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THE CICATRIZATION OF WOUNDED CITRUS LEAVES

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Since the independent plant must expose green tissues to light, massive opaque coverings may not be employed by foliage leaves. Their exposed position and necessarily delicate structure render them peculiarly liable to injury. The writer has been interested in noting some of the responses of foliage leaves to injuries. A paper now in press summarizes the literature of this subject and describes the healing structures developed by certain mesophytic leaves following wounding.

The leaves of the lemon, *Citrus limonia,* offer structurally rather sharp contrast to our deciduous foliage. A general examination of these leaves showed that their differences in wound responses were quite as marked as their morphological divergences from the mesophytic structure. An outline report on the cicatrization of lemon leaves was given at the meeting of the Botany section of the Iowa Academy three years ago. No paper was offered for publication at that time, however, as that study was based wholly on experiments with local plant house material. It seemed important that one first compare such results with conditions found in regions of the successful culture of this species out of doors.

During the summer of 1925 the writer spent several weeks experimenting with the leaves of native and cultivated plants in the vicinity of Los Angeles in southern California.[1] The material then secured included a series of stages for this species, giving the cicatrice development following wounding of lemon leaves.

The Iowa City material, earlier studied, was secured from a large potted lemon tree which was kept winters in the University plant house and placed outdoors from spring to autumn. During the period of study (July) the plant stood at the exposed corner of Old Science Hall, where it received full sunshine the greater part of the day. Only obviously mature leaves were wounded. The western leaves were secured from a medium-sized lemon tree growing under irrigation near the campus of the University of Southern Cali-

1 These experiments were aided by a moderate grant from the research funds of the American Association for the Advancement of Science.

fornia. The experiments were carried through the month of August.

Leaves in both series were wounded by cutting across the outer portion of the blade with scissors. A considerable number of leaves was wounded in each experiment, so that material would be adequate for the study of various stages. Frequent collections were made for the first few days, then at weekly intervals for a month or more. In making collections strips were cut parallel to the wounded margins and preserved in formalin for histological study. Freehand and freezing microtome sections were useful in outlining events but the critical study was based primarily upon material imbedded in paraffin. For such study sections twelve microns in thickness have proved most satisfactory. Microchemical tests were carried out on sections, attention being given to the occurrence of cutin, suberin and lignin.

The foliage of *Citrus limonia* is very attractive, the leaves being thick, firm and glossy. Transverse sections reveal certain characteristics of the broadleaved evergreen structure — heavy cuticle, sturdy epidermis, usually multiple palisade and relatively reduced volume of air space. (Plate 1.) A general comparison of the Iowa and California material is given below. In all instances the figures given for any structure are the average of several measurements (usually ten) and from sections of different leaves. The third line shows, for purposes of comparison, the average measurements of leaves from twenty-eight deciduous trees and shrubs growing in Iowa.

	Total Thickness	Upper Epidermis	Palisade	Spongy Mesophyll	Lower Epidermis	Upper Cuticle
Citrus limonia (Calif.)	263	17.1	69	161	12.7	2.1
Citrus limonia (Iowa)	233	11.4	50	160	10.1	1.9
Average of twenty-eight deciduous leaves (Iowa)	144	21.13	54	54	14.9	0.9

The above comparison of Citrus leaves grown in the two stations shows numerous differences. The California leaves are considerably thicker and have more palisade, about equal amount of spongy mesophyll, distinctly heavier epidermal layers and somewhat increased thickness of cuticle. The drawings originally prepared from Iowa material are used in this paper, and comparisons with the California material are discussed in the text.

The Pseudocicatrice — In an earlier paper (1) the writer has summarized the importance of this preliminary barrier promptly set up by wounded leaves. Initial protection is afforded by the collapse of tissue which dies following lesion, partly due to crushing but primarily resulting from water loss. This buffer covering to the wounded leaf margin has been termed by the writer the *pseudocicatrice.* It is an aggregate resulting from the collapsed mesophyll and the incurved epidermal layers, together with any secretions that may be added by the wounded leaf.

In Citrus leaves the death of tissue after wounding is marked; a zone of mesophyll involving the width of 30 to 50 or more layers of palisade dies. These cells partly collapse, their contents dry up and the whole mass shrinks inward against the leaf margin. Because of this collapse and shrinkage of interior cells the epidermal layers are brought inward and soon give the wounded margin a considerable protection since the heavy cuticle is retained on the outer wall.

The pseudocicatrice of Citrus is somewhat longer in the Iowa material with an average closer approach of the incurved epidermal layers. They are sometimes found in contact, though generally the upper epidermis curves inside the lower. (Plate 1.) In the California material the epidermal protection is less complete, since the upper epidermis usually curves rather sharply downward or inward while the lower epidermal layer protrudes considerably. The only suggestion of infection noted in any of these Citrus leaves was the occurrence of fungal spores on the pseudocicatrice of one of the western leaves; a few of the spores had germ-tubes, but no mycelium was found.

