Detection of Chromatographic Spots in Paper

Many substances which show no fluorescence or phosphorescence at room temperature do so on cooling. Accordingly, it was found that many substances which give no visible chromatographic spots do so if the paper is cooled in liquid nitrogen. The method of detection consists of simply dipping the paper into liquid N_2 and then viewing it in the dark in near-ultraviolet light. Since the paper itself becomes weakly phosphorescent under these conditions, spots can also be detected as dark areas if the substance in question quenches the phosphorescence of the paper. Many substances show an afterglow.

If no liquid N_2 is available, Dry Ice can be used in some cases. The paper is placed in a glass cylinder so that it touches the glass. The cylinder thus formed by the paper is then filled with crushed Dry Ice, and the paper is then viewed without removing it from the container.

This method of detection has the advantage that it does not entail the chemical alteration of the substance to be tested. The color and intensity of the phosphorescence may help in identification.

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Pink Discoloration in Eggs Caused by Sterculic Acid

Pink discoloration of eggs during cold storage is associated with the feeding of cottonseed oil or cottonseed meal to laying hens. This discoloration is a result of the combination of conalbumin of the white with ferrous ion of the yolk to form a pink complex (*1*). Increased permeability of the vitellin membrane allows diffusion of proteins and water into the yolk. Reverse diffusion of the complex into the white accounts for the pink color of the white. The yolk enlarges and becomes apricot-colored as a result of the blending of the pink color with the natural yellow of the yolk. The causative agent present in cottonseed has not been positively identified.

Lorenz (*2*) first observed that the component causing pink discoloration of eggs and the component responsible for the Halphen reaction (*3*) might be identical. The Halphen test is used to identify cottonseed oil since no other common oils give a positive test. Several uncommon oils, such as Kapok seed oil and *Sterculia foetida* oil, are also known to give a positive test. Kapok seed oil also causes pink discoloration of eggs. The fatty-acid composition of *S. foetida* oil was investigated recently. In addition to minor quantities of oleic, myristic, and palmitic acids, this oil contains a large proportion (70 percent) of an unusual C_{19} acid called sterculic acid (*4*). The structure of this acid (I) was first elucidated by Nunn (*5*). Verma *et al.* (*6*) disagreed with this assignment and proposed structure II. Other evidence supporting structure I has been presented by Faure (*7, 8*), who has shown also that pure sterculic acid gives a positive Halphen reaction (Fig. 1).

In this laboratory *S. foetida* oil (*9*) has been fed to laying hens and found to cause pink discoloration of eggs. When the *S. foetida* oil was hydrogenated sufficiently to eliminate double bonds but not to disrupt the cyclopropane ring structure, it did not cause pink discoloration. Six individually caged White Leghorn laying hens were divided into three groups and fed *ad libitum* for 15 days. The feed was the usual complete ration mixed with or without *S. foetida* oil in corn oil as follows: group I, 1.5 percent corn oil; group II, 1.5 percent corn oil and 0.09 percent *S. foetida* oil; and group III, 1.5 percent corn oil plus 0.09 percent hydrogenated *S. foetida* oil. At the end of 1 month of storage, the eggs were opened. The eggs from groups I and III were all normal, while seven of 11 eggs from group II showed definite pink discoloration.

Sterculic acid is the major constituent fatty acid in *S. foetida* oil. It is known that the other component fatty acids do not cause pink discoloration of eggs. Sterculic acid is, therefore, apparently responsible for the increase in permeability of the vitellin membrane which leads to pink discoloration of eggs (*10*).

Note added in proof: In a subsequent experiment, pure sterculic acid, $n_D^{28.3}$ 1.4632 [$n_D^{24.8}$ 1.4643 (*8*)], prepared by the urea complex method of Nunn (*5*) was fed to White Leghorn laying hens. Six individually caged birds were divided into three groups and, in addition to the basal ration, were fed daily by pipette the following: group I, 1 ml of corn oil; group II, 1 ml of corn oil and 0.10 g of pure sterculic acid; group III, 1 ml of corn oil and 0.025 g of pure sterculic acid. After 1 month of cold storage, eggs from groups II and III showed definite pink discoloration, while eggs from Group I were normal, thus confirming the conclusion that sterculic acid causes pink discoloration of eggs.

