SURFACE ANATOMY OF WEED LEAVES WITH PARTICULAR REFERENCE TO STOMATA

by

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ABSTRACT

Thirty-five species of weeds were grown in a field nursery in order to obtain comparable data on the number, size, and surface distribution of their stomata. Mature leaves were cleared, stained, and mounted for microscopic examination The mean numbers of stomata per mm^2 of leaf surface ranged from 2.3 to 294.9. The mean lengths varied from 21.6 μ to 59.0 μ. Twenty species had more stomata on the lower than on the upper surface, while only 3 species had significantly more on the upper than on the lower surface. Between species there was negative correlation of stomatal density with length, but between surfaces within species, the lengths remained fairly constant despite differences in stomatal densities. Plants grown in partial shade had relatively more stomata/mm^2 than those grown in full sun. Similarly, leaves taken in July had a greater density of stomata than those harvested in June. In neither case could the differences be attributed to environmental factors, alone.

A slight but significant correlation of stomatal density on the upper surface with known susceptibility to 2,4-D was detected. A greenhouse experiment using picloram sprays on 3 of the species, indicated greater penetration associated with dense stomata on the lower surface than with sparse stomata on the upper surface.

A series of drawings were done depicting the different stomatal characteristics and types of trichomes. It was estimated that the leaves of 9 species were sufficiently hairy to influence retention or penetration of sprays.

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INTRODUCTION

Modern agriculture is concerned with the efficient production of the maximum quantity and quality of food from a given land area. Among the products of science which are helping to achieve this goal are chemical fertilizers, growth regulators, and pesticides. When properly used, these materials increase yields by stimulating growth of crop plants while reducing competition from weeds, insects, and pathogens. These chemicals may be applied to the soil or to the foliage of plants. In some cases they must be absorbed into the plant before being effective while in others the chemicals act externally. The effectiveness of most foliar applied fungicides and insecticides depends on their ability to adhere to the surfaces of crop plants. Penetration into the plant is incidental, although it may have important residue implications. Foliar applied nutrients, systemic insecticides, and herbicides on the other hand, must penetrate the plant surface and enter the protoplasm in order to be effective.

In this study, the emphasis is on the foliar penetration of herbicides and the plant factors which affect that penetration.

The route followed by foliar applied chemicals in entering plant leaves has long been a subject of controversy. Thousands of hours of research have been devoted to the subject and it is a tribute to the complexities of nature that no simple explanation is available.

The exact mode of entry of foliar applied herbicides is an academic problem, with important practical applications. For example, if foliar penetration of a given compound is shown to occur mainly through the leaf cuticle over veins, then species susceptibility could be influenced by the presence or absence of bundle sheath extensions. If the exact route of entry of a herbicide were known, then information concerning the factors affecting that route could be used to obtain greater selectivity and efficiency of the chemical.

Much of the confusion and disagreement in the penetration question has been due to the attempts of scientists to reduce to simple terms, a highly complex biological relationship. No general statement regarding penetration will ever be valid for all possible situations. Among the factors which may be expected to influence the penetration of chemicals into plant leaves are the following:

Plant Factors
1) Thickness and composition of cuticle.
2) Presence of superficial wax.
3) Number, size, and distribution of stomata.
4) Presence and distribution of trichomes.

Environmental Factors
1) Temperature.
2) Relative humidity.
3) Wind velocity.
4) Light intensity and quality.
5) Precipitation.
6) Mechanical damage by insects, animals, man, and machinery.

Chemical Factors
1) Formulation.
2) Volatility.
3) Polarity.
4) pH.
5) Surface tension
6) Size of spray droplets.

Each of the factors listed has been studied but difficulty arises in co-ordinating the results of experiments carried out under different conditions.

There are two logical ways to attack the problem as it now stands. The first is to perform large factorial experiments. The other is to accumulate as much information as possible about each factor. This latter approach has been used to varying degrees in the study of plant cuticle, leaf surface wax, and physical and chemical properties of herbicidal solutions.

The present work consists of a quantitative study of the size, number, and distribution of stomata in the leaves of common weed species and a qualitative survey of the trichomes present on the same leaves. In addition to the survey for the above-mentioned characters, three weed species were used in a herbicide spray experiment designed to detect differences in susceptibility related to these characters.

REVIEW OF LITERATURE

The key to the purpose of the present study is given by the following statement by Currier and Dybing in 1959 (8).

> " Comparative studies of structure and function of the leaves of a wide variety of weed and crop plants are necessary, because the species variable confounds all other variables in the penetration complex."

Five years later, Holly (31) observed that in the few species studied, differences in penetration showed little correlation with differences in susceptibility. Nevertheless, he made the following statement.

> "Despite the lack of direct evidence of an effect of penetration on selectivity, it is to be hoped that penetration studies will be made on a wider range of species and that the results obtained can be related to stomatal characteristics and other aspects of leaf structure."

As these two statements suggest, there have been very few studies using a large number of species. To evaluate the significance of the present study and the methods used, the literature of foliar penetration, stomata, and trichomes will be reviewed.

I. FOLIAR PENETRATION

The volume of literature available under this general topic is truly amazing, considering that it has been an active field of study for only about twenty years. There are a number of general reviews on the subject (3,6,8,15,18,21,48,55, 69,72,74). Others are mainly concerned with the plant cuticle (7,40,53), leaf surface wax (59), retention of sprays (31), and surfactants (2,33).

Whereas early workers in this field often made erroneous generalizations based on their experimental results, it is now realized that all possible factors must be considered when discussing penetration of foliar applied chemicals. With this in mind, a logical way to summarize the current knowledge is under the individual factor headings.

1. Leaf Cuticle

The cuticle forms the outer covering of all young plant organs except, possibly, the root hair (20). It occurs as a separate layer or variously merged with the outer epidermal wall. In fact, it is more accurate to refer to the cuticular layers, one of which is the cuticle. The cuticular layers are those outer surface lamellae which are embedded with cutin and wax, including the outer portion of the epidermal wall (7).

Cutin is a mixture of long chain unsaturated fatty acids and alcohols which are produced by cell metabolism (7). These products migrate via cell walls to the nearest air/water interface where their unsaturated linkages are oxidized and polymerization occurs (15). The cuticle waxes consist mainly of fatty acids esterfied to monohydric alcohols of 24-36 carbons but they also contain free alcohols, alkanes, ketones, and aldehydes (7,40,69). Pectins are composed of long chain polygalacturonic acid molecules with carboxyl side groups which may be methyl esterified (7,60). The cuticle is like a sponge made of cutin in which the holes are filled with wax (69). The outer layers tend to have more wax while the inner layers

also contain pectin and cellulose.

The quality and thickness of cuticle are influenced not only by species differences, but also by the environment. Wax deposition is greatest under hot, dry, sunny conditions (31). Thus, a desert succulent would be likely to have a thick cuticle of high wax content, whereas a shade plant in a rain forest would probably have a thin, hydrated cuticle (7).

The waxy cuticle of a mature leaf is a serious barrier to the penetration of externally applied substances (69). Recent studies have proven that the penetration of most substances through the intact cuticle does occur, although doubt remains in regard to some inorganic compounds (61). It has been proposed that polar substances penetrate mainly via the hydrophilic cutin and pectin, while non-polar compounds prefer a lipoidal pathway (7). In any event, all foliar absorbed compounds must somehow traverse the cuticle. There are many conflicting reports on the speed of penetration, due in part to differences in species and chemicals used.

Areas which may permit more rapid penetration include cracks in the cuticle, thin areas over veins, thin areas over dividing epidermal cells, the thin cuticle lining sub-stomatal chambers, and the bases of trichomes (69). The latter two will be discussed below. Recently the existence of pores in the cuticle has been shown for a few species but it is too early to speculate as to whether they are involved in penetration (40).

2. Surface Wax

In addition to that which is embedded in the cuticle, many species have wax of similar composition forming a surface covering or bloom (31). This wax generally has the appearance of a dense mat of rods as revealed by the electron microscope (59). The deposition of surface wax continues only until the leaf is mature and then any wax deposition occurs below the mature cuticle, indicating that the wax passes through the immature cuticle. Special wax channels through the cuticle have been doubted (31), but recently they have been shown for a few species (40,55).

These waxes are highly repellent to aqueous solutions and thus provide the conditions necessary for selective post-emergence weed control in such waxy leaved crops as peas, onions, and cereals. In some species, the wax deposits are absent from certain areas so that penetration could occur preferentially in those areas. For example, in pea leaflets, wax is absent over the stomata (31).

The structure of the wax rodlets is important in determining water repellancy as shown by the fact that wax dissolved in ether and redeposited on a glass slide is not repellant (19).

A survey of a miscellaneous assortment of plants for

surface wax included the following weed species (59):

Wax Bearing	Non-Wax Bearing
Setaria viridis	*Solanum nigrum*
Setaria faberii	*Ambrosia trifida*
Setaria lutescens	*Ambrosia elatior*
Digitaria sanguinalis	*Polygonum persicaria*
Polygonum aviculare	*Convolvulus arvensis*
	Cirsium vulgare
	Plantago lanceolata
	Plantago major

3. Stomata

It has generally been agreed that vapors and oils easily penetrate stomata (8). The role of stomata in penetration of aqueous solutions has been a subject of controversy (8,31,55). Some reports indicate that stomatal penetration did not occur (22,68,70), or at least not through the open pore (25). Others suggest that it does occur if adequate surfactant is included (62,69). For a few species, the stomatal pore has now been demonstrated to be a major point of entry (9,14). Other experiments have shown correlation between stomatal numbers and entry of compounds but little difference between the open and closed conditions (56). Also the fact that guard cell walls are believed to contain a large concentration of ectodesmata (25) and may be preferentially stained (14), has lead to suggestions that the guard cells and accessory cells may be important areas of penetration (21,56). If this is true, it would seem reasonable that penetration would be most rapid when the stomata are opening and the guard cells are imbibing.

While there have been no comprehensive studies on the

effect of stomatal size on entry via the open pore, the poor penetration into bean leaves relative to nine other species was attributed to small stomatal size (14). A systemic study along these lines might well resolve much of the conflicting data.

When a liquid enters the sub-stomatal chamber it still must penetrate the cuticle of the mesophyll cells before becoming available for effective action or translocation. However, stomatal penetration suggests two important advantages over penetration of external cuticle. One of these is that the internal cuticle is much thinner and more easily penetrated and the other is that the high humidity of the sub-stomatal chamber prevents the spray solution from drying out, thus enabling penetration over a longer period of time (6).

In summary, stomata are definitely involved in foliar penetration. Their exact role in the penetration of different species under varying environmental conditions and with sprays of differing chemical and physical characteristics is not well understood.

4. Trichomes

Leaf trichomes (hairs) may have an important bearing on the wettability of leaf surfaces and thus on their ability to retain aqueous sprays. Even when retention occurs, hairs which are densely packed and hydrophobic may effectively prevent penetration by holding the spray droplets away from the leaf surface. On the other hand, loosely matted hairs may hold greater spray deposits than glabrous leaves and thus increase

penetration (31).

A further effect of trichomes on penetration may depend on the existence of ectodesmata in their walls, especially near the base (25). Ectodesmata are believed to be protoplasmic threads penetrating outer epidermal cell walls, similar to the plasmodesmata which connect adjacent protoplasts (20).

5. Ectodesmata

The existence of high concentrations of ectodesmata in the walls of guard cells, and trichomes, and the anticlinal walls of epidermal cells over veins has been shown (25). It has also been shown that penetration of dyes and excretion of previously absorbed substances may occur preferentially in these areas (14,25,27). Thus it has been proposed that penetration may be enhanced by the presence of ectodesmata (25,26,40). This would agree with the theory that cuticular penetration occurs most rapidly where an internal transport mechanism maintains a steep concentration gradient (55).

At the present time, prevailing interest in the role of ectodesmata is being shown by European workers (40), while until recently the American attitude in regard to their importance has been one of skepticism (7).

6. Temperature

Temperature has both direct and indirect effects on foliar penetration. Indirectly, high temperature over a long period reduces penetration by stimulating the formation of a thick cuticle of high wax content (62). Directly, high

temperatures increase penetration by lowering the viscosity of the applied solution and the cuticular barriers (8), and by accelerating metabolic processes concerned with uptake and translocation (22,36,56). For chemicals with high vapor pressures, high temperatures may result in volatilization of the solution from the leaf surface, thereby reducing the amount available for penetration (40). In a recent study, most but not all of the differences in susceptibility to herbicides of plants under different temperatures, could be explained on the basis of differences in absorption and translocation (49).

7. Relative Humidity

Humid conditions tend to favour development of thin cuticle of low wax content (7). Such leaves should be relatively permeable. It has been shown that high humidity at the time of application increases uptake of both organic (5,8) and inorganic compounds (47). This may be due to delayed drying of the spray deposit (8), stimulation of stomatal opening (52), and hydration of the cuticle with resultant wider spaces between wax platelets (69).

8. Light

Bright sunlight stimulates the production of thick cuticle of high wax content and thus, indirectly reduces penetration of foliar sprays. It is probable that sunlight acts mainly by raising leaf temperature. Shade grown plants are relatively easily damaged by foliar herbicides (37). Light on the other hand, may also promote penetration by causing opening of stomata (8,56), and by maintaining a concentration

gradient due to translocation of photosynthate out of the leaves (8,71). Recent findings (57), that dark followed by light promotes faster penetration than continuous light requires further study. As previously suggested, it may be that the swelling of guard cells during opening causes rapid uptake.

9. Rain and Dew

Rain may wash off spray deposits before they have had time to penetrate or become absorbed by the cuticle. A light rain may promote uptake by keeping the deposit in solution. Similarly, a dried spray deposit may redissolve in night dew and be absorbed with the dew droplet when the stomata open in the morning (46).

10. Spray Droplet

The size, surface tension, and velocity of application of spray droplets influences retention and penetration. As a rule, the smaller the droplet, the better its retention on the leaf. In peas, retention fell from 100% to zero as droplet diameter increased from 100 to 250 microns (31). Particles smaller than 20 microns seldom settle onto a leaf surface on a sunny day (15). On an overcast day, such small droplets may enter stomata directly, provided that a high velocity spray is used (8). Large droplets remain in solution longer so that cuticular penetration may occur over a longer period of time.

11. Formulation of Chemical

Chemicals may be formulated in many different ways,

each having its own characteristic properties. Herbicides may be formulated as solutions of water or oil, emulsions, wettable powders, granules, and dusts (39). For foliar applications, solutions or emulsions are generally used. It is believed that different formulations of a herbicide have the same action in the plant but differ in their speed and path of penetration (74). Work with 2,4-D showed the following order of toxicity to one species: Na salt - NH4 salt - amine - ester (least to greatest) (8).

The effectiveness of ester formulations is largely due to their low polarity and consequent ease of penetration of the cuticle (56). Low molecular weight esters such as methyl, ethyl, and propyl tend to be volatile so that much of the herbicide may be lost before penetration can occur. The use of high molecular weight esters overcomes this (39).

