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DEVELOPMENT AND PRODUCTION OF PATHOGEN FREE PROPAGATIVE MATERIAL OF ORNAMENTAL PLANTS

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PLANT DISEASE REPORTER SUPPLEMENT

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PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Horticultural Crops Research Branch Plant Industry Station, Beltsville, Maryland

DEVELOPMENT AND PRODUCTION OF PATHOGEN-FREE PROPAGATIVE MATERIAL OF ORNAMENTAL PLANTS

Ornamental Crops Subcommittee[1] of the Committee on Seed and Plant Material Certification, The American Phytopathological Society

Plant Disease Reporter Supplement 238 June 15, 1956

CONTENTS

[1] K. F. Baker (Chairman), J. G. Bald, P. Brierley, A. W. Dimock, E. W. Lyle, D. E. Munnecke, C. J. Olson, J. Tammen, S. Wilhelm.

I. INTRODUCTION

The activities of the new Ornamental Crops Subcommittee necessarily differ somewhat from those of the parent Committee on Seed and Plant Material Certification. Since there are almost no certification schemes in this country for this group of crops, a study of existing programs was not possible. However, it was considered worth while to study some of the methods devised for the determination, development, and production of pathogen-free propagative material of ornamental plants. This is the fundamental basis of a certification program, should one later be evolved. There is a considerable body of information, much of it unpublished, in obscure trade papers, or buried in papers on other subjects, concerning this aspect of the pathology of ornamentals.

It is the purpose of this series of papers to present selected examples of the successful production of pathogen-free stock, giving the following information for each:

1. Methods for detection of infected or infested propagative material.
2. Methods for obtaining the original stock of pathogen-free material.
3. Methods for maintaining the pathogen-free status of the stock under conditions of commercial propagation.
4. The degree of success attained in commercial practice by both the propagator and the man who finishes growing the stock for sale. Is the program economically worth while for the propagator, and does the stock enable the grower to produce better or cheaper plants than before?

It is hoped that this first attempt at such a summation will prove useful in at least the following ways:

1. To make available to floripaths, and to other pathologists who occasionally advise on diseases of commercial ornamentals, a partial summary of proven control procedures available in 1955. This may serve to focus attention on the possibility of reducing the severity of diseases of many ornamental crops to such a level that expensive control programs by the grower are reduced or rendered unnecessary. This approach is perhaps more feasible with ornamentals as a group than with most other crops. Ornamentals are grown in an isolated soil mass (e. g., pots, flats, benches, or beds) commonly treated to free it of pathogens. The recontamination hazard is reduced by the protection afforded by glasshouses and similar structures, and by the compactness of the production units, which are relatively isolated from each other. The controlled climate of the glasshouse also minimizes many diseases. Finally, the relatively high financial return from ornamental crops makes economically possible many control procedures not feasible for most vegetable, agronomic, or fruit crops. However, the treatment of propagating material also provides a method for the reduction of disease under field conditions, as is shown by the results reported for bacterial blight of stock, Heterosporium disease of nasturtium, Fusarium yellows of gladiolus, and black spot of rose.

2. To bring to the attention of other plant pathologists unfamiliar or untried techniques that might be adapted to other types of crops.

3. To make available to commercial propagators information that should enable them to incorporate some of the methods into their own programs. It is recognized that, due to man's inherent imperfections, no practical programs of the types outlined here will be faultlessly executed. However, this does not justify recommending a compromise method. The system proposed can and should be perfect.

4. To publicize proven methods for practically eliminating plant diseases in some crops, and thus indicate that eventual certification of such crops is both possible and reasonable.

An accessory benefit from the preparation of these papers was the clarification and evaluation by the several authors, of basic philosophies, objectives, and methods with respect to pathogen-free propagative material.

This series of papers has included only representative commercially tested programs. Many other schemes are being devised by pathologists or tried by growers; it was thought best not to include such commercially undemonstrated methods at this time. There are several instances of production of a pathogen-free floricultural crop that have had adequate scientific demonstration of their feasibility, but still lack commercial sponsors to place them in practical use. Publication in such a series as this would be an excellent means of bringing the possibilities to the notice of commercial propagators. However, the Subcommittee decided not to seize this attractive opportunity. Perhaps some future Committee may wish to include them in a later summation when they have become established procedures. This series of papers may then be viewed as an attempt to evolve a useful means of focusing attention on a rapidly developing, but often unrecognized, phase of phytopathology.

There are many evidences among pathologists and growers of a growing awareness of the desirability for preventing the initiation of a disease, rather than relying on spray programs to suppress it after it is established. These papers will have served their purpose if they advance this trend. For the present, a guiding principle of many floripaths with reference to diseases will continue to be, "Don't fight 'em, eliminate 'em."

II. PRODUCTION OF CHRYSANTHEMUM PROPAGATING MATERIAL FREE FROM CERTAIN MAJOR PATHOGENS

A. W. Dimock[1]

Certification of plant material is practical only when one or the other of the following possibilities exists: (a) the presence of the pathogen on or in infected material can be determined with certainty by some practical means (see below); (b) a practical method of controlling the disease in propagative material is known. We will consider here only those chrysanthemum diseases, other than viruses, to which the second of the above conditions applies. These include Septoria leafspot, foliar nematode disease, rust, Mycosphaerella ray blight, and Verticillium disease. Powdery mildew will not be treated since it presumably is caused by a non-specific Erysiphe which occurs widely on many weed hosts, and may be controlled easily by the grower. Bacterial blight will be omitted since neither detection nor control is reliable by currently available methods.

Detection of Infected or Infested Propagative Material

From the point of view of a certification program, the detection of infected propagative material would play a part only in eliminating obviously unsatisfactory sources. The presence of any spots, flecks, or other abnormalities of the foliage would place the material under suspicion. But while the demonstration of fungus sporulation or nematodes in the abnormal tissues might definitely indicate disease, inability to demonstrate organisms would not assure the absence of spores, latent infections, or viruses. Having observed chrysanthemum diseases and chrysanthemum production rather closely for many years, the writer feels that although certification by inspection might be better than nothing, the emphasis must be on elimination of diseases at the source of plant production by the employment of the tested programs outlined below.

Methods of Obtaining Initial Pathogen-free Plants

Septoria Leafspots (S. obesa and S. chrysanthemella). -- Near-perfect control of these diseases may be achieved either under glass or in the open.

A. Under glass. Since the Septoria spores are disseminated almost exclusively by splashed water, control under glass may be achieved by eliminating splashing. Water the stock plants by subirrigation or by some method of careful surface flooding which will at no time permit splashing of the foliage. Take short tip cuttings only from shoots which have grown at least 10 to 12 inches since the start of the control program. Such cuttings will be free from Septoria infections and spores.

B. In the open or under glass. Complete control under conditions where splashing occurs may be achieved by spraying weekly with most any good fungicide. Zineb is recommended, but any other carbamate, or captan, will do as well. As soon as shoot growth begins, spray weekly with zineb at 1 pound in 100 gallons, with enough detergent to insure good wetting. Direct the spray upward so as to cover the lower surface of the leaves. This is essential. Take short cuttings from shoots which have grown at least 10 to 12 inches since the start of the spray program.

Foliar Nematode Disease (Aphelenchoides ritzema-bosi). -- May be completely controlled either under glass or in the open.

A. Under glass. Since movement of the nematodes from leaf to leaf depends upon either splashing or a film of moisture on the plants, nematode-free cuttings can be obtained by following procedure A under Septoria leafspots.

B. In the open or under glass. Two methods are possible:

1. Immunization of the Plant by Soil Treatment with Systemics.

Systemic treatment with sodium selenate: About 2 to 3 weeks after the stock plants have been planted, drench the soil of the beds with a solution containing 1 ounce pure sodium selenate crystals for each 15 gallons of water. Apply at the rate of 1 pint to each square foot (15 gallons would treat 120 square feet). After treatment, water thoroughly. Repeat treatment

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[1] Professor, Department of Plant Pathology, Cornell University, Ithaca, New York.

in 10 to 14 days. Take only short tip cuttings from shoots which have grown at least 6 inches following the second treatment.

Systemic treatment with demeton: Apply 1/2 pound technical demeton per 1000 square feet. The demeton (Systox) should be diluted in enough water to apply at least 1 pint of solution per square foot. After treatment, water the area thoroughly.

2. Spraying with Parathion or Demeton. Spray new growth every 10 to 14 days with parathion (1 1/2 pound 15% parathion wettable powder per 100 gallons) or with demeton (1 quart Systox per 100 gallons). Take short tip cuttings only from shoots which have grown 6 inches or more since the start of the spray program.

The spray programs have the advantage that the zineb, or other fungicide, for Septoria leafspot may be combined with the parathion or Systox, hence controlling both diseases with a single spray program.

Rust (Puccinia chrysanthemi). -- The zineb spray program outlined for Septoria leafspot will also give near-perfect control of rust. The program should be employed whether the stock plants are grown under glass or out-of-doors.

