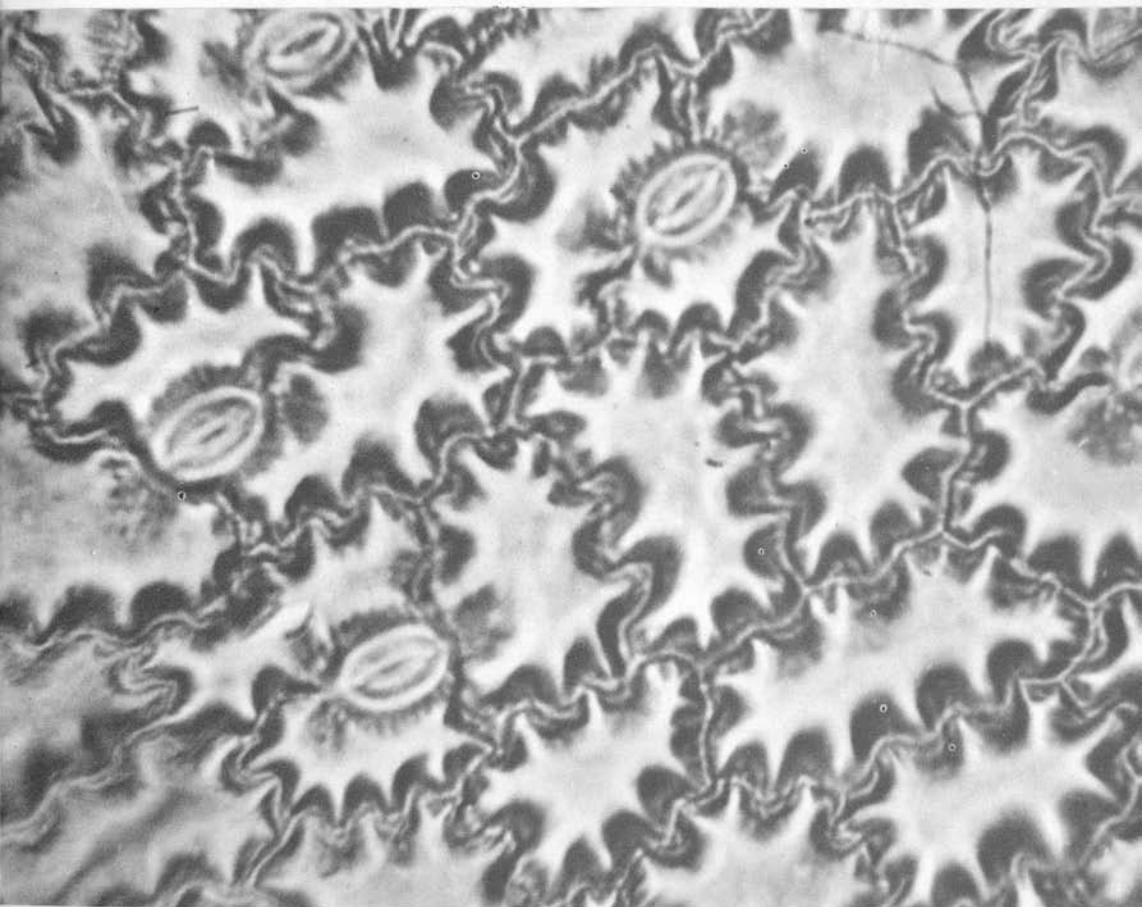


Identifying Plants By Leaf Epidermal Characters

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Leaf cell pattern of the upper surface of an alfalfa leaf.

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The method of plant identification presented here is based on the pattern of the epidermal cells on the upper surface of either the cotyledons or the true leaves. On either the cotyledons or the true leaves, the pattern is a specific and constant character of a species and of varieties within a species. The fact that the pattern of the epidermal cells differs is not new knowledge, but heretofore this character of plants has not been used as a means of identification.

Presently the keys for identification of plants are based largely on floral characters thus making the identification of a plant with no flowers a difficult problem. The seed analyst can identify the species of many cultivated plants by the seeds but in many instances the variety cannot be identified. This again poses a problem. If it is necessary to determine the variety, the mature plant must be grown from the seed in question. By the time this has been done, the usefulness or even the need for the identification may have long since passed. Identification by the cell pattern of the true leaves can solve the first problem, and identifying the plants at the cotyledon stage can solve the second problem.