The pH, degree of dissociation, and presence of other ions influences penetration (31). 2,4-D and dalapon evidently penetrate most rapidly in the undissociated form as low pH favors their entry (8,23,51). The permeability of the cuticle and rate of metabolic uptake may also be affected by pH (8,31). Ions such as NH_4^+ and PO_4^{---} definitely influence uptake but the direction of the effect is disputed (8,51,66).

More study on the effects of formulation on selectivity and penetration could lead to much more sophisticated and efficient use of the chemicals which are now available.

12. Surfactants

The value of surfactants in increasing the effectiveness

of herbicides is well known. Increased response of up to fourteen times has been recorded for 2,4-D applied with surfactant (8). The ways in which surfactants may increase penetration are summarized by Sargent (55):

a) by increasing the area of contact through spreading the solution.
b) by eliminating air films between the solution and plant surface.
c) by acting as cosolvents or solubilizing agents in cuticular penetration.
d) by easing entry through open stomata and subsequent movement through intercellular spaces.
e) by acting in a secondary capacity as humectants by retarding drying out of the solution.

Most of the effects of surfactants appear thus to be due to a lowering of surface tension. In some cases, however, herbicidal enhancement may occur without change in surface tension (55). An ideal surfactant molecule has both relatively lipophilic and hydrophilic portions and is not highly toxic in itself (8).

The effects of surfactants may not all be beneficial. For example, where selectivity in a crop depends on its waxy leaves, a surfactant may cause wetting and damage to the crop. Use of a surfactant on a highly wettable leaf surface may result in a thinner film of solution and thus a smaller spray load on the leaf (55).

Surfactants may be classified as non-ionic, cationic, or anionic (39). All surfactants do not give equally satisfactory results with all spray chemicals so that experimentation and selection is required to avoid difficulties such as precipitate formation (8).

Recent research in the field of surfactant use is extensive (33). Studies have involved the physical and chemical characters of surfactants themselves (2,35), as well as the relationship of those characters to herbicidal effects and entry of tracers into leaves (24,32). From the practical approach, extensive studies on the interactions of surfactants, surfactant concentrations, and various herbicides have been carried out (34,43,44,67).

II STOMATA

Stomata may be considered as openings in the epidermis bounded by two guard cells (20). In this discussion, the stomata are taken to include the pore and the guard cells. There may also be adjacent modified epidermal cells known as accessory cells. Stomata are organs of gaseous exchange in higher plants and are especially numerous in green leaves. Previous mention was made of the possible role of the stomatal pore, guard cells, and accessory cells in penetration of foliar chemicals.

The study of stomata from an anatomic and taxonomic viewpoint has been of intermittent interest since the turn of the century. Since the first comprehensive survey of greenhouse plants (16), there have been relatively few systematic studies on the size and number of stomata (46,54,65). A few studies have dealt with correlation of stomatal numbers with leaf size while others have been concerned with the stomatal complement of a restricted plant group (28,68). The orientation of stomata has been studied and it has been found that there is

a tendency for them to be aligned with their long axis parallel with underlying veins (63). This is consistent with the parallel orientation common in monocot leaves and the apparently random orientation in dicot leaves.

The taxonomic study of stomata has led to the recognition of four main types in regard to accessory cell number and arrangement (45).

The physiology of stomata, especially the opening and closing process has received particular attention. In conjunction with this there have been many methods of observing stomata published from time to time. These include: horn and yucca hygroscopes (11); resistance thermometer to study changes in leaf temperature (12); epidermal stripping and fixation in alcohol (41); porometer to measure air flow through leaf (13); cobalt-chloride hygrometric paper (42); infiltration with alcoholic dye solution (73); new resistance thermometer method (64); infiltration with various solvents (1,10); cellulose acetate films (50); scotch tape method (58); and time lapse photography of intact leaves (17). None of these methods is entirely satisfactory but used in combination with visual observation of live leaves, they yield useful information.

The earliest comprehensive studies of stomatal physiology are those of Lloyd in 1908 (41), and Loftfield in 1921 (42). Recent reviews are by Heath in 1959 (30), and Ketellaper in 1963 (38). The physiology of stomata is, even now, not completely understood.

Because open stomata may be an important site of

penetration of foliar applied chemicals, it is necessary to be familiar with the factors affecting opening and closing. The following summary is a modification of the review by Ketellapper (38). It has long been known that guard cells are peculiarily adapted for opening by combinations of cell wall characters and internal turgor pressure. In most dicots, the primary wall next to the pore has the cellulose micelles oriented in such a way as to permit minimal extension. To further minimize extensibility, secondary thickenings may be added. The remainder of the guard cell wall is relatively extensible so that when turgor pressure is increased, the guard cell tends to bend away from the pore. In most monocots, the guard cells are dumbbell shaped and the bulbous tips swell to open the pore.

The environmental factors which regulate stomatal aperture are light, CO_2, water stress, and temperature. Ordinarily, morning light causes photosynthesis in the mesophyll and guard cells accompanied by a reduction of CO_2 in the guard cells and opening of the stomata. A water deficit will over-ride all other effects and cause closure. Temperatures above 30°C. cause increased CO_2 content of the guard cells and stomatal closure. This may explain the commonly observed mid-day closure.

The classical mechanism for stomatal opening was that the reduced internal CO_2 caused increased guard cell pH which favoured a conversion of starch to soluble sugar with resultant increase in osmotic pressure and turgor. It is now

known that this explanation is incomplete. As a result, it has been proposed that an active, energy requiring, transport system is involved which supplements the classical mechanism. Whether the active transport applies to solute or water and if opening or closing or both is the active process is not known. Further research into the biochemistry of photosynthesis will probably provide an eventual explanation.

III TRICHOMES

The majority of vascular plants possess trichomes or epidermal hairs on at least some of their aerial organs. A trichome is initiated as a protruberance from an epidermal cell which elongates and may undergo various divisions (20). A unicellular trichome may resemble a simple hair or it may branch to form a forked hair or a T-shaped hair closely appressed to the leaf surface. Great diversity is possible in multicellular hairs. They may ascend for some distance and then divide in a single plane to produce stellate (star-shaped) or peltate (scale-like) tops. By combinations of periclinal and anticlinal divisions, dendroid (tree-like) or candelabra hairs may be produced. Those which divide periclinally at the base may form massive multicellular hairs.

The great diversity of trichomes has led to their systematic study and subsequent use in taxonomy (4). While they are not useful for the identification and classification of all plants, they are used in many keys and taxonomic monographs. They may be useful at the family, genus, species, and even species hybrid level (45).

Trichomes may retain living protoplasts or they may lose their protoplasmic contents (20). They may persist throughout the life of a leaf or they may be ephemeral, being discarded as the leaf matures (20). This corresponds with the proposed function of simple leaf trichomes in reducing transpiration by lowering surface temperature and maintaining a humid micro-environment at the leaf surface (29). They may also reduce the destruction of chlorophyll by sunlight when the leaf is young and the cuticle is thin (29).

The more complex or phylogenetically advanced (4) trichomes have special properties which equip them for various functions. The peltate trichomes are probably effective in reducing transpiration. Some trichomes have secretory glandular heads; others such as nettles, have brittle tips with bulbous, toxin-filled bases which eject poison into the unfortunate animal making contact. Some hairs have greatly thickened walls and sharp tips containing lignin, silica, or calcium carbonate (20). These structures protect the leaf surface from browsing animals and insects (29).

As was already mentioned, trichomes may contain a large number of ectodesmata (25). Thus they may serve as areas of uptake of foliar applied chemicals. It is also possible that glandular trichomes may have special absorptive ability. The ruptured basal cells or cavity left when a trichome is shed may be points of chemical penetration, although it is known that in some species cell wall growth and cutinization rapidly fill the openings (29). In general,

the study of trichomes in relation to agricultural sprays has been confined to macroscopic observations.

MATERIALS AND METHODS

I Establishment of the Weed Nursery.

In order to reduce environmental variability, a weed nursery was established in which all of the species studied were grown in an essentially uniform environment. The site was prepared on a turfed area of the Plant Science plots at the University of British Columbia. Two by five foot plots were dug out of the turf as shown in figure 1.

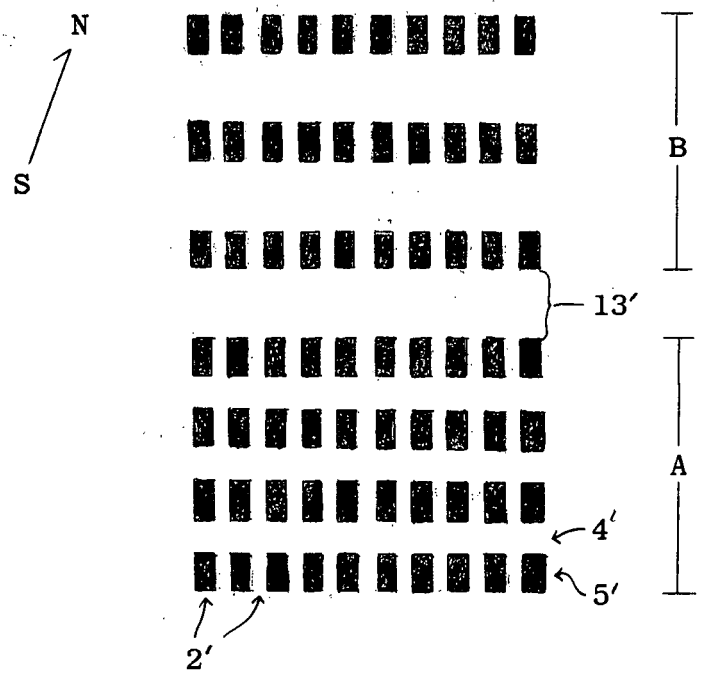

Figure 1. Weed nursery plot plan.

The weeds were introduced into their respective plots between May 26 and May 31, 1965. Most seedlings were obtained from various places on campus. Redroot pigweed, yarrow, Canada thistle, hemp nettle, and broadleaf dock were found at Langley, B.C. Spotted knapweed and toadflax came from Westwold, B.C. White cockle, Russian knapweed and leafy

spurge were from Campbell Creek and diffuse knapweed from Pritchard, B.C. Blue bur, wild oats, and barnyard grass were grown from seed in the greenhouse prior to transplanting in the field.

Table 1. List of weeds studied.

Family Common Name	Scientific Name
Amaranthaceae	
*red root pigweed	*Amaranthus retroflexus* L.
Boraginaceae	
*blue bur	*Lappula echinata* Gilib. (seed)
Caryophyllaceae	
*chickweed	*Stellaria media* (L.) Cyrillo
*mouse-ear chickweed	*Cerastium vulgatum* L.
*white cockle	*Lychnis alba* Mill.
Chenopodiaceae	
*lambsquarters	*Chenopodium album* L.
Compositae	
yarrow	*Achillea millefolium* L.
diffuse knapweed	*Centaurea diffusa* Lam.
*spotted knapweed	*C. maculosa* Lam.
Russian knapweed	*C. repens* L.
*Canada thistle	*Cirsium arvense* (L) Scop.
*cat's ear	*Hypochaeris radicata* L.
pineappleweed	*Matricaria matricarioides* (Less) Porter
*groundsel	*Senecio vulgaris* L.
annual sow thistle	*Sonchus oleraceus* L.
dandelion	*Taraxacum officinale* Weber
Cruciferae	
wild radish	*Raphanus raphanistrum* L.
*hedge mustard	*Sisymbrium officinale* (L.) Scop.
*shepherd's purse	*Capsella bursa-pastoris* (L.) Medic.
stinkweed	*Thlaspi arvense* L.
Euphorbiaceae	
*leafy spurge	*Euphorbia esula* L.
Gramineae	
*couch grass	*Agropyron repens* (L.) Beauv.
*wild oats	*Avena fatua* L.
*barnyard grass	*Echinochloa crusgalli* (L.) Beauv.
Labiatae	
hemp nettle	*Galeopsis tetrahit* L.
Plantaginaceae	
*narrow leaf plantain	*Plantago lanceolata* L.
broad leaf plantain	*P. major* L.
Polygonaceae	
*wild buckwheat	*Polygonum convolvulus* L.
*lady's thumb	*P. persicaria* L.
*prostrate knotweed	*P. aviculare* L.

*sheep sorrel	Rumex acetosella L.
*broadleaf dock	R. obtusifolius L.
Portulacaceae	
*western spring beauty	Montia sibirica L.
Ranunculaceae	
*creeping buttercup	Ranunculus repens L.
Scrophulariaceae	
*toadflax	Linaria vulgaris Hill

* Species which were grown in area B as well as in area A (figure 1)

Western spring beauty, Montia sibirica, although not considered a weed, was included as a representative of the succulent leaved Portulacaceae.

Each plot contained ten plants in a row, thus the spacing between plants was 6 inches. The plots were kept free of other plants by hand weeding at intervals throughout the summer. The grass between the plots was mowed regularly. As it was felt that such a lack of competition and shading was unnatural for weeds, thirty wooden slat shades were constructed and erected over the plots in area B. These shades consisted of wooden lathes approximately $1\frac{1}{2}$ inches wide spaced one lathe width apart on a 3 by 6 foot frame. It was intended that they would simulate a growing crop by reducing solar radiation and air and ground temperature. In all other respects, the plots in area B were identical to those in A. Henceforth, areas A and B will be referred to as sun and shade, respectively. Figure 2 shows the plot area with shades in place in mid-summer.

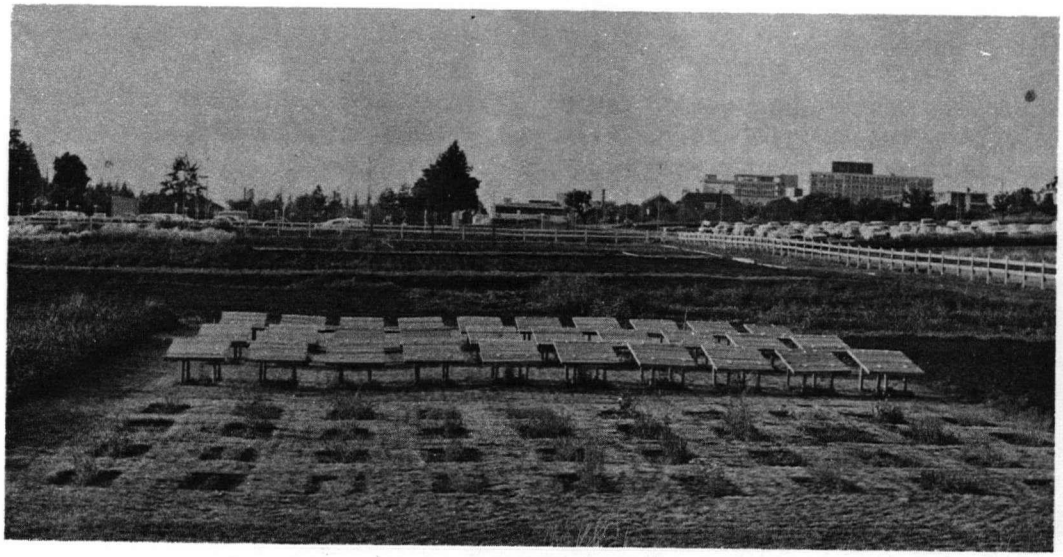

Figure 2. The weed nursery in mid-summer.

II Leaf Sampling Procedure.

