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# Indexing Systems for Producing Clean Stock for Disease Control in Commercial Floriculture

Most floriculture crops are propagated vegetatively. Because most viruses and viroids affecting ornamental crops are mechanically transmitted, only a small number of infected plants in a production area can adversely affect the quality of a large number of end products, whether foliage plants, cut flowers, or potted flowering plants. Similarly, vascular pathogens (fungi and bacteria) can severely affect yield and quality of vegetatively propagated crops.

This article describes the historical development and current "state of the art" of culture indexing procedures for clonally propagated floriculture crops. Emphasis is on the work done with the florist's chrysanthemum (*Chrysanthemum morifolium* Ramat.), one of the most popular floriculture plants.

Chrysanthemum became a major year-round crop following the introduction in the 1930s of artificial lighting to control the photoperiod. In the late 1940s and 1950, devastating losses from Verticillium wilt and chrysanthemum stunt necessitated new disease control methods to ensure the future commercial reliability of this crop. It has been said that chrysanthemum growers remember and relate to growing the crop before Verticillium wilt and chrysanthemum stunt were problems and after they were controlled, rather than to when these diseases were causing losses.

## Culture Indexing

Culture indexing to control ornamental plant diseases was first proposed by Mangin (11) in 1899. It was not until 1940, however, that the use of culture indexing for commercial control of Verticillium wilt of chrysanthemum was suggested by Dimock (5). During 1940, some beautiful and productive chrysanthemum cultivars were lost as a result of their susceptibility to Verticillium wilt. Until 1942, Verticillium wilt was the most serious disease of greenhouse and field-grown chrysanthemums and caused stunting, foliar necrosis, loss of flower color, and wilting.



Dimock set up a large block of fungi- and bacteria-free *Verticillium*-sensitive chrysanthemum cultivars produced by culture indexing techniques and a similar block of plants propagated in the normal manner from the same stock plants. None of the plants in the culture-indexed block had Verticillium wilt, whereas a high percentage of plants showed disease in the noncultured block. Cloy M. Miller of Yoder Brothers, Inc., observed these experiments at Cornell University in Ithaca during 1940 and recognized the commercial significance of initiating a "culturing" system. Yoder Brothers, Inc., was already producing mushroom spawn, and since the requirements and procedures for culturing chrysanthemum appeared similar, they initiated a pilot system. They soon realized, however, that developing a program for mass culture of support stock of more than 300 chrysanthemum cultivars would not be an easy task. Many standard cultural procedures had to be changed, requiring trained specialists to handle the cultured stock program. At this point, E. C. Stakman of the University of Minnesota arranged for H. G. Johnson to come to Yoder Brothers, Inc., to develop a commercially feasible culturing system for chrysanthemum.

Johnson initiated the culture program, first on a desk top, then in a spare room of an old mushroom spawn plant, and finally in a modest laboratory area. Small-scale culture blocks were enlarged and the results continued to look promising for control of Verticillium wilt. Although there were skeptics within and without the company, in 1941 "cultured" plants in trial blocks grown in various locations in the United States showed no wilt symptoms. Although World War II forced a halt to the program and cuttings from cultured blocks could not be supplied to growers until after the war because of restrictions on supplies, materials, and greenhouse space, there was no question that the "culture-indexed

