HARRY C. BURNETT

The Color Test for Exocortis Indexing in Florida

THE FLORIDA Budwood Registration Program was established in 1952 to enable growers to obtain a source of citrus budwood free from several serious virus diseases known to be present in Florida. Citrus trees become registered when they have been screened for tristeza, psorosis, xyloporosis, and exocortis. Indexing for tristeza can be completed within 6 months; for psorosis, 2 years are required; for xyloporosis, 4 years; but for exocortis, 8 years.

Since the period required for accurate detection of exocortis by nursery indexing methods only was so prolonged, a color test was developed by Childs, Norman, and Eichhorn (1) and is now a part of the indexing program. It is used in conjunction with other tests for exocortis in *Poncirus trifoliata* in the Division of Plant Industry.

Method of Using the Color Test in the Florida Program

The steps involved in screening trees for exocortis in the Florida Budwood Registration Program are as follows:

1. Buds from each candidate tree in the Budwood Registration Program are inserted in two *Poncirus trifoliata* seedlings. Both seedlings are given the same accession number, but one is designated as W (west) tree and the other as E (east) tree. The color test for exocortis is made on seedlings budded at least two years previously in the Florida Program.

2. A piece of bark approximately 3/4-inch long by 1/4-inch wide is cut from the tree. If one of the seedlings is not sufficiently large in diameter that the bark sample can be taken without endangering the

life of the tree, the bark specimen is taken only from the larger of the two trees. Childs *et al.* (1) suggest taking these samples at the bud union. However, Sinclair and Brown (3) have reported quite recently that scaling due to exocortis first appears on the side of the large roots near the soil line and spreads during the next 4 to 6 years over the entire rootstock, up to the bud union. Since publication of their paper, our bark samples have been taken below the bud union, near the soil line. As yet there is little evidence that any one point between the soil line and the bud union is to be preferred more than another. Positive readings have been made from all areas within the above limits. Perhaps the chief advantage in taking the bark sample near the ground level is that there is more likelihood of getting only *Poncirus trifoliata* bark and none of the scion top, since it is often difficult to determine the location of the bud union on young budded trees.

Immediately after excision, the bark samples are placed in a formaldehyde-acetic acid-alcohol solution, FAA No. 2 (2), for killing and fixation.

3. Transverse sections of bark samples about 20 to 30 microns thick are made on the sliding microtome. Ten sections are placed on a microslide.

4. A few drops of phloroglucinol-HCl reagent are added to the bark sections and a cover glass is eased into place. The staining process is complete within 3 or 4 minutes and results remain substantially unchanged for 4 hours. The phloem ray cells of the bark are the chief objects of study. The phloroglucinol-HCl reagent produces a red color reaction in some to many of the ray cells in *Poncirus trifoliata* bark if exocortis infection is present.

An arbitrary system of evaluating the sections was arrived at after consultation with Dr. J. F. L. Childs. When 3 or more cells are stained red in 10 sections, a positive diagnosis is given. When 2 are found, the diagnosis is called "probably positive" or "positive?". A "probably negative" designation is given when only one cell is stained in 10 sections, and, of course, a negative determination is given when no cells are found stained red.

Results Obtained with the Color Test

As of September 15, 1960, a total of 570 samples involving 322 candidate trees has been examined for the presence of exocortis. Of these,

150 were diagnosed as positive, 45 as probably positive, 333 as negative, and 42 as probably negative.

All except 6 of the trees were budded between April, 1955, and June, 1956. The 6 exceptions were budded in 1957. Final field determinations cannot be made until April, 1963, and June, 1964, for most of the trees under study. However, a correlation of the results obtained with the color test with the corresponding plants in the test plots is as follows:

Of the plants that were diagnosed as negative or probably negative 325 are still symptomless in the test plots; 98 plants that were diagnosed as positive or probably positive are now showing bark scaling in the test plots; and 97 read as positive are still symptomless in the nursery test plots. In 22 of the latter cases, the corresponding west or east tree is showing exocortis scaling symptoms. Color test results showed most of these to be positive 3 or 4 years after they were budded, yet after 4 or 5 years they still are without visible bark symptoms in the test plots.

There are 50 cases where a negative diagnosis by the color test was given, and the plants later showed positive bark symptoms in the nursery. The plants in question had been budded 3 or 4 years, and in most cases the field symptoms did not show until a year after the use of the color test. Tests were rerun on 17 of these 50 trees soon after field symptoms were noted. All 17 gave a positive reaction when retested. For 10 of these 50 trees, the color test gave a positive reaction for the adjoining east or west tree approximately one year before the field symptoms were noted.

Childs *et al.* (1) report that (a) the reaction to phloroglucinol is not exhibited uniformly in all the rays, (b) in a given specimen, particularly from a young tree, there may be only a few rays with reactive cells, and (c) there is some chance that reactive ray cells will not be present in a given bark specimen. It seems probable that, in the case of these 50 trees, if another sample had been taken, either at the same time or a few months later, we might have secured a positive test.

As pointed out earlier in this paper, the limiting factor for resampling a given tree is the danger of girdling too large a part of the trunk. However, since it was found that a color reaction may be obtained at any point from the soil line to just below the bud union, we have a much larger area from which we may secure our bark samples for testing. Therefore, if bark samples could be taken in the summer of the third year after budding, and again in the spring and summer of the fourth year, the chances for correct diagnosis, both negative and positive, would be greatly increased.

This, of course, can be confirmed only by waiting until 1963 and 1964, the end of the 8-year test period. If the results obtained from the application of the color test still correspond to the symptoms in their counterparts in the test plots, then we will know that indexing candidate citrus trees for exocortis can be completed by use of the color test within 4 years from the date of the original budding in *Poncirus trifoliata.*

Literature Cited

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2. Rawlings, T. E. 1933. Phytopathological and botanical research methods. John Wiley & Sons, Inc., New York. 156 p.
3. Sinclair, J. B., and R. T. Brown. 1960. Effect of exocortis disease on four citrus rootstocks. Plant Disease Reptr. 44:180-183.