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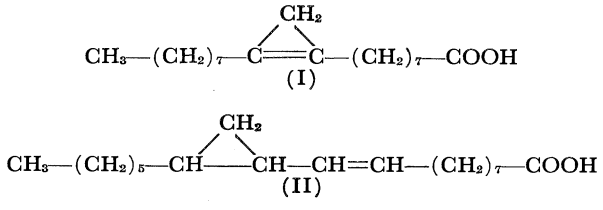

Fig. 1. Structures of sterculic acid proposed by Nunn (I) and by Verma *et al.* (II).

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9. The oil was extracted from seeds provided through the courtesy of F. R. Amos, director of forestry, Manila, Philippines.
10. This report is Arizona Agricultural Experiment Station technical paper No. 430. This work was supported in part by the National Cottonseed Products Association, Inc.

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Rapid Symptoms in Seedling VII Sweetpotato of a Virus Always Associated with Internal Cork

The rapid mechanical transmission of a virus (*1*) consistently associated with sweetpotato cork virosis to Scarlett O'Hara morning glory (*2*) opened up a new approach to study of this virus by reducing the incubation period from about a year on sweetpotato to a week on the morning glory. This discovery posed the possibility of finding a sweetpotato plant that would respond as promptly as the morning glory with distinctive symptoms to the same method of inoculation.

After numerous transmission experiments in 1955, there was no doubt that the same mechanical technique transmitted the virus from sweetpotato or other suscepts to the various sweetpotato varieties and seedlings under test, but the expression of symptoms in sweetpotato was poor, and the incubation

period was longer than a month. Besides, the rapid indexing technique revealed that practically all the sweetpotato varieties in our collection were virus-contaminated. This was checked and verified by the "flush of growth" technique in the greenhouse and by the "surge of growth" technique in the field (*3*).

In the search for a satisfactory sweetpotato indicator plant, the first problem was to find virus-free clones. Since practically all the commercial sweetpotato varieties are virus-infected, the next step was to examine and study seedling clones. Seeds of the Porto Rico-type sweetpotato were obtained from the Agricultural Experiment Stations of Louisiana and Georgia for this purpose. Thirty such seedlings tested in 1955 were found to be virus-free, but none was satisfactory as an indicator plant.

In 1956 over 300 seedlings were produced and tested. The seeds were from open-pollinated Porto Rico plants grown in Louisiana and Georgia. To expedite this work, we used only graft transmission. Of several methods of grafting, the "chip-bud" method (*4*) was found to be superior and it was adopted as standard practice in these studies. Again, all the seedling clones were found to be virus-free.

In two experiments involving 129 seedling clones, one designated as clone VII (from seed from Louisiana) exhibited clear-cut chlorotic spot leaf symptoms 3 weeks after it had been grafted with diseased buds; in contrast, longer periods were required for all other clones of seedlings and of the named varieties that were tested. The clones had been grown for 2 months in 4-in. pots and then pruned back to eight nodes and transplanted to 6-in. pots at the time of "chip-bud" inoculation.

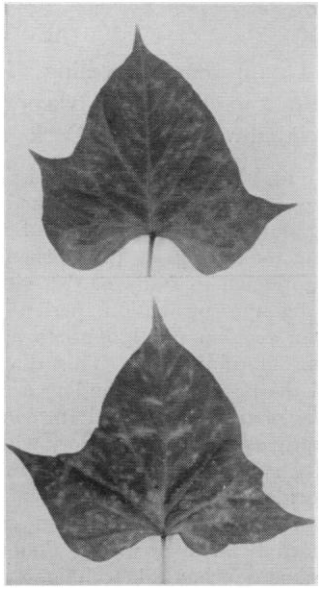

Fig. 1. Chlorotic spot symptoms of a virus associated with internal cork on sweetpotato clone VII (bottom) 24 days and (top) 12 days after mechanical inoculation by the rapid transmission method.

