A METHOD FOR THE STUDY OF FOLIAR ABSCISSION *IN VITRO*[1]

FREDRICK T. ADDICOTT, RUTH STOCKING LYNCH,
GEORGE A. LIVINGSTON[2], and JEAN K. HUNTER

(WITH EIGHT FIGURES)

Received December 27, 1948

During a series of experiments on abscission of leaves a method was devised which made possible the examination of this process in the laboratory under controlled conditions. Essentially, this method consists of excising small pieces of tissue including one or more abscission zones and using these pieces, called explants, for experimentation. Such explants have been made from Citrus (Valencia orange), Phaseolus (Black Valentine bean), and Coleus.

The Citrus explant includes ten millimeters of midrib and six millimeters of attached petiole (fig. 1). The most favorable bean explants are taken from trifoliolate leaves and are of two kinds: "a," the pulvinus and ten millimeters of the stalk of the terminal leaflet, and "b," the two pulvini of the lateral leaflets and ten millimeters of the attached proximal part of the leaf stalk (fig. 4). The Coleus explant consists of a node with the pair of attached petioles, each cut to six millimeters in length, and ten millimeters of the stem proximal to the node.

The presence of molds or bacteria on or near the explants can seriously affect a process as sensitive as abscission. Aseptic conditions are maintained during experiments by the use of sterilized solutions and glassware, by flaming instruments, and by setting up and examining the explants in a small transfer room that is sterilized by means of an ultraviolet lamp (Westinghouse Sterilamp). A number of disinfectants were tested for their ability to sterilize explants without injury to the tissues and without affecting the rate or incidence of abscission. These included bromine water, sodium hypochlorite, tergitol #7[3], roccal, zephiran chloride, lignasan, calcium dimethyldithiocarbamate, calcium ethylene-bisdithiocarbamate, sodium ethylene-bisdithiocarbamate, and phenylmercury oleate. None was fully satisfactory; roccal gave the best results. The optimum treatment was found to be a ten minute immersion in a 0.006 per cent. roccal followed by blotting.

[1] This paper is based upon work sponsored in part by the Biological Division, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W–18–035–CM–208 with the University of California.

[2] Present address: Department of Biology, Reed College, Portland, Oregon.

[3] Sources of these reagents were as follows:
Tergitol manufactured by Carbide and Carbon Chemicals Corporation, New York;
Roccal obtained from Winthrop-Stearns, Inc., New York;
Ziphiran chloride manufactured by Winthrop Chemical Company, New York;
Various thiocarbamates and phenyl-mercury oleate obtained from Dr. W. H. Tisdale, Pest Control Research Station, E. I. duPont deNemours and Company, Wilmington, Delaware.

The explants were treated by mounting them on agar containing test substances, by immersion, by injection, by the application of small drops. Various methods of support were used. In the experiments with Citrus and Coleus and the early experiments with beans, the explants were mounted on 4 per cent. agar in Petri dishes. After the agar had set, an inch wide strip of it was removed along a diameter of the plate, leaving a narrow bridge of agar across one end to hold the agar in place and to serve as a marker. Ten explants were mounted on the agar of each dish, the shorter part of each explant (pulvinus or petiole) overhanging the central channel (figs. 2, 3).

