Experimental Attempts to Transmit Citrus Viruses Through *Phytophthora* spp.

L. J. KLOTZ and T. A. DeWOLFE

SEVERAL INVESTIGATORS have successfully transmitted virus diseases of herbaceous plants by means of zoospores of the fungus *Olpidium brassicae* (1, 2, 3, 6). Previously, attempts were made (5) to transmit psorosis virus with *Phytophthora* spp. This paper reports recent attempts to transmit four citrus viruses with two species of *Phytophthora*.

Materials and Methods

Three 28-year-old navel orange trees infected with psorosis, concavegum disease, and blind pocket virus, respectively, and a grapefruit tree of the same age infected with infectious variegation virus were selected as sources of virus inocula. These trees were inoculated March 8, 1966, with glucose potato agar (GPA) cultures of *Phytophthora parasitica* Dast. and *P. citrophthora* (Sm and Sm) Leon. Areas to be inoculated on the tree trunks were cleaned with a suede shoe brush and 95 per cent ethanol. Disks of bark, 5 mm in diameter, were removed with a steel cork borer. Inoculation was effected by inserting directly upon the wood cambium, 5 mm disks of cultures that had produced a fringe of mycelial growth in glucose potato broth during the 24 hours prior to inoculation. To take advantage of the influence of the sun's heat on growth of the two fungi, *P. citrophthora* (optimum growth temperature 25°C) was placed on the north and east sides of the trunk, and *P. parasitica* (optimum growth temperature 32°C) was placed on the south and west sides. All 4 inoculations and a check, consisting of a disk of GPA without fungus, were made on the lower 38 cm of the trunk. Each inoculation and check was covered with a 12 mm disk of waxed paper and with a 25 x 75 mm piece of adhesive cloth tape. Highly polished and reflective aluminum sheeting was wrapped around the tree trunk to a height of 38 cm to protect the fungus inoculations from the direct rays of the sun.

Thirty-five days later, the inoculation points were uncovered, and the margins of the *Phytophthora* infections were revealed by lightly scraping the bark surface with a sterile scraper. With a sterile knife, 10 horizontal, parallel cuts, about 2 mm apart, were made through the bark and wood cambium of the upper 25 mm of each lesion to permit the protoplasm of the fungus mycelium and diseased bark to mingle. The lesions

were again covered with waxed paper and adhesive cloth tape, and the aluminum wrapper was replaced.

A week later, the coverings were again removed. Two fragments of fungus-infected bark were removed from the advancing margin of the lesion above the parallel cuts and placed in a cylindrical hole in an apple. The cylindrical plugs were replaced in the apple and sealed over with masking tape. The inoculated apples were incubated at 24°C. Seven days later, cultures of the 2 fungi were isolated from the advancing margin of infection in the apples and placed on GPA slants (4).

On April 28, 1966, using GPA disk cultures of isolates from the apples, 16 one-year-old Bessie sweet orange [*Citrus sinensis* (L.) Osb.] seedlings were inoculated. The checks consisted of 6 seedlings inoculated with fungi, without virus, and 6 that received only GPA disks. One week later, the resulting *Phytophthora* lesions were cut, as described for the old orchard trees, to mix the protoplasm of fungus and seedling bark. On May 13, the diseased bark was excised to prevent the disease from girdling and killing the seedlings. The procedure was repeated with 16 two-year-old Madam Vinous sweet seedlings. The dates for inoculation, wounding, and excisions of diseased bark were respectively, May 19, 28, and 30, 1966.

Results and Conclusions

The new foliage that developed from these seedlings in the ensuing three months has been examined daily. To date, typical leaf or bark symptoms that indicate transmission of any of the four viruses by the fungi failed to developed in any of the seedlings.

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