C. A. SCHROEDER

Aspects of Citrus Fruit Growth studied in tissue cultures

Vegetatively propagated clones of tissues from citrus fruits in cultures are being used to study the factors controlling fruit growth and development. Light, aeration, temperature, and nutritional factors can be adjusted individually for study of their effects on each kind of tissue.

The fruit is surface-sterilized, and all materials are handled in a sterile transfer chamber. Uniform disks of tissue about one-twelfth inch thick and five-eighths inch in diameter are planted on nutrient agar—a sterilized culture medium—in glass vials.

Cell masses grown in tissue cultures are more or less homogeneous, with little differentiation into specific tissue types. Bits of such proliferating tissue are transferred periodically to fresh media to promote continued growth. Older cultures that have not been transplanted may give indications of extreme aging. Cell walls become woody and pitted, and growth is retarded.

Citrus rind consists primarily of parenchyma — fundamental tissue — with vascular conducting tissues scattered throughout. The undifferentiated parenchyma from the innermost tissues of the rind is suitable for culturing; the more specialized oil-bearing tissue and other cells of the outer rind have not been grown in cultures thus far.

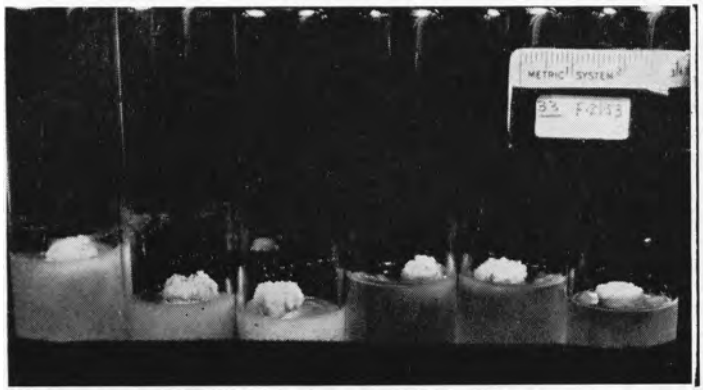
Tissue disks from the rind of nearly mature citron fruits begin to grow by cell division at their upper surfaces. In 3–5 weeks a disk may increase slightly in diameter and 4–5 times in thickness. Continuing new growth may envelop the original disk and spread over the agar surface, more than ¾″ in diameter. In 4–6 months the tissue may increase in weight up to 1,500%.

***Left*—Proliferating cell mass from stalk of lemon juice vesicle. *Right*—Juice vesicle which did not grow.**

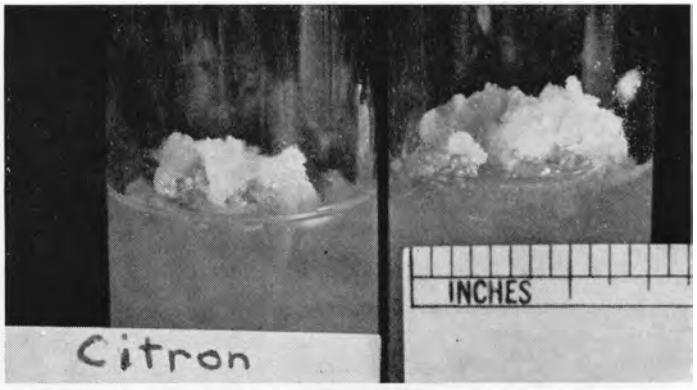
Rind parenchyma of lemon, citron, shaddock, and other citrus fruits has been cultured successfully. Continuous cultures of citron rind have been maintained for nearly four years. The tissues are still vigorous and appear to have an indefinite life if moved periodically to fresh agar.

The central pith or axis of citrus fruits is composed of well developed and prominent vascular strands, to which the seeds are attached. A considerable amount of parenchyma tissue separates the vascular strands and forms the bulk of the axis. Disks of pith parenchyma proliferate readily.

Each of the pulp segments is filled with juice vesicles—sacs in which the juice is produced—composed of many cells but simple in structure. The vesicles are attached by threadlike stalks, which reach to the outer portion of the surrounding membrane, just under the peel.



Citron rind tissue growing on agar nutrient medium.



Portion of tissue from citron rind grown 2½ years in culture.

Growth of stalk cells in cultures is apparent in two or three weeks, and within six months the tissue may increase in weight up to 26,000%. Cultures of stalk tissue from lemon have been maintained for more than a year and seem to possess unlimited growth potential, if transplanted at intervals. The enlarged sac portion of the vesicle develops less readily in cultures and its growth seems to depend on the presence and growth of the stalk.

Juice vesicle cells can grow and multiply on an almost entirely inorganic medium. This fact allows a study of the fundamental function of each chemical constituent of the nutrient medium in the development and nutrition of juice vesicle tissue.

Indoleacetic acid and gibberellic acid increase the growth of citron rind disks in culture. Either substance stimulates cell division when applied alone, and the two used together are additive in effect.