Mycosphaerella Ray Blight (M. ligulicola). -- Cuttings produced in areas where ray blight occurs might very well carry the pathogen as spores or as latent infections. The spray program suggested under Septoria leafspot would insure against this.

Verticillium Disease (V. albo-atrum). -- Near-perfect control of Verticillium disease could be achieved only if the stock plants were started from indexed cuttings and grown in sterilized soil in raised benches or concrete-bottom ground beds. An indexing procedure for establishing basic nucleus stock follows:

1. Take terminal cuttings about 6 inches long from vigorous shoots on the most healthy-appearing plants available.

2. Cut off the basal 2 inches to use for virus indexing (scion of virus indicator variety grafted to this basal segment, and portions of leaves used for juice inoculation of certain test plants).

3. Strip the leaves from the basal inch of the remaining 4-inch terminal cutting and secure similarly marked labels to the base and to the top of each cutting.

4. With a flamed scalpel or razor blade, cut off the basal inch of each cutting and immerse in a vessel containing freshly prepared Clorox solution (1 volume Clorox to 4 of water) for at least 1 minute, then remove and place on a clean paper towel.

5. When the segments have dried somewhat, cut a 1/4-inch piece from one end and discard, then cut 4 serial sections not over 1/32 inch thick from the remaining portion of the segment. While cutting, hold the segment on the spot of the towel which absorbed the excess Clorox solution. Flame the scalpel or razor blade before the first cut in each segment.

6. With a needle, transfer the 4 thin sections to a long potato dextrose agar slant in a test tube, placing one cut surface on the agar, and spacing the sections about 1/2 inch apart. To hasten results, place the tubes at 75° to 80° F (24-27°C).

7. The labeled tops of the cuttings may be handled by either storing or rooting immediately:

(a) If the tops are to be stored they should be spaced on a strip of waxed paper and rolled up, keeping the bases of the cuttings well separated. A rubber band is placed around each roll and the rolls placed in polyethylene bags at 31-34°F.

(b) Least delay in getting cuttings rooted is achieved by putting them at once in separate sterilized vials, Dixie cups, or other containers of moist, sterilized rooting medium. These may best be placed in racks under lights or in the greenhouse for rooting. The containers should have sufficient volume to retain a considerable water supply and should have tops large enough so that any required renewal of water is easy.

8. After at least 10 days examine the potato dextrose agar test tubes in good light and record the index number of all those which show any fungus or bacterial growth at any of the 4 stem slices.

9. Discard all of the cuttings which gave any growth in the tubes. Pot or bench the remaining healthy ones (after they have been indexed for virus) in sterilized soil. These mother-block plants must be in solid bottom beds or raised benches, and these must be sterilized even if pot culture is employed.

After the nucleus block has been established, cuttings may be taken from the indexed plants, without further culturing, to establish increase or production blocks. These blocks must be

grown in sterilized solid-bottom ground beds or raised benches and must be frequently renewed from the indexed nucleus blocks. Each nucleus and increase mother-block plant should be permanently labeled and, if possible, the progeny of each should be planted as a labeled unit in the production block. If at any time any plant in a given unit shows symptoms of Verticillium or any other systemic disease or undesirable horticultural character, the entire unit and its mother plant should be discarded or withdrawn from production until thoroughly rechecked. Obviously, a proportion of the progeny of each nucleus plant should be allowed to flower frequently in order to insure horticultural purity of the variety.

Summary Program for All the Above Diseases. --

All stock plants for cutting production should be grown from Verticillium-indexed (and virus-indexed) nucleus blocks.

All nucleus blocks and increase blocks must be grown in sterilized solid-bottom ground beds or raised benches.

All increase blocks should be thoroughly sprayed every 7-10 days with zineb at 1 pound per 100 gallons of water.

Either the soil of the nucleus blocks and increase blocks must be treated with sodium selenate or demeton, or parathion (1 1/2 lb. 15% wettable powder per 100 gallons) or demeton (1 quart Systox per 100 gallons) must be added to every second zineb spray treatment.

For propagation, only short terminal cuttings from shoots which have grown 10 to 12 inches since the start of the control program may be used.

The rooting medium must be sterilized between each batch of cuttings.

Methods of Maintaining Disease-free Production Blocks

There should be no possibility of the Septoria or leaf nematode diseases appearing if all increase block plants come through the nucleus-block program. Additional insurance would be provided by periodic zineb-parathion (or demeton) sprays. Such sprays would also insure against entry of rust or ray blight from wind-blown spores.

Continued freedom from Verticillium could be assured by constant surveillance to detect escapes, and by strict prohibition of practices which would permit recontamination of the soil. The latter would require that ends of hoses be kept off the walks at all times, that tools used in planting, cultivating, etc., be sterilized, and that neither workmen nor visitors be allowed to put their feet on the benches.

Are the Suggested Procedures Practical?

That the above procedures are practical for large propagators has been amply demonstrated by the success of one of the largest firms in the country in virtually eliminating the Septoria and nematode diseases from their stock, and reducing Verticillium from the major disease problem of the industry, to one of only occasional importance under glasshouse conditions. The fact that one or more of these diseases has occasionally reappeared in their stock when controls were relaxed is further evidence that the controls, when applied, are effective. It is significant that, although the disease control program has been very expensive, this firm has continously expanded, and other firms have instituted similar programs. For what significance it may have, it may be noted that a somewhat misguided organization, hoping to break into chrysanthemum cutting production, seriously attempted to employ a pathologist as a "front" simply because all the competitors had pathologists!

Is the expense of the program justified? Perhaps this can best be answered by the fact that a suit for $50,000 recently was instituted against a chrysanthemum cutting distributor for introducing disease into the plaintiff's growing beds. Had the plaintiff won and had his success become widely known, could any propagator then have afforded not to follow the best available disease elimination program?

Value of the Program to the Cut-flower Grower

Ability to purchase stock free of Septoria, leaf nematode, rust, and ray blight is of psychological advantage to the propagator and is not without value to the cut-flower producer. The propagator whose cuttings carry these diseases definitely loses sales to his more careful competitors. The cut-flower grower profits by not having infection sources introduced into his es-

tablishment, and thus is less likely to suffer seriously if he becomes lax in his spray program. If the grower could be sure that these diseases were not being introduced on planting stock he probably would not need to spray for them -- providing they had not been introduced previously. However, the spray program given above, which most growers should follow, would give excellent control of the four foliage and flower diseases mentioned, whether they were being introduced on the cuttings or not.

In contrast, cuttings free from Verticillium are of utmost importance, since no means of current-season control exists. If certified Verticillium-free cuttings are employed, the grower may provide positive insurance against the disease by adequate sterilization of the benches or beds. This has been amply demonstrated over the past decade -- has, in fact, reduced Verticillium to a disease of only occasional importance in greenhouse-grown chrysanthemums.

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III. DEVELOPMENT AND PRODUCTION OF VIRUS-FREE CHRYSANTHEMUM PROPAGATIVE MATERIAL

Philip Brierley[1] and C. J. Olson[2]

Prior to 1945 chrysanthemums were considered to be one important florists' crop not subject to any virus disease of major importance. Of course, aster yellows had been shown to affect mums as early as 1926 (8) and tomato spotted wilt had been recognized in chrysanthemums in Europe in 1935 (11) and later in our West Coast States. These diseases, although important in other crop plants, were too infrequent in chrysanthemums in this country to assume major importance in this crop. This picture changed radically with the appearance of chrysanthemum stunt about 1945 (4, 6). Successful control of stunt in florists' chrysanthemums stabilized the position of the propagation specialists who accomplished it. Chrysanthemum was the first florists' crop to be serviced by specialty firms who undertake to produce disease-free planting stock.

As a sequel to the research on stunt, and almost as a by-product of this work, mosaic (4, 7), rosette (2, 4), tomato aspermy (4), and flower-distortion (3) were detected in American chrysanthemums in the years 1950 to 1954. The tomato aspermy virus was first described in England in 1946 (1); this virus and the flower-distortion virus are apparently more common in European than in American chrysanthemums.

In this paper we shall describe briefly the symptoms and other characteristics useful in recognizing the virus diseases of chrysanthemums, explain how the viruses are detected and distinguished, tell what control measures are in use and how these measures are succeeding.

1. Chrysanthemum Stunt

Stunt was first recognized in 1945 (6), and became general in 1946 and alarming by 1947. The origin of the causal virus is still a mystery, but probably before it became evident in chrysanthemums it persisted in some of the several Compositae hosts which can carry it without showing symptoms. The delayed appearance or ill-defined symptoms in vegetative material permitted the stunt virus to go unnoticed until it was rampant. It was common for stock blocks to show flower symptoms of stunt in only 1 or 2 percent of the plants, but for cuttings from these to show 20 percent stunt at flowering. Such increases in stunt percentage indicate spread in spite of roguing.

