of 4.4 ovarioles per ovary. Three individuals had spermatheca much larger than those of normal workers, but all other characteristics of these bees were workerlike.

In the studies of dimorphism in the honeybee that have been conducted in this laboratory, high mortality has always occurred in the treatments from which intermediates between queens and workers were produced. Furthermore, most individuals have been predominately queenlike or predominately workerlike, and those structures that have been intermediate in form or size have usually been rather near the range of variation of either queens or workers. These facts make it difficult to assess the potential of any treatment from a study of the resulting adults.

It has been proposed (*4*) that partial starvation of worker larvae could be an initiating mechanism in dimorphism. Since these laboratory-reared larvae had all the food they could ingest at all times, quantitative starvation could not be the determining mechanism. It is evident that there is a substance or substances in royal jelly that initiates or controls differentiation of queens, and that at least some essential part of it is either highly labile or is not available to larvae after the jelly has been stored for a time. Until methods are developed for preserving the biological activity of royal jelly for honeybee larvae, studies of the effect of royal jelly on other animals (*5*) may yield misleading results.

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Use of Plants as Biological Indicators of Smog in the Air of Los Angeles County

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Intensive investigation of crop damage by smog in the Los Angeles area began in 1947 (*1, 2*). At present, smog is understood to be a complex of liquids, solids, and gases, comprising more than 50 chemical elements and compounds and producing, among other effects, low visibility, eye, nose and throat irritation, crop damage, excessive rubber cracking and odor nuisances. Certain species of vegetables, ornamentals, and weeds were shown to be singularly sensitive (*3*). Damage symptoms differ from those ascribed to any previously studied gases, including SO_2 (*4*), hydrogen fluoride (*5*), and illuminating gas and smoke from industrial stacks (*6*). Field damage is considered to be due to certain intermediate products resulting from the chemical combination of unsaturated hydrocarbons and ozone in the atmosphere (*7*); the exact chemistry of the phytotoxic products is unknown.

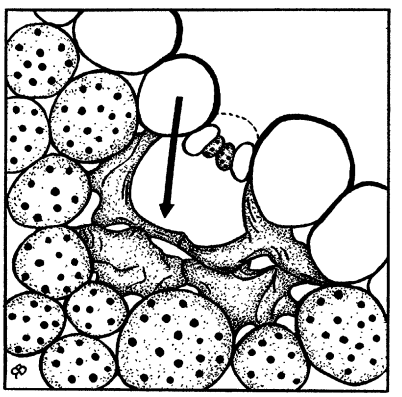

Fig. 1. Smog damage in *Poa annua* leaf, showing dehydrated cells (arrow) directly beneath stomata and surrounding substomatal chamber.

Through the courtesy of F. W. Went of the California Institute of Technology, plants were grown smog-free in the specially filtered greenhouses of Earhart Laboratory (*8*). Smog damage has been duplicated experimentally in the laboratory at Air Pollution Control District headquarters by subjecting normal plants to the reaction products of ozone and gasoline vapors (*9*). The living plant cell appears to be an excellent biological indicator for smog, and annual bluegrass, *Poa annua* (L), has been singled out as one of the most sensitive plants yet observed.

Gross symptoms of smog damage in the field vary with each crop from a permanent water-logged appearance of the leaf undersurface to complete necrosis. In dicotyledons, smog damage may be recognized as "silvering" in spinach, "bronzing" in romaine lettuce, brown-black mottling in tomato, or an increase in anthocyanin in table beet. In monocotyledons, tan banding is the characteristic response in barley and *Poa* (*3*), and longitudinal streaking in oat (*10*), corn, and many grasses.

Microscopic examination reveals the nature of histological damage. Most accurate results were obtained by studying fresh leaf material. Damaged cells were readily distinguished by staining thin hand sections with simple microchemical reagents, such as thionin in weak aqueous solution, Sudan III, and Sudan black. Damaged cells took stain readily, whereas normal ones resisted it. Permanent sections were not made, but data were kept by means of microphotographs.