Leaf samples were taken at two dates. The first time of sampling was June 28-30 and consisted of taking a minimum of 6 mature, healthy leaves from each of the 2nd, 4th, 6th, and 8th plants in each plot. These four plants per plot are henceforth referred to as replications 1 to 4. The second harvest was made on July 29-30 from plants 3,5,7, and 9.

As the leaves of each plant were removed they were placed in individual vials containing FAA killing and fixing solution (90:5:5 - 50% ethanol, formaldehyde, acetic acid). These vials were left overnight or for not more than 24 hours and then the FAA was replaced with 70% ethanol. They were then stored until needed for slide-making.

III Slide Preparation.

Approximately half of the leaves from each vial were placed in small beakers or petri dishes in which they were

cleared and stained prior to mounting on glass slides in a permanent mounting medium ('Kleermount' - Carolina Biological Supply Co.). This method was used because it allows microscopic observation of both the upper and lower epidermis in any given field and it also allows one to select definite fields in relation to distance from leaf tip, base, mid-rib, and margin. It has the disadvantage that stomatal aperture may be affected by the various steps.

The clearing procedure results in dissolution of the protoplasmic contents of the leaf so that only the cell walls are left to take up the stain. Beginning with the leaves taken from 70% ethanol, the clearing method involved the following steps:

a. wash thoroughly with water (in theory running water should be used but in practice 5-6 changes over 2-3 hours is adequate).
b. immerse in $2\frac{1}{2}$% aqueous NaOH until white (about 7 days average).
c. wash well in water.
d. immerse in aqueous chloral hydrate (250g./100cc.) overnight or up to 24 hours. The leaves should now be completely transparent.
e. wash well in water.

Several stain combinations were tried and of these, safranin plus tannic acid-ferric chloride, and crystal violet were most useful. Those which were of little value in epidermal staining included fast green, acid fuchsin, and acid fuschin plus tannic acid-ferric chloride. The safranin-tannic acid-ferric chloride combination actually gave the best differentiation but the crystal violet gave adequate results with fewer time consuming steps, so that it was finally selected. The procedure for both of these methods is outlined below.

1. Safranin-tannic acid-ferric chloride.

   a. 1% aqueous tannic acid - 10 minutes.
   b. wash well in water.
   c. 3% aqueous ferric chloride - 5 minutes.
   d. wash well in water.
   e. 30% ethanol - 5 minutes.
   f. 50% " "
   g. 70% . " "
   h. 95% " "
   i. 100% ethanol - "
   j. 1:1-xylene and 100% ethanol - 5 minutes.
   k. safranin (1g./100cc 1:1 xylene-ethanol) several hours.
   l. wash with 1:1 (xylene-ethanol)
   m. xylene - change at least once, no maximum time.
   n. mount.

2. Crystal violet.

   a. ½% aqueous crystal violet - several hours.
   b. wash well in water.
   c. take up the alcohols (e-h, above)
   d. decolourize in 100% ethanol until desired colour is reached.
   e. 1:1 - 5 minutes.
   f. xylene - at least 10 minutes, no maximum time.
   g. mount.

When possible at least three leaves of each replicate were mounted on a slide. In the case of large leaves, portions were cut out midway between the base and tip of the leaf. In all cases the leaves were mounted with their upper surfaces uppermost. In order to flatten the mounted specimens, lead weights of up to 150 grams were placed on each cover slip during the initial drying time of 1-2 weeks. Figure 3 shows the final appearance of a few of the slides.

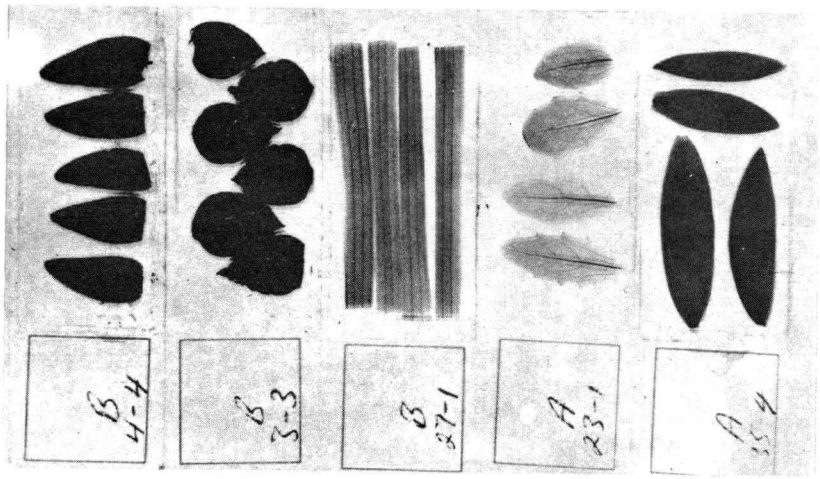

Figure 3. Mounted leaves ready for microscopic examination.

IV Counts, Measurements and Pictorial Record.

After slides for both sampling dates had been made, counting and measuring stomata was begun. Ten microscope fields (x500) were counted for each slide. In making counts care was taken to avoid the base, tip, midvein and margin where irregular numbers and/or immature stomata were commonly observed. Upper and lower surfaces of the same field were counted by focussing up and down.

Measurements were made with the aid of a calibrated eyepiece micrometer. Twenty stomata were selected at random during the counting procedure and were measured for length from tip to tip of the guard cells. It was decided to measure only the length of the guard cells as it was faster and less subject to variability then measurements of width or size of aperture.

A total of over 450 slides representing 33 species were studied. Altogether, approximately 75,000 stomata were counted and about 19,000 were measured.

Pen and ink drawings were made to illustrate the epidermal features of the various species. Photo-micrographs were taken to illustrate specific features but they were not used extensively because of their inability to show depth and the fact that some of the leaves were not perfectly flat so that only a part of the field was in focus at a time. In addition, not all of the species had enough contrast in staining to permit good photographs. Photographs were used to illustrate the appearance of trichomes under fairly low magnification. In all cases, a Zeiss Photomicroscope was used.

V. Herbicide Susceptibility Trial.

After some data on stomata counts were available, three species were selected for a simple herbicide susceptibility experiment in the greenhouse. The three species were white cockle which was selected for its relatively glabrous leaves with most stomata on the lower surface; hemp nettle selected for pubescent leaves with most stomata on the lower surface; and lambsquarters which has a thick coating of foliar wax on both surfaces and most stomata on the lower surface.

For this experiment, seeds which had been previously harvested from the field plots were sown in 3 by 3 inch plant bands in greenhouse flats. In order to obtain satisfactory growth following planting in December, 1965, the flats were

placed under a bank of cool white fluorescent lights which provided an intensity of about 900 foot candles at plant level for a daylength of 16 hours. After 6 weeks, growth was very good. At that time, the individual bands containing 3, 4, or 5 plants of white cockle, lambsquarters, or hemp nettle respectively, were removed from the flats and the treatments were applied.

The treatments consisted of 0, 1, 10, 100, and 1000 ppm. aqueous solutions of picloram (4-amino-3,5,6-trichloro-picolinic acid) applied to the leaves as a fine spray in a volume of 5 ml. per plant band. Runoff was negligible. Variables besides herbicide concentration included application to upper versus lower leaf surface and to plants with relatively closed stomata versus those with relatively open stomata. The latter conditions were achieved by applying the herbicide prior to exposure to morning light and after 2 hours of sunlight, respectively. In order to avoid the introduction of artificial conditions, applications were made by simply spraying from above or below the leaves for upper and lower surface application, respectively. The methods used approximate those which would be possible under field conditions.

Following the spray applications on January 16, 1966, the plant bands were randomly arranged on a greenhouse bench without supplementary light. Two weeks later, the plants were harvested at ground level and fresh and dry weights were taken. These weights were subjected to analysis of variance in an attempt to detect differences in harvest weight associated with the treatments.

RESULTS

The data pertaining to the 8 species which were grown only under sunny conditions were analyzed separately from those of the large group of 25 species, grown under shade and in sun. For convenience, the results of the two groups will be given separately and then a final summary table will be presented. All of the large statistical analyses, both for the herbicide experiment and the counts and measurements, were carried out using the I.B.M. 7040 Computer at the U.B.C. Computing Centre.

I Stomatal Number and Size of Eight Species Grown in Sun Only.

Table 2. Number and size of stomata of eight species, mean of both surfaces and sampling dates (in order of decreasing number).

Species	No./mm.^{2}	Size in Microns
dandelion	- 216.6a*	26.6
wild radish	- 215.4a	25.5a
b.l. plantain	- 190.8	25.7a
stinkweed	- 144.5	25.4a
spotted knapweed	- 137.3	29.2
hemp nettle	- 124.5	27.6
pineappleweed	- 88.5	34.5
yarrow	- 83.8	37.7

* In this and in all further presentations of data, means within columns followed by the same letter were not significantly different at the 5% level according to Duncan's new multiple range test (DMRT).

1. Stomatal Numbers

The concentrations of stomata varied greatly with species, harvest date, and surface (appendix table 1). Taking the means of both upper and lower surface and both sampling dates, the 8 species had the numbers of stomata per mm.^{2} of leaf surface shown in table 2.

Table 3. Number of stomata per mm.^{2} on upper and lower leaf surface at two sampling dates. (in order of decreasing grand mean).

	June		July	
Species	Upper	Lower	Upper	Lower
dandelion	141.3ghi	241.0abc	155.3efgh	328.6
wild radish	219.3abcd	195.3cdef	201.3bcde	231.0abc
b.l. plantain	125.3jkl	172.0defgh	217.9abcd	247.9ab
stinkweed	100.6jklm	148.0fghi	121.3jkl	207.9bcd
spotted knapweed	137.3ghi	128.0jk	131.3ij	152.6fgh
hemp nettle	11.3n	172.0defg	53.3mn	261.3a
pineappleweed	118.6jkl	60.0mn	120.6jkl	54.6mn
yarrow	81.3klmn	41.3n	136.0hi	76.6lmn
mean	116.9	144.7	138.2	197.2

(DMRT comparisons within the body of the table, only. The four surface means are significantly different from one another).

As shown in table 3, the mean number of stomata per mm.^{2} was much greater on the lower surface than on the upper at both harvest dates. Taking the average of both dates, the mean densities were 171.8/mm.^{2} on the lower and 127.2/mm.^{2} on the upper surface. This difference was significant at the 0.5% level. In the following text, only those differences which were significant at the 5% level or more highly significant are mentioned. Of the 8 species, only pineappleweed and yarrow had a greater density of stomata on the upper than on the lower surface. Taking the mean of both surfaces, the density of 130.8/mm.^{2} at the June sampling date was considerably less than the July mean of 169.5/mm.^{2} Not all species reacted in the same way but the interaction was much less than that with surface. There was significant interaction between leaf surface and sampling date. At the first harvest, the upper surface mean was 116.9/mm.^{2} and that of the lower was 144.7/mm.^{2} At the second harvest there was a greater spread, from 138.2/mm.^{2}

on the upper to 197.0/mm.^{2} on the lower surface. There was no significant species x surface x date interaction, indicating that all species had a similar surface x date interaction.

2. Stomatal Sizes

The lengths of stomata varied considerably between species and sampling dates but very little between surfaces (appendix table 2). The mean lengths of stomata from both leaf surfaces and sampling dates were given previously (table 2).

Table 4. Length of stomata in microns, on upper and lower surface at two sampling dates. (in order of decreasing grand mean).

	June		July	
Species	Upper	Lower	Upper	Lower
yarrow	39.7	45.3	31.0cd	35.0a
pineappleweed	32.4b	35.1a	34.1a	36.5
spotted knapweed	27.9ef	27.0fh	31.6bc	30.2d
hemp nettle	30.3d	30.4d	26.1hijkl	23.6m
dandelion	26.6hi	28.3e	26.0hijkl	25.5ijklm
b.l. plantain	26.8fh	25.2klm	25.3ijklm	25.3ijklm
wild radish	24.9lmn	24.4lmn	26.1hijkl	26.5hij
stinkweed	26.3hijk	26.4hij	25.2jklm	23.8m
grand mean	29.4	30.3	28.2a*	28.3a

*DMRT comparisons between grand means.

At the June harvest, the mean length of stomata on the lower surface was greater at 30.3μ than on the upper at 29.4μ (table 4). Examination of species x surface interaction shows, however, that much of this difference is due to a single species, namely yarrow. Other species tend to have slightly longer stomata on one surface or the other but these differences, although statistically significant, are so slight as to be of no practical consequence.

Mean length at first harvest was 29.8μ compared to 28.2μ

at the second harvest. Examination of species by date interaction shows that the differences between dates within individual species were quite large. Of the 8 species, 4 had longer stomata in June (mean of both surfaces); 3 had longer stomata in July; and 1 showed no difference.

3. Relationship of Number and Size of Stomata

Except for 2 species, there was no close relationship between stomatal number and size. As shown in table 2, yarrow and pineappleweed had by far the fewest and the largest stomata. The other species had a wide range of stomatal densities but very similar sizes.

II. Stomatal Number and Size of Twenty-five Species Grown under Sunny and Shady Conditions.

1. Stomatal Numbers

In this group, the greatest variation in stomatal density was found between surfaces. There were also significant differences between species, dates, and light conditions (appendix table 3). Taking the mean of both leaf surfaces, sampling dates, and light conditions, the various species exhibited a wide range of stomatal densities. Approximately the same number of species had fewer than 75, as had 75-150, or more than 150 stomata per mm.^{2} of leaf surface (table 5).

Table 5. Number of stomata per sq.mm. of leaf surface, mean of all factors.

Very Dense	
shepherd's purse	268.7
hedge mustard	241.0
Dense	
n.l. plantain	198.5
cat's ear	183.0a
leafy spurge	173.6a
lady's thumb	171.1ab
diffuse knapweed	161.2b
blue bur	156.8b
redroot pigweed	139.5
Medium	
prostrate knotweed	114.9c
barnyard grass	104.2cd
white cockle	98.8de
toadflax	96.7def
couchgrass	90.1ef
lambsquarters	87.2ef
Canada thistle	84.6f
Sparse	
broad leaf dock	75.6fg
groundsel	67.9gh
creeping buttercup	67.2ghi
wild buckwheat	64.3ghi
chickweed	58.6hij
mouse-ear chickweed	57.6ij
sheep sorrel	45.6jk
wild oats	44.7k
Very Sparse	
montia	24.9

Table 6. Numbers of stomata per sq.mm. of leaf surface (in order of decreasing density).