Detection of Infected Plant Material. -- The most typical stunt symptom is a general reduction in size of the plant without mottling or severe distortion of any kind, but the foliage is usually paler than that of normal plants. On many varieties of chrysanthemum the symptoms are ill-defined, but a few sorts have marked symptoms which make them useful as indicators. The symptoms are recognized best during periods of rapid growth. At this stage the leaf margins of stunted plants may fail to enlarge and therefore the foliage has a drawn, upright appearance. It is common for the normal plants to have foliage with a downward reflex. Infected plants usually bloom prematurely and the normally red- or bronze-colored flowers of some varieties have a bleached appearance. In the greenhouses during winter, stunted plants may fail to flower because of their reduced vigor. Normal flowering, however, does not necessarily indicate absence of stunt.

Obtaining Disease-free Stock. -- Because the symptoms of stunt are poorly defined on many varieties and may not appear for 4-6 months following infection, most propagators now rely upon a graft-index procedure such as outlined by Brierley (2) in 1952 to produce foundation stock which is later built up to commercial level. To make this procedure effective it is necessary to isolate the plants and exercise precautions in any cutting removal and pruning. Any operation which causes wounding can transmit this highly infectious virus. Therefore all soft growth is removed with the aid of tissue paper shields and all cutting operations are done with tools which are plunged in alcohol and flamed before use on each individual plant. No insect vector is known.

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[1] Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland

[2] Plant Pathologist, Yoder Brothers, Inc., Barberton, Ohio.

In the indexing routine as practiced by the junior writer, the graft-inoculations are made as soon as practical on the indicator variety Blazing Gold. This variety reliably produces a diffuse yellow veining in the young foliage 6 to 8 weeks after inoculation. The Mistletoe varieties, and also Dauntless and Blanche, produce a distinctive mottle in the same period. However, the mottle disappears from the Mistletoe varieties during periods of low light intensity and is almost unrecognizable in Dauntless during the summer. The variety Blanche produces mottle reliably, but all stocks available carry either mosaic virus or a combination of mosaic and rosette viruses without symptom expression. This variety is useful in detecting stunt in the presence of either or both of these viruses.

In routine practice the scion sample is taken from a newly established plant at the time of the first terminal removal (first pinch). If two indicators are grafted simultaneously, the first laterals from the pinch are used as scions. This reduces the sampling error to a minimum. Such a practice is advisable since partially infected plants do occur and complicate the indexing procedure. By means of simple splice grafts the scions are attached to the soft portion of the stems of the indicator plants just as near the growing point as the texture will permit. To prevent mechanical spread of the virus in the procedure, scions are removed with tissue paper shields, and flamed double-edge razor blades are used to make the necessary cuts. After being trimmed, the scions, while supported with tissue paper or a flamed forceps, are attached to 3/4-inch cellulose tape. The tape is then drawn loosely around the scion and the stock, and the adhesive surfaces are placed together. It is a simple matter to match cambiums on at least one side, because of the transparency of the tape. Two (1 1/2-inch) strips of tape will usually hold the scion in position after tightening by pressing the thumb and the index finger along the outside surface of the tape where the adhesive surfaces meet. In bright, warm weather a canopy of cheesecloth may be required to shield the scions for a week to 10 days. After this period the scions have usually regained turgor.

Since there is variability in the growth of the scions, and since the foliage produced by the receptor is used for interpretation of results, a check of scion survival and a choice of 2 receptor laterals closest to the graft are made 1 month after grafting. The chosen laterals are pinched to reduce height and induce expression of symptoms in the resulting growth. Plants that induce no symptoms in the receptor varieties after 3 months are propagated as foundation stock. In another procedure, the scions that induce no symptoms on the receptor varieties are removed, rooted, and used as foundation stock.

Maintaining the Disease-free Stock. -- Vigilance must be constant to prevent recontamination of the foundation stock and the plants propagated from it. Great care as to placing and assembling of materials at the time of propagation is essential. The critical period occurs when stocks are expanded to large-scale commercial production. One simple precaution consists in beginning all daily operations on selected material in the morning and moving on to the older plantings. Separate handling of cuttings removed from the newly indexed stock is essential. Building the reselected stock up to adequate size will eliminate the need for holding older exposed material to supply existing demands.

It is also necessary to maintain the stock at a high cultural level. Disease-free color reversions and other undesirable sports or mixtures are worthless. To reduce the chance of such aberrations occurring, the foundation stock must be flower-indexed periodically. The foundation planting must be as small as practical to reduce the amount of work in the indexing procedures. A single clone carried in error can produce a high percentage of undesirable plants in the expanded commercial stock. Annual indexing of the foundation stock appears necessary to maintain the high level of control required. Anything less than complete freedom from disease in the foundation stock vitiates the program of producing virus-free commercial stocks because of the highly infectious nature of the virus.

Success Attained in Practice. -- The program described has been highly effective. The sudden nationwide appearance of the disease produced an incipient panic in 1947-48. It was common to find 90 percent of the stock in a planting stunted. Since the control program was put into effect there has been a tremendous reduction in the amount of stunt in greenhouse plantings. By 1949 it was reduced to relatively minor importance in the florists' chrysanthemum industry. Increased interest in the use of chrysanthemums has paralleled the release of indexed stocks. Stunt is still prevalent in garden chrysanthemums, which are propagated by nurserymen rather than by florists. In recent years Neal Brothers of Toledo, Ohio, have been indexing the garden varieties by the methods described above. Reselected stocks of these sorts are now becoming available.

2. Chrysanthemum Mosaic

Mosaic is prevalent in chrysanthemums in this country and also in Europe (4, 5). The mosaic virus, first noticed in New York by Keller (7), is widely distributed in outdoor and florists' varieties. It is not unusual to find it present in unnamed seedling stocks prior to release. Since interest in new varieties is great and some important established varieties are evidently completely infected with mosaic virus, some propagators feel that they must tolerate it in their stocks in order to carry complete listings of the varieties in demand. As new seedling material becomes available this picture may well change.

Detection of Infected Plant Material. -- Mosaic virus does not ordinarily produce symptoms that detract from the performance of the plant. However, recent findings indicate that it may cause a brown breakdown of ray florets of varieties that show mild or no leaf symptoms. (3). Aphids can transmit the mosaic virus, but sap transmission is difficult, and very few instances of mosaic are found in indicator varieties in exposed commercial plantings of florists' varieties. This virus is infectious to petunia.

Obtaining Disease-free Stock. -- Though mosaic may pass unnoticed in many varieties it is very damaging to some. These may be used as indicators in a graft-index procedure similar to the one suggested for stunt. The Good News variety displays a graded series of symptoms, from the milder type, with only a light mottling of the leaves, to the extreme type with crinkling or dwarfing and often complete blasting of leaves and flower buds. This variation in symptoms is not fully explained, but a complex of viruses and virus strains may be involved. Infected Mistletoe, Dynamo, and Pandora varieties also produce graded degrees of veining and mottling and sometimes the foliage is completely blasted.

Maintaining Disease-free Stock. -- Under greenhouse conditions there is little difficulty in maintaining the stock mosaic-free. Rarely, a mosaic-free plant appears in propagation from mosaic-infected parent stock. Any program devised to control stunt should control mosaic provided a suitable indicator such as Mistletoe is used in graft-indexing.

Success Attained in Practice. -- Up to the present, practical control has not been difficult. Detection of mosaic in seedling stocks maintained in the greenhouse is very rare, but such stocks often give some positive mosaic reactions upon graft-indexing if they have grown outdoors for an an extended period during preliminary trials.

3. Chrysanthemum Rosette

Rosette, first described by Brierley (4) as Ivory Seagull mosaic, was noticed by chance when graft-indexing on the variety Blazing Gold was first used to eliminate stunt. It is a rare disease and the causal virus is carried without symptoms by three varieties in a collection of 1300 varieties and seedlings which are periodically indexed by the junior writer. The original detection was made on a selection of Ivory Seagull in which the rosette virus did not cause any change in performance as compared with that of the uninfected parent variety. The rosette virus has been found in samples supplied from Ohio, Michigan, New York, and Europe.

Detection of Diseased Material. -- Since rosette virus is masked in many varieties and causes severe symptoms in others, any discussion regarding detection applies only to the susceptible types now used in graft-indexing. The symptoms expressed in Blazing Gold vary from enlargement of veins to crinkling of foliage and rosetting of the terminal growth. In Good News, distinct dull yellow mottling develops after 2 months. Although mosaic-like symptoms accompany the rosette reaction, the rosette virus, unlike the mosaic virus, is not infectious to petunia (5). The reaction of Blazing Gold is distinct.

Maintaining the Disease-free Stock and Success Attained. -- Since rosette is rare and the causal virus is masked in several varieties, there is perhaps some doubt as to what degree of control is achieved. Manual transmissions are difficult. Under commercial conditions Blazing Gold often has been planted adjacent to varieties that carry rosette, but natural infections have never been observed. Therefore little trouble from this virus may be expected when stock has been indexed on Blazing Gold and precautions are taken against the spread of stunt and mosaic.

