to the plastic, they were decolorized by soaking in bleach before mounting. This produced a peel that was thick enough to handle easily and could still be flattened under a cover slip. Stem current annual growth was best replicated by suspending it from the tip of a pin and applying the liquid plastic from above and allowing it to run to the bottom.

Replicas are best mounted in air. A piece of plastic peel from each replicated surface of a plant can be mounted on the same slide. The most satisfactory method for holding the cover slip down was the use of a dri-mount of the type used to mount photographs. Strips of dri-mount, which are sticky on both sides and do not require the application of heat, were placed around the edge of the cover slip which was inverted over the plastic peels arranged on the slide. This provided a seal around the entire cover glass so that a peel could not slip out.

Cell outlines are usually visible on the plastic peels because the plastic flows into the crevices above the vertical walls of the epidermal cells. This produces relief on the lower side of the peel. Appendix D, Figures 11, 23, 45, indicate the detail that can be achieved. Important details of hairs can be depicted in plastic (Appendix D, Figure 61). However, hairs are more likely to cause bubbles in the plastic or otherwise distort the image by causing globs of plastic to form. Stiff hairs leave holes in the plastic when the leaf is removed; and an abundance of hairs may make it impossible to replicate the cell outlines. Some plant surfaces must be very smooth with little difference between the depth of grooves over cell walls and striations over the rest of the surface. The resulting plastic peel is therefore difficult to interpret (Appendix D, Figures 74, 76). Additional problems with plastic peels are encountered while taking photomicrographs. Chief among these is difficulty in focusing on the surface. The difference in vertical position of various features, i. e. stomata, guard cells and the surrounding epidermal surface, may be great enough that one or the other may be out of focus. These differences are not as noticeable in mounts of the actual cuticle. Example of this difficulty can be seen in Appendix D, Figure 39, where parts are either in or out of focus.

Preparing Fecal Material for Microscopic Inspection

Various methods of preparing fecal material are available. They all involve teasing apart or otherwise separating the fecal material. This is followed by additional separation and clearing of particles and then mounting them stained or unstained, temporarily or permanently, on a slide.

Hercus (1960) stored sheep feces in formalin-acetic-alcohol and then made a three-gram sample up to 100 ml with water. Three subsamples were then drawn for microscopic analysis with no further preparation. Baumgartner and Martin (1939) and Dusi (1949) used Hertwig's clearing agent² for preparing stomach and fecal material, respectively. The material was placed on a slide and heated to boil off the solution. Hegg (1961) heated feces for five minutes in ten percent potassium hydroxide over a water bath. The solution and feces were then shaken vigorously to loosen the fragments of epidermis and then washed and decanted to separate single cells, pollen and spores from the larger epidermal particles and coarser pieces. Urness and Zyznar (1968) boiled fecal pellets for 5-15 minutes in ten percent sodium hydroxide, then crushed the pellets, rinsed the material and spread it out to be examined. Storr (1960)

² 19cc HCl added to 150cc H₂O, 60cc glycerine, 270 gm chloral hydrate crystals.

dried and ground the fecal material, boiled it in Jeffrey's solution for one minute, washed it in water, then stained and mounted the fragments. Stewart (1967) prepared fecal material for storage by first heating it in nitric acid. Steps of his procedure were designed for producing several slides from the same source material for experiments concerning quantitative measurements of diet components.

The procedure that was adopted for this work was essentially that of Stewart's (1967). The steps are as follows: (1) break apart a pellet by crushing; (2) take 0.5 gm sample, tease apart thoroughly in 4 ml of concentrated HNO_3 ; (3) heat in a water bath 2-3 minutes; (4) make up to 100 ml with water, boil and stir; (5) make up to 800 ml with water in a bowl (diameter = 2X height); (6) stir and quickly dip out a 40 ml sample; (7) centrifuge to concentrate in 1 ml; (8) prepare six microscope slides by covering half of each with a film of Mayer's albumen adhesive (Conn, Darrow and Emmell, 1960); (9) divide the material equally among the slides and spread it as thin as possible; (10) heat gently over a bunsen burner to coagulate the albumen; (11) stain with safranin, dehydrate quickly through an ethyl alcohol series and dry. The slides produced in this manner can be covered later if desired.

Photomicrography

The following equipment was used: Wild M20 Binocular

Microscope with photo tube, Wild floutar objectives (10/.45, 20/.60 and 40/.75), Wild achromatic aplanatic condenser, Wild low voltage built-in microscope light with transformer, Makam camera by E. Leitz Wetzlar, and "Remiphot" Photo-Electric Exposure meter by Reichert. Kodak Contrast Process Panchromatic 3-1/4 X 4-1/4 sheet film was used.

The illumination system provided Kohler illumination which focuses the light source through a ground glass onto the plane of the object being viewed. This is important to gain high resolution. A procedure in the form of a check list was developed to standardize the steps to be followed prior to picture taking. They are as follows: (1) select area of slide to photograph; (2) select desired magnification; (3) establish Kohler illumination for that magnification; (4) select proper condenser front lens to correspond with objective; (5) focus image on ground glass of the camera and adjust light intensity; (6) determine exposure from light reading; (7) record all pertinent data as (a) subject, (b) subject position according to abscissa and ordinate vernier scales of the mechanical stage, (c) voltage reading on transformer, (d) filter and objective, (e) exposure time, (f) film holder number; (8) expose the film.

By following the above procedure, uniformity among pictures increased with experience. Actual magnification at the film level was determined by photographing a stage micrometer through each

objective. These negatives were used during printing to determine further changes of magnification.

Pictures were used as part of the key (Appendix B). Comparison of pictures is facilitated by taking the pictures at the same magnification and the majority of pictures were taken with the 20X objective. Higher and lower magnifications were used when necessary to show the desired detail.

Film size eliminated the need for further enlargements. Prints were made using a Simon Omega D2 enlarger, allowing "printing in" and "dodging" with greater ease than is encountered when using a contact printer.

KEY DEVELOPMENT

The key that has been constructed is a first approximation at separating specific eastern Oregon range plant species according to their cuticular characteristics. As such it might or might not group other plants with their close relatives contained in the key. The key is in Appendix B.

Considerations for Making a Key

A usable key must be constructed in light of its purpose. Identifying cuticle fragments from fecal material involves problems that would not be met identifying a whole leaf or stem by its cuticular characteristics. Problems arise from the possibility that a cuticle fragment may or may not be representative of the surface from which it came. The key must therefore make use of dependable characters existing and apparent in feces.

The key is concerned with biological materials--living organisms that participate in dynamism of evolution. They are changing now and represent past changes. Their characters are present in gradients of size, shape, distribution, density, etc. Therefore, when considering the entire array of characters available for use in the key, the dependability of each character must be assessed in terms of uniformity and variability within an individual and within a

species. The scope of this work was not meant to include such an undertaking for the 69 plant species handled. Previous work, notably Metcalfe and Chalk (1950) and Metcalfe (1960), provide valuable information for basing decisions. Established terminology is found in these sources. Definitions of terms and descriptions of structures are also found in Esau (1965). The use of established terminology used by prominent persons such as these is important. Standardization and universal usage of terms is thereby advanced.

The obvious features readily seen on cuticle fragments in feces include cell arrangement, cell outline, and presence or absence of trichomes and stomata. Other features can be detected if present, but may not be as obvious. Cell outline was considered the most variable of those four characters. Metcalfe and Chalk (1950) state that cell outline is subject to variation according to environmental conditions, but that it provides good confirmatory evidence for other more dependable characters. Trichome structure, surface characteristics and function are more dependable than length, density and distribution. Arrangement of stomata subsidiary cells and extremes in size are useful characters. Cell arrangement is the primary means for easily separating monocot and dicot leaves with few exceptions. The use of the term "absent" as it appears opposite "present" must be taken to mean "not seen". A character may have been absent on the material examined for this work; however, this

does not preclude the possibility that the character might be found on some other representative of that species.

When approaching the construction of the key, a variable had to be considered that had not been assessed. This was the differential digestion of species, which may be chiefly a function of the plant species (Stewart, 1967) or of the animal species, age, etc. (Crocker, 1959). Considering this variable with those of trichome and stomata density and distribution leads to the following academic exercise. A plant surface may exist that possesses both trichomes and stomata. According to the variables, fragments of cuticle from the surface could conceivably fall into four groups: (1) trichomes and stomata present, (2) trichomes present, stomata absent, (3) trichomes absent, stomata present, and (4) trichomes and stomata absent. It is reasonable to prepare the key to handle some plants in all of their possible variations. A plant handled this way will key out at more than one location in the key. This increases the complexity of the key and produces some couplets which contain several species surfaces. These couplets can be analyzed by considering which species are most likely to arrive at that place in the key. This has been done for couplet 55.

Trichomes have been used extensively in the key and include both glandular and non-glandular hairs and peltate hairs. The structure of some non-glandular hairs, referred to as "hairs", may

not be apparent in surface view. Chrysothamnus nauseosus is suspected of presenting this problem. The Compositae characteristically have multicellular hairs, including one type having a short basal cell(s) and very long terminal cell (Metcalf and Chalk, 1950). The hairs on this species appear very long, unicellular in surface view. A transverse section through the base of the hair would be necessary to show the short cell(s) suspected of being there. Dri-mounts of trichomes are easily made and usually distinctly reveal characteristics.

Plant epidermal characteristics vary from one position to another on the plant. Picture sequences show the extent of this variation for a grass, Danthonia unispicata (Appendix D, Figures 13, 14, 15 and 16), a forb, Fragaria chiloensis (Appendix D, Figures 11 and 12), a shrub, Symphoricarpus albus (Appendix D, Figures 85, 86 and 87) and a tree, Populus tremuloides (Appendix D, Figures 88, 89 and 90). If the variety includes a difference in the presence or absence of trichomes or stomata, or cell arrangement, then each surface must be handled in a separate section of the key. However, the variations may be ignored in the presence of one stable character as is the case for Danthonia unispicata, with the exception of the sheath adaxial epidermis.

IDENTIFICATION OF CUTICLE FRAGMENTS IN FECAL MATERIAL AS A RESEARCH PROCEDURE

Successful use of a key to identify cuticle fragments found in fecal material and thence a qualitative reconstruction of an animal's diet, depends upon the integration of several pieces of knowledge. At the outset, the user needs a comprehensive familiarity with the vegetation, its floristics, grouping and distribution over the landscape. This knowledge will provide a sound basis to estimate which plants will possibly occur together in an animal's diet. This basis is further modified by a complete understanding of the biology of the animal under study. A thorough knowledge of the habitat and the animal begins to limit the variables which would otherwise attenuate the use of a cuticular key. Further helpful constraints are: accurately recording the time of year fecal material is voided and collecting only fresh material.

Guidelines to the Use of a Key for Identifying Cuticle Fragments Found in Fecal Material

The following guidelines are proposed as a logical ordering of considerations necessary for the successful application of a key to cuticular identification of plants when used within the setting described above. Prepare a species list of plants available and note those plants known to be eaten by the animal being studied. Modify

the key to include only plants on the species list. This requires deleting some from the key prepared in this study and adding some to it. This is essential to make the key applicable to the study. Preparation of additional material is best accomplished by the following steps. (1) Prepare fresh material of herbarium mounts of the plant by brushing with a soft paint brush to remove pollen, fungi mycellia and fruiting bodies and other debris. (2) Record the prominent features as visible with a dissecting microscope such as details of trichomes, papillae, stomata, etc. (3) Based on the nature of the plant surface, choose the best method for preparing a reference slide.

After a species list and reference slide collection are completed, practice with the key is essential to acquaint an investigator with the terminology used and the appearance of specific features.

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APPENDICES

APPENDIX A

Species list of plants included in key, Appendix B.

Numbers of terminal couplets in which each species occurs in key,
Appendix B.

Numbers of Figures representing species in Appendix D.

SCIENTIFIC NAME	TERMINAL COUPLETS	FIGURES
GRASSES AND GRASS-LIKE		
<u>Agropyron spicatum</u> (Pursh) Scribn. & Smith	10, 12, 18	1, 2
<u>Bromus marginatus</u> Nees.	19, 22	6
<u>Calamagrostis rubescens</u> Buckl.	15, 18, 19	7
<u>Danthonia unispicata</u> (Thurb.) Munro ex Macoun.	5	13, 14, 15, 16
<u>Festuca idahoensis</u> Elmer.	8, 10, 11	4
<u>Koeleria cristata</u> (L.) Pers.	16	8
<u>Poa secunda</u> Presl.	15, 16	5
<u>Sitanion hystrix</u> (Nutt.) J. G. Smith	8, 10, 12	9
<u>Stipa thurberiana</u> Piper.	5	17
<u>Carex geyeri</u> Boott.	20	18
FORBS		
<u>Achillea millefolium</u> L.	29, 38, 54, 60	34, 38
<u>Actaea rubra</u> (Ait.) Willd.	38, 48, 84, 85	28, 50
<u>Adenocaulon bicolor</u> Hook.	27, 43, 84	27, 53
<u>Arnica cordifolia</u> Hook.	37, 54, 60, 85	26, 62
<u>Astragalus canadensis mertonii</u> (Nutt.) Wats.	31, 46, 71, 73	36, 61
<u>A. purshii lagopus</u> (Rydb.) Barneby	43, 47	56
<u>A. stenophyllus</u> T. & G.	36, 47, 62	25, 55
<u>Balsamorhiza serrata</u> Nels & Macbr.	24, 52	40, 44
<u>Camassia quamash</u> (Pursh) Greene	22	49
<u>Clintonia uniflora</u> (Schult.) Kunth	22	10
<u>Erigeron bloomeri</u> Gray	52, 73	31, 51
<u>Eriogonum umbellatum</u> Torr.	27, 43	57
<u>Eriophyllum lanatum</u> (Pursh) Forbes	27, 43	35, 47
<u>Fragaria chiloensis</u> (L.) Duchesne	50, 75, 78	11, 12
<u>F. vesca</u> L.	34, 50, 73, 78	42
<u>Geum macrophyllum</u> Willd.	37, 48, 68	33, 64
<u>Hieracium albiflorum</u> Hook.	38, 51, 69, 83	52
<u>Horkelia fusca parviflora</u> (Nutt.) Peck	35, 48, 73	66
<u>Lactuca serriola</u> L.	51, 74	60
<u>Limnaea borealis</u> L.	35, 69, 80, 81	59
<u>Linum perenne</u> L.	24	45
<u>Lomatium leptocarpum</u> (T. & G.) Coult. & Rose	35, 36	43
<u>L. triternatum</u> (Pursh) Coult. & Rose	35	41
<u>Lupinus lepidus aridus</u> (Dougl.) Jeps.	32, 47	58
<u>Osmorhiza purpurea</u> (Coult. & Rose) Suksd.	38, 48, 74, 85	37

