Virus-Specific ^{125}I-Labeled Antibodies as a Possible Tool for Indexing Cantaloupe Seeds for Squash Mosaic Virus

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ABSTRACT

Small pieces of seeds from healthy and squash mosaic virus-infected seed lots were treated with ^{125}I-labeled antibodies. Virus-infected seed lots could be distinguished from healthy seed lots; however, the method could not be used to predict the percentage of virus transmission. Phytopathology 60:1854-1855.

The transmission of squash mosaic virus (SqMV) through seeds from infected plants is a major source of its introduction into commercial plantings of cantaloupe in California (3). Since this virus has a very narrow host range (1), prevention of widespread field infections would be possible if clean seed were available. Standard indexing techniques for detecting seed-borne viruses are tedious and time-consuming, entailing the planting and examining of enormous numbers of seedlings. Any method capable of detecting virus in individual seeds would greatly facilitate indexing procedures and aid in conducting detailed studies of the virus-seed relationships.

Immunolabeling has recently been used to localize small amounts of viral antigen (4, 8), and it seemed possible that these techniques could be applied to seeds as a test for the presence of virus. The present paper describes experiments in which immunolabeling methods were applied to cantaloupe seeds in an effort to identify individual seeds and seed lots infected with SqMV.

Cantaloupe seed or leaf tissue was stabilized by fixation and infiltration with gelatin (5, 7). One-mm squares of this tissue were then treated with iodinated antibody. After thorough washing, the amount of radioactivity retained was determined in an effort to identify the diseased seeds. In earlier studies, the nonspecific label was found to be too great to permit detection of the small amounts of virus in nongerminated infected seeds (7). The following modifications of the labeling procedure (5) increased the specific binding of labeled antibody by infected tissues about 100-fold: (i) Prior to iodination the antibodies were purified from whole serum by salting out with 33% ammonium sulfate; (ii) the tissue was labeled in 0.01 M citrate-phosphate buf-

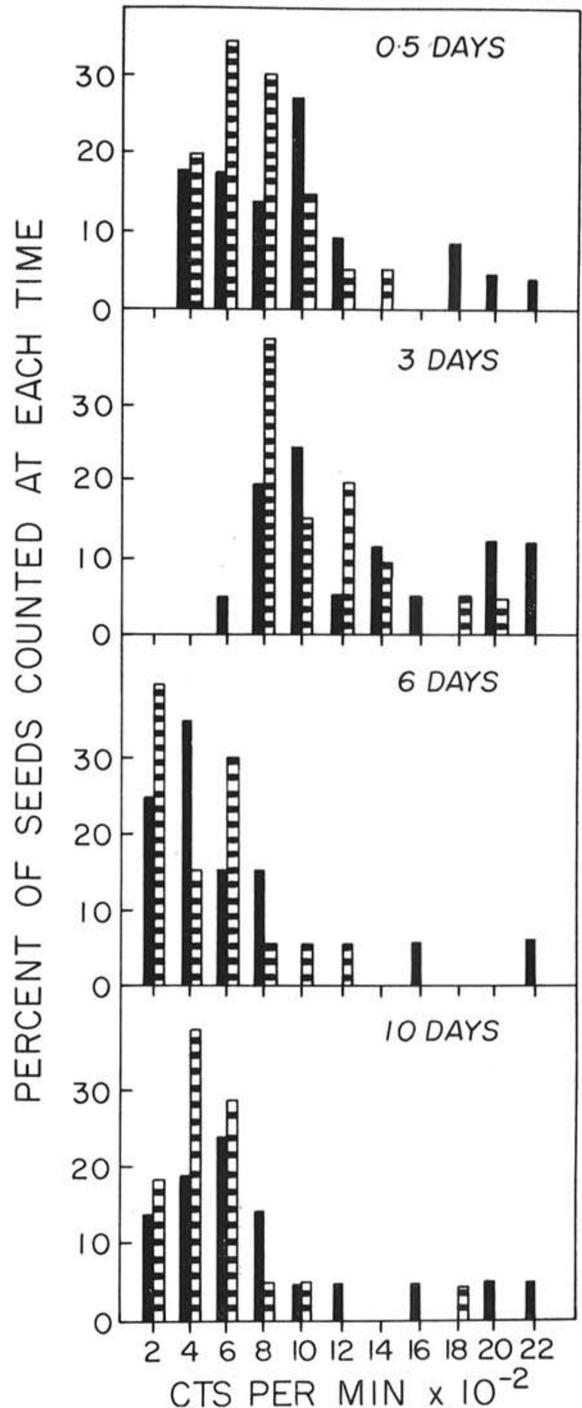

Fig. 1. Distribution of immunolabel in cantaloupe seeds from healthy and squash mosaic virus-infected seed lots. At different times after placement in germination plates, 20 seeds were removed from each lot and 1-mm squares of tissue were removed. These were treated with ^{125}I-labeled antibody as described in the text. The amount of label retained by the tissue was determined by scintillation counting. Seeds from healthy lot = dashed bars; seeds from a SqMV-infected lot = solid bars.

fer at pH 6.5; (iii) the excess antibody was washed from the tissue for at least 2 hr with 50 ml of 0.1 M phosphate buffer at pH 8.0. These modifications were designed to reduce nonspecific labeling from ionic interaction of antibody and host protein (2).

