SPECIAL ARTICLES

THE ULTRACENTRIFUGAL CRYSTALLIZATION OF TOBACCO MOSAIC VIRUS PROTEIN

WHEN the clear juice pressed from plants[1] infected with tobacco mosaic virus is centrifuged at 25,000 r.p.m. (maximum field = 40,000 gravity), a pellet separates at the bottom of the tube. Under the polarizing microscope this solid is obviously crystalline. Tests show it to contain all but a small fraction of a per cent. of the virus initially present in the juice; we have accordingly sought to determine whether such crystals, obtained by high-speed centrifuging, are the same as those of the chemically[2] prepared virus protein.

One of the most distinctive characteristics of a crystalline species is its x-ray diffraction pattern. That of the chemically purified virus protein is known[3] to be well defined and to consist of many sharp lines. The x-ray method has therefore been chosen to compare the crystals made chemically and by ultracentrifugal precipitation. To do this, 5 cc portions (1) of plant juice clarified by filtration through Hyflo Supercel followed by freezing and a subsequent low-speed centrifuging and (2) of solutions of the chemically purified virus protein were centrifuged for three hours in an air-driven ultracentrifuge.[4] The quantity head containing the tubes of liquid resembled one recently described[5]; it had previously been used for the concentration of pneumococcic antibodies in serum.[6] Crystalline pellets from the bottom of the tubes after centrifuging were mounted directly in the holder of a gas-tight x-ray camera and attached to the usual diffraction outfit[7] installed in a cold room maintained at 36° C. Desiccation or deliquescence of the sample was prevented by filling the camera with helium containing an appropriate amount of water vapor. Measurements of the spacings and estimates of the relative intensities of the principal diffraction lines obtained from crystalline masses prepared (1) by chemical means, (2) by ultracentrifuging a solution of the chemically purified virus protein and (3) by ultracentrifuging the clear plant juice itself are listed in Table I. As the data suggest, the three patterns are indistinguishable one from another.

TABLE I

OBSERVED SPACINGS AND RELATIVE INTENSITIES OF X-RAY DIFFRACTIONS FROM CRYSTALLINE TOBACCO MOSAIC VIRUS PROTEINS

Intensities	Specimens of lines from samples crystallized by Ultra-centrifugation		
	Chemical means*	From solution of purified protein	From plant juice
Faint	37 A.	35 A.	36 A.
Very faint	28	....	....
Medium	20.8	20.7	20.6
Very faint	16.2 }	14.5	14.6
" "	14.2 }		
Strong	11.0	11.2	11.1
Faint	10.2	....	....
Medium	9.2	9.2	9.2
"	7.44	7.43	7.43
Faint	6.5	6.4	....
Very faint	5.7	5.7	....
Faint	5.44	5.45	5.44
Medium (probably not single)	4.95	5.02	4.94
"	4.71	4.72	4.71
"	4.44	4.46	4.46
Faint	4.08	4.10	4.10
Medium	3.88	3.88	3.88

* Data of reference 3. The camera used in photographing the ultracentrifuged samples did not have a sufficient radius and resolving power to record the spacings larger than 40 A.

CONCLUSION

From these observations it is apparent that a crystalline virus protein can be obtained directly by ultracentrifuging the juice of plants infected with the tobacco mosaic disease. The x-ray patterns of this crystalline material and of the protein prepared from the juice by chemical means are indistinguishable; the two substances, must, therefore, be substantially identical.

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A RELATION BETWEEN SEED ATTACHMENT AND CARPEL SYMMETRY AND DEVELOPMENT IN PRUNUS[1]

IN connection with more detailed studies of developmental morphology of deciduous fruits[2,3] and of the effect of embryo development upon fruit development,[4] a relation has appeared between carpel development and the position of seed attachment which is of some interest both in relation to fruit development and to the general topic of symmetry.

The observations cover several hundred specimens from different varieties of peach (*Prunus Persica* Batech), plum (*P. domestica* L., *P. insititia* L., *P. salicina* Lindl., and *P. americana* Marsh.), the sweet cherry (*P. avium* L.), the sour cherry (*P. Cerasus* L.), the apricot (*P. Armeniaca* L.), and the nectarine (*P.*

[1] We are indebted to W. M. Stanley, of the Department of Animal and Plant Pathology of the Rockefeller Institute, for the plant materials used in this study.

[2] W. M. Stanley, SCIENCE, 81: 644, 1935; *Jour. Biol. Chem.*, 115: 673, 1936.

[3] R. W. G. Wyckoff and R. B. Corey, *J. Biol. Chem.*, 116: 51, 1936.

[4] J. Biscoe, E. G. Pickels and R. W. G. Wyckoff, *Jour. Exp. Med.*, 64: 39, 1936.