SCIENTIFIC NAME	TERMINAL COUPLETS	FIGURES
<u>Pedicularis racemosa</u> Dougl.	38, 41, 60, 85	39
<u>Rudbeckia occidentalis</u> Nutt.	38, 54, 60, 85	46
<u>Scrophularia lanceolata</u> Pursh.	26, 69, 74	63
<u>Sidalcea oregana</u> (Nutt.) Gray	35, 48, 66, 68	48
<u>Thermopsis montana</u> Nutt.	37, 47, 69, 80	54
<u>Trifolium latifolium</u> (Hook.) Greene	32, 47, 71, 74	65
SHRUBS		
<u>Arctostaphylos patula</u> Greene	26, 61	83
<u>Artemisia arbuscula</u> Nutt.	29, 53	79
<u>A. cana</u> Pursh.	29, 53, 64	81
<u>A. tridentata</u> Nutt.	29, 53	80, 82
<u>Ceanothus velutinus</u> Dougl.	35, 79	84
<u>Cercocarpus ledifolius</u> Nutt.	24, 48, 79	30
<u>Chrysothamnus nauseosus</u> (Pall.) Britt.	24, 54	67
<u>C. viscidiflorus</u> (Hook.) Nutt.	38, 66	68
<u>Holodiscus discolor</u> (Pursh) Maxim.	33, 48, 49, 83	32
<u>Lonicera utahensis</u> Wats.	38, 50, 69, 85	76
<u>Purshia tridentata</u> (Pursh) DC	30, 43, 48, 78	70
<u>Ribes lacustre</u> (Pers.) Poir.	35, 73, 81	78
<u>R. viscosissimum</u> Pursh	35, 41, 48, 68, 81	71
<u>Rosa gymnocarpa</u> Nutt.	24, 73, 83	72
<u>Rubus parviflorus</u> Nutt.	38, 73, 79	69
<u>Sambucus cerulea</u> Raf.	38, 60, 79	77
<u>Spiraea betulifolia lucida</u> (Dougl.) C. L. Hitchc.	38, 75, 83	73
<u>Symphoricarpos albus</u> (L.) Blake.	38, 68, 83	85, 86, 87
<u>Vaccinium membranaceum</u> Dougl.	24, 58	74
<u>V. scoparium</u> Leiberger	24, 59, 83	29, 75
TREES		
<u>Acer glabrum</u> Torr.	24, 85	23
<u>Juniperus occidentalis</u> Hook.	20	19
<u>Pinus contorta</u> Loud.	20	20
<u>P. ponderosa</u> Dougl.	20	21
<u>Populus tremuloides</u> Michx.	24, 58, 81	88, 89, 90
<u>Salix</u> sp.	24, 48, 59, 79	
<u>S. scouleriana</u> Barratt	24, 48, 59, 79	24
<u>Taxus brevifolia</u> Nutt.	20	22

APPENDIX B

A Key to Cuticular Identification of
69 Species of Eastern Oregon Range Plants

Figure numbers refer to figures in Appendix D.

- | | | |
|----|---|------|
| 1. | Cells showing a linear arrangement. | (2) |
| 1. | Cells grouped not in a linear arrangement; dicot leaves | (39) |
| 2. | Cells rectilinear, sometimes nearly square, in distinct parallel rows. | (3) |
| 2. | Cells not rectilinear, but long axes of cells nearly unidirectional or cuboidal cells in somewhat parallel rows; dicot stems. | (23) |
| 3. | Two sizes of cells present: long cells and two types of short cells - silica and suberose; grasses. | (4) |
| 3. | Long cells only (other than stomata and subsidiary cells). | (20) |
| 4. | Silica bodies over veins dumbbell or nodular (Figures 14, 16 and 17). | (5) |
| 4. | Silica bodies not as above. | (6) |
| 5. | Short cells over veins in long rows of alternating pairs of silica and cork cells.
<u>Danthonia unispicata</u> lamina adaxial, abaxial epidermis; sheath abaxial epidermis (Figures 15 and 16) | |
| 5. | Short cells over veins solitary, paired silica and suberose cells or in short rows of up to five pairs.
<u>Stipa thurberiana</u> lamina adaxial, abaxial epidermis; sheath abaxial epidermis (Figure 17) | |
| 6. | Short cells over veins primarily in pairs and silica body fitting into a concavity in the cork cell; occasionally a solitary short cell. | (7) |
| 6. | Short cells over veins primarily solitary. | (13) |
| 7. | Paired short cells over veins only. | (8) |
| 7. | Paired short cells over veins and interveins. | (9) |
| 8. | Silica bodies round; stomata present in intervein; macrohairs over entire surface.
<u>Sitanion hystrix</u> lamina adaxial epidermis (Figure 9) | |

8. Paired and solitary short cells present. Silica bodies in paired short cells, round, oblong and (or) nodular. Silica bodies in solitary short cells, nodular and elongated-sinuuous. No silica bodies or stomata in intervein, no macrohairs.
Festuca idahoensis sheath abaxial epidermis
9. Stomata present in the intervein. (10)
9. Stomata not present in the intervein. (11)
10. Macrohairs present over entire surface.
Festuca idahoensis lamina adaxial epidermis
10. Macrohairs absent
Agropyron spicatum sheath abaxial epidermis (Figure 2)
Sitanion hystrix sheath abaxial epidermis
11. Oblique lines extending from the situations into or across the short distance of the cell. Prickles present, infrequent.
Festuca idahoensis lamina abaxial epidermis (Figure 4).
11. Oblique lines not present, prickles not seen. (12)
12. Papillae present on vein and intervein.
Sitanion hystrix lamina abaxial epidermis
12. Papillae not seen.
Agropyron spicatum lamina abaxial epidermis (Figure 3)
13. Silica bodies over veins elongated - sinuous and (or) nodular. (14)
13. Silica bodies over veins elongated - smooth. (17)
14. Short cells paired in intervein. (15)
14. Short cells solitary or not seen in intervein. (16)
15. Silica bodies crescent-shaped to elliptic; macrohairs of two different sizes.
Calamagrostis rubescens sheath abaxial epidermis (Figure 7)
15. Silica bodies round to saddle shaped; macrohairs not seen; very few prickles.
Poa secunda sheath abaxial epidermis
16. Macrohairs present in vein and intervein.
Koeleria cristata (Figure 8)
16. Macrohairs absent; prickles present in intervein.
Poa secunda lamina (Figure 5)

17. Macrohairs present. (18)
17. Macrohairs absent. (19)
18. Macrohairs of two distinct sizes, short and very long.
Calamagrostis rubescens lamina adaxial epidermis
18. Macrohairs not as above.
Agropyron spicatum lamina adaxial epidermis
19. Long cells straight walled, those especially in the intervein being wider at their middle than ends.
Bromus marginatus lamina adaxial, sheath abaxial epidermis (Figure 6)
19. Cells finely sinuous, prickles present over the veins.
Calamagrostis rubescens lamina abaxial epidermis
20. Stomata with several subsidiary cells above sunken guard cells.
Juniperus occidentalis (Figure 19)
Pinus contorta (Figure 20)
Pinus ponderosa (Figure 21)
Taxus brevifolia (Figure 22)
20. Stomata not as above. (21)
21. Stomata with two subsidiary cells, (2-3) 4 papillae per cell confined to one row over the vein; cell walls sinuous.
Carex geyeri (Figure 18)
21. Not as above. (22)
22. Stomata present, subsidiary cells absent, cells walls straight.
Camassia quamash (Figure 49)
Clintonia uniflora lamina abaxial epidermis (Figure 10)
22. Stomata absent, long cells only.
Bromus marginatus lamina abaxial epidermis
Grass leaf sheath adaxial epidermis (Figures 1 and 13)
Clintonia uniflora lamina adaxial epidermis
23. Cells cuboid or nearly so. (24)
23. Cells not as above. (25)
24. Stomata present and paracytic.
Linum perenne (Figure 45)
Vaccinium membranaceum
V. scoparium (Figure 29)

24. Stomata absent or not paracytic.
Acer glabrum
Balsamorhiza serrata petal (Figure 40)
Cercocarpus ledifolius (Figure 30)
Chrysothamnus nauseosus
Populus tremuloides (Figure 90)
Rosa gymnocarpa
Salix scouleriana
S. sp.
25. Trichomes present. (26)
25. Trichomes absent. (36)
26. Trichomes are uniseriate capitate glandular hairs.
Arctostaphylos patula
Scrophularia lanceolata
26. Trichomes are nonglandular hairs. (27)
27. Hairs forming a mat.
Adenocaulon bicolor (Figure 27)
Eriogonum umbellatum
Eriophyllum lanatum (Figure 35)
27. Hairs not forming a mat. (28)
28. Hairs multicellular. (29)
28. Hairs unicellular. (30)
29. Hairs two-armed, having uniseriate stalk and unicellular head.
Artemisia arbuscula
A. cana
A. tridentata (Figure 82)
29. Hairs with short basal cells and very long terminal cell.
Achillea millefolium (Figure 34)
30. Hairs profuse and intertwining.
Purshia tridentata
30. Hairs not as above. (31)
31. Hairs two-armed.
Astragalus canadensis mortonii (Figure 36)
31. Hairs not two-armed. (32)

32. Hairs tuberculate. (33)
Lupinus lepidus aridus
Trifolium latifolium
32. Hairs not tuberculate. (33)
33. Hairs of two distinct sizes; one size $> 650 \mu$, the other much shorter.
Holodiscus discolor
33. Hairs not as above. (34)
34. Hairs over 650μ and smooth.
Fragaria vesca
34. Hairs shorter than 650μ (35)
35. Hairs with rough surfaces.
Lomatium triternatum (Figure 41)
Ribes lacustre
Sidalcea oregana
35. Hairs with smooth surfaces.
Ceanothus velutinus
Horkelia fusca parviflora
Linnaea borealis
Ribes viscosissimum
36. Anomocytic stomata abundant.
Astragalus stenophyllus (Figure 25)
Lomatium leptocarpum (Figure 43)
36. Anomocytic stomata absent or rare. (37)
37. Ratio of cell length to width 8:1 or greater.
Arnica cordifolia (Figure 26)
Geum macrophyllum (Figure 33)
Thermopsis montana
37. Ratio smaller. (38)
38. Ratio of cell length to width 4:1 to 8:1.
Achillea millefolium (Figure 34)
Actaea rubra (Figure 28)
Chrysothamnus viscidiflorus
Hieracium albiflorum
Lonicera utahensis
Osmorhiza purpurea
Pedicularis racemosa
Rudbeckia occidentalis

38. Ratio of cell length to width is less than 3:1; cell sometimes flattened so that width is greater than length.
Rubus parviflorus
Sambucus cerulea
Spiraea betulifolia lucida
Symphoricarpos albus (Figure 87)
39. Trichomes present. (40)
39. Trichomes absent. (55)
40. Trichomes are glandular hairs. (41)
40. Trichomes are nonglandular hairs. (42)
41. Multiseriate shaggy with round head, on veins.
Ribes viscosissimum (Figure 71)
41. Peltate without stalk.
Pedicularis racemosa (Figure 39)
42. Hairs forming a mat of long, fine, kinky, complexly intertwined hairs. (43)
42. Hairs not forming a mat. (44)
43. Hairs of two types: those forming the mat and a few, short-robust, unicellular, smooth hairs.
Purshia tridentata (Figure 70)
43. Only one hair type and those forming a mat.
Adenocaulon bicolor lamina abaxial epidermis (Figure 53)
Astragalus purshii lagopinus (Figure 56)
Eriogonum umbellatum (Figure 57)
Eriophyllum lanatum (Figure 47)
44. Hairs unicellular. (45)
44. Hairs multicellular. (51)
45. Hairs tuberculate. (46)
45. Hairs not tuberculate. (48)
46. Hairs unequal two-armed.
Astragalus canadensis mortonii (Figure 61)
46. Hairs not as above. (47)

47. Hairs tuberculate their full length, only slightly lessening toward their proximal end.
Astragalus purshii lagopinus (Figure 56)
47. Hairs with tuberculae definitely increasing distally.
Astragalus stenophyllus (Figure 55)
Lupinus lepidus aridus (Figure 58)
Thermopsis montana (Figure 54)
Trifolium latifolium (Figure 65)
48. Hairs long, usually over 650 μ . (49)
48. Hairs of varying lengths, under 650 μ
Actaea rubra (Figure 50)
Cercocarpus ledifolius
Geum macrophyllum (Figure 64)
Holodiscus discolor
Horkelia fusca parviflora (Figure 66)
Osmorhiza purpurea (Figure 37)
Purshia tridentata (Figure 70)
Ribes viscosissimum (Figure 71)
Salix scouliana (Figure 24)
S. sp.
Sidalcea oregana (Figure 48)
49. Hairs straight, robust. (50)
49. Hairs slender, wavy.
Holodiscus discolor
50. Hairs mostly smooth.
Fragaria chiloensis (Figures 11 and 12)
F. vesca (Figure 42)
50. Hairs rough, sometimes rugose and profile appearing tuberculate.
Lonicera utahensis (Figure 76)
51. Hairs multiseriate.
Hieracium albiflorum (Figure 52)
Lactuca serriola
51. Hairs uniseriate. (52)
52. Epidermal cells specialized to form an obvious pattern around the base of the trichome.
Balsamorhiza serrata (Figure 44)
Erigeron bloomeri (Figure 51)
52. Epidermal cells not obvious as above. (53)

53. Hairs two-armed, having uniseriate stalk and a unicellular head; may appear unicellular. Head is long and repeatedly bent along its length; hairs intertwining.
Artemisia arbuscula (Figure 79)
A. cana (Figure 81)
A. tridentata (Figure 80)
53. Hairs not as above. (54)
54. Hair with short basal cell(s) and long, repeatedly bent, twisted, flattened terminal cell; may appear unicellular.
Achillea millefolium (Figure 38)
Chrysothamnus nauseosus (Figure 67)
54. Hairs not as above.
Arnica cordifolia (Figure 62)
Rudbeckia occidentalis (Figure 46)
55. Stomata present.¹ (56)
55. Stomata absent.¹ (76)
56. Stomata paracytic. (57)
56. Stomata anomocytic. (60)
57. Stomata large, usually longer than 30 μ (58)
57. Stomata smaller. (59)
58. Cell walls deeply undulating.
Vaccinium membranaceum lamina abaxial epidermis (Figure 74)
58. Cell walls shallowly undulating.
Populus tremuloides (Figures 88 and 89)
59. Stomata 20 - 24 μ long, cell walls irregularly and deeply undulating.
Vaccinium scoparium lamina abaxial epidermis (Figure 75)
59. Stomata 10-12 μ long.
Salix scouleriana lamina abaxial epidermis (Figure 24)
S. sp. lamina abaxial epidermis

¹ See further analysis of couplet 55 at end of key.