4. Tomato Aspermy

Aspermy was detected first in the United States in chrysanthemums from California. It has been indexed from chrysanthemums from Pennsylvania grown adjacent to tomatoes and has also been present in samples from Maryland, Michigan, New York, and Ohio. It is also widely distributed in Europe (4, 5) and it seems likely that it has been introduced into North America in plant materials from European sources.

Tomato plants infected with aspermy virus produce seedless fruits as well as exhibit other harmful effects. The virus, named from this characteristic, is aphid-borne and infectious to tomatoes, spinach, lettuce, pepper, and other plants, some of which it severely damages. Tobacco, petunia, and Nicotiana glutinosa have been used as test plants. Since aspermy virus may injure some varieties of chrysanthemum and is widely distributed in Europe, it seems likely that it may cause concern in America. In the present search for varieties suitable for pot plant culture, there has been considerable interest in the European varieties. It would appear that suitable indexing procedures should be employed for such material.

Detection of Infected Material. -- English workers mention flower breaking, stunting of plants, and distortions of flowers as symptoms of tomato aspermy on chrysanthemum. In the material observed in America thus far foliar symptoms are rare, but some affected varieties produce wavy ray florets and smaller blooms than normal plants. Indexing is necessary for detection of the aspermy virus.

Obtaining Disease-free Stock. -- Either petunia or tobacco is a suitable indicator. The most characteristic symptom on the Turkish tobacco variety Samsun is a yellow mottling which appears in 6 to 14 days. White etching and occasional chlorotic rings and green blisters also appear. In petunias of the variety Blue Ball the symptoms are similar.

In actual practice the entire chrysanthemum plant to be indexed is sampled by removing immature leaves from all growing points. This sample is placed in small beverage, or "shot" glasses, which may be used as individual mortars in which grinding is done with test tubes having suitably rounded bottoms. The glasses and test tubes withstand exposure to boiling or steaming. The pulverized leaf material is covered with distilled water and the slurry is immediately brushed onto young tobacco plants having 3 to 5 leaves previously dusted lightly with carborundum powder. Moistened cotton swabs are used in making abrasions on 2 half-leaves and the terminal of the plant. Commonly 3 plants are placed in a single 7-inch pot and one of these is used as an abrasion check while the others are used for inoculation. During inoculation the leaves are supported by holding several thicknesses of tissue paper against them. Appropriate discarding of plants is done to conform with results of the inoculations. When aspermy virus is found in a variety, additional plants of it are rechecked by repetition of the procedure outlined

Maintaining the Disease-free Stock and Success Attained. -- When rechecks have been made there have been no instances of positive symptoms of aspermy appearing in the plants which were negative in the first indexing. However, aspermy has been infrequently detected; 23 clones have yielded it in a 3-year period in screening approximately 1400 incoming clonal lines. The clones found infected with aspermy virus were from Pennsylvania, Ohio, Europe and Canada. Seventeen clonal lines which have been rechecked one or more times have yielded no aspermy. The procedure would appear to be adequate.

5. Aster Yellows

Since first reported by Nelson (9) and transmitted by Kunkel (8) in 1926, aster yellows has been widely observed in chrysanthemums in the United Sattes. The affected plants are severely damaged, but usually only low percentages are infected in any planting. The disease persists in perennial weeds and is transmitted by leafhoppers from these to asters and chrysanthemums planted outdoors. Actual infection of plants in greenhouses is rare or lacking, but chrysanthemums infected outdoors and later brought to a greenhouse may show aster yellows there.

Green flowers are a distinctive symptom of aster yellows in chrysanthemums. The laterals produced on diseased plants may be weak and spindly. Thin, weak basal shoots having shortened internodes are a delayed symptom on infected plants which may have flowered normally. Infected plants usually die in a few months, but cuttings from recently infected plants may show weak terminal growth and shortened internodes after setting. Propagations from typically infected plants are usually not successful and the disease tends to eliminate itself. Since the aster yellows virus is spread in nature only by leafhoppers, it does not present a serious problem in florists' chrysanthemums. In field

plantings of garden varieties it can cause commercial loss. Plants infected late in the season may express no distinctive symptoms, but furnish no salable propagations. Effective control of leafhoppers is important in the field culture of chrysanthemum.

6. Tomato Spotted Wilt

Tomato spotted wilt has long been known in chrysanthemums in England (1) and has been reported also from California and Washington (4). Repeated index tests on tobacco by the senior writer have been consistently negative. Spotted wilt has thus far never been prevalent enough in American chrysanthemums to cause concern. Symptoms described by English workers include ring and line patterns, pale areas and necrotic spots in the foliage, death of stems and leaves, and also reduced growth. Symptoms are more readily expressed in young plants than older ones. Overwintering chrysanthemums may serve as a source of infective material for thrips, which carry this virus to other crops.

7. Chrysanthemum Flower Distortion

The flower-distortion virus (3, 10) is highly virulent to chrysanthemum. It occurred in White Wonder chrysanthemum free from other known viruses, received from a Pennsylvania nursery, and caused marked dwarfing and distortion of the flowers of Friendly Rival 4 months after grafting. No leaf symptoms appeared in Friendly Rival and none in leaves or flowers of Good News 4 months after grafting to White Wonder. The flower-distortion virus was recognized so recently that little is known about means of transmission or even about its prevalence in this country. We assume that flower distortion is uncommon here because no complaints of severe dwarfing and distortion of chrysanthemum blooms have come to our attention.

It seems not to be transmissible by manual methods, but its appearance in American varieties suggests that a natural agent of transmission exists here. Apparently still rare in the United States, flower distortion, like aster yellows, seems to offer a potential threat to field-grown chrysanthemums rather than to florists' chrysanthemums. Blazing Gold, the best test variety known, shows young shoots rosetted 2 to 3 months after graft-inoculations, with the tip leaves smaller and paler than normal. Large-flowered standards, such as Blazing Gold and Friendly Rival, produce small flowers shaped like an unopened bud with ray florets short, narrow, and incurved. Since Blazing Gold is already in use as a test variety for stunt and rosette viruses, the indexing procedure outlined above should screen out the flower distortion virus also.

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IV. DEVELOPMENT AND PRODUCTION OF PATHOGEN-FREE SEED OF THREE ORNAMENTAL PLANTS

Kenneth F. Baker[1]

Three diseases in which the pathogen is seed borne have been selected to illustrate variations in the techniques and in present objectives of the treatments employed. They also exemplify different types of economic loss. The first disease is destructive in both seed and cut-flower plantings in California, but is apparently rare in eastern greenhouses. The second is seldom observed in California seed fields or home yards, but is very important in plantings in rainy areas. The third is destructive in California seed fields and home plantings, and is known slightly in few other areas.

1. Bacterial Blight of Stocks

This disease, caused by Xanthomonas incanae (Kendr. & K. Baker) Starr & Weiss, has produced heavy losses in large commercial seed and cut-flower fields, and in home yard plantings of Matthiola incana R. Br. in California (5). While it may kill seedlings, the worst losses occur in more mature plants. The most characteristic symptom is the blackening of the leaf scars at the base of the plant.

Detection of Infected or Infested Propagative Material. -- The bacteria are carried both externally and internally by a low percentage of the seed. If one can examine the field where seed is produced (as in a certification program), it is usually possible to detect symptoms of the disease when present. However, there is some evidence that plants infected tardily and therefore externally symptomless may produce infected seed. For these reasons, it is considered safest to index uncertain seed lots by planting them in flats of steamed soil and growing in a cool place. In coastal California it is preferable to place these flats outdoors. Under favorable, cool, moist conditions the symptoms may be seen when the plants are 3-4 inches high. Laboratory methods for detecting seed-borne infection by this pathogen have not been developed, since the disease has now been reduced to commerical unimportance.

Obtaining Pathogen-free Propagative Material. -- Seed to be planted for commercial seed production is now regularly treated with hot water each year before planting. The seed is placed in plastic screen bags for treatment[2] rather than in cheesecloth as recommended earlier (5). These bags are made by double-stitching two pieces of plastic netting (15-22 meshes per inch) on three sides, and fixing a hem for a draw string at the top. All raw edges of the material should be kept on the outside of the bag to reduce sticking of seed. The bags used by various companies vary from 30 x 30 to 18 x 30 inches in size. The quantity of seed placed in the bag also varies; about 3 ounces is placed in the larger and 4 to 6 ounces in the smaller size. One company ties the top of the bags with wire rather than a draw string. Wire labels are ordinarily used to mark each bag.

The outer walls of the epidermal cells of stock seed swell and rupture when wetted, releasing their mucilaginous contents (4). Because this copiously extruded material causes wetted seeds to stick to each other and to materials on which they are placed, the hot-water treatment of stock seed is unusually difficult. However, in the plastic screen bags this troublesome feature is reduced to unimportance.