[5] J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 64: 503, 1936.

[6] R. W. G. Wyckoff, SCIENCE, 84: 291, 1936.

[7] R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, 7: 35, 1936.

[1] New York State Agricultural Experiment Station Journal Series No. 165.

[2] H. B. Tukey, *Proc. Am. Soc. Hort. Sci.*, 30: 209–318, 1933.

[3] H. B. Tukey, *Proc. Am. Soc. Hort. Sci.*, 31: 125–144, 1934.

[4] H. B. Tukey, *Bot. Gaz.*, 98: 1–24, 1936.

Persica var. *nucipersica* Schneid). In all, 24 varieties have been examined.

The fruit of these species consists of a single carpel. On each carpel an anatropous ovule is borne, which is attached near the distal end of the ovarian cavity. Just before full bloom and prior to fertilization, one ovule aborts, although occasionally both may persist, more commonly in some varieties than in others. The remaining ovule develops into a seed.

It is common observation that many fruits of these species are asymmetric or unequally sided, the one side or "half" of the fruit being more fully developed than the other. Furthermore, the larger side may often be more highly colored, more highly flavored and may soften and ripen earlier. In some varieties this situation is pronounced, as in the Imperial Epineuse plum, where in some seasons the smaller side of the carpel remains hard and green and fails to develop to good edible and marketable condition, although the larger side matures and ripens properly. Likewise, the Crawford type peaches and fruits, with a pronounced bulge or "belly" along the ventral suture, show an uneven development of sides to a marked degree. In other varieties, with fruit smooth in contour, and more round or oval in shape, the unevenness of sides is less noticeable.

The pit is similar to the flesh in being more fully developed on the side which shows greater flesh development. This relation between pit and flesh is to be expected since the pit and the flesh together constitute the carpel, the inner portion of which becomes schlerenchymatous and hard and separates as the stony pericarp or pit, while the outer portion becomes soft and is the fleshy pericarp or edible portion of the fruit.

In cases of asymmetric fruits, examination of the stony pericarp and the point of attachment of the seed shows that the seed is attached to the larger and better developed side of the carpel (Fig. 1). In instances where both seeds develop, this difference between sides is not apparent and the fruits appear symmetric. It is possible to forecast the side to which the seed is attached by observing the larger and better developed side either of the fleshy pericarp or the stony pericarp. The fact that the symmetry of the stony pericarp is affected in the same manner as the fleshy pericarp indicates that the influence of seed attachment occurs early in the development of the peach, since the stony pericarp is fully outlined in the carpel at an early date and the stony pericarp has become hard by 49 days after fertilization[2] in a variety which requires 144 days to fruit ripening.

The arrangement of the vascular system of fruits of these species offers a ready explanation for this situation. Using the peach as an example, the dorsal side of the carpel (identified as a single line running from proximal to distal end) is homologous with the midrib portion of a folded leaf. The ventral suture (identified by its broadness and double fold appearance) is the region where the two edges of the carpel meet. It has been demonstrated[5] that although the edges of the carpel are adjacent, yet there is no actual union of edges along the ventral suture of the pit at the distal end. Accordingly, while the two ovules within the ovarian cavity are adjacent, they are separated morphologically from each other a distance equal to the transverse circumference of the fruit.

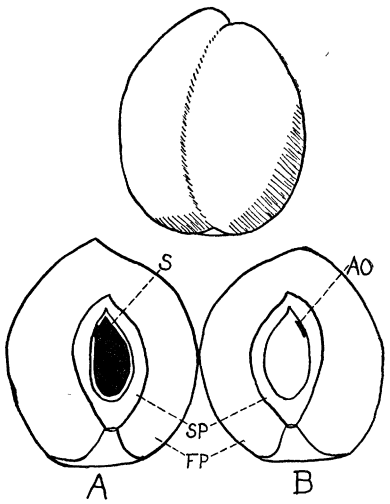

FIG. 1. Unequally sided peach fruit. The side of the carpel (A) to which the seed (S) is attached is more fully developed than the side (B) to which the abortive ovule (AO) is attached. SP = stony pericarp; FP = fleshy pericarp.

The two ventral bundles, which supply vascular connection to the ventral region of the fruit, are thus widely separated morphologically, although adjacent in their position in the fruit. Since a growing embryo establishes a deficiency gradient towards which food materials move and favors the development of the associated vascular system, it might be expected that the side of the carpel to which the embryo is attached would be the side more fully developed.

Finally, it is well known that the set of fruit is affected by fertilization and seed formation.[6] Moreover, seed development[7] affects the entire plant and may even result in the failure of female reproductive organs to develop in subsequently developing flowers, thus bringing about sterility of those flowers. More recently it has been shown that the development of an embryo within a developing fruit may have an even more local influence and may affect the development of that fruit as a whole.[3] Now it appears that

[5] C. H. Ragland, *Proc. Am. Soc. Hort. Sci.*, 31: 1–21, 1934.