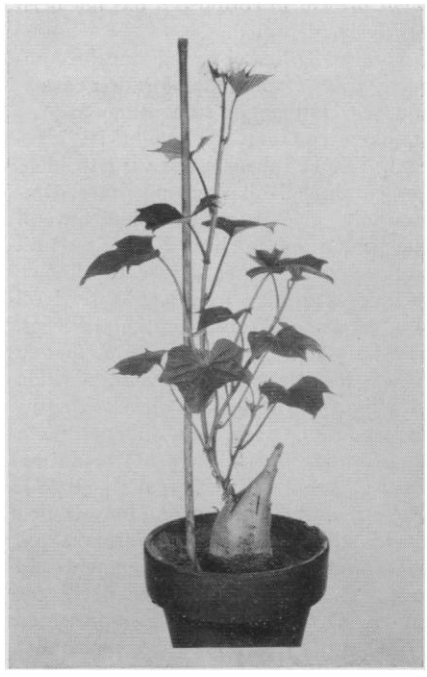

Fig. 2. A stab-graft at the end of 24 days. The graft was of a healthy clone VII shoot on a diseased Porto Rico root. Chlorotic spot symptoms had begun to show on the new growth 17 days after grafting.

Following this discovery, a third experiment was set up involving clone VII and a mechanically inoculated Scarlett O'Hara morning glory check, both replicated five times. Chip-buds were placed at the third nodes, and the sweetpotato plants were pruned back to the fourth nodes to stimulate a spurt of growth.

Chlorotic spots appeared on the new growth from the buds in the axils of node 4, beginning on the seventh day and becoming more distinctive later. Figure 1 illustrates these symptoms on the 12th and 24th days. The mechanically inoculated Scarlett O'Hara check also showed typical vein-banding mottle on the seventh day and vein-clearing by the 12th day.

Another experiment compared the merits of the graft and mechanical methods of inoculation of clone VII, with Scarlett O'Hara as a check, when diseased clone VII was used as the source of inoculum. The grafted plants showed symptoms on the seventh day, as in the previous experiment, but clone VII and Scarlett O'Hara, when inoculated mechanically, showed no symptoms until the 11th day. The delay in symptom expression following the latter method of inoculation was attributed to the probability that sweetpotato clone VII contained less virus than did infected plants of Scarlett O'Hara which had been used in previous experiments.

The fifth experiment tested the mechanical method of inoculation on clone VII plants having five leaves unfolded and on the Scarlett O'Hara check. This time, three sources of cork virus were tested in triplicate on three conditions of plants of clone VII: (i) plants unpruned, (ii) plants with two leaves removed, and (iii) plants with four leaves removed. The bottom leaf was inoculated in all cases. The three virus sources were sweetpotato clone VII, Scarlett O'Hara containing virus isolated from symptomless Nemagold sweetpotato roots, and Scarlett O'Hara containing virus from Porto Rico roots with symptoms of internal cork.

On the seventh day, symptoms of vein-banding began to show on Scarlett O'Hara plants that had been inoculated with inoculum 3. These symptoms became more marked with time. On the same day, symptoms produced by inoculum 3 were most advanced on the unpruned clone VII plant, but they were also present on the plant that had had two leaves removed. With inoculum 1, the Scarlett O'Hara check showed the first vein-banding symptoms on the 21st day, a week later than the appearance of chlorotic spots on clone VII. Thus, clone VII appeared to be equal to if not better than Scarlett O'Hara as an indexing host when small amounts of inoculum were present. In all cases, the shock of removal of all but the inoculated leaf (the standard procedure for Scarlett O'Hara) interfered with early symptom expression on clone VII. The removal of two leaves caused a short delay in symptom expression. The unpruned clone VII plants, with five leaves, were in a good growth status for the prompt appearance of symptoms.

Clone VII has been found valuable in other studies on internal cork. Scions of clone VII, when stab-grafted (*5*) into suspected diseased sweetpotato roots, demonstrated the presence of virus by positive chlorotic spot symptoms on the first new scion growth (Fig. 2). Clone VII also proved to be excellent for use in insect transmission experiments. It served just as well as Scarlett O'Hara in aphid transmission studies (*6*).

In conclusion, these studies revealed clone VII (a sweetpotato seedling from open-pollinated Porto Rico seed from Louisiana) as an excellent indicator plant or indexing host for the virus associated with internal cork whether used in mechanical, graft, or insect transmission experiments.