Species	Surface (mean of both harvests and light conditions) Upper	Lower	Harvest (mean of both surfaces and light conditions) June	July	Light (mean of both surfaces and harvests) Shady	Sunny
shpd's purse	242.6b*	294.9a	281.5	256.0	272.5	265.0
h. mustard	185.7d	296.4a	218.6	263.5	242.4	239.7
n.l. plantain	189.5cd	207.5c	190.3	206.6	208.0	189.0
cat's ear	197.7cd	168.4e	163.3	202.8	110.1	123.0
leafy spurge	158.3ef	188.9d	177.0	170.1	171.3	175.9
lady's thumb	101.6ijk	240.6b	138.0	204.2	171.2	170.9
dif. knapweed	155.8ef	166.7e	156.9	165.6	155.3	167.2
blue bur	147.6fg	165.9ef	122.5	191.0	167.9	145.6
pigweed	113.7hi	165.2ef	136.0	143.0	152.8	126.2
knotweed	98.9ijk	130.8gh	100.2	129.5	125.6	104.7
bynd. grass	105.4ij	103.1ijk	91.3	117.2	111.2	97.2
wh. cockle	66.1mno	131.5gh	96.9	100.7	100.2	97.4
toadflax	2.3s	191.0cd	96.1	97.2	95.1	98.2
couch grass	84.4klm	95.8ijk	65.5	114.7	72.6	107.6
lambsquarters	68.5lmn	105.9ij	86.1	88.3	91.8	82.6
Can. thistle	43.9pqr	125.4h	102.2	67.0	84.5	84.8
b.l. dock	25.6r	125.7h	75.5	75.8	76.3	75.0
groundsel	43.6pqr	92.3ijk	82.3	53.5	65.2	70.6
cr. buttercup	49.2op	85.3jkl	61.5	73.0	72.0	62.5
w. buckwheat	27.4qr	101.2ijk	64.0	64.7	81.6	47.0
chickweed	50.0nop	67.2lmno	32.3	84.9	63.5	53.7
mouse-ear	44.6pqr	70.6lm	53.5	61.7	55.4	59.8
sheep sorrel	40.6pqr	50.7nop	41.4	49.9	48.0	43.2
wild oats	44.7pqr	44.7pqr	35.4	54.0	47.9	41.6
montia	4.1s	45.7pq	27.9	21.9	19.8	30.1
average	91.7	138.5	107.9	122.3	119.8	110.3

* Duncan's multiple range test carried out for surface, only.

The mean density was greatest on the lower surface, at the second sampling date, and under shady conditions (table 6). There were significant species x date; species x surface; and species x sunlight interactions (appendix table 3). Altogether, 22 of the 25 species had more stomata per sq.mm. on the lower than on the upper surface (figure 4); 18 had more under shady than under sunny conditions; and 20 had a greater density at

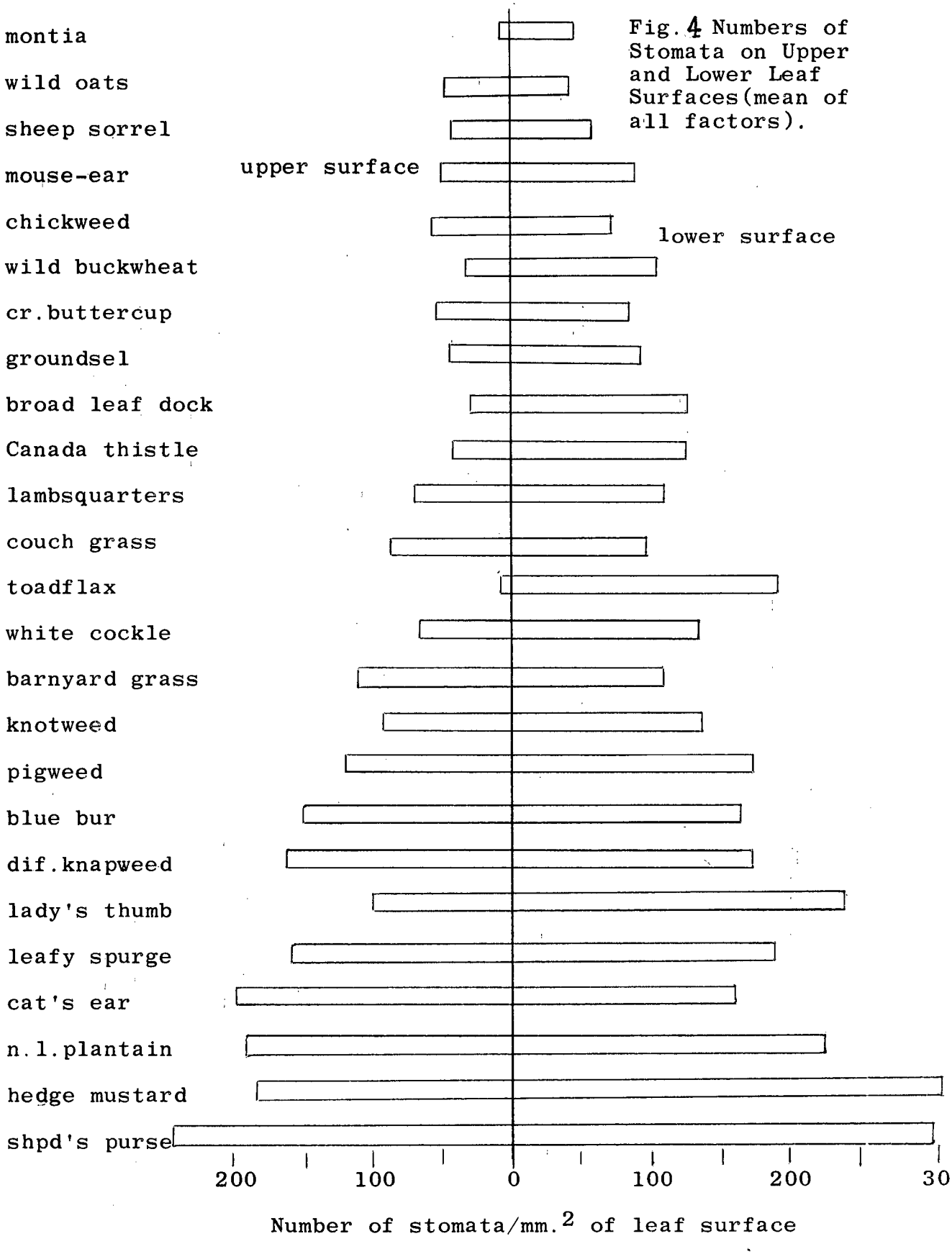

Fig. 4 Numbers of Stomata on Upper and Lower Leaf Surfaces (mean of all factors).

the second than at the first harvest. Other significant interactions showed that not all species had the same relative stomatal density on upper and lower surface at both dates or under both sun or shade; and all species did not react to light conditions in the same way at both dates, i.e. 14 of the 25 species reacted in the opposite way to shade in July as in June.

2. Stomatal Sizes.

Table 7. Length of Stomata in microns, mean of all factors.

Very Short	
Shepherd's purse	22.0
hedge mustard	23.4
Short	
lady's thumb	25.2g
diffuse knapweed	25.5fg
leafy spurge	25.7f
pigweed	25.9f
blue bur	27.6e
cat's ear	27.7de
toadflax	28.1d
n.l. plantain	28.6
Medium	
prostrate knotweed	31.0
barnyard grass	32.1
Canada thistle	33.0c
b.l. dock	33.3c
white cockle	33.8
lambsquarters	34.8
Long	
wild buckwheat	39.3
mouse-ear chickweed	42.5b
groundsel	42.5b
couch grass	43.4a
sheep sorrel	44.3a
creeping buttercup	44.8a
chickweed	45.1
Montia	48.9
Very Long	
wild oats	59.0

The lengths of stomata varied mainly between species and between light conditions (appendix table 4). As shown in table 7, the mean lengths of stomata for all factors ranged from 22.0μ in shepherd's purse to 59.0μ in wild oats. The

species are grouped into general categories from very short to very long. In table 8, the species means are broken down to show how they were influenced by surface, date, and light conditions.

Table 8. Average length of stomata in microns (listed in order of increasing mean size).

	Surface (mean of both harvests and light conditions)		Harvest (mean of both surfaces and light conditions)		Light (mean of both surfaces and harvests)	
Species	Upper	Lower	June	July	Sunny	Shady
shpd's purse	21.6	22.4	21.9	22.1	22.1	22.0
h. mustard	23.3y	23.5y	23.9	23.0	23.7	23.1
lady's thumb	25.7uv	24.7	26.0	24.4	24.8	25.6
dif. knapweed	25.3v	25.7uv	23.9	27.1	28.3	22.8
leafy spurge	25.6uv	25.9uv	26.5	25.0	26.3	25.2
pigweed	25.5v	26.2u	26.5	25.2	25.9	25.8
blue bur	27.8rs	27.3st	28.8	26.3	27.2	27.9
cat's ear	26.9t	28.6pq	26.8	28.6	25.7	29.7
toadflax	28.6pq	27.5st	28.2	28.0	28.5	27.6
n.l. plantain	28.9p	28.2qr	28.3	28.8	28.0	29.1
knotweed	31.4mn	30.5o	32.2	29.7	33.5	28.4
bynd. grass	31.7m	32.5l	32.9	31.2	31.8	32.3
Can. thistle	35.1i	30.9no	29.0	37.0	33.0	33.0
b.l. dock	34.3k	32.3l	32.2	34.4	34.4	32.1
white cockle	33.2	34.5jk	32.4	35.2	35.9	31.7
lambsquarters	34.9ij	34.6ijk	34.7	34.8	36.0	33.5
w. buckwheat	39.8h	38.9	37.7	40.9	37.9	40.8
mouse-ear	41.6	43.3fg	41.5	43.4	43.1	41.9
groundsel	45.5bc	39.5h	37.6	47.4	44.4	40.6
couch grass	43.9de	42.8g	44.5	42.2	44.5	42.3
sheep sorrel	45.0c	43.7def	46.2	42.5	43.7	45.0
cr. buttercup	43.5efg	46.0b	45.4	44.2	42.7	46.8
chickweed	44.2d	46.0b	49.8	40.3	44.4	45.8
Montia	45.9b	51.9	46.8	51.0	51.1	46.6
wild oats	50.9a	59.0a	64.1	54.0	59.9	58.2
average	34.7	34.7	34.7	34.7	35.1	34.3

* Duncan's multiple range test carried out for surface only.

There was no mean difference in length between upper and lower surface; however, there were differences within individual species (table 8). Similarly, sampling date means were not

significantly different but there was a significant species by date interaction (appendix table 4). The mean lengths for each surface and sampling date were the same at 34.7μ. The mean length of stomata under full sun was 35.1μ which was significantly greater than 34.3μ, the mean for shade. Again, not all species reacted in the same way to sunlight.

There was an interaction of light conditions with sampling date, the differences between sun and shade being greater in June than in July. There was also a surface x light interaction. Under full sunlight, the upper surface had slightly larger stomata than the lower, while under shade, the reverse was true. All of the three factor interactions and the four factor date x species x light x surface interactions were significant. The small magnitude of the differences involved, however, does not warrant emphasis of these interactions.

1. Relationship of Number and Size of Stomata.

By comparing tables 5 and 7, it can be seen that there was an apparent inverse relationship between stomatal number and length. With minor deviations, the more stomata present per sq.mm., the smaller their size and vice-versa. In order to obtain a valid statement regarding the apparent relationship between number (x) and size (y), the 200 pairs of date x surface x light x species interaction means were used to calculate a correlation coefficient. A highly significant negative value of r=-.71 was obtained. This indicates strong negative correlation of stomate number and length, taking all 25 species together. The coefficient of determination or r^2

suggests that 50.3% of the variation in length of stomata can be attributed to the linear relationship between length and number.

By multiplying the mean stomatal length for each species (table 7) with the corresponding density (table 5), a figure is obtained which is closely related to the total area of the epidermis which is composed of guard cells. While it is not a measure of area, the fact that stomatal width varies much less than length makes it valid for purposes of comparison. Calculation of this figure showed a range from 7220 (total length of guard cells /mm.^{2}) in shepherd's purse to 1217 in montia. In general, those species with the greatest density of stomata had the largest value for this area index and those with the lowest density had the smallest.

4. Relationship of Stomatal Characters with Taxonomic groups.

Examination of the data for all 33 species (sun only and sun plus shade), shows some possible trends in regard to plant families. As the number of species examined in each family was relatively small, however, the differences cannot be considered significant. In this regard, no statistical analysis was carried out.

Of the 5 families represented by 3 or more species, the Cruciferae was characterized by consistently large numbers of small stomata. Members of the Caryophyllaceae and Gramineae had uniformly small numbers of large stomata. Representatives of the Compositae and Polygonaceae had a wide range of densities and corresponding sizes. The ratio of stomatal numbers on upper

Table 9. Relationship of stomatal density and size with plant families (densities and lengths are means of two sampling dates under sun only).

Family	Species	Number/mm.^{2} Upper	Number/mm.^{2} Lower	Length (mean of both surfaces,)
Caryophyllaceae				
	white cockle	65.5	135.0	35.9
	mouse-ear	45.6	65.2	43.1
	chickweed	51.6	75.4	44.4
	mean	54.2	91.9	41.4
Compositae				
	yarrow	108.6	59.0	37.7
	diffuse knapweed	143.3	167.2	28.3
	spotted knapweed	143.3	140.3	29.2
	Canada thistle	45.9	123.0	33.0
	cat's ear	135.7	132.5	25.7
	pineappleweed	119.6	57.3	34.5
	groundsel	40.9	89.4	44.4
	dandelion	148.3	284.9	26.6
	mean	107.0	110.2	32.4
Cruciferae				
	shepherd's purse	229.8	315.2	22.1
	hedge mustard	176.6	308.3	23.7
	stinkweed	111.0	178.0	25.4
	mean	172.5	267.2	23.7
Gramineae				
	couch grass	71.8	74.5	44.5
	wild oats	46.6	49.2	59.9
	barnyard grass	110.7	111.7	31.8
	mean	76.4	78.5	45.4
Polygonaceae				
	wild buckwheat	41.9	121.4	37.9
	lady's thumb	110.7	231.7	24.8
	sheep sorrel	39.6	56.5	43.7
	knotweed	107.4	142.6	33.5
	broad leaf dock	29.6	123.0	34.4
	mean	65.8	135.0	30.9

and lower surface was highly variable in Compositae. All members of Polygonaceae and Cruciferae had by far the most stomata on the lower surface. The other three families, especially Gramineae, showed smaller differences between surfaces.

III Illustrations of Weed Leaf Anatomy.

The following pages contain plates I-IX. Plates I-VI contain drawings showing the upper (left) and lower (right) leaf surfaces of the various weed species, all approximately x 115. The fields were chosen from intercostal areas and do not necessarily show the average number and size of stomata for each species. Plates VII - IX consist of photomicrographs illustrating both surface and internal features of leaf anatomy.