The bags of seed are plunged into a large (100-200 gal.) tank of circulating water held at 129.2°-131.0°F (54-55°C) and immediately kneaded gently with the fingers to drive out trapped air and aid penetration of water. After exactly 10 minutes the bags are removed, plunged immediately into fresh cold water, and again kneaded to facilitate rapid uniform cooling. One company then places the bags in a centrifugal drier to drive off surface moisture. The bags are then laid on a table covered with newspaper, and gently smoothed out flat to distribute the seed in a uniformly thin layer. Clean paper should be used for each lot of seed. They are then clipped on a line with clothes pins to dry in a warm room with circulating air. Avoid placing bags on hot objects or in open sun on very hot days. The seed should dry within 4-6 hours. When dry, the bags are turned inside out and any adherent seeds loosened by rubbing with the

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[1] Professor, Department of Plant Pathology, University of California, Los Angeles 24, California.

[2] Modified method developed by Mr. Warren F. Locke, Waller Flowerseed Co., Guadalupe, California.

fingers or with the bag. The dried seed is passed through a 6/18-inch screen and caught on a 1/16-inch screen to separate out seeds that are stuck together and let the fine chaff pass through. The adherent seeds may be separated by gently rubbing with the fingers against the screen.

Since the mucilaginous particles from the seeds easily scatter in handling, all operations involving untreated seed must be done in a building or room other than that used for the treatment, and care exercised to prevent blowing of any infested material onto treated seed. Similarly, workmen must not handle treated seed after untreated seed without carefully washing their hands. The seed should not be stored in unsterilized old used bags nor placed in storerooms with infested seed.

Maintaining the Pathogen-free Status of Propagative Material. -- The treated seed is sown in land not planted to stocks for 2, preferably 3, years. Care must be exercised that the treated seed is not used in a planter previously used for untreated seed, since the mucilaginous particles, very difficult to clean out of equipment, will cause recontamination. As an additional precaution the treated seed should be sown on sandy, rather than heavy, land so that rain water will not run from plant to plant. Ditch, rather than overhead, irrigation should be practiced.

Only single-flowered stocks produce seed, and the doubles are sterile. To maintain the required percentage of plants with double flowers, it is necessary to make single-plant selections and to grow seed from these as separate lines. Those lines with a satisfactory percent of doubles are carried through for seed, which is used in the next year to plant the production field. Single-plant selections are again made, and the rest of the seed is sold for cut-flower plantings. These facts mean that the seed from single-plant selections, of which there are often thousands, must be handled separately in heat treatment each year. In addition, the seed sown in production fields must be treated each year.

Success Attained in Practice. -- This disease formerly caused heavy losses in California seed and cut-flower fields. For example, one seed company nearly lost many of its varieties in the 1939 crop because the disease had reduced yield to a level insufficient for their own planting needs. During the past 10 years the disease has become almost rare, due largely to the above control program of seed producers. Thus, no disease has been found in the seed fields of one company during the last 5 years. Cut-flower growers have ceased treating the seed they plant. Seedsmen deserve great credit for the splendid record achieved. They are continuing to treat seed each year to maintain the disease-free status, and it is possible that the disease will essentially disappear in California.

2. Alternaria Disease of Zinnia

This disease of Zinnia elegans Jacq., caused by Alternaria zinniae Pape, is peculiar in that it causes practically no symptoms on plants in semi-arid coastal California seed fields, even though seed from them is infected by the fungus (1, 3). When this seed is planted in rainy areas, severe losses are sustained from leaf and petal spot and stem and root lesions.

Detection of Infected or Infested Propagative Material. -- It is not possible to determine by inspection whether the fungus is present in California seed fields. There is, furthermore, considerable difficulty in detecting infected seed by microscopic examination. An indexing method has, therefore, been devised (1). Seed is sown thickly (about 1000 per 18 x 18 inch flat) in soil that has been pasteurized in the flats, and is thinly covered with additional pasteurized soil. The soil is kept very wet for 20 days in the glasshouse. If the fungus is present, black lesions girdle the seedling stems at soil level or slightly above; on these lesions Alternaria may sometimes sporulate. Cotyledons frequently exhibit infections that appear to originate from the seed coat which remains attached to them for some time. This method gives a reliable means of determining infected seed lots that should be treated, but may give an exaggerated picture of the amount of infection, unless data are taken before secondary infection occurs. No laboratory indexing method has been devised.

Obtaining Pathogen-free Propagative Material. -- It has been demonstrated (1) that treatment of fresh seed with hot water will kill the pathogen without seriously reducing germination. The seed is placed loosely in cheesecloth, nylon screen, or plastic screen bags. The bags are plunged, without presoaking, into circulating hot water at 125°F (51.7°C) and promptly kneaded with the fingers to expel air bubbles. After 30 minutes the bags are removed and dipped at once into cold

water, with mild kneading, to cool. The seed may then be dried in thin layers on wire screens or clean newspapers. Old seed may give marked reduction in germination. Thus, treated 1-year old seed of several varieties averaged 15.6 percent germination reduction below the checks, 2-year seed averaged 38.9 percent, and 3-year seed 78.8 percent reduction (1). The same sanitary precautions in handling and storing the seed outlined above for stock should be taken.

This treatment is largely used by seed companies for seed used to plant fields for commercial production, and by bedding plant growers who suspect that the seed used may be infested.

Maintaining the Pathogen-free Status of Propagative Material. -- The treated seed is sown in land not planted to zinnias for several years. The fields should not be near zinnias grown from infected seed or in infested soil, because of the hazard of spores being blown to the clean stock. It is necessary that the seed crop be produced in areas that are rain-free, have low humidity, and are largely free of condensation (dew) and fog during the growing and harvest seasons. For example, zinnia seed produced in the coastal and inner-coastal areas of California, in Rocky Ford, Colorado, and in Doylestown, Pennsylvania was found by indexing to be infested with A. zinniae, whereas that grown in the San Joaquin Valley, California, was free from infection (1). Planting treated seed in hot dry areas, such as the San Joaquin, Coachella, Antelope, Hemet, and Imperial Valleys, probably will produce seed crops free of Alternaria, although it is not yet certain that the yields will be as high as in cooler areas. It is also important that harvesting be completed before foggy weather and winter rains set in. Machine threshing of standing plants may give greater freedom from Alternaria, as well as "molds" such as Botrytis and Cladosporium, than will hand picking where seed heads are piled on canvas on the ground for subsequent threshing.

Success Attained in Practice. -- Several seed companies are hot-water treating the seed before it is planted in their fields. Most of the California zinnia seed fields are now in either the inner-coastal strip or in the interior valleys, and almost none remain in coastal fields. There still is some infected seed produced in fields not in an interior valley, but the trend is to produce them as far inland as economically feasible. There is some increased growing cost, due to distance from the coastal production areas of the companies.

There is no comparative data on the present and past incidence of the disease, but there is reason to believe that the fungus is less prevalent on California seed than formerly. Much more could be done if seedsmen were fully convinced of the necessity of curbing this disease in order to prevent the decline in popularity of the zinnia -- one of their "bread and butter crops".

3. Heterosporium Disease of Nasturtium

This disease of Tropaeolum majus L., caused by Heterosporium tropaeoli Bond, formerly caused severe losses in seed fields in coastal California. It produces yellowing and death of the leaves after mid-season and thus reduces yield (2). Outside of California the disease has been reported only from New York, Guatemala, Ceylon, Tanganyika Territory, New South Wales, and Mauritius. Since the disease apparently is of economic importance only in California, control procedures have centered on reduction of losses in seed fields, and only secondarily on production of pathogen-free seed for the market. The techniques used for reducing losses in the seed fields would, however, be applicable should a demand develop for pathogen-free seed.

Detection of Infected or Infested Propagative Material. -- The presence of this disease may easily be detected in the seed fields. Inspection of the seed to determine the presence of the fungus is difficult because other fungi (e.g., Alternaria) also commonly cause black discolored areas of the pericarp. An indexing method similar to that used for Alternaria zinniae was devised (2) to determine whether seed lots are infected. Pasteurized soil in a flat is planted with 100 seeds and kept very moist in a glasshouse. Small infected seedlings will die in 2-3 weeks, and in 3-5 weeks stem lesions develop on larger plants. The number of infected dead seeds is not indicated in this test, and data must be taken before secondary infections occur. The total number of infected seeds, germinable or dead, can be determined in the laboratory by placing them on sterile, moist, finely ground black peat held under fluctuating temperatures, and observing Heterosporium sporulation on the pericarp.

Obtaining Pathogen-free Propagative Material. -- The pathogen may be eradicated from the seed by the use of a hot-water treatment (2). Seed is soaked in cool tap water in large galvanized cans for 1 hour in order to displace the air between the loose pericarp and the seed. Weighted screens are placed over them to prevent floating. As there is marked swelling of the seed, the cans should not be more than two-thirds full. The water is drained off and the seed placed in wire or plastic screen boxes that are plunged into a large tank of circulating water held at 125°F (51.7°C) and frequently turned over during the 30-minute treatment. The containers are then removed and immediately cooled by flooding with tap water. The seed is then spread out in thin layers on screens to dry outdoors or in a heated room or dehydrator. Drying should be accomplished in 12-20 hours without overheating. An average germination loss of 2.9 percent (ranging from a 29.0 percent reduction in 3-year-old seed to an apparent increase of 12.8 percent in one lot) was found in 13 lots of seed experimentally treated. A commercial seed company treated 1650 pounds of seed of 29 lots with an average germination reduction of 6.3 percent and a maximum loss of 32.5 percent in one lot. Germination loss from the treatment has been negligible in the decade that it has been used by seed producers.