60. Stomata usually larger than 35 μ , elliptic to oblong shaped; cell walls deeply undulating. (61)
- Achillea millefolium lamina abaxial, adaxial epidermis
Arnica cordifolia lamina abaxial epidermis (Figure 62)
Pedicularis racemosa lamina abaxial epidermis (Figure 39)
Rudbeckia occidentalis lamina abaxial epidermis (Figure 46)
Sambucus cerulea lamina abaxial epidermis (Figure 77)
60. Not as above. (61)
61. Stomata equal to or larger than the epidermal cells; epidermal cells uniform and smooth walled - having a very regular appearance. (62)
- Arctostaphylos patula (Figure 83)
61. Not as above. (62)
62. Stomata large, longer than 32 μ (63)
- Astragalus stenophyllus lamina adaxial epidermis
62. Stomata smaller. (63)
63. Stomata 26-30 μ long. (64)
63. Stomata smaller. (70)
64. Cells elongated and curved, walls smooth, stomata nearly orbicular. (65)
- Artemisia cana
64. Not as above. (65)
65. Cell walls nearly regular - straight. (66)
65. Not as above. (67)
66. Cuticle striated. (68)
- Chrysothamnus viscidiflorus lamina abaxial, adaxial epidermis
(Figure 68)
66. Cuticle not striated. (69)
- Sidalcea oregana lamina adaxial epidermis
67. Cell walls irregularly and shallowly undulating. (68)
67. Not as above. (69)
68. Stomata nearly orbicular. (68)
- Ribes viscosissimum lamina abaxial epidermis (Figure 71)
Sidalcea oregana lamina abaxial epidermis (Figure 48)

68. Stomata oblong.
Geum macrophyllum lamina adaxial, abaxial epidermis (Figure 64)
Symphoricarpos albus lamina abaxial epidermis (Figure 86)
69. Cell walls regularly, repeatedly and shallowly undulating; stomata nearly orbicular.
Linnaea borealis lamina abaxial epidermis (Figure 59)
69. Cell walls irregularly and deeply undulating.
Hieracium albiflorum lamina abaxial epidermis (Figure 52)
Lonicera utahensis lamina abaxial epidermis (Figure 76)
Scrophularia lanceolata lamina adaxial epidermis
Thermopsis montana lamina abaxial epidermis (Figure 54)
70. Stomata 19-25 μ long. (71)
70. Stomata 16-17 μ long. (75)
71. Cell walls nearly regular - straight.
Astragalus canadensis mortonii lamina adaxial epidermis
Trifolium latifolium lamina adaxial epidermis
71. Not as above. (72)
72. Cell walls irregularly and shallowly undulating. (73)
72. Not as above. (74)
73. Stomata usually orbicular.
Erigeron bloomeri lamina adaxial, abaxial epidermis (Figure 51)
Ribes lacustre lamina abaxial epidermis
73. Stomata elliptic to oblong.
Astragalus canadensis mortonii lamina abaxial epidermis (Figure 61)
Fragaria vesca lamina abaxial epidermis
Horkelia fusca parviflora lamina adaxial, abaxial epidermis (Figure 66)
Rosa gymnocarpa lamina abaxial epidermis (Figure 72)
Rubus parviflorus lamina abaxial epidermis (Figure 69)
74. Cell walls irregularly and deeply undulating.
Lactuca serriola lamina adaxial, abaxial epidermis (Figure 60)
Osmorhiza purpurea lamina abaxial epidermis (Figure 37)
Scrophularia lanceolata lamina abaxial epidermis (Figure 63)
74. Cell walls nearly regular - shallowly undulating.
Trifolium latifolium lamina abaxial epidermis (Figure 65)

75. Epidermal cells protruding to a rounded peak,
Spiraea betulifolia lucida lamina abaxial epidermis (Figure 73)
75. Not as above,
Fragaria chiloensis (Figures 11 and 12)
76. Cell walls nearly regular (constancy of straightness of undulation). (77)
76. Cell walls irregular. (82)
77. Cell walls straight between junctures. (78)
77. Cell walls not straight. (80)
78. Cells uniform. (79)
78. Cells variable,
Fragaria chiloensis lamina adaxial epidermis (Figure 11)
F. vesca lamina adaxial epidermis
Purshia tridentata lamina adaxial epidermis (Figure 70)
79. Cuticle striated, and (or) with large parallel ripples.
Salix scouleriana lamina adaxial epidermis
S. sp. lamina adaxial epidermis
79. Cuticle not striated and not having ripples.
Ceanothus velutinus lamina adaxial epidermis (Figure 84)
Cercocarpus ledifolius lamina adaxial epidermis
Rubus parviflorus lamina adaxial epidermis
Sambucus cerulea lamina adaxial epidermis
80. Cell walls shallowly undulating. (81)
80. Cell walls deeply undulating.
Linnaea borealis lamina abaxial epidermis (Figure 59)
Thermopsis montana lamina adaxial epidermis
81. Cells uniform,
Linnaea borealis lamina adaxial epidermis
Populus tremuloides lamina adaxial epidermis (Figure 88)
Ribes viscosissimum lamina adaxial epidermis
81. Cells variable,
Ribes lacustre lamina adaxial epidermis (Figure 78)
82. Cell walls shallowly undulating. (83)
82. Cell walls deeply undulating. (84)

83. Cells uniform.
Holodiscus discolor lamina adaxial epidermis
Vaccinium scoparium lamina adaxial epidermis
83. Cells variable.
Hieracium albiflorum lamina adaxial epidermis
Rosa gymnocarpa lamina adaxial epidermis
Spiraea betulifolia lucida lamina adaxial epidermis
Symphoricarpos albus lamina adaxial epidermis (Figure 85)
84. Cuticle striated.
Actaea rubra lamina adaxial epidermis
Adenocaulon bicolor lamina adaxial epidermis
84. Cuticle not striated. (85)
85. Surface papillose.
Pedicularis racemosa lamina adaxial epidermis
85. Surface not papillose.
Actaea rubra lamina abaxial epidermis (Figure 50)
Acer glabrum lamina adaxial, abaxial epidermis (Figure 23)
Arnica cordifolia lamina adaxial epidermis
Lonicera utahensis lamina adaxial epidermis
Osmorhiza purpurea lamina adaxial epidermis
Rudbeckia occidentalis lamina adaxial epidermis

Further Analysis of Couplet 55

Different cuticle fragments from the same surface of some plants may satisfy both choices in couplet 55. However, other plant surfaces could yield cuticle fragments satisfying only one of the choices. This couplet includes plant surfaces with trichomes absent or having very low density.

Stomata present

The following plant leaf surfaces have trichomes absent and stomata density greater than 15 per one-tenth square mm. Thus, of all the possibilities, these are the most likely to occur at this point in the key.

Chrysothamnus viscidiflorus, adaxial epidermis
Lactuca serriola, adaxial epidermis
Ribes lacustre, abaxial epidermis
Rubus parviflorus, abaxial epidermis
Scrophularia lanceolata, abaxial epidermis
Spiraea betulifolia lucida, abaxial epidermis
Symphoricarpos albus, abaxial epidermis
Vaccinium scoparium abaxial epidermis

Stomata absent

The adaxial epidermis of the following plant leaves have trichomes and stomata absent, and thus, are the most likely to occur at this point in the key.

Acer glabrum
Adenocaulon bicolor
Ceanothus velutinus
Fragaria chiloensis
Lonicera utahensis
Osmorhiza purpurea
Pedicularis racemosa
Populus tremuloides
Ribes lacustre
Rosa gymnocarpa
Spiraea betulifolia lucida
Symphoricarpos albus
Thermopsis montana
Vaccinium membranaceum

APPENDIX C

Definitions and Diagrams of Terms
Used in Key, Appendix B

(Esau, 1965; Metcalfe, 1960; Metcalfe and Chalk, 1950)

ABAXIAL. Directed away from the axis.

ADAXIAL. Directed toward the axis.

ANOMOCYTIC STOMA. A type in which no subsidiary cells are associated with the guard cells.

DEEPLY UNDULATING. Having the depth of an undulation greater than its width.

LAMINA. The blade or expanded part of a leaf.

MACROHAIR. A unicellular hair usually visible to the naked eye.

Macrohairs are probably homologous with prickles and the two are sometimes difficult to distinguish.

MAT. A dense covering of fine and repeatedly twisted, bent and intertwining hairs, having unity when pulled from the surface.

MULTISERIATE. Consisting of many layers of cells.

PAPILLAE. Various shaped protrusions from the outer walls of epidermal cells, sometimes highly cutinized.

PARACYTIC STOMA. A type in which one or more subsidiary cells flank the stoma parallel with the long axis of the guard cells (Appendix D, Figures 45 and 75).

PELTATE. A trichome, consisting of a discoid plate of cells, borne on a stalk or attached directly to the foot.

PRICKLE. A robust, sharply but shortly pointed structure with a swollen base.

SHALLOWLY UNDULATING. Having the width of an undulation greater than its depth.

SILICA CELL. A short cell filled with silica solidified into variously shaped bodies; as in the epidermis of grasses.

body shapes:

distal end of sheath or lamina (to the right)

dumbbell and intermediate between cross and dumbbell



elongated - sinuous



elongated - smooth



nodular



saddle-shaped



SUBEROSE CELL. A cork cell having suberized walls.

TRICHOME. An outgrowth of the epidermis, variable in shape,
size and function.

UNISERIATE. Consisting of one layer of cells.

VEIN, INTERVEIN. The regions of the Gramineae epidermis over
the vein and over the region between veins respectively.

APPENDIX D

FIGURES

Figure 1. Agropyron spicatum sheath adaxial epidermis.
Archer Adhesive plastic peel. X252.

Figure 2. Agropyron spicatum sheath abaxial epidermis.
Safranin stain. X252.

Figure 3. Agropyron spicatum lamina abaxial epidermis.
Safranin stain. X252.

Figure 4. Festuca idahoensis lamina abaxial epidermis.
Safranin stain. X252.

Figure 5. Poa secunda lamina abaxial epidermis.
Safranin stain. X252.

Figure 6. Bromus marginatus lamina adaxial epidermis.
Safranin stain. X252.