The pattern of the epidermal cells of the leaf is obtained by a technique not unlike fingerprinting, which has long been used for identification of persons. A thin film of clear nail polish (cellulose acetate) is spread on the leaf, allowed to harden, and then stripped from the leaf with forceps. The pattern on the film of dried nail polish is a replica of the leaf surface showing in relief all the features as they appear on the leaf itself. This print can be mounted *dry* on a slide for examination under the microscope with dark field illumination. It is necessary to mount the film dry as the addition of any liquid obscures many details of the pattern which are essential for identification.

Many plants, both dicotyledons and grasses, have been studied. The cell pattern of the upper surface of the true leaves and the cotyledons, or both, has consistently been characteristic of the species or variety. It is not possible in this Circular to publish photomicrographs and descriptions of all the plants studied. To illustrate the variations in cell patterns of species and varieties photomicrographs of prints* are shown of two varieties of marigold, *Tagetes patula*, four species of *Panicum*, four varieties of chrysanthemum, and four varieties of alfalfa.

The following schedule of characters of the upper surface of the leaf is a convenient method of describing the cell pattern in a concise series of numbers.

* All photomicrographs are of prints of the upper surface of the leaf x 380.

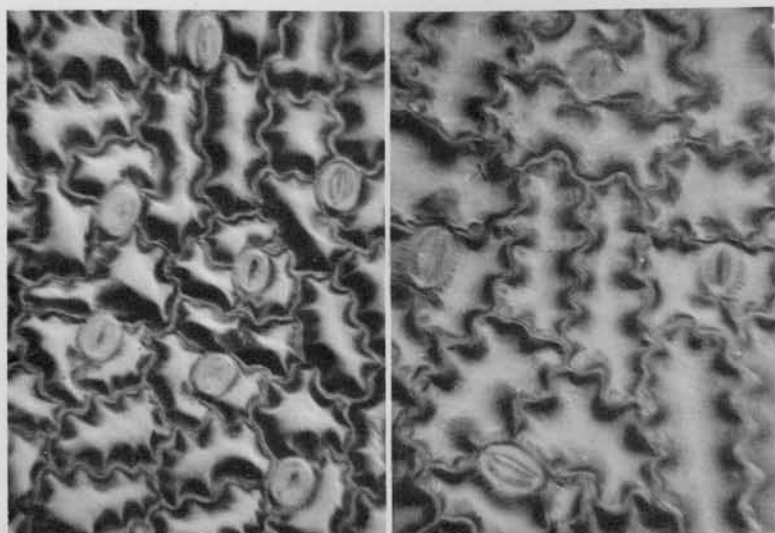


Figure 1. At left, photomicrograph of the cell arrangement on the upper surface of the Atlantic variety of alfalfa. At right, the variety Ranger.

DICOTYLEDONS

Cells

| <i>Situation of margin</i> | <i>Size</i> | <i>Shape and arrangement</i> |
|----------------------------|---------------------------------------|------------------------------|
| 1. deep | 6. very large, 250-700 per sq. mm. | 11. uniform |
| 2. very deep | 7. large, 701-800 per sq. mm. | 12. variable |
| 3. shallow | 8. medium, 801-900 per sq. mm. | 13. flat |
| 4. few | 9. small, 901-1000 per sq. mm. | 14. not flat |
| 5. many | 10. very small, 1001-1500 per sq. mm. | 15. orderly |
| | | 16. not orderly |

Stomata

| | |
|----------------|--------------------|
| 17. very large | > 32 x 19 μ |
| 18. large | 32 x 19 μ |
| 19. small | 28 x 17 μ |
| 20. few | 50-100 per sq. mm. |
| 21. many | |
| 22. none | |

It will be noted that all the categories can be determined by count, measurement, or accurate visual examination. Substituting the number of each category for the descriptive words, a plant can be described as shown in the discussion of the several plants.