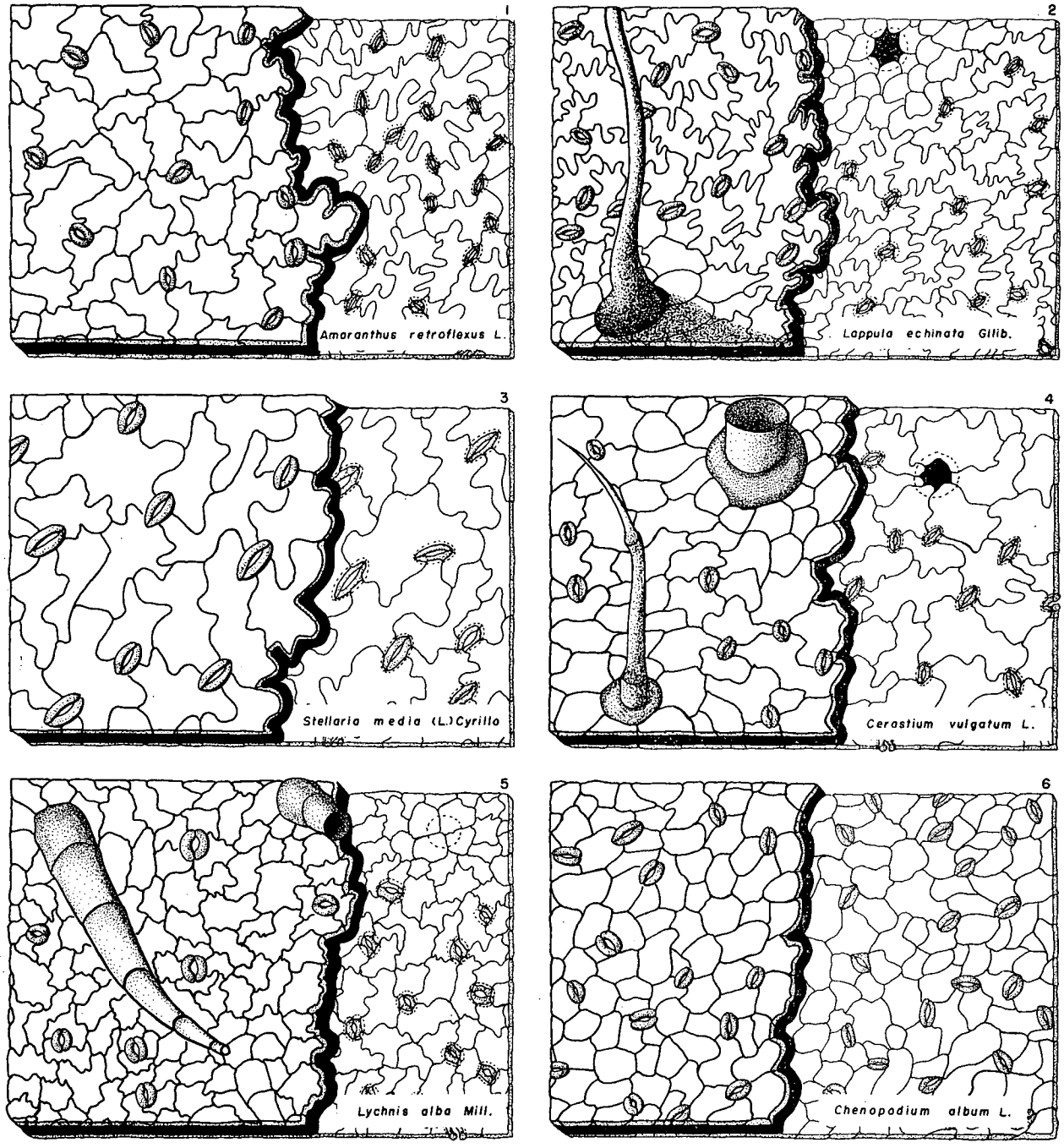

PLATE I. Epidermal Features of Amaranthaceae (1), Boraginaceae (2), Caryophyllaceae(3-5), and Chenopodiaceae(6): Note wavy anticlinal walls (1 and 2) compared to fairly regular epidermal cells (6). Slender, unicellular (2); and multicellular trichomes (4 and 5), are present on both surfaces. Black areas of lower surface (2 and 4) show trichomes embedded in the epidermis and dotted circles represent hairs raised above special cells (5). The guard cells are slightly raised in all species except 6, in which they are flush. The large structure on the upper surface of Cerastium(4) is the base of a trichome broken off during slide preparation. In Chenopodium(6), the surface feature of major importance to spray retention and penetration is probably the thick coating of surface wax or bloom (not shown).

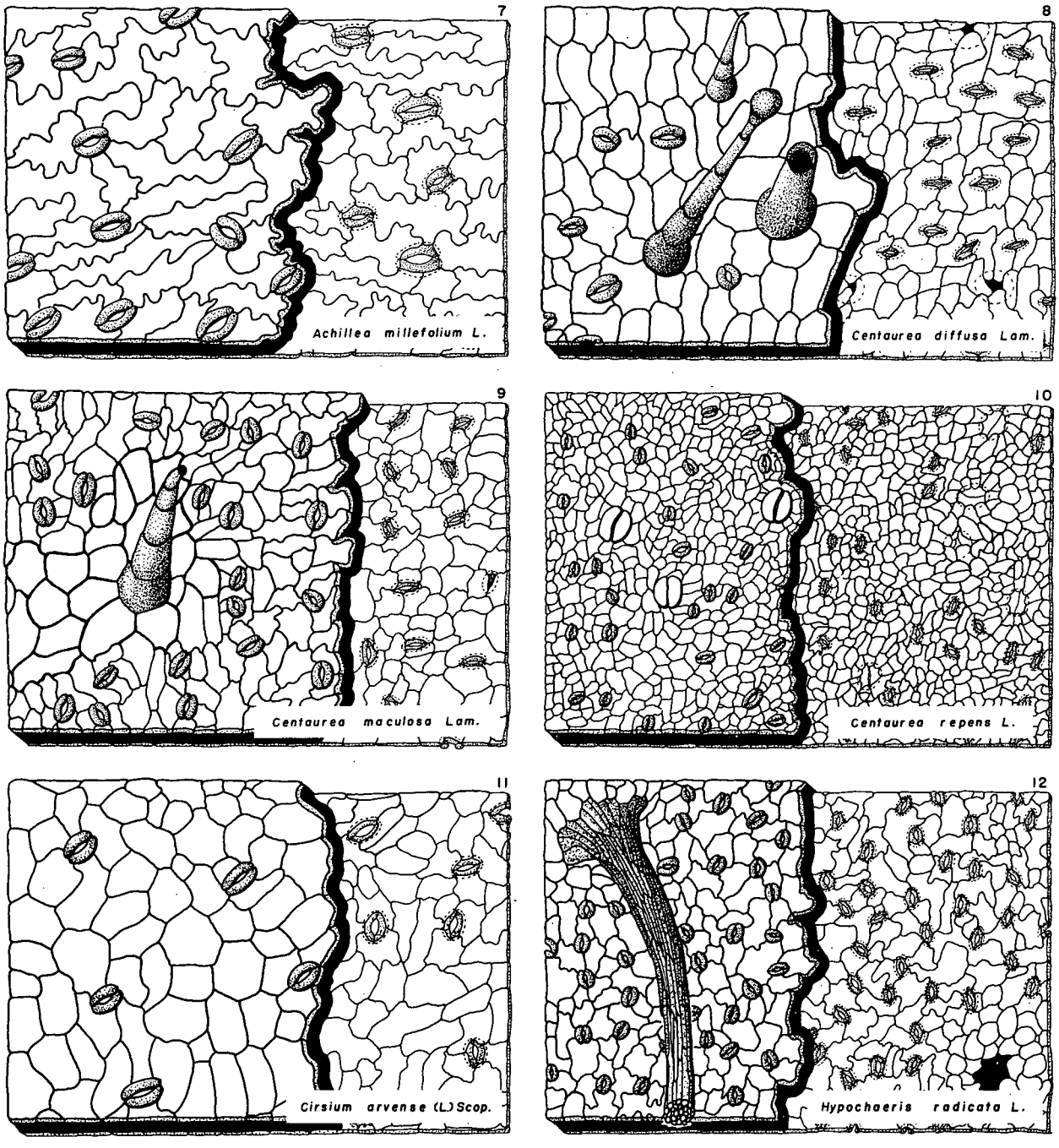

PLATE II. Epidermal Features of Compositae : Note the great diversity within the family and even within the genus Centaurea. Compare pointed, gland-tipped, and broken trichomes(8) with massive multicellular hair(12). Epidermal cells near base of trichome with thick walls(9) and stomata-like glands(10), contrast with extreme simplicity(11). The orientation of guard cells and other epidermal cells in Achillea(7) tends to be parallel with the long axis of the leaflet.

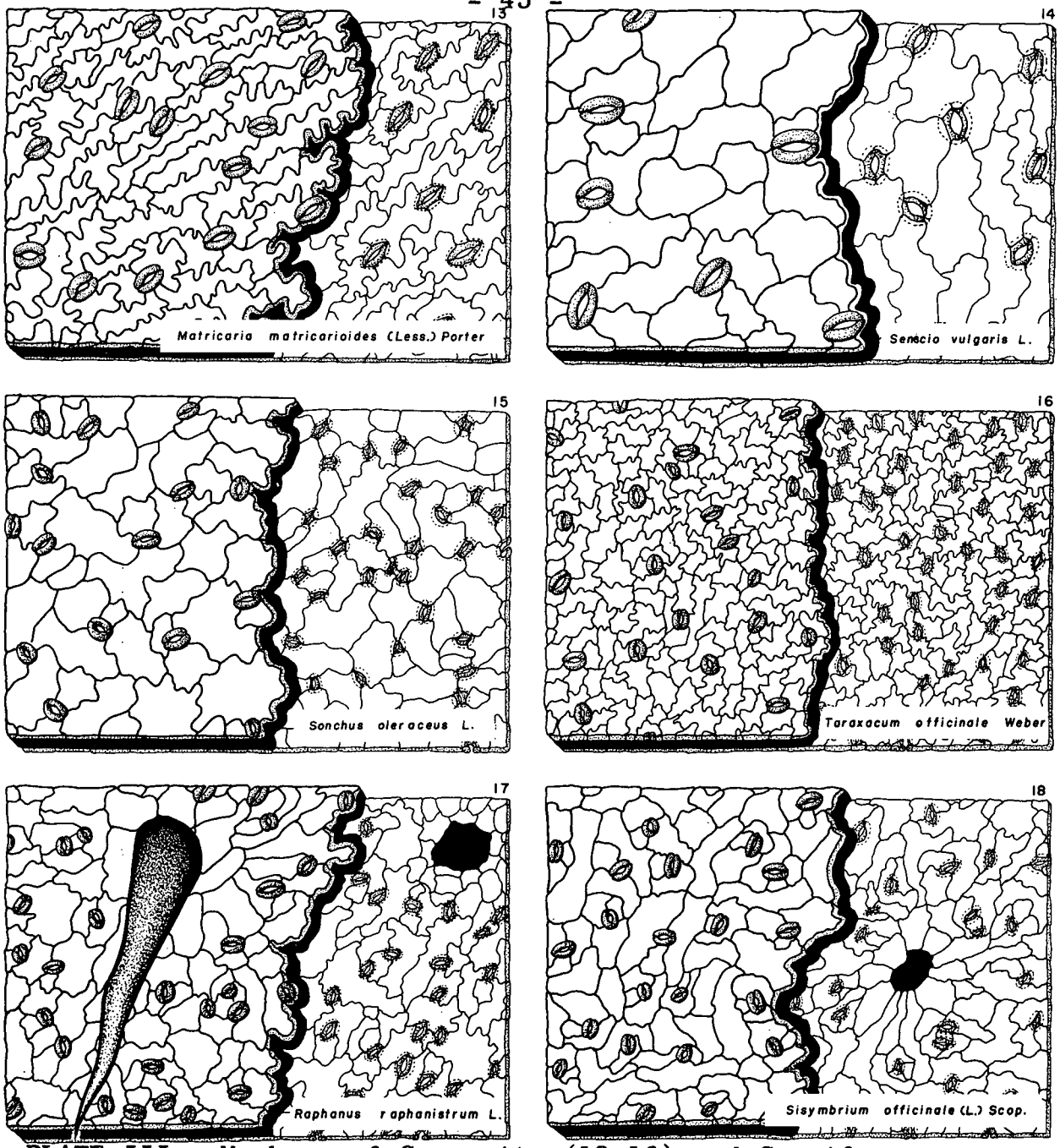

PLATE III. Members of Compositae(13-16) and Cruciferae (17 and 18): Guard cells arranged parallel to long axis of leaves(13). Contrast in cell size (14and 16), is a notable feature of Compositae. The Crucifers tend to have anisocytic arrangement in which three subsidiary cells, one much smaller than the other two, surround the stomata. The large trichomes in Raphanus(17) have greatly thickened walls and sharp-pointed tips.

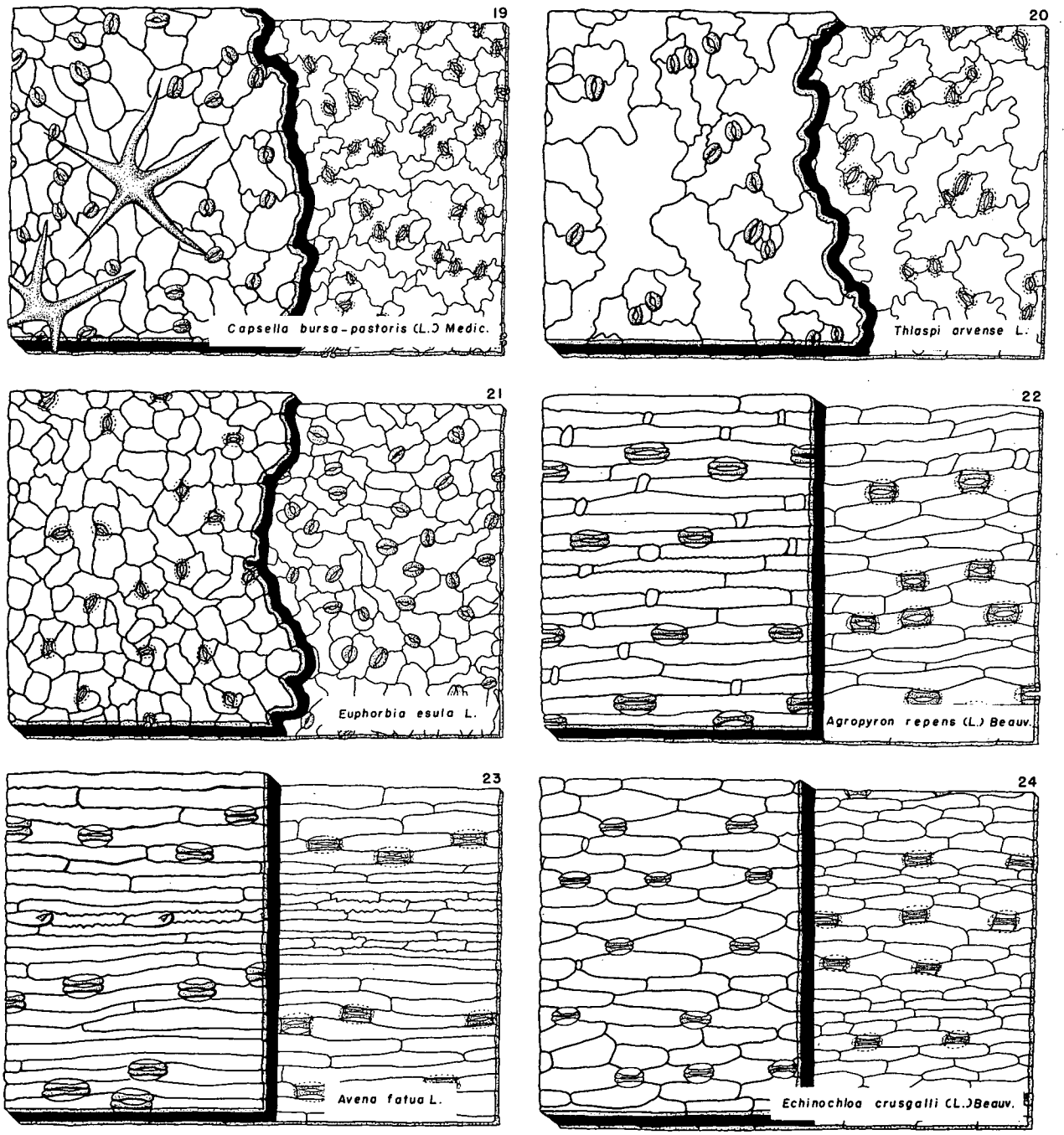

PLATE IV. Epidermal Features of Cruciferae(19-20), Euphorbiaceae (21), and Gramineae(22-24) : Stellate tricomes(19) and anisocytic arrangement of subsidiary cells(19 and 20) found in Cruciferae. Note sunken stomata in Euphorbia(21). The three grasses show typical monocot dumb-bell shaped guard cells with adjacent subsidiary cells. Short cells(22) and bristles over veins(23) are common in grasses. In each of these grasses, the stomata occur in rows in depressions between veins. A spray droplet landing on such a leaf would tend to be suspended on the epidermis over the veins and would be unable to contact the stomata. The pairing of stomata in Thlaspi(20) is characteristic of immature leaves in which cell division is not complete.