Maintaining the Pathogen-free Status of Propagative Material. -- Seed companies have utilized the hot-water treatment as one means of breaking the fungus carry-over cycle. This is possible because: (a) the fungus is limited to a single host; (b) the pathogen survives in soil only for about a year or until plant parts have decomposed; (c) a 1-4 year rotation is commonly practiced to eliminate volunteer nasturtiums from carry-over seed; (d) there is ample opportunity for planting treated seed in isolated fields. Since the crop is highly sensitive to both frost and high temperatures, and must therefore be grown in the coastal strip, control by selecting inland areas free from fog and dew is precluded. It is important, therefore, to eliminate the inoculum by isolation, seed treatment, and by careful removal of nasturtiums as weeds in nearby uncultivated fields and as ornamentals in adjacent home yards. Experience has indicated that satisfactory commercial control of the disease may be obtained in production fields by seed treatment in alternate years. By treatment of the seed each year and careful attention to isolation, the present limited control could be changed to the production of pathogen-free seed.

Success Attained in Practice. -- The hot-water treatment of seed planted for production is presently practiced by three companies. The leaf-blight phase has been almost completely controlled in plants grown from treated seed under isolated conditions, with development of only a few scattering late-season leafspots from air-borne inoculum. Even in some inadequately isolated fields the appearance of the disease was so delayed that it did not become a serious problem. With adequate isolation and rotation practices, plus treatment of the seed each year, there is no question but that pathogen-free seed could be produced.

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V. CONTROL OF CARNATION DISEASES THROUGH THE CULTURED-CUTTING TECHNIQUE

James Tammen, R. R. Baker, and W. D. Holley[1]

The causal organisms of two of the most devastating diseases of carnations, Fusarium wilt (Fusarium oxysporum f. dianthi) and bacterial wilt (Pseudomonas caryophylli), are systemic in the vascular system of the hosts (3, 4). Hence control of these diseases depends not upon sprays, dusts or chemical dips, but upon use of pathogen-free[2] propagative material.

Many of the early commercial carnation growers and plant pathologists sensed this fact, and recommended the selection of propagative material from symptomless plants which were far removed from disease centers. This method of selecting for disease-free stock, coupled with sanitary measures and soil fumigation or sterilization, was often successful and is used with fair success to the present time. The fact that the carnation plant may harbor these two organisms without showing either external or internal symptoms indicates, however, that disease control by the method of plant selection will be limited and in many cases ineffectual.

Recognizing the need of the carnation industry for a method that would eliminate both the Fusarium and bacterial wilt pathogens, rather than merely reduce the damage inflicted by them, Dimock (6) suggested application of the principle used previously (5) for the development of Verticillium-free chrysanthemum stock: the culturing of cuttings. This method, variously modified, was tried by a number of workers (1, 7, 8, 9, 10, 11, 12) and is presently used in certain carnation-producing areas in the United States and Europe for obtaining an original stock free from the systemic pathogens. It is easily followed, yet exacting in its requirements for handling the cuttings to be cultured and the pathogen-free stock after culturing. Competent, responsible personnel and strict adherence to the basic outlined procedures are essential to its efficacy.

Since culturing is a rather specialized technical operation calling for meticulous care, commercial flower growers have not achieved maximum success with its use. Though there is no real reason why such growers could not carry out the program successfully, it may be found best to leave the culturing of cuttings to specialist propagators, as is presently done with chrysanthemums.

Detection of Infected Propagative Material

Obtaining Original Cuttings. -- Alternaria branch rot, Heterosporium leafspot, rust, Fusarium stem-rot, and similar diseases usually may be detected and eliminated visually in any but the initial stages. Because of the difficulty of detecting affected plants in the early stages of infection, however, the stock from which the original cuttings will be taken must be grown under conditions which will eliminate or at least minimize these non-systemic diseases. Thus, the relative humidity in the greenhouse should be kept below the condensation level by increasing heat and opening vents when sudden drops in temperature occur. All wetting of the foliage, such as splashing while watering, should be avoided. Weekly protectant sprays of zineb (zinc ethylene bisdithio-carbamate) or captan (N-trichloromethylmercapto-4-cyclohexene-1, 2-dicarboximide) will also aid in the prevention of these diseases.

It cannot be overemphasized that the elimination of parasites within the cuttings does not guarantee pathogen-free stock because pathogens may also be carried on the outside of cuttings. This method of carry-over appears, for example, to be quite important in the development of Fusarium stem rot (1). Therefore, the plants from which cuttings are obtained should be periodically sprayed with captan. In some cases cuttings may carry so much inoculum that a 5-minute dip in a solution composed of 5 millileters of commercial Clorox in 95 milleters of water

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[1] Chief Ornamental Pathologist, State Plant Board of Florida, Gainesville, Florida; Assistant Plant Pathologist, and Associate Horticulturist, Colorado Agricultural Experiment Station, Fort Collins, Colorado, respectively. The authors wish to express their sincere appreciation to A. W. Dimock, Professor of Plant Pathology, Cornell University, Ithaca, New York, for criticisms and contributions in the preparation of this manuscript.

[2] Throughout this paper the term "pathogen-free" is used to mean free from specific stem and leaf pathogens such as Alternaria dianthi, A. dianthicola, Uromyces caryophylli, Heterosporium echinulatum, and Fusarium roseum f. cerealis, as well as free from systemic fungus and bacterial pathogens.

is necessary (2). Every precaution must be exercised in the ensuing propagative procedure, however, as this treatment is slightly phytotoxic.

Cuttings which are to be cultured should be broken, not cut, from the parent plant. A portion of each cutting will be used in culturing, so a cutting about 1 1/2-2 inches longer than normally selected for propagation should be taken. Cuttings taken from somewhat hardened plants are more satisfactory for culturing than those from plants which are too soft or succulent.

Immediately upon removing the cuttings from the parent plant, place them in a clean, dry, polyethylene bag. Do not at any time put cuttings into, or sprinkle them with, water. Such treatment may lead to uptake of saprophytic bacteria in the vascular system, with the result that cuttings may be unnecessarily discarded because of bacterial growth which develops in the cultures. Cuttings may be stored at 31°-40°F in the polyethylene bags for a short period before culturing.

The Culturing Procedure. -- The culturing procedure should be carried on in a dust-proof room well isolated from areas in which plants or cut flowers are handled. The room should be atomized with thymol 1-1000 before each culturing session. There are two general methods of handling the cuttings during the culturing procedure.

In the storage method, each cutting is numbered and the basal 1 1/2 inches is removed with a flamed scalpel[3] for culturing. The cuttings are then submerged in a solution of sodium hypochlorite containing 0.26 percent available chlorine (a 5% solution of fresh Clorox) for 5 minutes. They are then removed, aerated for 30 minutes, and stored in clean, dry, polyethylene bags at 31°-40°F. The hypochlorite treatment has been shown (2) to reduce the occurrence of Fusarium stem rot due to F. roseum f. cerealis (12). The cuttings should be kept separated by spacing them on a strip of waxed paper somewhat wider than the length of the cuttings. The paper should be rolled into a loose bundle with a rubber band around it before being placed in the storage bag. When the broth tubes (see below) are examined 10 days later, all cuttings corresponding to contaminated cultures are destroyed. The base of each cutting is broken off before rooting, to reduce the deleterious effect of the chemical. Thus, only clean cuttings are rooted in the sterile perlite-sand mixture.

In the paper cup method, the basal 1 1/2 inches is removed from the cutting with a flamed scalpel[3] for culturing. The remainder of the cutting is inserted in a sterile moist perlite-sand mixture in a numbered Dixie cup or similar waxed paper cup with 3 holes punched in the bottom. Each basal piece of cutting is numbered to correspond to the cup into which the cutting is placed. The cuttings in the paper cups are placed in racks in the greenhouse, and are not permitted to dry out during the rooting period. These bench-type wooden racks are fabricated with holes somewhat smaller than the top of the cups and spaced far enough apart that the cups are well separated. The sides of the rack should be high enough to keep the cup bases from touching the frame. The position of a given cup in the racks should correspond to that of the appropriate culture tube in a wire frame, to reduce errors in labelling. Care must be exercised when watering the cups so that there is no splashing. Readings are made on the broth tubes (see below) when the cuttings are adequately rooted, and all cuttings which correspond to contaminated cultures are destroyed.