Alfalfa

A more extensive study was made of 12 varieties of alfalfa as it seemed possible that this method of identification could be put to immediate practical use on this plant. The varieties are Vernal, Cayuga, Narragansett, Williamsburg, Buffalo, Atlantic, Dupuits, Ranger, Alfa, Caliverde, Culver, and Cardinal. The seed from which the plants were grown was from authentic samples of the varieties.

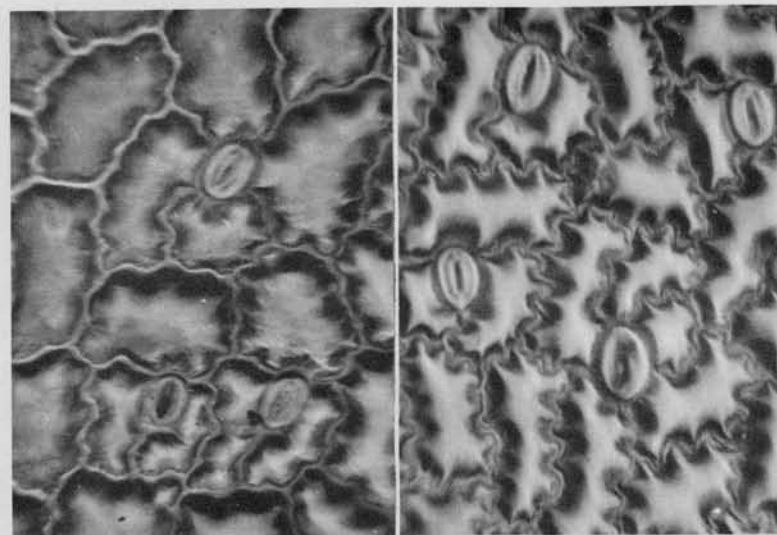
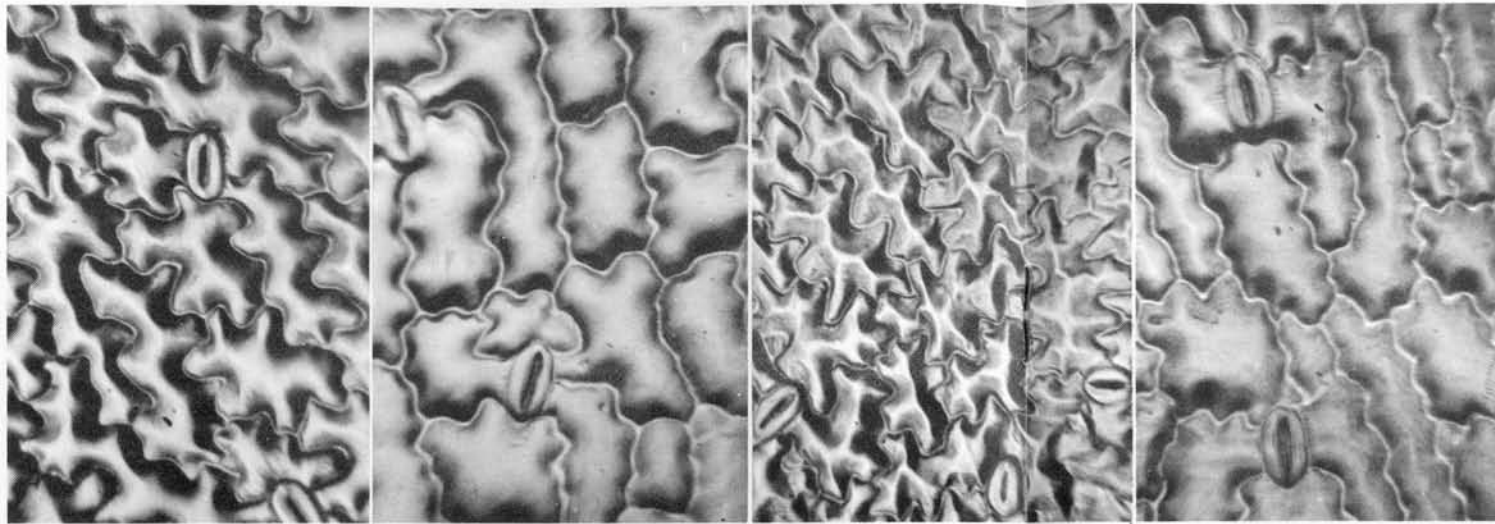


Figure 2. Leaf epidermal cell pattern of the alfalfa variety Buffalo (left) and the variety Caliverde (right).