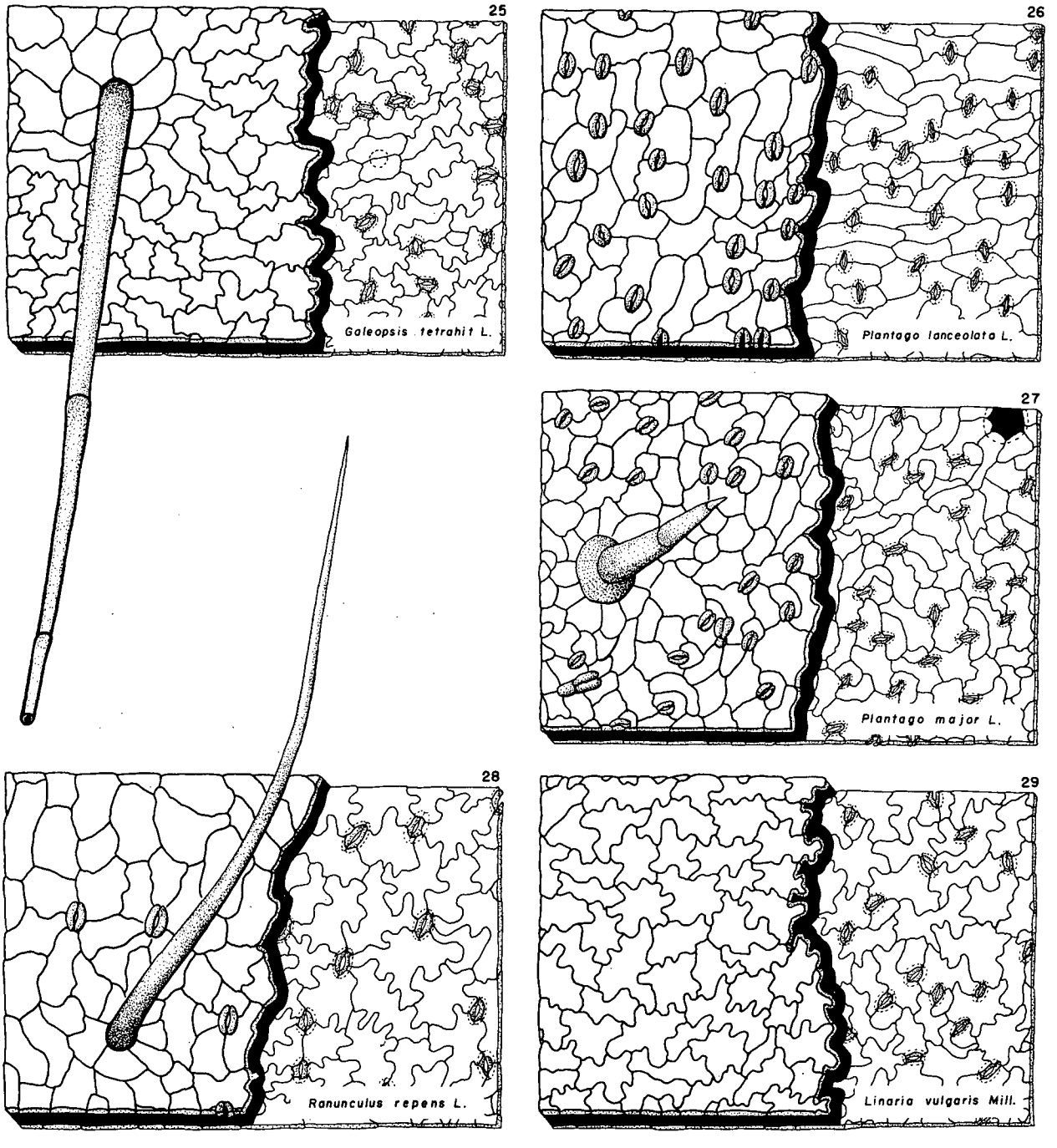

PLATE V. Epidermal Features of Labiatae(25), Plantaginaceae (26-27), Ranunculaceae(28), and Scrophulariaceae(29) : Upper surfaces apparently free of stomata in 25 and 29 but examination of several fields reveals their presence. Within the genus Plantago, P.lanceolata(26) tends to have diacytic and P.major(27), anisocytic arrangement of subsidiary cells. Note the small stalked glands on upper surface of P.major. In P.lanceolata, the stomata are oriented parallel with the long axis of the leaf.

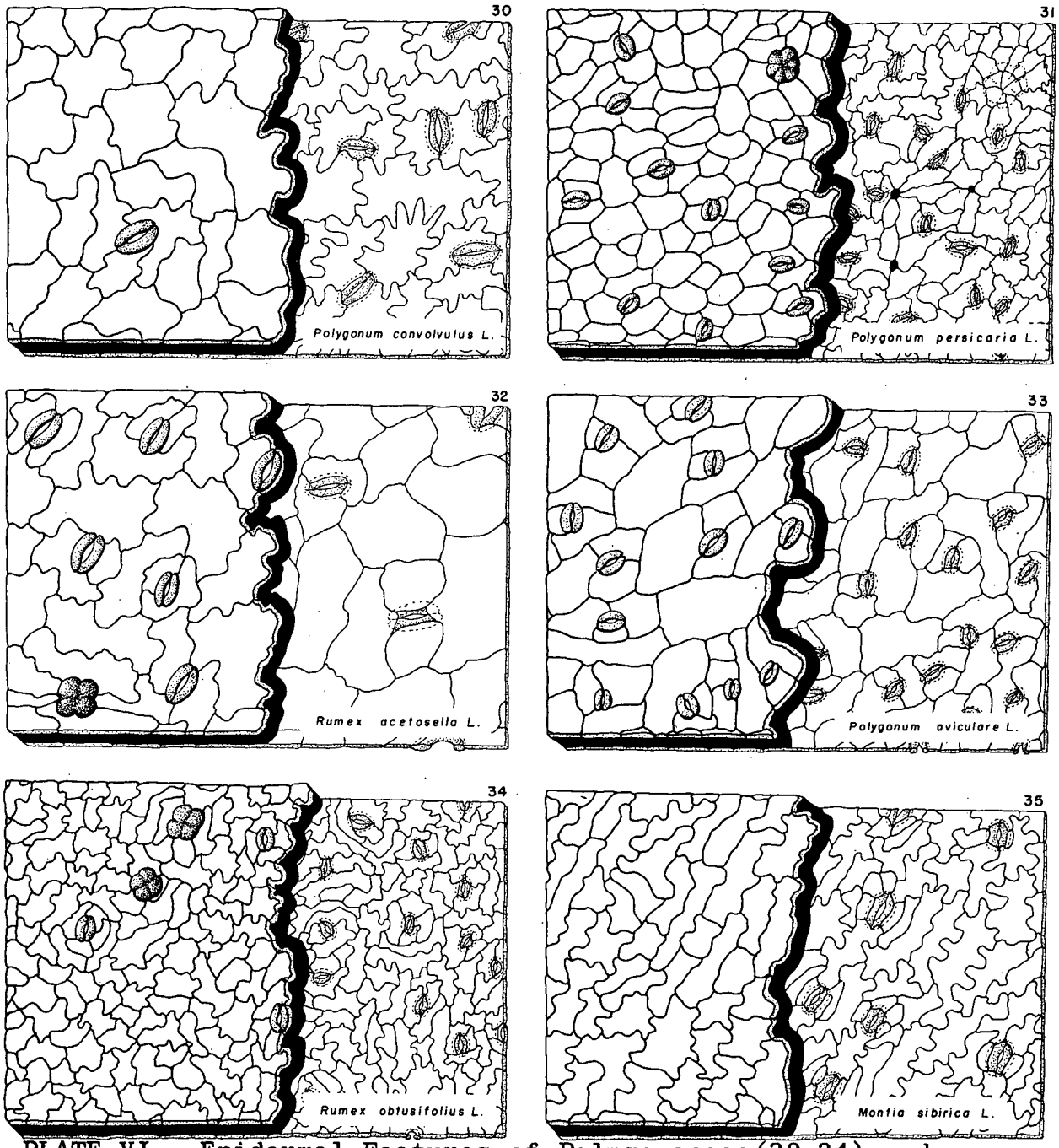

PLATE VI. Epidermal Features of Polygonaceae(30-34) and Portulacaceae(35): Leaves of Polygonaceae tend to be glabrous but bases of small hairs can be seen (lower surface of 31). Glands are common in this family(31,32,34), as are crystals(plateIX). Montia(35) has wavy, elongated epidermal cells with very few stomata on the upper surface. The arrangement of subsidiary cells in Montia is a variation of paracytic.

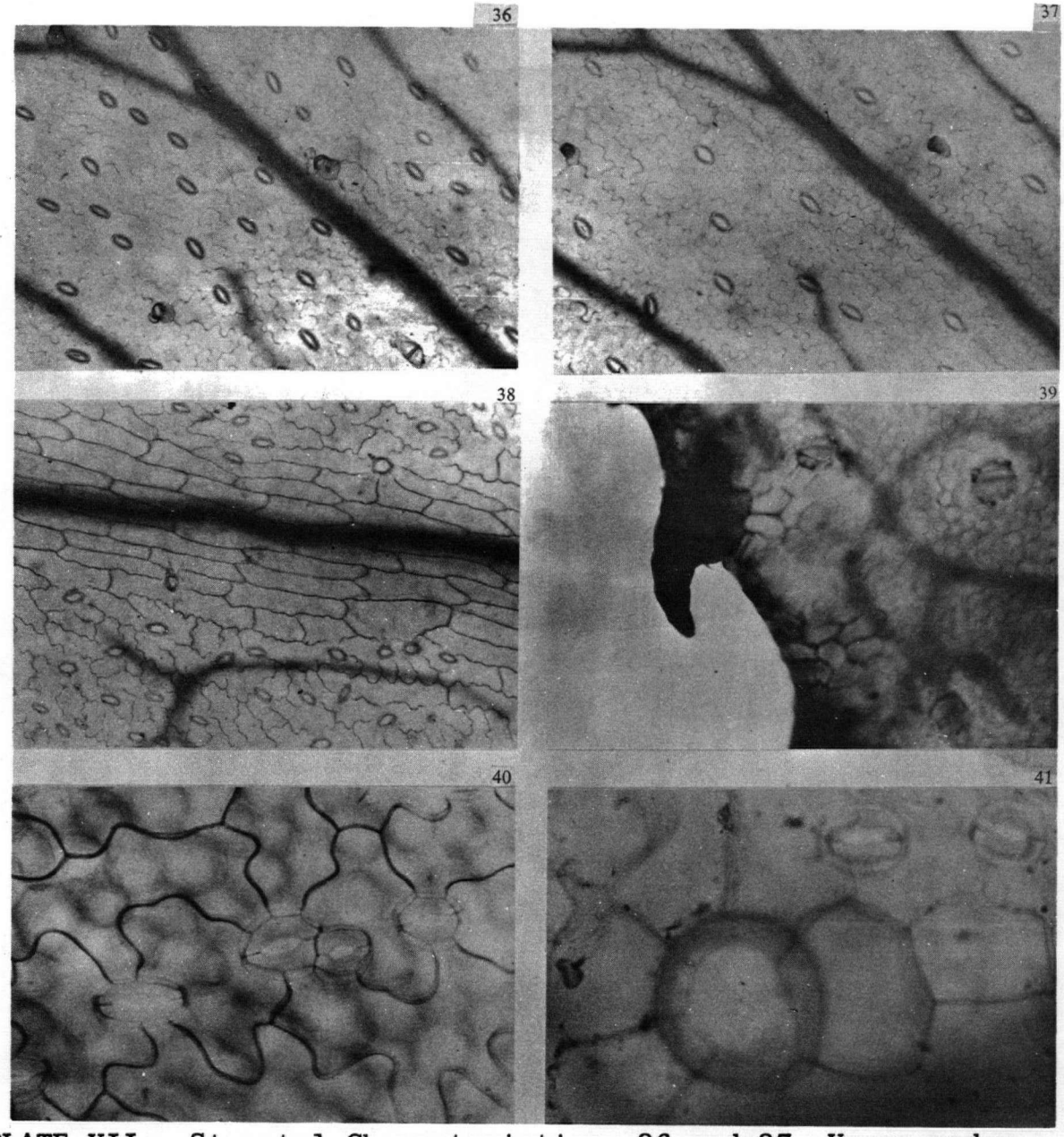

PLATE VII. Stomatal Characteristics: 36 and 37. Upper and lower surfaces, respectively, of yarrow (x56), demonstrating the direct comparison of surfaces made possible by using cleared and stained leaves. 38. Lower epidermis of groundsel (x56), showing absence of stomata over main vein. 39. Leaf margin of spotted knapweed (x150) showing stubby, multicellular hair and three glands. 40. Lower epidermis of chickweed with abnormal, adhering stomata (x220). 41. Comparison of size of stomatal pores with cavity left by a trichome in white cockle leaf (x360).