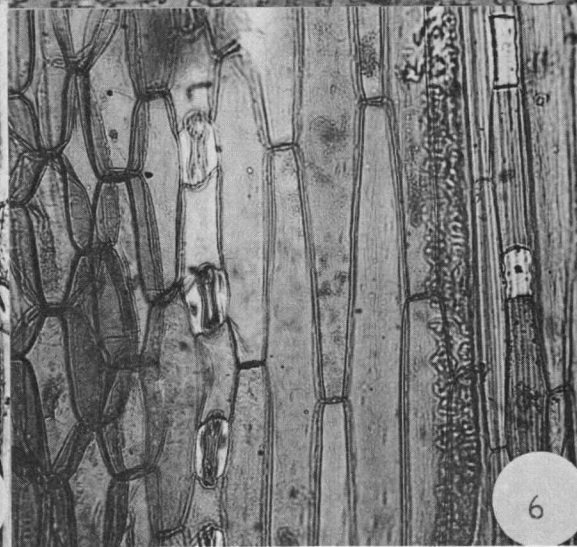
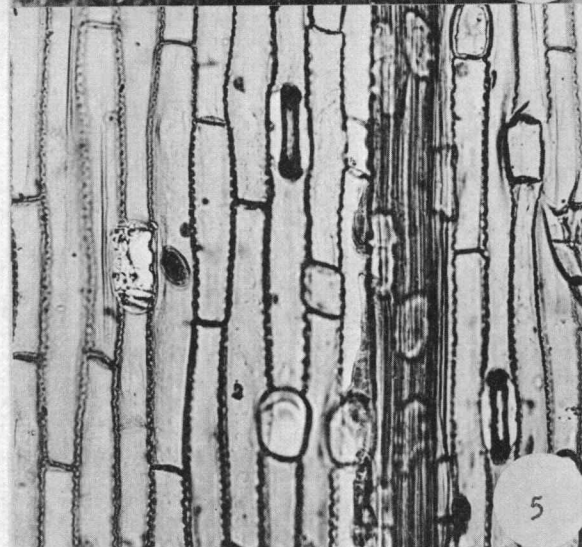
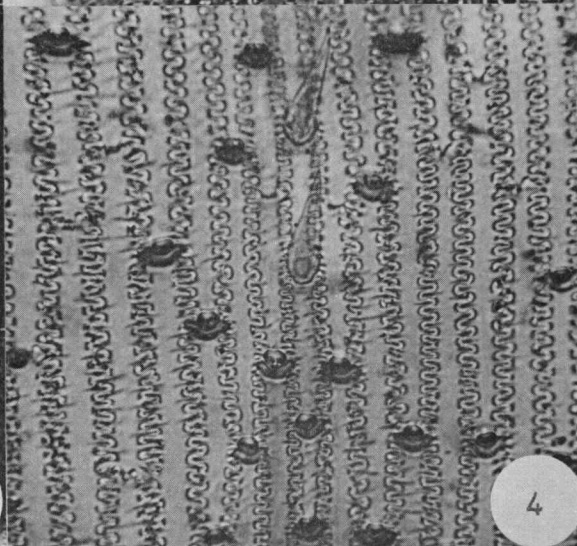
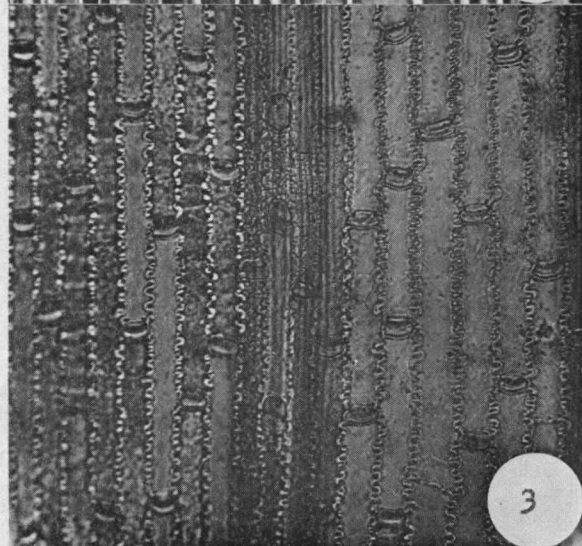
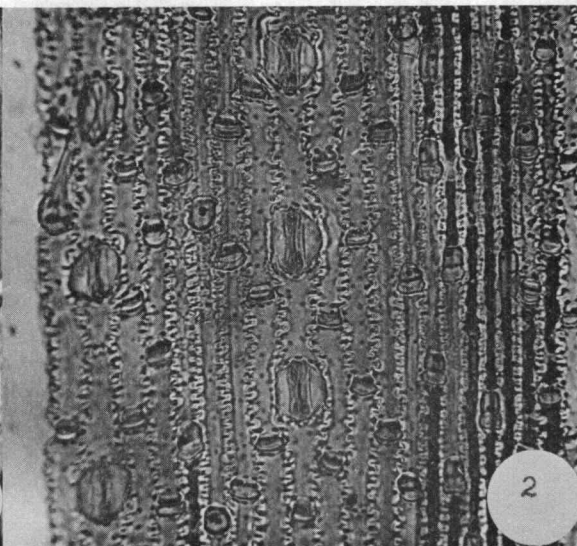
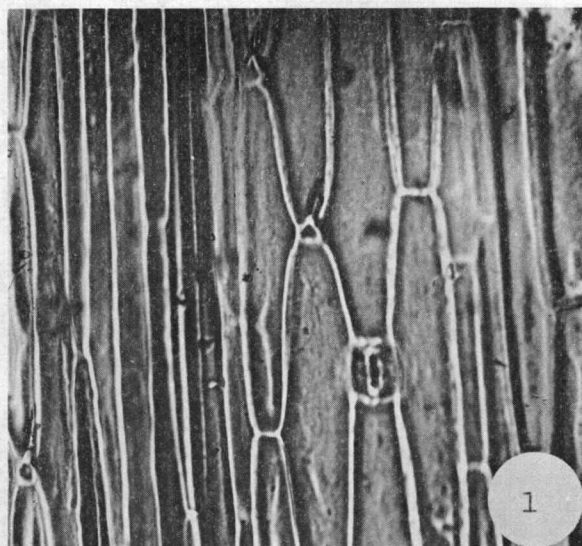


Figure 7. Calamagrostis rubescens sheath abaxial epidermis. Safranin stain. X252.

Figure 8. Koeleria cristata sheath abaxial epidermis. Safranin stain. X252.

Figure 9. Sitanion hystrix lamina adaxial epidermis. Safranin stain. X252.

Figure 10. Clintonia uniflora lamina abaxial epidermis. Archer Adhesive plastic peel. X126.

Figure 11. Fragaria chiloensis lamina adaxial epidermis. Archer Adhesive plastic peel. X252

Figure 12. Fragaria chiloensis lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

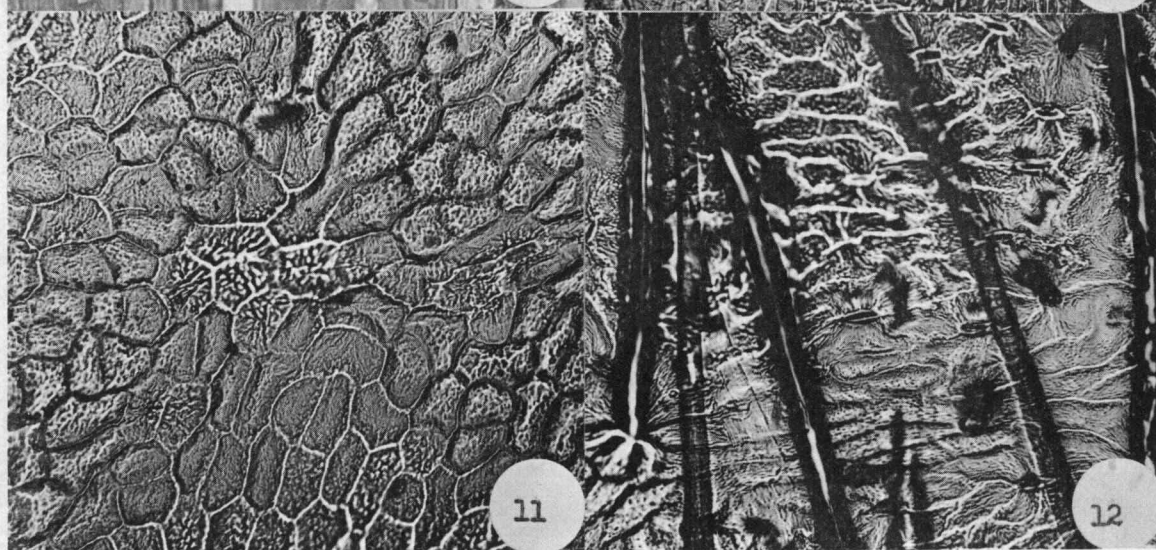
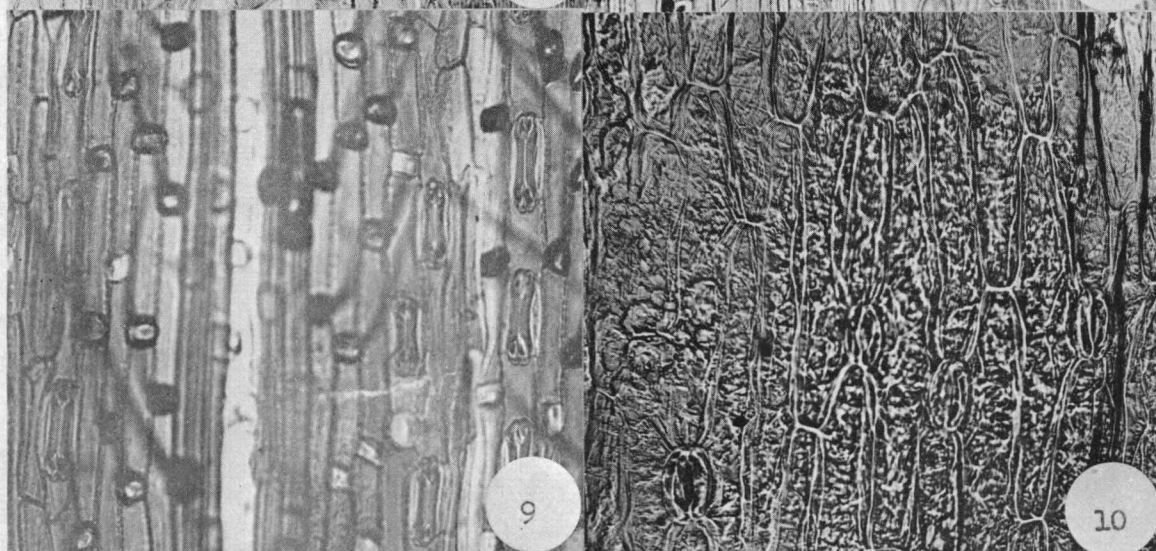
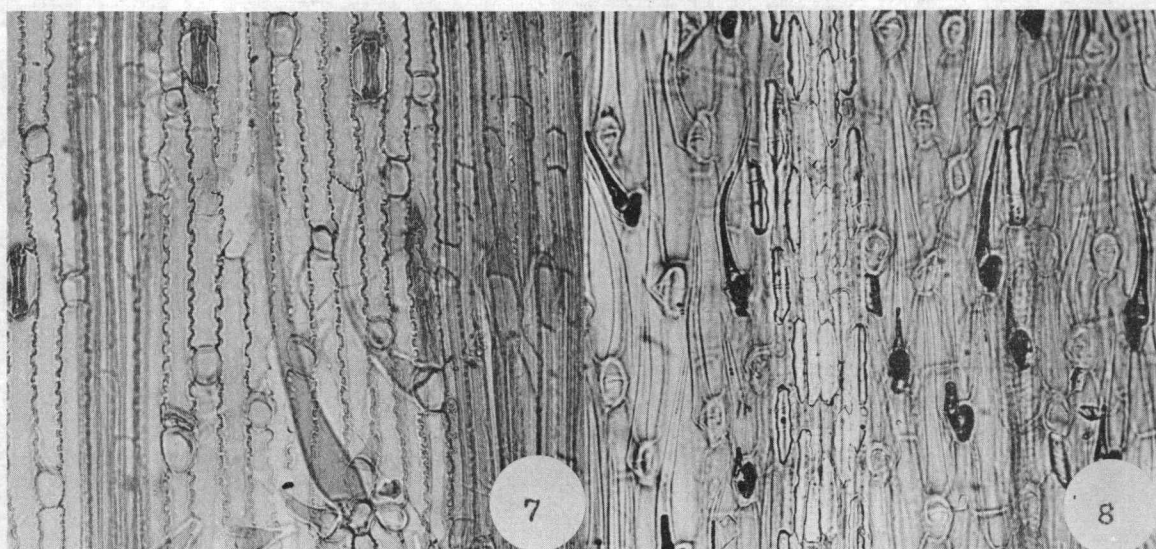


Figure 13. Danthonia unispicata sheath adaxial epidermis.
Archer Adhesive plastic peel. X252.

Figure 14. Danthonia unispicata sheath abaxial epidermis.
Safranin stain. X252

Figure 15. Danthonia unispicata lamina adaxial epidermis.
Safranin stain. X252.

Figure 16. Danthonia unispicata lamina abaxial epidermis.
Safranin stain. X252.

Figure 17. Stipa thurberiana sheath abaxial epidermis.
Safranin stain. X252.

Figure 18. Carex geyeri lamina adaxial epidermis.
Safranin stain. X252.

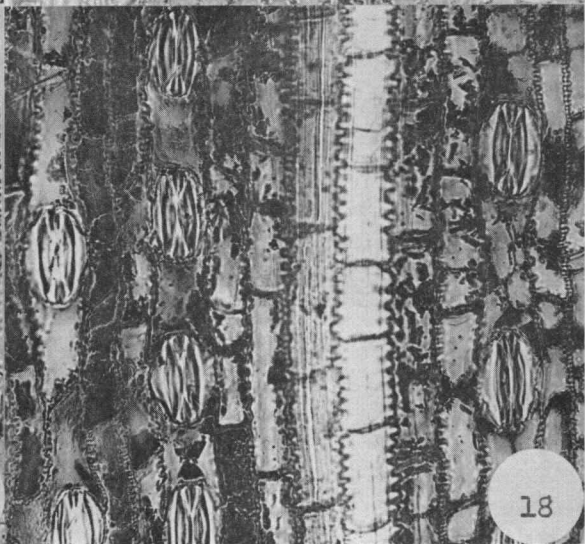
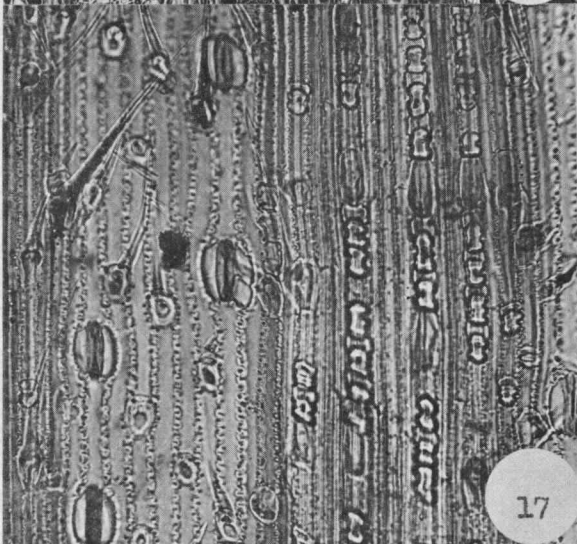
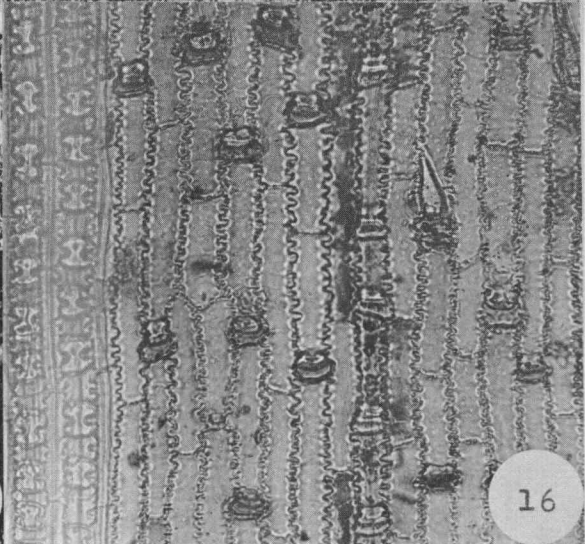
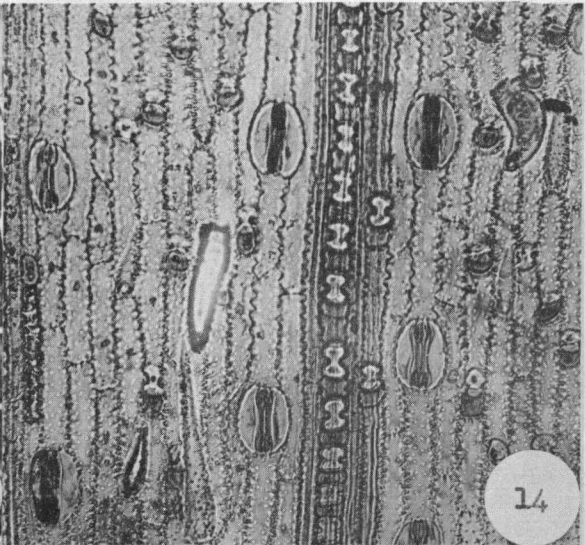
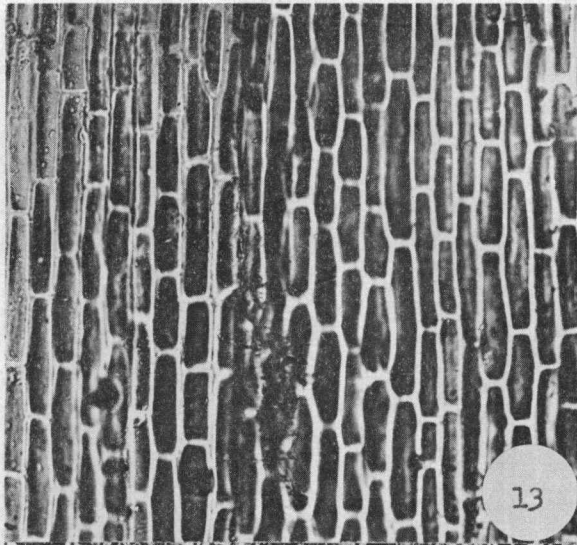