Six lots of 100 seedlings of each variety were grown in a sand culture at different times and under varying conditions of light and moisture. The conditions under which the plants were grown did not affect the cell pattern. Prints were made from the center section of the upper surface of ten cotyledons taken at random from each variety in each of the six lots of plants. The characteristic pattern for each variety was determined by direct microscopic examination of the prints and from photomicrographs. For purposes of checking the accuracy of the method, camera lucida drawings were made as well as many measurements of cell and stomata size, but as cell size can be determined by the number of cells in a given area and the frequency of stomata in the same manner, the details are not given here. The numerical description of the varieties of alfalfa according to key for dicotyledons is given below.

| | |
|--------------|-----------------------|
| Vernal | 3-5-8-11-13-19-21 |
| Cayuga | 3-4-8-12-15-18-20 |
| Narragansett | 3-5-9-12-14-16-19-21 |
| Williamsburg | 3-5-7-12-14-16-18-20 |
| Buffalo | 3-4-7-11-13-16-18-21 |
| Atlantic | 3-5-10-12-14-16-19-21 |
| Dupuits | 3-5-8-12-14-16-18-20 |
| Ranger | 1-5-7-12-14-16-18-20 |
| Alfa | 3-5-7-11-13-16-18-20 |
| Caliverde | 1-5-8-14-16-18-20 |
| Culver | 3-4-8-12-14-16-18-20 |
| Cardinal | 1-5-9-12-16-18-20 |

For example, Atlantic is described as having (3) shallow situations of cell margins, (5) many, (10) very small cells, (12) variable, (14) not flat, (16) not orderly, (19) small stomata, (21) many stomata.



Marigolds

The photomicrographs of the prints of two varieties of marigolds *Tagetes patula* show not only varietal differences in the patterns of the true leaves and cotyledons but wide differences between the patterns of the true leaves and the cotyledons in the same variety. The placement of the stomata in the cotyledons between a typically shaped cell appears to be characteristic of this species of *Tagetes*. According to the key for dicotyledons the numerical description is as follows:

| | | |
|-----------|----------------------|-------------------------|
| | <i>Var. SPRY</i> | <i>Var. PETITE GOLD</i> |
| True leaf | 1-5-6-12-14-16-18-20 | 2-5-9-12-14-16-19-20 |
| Cotyledon | 3-4-6-11-13-15-16-20 | 3-5-6-12-13-16-17-20 |

Chrysanthemums

In the chrysanthemums a greater difference is found between varieties than in the marigolds. The immediate ancestry of these varieties is known and it is interesting to note that the cell pattern is not inherited either in a seedling or a sport. Headliner is a seedling of Coppersmith, and White Wonder is a sport of Improved Early Wonder. The numerical description of the varieties follows:

| | | | |
|-------------|-------------------|-------------------|----------------------|
| Coppersmith | 3-4-9-11-15-18-20 | Imp. Early Wonder | 3-4-7-12-13-16-17-20 |
| Headliner | 2-5-6-11-14-16-22 | White Wonder | 3-5-9-12-13-16-17-20 |

Figure 3. Photomicrographs of leaf and cotyledon epidermal cells of two varieties of marigold. From left: True leaf of the variety Spry, cotyledon of the variety Petite Gold, true leaf of that variety, cotyledon of the variety Spry.