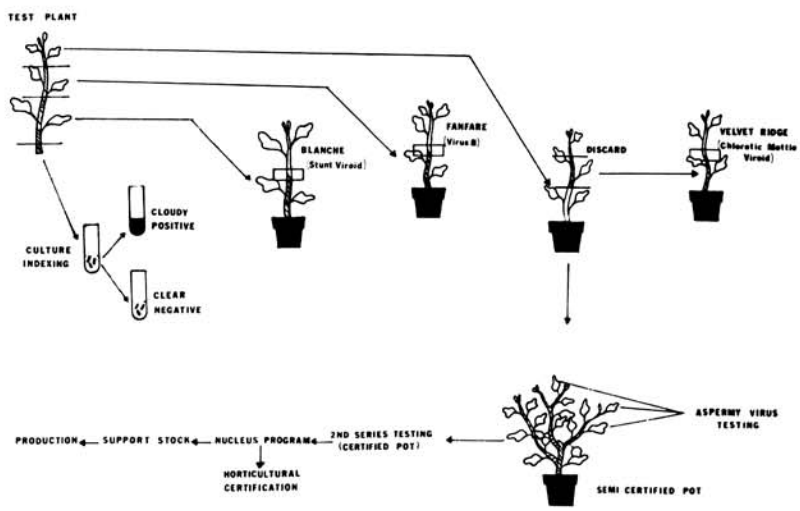


**Fig. 1. Culture, virus, and viroid indexing system used for pathological certification of chrysanthemum. In the culture indexing program, cloudy or turbid medium in a test tube is considered positive and the test plant is discarded, whereas clear medium is considered negative and the test plant is used in the virus and viroid indexing program. Chrysanthemum cultivars Blanche, Fanfare, and Velvet Ridge are used for detection of stunt viroid, virus-B, and chlorotic mottle viroid, respectively. Aspermy virus testing is done by ELISA.**


**Fig. 2. Indicator chrysanthemum cultivars: (A) Symptoms of stunt viroid on Blanche include leaf distortion, curling, and spotting. (B) Symptoms of virus-B on Fanfare include leaf depressions and spotting. (C) Symptoms of chlorotic mottle viroid on Velvet Ridge include mosaic and leaf spotting.**

cutting" had been successful and the method had been proved practical. Since Yoder Brothers, Inc., supplied most of the planting stock for the United States, nearly all chrysanthemum growers later enjoyed the benefits of the program.

The culture indexing program removed Verticillium wilt as a serious disease of chrysanthemum in commercial greenhouses across the United States, and several of the previously discarded beautiful chrysanthemum cultivars were reintroduced. Culture-indexed cuttings also helped to reduce, and more recently to eliminate, Fusarium wilt of chrysanthemum stock plants, first reported in 1970, as a major problem of flowering chrysanthemum (16).

*Fusarium*, *Verticillium*, and other systemic fungal pathogens and bacteria are detected by the following culture indexing procedures: A stem section 1.5–2.0 cm long is taken from the base of a test cutting/plant with a sterile razor blade and surface-sterilized by standard procedures. The stem piece is then rinsed in sterile water, cut into three to five small pieces, placed in a test tube containing previously sterilized potato-dextrose broth, and incubated at room temperature for 10–12 days. When the inoculated medium in a tube is cloudy or turbid, indicating microbial growth, the test cutting is considered positive for culture and is discarded. When the inoculated medium is clear and free from fungal or bacterial growth, the test is considered negative and the cutting is used in the virus and viroid indexing program (Fig. 1).

The principles of the culture indexing system developed for chrysanthemum were later used for carnation (*Dianthus caryophyllus* L.) (7), geranium (*Pelargonium* sp.) (13), and other floriculture crops. For many years, the vascular wilt diseases of carnation incited by *Fusarium oxysporum* f. sp. *dianthi* and *Pseudomonas caryophylli* caused serious economic losses to carnation growers. It has been generally accepted that the culture indexing system is responsible for eliminating the commercial problem of carnation bacterial wilt caused by *P. caryophylli*. Because *F. oxysporum* is soilborne and can survive in soil for several years, culture indexing alone was not able to control this wilt problem, considered one of the most important diseases of the perpetual-flowering carnation worldwide. With the use of a culture indexing system by the major geranium producers, the incidence of bacterial blight caused by *Xanthomonas pelargonii* and of Verticillium wilt caused by *V. albo-atrum* has been greatly reduced or eliminated in several commercial geranium operations.

## Virus and Viroid Indexing

Because of government restrictions during World War II, Yoder Brothers, Inc., arranged with C. Delworth to maintain their culture-indexed chrysanthemum stocks in Canada. Delworth observed that some plants of the cultivars Minuet and Cordova showed uneven growth and shortened flowering stems and that a bronze sport of Minuet showed severe flower streaking. When the stock was returned to the United States, several other cultivars that had been planted adjacent to Minuet and Cordova also showed these symptoms. Shortly, general observations of all stocks raised questions as to whether any plant was free from the disorder. Some affected plants showed mottling of leaves in addition to general stunting and pale green stems and leaves. Even though affected plants were growing vigorously, the flowers formed prematurely and were small and the yield was reduced. In addition, flower stems were unable to absorb water after harvest, making these flowers unacceptable for the cut-flower market.

Growers were alarmed by these developments, and most responded by reselecting their stock from flowering plants that appeared completely normal. In some cultivars only a few plants flowered normally, while in other cultivars only a few unaffected stems could be found. These symptoms were not similar to those of any other disease previously described in an herbaceous floral crop, and it soon became evident they were the result of a new disease. Various causes, such as continuous use of artificial light, pesticides, and rooting hormone, were proposed. By 1945, it was clear that a rapid solution was essential if commercial chrysanthemums were to be saved.

In 1947, Dimock (6) described this new disease of chrysanthemum and suggested it was caused by a stunt "virus." The graft-transmissibility of stunt was shown by Brierley and Smith in 1949 (2). Later, Diener and Lawson (4) showed that stunt is caused by a viroid rather than by a virus. Because most of the chrysanthemum stock was affected by stunt, finding stunt-free material to run experimental plots was difficult. It became necessary to maintain clonal lines to give linkage that would account for the final health of the plant. During 1947–1948, isolated plots in protected areas, sterilized tools, and complete insect control were used to clonally multiply "virus-free" material, and the plants were flowered twice to verify the stunt-free status of the stock. Individual clonal buildup of a variety offered vegetative comparison of the typical "healthy" plant to other clones. Any questionable clone was discarded and the linkage was retained. Stock derived in this fashion reduced stunt remarkably in the early cutting harvest, but the percentage of stunt-affected cuttings in later harvests increased from 5 to 50 over a period of several months. Obviously, to control stunt commercially, a zero tolerance level had to be achieved.

Grafting had been used successfully in virus testing programs of fruit crops, and this method was tested for chrysanthemum stunt indexing. Approach grafts gave only a low percentage of scion survival. Simple splice grafts made by trimming the tip off the test plant and the base off the indicator scion to form a wedge and sealing them together with adhesive tape gave good results when the plants were held in isolated areas under mist propagation for 2 weeks. Rooted cuttings of the test plant were then individually removed and planted in large pots. The terminal of the test plant was rooted separately and used to produce the tracer stock plant. The virus and viroid indexing system is outlined in Figure 1.

The cultivar Mistletoe was used by many researchers as an indicator for stunt detection but presented some practical problems under low-light winter conditions. C. J. Olson of Yoder Brothers, Inc.,

conducted several experiments to find a reliable indicator and selected the cultivar Blanche because it showed severe leaf curling and spotting symptoms (Fig. 2A). It was soon learned, however, that Blanche had chrysanthemum virus-B, which caused flower abnormalities (1) and killed many susceptible understock test plants; in these cases, Dauntless or Blazing Gold was used for stunt indexing. Our recent studies indicate that in Blanche, virus-B is essential for the development of stunt symptoms. Without virus-B, the stunt viroid multiplies in Blanche but no visible symptoms result (Raju, *unpublished*). We have had nearly perfect success in detecting stunt viroid by using one graft of Blanche and reading it after 4 months. In a very few cases, however, stunt was found even after one graft. Therefore, a double indexing system with two Blanche grafts, done at 2-month intervals, was developed and is still in use at Yoder Brothers, Inc.

For virus-B detection, Olson grafted test plants to Fanfare indicators, which showed leaf curling, mosaic, and leaf spot symptoms (Fig. 2B). Grafting techniques and time requirements are the same as described for stunt viroid detection. Enzyme-linked immunosorbent assay (ELISA) has been shown to reliably detect virus-B in Europe (9), and our recent research has confirmed this (Raju, *unpublished*).

During 1955, tomato aspermy virus (TAV) was found to cause considerable loss of chrysanthemum in England. The same virus was found in the United States (1). To detect TAV, leaf inoculation on *Nicotiana glutinosa* L. 'Samsun' (tobacco) was included in the virus indexing procedure. It was later found, however, that TAV concentration in infected chrysanthemum gradually drops below the limit of detectability during the summer under Florida conditions (Raju, *unpublished*). As a result, virus certification using tobacco was not performed between June and August. We recently found, however, that ELISA reliably detects TAV in chrysanthemum throughout the year (Raju, *unpublished*) and have replaced tobacco inoculation with ELISA in our indexing programs. We developed a direct ELISA system for detecting virus-B and TAV in chrysanthemum based on procedures described previously (15), using flat-bottom micro ELISA plates and an alkaline phosphatase enzyme system.

In 1971, a new disease, chrysanthemum chlorotic mottle (CClMV), was observed in the United States, and a viroid etiology was suggested (17). Since 1971, grafting to the cultivar Velvet Ridge was added to the virus indexing program for the detection of CClMV. In less than 4 weeks, Velvet Ridge shows chlorotic blotches and yellowing of leaves when CClMV is present in the test plant (Fig. 2C).

In addition to indexing procedures, heat therapy techniques used to obtain virus-free budwood of fruit trees (12) were found to be useful in eliminating TAV and virus-B from chrysanthemum. Infected plants were placed in a heat chamber (35–38 C) for 4–5 weeks; meristems were then removed and grown on standard tissue culture media and the survivors were reindexed. The combined use of heat therapy, meristem tip culture, and virus indexing has helped the chrysanthemum industry to select virus- and viroid-free material from an infected parent plant.

## Pathological Certification

With the control of Verticillium and bacterial wilts of chrysanthemum by culture indexing and the elimination of stunt as a commercial problem by virus and viroid indexing, the programs were combined into what is now called "pathological certification," which may take 10–12 months. These carefully indexed plants are first tissue-cultured using the meristems, then released to a certified nucleus house that is insectproof and under positive air pressure. In this house, all the clonal linkage of a variety is individually maintained and horticultural content is verified by clone flowering before the plant is released for production stock. Plants derived in this fashion are free from systemic pathogens and therefore grow and flower uniformly (Fig. 3). To achieve the full benefits of indexing, it is extremely important to keep this clean stock free from pests by disease scouting and preventive spray programs, since the certified plants are not immune to pest infestation when grown in the field.

Even though initial efforts were largely concentrated on chrysanthemum indexing, it should be emphasized that parallel programs for carnations and geraniums have also been established. Virus testing and generation of virus-free plants with meristem tip culture have been developed for several bulb crops in Europe, and the increased yields with virus-free plants have been demonstrated. With the use of vascular wilt (13) and virus indexing programs (8) combined with tissue culture, Oglevee Associates, Inc., have developed commercially available clean geranium stock. Improvements in foliage and flowers have been suggested (8) with virus-free geraniums. Unlike chrysanthemum, however, most geraniums today are probably still virus-affected, since it has been difficult to detect all the geranium viruses using indicator hosts.

In a recent survey of carnations in California (10), carnation mottle virus, carnation necrotic fleck virus (CNFV), and carnation etched ring virus (CERV) were found in the stock of several commercial propagators, although carnation ringspot virus was not found. These results indicate that totally virus-free carnation stock is not being planted even though carnation viruses were described as early as 1957 (3) and the effects of viruses on carnation cutting yield and flower quality were well documented. These viruses may be present because of detection problems


**Fig. 3. Uniform plant growth and flowers of chrysanthemum derived from certified stock: (A) Plants before flowering and (B) flowers of clean stock plants.**

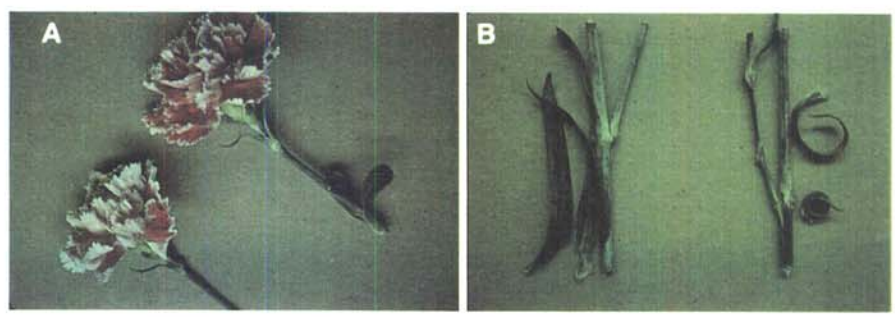


**Fig. 4. Effect of mottle and necrotic fleck viruses on the carnation cultivar Elegance: (A) Affected (bottom) and nonaffected (top) flowers and (B) affected (right) and nonaffected (left) stems and leaves.**

and the lack of lethal effects of carnation viruses on the host. For example, there is no reliable indicator host for the detection of CNFV, and viral antiserum is scarce. Similarly, it has been difficult to detect CERV using *Saponaria vaccaria* L. as a test plant in commercial greenhouses even though it works well for research purposes. Heat therapy and tissue culture systems have been used commercially to obtain virus-free material, but the lack of reliable detection methods for some viruses has made it difficult for the program to work. Hence, not much emphasis has been placed on virus-free carnations commercially. Recently, ELISA has been shown to be very effective for detecting carnation viruses (10), and our independent research results with direct ELISA confirm this (Raju, *unpublished*). This should greatly improve the virus indexing methods for carnation and facilitate commercial availability of virus-free cuttings. Some of our own observations indicate that virus-free carnations produce better quality flowers and increase plant growth (Fig. 4).

## Future Directions

Such new techniques as double-stranded RNA detection and "dot" blot hybridization (14) will be of great value in the detection of viruses and viroids of floricultural crops. In recent years, ELISA has significantly helped in detecting low concentrations of such viruses as TAV in chrysanthemum and has proved to be a valuable tool in carnation virus indexing. Similarly, ELISA can play a major role in virus detection in geranium, hibiscus, kalanchoe, bulb crops, and other ornamental plants. With the recent development of monoclonal antibodies to several of the viruses affecting floricultural crops, the animal-to-animal variation seen with the polyclonal antisera can be avoided, and the antisera produced by monoclonal antibodies is extremely sensitive in ELISA. However, the ELISA system should be checked with all available virus strains before being used commercially because some of the antisera batches may not detect all of the strains.

Development of a quick diagnostic test for CCIMV and standardization of virus detection in geranium will be of great value in chrysanthemum and geranium indexing programs. The use of polyacrylamide gel electrophoresis (PAGE) for detection of stunt viroid in chrysanthemum has been of great value in clean stock programs. However, grafting with indicator host is still needed to ensure stunt-free nucleus stock. Modifications in PAGE or development of a highly sensitive technique like ELISA is essential for the detection of chrysanthemum stunt.

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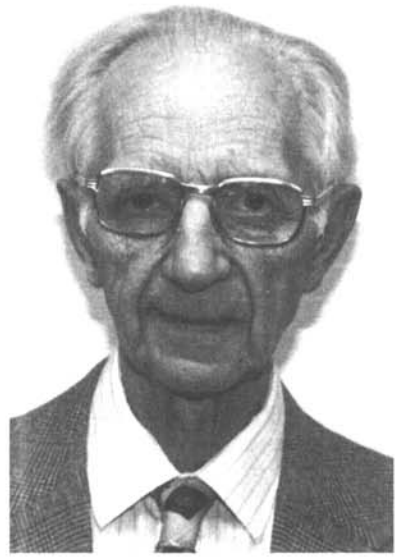
Dr. Raju is head of the plant pathology department in the Technical Branch of Yoder Brothers, Inc., in Alva, Florida. His research interests include virus and viroid detection and indexing of ornamental plants, in vitro isolation and characterization of bacterial pathogens and Mollicutes, serology of plant pathogens, and biocontrol of soilborne diseases of ornamentals. He received an M.S. in botany and plant virology from Sri Venkateswara University, India, in 1972 and a Ph.D. in plant pathology from Rutgers University in 1978.



**Conrad J. Olson**

Mr. Olson was the chief plant pathologist and entomologist in the Technical Branch of Yoder Brothers, Inc., until his retirement in 1981. During his 42-year career with the company, his research interests included the development and production of pathogen-free ornamental stock plants, the spread of virus and viroid diseases, and the control of ornamental insects and pathogens. In 1949, he received the Foundation for Floriculture award from the Society of American Florists for his contribution to development of indexing systems. He received a B.S. in plant pathology in 1940 from the University of Minnesota.

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