Figure 19. Juniperus occidentalis leaf epidermis.
Archer Adhesive plastic peel. X63.

Figure 20. Pinus contorta leaf epidermis.
Archer Adhesive plastic peel. X252.

Figure 21. Pinus ponderosa leaf epidermis.
Archer Adhesive plastic peel. X252.

Figure 22. Taxus brevifolia leaf epidermis.
Archer Adhesive plastic peel. X126.

Figure 23. Acer glabrum lamina adaxial epidermis.
Archer Adhesive plastic peel. X252

Figure 24. Salix scouleriana lamina abaxial epidermis.
Archer Adhesive plastic peel. X252.

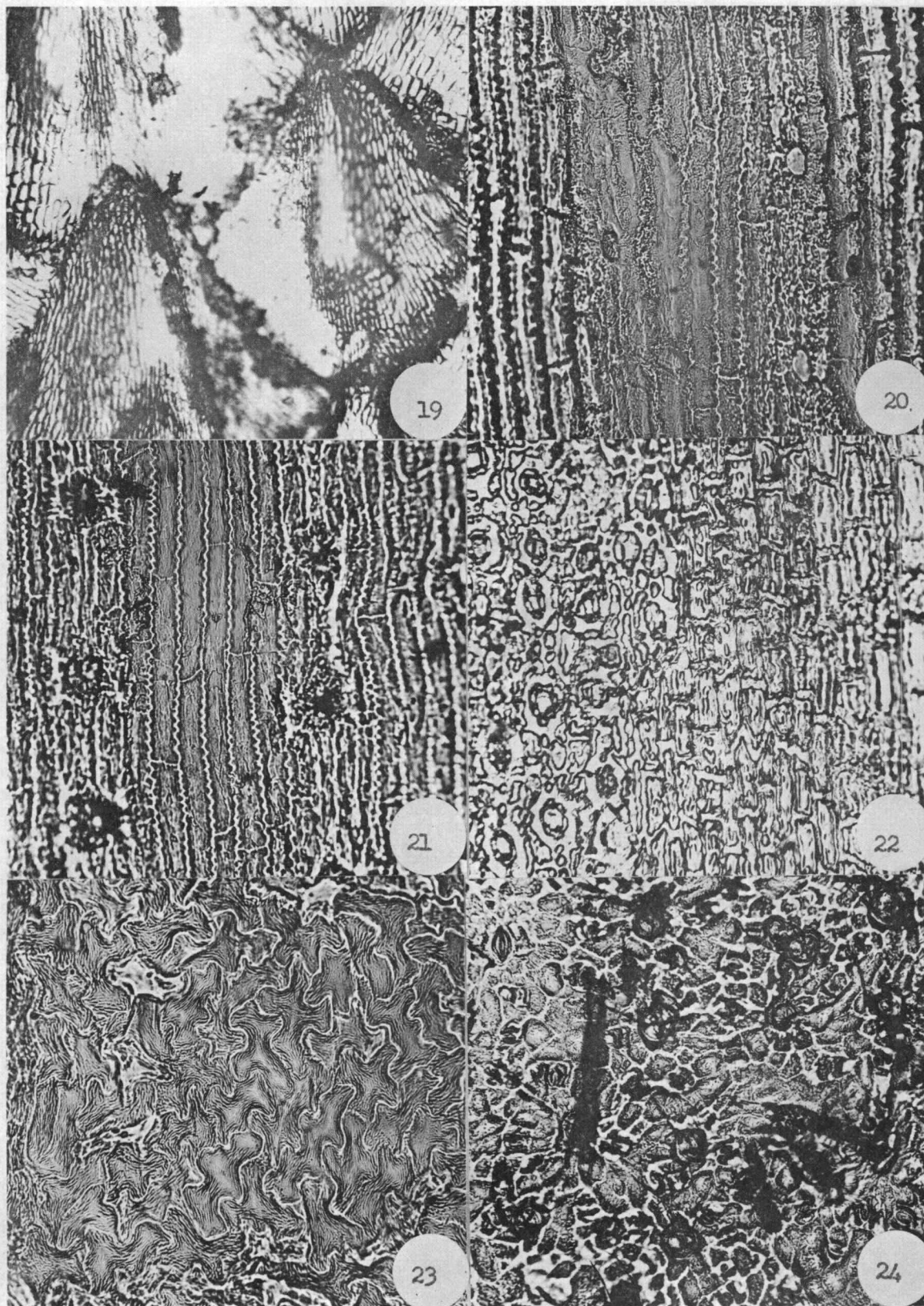


Figure 25. Astragalus stenophyllus stem epidermis.
Safranin stain. X252.

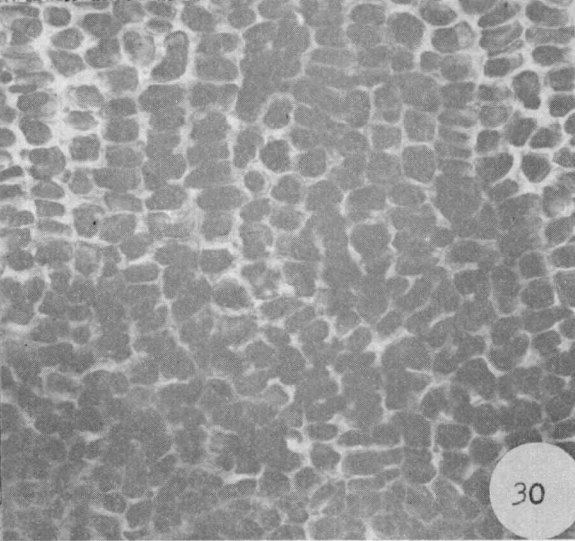
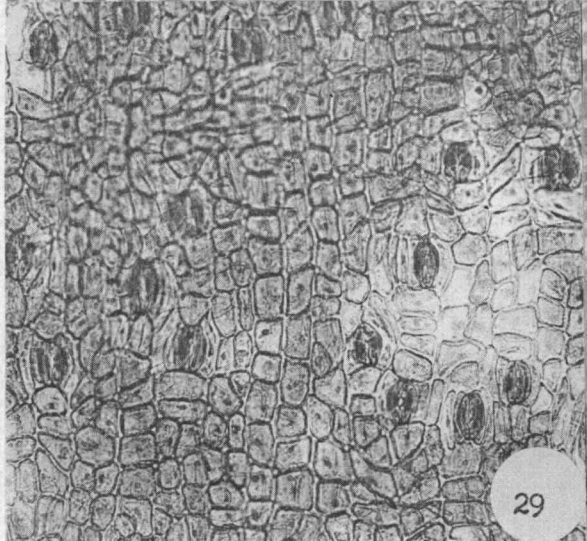
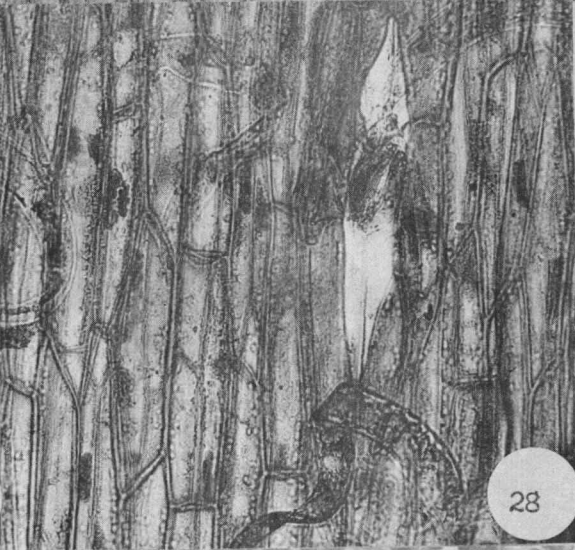
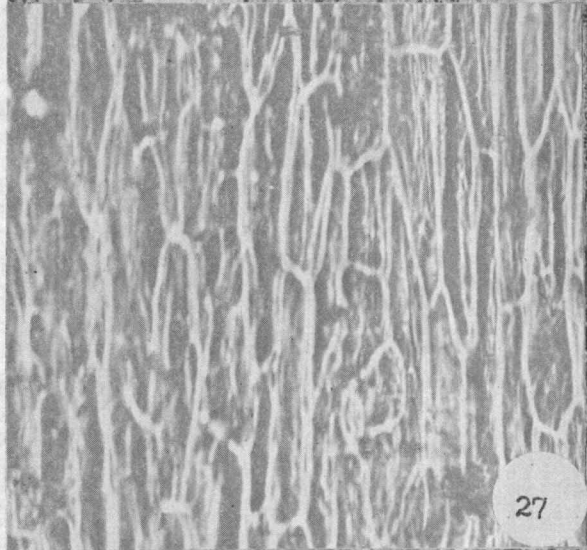
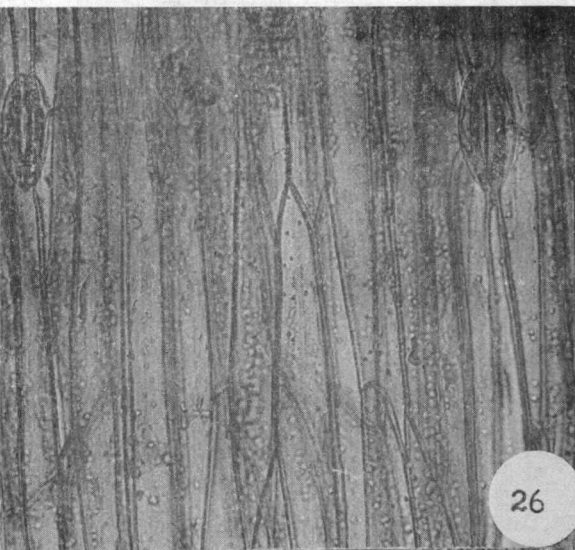
Figure 26. Arnica cordifolia stem epidermis.
Safranin stain. X252.

Figure 27. Adenocaulon bicolor stem epidermis.
Archer Adhesive plastic peel. X252.

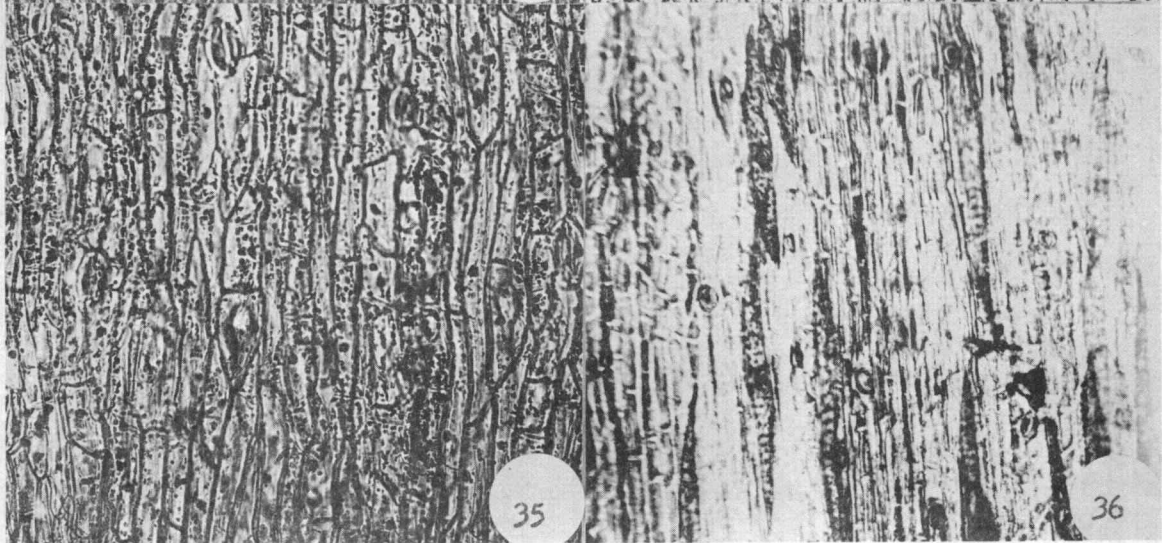
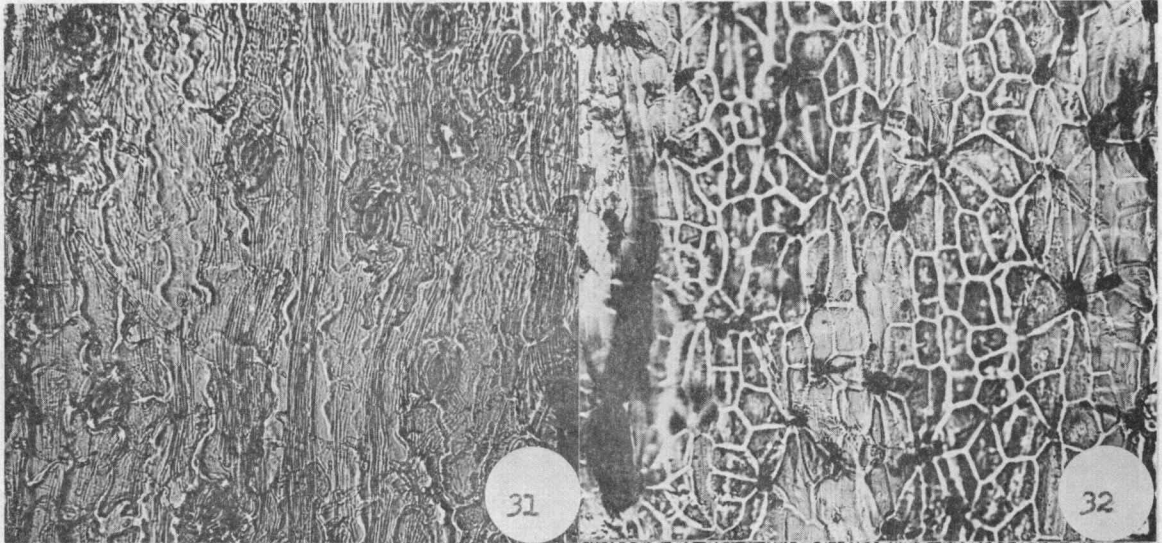
Figure 28. Actaea rubra stem epidermis.
Safranin stain. X252.