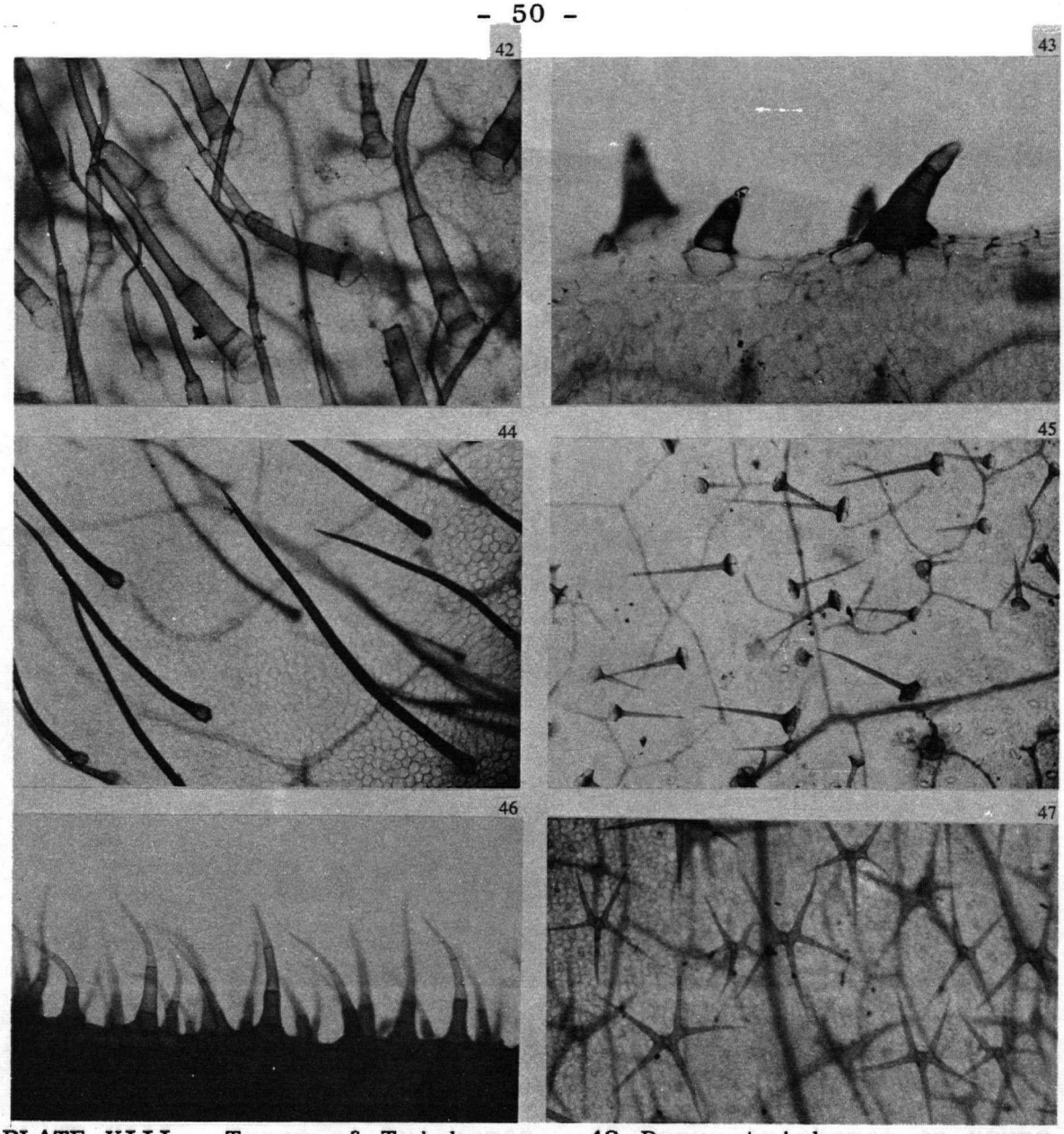

PLATE VIII. Types of Trichomes: 42.Dense trichomes on young leaf of mouse-ear chickweed (x56). 43.Leaf margin of diffuse knapweed showing stubby, multicellular trichomes (x150). 44.Slender, unicellular hairs of buttercup(x56). 45.Leaf hairs of hemp nettle (x56). 46.Margin of white cockle leaf (x56). 47.Stellate pubescence of shepherd's purse (x56). The margin of shepherd's purse leaves have predominantly simple, unicellular trichomes with a few stellate trichomes intermixed. In most other species the marginal hairs differ from those of the leaf surface only in being closer together.

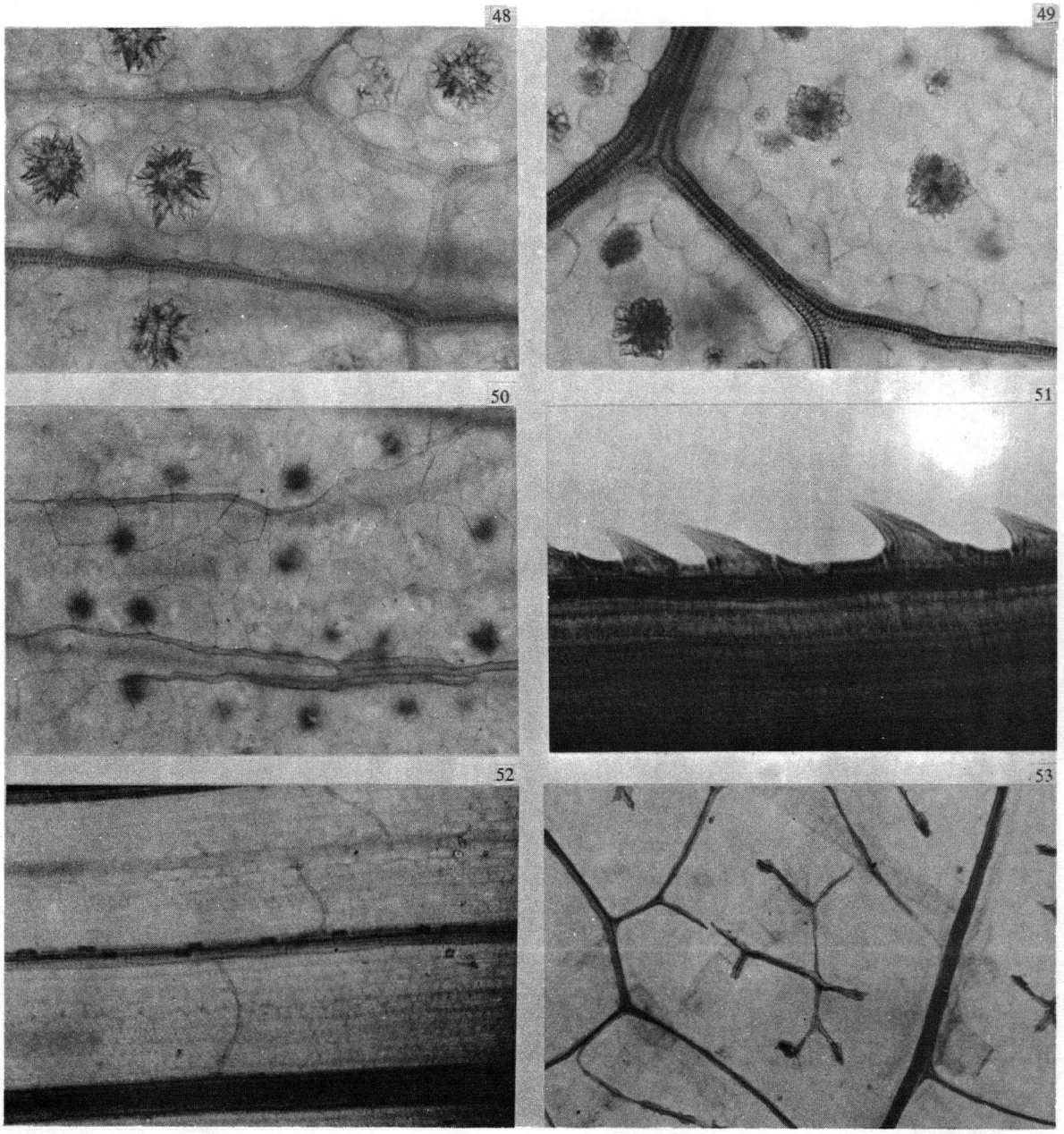

PLATE IX. Miscellaneous Anatomical Feature: 48.Crystals in leaf of prostrate knotweed (x150), typical of Polygonaceae. 49.Crystals and bundle sheath parenchyma in redroot pigweed (x150). 50.Laticifers in leaf of knotweed with crystals in background (x70). 51.Scabrous margin of wild oat leaf (x150). 52.Venation in couch grass without blind vein endings, common to all monocots (x56). 53.Blind vein endings common to all dicots in white cockle (x56). In the living condition, the secondary walls of the free veins in the center would soon join up with the main vascular system.

IV Herbicide Susceptibility Experiment.

In this experiment, it was expected that the reduction in weight of the hemp nettle, lambsquarters, and white cockle would give an indication of the relative damage caused by the picloram treatments. Both fresh and dry weights were taken.

Table 10. Number and size of stomata of greenhouse grown plants (mean of 10 leaves).

Species	Number/mm^2		Size (μ)	
	Upper	Lower	Upper	Lower
hemp nettle	79.8	266.0	27.5	25.0
lambsquarters	93.1	159.6	35.0	31.9
white cockle	66.5	159.2	37.5	34.0

Table 10 shows the stomatal densities and lengths of the 3 species just prior to spraying. Measurements were made by direct microscopic observation of fresh leaves or epidermal strips from 10 plants of each species. The figures agree with those obtained from the field except that the greenhouse grown plants had slightly more stomata/mm.^{2}

The results of the analyses of variance of fresh weight data (appendix table 5) and dry weight data (appendix table 6) were slightly different. In both cases, the largest differences in weights were due to the inherent differences between species.

1. Fresh Weights

The fresh weight analysis failed to show differences in weight due to relative stomatal opening or to surface of application. There were differences due to treatments (rates of picloram). The fresh weights in grams, mean of all species, stomatal conditions, and surfaces of application were:

0	1	10	100	1000 ppm. picloram
87.0	94.7a	93.6a	83.1	73.2

All species did not show the same response to the different levels of picloram.

Table 11. Fresh weights of 3 species two weeks after application of picloram sprays (in grams).

rate of picloram	0	1	10	100	1000ppm.
hemp nettle	72.9ab	87.2de	75.4abc	73.0ab	64.5a
lambsquarters	81.9bcd	89.0de	94.5e	85.5cde	80.7bcd
white cockle	106.3f	107.8f	110.9f	90.8de	74.4abc

Hemp nettle and lambsquarters showed significant increases of weight above the control but white cockle did not (table 11). The weights were highest at the 1ppm. treatment in hemp nettle and at 10ppm. in lambsquarters. With white cockle, weights were reduced below the control at both 100 and 1000 ppm. but in the other species there were no significant reductions in weight below the control regardless of treatment.

Table 12. Effect of relative degree of stomatal opening at time of picloram applications on fresh weight of weeds (mean of all species and surfaces, in grams).

rate of picloram	0	1	10	100	1000 ppm.
open	90.0bcd	94.8d	96.0d	79.4a	65.8
closed	84.0abc	94.5d	91.2cd	86.8abcd	80.7an

There was significant interaction between treatment and relative stomatal opening. At the highest rate of picloram, fresh weights were reduced more in plants with relatively open stomata than in those with their stomata relatively closed (table 12). Examination of species x stomata x treatment interaction showed that hemp nettle and lambsquarters agreed with the average effect but white cockle showed just as much weight reduction following application to closed stomata.

2. Dry Weights

There were differences in dry weights depending upon the surface of application. The mean weights for all species and rates of picloram were 1241 mg. for upper surface and 1149 mg. for lower surface application. There was no significant species x surface interaction. No differences due to relative stomatal opening were detected. Differences due to rate of picloram were smaller than those found in the fresh weight data. They were as follows :

0	1	10	100	1000 ppm.
1217a	1305a	1263a	1163ab	1026b

As with fresh weights, all species did not show the same response to the different levels of picloram but in this instance, the interaction was not significant.

Table 13. Dry weights of 3 species two weeks after application of picloram sprays (in milligrams).

rate of picloram	0	1	10	100	1000 ppm.
hemp nettle	794a	961ab	770a	761a	675a
lambsquarters	1131cd	1358cde	1461de	1359cde	1276c
white cockle	1546de	1596e	1559de	1371cde	1128bc

The dry weight means for the various treatments (table 13) agree closely with the fresh weight means (table 11). The % dry weights obtained from the species means of all treatments were :

hemp nettle	- 10.6%
lambsquarters	- 15.7%
white cockle	- 14.7%

There were no consistent differences in % dry wt. within species due to rates of picloram. There were no significant two factor interactions associated with dry weights. There were significant interactions of species x stomatal opening x treatment and stomatal opening x surface x treatment.

DISCUSSION

Thirty-three weed species grown in full sunlight were examined and it was found that they varied widely in density (number/mm.^{2}), length, and distribution of stomata. This is in agreement with previous surveys (16,46,54,65). Variation between leaves within a plant and between plants within a species was kept low by sampling similar sized leaves and making counts from corresponding areas of the lamina. The close negative correlation of stomatal number and size which was observed was not a peculiarity of stomata but applies to all epidermal cells as shown in the drawings.

Of the 25 species which were grown under both full sun and 50% shade, some varied greatly in the density of stomata under the different environments. This, plus the variation between sampling dates within species, indicates that data on stomatal densities obtained under one set of conditions can be applied in only a very general way to other situations.

The larger number and smaller size of stomata developed under partial shade was opposite to the expected effect. It has generally been accepted that growing plants in shade results in larger than normal leaf areas and epidermal cells (28). This is due to reduction in the inhibition of cell enlargement caused by excess light intensities. It is also known, however, that cell division may be inhibited by bright sunlight and that this inhibition may occur at lower solar radiation than that which results in the inhibition of cell enlargement (46). Thus the shade effect of increasing stomatal numbers can be explained

on the basis of a reduction in the inhibition of cell division caused by sunlight. It should be stressed, however, that due to the nursery plot design (figure 1), it is not certain that the differences between sun and shade grown plants were due to differences in sunlight. Because of the lack of randomization of the sun and shade plots we cannot entirely rule out the possibility that some other environmental factor, such as soil differences, caused the variation in stomatal density.

The average increase in stomatal density from June to July further illustrates the different results which may be obtained from sampling under slightly different conditions. The differences cannot be explained on the basis of weather alone, because the two months were very similar in that regard (table 14).

Table 14. Weather data for June and July, 1965 (U.B.C.)

	June	July
mean temp. (°F)	57.8	63.3
sunshine (hours)	315.5	296.9
precipitation (inches)	0.67	0.28
irrigation (inches)	ca 1.5	ca 1.5

Both months were unusually dry and sunny. The densities of stomata were greatest in July when the total hours of sunshine were lowest. This was the same response to reduced sunlight as was shown by the shade grown plants. Because of the small differences in hours of sunshine, however, it would be presumptuous to attribute the differences in stomatal density to this factor. It is more likely that the mean temperature difference of 5.5°F had an effect. It should also be remembered that other factors such as soil moisture content

and differences in sampling procedure between the two dates could have contributed to the results. Perhaps smaller leaves were unintentionally harvested in July than in June. Such leaves would probably have relatively concentrated small cells as total leaf area and stomatal density have been shown to be negatively correlated within some species (28). Measurement of leaf areas would have permitted evaluation of this possibility.

The finding that most species had more stomata on the lower than on the upper surface agrees with other surveys (16,46,65). The 3 species which had significantly more stomata on the upper than on the lower surface were all members of the Compositae. Two of these, yarrow and pineappleweed have very finely divided leaves in which the lobes may be so oriented that the lower surface is turned uppermost. The differences found in stomatal sizes have been reported before but not for these species (65).