With either method, the basal 1 1/2-inch piece which had been removed is placed in a small glass or beaker containing a sodium hypochlorite solution of about 1% available chlorine (20 milliliters commercial Clorox in 80 milliliters water) for 5 minutes. The piece is then removed from the solution and placed on a paper towel to drain for a few minutes. A 1/4-inch piece is then cut from one end and discarded, 2 slices 1/32 inch thick are cut and retained; another 1/4-inch piece is then cut off and discarded and 2 more 1/32-inch pieces are cut and retained. The 4 thin sections should be cut and held in areas of the paper toweling onto which the excess Clorox has drained. They are then transferred with a flamed, long-handled needle into a test tube containing sterile Bacto Nutrient Broth plus 1.5% glucose. The broth method of culturing cuttings is at present used in Denmark (10) and current tests[4] indicate that it is more sensitive for the detection of bacterial infections than the previously used potato dextrose agar slants or plates.

The test tubes containing the stem sections are given numbers identical to the cuttings from which they were removed. They are then stored at room temperature and agitated slight-

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[3] All implements used in this procedure must be sterilized by dipping in alcohol and flaming prior to use on each different cutting being cultured.

[4] Personal correspondence with Paul E. Nelson, Assistant Professor of Plant Pathology, Cornell University, Ornamentals Research Laboratory, Farmingdale, New York.

ly every few days for 10 days or until the cuttings in the paper cups are rooted. At this time each tube is carefully examined, preferably with a dissecting microscope. If any tube shows evidence of either fungus development or bacterial turbidity, the number is noted and the corresponding cutting destroyed. Even if the growth is obviously a contaminant, the cutting should be destroyed, since the contaminant may be producing a volatile or diffusible antibiotic which restricts growth of the pathogen sought. Cuttings are retained only if the tubes are entirely free of fungus or bacterial growth.

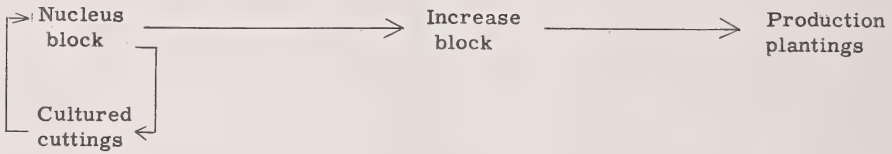
Maintenance of the Pathogen-free Status of Propagative Stock

As it is not feasible to use a cultured cutting for each plant in commercial flower production, the mother-block system of propagation has become increasingly popular among carnation growers. By this system a large supply of pathogen-free cuttings may be continously available from a small number of cultured cuttings. Two types of mother blocks, the nucleus block and the increase block, are used in these operations. They must be clearly distinguished. The progressive flow of propagative stock is in one direction only, once the system is in operation:

(a) Initiating the operation.

Healthiest plants obtainable → Cultured cuttings → Nucleus block → Increase block → Production plantings

(b) Continuing the operation.



The commercial production of flowers and the production of pathogen-free nucleus stock at least, if not the increase block as well, must be handled as separate operations not utilized for cut-flower harvest.

The Nucleus Block. -- This consists of a small number of plants grown directly from cultured cuttings in an isolated area and handled with infinite care to avoid reinfection. The glasshouse containing the nucleus block should be accessible to only a few reliable persons. If a relatively small number of production cuttings are needed, the nucleus block may directly produce the rooted cuttings planted for flower production. In larger operations, cuttings from it are grown in an increase block. Because of the small size of the nucleus block, it is easily checked for disease and undesirable mutations.

The nucleus stock may be grown in large pots placed on concrete blocks on the glasshouse floor (Fig. 1). In steaming such an arrangement, a plastic cover is placed over the pots and blocks on the floor. The pots may be placed on a glasshouse bench, but should then be placed on inverted pots. Each cultured plant is thus maintained as a single unit. Should a plant become diseased, it may be removed and destroyed, with little chance of infection of surrounding plants. Soil and containers must be sterilized in all cases. All equipment used in handling and planting the nucleus and increase blocks should be sterilized before use. If watering is by hose, the nozzle should never touch the soil, either in the benches or under them. Periodic checks should be made of the nucleus block in order to ascertain their freedom from the vascular pathogens. If, upon culturing, infected plants are discovered, they should be destroyed.

The Increase Block. -- In this the cuttings to be sold or to be planted for flower production are produced. An increase block is planted only with cuttings from the nucleus block. It is larger than the nucleus block and generally is less carefully shielded from reinfection. It must be given every economically feasible protection, however, if the program is to be successful. To facilitate later checking for disease and for genetic purity, groups of plants in the increase block derived from a given nucleus plant should be kept together as a unit and numbered the same

Fig. 1. Nucleus carnation stock in a Colorado glasshouse. Each plant is in a separate pot on an individual concrete block on the concrete floor. In steaming, the whole series is covered by a tarp.

as the nucleus plant. Mechanical barriers may be used between selection units so that any pathogens carried in or reintroduced may be localized.

The increase blocks are planted directly in benches and as a rule a spacing of 8 x 8 inches is best. A greater yield of cuttings in late plantings may be obtained from a 6 x 8 inch spacing. The first crop of cuttings may be soft due to extreme vigor of the plants; decreased watering will reduce this tendency. Cuttings should be broken off above the second pair of basal leaves, so as to leave 2 growing points for future cutting production. No heel cuttings should be taken until about 2 months before the block is discarded.

Plants in the increase block (foundation stock) must be scrutinized carefully for mutations or sports. It is essential that each plant flower so that undesirable individuals or clones can be eliminated. This may be accomplished by three different methods.

(a) The performance of each plant can be checked by letting the 2 top breaks flower after the first pinch. While these are producing flowers, the lower breaks should be pinched to produce a heavy plant. Plants which produce faded, hollow-center, off-color, or small flowers should be discarded. Grassy or vegetative individuals must be eliminated. Under this system the 2 top breaks of the plants of the increase block will have flowered, and cuttings can be taken 4 1/2 months after benching.

(b) In intensive and critical operations, plants derived from each clone in the foundation stock may flower in small blocks over a year's time. Careful records on the performance of these plants can supply a basis for the elimination of undesirable individuals in the nucleus stock.

(c) A third method is now commercially used, but is highly undesirable. In some sections of the country, abundant cutting production is desired beginning in January. Increase-block plants are allowed to flower for the Christmas market and are then utilized for propagative material. In Colorado, flowers may be produced for this market from a single pinch if plants are benched in mid-June. An essential practice in this system involves the pinching of any breaks in November which will not flower by Christmas. Cuttings from such a pinch can be taken for rooting in January. The inherent defect in this method lies in the mixing of commercial flower production and the propagation of pathogen-free cuttings. Neither is likely to be adequately handled.

Periodic checks should be made on the health of plants in the increase block. About 10 percent of each flush of cuttings from each clone unit should be cultured. If an individual plant gives a positive reading it should be rechecked, and if still positive should be discarded. If more than one plant in a clone unit remained positive on rechecking, the entire clone unit and its nucleus plant should be renewed.

Commercial Feasibility of the Program

To a commercial carnation producer losing 10 to 20 percent of his marketable flower crop from disease, there can be no question as to the need of pathogen-free stock. Culturing in itself will not completely eliminate the Fusarium and bacterial wilt organisms from commercial plantings. However, it has been demonstrated that losses due to these two diseases can be reduced to a negligible level by culturing, when coupled with proper cultural practices. It must be recognized, however, that lack of care in the culturing procedure, improper care of mother blocks, or the use of incompetent personnel will spell disaster.

The cost of a culturing program will vary over wide limits depending upon the efficiency of the personnel, volume of stock needed for indexing, and percentage of stock which is originally free from disease. Some idea, however, of the probable cost of such a program as performed by competent personnel may be obtained from the following data:

Making up and sterilizing broth	Man hours per 500 cuttings
Making up and sterilizing broth	2-3
Culturing	8-12
Examination of sections in tubes	1-2
Washing of glassware	2-3
Total	13-20

The expense of such a culturing program is additional to the usual cost of producing cuttings. In Colorado it is estimated that commercial production of a carnation cutting costs about $.03, and $.02 additional to root it. Figures on the total cost of producing a pathogen-free cutting are not available.

In considering the program, it should be borne in mind that, in addition to the potentialities for disease control, there are other tangible benefits of the cultured mother-block system. Experience has demonstrated the following advantages:

1. Cuttings produced from increase-block plants are more vigorous than those from flowering plants, due to better light and nutritional conditions, as well as freedom from disease. The increased use of stored cuttings makes this an even more important point.
2. Cuttings taken from increase blocks are more uniform, and are more easily taken and prepared than are those taken from flowering stock. One commercial operator has stated that the man-hours saved in such a system pays for the area diverted to increase-block population.
3. The nucleus blocks and increase blocks can be scrutinized carefully for undesirable growth characteristics, and "running out" of varieties can thus be prevented.