Figure 29. Vaccinium scoparium stem epidermis.
Safranin stain. X252.

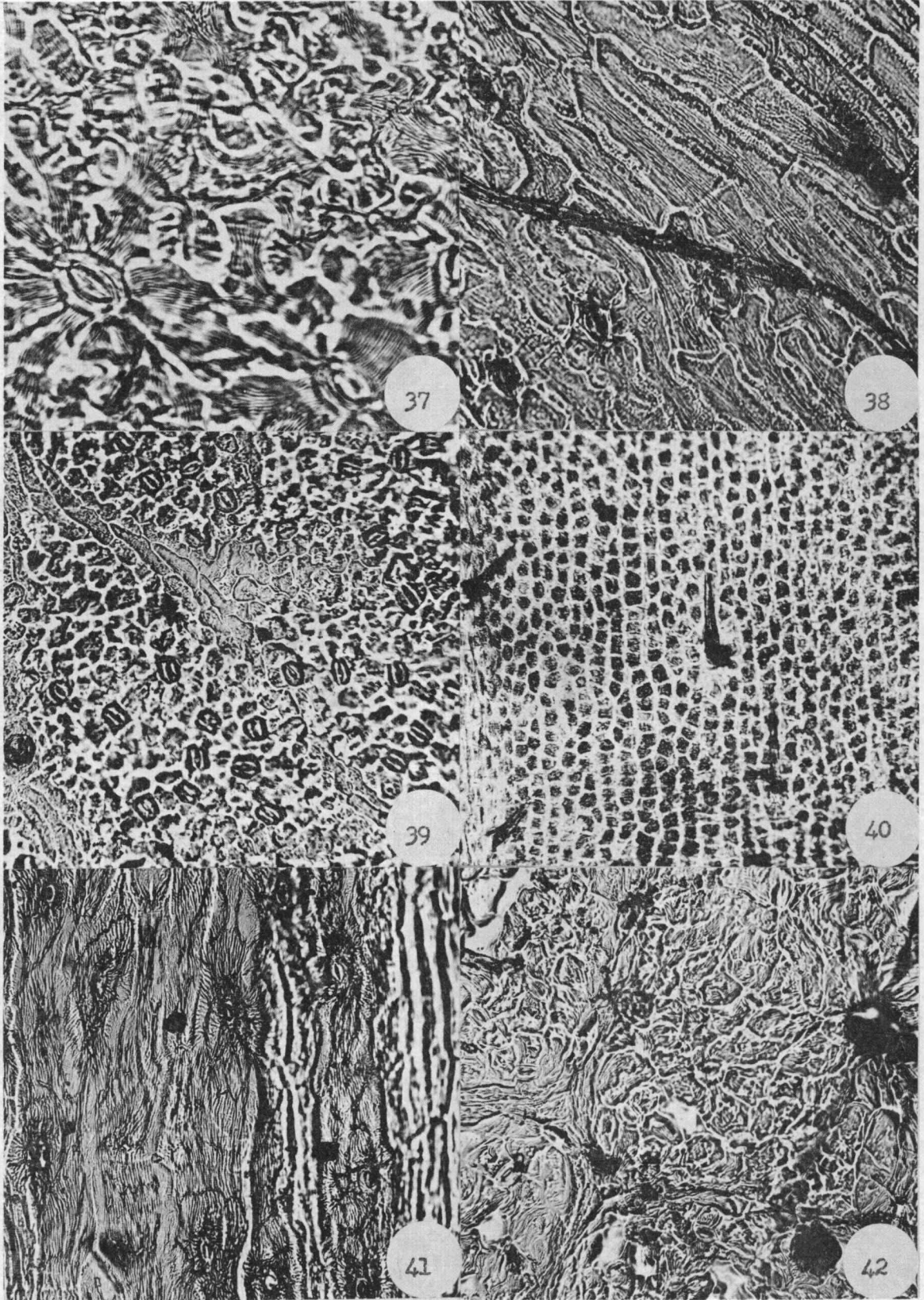
Figure 30. Cercocarpus ledifolius stem epidermis.
Safranin stain. X252.



- Figure 31. Erigeron bloomeri stem epidermis.
Archer Adhesive plastic peel. X252
- Figure 32. Holodiscus discolor stem epidermis.
Archer Adhesive plastic peel. X252.
- Figure 33. Geum macrophyllum stem epidermis.
Safranin stain. X252.
- Figure 34. Achillea millefolium stem epidermis.
Unstained cuticle. X252.
- Figure 35. Eriophyllum lanatum stem epidermis.
Safranin stain. X252.
- Figure 36. Astragalus canadensis mortonii stem
epidermis. Unstained cuticle. X126.



- Figure 37. Osmorhiza purpurea lamina abaxial epidermis. Archer Adhesive plastic peel. X503.
- Figure 38. Achillea millefolium lamina abaxial epidermis. Unstained cuticle. X252.
- Figure 39. Pedicularis racemosa lamina abaxial epidermis. Archer Adhesive plastic peel. X126.
- Figure 40. Balsamorhiza serrata petal abaxial epidermis. Archer Adhesive plastic peel. X126.
- Figure 41. Lomatium triternatum lamina abaxial epidermis. Archer Adhesive plastic peel. X252.
- Figure 42. Fragaria vesca lamina abaxial epidermis. Archer Adhesive plastic peel. X252.



- Figure 43. Lomatium leptocarpum lamina epidermis.
Safranin stain. X252.
- Figure 44. Balsamorhiza serrata lamina abaxial epidermis. Safranin stain. X252.
- Figure 45. Linum perenne lamina adaxial epidermis.
Archer Adhesive plastic peel. X126.
- Figure 46. Rudbeckia occidentalis lamina abaxial epidermis. Archer Adhesive plastic peel. X252.
- Figure 47. Eriophyllum lanatum lamina epidermis
Safranin stain. X252.
- Figure 48. Sidalcea oregana lamina abaxial epidermis.
Safranin stain. X252.

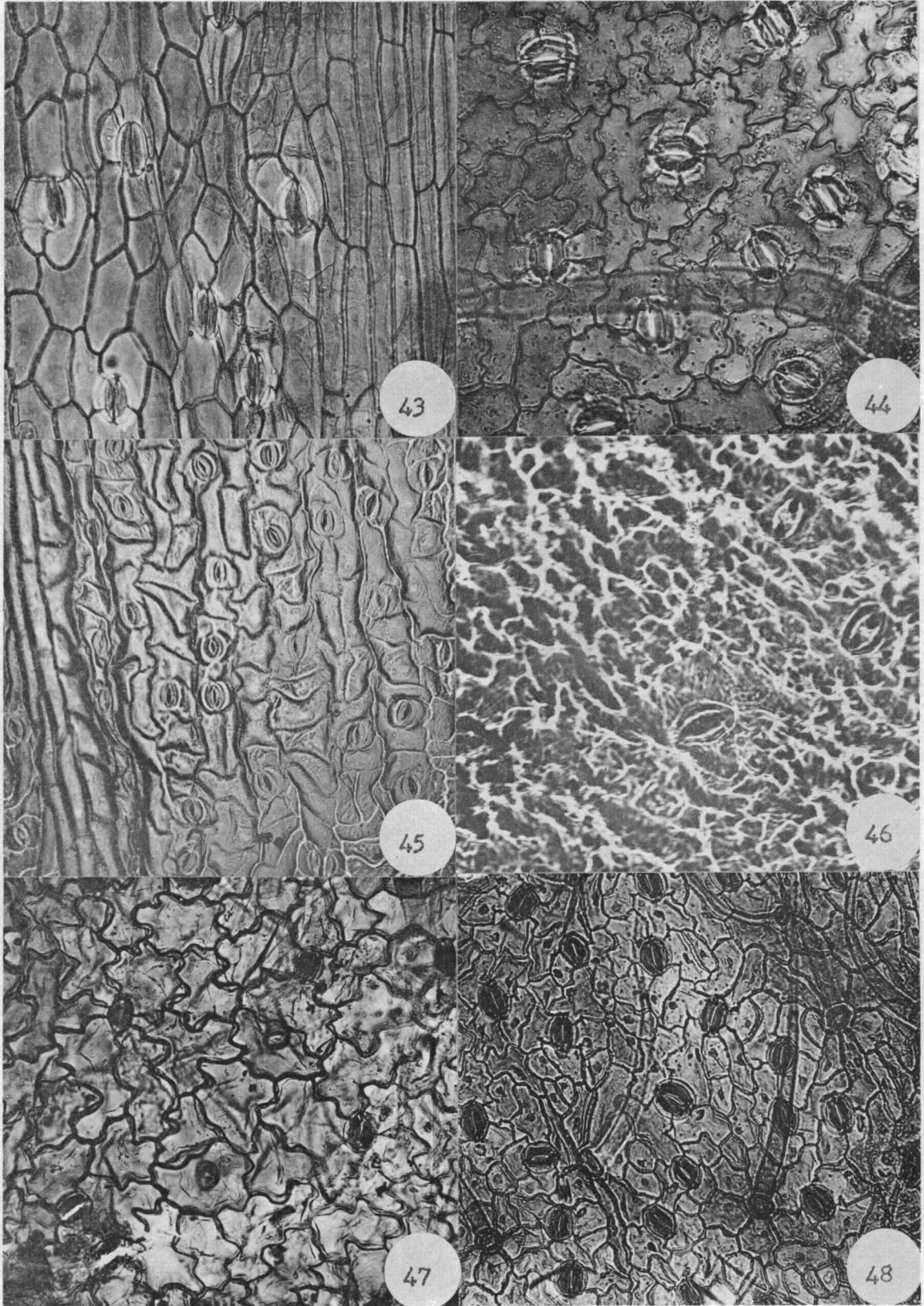


Figure 49. Camassia quamash lamina adaxial epidermis.
Safranin stain. X252.

Figure 50. Actaea rubra lamina abaxial epidermis.
Cellulose acetate peel. X252.

Figure 51. Erigeron bloomeri lamina epidermis.
Safranin stain. X252.

Figure 52. Hieracium albiflorum lamina abaxial epidermis.
Unstained cuticle. X503.

Figure 53. Adenocaulon bicolor lamina abaxial epidermis.
Safranin stain. X252.

Figure 54. Thermopsis montana lamina abaxial epidermis.
Safranin stain. X252.