In discussing the possible implications the data may have for herbicide penetration, it must be remembered that most herbicides are applied at low pressure and thus the bulk of the spray deposit occurs on the upper surface of the leaf. One must also consider whether stomatal density or size is the more important factor. Except for the rare instances in which spray droplets may directly enter large, open stomata (8), density is probably more important. If stomata do have a special role in penetration, then the more stomata per unit area, the faster will be penetration, regardless of whether it occurs via the open pore

Table 15. Relationship of stomatal density on the upper leaf surface and susceptibility to 2,4-D.

Species	Number of Stomata/mm.^{2}	Susceptibility to 2,4-D (72,76).
shepherd's purse	229.8	2*
wild radish	213.3	2
n.l. plantain	194.8	1
hedge mustard	176.6	1
b.l. plantain	171.6	2
blue bur	163.3	2
leafy spurge	159.9	4
dandelion	148.3	3
spotted knapweed	143.3	4
diffuse knapweed	143.3	4
cat's ear	135.7	2
redroot pigweed	131.0	3
pineappleweed	119.6	3
stinkweed	111.0	1
lady's thumb	110.7	3
yarrow	108.6	4
prostrate knotweed	107.4	4
lambsquarters	79.8	1
white cockle	65.5	4
creeping buttercup	58.9	4
chickweed	51.6	3
Canada thistle	45.9	3
mouse-ear	45.6	3
wild buckwheat	41.9	3
groundsel	40.9	3
sheep sorrel	39.6	3
hemp nettle	32.3	4
broad leaf dock	29.6	2
toadflax	2.3	4

* 1.=susceptible
2.=moderately susceptible
3.=moderately resistant
4.=resistant

or through ectodesmata in the guard cell walls (56). With this in mind, the species were ranked in order of decreasing number of stomata/mm.^{2} on the upper surface and rated according to 2,4-D susceptibility (72,76).

In constructing table 15, the data used were the means of both harvests for the 8 species grown in sun only and the means of both harvests in full sun from the species x surface x light conditions interaction table (appendix table 7) for the other species. As a result all means are equivalent in regard to sampling procedure. The 3 grasses were not included because of their well-known physiological resistance to damage by 2,4-D.

If stomata are an important site of herbicide entry, then it could be expected that the range found in table 15 would give an indication of probable herbicide damage. By careful inspection of the table, it can be seen that there was a slight tendency for those species with the greatest concentrations of stomata to be more susceptible to 2,4-D. For example, 6 of the 10 species rating 1 or 2 in susceptibility appear at the top. There are exceptions to the trend. For example, leafy spurge and the knapweeds have a high stomatal density but they are resistant to 2,4-D. At the other extreme, broad leaf dock is moderately susceptible despite having few stomata on the upper surface. In order to determine if there was a significant relationship between stomatal density and 2,4-D susceptibility, the Spearman rank correlation coefficient was calculated. The value obtained was $r_s = .38$, indicating a significant positive correlation. This suggests that about 14% of the variation in 2,4-D susceptibility of the species may be due to differences in stomatal concentration on the upper leaf surface.

Table 16. Weed species listed in order of decreasing total length of guard cells per mm.^{2} of upper leaf surface with 2,4-D susceptibility ratings.

Species	Mean Length X Mean Number of Stomata/mm.^{2}	Susceptibility to 2,4-D (72,76)
cat's ear	6510	2*
n.l. plantain	5533	1
spotted knapweed	5439	4
shepherd's purse	5004	2
blue bur	4539	2
pineappleweed	4479	3
stinkweed	4526	1
hedge mustard	4156	1
leafy spurge	4150	4
diffuse knapweed	4093	4
b.l. plantain	3971	2
yarrow	3953	4
wild radish	3844	2
prostrate knotweed	3691	4
redroot pigweed	3392	3
lambsquarters	2992	1
dandelion	2864	3
lady's thumb	2816	3
creeping buttercup	2500	4
chickweed	2308	3
white cockle	2298	4
groundsel	1928	3
mouse-ear	1912	3
sheep sorrel	1756	3
Canada thistle	1642	3
wild buckwheat	1596	3
broad leaf dock	1037	2
hemp nettle	911	4
toadflax	76	4

* 1. = susceptible
2. = moderately susceptible
3. = moderately resistant
4. = resistant

An even better indication of the potential penetration area is obtained by multiplying stomatal concentration by mean length. This was done in table 16. The same numbers as in the previous table and their corresponding lengths were used. Although the species rank is slightly different in this table, the same general relationship of stomata with 2,4-D damage is apparent. A significant Spearman rank correlation coefficient of $r_s = .35$ was calculated. This indicates slightly less correlation of 2,4-D susceptibility with stomatal length index than with stomatal density.

In considering the practical implications of the observed correlation, a number of facts should be reviewed. First, susceptibility to 2,4-D does not imply similar susceptibility to all other herbicides. In fact, many herbicides have totally different spectrums of susceptible species. 2.4-D was chosen because it is the only herbicide for which so much information is available. It is not an ideal herbicide for the purpose because the commonly used amine formulations are absorbed equally well by the roots and the ester formulations are known to penetrate leaf cuticle (39). Furthermore, the information on susceptibility is in no way quantitative, being based mainly on field observations over a number of years.

Having considered the foregoing qualifications, it is still not unreasonable to believe that about 14% of the differences in susceptibility could be due to stomatal differences. It is possible to explain some of the discrepancies in the tables on the basis of leaf morphology. Pineappleweed and yarrow are

both more susceptible to 2,4-D than would be expected on the basis of their upper surface stomatal density. As previously noted, their leaves are very finely divided and the narrow leaflets often have their margins or lower surfaces uppermost. Thus, there is little leaf area available for spray penetration even if wetting occurs. At the other extreme, the greater than predicted susceptibility of broad leaf dock can be explained by the unusually large and easily wetted leaves. The resistance of leafy spurge and the knapweeds cannot be accounted for on the basis of leaf morpholgy and must be physiological.

As susceptibility information is accumulated for additional herbicides, it will be interesting to see if the correlation with stomatal density detected in this study holds true for compounds other than 2,4-D. Examination of more weed species for stomatal characteristics would also serve to strengthen or weaken the hypothesis that the number of stomata on the upper leaf surface is positively correlated with susceptibility to foliar applied herbicides.

The experiment carried out with picloram failed to show conclusive results regarding entry associated with stomata. This is not surprising in view of the relatively simple methods used and the imperfect correlation of stomatal number and length with susceptibility to 2,4-D. The most positive results showed that dry weights were reduced more by lower than by upper surface applications. This agrees with the much greater numbers of stomata on the lower leaf surfaces but it does not distinquish between cuticular and stomatal penetration. The increases in

weight with low rates of picloram indicate a growth regulator effect which complicates interpretation of the results. The reduction in weight following application to the lower surface could just as well be due to less picloram absorption at 1 and 10 ppm., as to more at 100 and 1000 ppm.
Because of the high variability between replications, the differences detected in fresh weights due to degree of stomatal opening were not detected in the dry weights. In summary, it was found that all species were damaged more by lower surface than by upper surface application. Differences due to degree of stomatal opening were indicated but not conclusively shown. More precise control of surfaces sprayed and stomatal opening would probably have yielded more conclusive results.

The drawings and photomicrographs illustrate a number of interesting differences and peculiarities among the species studied. In regard to trichomes, it would appear that, of the species studied, only blue bur, mouse-ear, white cockle, cat's ear, diffuse knapweed, wild radish, shepherd's purse, hemp nettle, and buttercup have sufficient hairiness to influence retention or penetration of sprays. The whole mount method of slide preparation has fairly damaging effects on large trichomes and the broken and missing trichomes shown in the plates are not necessarily a normal occurrence. However, it is known that, many leaves shed their trichomes early in life (20), and the plates illustrate the relatively large cavities which may be left.

Illustrations V-29 and VI-35 show the apparently

stomata-free upper surfaces of toadflax and montia. The data revealed that stomata do exist on these surfaces but in very small numbers per unit area. This illustrates the advantages of the whole mount method over epidermal stripping. Several epidermal strips could easily be taken from these species without encountering stomata. The function of these rare upper surface stomata is difficult to explain. They are far too sparse to be of significance in the total gaseous exchange of the leaves. Perhaps we are witnessing the last stages of an evolutionary process which will result in completely hypostomatous leaves for these species.

CONCLUSION

The survey of mature leaves of 33 weed species has revealed stomatal densities ranging from 294.9/mm.^{2} in the lower epidermis of hedge mustard to 2.3/mm.^{2} on the upper surface of toadflax. Differences in length of stomata were less, ranging from 59μ in wild oats to 21.6μ in shepherd's purse. Size appears to be negatively correlated with density. In some botanical families (Cruciferae, Caryophyllaceae, Gramineae), the various genera appear to have similar stomatal characters, while in others (Polygonaceae, Compositae), variability appears to be a family trait.

The drawings and photomicrographs present a visual summary of the data as well as providing a record of other features. The species can be grouped in a number of ways based on leaf surface anatomy. For example, the stomata were sunken in leafy spurge (Euphorbia esula), flush with the surface in lambsquarters (Chenopodium album), and raised slightly above the other epidermal cells in all other species. Twelve species had by far the most stomata on the lower surface and only 3 had most on the upper surface. No completely hypostomatous leaves were found but those of toadflax (Linaria vulgaris) and montia (Montia sibirica)were nearly so. Ten species were appreciably pubescent while the others were essentially glabrous at maturity. Gland-tipped hairs and sessile glands were found in the knapweeds (Centaurea spp.). Short, stalked, mushroom-like glands were common in Polygonaceae.

Small, fragile glands were found on the leaves of broad-leaf plantain (Plantago major).

A positive correlation was indicated between density of stomata on the upper leaf surface and susceptibility to 2,4-D. The correlation was only slight, so that stomatal density would be unlikely to decide the success or failure of 2,4-D, but rather could influence its relative efficiency against different species.

Evidence obtained from the greenhouse experiment using picloram showed that 3 species having many more stomata on the lower than on the upper surface were all damaged more by sprays to the lower than to the upper surface. This indicates penetration associated with stomata but does not rule out cuticular penetration. The effects of relatively open versus relatively closed stomata on entrance by picloram were inconclusive.

The usefulness of the information obtained in the survey of weed leaf anatomy has not been shown in the present study. However, there are indications that as more information on herbicide susceptibility is accumulated, the data obtained may prove to be very useful from the point of view of weed control.

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APPENDIX

Table 1. Analysis of variance of stomatal numbers of 8 species.

Source	Degrees of Freedom	Mean Square	F
Species	7		46.70**
Dates	1		50.60**
Leaf Surface	1		66.88**
S x D	7		3.60**
S x L	7		29.57**
D x L	1		4.66*
D x S x L	7		1.33n.s.
Error	608	26.71	
Total	639		

Table 2. Analysis of variance of stomatal lengths of 8 species.

Source	Degrees of Freedom	Mean Square	F
Species	7		619.57**
Dates	1		142.19**
Leaf Surface	1		14.95**
S x D	7		128.14**
S x L	7		32.42**
D x L	1		8.34**
D x S x L	7		3.98**
Error	2528	1.78	
Total	2559		

n.s. = not significant at 5% level
* = significant at 5% level
** = significant at 1% level

Table 3. Analysis of variance of stomatal numbers of 25 species grown in sun and shade.

Source	Degrees of Freedom	Mean Square	F
Species	24		227.14**
Dates	1		72.21**
Environments	1		30.82**
Leaf Surfaces	1		759.62**
S x D	24		10.41**
S x E	24		10.13**
S x L	24		32.30**
D x E	1		3.46n.s.
D x L	1		1.68n.s.
E x L	1		0.01n.s.
D x S x L	24		2.23**
D x S x E	24		9.46**
D x L x E	1		0.14n.s.
E x S x L	24		1.80**
E x S x L x D	98		3.60**
Error	3726	16.288	
Total	3999		

Table 4. Analysis of variance of stomatal lengths of 25 species grown in sun and shade.

Source	Degrees of Freedom	Mean Square	F.
Species	24		3683.46**
Dates	1		0.64n.s.
Environments	1		148.29**
Leaf Surfaces	1		1.42n.s.
S x D	24		194.96**
S x E	24		70.63**
S x L	24		51.49**
D x E	1		58.05**
D x L	1		0.16n.s.
E x L	1		52.82**
S x E x L	24		8.45**
D x S x E	24		124.96**
D x S x L	24		5.91**
D x E x L	1		5.15*
D x S x E x L	98		36.74**
Error	15726	2.48	
Total	15999		

n.s. = not significant at 5% level
* = significant at 5% level
** = significant at 1% level

Table 5. Analysis of variance of fresh weights of weeds after application of picloram sprays.

Source	Degrees of Freedom	Mean Square	F
Species	2		46.24**
Aperture	1		1.27n.s.
Leaf Surface	1		2.76n.s.
Treatment	4		15.42**
S x A	2		2.21n.s.
S x L	2		0.77n.s.
S x T	8		3.49**
A x L	1		3.22n.s.
A x T	4		3.94**
L x T	4		2.46*
S x A x L	2		0.67n.s.
S x A x T	8		2.08*
S x L x T	8		1.61n.s.
A x L x T	4		1.98n.s.
S x A x L x T	34		1.95n.s.
Error	154	237.83	
Total	239		

Table 6. Analysis of variance of dry weights of weeds after application of picloram sprays.

Source	Degrees of Freedom	Mean Square	F
Species	2		81.66**
Aperture	1		0.19n.s.
leaf Surface	1		4.13*
Treatment	4		4.64**
S x A	2		0.55n.s.
S x L	2		0.17n.s.
S x T	8		1.24n.s.
A x L	1		0.84n.s.
A x T	4		2.06n.s.
L x T	4		1.70n.s.
S x A x L	2		0.15n.s.
S x A x T	8		2.05*
S x L x T	8		1.02n.s.
A x L x T	4		2.38*
S x A x L x T	34		1.72n.s.
Error	154	1210.9	
Total	239		

n.s. = not significant at 5% level
* = significant at 5% level
** = significant at 1% level

Table 7. Densities and lengths of stomata on the upper leaf surface of 25 species. (mean of 2 harvests, grown in full sun).

Species	Number of Stomata/mm.^{2}	Length of Stomata(μ)
redroot pigweed	131.0	25.8
blue bur	163.3	27.5
chickweed	51.6	44.4
mouse-ear	45.6	42.3
lambsquarters	79.8	37.4
diffuse knapweed	143.3	28.4
white cockle	65.5	35.2
Canada thistle	45.9	35.2
cat's ear	135.7	25.4
groundsel	40.9	46.6
wild buckwheat	41.9	38.8
hedge mustard	176.6	23.6
shepherd's purse	229.8	21.7
leafy spurge	159.9	26.1
couch grass	71.8	45.5
wild oats	46.6	59.7
barnyard grass	110.7	31.8
n.l. plantain	194.8	28.6
lady's thumb	110.7	25.5
sheep sorrel	39.6	43.9
prostrate knotweed	107.4	34.2
broad leaf dock	29.6	35.5
creeping buttercup	58.9	42.9
montia	4.7	42.3
toadflax	2.3	29.1