After treatment and washing, four squares of the tissue were placed in 10 ml of scintillation fluid in a vial (5 g of 2.5 diphenyloxazole [PPO, Sigma Chemical Co., St. Louis, Mo.] and 0.3 g of POPOP dimethyl ester [Sigma Chemical Co.] per liter of toluene). Aqueous and nonaqueous phase differences, which were present because the tissue had not been solubilized prior to the addition of the toluene-based fluid, caused a gradual increase in counts. It was therefore necessary to allow the vials to stabilize for at least 12 hr prior to counting and recording. The magnitude and variability of quenching due to lack of solubilization in vials containing single tissue squares was determined by a channels-ratio comparison and correction. The lack of solubilization did not appear to have an appreciable effect on either the relationship of infected to healthy samples or variability from one replicated vial to another within the two samples.

Several experiments were run to test the effectiveness of immunolabeling in the detection of virus-infected seeds in a seed lot. In the first series of experiments, a seed lot that resulted in about 25% virus transmission in four greenhouse trials was used. Samples of the seed lot were dampened and allowed to germinate for varying periods before labeling. Twenty seeds were used at each time period. The distribution of the labeling levels of the individual seeds making up the virus-infected sample was skewed when compared with the distribution for healthy seeds at every time period assayed (Fig. 1).

It can be seen that although the absolute levels of labeling generally decreased with seedling age, at each time interval the most heavily labeled seeds were from diseased lots.

A previous radioautographic study of similar seed material had shown an increasing but highly localized specific labeling as the age of the seedlings increased (7). Such a result was not evidenced here because this assay averaged all the labeling from the four tissue squares into one reading. Thus, minute areas of viral increase would be offset by the overwhelming numbers of cells in which no increase was occurring. This localized nature of viral presence or increase would also account for the fact that better separation of counts between diseased and healthy seedlings could not be achieved.

A second series of experiments was conducted with a new preparation of labeled antibody and a seed lot that produced about 15% virus-infected plants in repeated assay. Similar skewed labeling distributions were obtained with these samples (20 seeds/sample), which were studied at 0.5 and 7 days after the start of germination. Prior to fixation, the distal end of each seed or seedling was removed and assayed on zucchini squash. All positive assays were from seeds that were heavily labeled. In the 0.5-day-old sample, 12 seeds were labeled heavily, but only 4 gave a positive assay on squash. In the 7-day-old sample, 2 out of 10 that were heavily labeled assayed positive. Although the seeds that assayed positive were all in the heavily labeled group, they were not always the most heavily labeled.

Thus, liquid scintillation analysis of immunolabeled seed samples from infected plants distinguished virus-infected seed samples from healthy samples when the percentage virus-transmission was substantial (15-25%). In these instances, a general assessment of the diseased condition of unknown seed lots could be quickly carried out. Indeed, such data can be obtained within 1 day of receipt of the seed sample. These studies could not be used, however, to predict the percentage of virus transmission in each seed lot. Our inability to make this prediction serves to emphasize the results of a previous study (6) in which it was shown that seeds often contained virus which could not be detected either by direct assay or planting. The results of that study suggested that successful seed transmission resulted from the action of many variables that affect the virus-host relationship after initial invasion of the seed by virus.

LITERATURE CITED

1. Freitag, J. H. 1956. Beetle transmission, host range and properties of squash mosaic virus. Phytopathology 46:73-81.
2. Grossi, C. E., & H. Mayersbach. 1964. Technical factors affecting immunohistological methods. I. The influence of different pH and buffer salts for unspecific reactions. Acta Histochem. 19:382.
3. Kendrick, J. B. 1934. Cucurbit mosaic transmitted by muskmelon seed. Phytopathology 24:820-823.
4. Langenberg, W. G., & D. E. Schlegel. 1969. Localization of tobacco mosaic virus protein in tobacco leaf cells during the early stages of infection. Virology 37:86-93.
5. Powell, C. C., Jr. 1969. Virus-host relationships in the seed transmission of squash mosaic virus in cantaloupe. Ph.D. Thesis, Univ. Calif., Berkeley. 169 p.
6. Powell, C. C., Jr., & D. E. Schlegel. 1970. Factors influencing seed transmission of squash mosaic virus in cantaloupe. Phytopathology 70:1466-1469.
7. Powell, C. C., Jr., & D. E. Schlegel. 1970. The histological localization of squash mosaic virus in cantaloupe seedlings. Virology 42:123-127.
8. Schlegel, D. E., & M. Hudson. 1969. Increasing sensitivity of immunodiffusion reactions with radioautography. Phytopathology 59:886.