High temperature limits growth of tissues in cultures. Tissue disks grow much less at 90°F than at 77°F. The most favorable temperature conditions and the limiting temperature range for specific tissues are being determined.

Compared with the rate of growth in complete darkness, strong fluorescent light up to 400 foot candles appears to exert a slight depressing effect on the growth of rind tissue.

Such laboratory studies on tissue cultures may help to interpret various phenomena of fruit growth in the orchard.

C. A. Schroeder is Professor of Subtropical Horticulture, University of California, Los Angeles.

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WIND

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were compared: one, with 60% perviousness, 1½″ slats at 2″ spacing; another, with 50% perviousness, 2″ slats spaced at 2″; and the third, with 25% perviousness, had 2″ slats at ¾″ spacing.

The anemometer results were recorded continuously, but only periods when the wind was perpendicular or nearly perpendicular—10° off—to the fence line were used for the comparison. The average of four tests showed a close similarity of results. In all three surveys the wind velocity at the distance of 100′ downwind from the fence approached 100% of the upwind velocity, and more than 90% of the upwind velocity was beyond 60′ downwind from the fence. Nearer the fence the downwind velocities separated according to fence perviousness, but not a great deal.

The plotted curves of the downwind velocities should not cross on a graph because the three fences used did not represent extreme cases of perviousness. Only when a solid wall—known to protect very well immediately behind it but not at all beyond distances of ten times the wall height—is compared with a rather pervious obstacle, whose protection is mild near the fence, can the curves be expected to cross.

The rather small spread of the plotted velocities might suggest that the most pervious type of slatted fence windbreak —when lower cost and possibly less structural support because of lesser wind pressure are considered—should be the most reasonable for wind protection.

Herbert B. Schultz is Specialist in Agricultural Engineering, University of California, Davis.

Clarence F. Kelly is Professor of Agricultural Engineering, University of California, Davis.

Wilbor O. Wilson, Professor of Poultry Husbandry, and Lee Femling, Gerald Henderson, David van Rest, and C. R. Miller, Department of Engineering, University of California, Davis, assisted in the slatted fence windbreak experiments.

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TORTRIX

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the closeness of the fruits and feed from within this protection.

The orchard chosen for the trials suffered considerable damage from orange tortrix during 1958. In the winter of 1958–59, the larvae found feeding within mummified fruit on the trees were so numerous they indicated a potentially damaging population for the fruit season.

Orange Tortrix Test Plots in Yellow Newtown Pippin Apples. Watsonville, 1959.

Material	Dosage per acre*	Infested fruit %
TDE	10.6 lbs. 50% WP**	0.5
Sevin	7.6 lbs. 50% WP	1.8
Methyl Trithion	10.6 lbs. 25% WP	6.5
Ethion	10.8 lbs. 25% WP	10.3
Ethyl Guthion	7.7 lbs. 25% WP	11.3
DDT	10.4 lbs. 50% WP	15.3

* Application dates: May 5, June 11, July 28.
** WP: Wettable powder.

Materials chosen for trial—Guthion, Sevin, Ethion, and Trithion—had shown promise in previous codling moth trials. TDE was used as the standard insecticide and DDT was used as a check because it is not effective against orange tortrix. The ethyl formulation of Guthion, rather than the methyl form, and the methyl form of Trithion instead of the standard ethyl formulation were used.

Each treatment was applied to four trees with two replications in a randomized block plot design. Materials were applied with a conventional high pressure rig and orchard spray guns at an average of 500 gallons per acre per spray.

In an attempt to time the treatments, bait pans and a light-trap were used to capture adults, but so few were trapped the sprays were timed according to the local standard codling moth schedule.

At harvest, the test plots were evaluated by examining 300 fruits per replicate picked at random from the treated trees. Fruits with typical orange tortrix feeding scars were recorded as infested.

TDE gave the best control in the Watsonville experiment and Sevin was the only one of the other compounds that yielded commercial control. Ethyl Guthion, Methyl Trithion, and Ethion did not prevent damage in excess of the generally accepted economic level of 5% infested fruit.

During the experiment, the compounds were studied for their effectiveness on other pests. Ethyl Guthion and Ethion provided good control of European red mites, but it was necessary to add an acaricide to the other materials for the June 11 spray.

A light infestation of codling moth was noted on the fruit treated with Methyl Trithion and Ethion. None of the other plots showed any codling moth damage. Apple aphid and woolly apple aphid were present, but infestations remained at a low level because of extremely hot weather during June and July. The DDT and the TDE plots were the only ones to show a potentially damaging aphid population, and an aphicide was added to the June 11 treatment.

Fruit treated with Methyl Trithion showed russet spots on the skin beneath the dried spray droplets, but injury was superficial. None of the other compounds gave adverse effects.

Harold F. Madsen is Associate Entomologist, University of California, Berkeley.

Louis A. Falcon is Research Assistant in Entomology, University of California, Berkeley.