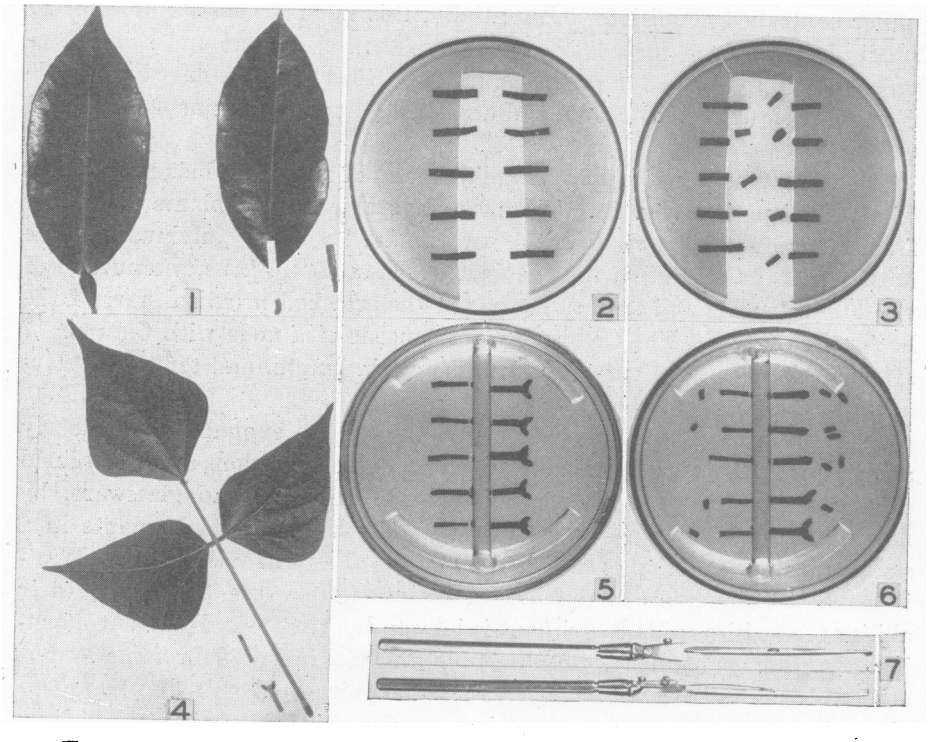

FIG. 1. Citrus leaves showing portion removed for an explant.
FIG. 2. Citrus explants on agar at the start of an experiment.
FIG. 3. Citrus explants on agar showing the effects of an experimental treatment.
FIG. 4. Bean trifoliolate leaf with explants.
FIG. 5. Bean explants on holders at the start of an experiment.
FIG. 6. Bean explants on holders showing the effects of an experimental treatment.
FIG. 7. Abscissor, two views.

In later experiments, bean explants were mounted on Pyrex glass racks (explant holders) within Petri dishes (figs. 5, 6, 8). A holder consists essentially of a horizontal glass rod supported by feet and with a row of five glass pins projecting from each side. Two per cent. agar is poured around the feet of the holder to prevent displacement and to maintain a high relative humidity within the Petri dish. Dishes of explants are kept in the dark at 25° C except for the short daily period of examination.

In some cases the explants are examined through the cover of the Petri dish. In other cases the cover is raised slightly and a standard pressure applied to the free end of each explant. This pressure is applied with an abscissor (fig. 7), a device employing the principle of the spring balance. By means of the calibrated displacement of its straight spring the investigator is able to apply a known force to the explant. The instrument was developed in order to produce stresses on the abscission zone similar to those ordinarily present under field conditions, and to provide a means for apply-

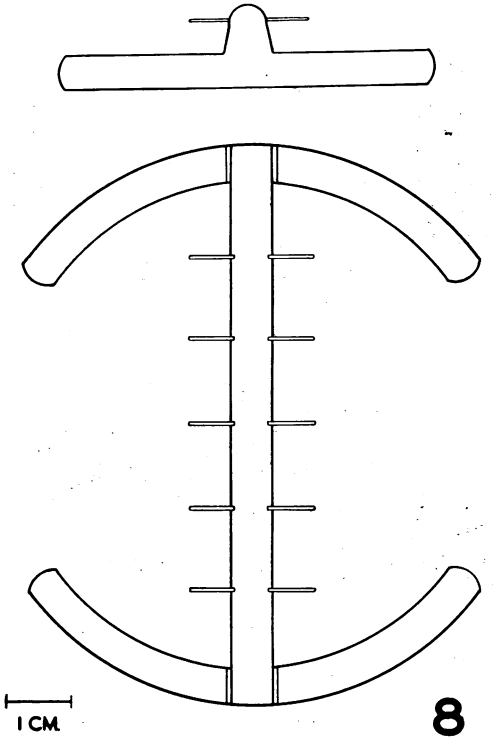

FIG. 8. Pyrex glass explant holder, two views.

ing a known force to the explants at regular intervals. Since, in the beans investigated, the leaflet weight is about one gram, the abscissor was adjusted to give a force of two grams, thus approximating the stress on the abscission zone in the field.

The technique described is a valuable tool for the investigation of leaf abscission under controlled conditions. In addition it gives promise of being adaptable to experiments with the abscission of flowers, fruits and stems.

We wish to acknowledge with gratitude the capable cooperation of Mr. H. Wilder Tomlin who constructed the explant holders and abscissor.

DIVISION OF BOTANY
UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA