A New Adaptation of Tissue Implantation for the Study of Virus and Mycoplasma Diseases

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ABSTRACT

A newly recognized disease of chrysanthemum, chlorotic mottle, presumably of virus etiology, was efficiently transmitted by inserting cores of tissue removed from stems of diseased plants with a surgical cannula into stems of healthy plants from which similar cores had been removed with a slightly smaller cannula. The technique enabled extremely rapid tissue implantation, and consistently resulted in nearly 100% disease transmission. Phytopathology 61:429-430.

Transmission of plant viruses by tissue implantation has been reviewed briefly by Fulton (5) and Bawden (1). Apparently, the earliest reported trials of tissue implantation, as distinguished from conventional grafting with apical portions of stems, budding with lateral buds containing primary meristems, or conjoining matched halves of bulbs, were those of Quanjer et al. (10) in 1916. They were unsuccessful in attempts to transmit potato leaf-roll virus by implanting in healthy tubers cylindrical cores without "eyes" taken from infected tubers. Ten years later, Goss (6) and Murphy & McKay (8) reported successful transfer of potato viruses, including leaf roll, employing the same technique. In these instances, cores were removed from healthy tubers with cork-borers of 8 to 13 mm diam, and cores taken from diseased tubers with the next larger cork-borers were forced into the holes.

Successful transfer of a tree virus by implanting pieces of infected leaves or infected stem tissues devoid of primary meristem under bark flaps of healthy trees was reported by Sreenavasaya (11) in 1930, working with spike disease of sandal. This technique later was used by Cochran & Rue (3) with peach mosaic, by Wallace (13) with citrus psorosis, and by Stouffer (12) with apple viruses. Cochran & Rue (3) also obtained successful virus transfer when diseased fruit tissues were similarly implanted; Moore, according to Fulton (5), was successful in transmitting cherry necrotic ringspot virus when diseased sepal, pistil, anther, and filament tissues were used, and Stouffer (12) was successful in transmitting apple chlorotic leaf spot virus from leaves of chenopodium to apple using this technique. Successful transfer of a virus by implantation of diseased tissue devoid of primary meristem into stems of herbaceous plants was reported by Blattny & Limbeck (2). They successfully transmitted stolbur, now considered to be caused by a mycoplasma-type organism (9), by implanting thin stem sections and leaf fragments from infected Convolvulus in stems of tomato and tobacco plants. Jorgensen (7) reported transmission of strawberry viruses by implanting slivers of infected petiolar tissues in slits in the petioles of indicator varieties.

Successful transmission of chlorotic mottle of chrysanthemum (4), a previously undescribed disease presumably of virus etiology, was initially obtained only by conventional stem grafting, and we felt that tissue implantation, if successful, might be useful in studying various aspects of the disease and in indexing plants. For our first tests, segments cut from diseased leaf tissue were inserted in longitudinal slits in stems of healthy plants, the wound then being covered with paraffin film. Leaf tissue from healthy plants was implanted in slits in stems of healthy control plants. Chlorotic mottle symptoms became evident in 19 to 24 days in all plants implanted with infected leaf tissue, but in none of the controls.

This success led us to look for improvements. We wondered whether a modification of the cork-borer technique, using a small surgical cannula designed for obtaining biopsy samples, might enable more precise manipulation and more rapid implantation with less injury to either donor or acceptor tissues. A No. 13, 2-mm diam cannula, (Becton-Dickinson Division, Becton, Dickinson Co., Rutherford, N.J. 07070) was obtained and a series of implantations initiated. Discs of donor tissue taken with the cannula from chlorotic leaves and cores taken from various locations along the stems and roots of infected Blue Ridge chrysanthemums were implanted in holes made by removal of similar cores from stems of healthy Blue Ridge plants at a locus about 30 mm below the apex, as close as a core could be removed without severing the stem. A core of tissue was easily removed by holding a finger behind the stem and pressing the cannula forward with a slight rotating motion (Fig. 1). The donor core was implanted in the hole in the acceptor stem by holding the tip of the cannula against the stem and pushing the core into place with the stylet. No attempt was made to align or match tissues of donor and acceptor. Six plants were implanted with cores of each category of donor tissue.

The results of this test were striking. Pronounced typical chlorotic mottle symptoms became evident in about 14 days in every plant in which an implant was made with donor tissue taken from as near as possible to the apex of the diseased plant. Symptoms developed more slowly when implants were made with donor tissue from lower on the stem, from leaves, or from roots, but successful transfer was achieved with tissue from all locations. Numerous repetitions of this test have given essentially similar results. All implantations made with subapical tissue have resulted in marked symptom expression in the acceptor in about 14 days.

Similarly successful results were obtained when this technique was used to transfer chrysanthemum stunt virus from diseased to healthy indicator plants of the variety Blanche. An initial attempt to transmit an established mosaic virus from infected to healthy Fanfare chrysanthemums, however, was less successful.

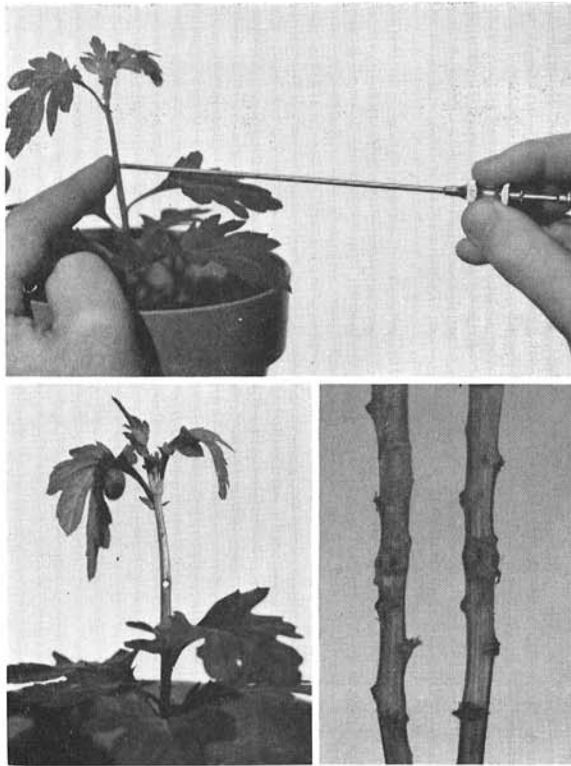

Fig. 1. (Above) Use of 2-mm cannula to remove or implant core of stem tissue. (Below, left) Hole in stem of acceptor prior to implantation of core of tissue from donor. (Below, right) Implantation sites several weeks after implantation.

Only 2 of 12 plants receiving implants became infected, even though donor cores were taken from and implanted in subapical stem locations. That this might have been due to inadequate matching of tissues of donor and acceptor was suggested by a second test in which seven plants were implanted using extreme care to match tissues of donor and acceptor, and seven plants were implanted with no attempt to obtain matching. In the matched group all plants became infected; in the random group only one or two became infected.

Cannulas of even smaller diam than those we used are available and with refinement of technique might be useful in obtaining very small samples from selected tissue zones within the donor plants (e.g., pith, cortex, apical meristem, phloem, etc.) and selectively implanting them within the acceptor. This technique is being used effectively both in basic study of chlorotic mottle and other "virus" diseases of chrysanthemums and in large-scale indexing of chrysanthemums by commercial propagators.

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