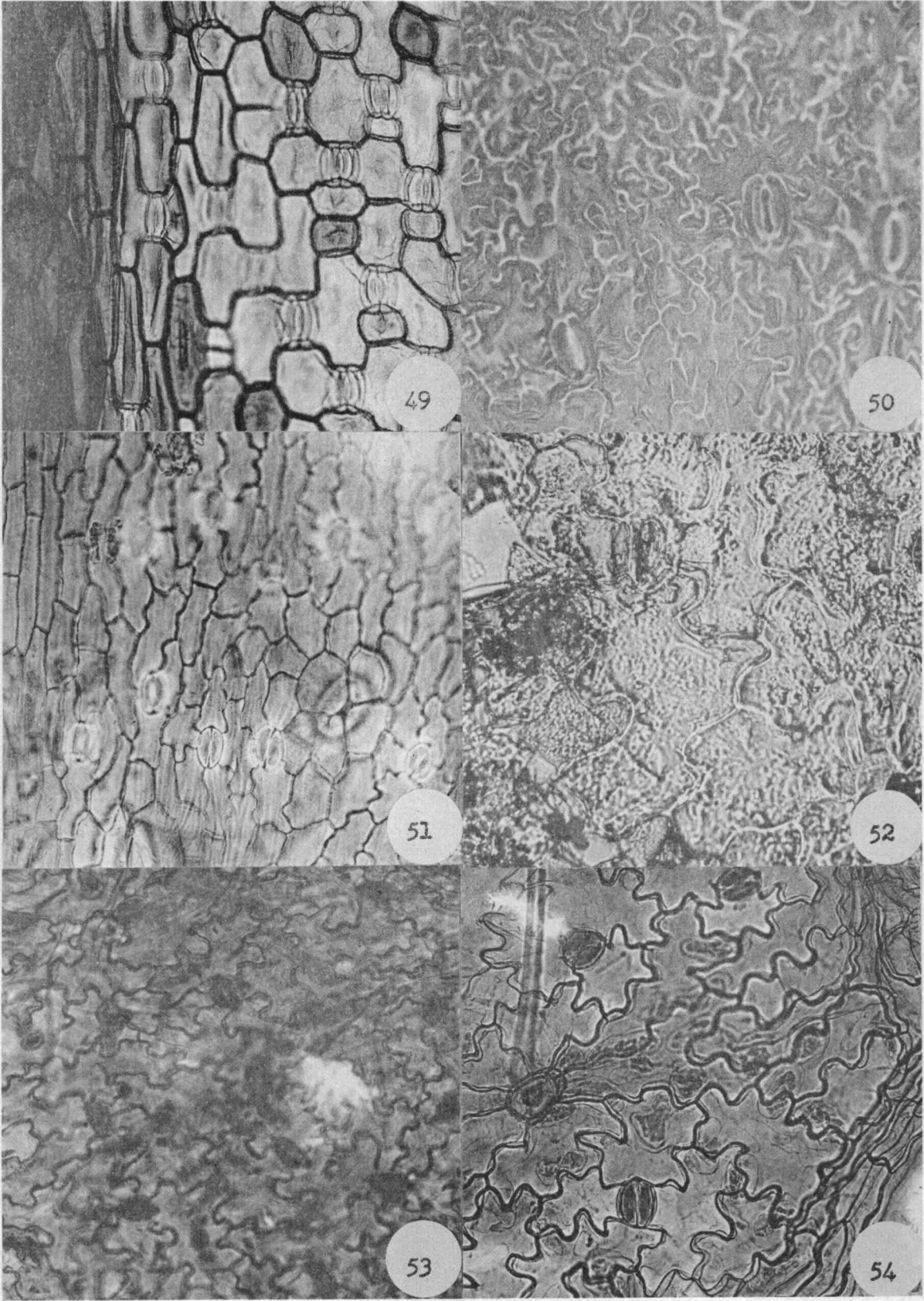


Figure 55. Astragalus stenophyllus lamina abaxial epidermis. Safranin stain. X252.

Figure 56. Astragalus purshii lagopinus lamina abaxial epidermis. Safranin stain. X252.

Figure 57. Eriogonum umbellatum lamina adaxial epidermis. Safranin stain. X252.

Figure 58. Lupinus lepidus aridus lamina adaxial epidermis. Safranin stain. X252.

Figure 59. Linnaea borealis lamina abaxial epidermis. Safranin stain. X252.

Figure 60. Lactuca serriola lamina adaxial epidermis. Archer Adhesive plastic peel. X126.

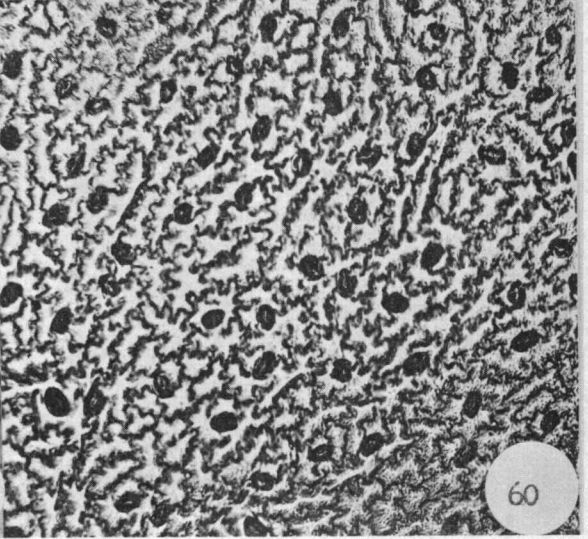
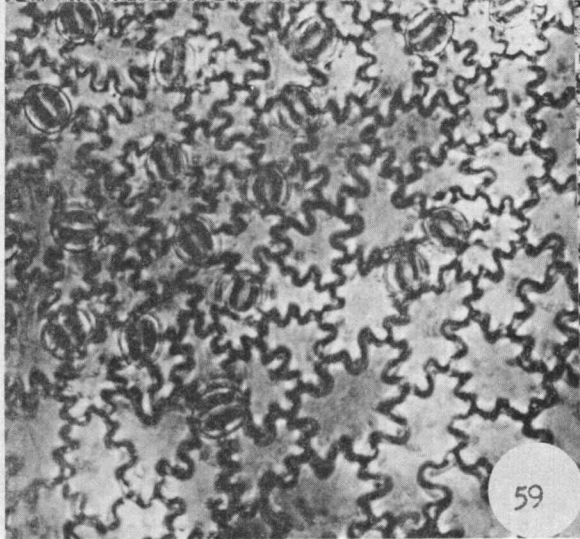
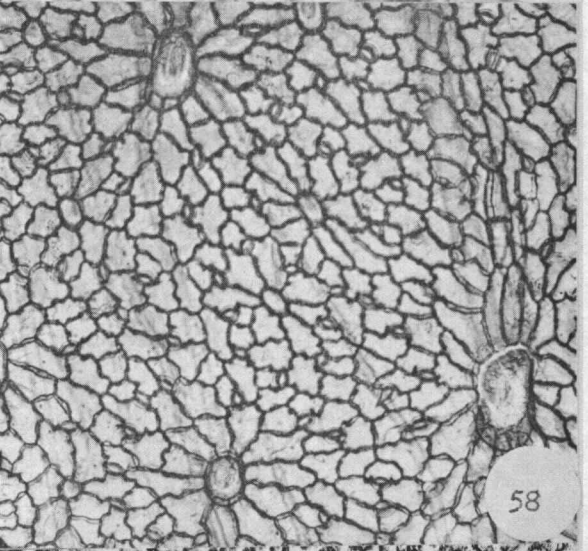
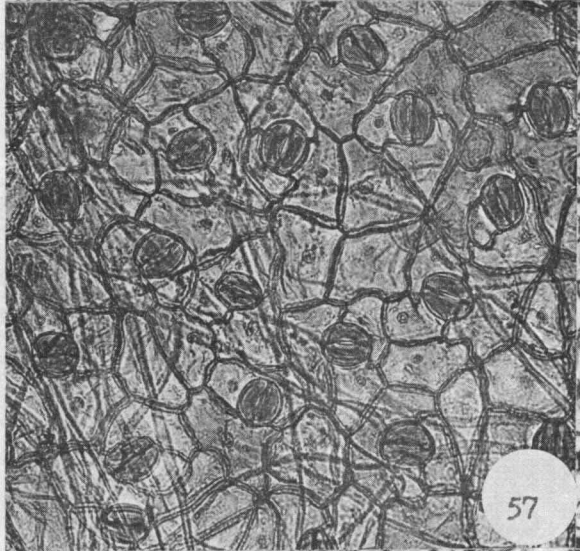
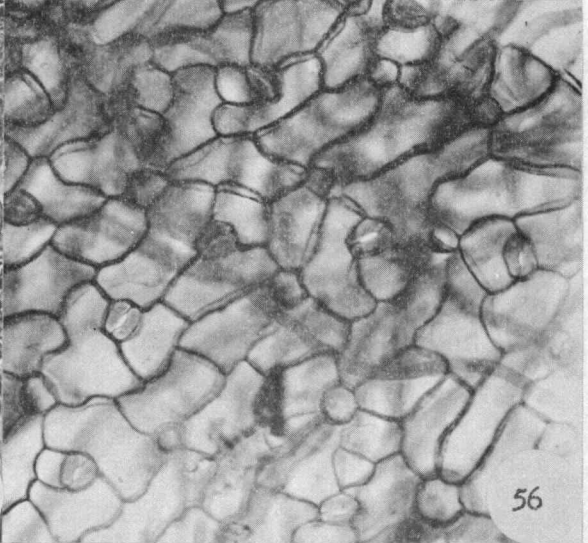


Figure 61. Astragalus canadensis mortonii lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

Figure 62. Arnica cordifolia lamina abaxial epidermis. Safranin stain. X252.

Figure 63. Scrophularia lanceolata lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

Figure 64. Geum macrophyllum lamina abaxial epidermis. Unstained cuticle. X252.

Figure 65. Trifolium latifolium lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

Figure 66. Horkelia fusca parviflora lamina abaxial epidermis. Safranin stain. X252.

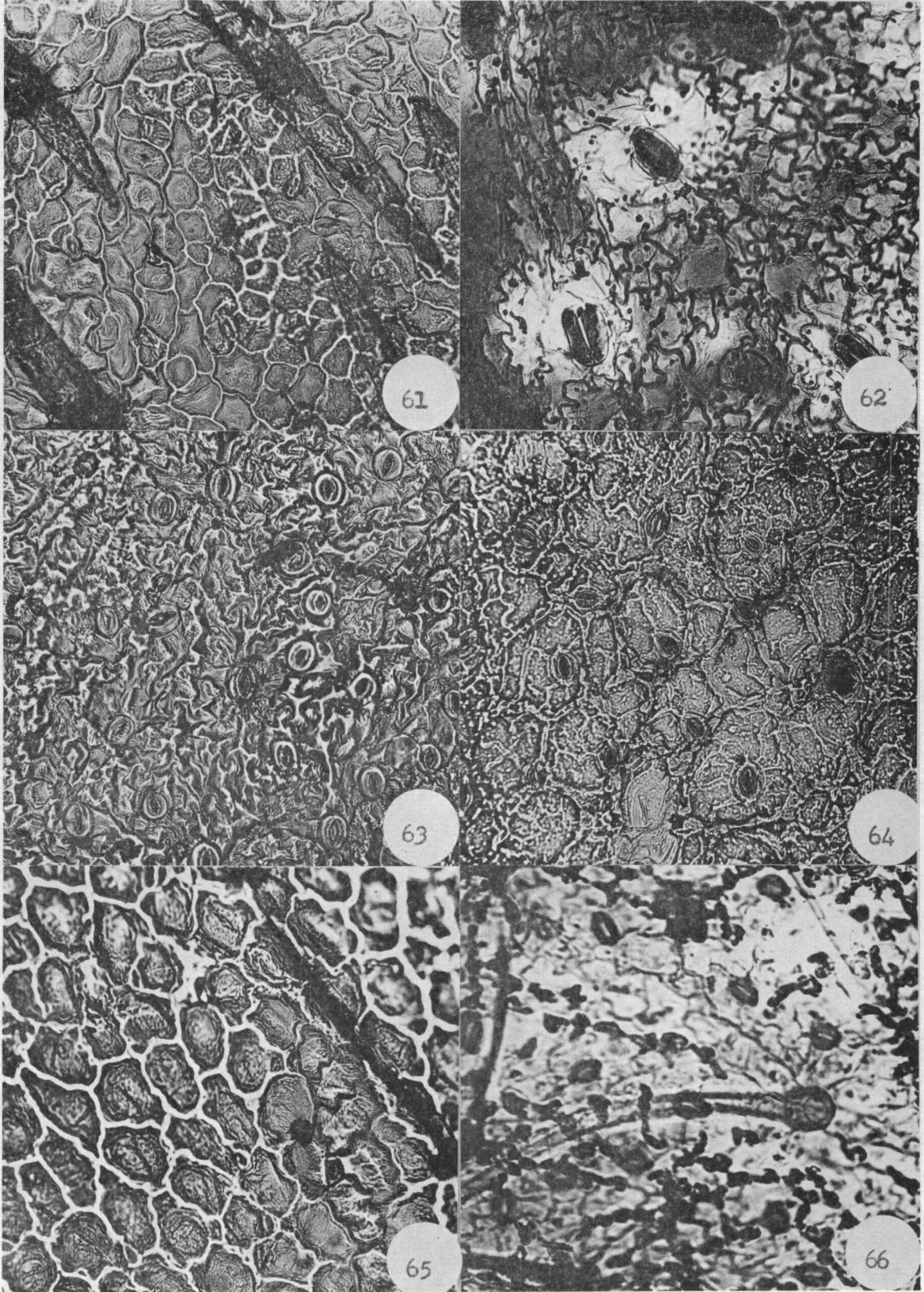


Figure 67. Chrysothamnus nauseosus lamina adaxial epidermis. Safranin stain. X503.

Figure 68. Chrysothamnus viscidiflorous lamina abaxial epidermis. Safranin stain. X252.

Figure 69. Rubus parviflorus lamina abaxial epidermis. Safranin stain. X252.

Figure 70. Purshia tridentata lamina adaxial epidermis. Safranin stain. X252.

Figure 71. Ribes viscosissimum lamina abaxial epidermis. Safranin stain. X252.

Figure 72. Rosa gymnocarpa lamina abaxial epidermis. Safranin stain. X252.