[6] A. E. Murneek, *Proc. Am. Soc. Hort. Sci.*, 33: 4–6, 1936.

[7] A. E. Murneek, *Plant Phys.*, 7: 79–90, 1932.

in the case of a simple fruit the position of the embryo in relation to the carpel and its point of attachment to that carpel markedly affects the shape and development of a portion of the fruit. Thus, the dominating effect of the embryo upon fruit development is extended to even a portion of a carpel.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED AUTOMATIC RECORDER FOR IONOSPHERIC HEIGHT MEASUREMENT

THE purpose of this article is to describe a satisfactory method of photographically recording virtual heights of the various layers of the ionosphere without the use of complicated equipment. Errors of considerable magnitude which are likely to be overlooked by investigators, regardless of the nature of the recording equipment, are also discussed.

The general method of photographing radio pulse reflections from the ionosphere as seen on a cathode ray oscilloscope screen is not new, but usually the equipment is of special design and often quite complicated. The accuracy of the method described herein is practically as good as that of some of the intricate and expensive methods now in use.

A diagram of the oscilloscope screen (Fig. 1) shows from left to right the ground pulse, an E reflection and an F reflection, respectively. It will be observed that the entire screen is masked, with the exception of the base or zero-signal line. When a synchronous pulse is received, it causes the sweep line to be deflected upward behind the mask, leaving a blank space in the line.

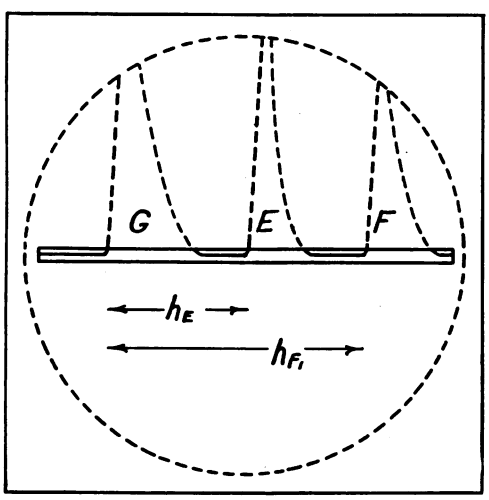

FIG. 1. Screen of oscilloscope showing ground pulse G with two reflected impulses E. and F.

The recording device consists of an ordinary camera focused on the oscilloscope screen and a hand-wound clockwork attached to the key for slowly winding up the film. It is obvious that if the clockwork runs at a constant rate, the time scale will not be linear, as the film will speed up with increasing roll diameter. After one film had been run through it was found that the speed at the end was practically double that at the beginning. In subsequent recordings, the governor on the clockwork was adjusted every half hour so that the film passed by the lens at nearly a constant rate. Also, a calibrating wave was introduced at half-hour intervals. Even this speed adjustment is an unnecessary refinement, since the time scale is marked off in desired intervals by the introduction of the calibrating wave or simply by closing the camera shutter for a few seconds.

The film from which the prints in Fig. 2 were made was driven at about six inches per hour, and each of the sections shown represents a fifteen minute observation. The distance between any two adjacent peaks on the 3,000 cycle calibrating wave shown at the end of the second recording represents a layer height of 50 km. The layer heights as measured at the beginning of the second recording are 115 km, 225 km and 355 km for the E, F-1 and F-2 layers, respectively. These particular runs were made soon after sunset on a frequency of 2,398 kc. Police stations operating on adjacent channels caused the five or six smears on the recordings. It is interesting to note that in the latter

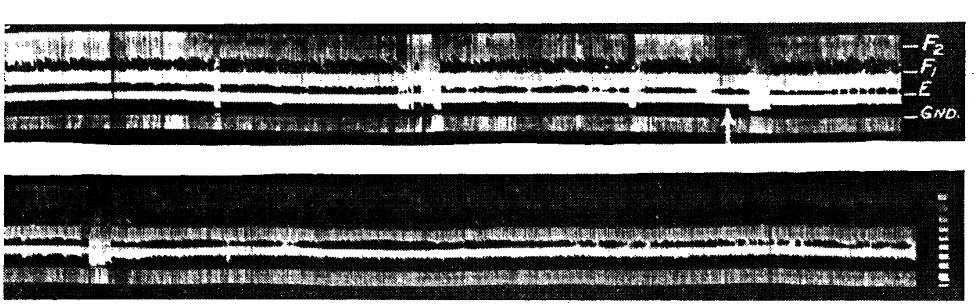

FIG. 2. Photographic records of ionospheric heights made shortly after sunset.