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Chemical Inactivation of Avocado Sunblotch Viroid on Pruning and Propagation Tools

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Introduction

By 1974, several lines of evidence suggested that the causal agent of the avocado sunblotch disease is a viroid rather than a virus (1, 2, 3). Because of this, we redoubled our efforts to mechanically transmit the disease agent, for the ability to do so would be useful in studying both the causal agent and the sunblotch disease.

Because the "rubbing" or "abrasion" technique widely used for mechanically transmissible viruses and some viroids was not successful (2, 3), we used the "slash" technique first described and successfully used by Semancik and Weathers for transmitting citrus exocortis viroid (8). This technique enabled us not only to transmit the sunblotch viroid directly from diseased to healthy seedlings, but permitted the assay for sunblotch viroid in various fractions extracted from diseased tissue (2, 3, 5) and the transmission of highly purified viroid (9).

Although in the earlier mechanical transmission studies the rate of transmission of the viroid from avocado directly to avocado was only 13.7%, the potential of mechanical transmission of the viroid by contaminated pruning and propagating tools does exist (2, 5). Because it was shown that sodium hypochlorite (chlorine bleach) inactivates citrus exocortis viroid, we recommended, from the start, that it be used to inactivate the sunblotch viroid by treating pruning and propagating tools used for avocado culture (4). We did, however, initiate experimental studies with sodium hypochlorite and two other chemical agents to demonstrate inactivation of sunblotch viroid. This paper is the report of the final results of these studies.

Materials and Methods

Chemical Agents. Three agents were tested. Sodium hypochlorite at a concentration of 20% of the commercial product was tried as we previously recommended (3). The second agent was an aqueous solution containing 2% formaldehyde and 2% sodium hydroxide. The third agent tested was a solution of 6% hydrogen peroxide which had been freshly diluted from a 30% commercial solution.

Test Plants. Fifty-four 'Hass' variety seedlings were utilized. Twelve seedlings were used for testing the efficacy of each of the three chemical agents. Twelve seedlings were used to test the effectiveness of simply washing the cutting surface with water. The remaining six plants were inoculated with the viroid for untreated controls.

Preparation of Inoculum. The sunblotch viroid inoculum was prepared by the method of Utermohlen and Ohr (10) which was modified slightly. The modification involved

dialysis of the resuspended second alcohol precipitate against TKM buffer (Tris, KCl and $MgCl_2$> pH 7.4 for 36 hours.

Inoculation Method. A new single-edged razor blade was used for each individual seedling. The razor blade was dipped in the viroid suspension, then "washed" with the chemical agent or with water, then used to inoculate the seedlings by the "slash" technique. This was repeated 10 times for each seedling. The procedure was the same for the six control seedlings except that the washing step was omitted.

Results and Discussion

The results of the research are summarized in Table 1. All three chemical agents prevented infection of the avocado seedlings by the slash technique. One should keep in mind that the actual slash procedure was repeated 10 times on each seedling in each group which means that had there been no inactivation, there would have been 120 opportunities to establish infection with each test group. The water wash treatment served as a non-chemical control in the experiment. Apparently just washing with water diminishes the chance, but does not completely eliminate the possibility, of infection.

We feel that symptomless carrier trees could play an important role in the accidental transmission of sunblotch viroid on contaminated pruning and propagating tools (5). Although gel electrophoresis tests indicate that the concentration of the viroid in symptomless carrier tree tissues is somewhat lower than that found in symptomatic tissue from symptomatic trees, the viroid is apparently more uniformly distributed in symptomless carrier trees than in symptomatic infected trees which have not gone through the recovery process (1, 7, 10). Because of this more uniform distribution, symptomless carrier trees might be the main source of the viroid in the spread of the viroid by mechanical transmission during cultural practices.

We have shown that symptomless carrier trees can serve as a source of pollen containing the viroid in pollen transmission of the viroid (6). We have also shown that the pollen recipient tree does not become itself infected during the course of pollen transmission (6). However, the fact that infected progeny seedlings resulting from pollen transmission can sometimes be symptomless carriers (6) would seem to increase the chances of such trees being a reservoir for the viroid in accidental mechanical transmission.

Based on the results described above, we recommend that pruning and propagating tools be treated with one of the chemical agents used in this work as an extra precaution to prevent accidental mechanical transmission of the sunblotch viroid.

TABLE 1. Effects of Chemical Agents on Sunblotch Viroid.

Treatment	Results	
	After 2 years	After 4 years
Sodium hypochlorite	0/12[a]	1/12
2% formaldehyde plus 2% NaOH	0/12	0/12
6% Hydrogen peroxide	0/12	0/12
Water wash	0/12	1/12[b]
Untreated control	6/6[c]	6/6

a. Numerator = Number of infected seedlings. Denominator = Number of plants inoculated.

b. The single infected seedling read positive after 2 years and 4 months.

c. All untreated controls read positive between 14 and 24 months after inoculation.

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