These results, as well as those from the study of other leaves of various types, emphasize the importance of the pseudocicatrice in dealing with foliar wounds. It develops quickly, retards traumatic water loss and lessens the dangers of infection. Its significance is probably more than merely protective since it may serve to bring about internal conditions favorable to mitosis in the underlying tissues.

Underneath this pseudocicatrice the true cicatrice develops relatively slowly. In thinner deciduous leaves mitosis begins within two days after lesion and the cicatrice is well established in ten days. In Citrus events move more leisurely. The first signs of cell enlargement were noted in about a week, seemingly a day or so earlier in California material. Enlarged cells in both palisade and spongy mesophyll gradually close off the air spaces in a zone next back of the pseudocicatrice. Mitoses are further delayed, beginning about the tenth day, and the new walls are laid down parallel with the wounded surface. Cell division now proceeds more rapidly with a tendency to arrange the newly formed cells in rows as in normal cork tissue. In two weeks the cicatrice layer averages about four cells in thickness for both Iowa and California material. This zone deepens to fifteen or twenty cells in width before specializing into the cicatrice tissue.

In its mature expression the cicatrice proper is differentiated into an outer zone of corklike tissue with thickened, strongly suberized walls, and an inner region of cells which retain their protoplasm and have walls that are but slightly thickened. The cicatrice is widest in the region of the spongy mesophyll. The epidermal cells divide freely and lengthen the epidermal cover of the cicatrice region both above and below. (Plate 2.)

The cells of the outer portion of the cicatrice enlarge considerably and the lack of a supporting tissue on the exposed side leads to a conspicuous outward bulging of the walls (Plate 2). With diminished turgor in these outer cells they in turn are impressed by the deeper-lying cells as they enlarge. This pressure usually leaves them somewhat flattened, with a tendency towards an outward convexity of all walls in the wound cork.

The walls of these cells are heavily suberized and respond also to tests for lignin but no cutin was noted in this region. In the cicatrice of lemon leaves injured by insects the cork zone, of unknown age, is sometimes thicker than that resulting from the experimental wounds. However, since the collections covered a period of less than thirty days, it may be that the cambium continues its activity for a longer period, gradually thickening the wound cork.

The interior zone of living cells is derived in part from the enlargement of original mesophyll cells, and in part from mitoses following wounding. This tissue has its cells compacted tightly together, offering sharp contrast to the normal leaf tissue but in Citrus shows no positive response to tests for suberin, lignin or cutin.

Bartholomew (2) in his study of the Alternaria rot of lemon (fruits) instances (pp. 26-27) the development of resistant layers of corklike cells which operated to block the progress of the mycelia. A comparison of his Figure 3 with the plates accompanying the present paper shows the close agreement of the barrier tissues set up by the leaf and by the fruit of this plant.

LITERATURE CITED

1. WYLIE, ROBERT B. Leaf Structure and Wound Response. Science 65: 45-50. 1927.
2. BARTHOLOMEW, E. T. Alternaria Rot of Lemons. Bulletin 408, University of California College of Agriculture Experiment Station, Berkeley, California. 1926.

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DESCRIPTION OF PLATES

(Based on Iowa material)

