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## **Pollen Transmission of Avocado Sunblotch Virus Experimentally Demonstrated**

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Transmission of avocado sunblotch virus from infected mother trees to progeny seed has been known for some time as has the fact that the rate of transmission of the virus is higher in seed from recovered, symptomless carrier trees (80 to 100 percent) than from trees showing symptoms (0.5 to 5 percent). In several instances, too, young avocado seedlings of various varieties have been found to be infected with the virus when the seed-mother trees were found free of the virus. Since the infected progeny seedlings had germinated and grown under conditions which precluded the possibility of transmission by natural root graft, the most logical explanation for their infection was that the virus was transmitted by pollen and, furthermore, since the progeny seedlings exhibited symptoms as seedlings, it appeared unlikely that their infection was the result of rare mechanical transmission.

As a result of these observations, research was initiated to demonstrate pollen transmission of the virus. Early in the studies we did demonstrate that the virus was present in pollen grains. This was accomplished by implantation of pollen grains, completely free of other floral parts, from flowers of infected trees under the bark of test seedlings. The virus was successfully transmitted to seedlings utilizing this technique.

This did not, however, conclusively prove that pollen transmission occurs in nature. When attempts to effect transmission by hand pollinations proved unsuccessful because of various difficulties, especially low fruit set, honey bee pollination was utilized with a single healthy Zutano variety tree serving as the pollen recipient. It was enclosed in an insect-proof cage in the field with four virus-infected trees (growing in 32-gallon metal cans) serving as pollen donors. In the first two years of the experiment (1977 and 1978 growing seasons), the virus-infected, pollen donor trees included two with sunblotch symptoms (Hass and Topa Topa varieties) and two symptomless carriers of the virus (Caliente and Ryan varieties).

### **'A' and 'B' flowers**

Bergh has shown that the flowers of avocado varieties are of two types, "A" and "B." In each type the male and female organs of the flower do not function at the same time. The stigma (female organ) of type "A" flowers will receive pollen in the morning of one day, but their stamens (male organs) will not shed pollen until the afternoon of the second day. In "B" type flowers, the stigma will receive pollen in the afternoon, but their stamens will not release pollen until the following morning. Therefore, the maximum fruit set, interplanting of varieties with both flower types is recommended. Because the

pollen recipient tree (Zutano) has "B" type blossoms, the pollen donor trees were selected to include complementary "A" type varieties to insure maximum cross pollination.

A healthy Zutano field tree was selected as the source of seed for controls. This tree was left uncaged for three reasons:

- (1) Caging the tree without bees would result in no fruit set,
- (2) Caging the tree alone with bees would probably result in low fruit set inasmuch as only "B" type blossoms would be present, and
- (3) Healthy flowering trees with complementary type blossoms were not available in moveable containers.

When the blossoms on the trees were at the proper stage, a colony of honey bees (*Apis mellifera* L., variety Italian) was introduced into the cage in the form of a 6-frame mini-hive. This consisted of 4 frames of brood (with about 7,500 bees) and one frame each of capped honey and stored pollen. Water was also provided. The honey, pollen, and water were replenished as needed. When blossom production had ceased, the bees were removed, the cage was disassembled, and the virus-infected trees were moved to another location.

### **Verifying symptoms**

During the 1977 growing season, 111 fruit set on the pollen recipient tree. Seed from these fruit were planted along with 120 seed from the healthy Zutano tree which served as controls. All were examined periodically for sunblotch disease symptoms. Two seedlings from the pollen recipient tree exhibited symptoms of sunblotch. To verify that the seedlings were infected with the virus, eight healthy avocado seedlings were inoculated with buds and bark patches from each seedling with symptoms. After two months, the 16 test seedlings exhibited sunblotch symptoms, thereby confirming the presence of the virus in the two seedlings.

The remaining 109 progeny seedlings from the pollen recipient tree which did not exhibit symptoms of sunblotch were budded with healthy Fuerte variety buds to see if they were carrying the virus in the manner of symptomless carrier trees. To date, these are all negative for sunblotch, but are still under observation. All of the seedlings in the 1977 control group have also been negative for sunblotch.

The whole experiment was repeated during the 1978 growing season, and a crop of 64 seed was harvested. These were planted along with 20 seed from the healthy Zutano control tree. Two seedlings grown from the 1978 crop exhibited symptoms of sunblotch and are known to be infected. The remaining 62 progeny seedlings from the pollen recipient tree and the 20 control seedlings have not exhibited symptoms. The 62 symptomless seedlings from the 1978 crop are also being tested to see if they are carrying the virus in the manner of symptomless carrier trees. So far, results are negative.

The experiment has been repeated during the 1979 growing season with the only essential difference being that the virus infected pollen donor trees are all recovered,

symptomless carrier trees.

Subsequent to the fruit harvests from both the 1977 and 1978 growing seasons, tests were made to determine whether the Zutano pollen recipient tree had become infected with the virus while pollen transmission was occurring. After the 1977 harvest, 12 healthy seedlings were inoculated with five buds each from the pollen recipient tree. Each bud was from a separate budstick, and thus 60 budsticks in all were utilized. So far, all of these test seedlings are negative.

Similar tests following the 1978 harvest are underway, and the test seedlings are still under observation. To date, there is no evidence that the virus has moved into the pollen recipient tree. However, because definitive evidence concerning possible transmission of the virus to the pollen recipient tree is of considerable importance, additional tests will be conducted following the harvest of fruit from the 1979 growing season.

Pollen transmission of sunblotch virus in avocado has thus been experimentally demonstrated. Fortunately, the indicated rates (1.8 percent for 1977 and 3.125 percent for 1978) were rather low, but the fact that it does occur is of considerable practical importance. It would appear to be of special importance in the Indexing Program to establish virus-free sources of seed and budwood. Perhaps the regulation regarding the minimum distance between a candidate tree and a known or suspected virus-infected tree should be reconsidered. The present minimum distance of 50 feet, which precludes natural root grafting, seems to be a rather small distance in view of possible pollen transmission of sunblotch virus.

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