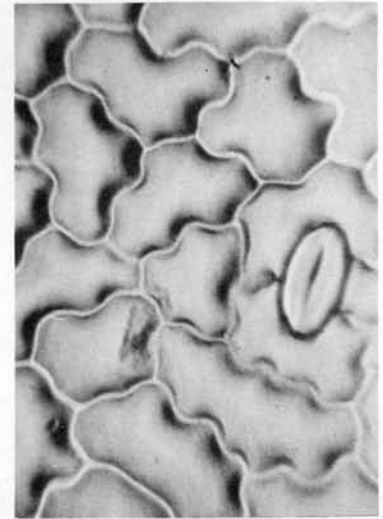
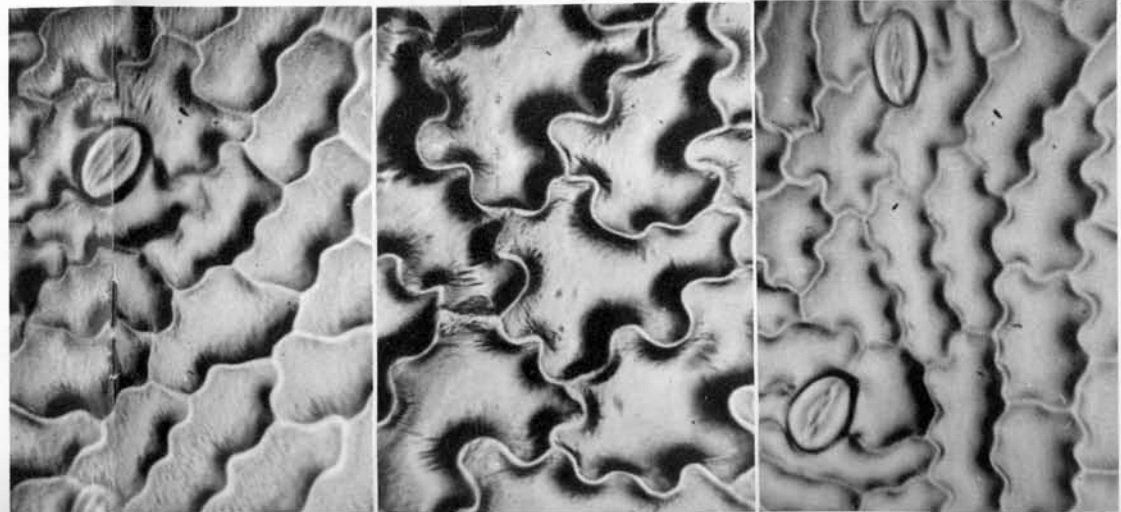


Figure 4. Leaf epidermal cell pattern of chrysanthemum. Clockwise from upper right: Improved Early Wonder, White Wonder (a sport of Improved Early Wonder); Headliner (a seedling of Coppersmith, and Coppersmith.



GRASSES

The differences among the four species of *Panicum* are quite apparent, but the complicated pattern of these species and of grasses in general make it more difficult to characterize them in a numerical key.

Cells

| <i>Sinuation of margin</i> | <i>Shape and arrangement</i> | <i>Spines</i> | <i>Motor Cells</i> |
|----------------------------|--------------------------------------|---------------|--------------------|
| 1. present | 3. long | 8. many | 17. chain-like |
| 2. none | 4. medium | 9. few | 18. not chain-like |
| | 5. short | 10. none | |
| | 6. interspersed with short cells | | |
| | 7. not interspersed with short cells | | |

Stomata

| | |
|-----------------------------|---------------------|
| 11. large | 14. elliptical |
| 12. small | 15. many |
| 13. diamond shaped | 16. few |
| <i>Panicum clandestinum</i> | 1-4-10-11-13-16-18 |
| <i>Panicum milacearum</i> | 1-3-10-12-13-15-17 |
| <i>Panicum capillare</i> | 2-5-6-9-12-14-15-17 |
| <i>Panicum Crus-Galli</i> | 2-5-8-11-14-16-18 |

Limited studies have been made of some of the lawn grasses and the species and varieties apparently can be identified by the "fingerprinting" technique.

Possible Usefulness of the New Technique

This method requires no more skill than is required for plant identification by the conventional taxonomic keys. The descriptions contain fewer variables than conventional keys and consequently fewer chances of error. From the studies made on many species and varieties it is believed that the method can be widely used to distinguish accurately species and particularly varieties of plants. It is suggested that this technique could be very useful to seed analysts when it is not possible to identify a variety from the seed.

We do not decry the time-tested keys or suggest this method as a substitute but rather present it as a useful addition to existing plant identification techniques.



Figure 5. Photomicrographs of leaf epidermal cell pattern of four grasses. Upper left, *Panicum clandestinum*; upper right, *P. milacearum*; lower left, *P. capillare*; and lower right, *P. Crus-Galli*.