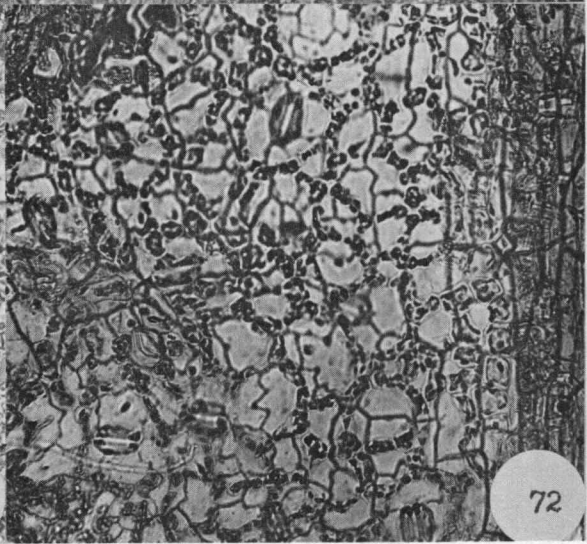
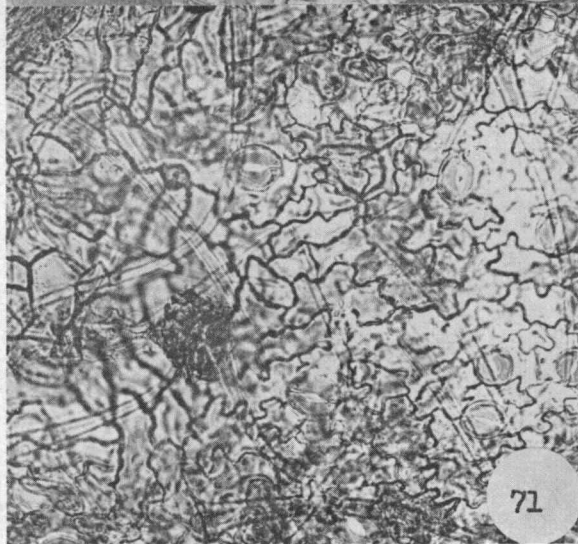
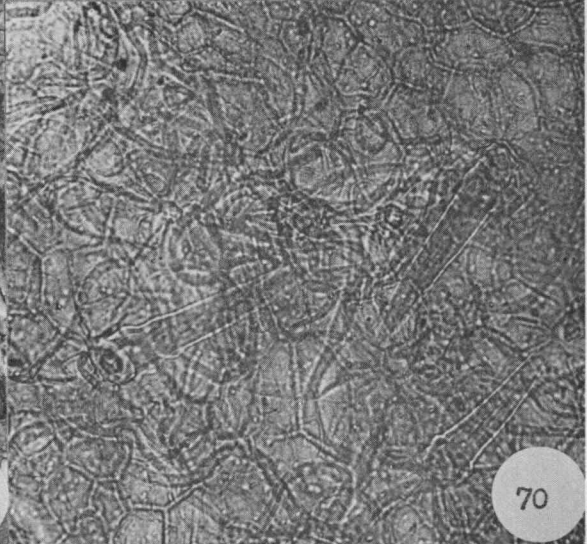
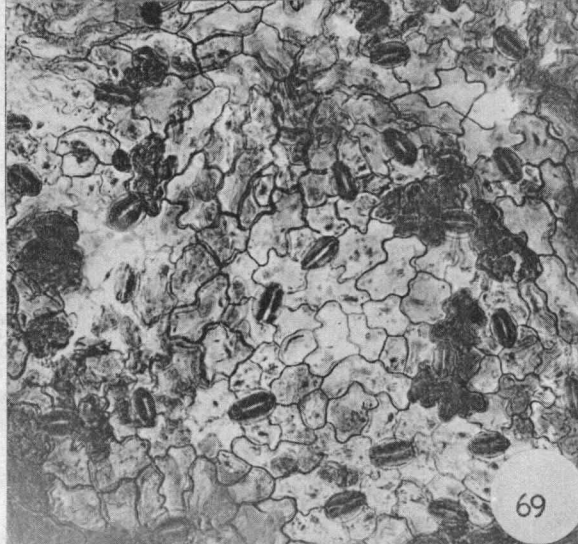
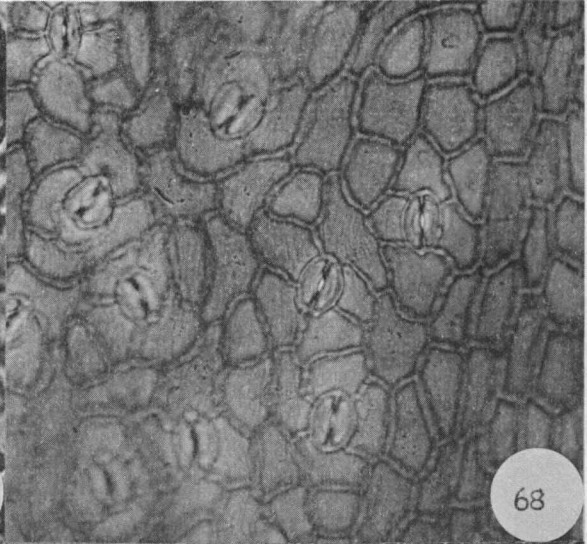
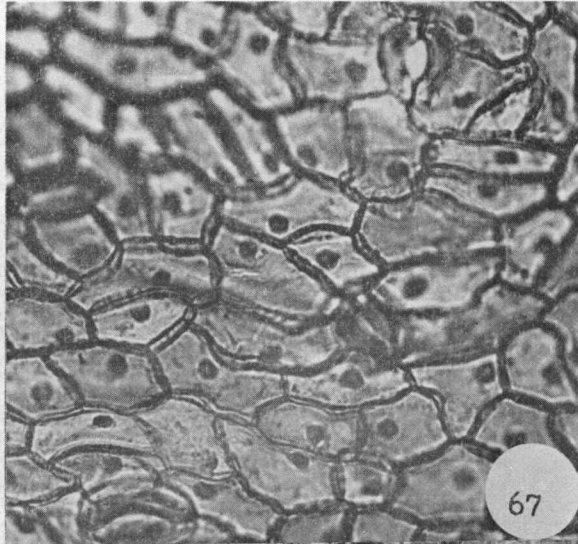


Figure 73. Spiraea betulifolia lucida lamina abaxial epidermis. Archer plastic peel. X252.

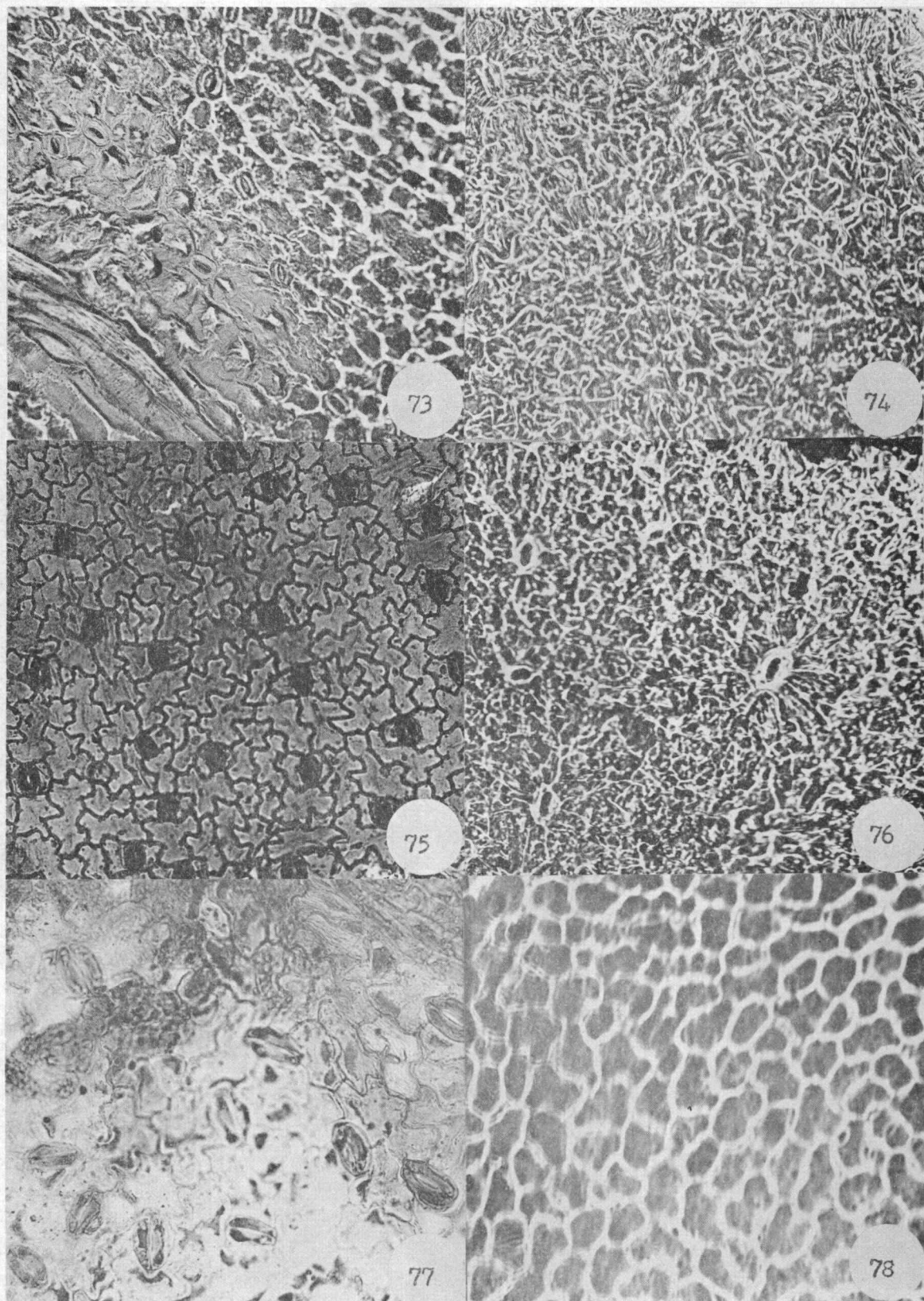
Figure 74. Vaccinium membranaceum lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

Figure 75. Vaccinium scoparium lamina abaxial epidermis. Safranin stain. X252.

Figure 76. Lonicera utahensis lamina abaxial epidermis. Archer Adhesive plastic peel. X252.

Figure 77. Sambucus cerula lamina abaxial epidermis. Safranin stain. X252.

Figure 78. Ribes lacustre lamina adaxial epidermis. Archer Adhesive plastic peel. X252.



- Figure 79. Artemisia arbuscula lamina adaxial epidermis. Safranin stain. X252.
- Figure 80. Artemisia tridentata lamina epidermis. Safranin stain. X252.
- Figure 81. Artemisia cana lamina abaxial epidermis. Safranin stain. X252.
- Figure 82. Artemisia tridentata stem epidermis. Safranin stain. X252.
- Figure 83. Arctostaphylos patula lamina abaxial epidermis. Archer Adhesive plastic peel. X252.
- Figure 84. Ceanothus velutinus lamina adaxial epidermis. Archer Adhesive plastic peel. X252.

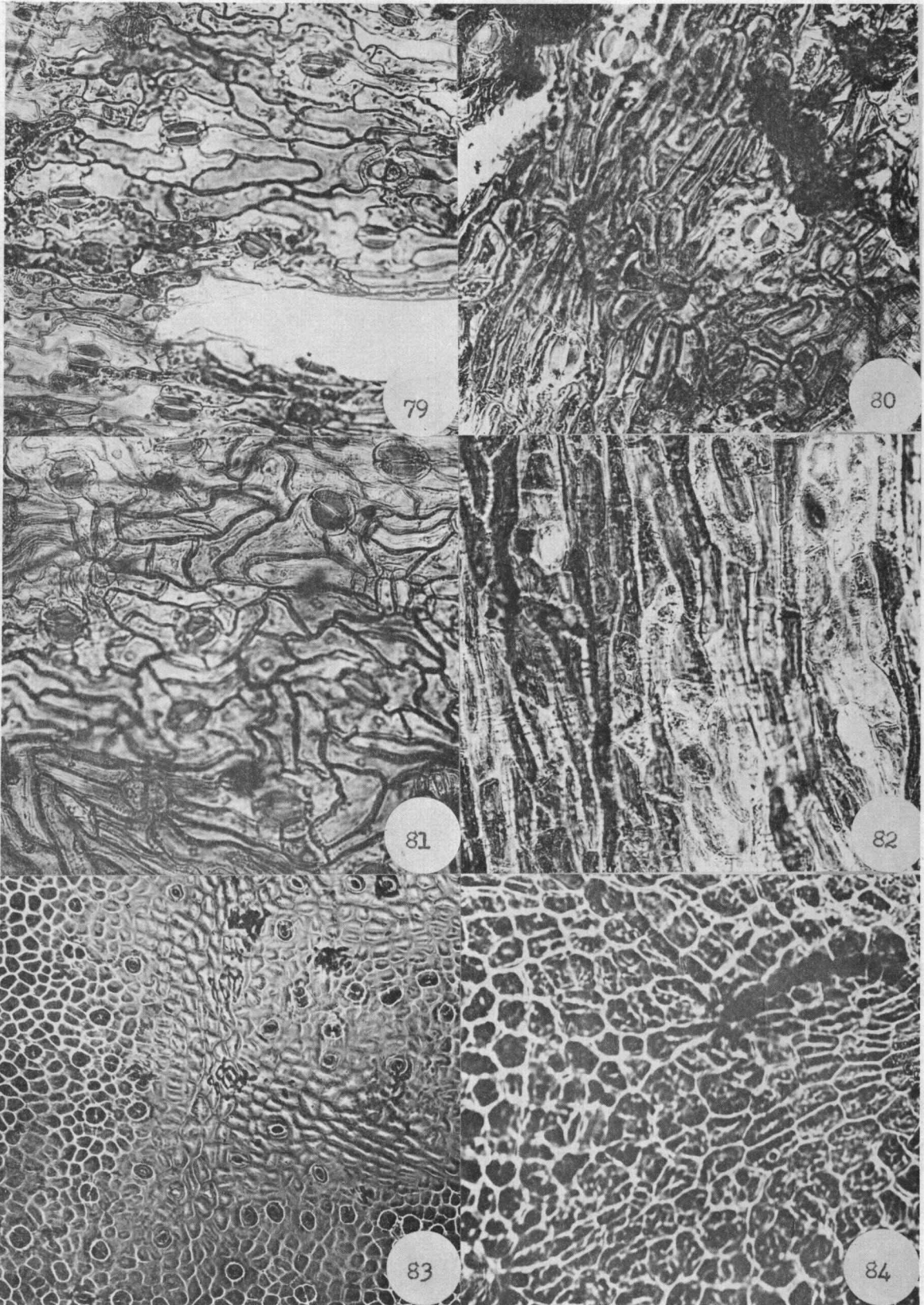


Figure 85. Symphoricarpos albus lamina adaxial epidermis. Safranin stain. X252.

Figure 86. Symphoricarpos albus lamina abaxial epidermis. Safranin stain. X252.

Figure 87. Symphoricarpos albus stem epidermis. Safranin stain. X252.

Figure 88. Populus tremuloides lamina adaxial epidermis. Safranin stain. X252.

Figure 89. Populus tremuloides lamina abaxial epidermis. Safranin stain. X252.

Figure 90. Populus tremuloides stem epidermis. Archer Adhesive plastic peel. X252.

