(*4*), and the hyaluronidase activity of the sperm suspension employed in the present control group was practically nil. However, the percentage of fertilized ova in the control group and in the experimental group was very similar. The motility of spermatozoa in the sperm suspension with or without hyaluronidase was the same; all showed active motility in the highly diluted form at room temperature for 6 hr. The role of hyaluronidase as determined by the observation of dispersing cumulus cells *in vitro* does not appear to be so important in fertilization as previous investigators have thought because fertilized ova were still observed in cumulus cells (*1, 2, 9*). The experimental findings here reported fail to support the claims made for the clinical use of hyaluronidase.

References

1. AUSTIN, G. R. *Nature*, Lond., 1948, **162**, 63.
2. BLANDAU, R. J., and ODOR, D. L. *Anat. Rec.*, 1949, **103**, 93.
3. CHANG, M. C. *Science*, 1946, **104**, 361.
4. ———. *Proc. Soc. Exp. Biol. Med.*, 1947, **66**, 51.
5. *Ibid.*, 1949, **70**, 32.
6. EMMENS, C. W., and SWYER, G. I. M. *Nature*, Lond., 1947, **160**, 718.
7. HADIDIAN, Z., and PIRIE, N. W. *Biochem. J.*, 1948, **42**, 260.
8. KURZROK, R. *Amer. J. clin. Path.*, 1948, **18**, 491.
9. LEONARD, S. L., PERLMAN, P. L., and KURZROK, R. *Proc. Soc. Exp. Biol. Med.*, 1947, **66**, 517.

A Simple Staining Technique for Detecting Virus Diseases in Some Woody Plants[1]

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During the course of biochemical studies on virus diseases affecting deciduous fruit trees, it was observed that virus infection induces the accumulation of phenolic compounds in affected tissues. A color test for these compounds was developed (*2*), which proved useful in the study of various stone-fruit virus diseases.[2] Further work suggested that if these phenolic compounds could be fixed in the tissue and subsequently stained, they might serve as a means of observing virus distribution in the plant. Such a test was devised for the staining of phenolic compounds in cleared leaf tissue. The stained compounds are not distributed uniformly throughout the tissue but are located in patterns which are characteristic for the type of virus and for the severity of infection. The technique is useful in detecting and studying what appear to be virus movement and distribution in plants capable of producing polyphenols similar to those produced by deciduous fruit trees.

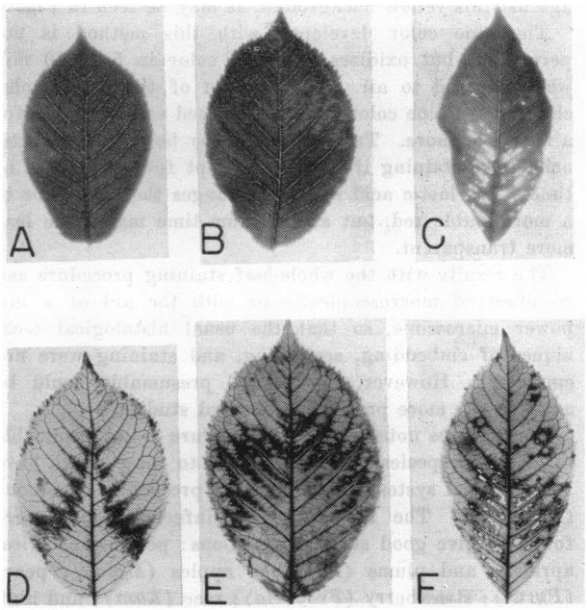

FIG. 1. Bing cherry leaves affected with ringspot. A, B, and C, before clearing and staining; D, E, and F, the same leaves after staining. The shot holes in C and F are caused by the virus.

The staining procedure consists of removing the chlorophyll, fixing the polyphenols, and developing blue-colored polyphenol compounds by treatment with NaOH. Whole leaves or sections of leaves were used. This procedure was also used with sections of petioles, stems, and roots. The decolorizing and fixing are performed in one operation by using a reagent composed of 700 ml of 95% ethyl alcohol, 20 ml of 37% formaldehyde, and 230 ml of distilled water. Samples may either be boiled in this solution under a condenser, or they may be heated in a water bath at 80° C until the chlorophyll is completely removed. When the clearing and fixing reagent becomes strongly colored, it should be discarded and fresh reagent added. One or two changes are usually sufficient. The chlorophyll from young leaves is removed in 5 to 10 min, but older leaves may take as long as 2 hr to clear. When decolorized, the leaves appear white or light yellow.

After decolorization, the samples are transferred to normal NaOH and heated at 80° C to 100° C until maximum color develops. The time of heating varies with the type and age of the sample but is usually from 2 to 10 min. A deep blue color indicates a test reaction between polyphenols and NaOH. Tissues in which these phenolic compounds are absent are yellow. The blue color of the phenolic compounds stands out sharply

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[2] A recent comment in *Science* (*1*), suggesting that our original color test for virus diseases was due to reducing substances, is in error. A small amount of copper sulfate was added to the reagent for the colorimetric test, to catalyze the oxidation of the polyphenols. The author of the comment assumed that a cuprous oxide precipitate was the basis for the test, whereas a red-colored solution was originally reported. Copper is not essential to color formation in this test.

against this yellow background, as may be seen in Fig. 1.