In all plants observed, both monocotyledons and dicotyledons, there is apparent a characteristic progression in smog damage. The first visible response is a shiny, water-logged appearance on the leaf undersurface. The lower epidermis is raised in tiny blisters, produced by the swelling of the epidermal cells closest

to the stomata. At the same time stomatal apertures enlarge as guard cells expand in width. The entire leaf at this time becomes turgid. Actively functioning leaf stomata are considered to be the portals of entry for atmospheric smog, since the cells that line the substomatal chambers show protoplasmic injury first and are always the most severely damaged (*11*). Chloroplasts disintegrate and plasmolysis follows. Cell walls shrink slowly, maintaining plasmodesmatal connections with neighboring cells. Damage is usually limited to a few cells surrounding the affected substomatal chamber. There is no rupture of cell walls or dissolution of middle lamella. Intercellular air spaces enlarge as affected cells shrink. Since dehydration is slow, cellular "mummification" of affected tissue is not complete until 1 to 2 days following exposure (Fig. 1). The extent of tissue involvement is, as in any gas damage, in proportion to the concentration and duration of pollution.

In the grass, *Poa annua* (L), the sensitivity of the leaf tissue is a function of its maturity (*3*). Damage in the youngest leaf appears only at the tip; in a leaf somewhat older, close to midblade; and in a fully matured leaf, only at the base. This localization of damage has been shown to be related to the gradient of cellular differentiation from tip toward base in the maturing leaves and is probably true also in broad-leaved plants, such as spinach and tobacco, in which cellular maturity is likewise progressive from tip toward base (*12, 13*). Only the cells that have just completed maximum expansion are smog sensitive. Young leaves are not susceptible, probably by virtue of their compact cellular nature, absence of well-developed intercellular air spaces and substomatal chambers, and nonfunctional stomata. Old leaves are not sensitive by virtue of their comparatively heavily suberized cell walls (*14*).

Whatever the gross picture of smog damage, anatomical studies indicate that in all sensitive vegetation the microscopic picture is the same. It is evident from this work, that the damage produced by smog differs from that produced by any other phytotoxic agent studied, for example, frost, ozone (*15*), SO_2, HF, fungus, and insect. Smog-attacked cells are not disrupted as they shrink, resulting in a tissue "skeletonization" in the limited regions of the substomatal chambers; damage in response to other gases is usually unlimited, spreading throughout the lamina and, more often than not, resulting in complete necrosis (SO_2, O_3) or affecting vascular elements (HF) (*5*). The anatomy of stems and roots and the vascular elements of leaves are never affected by smog, indicating that the phytotoxic constituents of smog are not translocated within the plant.

Poa annua (L) is considered a very reliable biological indicator for atmospheric smog for several reasons: (i) the extreme sensitivity of its cells to minute quantities of phytotoxic materials; (ii) its method of cellular differentiation from tip toward base in the linear leaf with resulting marked transverse leaf banding; and (iii) its ubiquity as a weed in Los Angeles County, making a naturally occurring check available in many and scattered areas. Work is in progress in an attempt to calibrate this plant as a quantitative, as well as a qualitative, bioassay material. A detailed account of the anatomy of normal and smog damaged *Poa* has been completed.

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Contamination of Nuclear Fractions of Thymus Homogenates with Whole Cells

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A reexamination of methods for the isolation of nuclei of cells of the thymus gland of the rat and calf has led to the observation of atypical reactions that suggest a gross contamination with intact whole cells. In view of the recent use of nuclear fractions of thymus homogenates in studies on the localization of enzymes (*1, 2*), it is desirable to report a method (*3*) for demonstrating such contamination.

The isolation of a pure nuclear fraction from the thymus gland is complicated by the presence of large numbers of small thymocytes, that is, small cells with large nuclei surrounded by a very thin layer of cytoplasm. Histologically, these cells are indistinguishable from small lymphocytes (*4*). It is frequently very difficult, if not impossible, to detect contamination of fresh nuclear fractions with these cells by routine examination with the phase contrast microscope, since the layer of cytoplasm is so thin as to be indistinguishable. The difference in reaction of isolated nuclei and whole cells to changes in ionic composition or osmotic pressure of the suspending medium offers a ready means of determining the extent of contamination.

For this study, the nuclear fraction of calf thymus was isolated by the method of Stern and Mirsky (*1*), which employs a solution of 0.0018*M* $CaCl_2$ in ap-