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References and Notes

1. The virus under investigation is one that has been found constantly associated with root and foliage symptoms of internal cork disease. This sweetpotato virosis received its name from the root symptom phase. Studies are in progress to determine the relationship of associated symptoms such as leafspot, chlorotic spot, ringspot, chlorotic ringspot, purple ringspot, feather, and oak-leaf with the typical root symptoms.
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4. A chip of diseased stem tissue with a bud in the middle, and measuring about ½ in. in length, was removed with a sharp razor blade and transplanted to an identical position on the healthy plant stem. It was held in place by a ½-in. strip of Stericrepe rubber.
5. With a scalpel, vertical stab wounds are made in the upper end of a sweetpotato root. The root is bedded upright in sand. Into each wound a snug-fitting, wedge-shaped scion is inserted. The scions usually establish vascular connections in 7 to 10 days.
6. E. M. Hildebrand, in preparation.

28 June 1957

Bone Crystallites as Observed by Use of the Electron Microscope

In 1949 Wolpers (*1*) observed, by use of an electron microscope, that the mineral portion of bone consists of needle-shaped crystals 30 to 60 A wide and 400 to 1000 A long. However, later electron microscopic studies by Robinson *et al.* (*2*) indicated that bone crystals are hexagonal platelets having average dimensions of 500 A long by 250 A wide by 100 A thick. Robinson's concept has been accepted by most workers in this country for several years. In 1953 Schwarz and Pahlke (*3*) interpreted electron micrographs to indicate that the calcareous (mineral) deposits in bone are spindle-shaped particles 150 to 1300 A long. From 1953 to 1957 Finean and Engstrom (*4*) presented evidence from x-ray diffraction studies indicating that bone contains rod-shaped, apatite crystallites 40 to 75 A wide and about 200 A long. Recently, Fernandez-Moran and Engstrom (*5, 6*) observed a predominance of rod- or needle-shaped apatite particles 30 to 40 A wide and about 200 A long in electron microscopic studies of undecalcified bone sections (human, rat, hen, and fish).

Electron microscopic studies of bone have been made in this laboratory (*7*). Sections (6 μ thick) of frozen-dried, methacrylate-embedded, undecalcified bone were obtained by routine methods (*8*). These were re-embedded in methacrylate with the desired orientation and sectioned at 90° to the plane of the 6-μ section. Satisfactory sections were obtained by using a diamond knife (*9*) in a Porter-Blum microtome (*10*). The sections were mounted on a grid coated with a carbon membrane and examined, using a 100-kv beam in an RCA EMU-3 electron microscope. The thickness of the sections was nominally 250 A. However, for best results it was necessary to study selected areas which may have been somewhat thinner than the average section thickness.

Inspection of many sections of normal, mature, cortical bone taken from humans and dogs showed an abundance of rod-shaped particles situated in groups or bundles in the plane of the section (Fig. 1). In other sites they appeared to be less regularly arranged, probably because of their oblique orientation. From considerations of relative densities, these are considered to be the inorganic crystallites. These crystallites were about 50 A thick and ordinarily 600 to 700 A long. Occasionally longer (about 1200 A) and frequently shorter (down to 200 A) particles were seen. Because the lengths of the particles may be several times the thickness of the section, it is apparent that the significance of these values (especially the smaller ones) is open to question until a method can be devised to demonstrate that the particle lies in, and not oblique to, the plane of the section. The volume occupied by the mineral component, from considerations of ash weights and densities of the organic and mineral components, is approximately 40 percent, a figure which is generally compatible with the appearance of these electron micrographs. The remainder of the volume is composed of areas of lower electron density in which the periodic banding that is typical of collagen fibers could sometimes be observed.

More recently it has been possible to section unembedded cortical bone, thus allowing examination shortly after sacrifice of the animal and without previous chemical treatment. Preliminary studies of such sections have shown structures

Fig. 1. Electron micrograph of a section of undecalcified cortical bone from the mid-shaft of the femur of an adult dog. The section shown is parallel to the long axis of the bone.

indistinguishable from those described above for sections embedded in methacrylate.

These electron micrographs are interpreted to show that the crystallites of bone are rod- or needle-shaped structures and not hexagonal platelets as reported by Robinson *et al.* (*2*). Fernandez-Moran and Engstrom (*6*) reported evidence of fine structure within these rod-shaped particles. However, our studies so far have failed to support this observation. Currently we are investigating the relationship of these crystals to the organic fibers of bone.

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10. The technical assistance of O. T. Minick of the Electron Microscope Laboratory is gratefully acknowledged.

5 August 1957