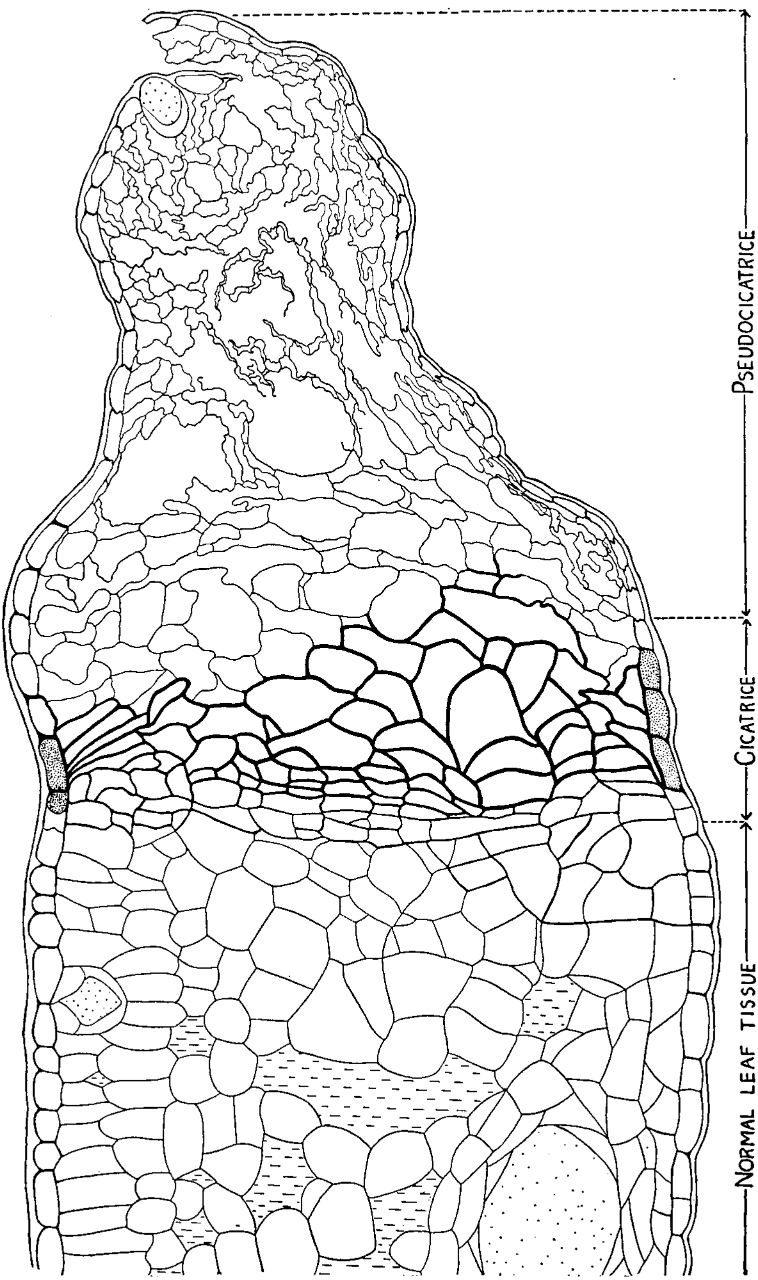

Plate 1. Transverse section of lemon leaf twenty-eight days after wounding. The pseudocicatrice and cicatrice are indicated by marginal legend.

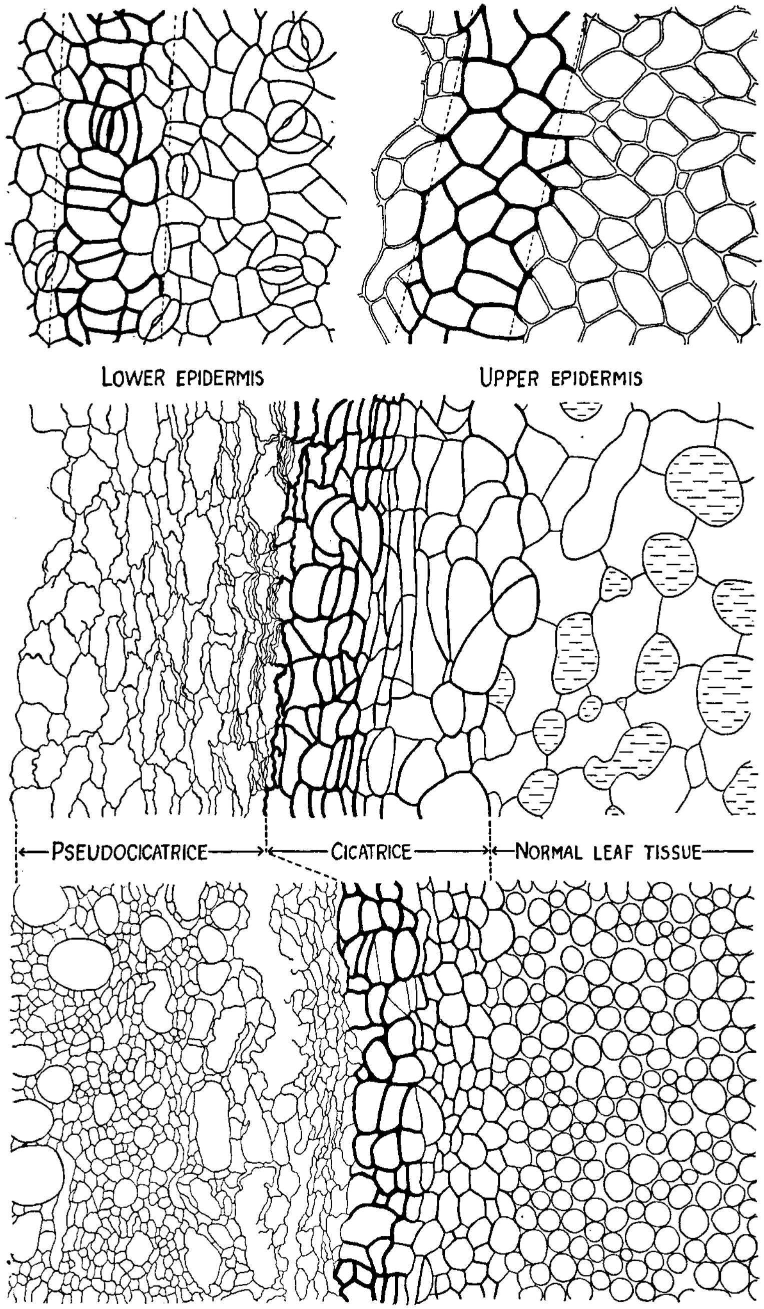

Plate 2. Drawings from sections cut parallel to epidermis. The upper figures, as labeled, show the epidermal layers above and below the cicatrice region. The central figure is drawn from a section through the spongy mesophyll, while the lowest figure shows a corresponding section in the region of the inner part of the palisade layer. All of the figures of this plate include normal leaf tissue, cicatrice and pseudocicatrice.