The blue color developed with this method is not permanent but oxidizes to a red color in 5 to 10 min when exposed to air. Acidification of the sample also changes the blue color to red. The red color persists for a month or more. Treated tissue may be placed in lactic acid after staining if it is to be kept for any length of time. The lactic acid not only changes the blue color to a more stable red, but at the same time makes the leaf more transparent.

The results with the whole-leaf staining procedure can be observed macroscopically or with the aid of a low power microscope, so that the usual histological techniques of embedding, sectioning, and staining were not employed. However, the method presumably could be adapted for more precise histological studies.

It should be noted that the procedure is not applicable to all plant species but is restricted to those which have a polyphenol system similar to that present in deciduous fruit trees. The following virus-infected plants were found to give good staining reactions: peaches, cherries, apricots, and plums (*Prunus*); apples (*Malus*); pears (*Pyrus*); strawberry (*Fragaria*); rose (*Rosa*); and high bush cranberry (*Viburnum*). Negative results were obtained with all the viruliferous herbaceous plants—other than the semiherbaceous strawberry—thus far tested.

Since this method is based upon the accumulation of certain polyphenols in virus-infected tissue, care should be taken in the sampling procedure and the interpretation of results, because conditions other than virus infection may induce accumulation of the same or similar phenolic compounds. Nectaries on the leaf normally stain very deeply. Any type of phloem blockage (girdling) may produce phenol accumulation. Similarly, mechanical injuries to the leaf often induce the accumulation of the phenolic compounds, as a wound reaction, immediately adjacent to the injury. Virus diseases, however, produce characteristic distribution patterns of the phenolic compounds and, therefore, can usually be distinguished from other more localized conditions.

The type of staining pattern produced in leaf tissue invaded by different viruses depends upon the kind of virus and the stage of development of the disease. In the case of western X-disease of peaches, a yellows-type virus disease, staining is confined to the phloem in the veins of the leaf during the early stages of the disease. As the disease progresses, localized areas of parenchyma tissue adjacent to the veins become involved, and finally most of the leaf stains deeply throughout. The ringspot diseases of cherries, either in latent or visible stages, produce striking staining patterns (Fig. 1). In the strictly latent stage the staining is confined to the phloem in the leaf veins. In those forms showing symptoms, even though so faint as to be nearly indiscernible, characteristic distribution patterns in the leaf parenchyma are produced that correspond to the symptom patterns.

Stem sections have not been as useful as those of leaves, because the nature of the tissue precludes the production of specific staining patterns. The staining is usually confined to the phloem, but xylem and phloem rays, pericyclic parenchyma, and sometimes cortical and pith parenchyma also will stain, depending upon the severity of the disease.

Genetic variegations may produce chlorophyll distribution patterns in a leaf that resemble virus disease symptoms. None of the genetic variegations tested has reacted to the stain test. Similarly, certain insecticides and other chemicals will induce viruslike symptoms in the leaf, and these also failed to give a stain reaction. The accumulation of polyphenols in virus-affected tissue is not completely understood, but apparently it is not related to the reactions involved in chlorophyll destruction. It seems likely that one or both of the following mechanisms is involved: (1) partial or complete blockage of the phloem, resulting in the dehydration of accumulated sugars to polyphenols; (2) mutual precipitation of virus protein and polyphenol, and by mass action a subsequent accumulation of polyphenol-virus aggregate. In the latter event, the staining reaction may be a test for the localization of inactivated virus particles.

References

1. KELLY, S. *Science*, 1949, **109**, 573.
2. LINDNER, R. C. *Science*, 1948, **107**, 17.

Biological Experiments on *Drosophila melanogaster* with Supersonic Vibrations

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Recently, supersonic waves have begun to be widely used by physicians to treat such diseases as rheumatism, ulcus cruris, etc. It is, therefore, necessary to make an exact examination of the biological effects of these vibrations. It is first of all important to know whether supersonic waves have any mutagenic influence on the genes.

Many biological experiments with supersonic vibrations (hereafter to be called S.V.) have been made, but mostly with the purpose of determining the killing effect against protozoa, bacteria, or small experimental animals such as mice. Hersh *et al.* (*2*), who made genetic experiments with *Drosophila melanogaster*, treated them in the air, not being aware that air cannot conduct sonic waves of high frequency. We have made some further genetic experiments by investigating the effects of S.V. on mutations and development in *Drosophila melanogaster*.

We used a wild stock of *Drosophila*, treating all stages of development, i.e., egg, larval, and pupal. The insects were placed in an isotonic NaCl solution in a tube of artificial resin, Trolitul, with a bottom of special thickness to let the waves through. This receiver was put on the vibrator head of a piece of equipment used by physicians (an ultravibrator) which has an intensity of 0.7–4 w/cm^2 (800,000 vibrations per sec). To exclude a heat effect, we cooled the receiver with water, thus allowing a treatment of over 25 min without a significant increase in the temperature of the NaCl solution.