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VI. PROGRESS IN THE DEVELOPMENT AND PRODUCTION OF VIRUS-FREE CARNATION VARIETIES

E. C. Gasiorkiewicz[1] and C. J. Olson[2]

Diseases of the carnation remain an economic problem even though cultural practices tend toward controlled continuous cultivation of the crop under glass, rather than the traditional method of growing plants in the field for the summer and then resetting in the greenhouse for flowering. Among these troubles are the ever-present virus diseases, mosaic, streak, and the more damaging yellows caused by a combination of the streak and mosaic viruses. These diseases exemplify different types of economic loss. Mosaic does not affect the productivity (5, 11) but reduces the quality of flowers produced. Streak lowers productivity without marked reduction in quality (4). Yellows is destructive to plants wherever carnations are grown. The identity and properties of the carnation viruses are still uncertain (1, 2, 3, 4, 6, 7, 9, 12, 13). The terminology used here is a tentative working interpretation of available information.

Growers generally still tend to accept these virus diseases in their plants. Plant breeders are interested in retaining the virus-free status of their seedling material for future propagation. Floripaths, on the other hand, may search for virus-free plants in existing commercial varieties to use as a foundation for their rehabilitation.

1. Mosaic

This disease, caused by the carnation mosaic virus, has reduced quality of flowers in commercial plantings (5, 11). It does not kill plants but affects the foliage and blooms.

Detection of Infected Plants. -- The most characteristic symptom is a slight mottling of the leaves, with light green, irregular to elongate blotches prominent during cool periods. The symptom can be more readily detected by using diffuse, strong, transmitted light on young foliage. Flowers of colored varieties, especially dark pink and red, may show a color breaking on the petals. The breaking consists of somewhat lighter streaks of the base color parallel to the veins. Since the mosaic symptoms are masked in warm weather and some varieties show few dependable symptoms, it is considered safest to index cuttings. Indexing for carnation mosaic virus in the United States was not practiced by commercial propagators prior to 1952.

Obtaining Virus-free Propagative Material. -- New seedlings are free from the virus. Three methods have been devised for recognition of mosaic-infected plants: bioassay indexing, serological indexing (9, 12), and ultraviolet radiation indexing (10). Only the first has been tested by us and is reported here.

Yoder Brothers Inc. of Barberton, Ohio initiated an indexing program employing carborundum inoculation of the Dianthus species reported by Brierley and Smith (2) to be good indicator plants. Dianthus barbatus L. (Sweet William) is commonly used, but results obtained in comparative trials by the junior author indicated that D. superbus L. is a more sensitive indicator species.

The prodedure used to index a stock of approximately 900 clonal lines was as follows: Three seedlings of Dianthus superbus and 3 of D. barbatus were planted in each pot of steamed soil. In each cluster, 2 plants of each species were inoculated with sap extracted from individual carnation plants when they had 4 to 5 fully developed leaves. One plant of each species was retained as a check to determine the amount of accidental spread of mosaic in the testing procedure. The sample of tissue used for inoculation was removed by pulling 5 to 10 immature terminal leaves with a forceps which had been dipped in alcohol and flamed. Samples were thus selected to weigh approximately 1 gram. The leaf sample obtained was pushed to the bottom of a "shot" size beverage glass and macerated with a test tube. About 1 millileter of distilled water was added to the macerated tissue. This test inoculum was brushed, with a sterile cotton-tipped applicator, over two leaves and the terminal portion of a seedling which had been dusted with carborundum powder. The leaves were supported during inoculation by holding several layers of tissue

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[1]Assistant Professor of Botany, University of Massachusetts Field Station, Waltham 54, Massachusetts. Contribution Number 1014 of the University of Massachusetts Agricultural Experiment Station, Amherst.

[2]Plant Pathologist, Yoder Brothers Inc., Barberton, Ohio.

paper under them. The paper was discarded after inoculations from a given source plant.

Dianthus barbatus produced some local lesions in 5 to 7 days, and systemic symptoms in new growth in 10 to 12 days. A selected line of D. superbus was favored because of its reliability in producing systemic symptoms in 14 to 21 days. In 1953, 7 positive reactors were found out of 730 clones. When incompletely positive readings were obtained in an inoculation series, the carnation variety was re-indexed. In such a repeat index of 20 plants, there were 12 cases in which D. barbatus failed to react but D. superbus gave 2 positive reactions in the same set of indicator clusters. In samples of 2 plants both indicators gave positive reactions, and in 6 cases, 1 D. barbatus seedling of the inoculation cluster yielded symptoms. Eight infected carnation plants out of 20 were revealed by D. barbatus, against 4 for D. superbus.

In 1954, 210 individual carnation plants suspected of carrying virus were indexed. In 80 inoculated clusters D. barbatus failed to react, while in the parallel inoculation series on the selected D. superbus, 1 or 2 seedlings of the cluster gave a positive reaction. In another 66 inoculated clusters, 2 inoculated seedlings of the D. superbus clusters reacted with systemic symptoms and in D. barbatus inoculated with similar virus isolates only 1 seedling per cluster gave positive reaction. Fifty-seven isolates gave positive reactions on both Dianthus indicators. Only in 7 cases did D. barbatus react more quickly than D. superbus. Of the total 420 D. barbatus plants used in the inoculation test, 175 positive readings were obtained; 109 local lesions; 64 systemic; 2 both local and systemic symptoms. Only 130 infected carnation plants were revealed by D. barbatus, against 210 for D. superbus.

Results indicate the need of uniform indicator plants. D. superbus proved more reliable and less erratic in manifestation of systemic mottle symptoms.

Maintaining Disease-free Stock. -- Extreme precautions against manual spread of the virus have been exercised during the last 2 years. It is suggested that plant foliage be dry during the removal of cuttings with paper shields. Flamed instruments should also be used for all pruning or disbudding in the isolation blocks. Even with these precautions, in a large commercial operation mosaic spread to 5 seedling blocks in a period of 18 months. However, no effort was made to eliminate mosaic from existing commercial propagative plantings because of the necessity of growing the varieties adjacent to infected stock. Florists presently grow the virus-free plants among infected ones and thus nullify the previous care. The maintenance of virus-free foundation stock by these methods would appear to be practical, however (see below). The full benefit from this program will come when growers are ready to capitalize on it by proper isolation and careful handling. It is considered that when a suitable collection of virus-free varieties are assembled, it should be practical to grow such a group under isolation for an extended period of time to retard the re-infection with mosaic. Because the virus is transmissible by the peach aphid (3), careful aphid control must be practiced in the disease-free stock.

Degree of Control Achieved by Bioassay Program. -- In 1952 the first screening for the presence of carnation mosaic virus included 41 varieties and seedlings which were represented by 275 clonal lines. In the first index, 140 clones gave positive readings. The 135 clones which gave negative results on the first index were rechecked and 17 positive reactions were obtained, giving a total of 57.09 percent virus infection of the original clones. From these results it would seem advisable to recheck virus-free selections from the bioassay indexing shortly after the initial tests to prevent the maintenance of any infected escapes.

In 1953 the clones were re-indexed, and no positive readings were obtained. The 1954 recheck yielded 3 questionable reactions. These results indicate that it is possible to select mosaic-free stock by this bioassay indexing and to maintain it virus-free in a mother block by isolation and annual indexing. It seems clear that this success can be carried on into large propagation blocks when there is sufficient commercial demand for such virus-free stock. For the present, a basic virus-free stock of new seedling varieties is being maintained.

2. Streak

Jones (6) identified streak as a component of the carnation yellows complex in 1945. This virus is not so widely spread as mosaic but is more damaging in its effects. Parts of Creager's (4) report on carnation mosaic were interpreted by Jones to be concerned with the streak virus. In the light of Jones' study, Creager (4) reported that streak reduced the flower cut in King Cardinal variety by 19 percent with a further reduction in quality of flowers. Lowered productivity is the main basis for interest in this virus (2). Natural infection of up to 30 percent under

field conditions in the variety King Cardinal was reported (4). Jones (6) reported the aphid, Myzus persicae Sulz., as the vector of carnation streak virus. Brierley and Smith (2) in 1947 stated "that we have been unable thus far to confirm Dr. Jones' conclusions that the green peach aphis transmits streak, but some insect more common in the field than in the greenhouse is evidently the agent of spread".

Detection of Infected Plants. -- The typical symptom is the appearance of white, yellowish brown, or purplish broken lines or streaks in the leaves, usually paralleling the veins. They are plainly expressed in old leaves of established plants in the spring, especially from March to May. Many of the older leaves thus infected may become severely spotted, turn yellow, and die. The streak virus appears to produce no distinguishing symptom on the flower.

Obtaining Disease-free Plant Material -- New seedlings are free from the virus. The chrysanthemum graft-indexing procedure has been adapted to select plants free from streak virus, whether it is alone or combined with mosaic. Splice-grafting resulted in 60 to 80 percent successful union, with reliable expression of symptoms in 2 months during spring or fall when greenhouse night temperatures were maintained at 60°F.

Seedling No. 115 was selected as the indicator for graft indexing following a screening of 79 varieties and seedlings for ability to express yellows and mosaic when grafted with diseased scions. The method of grafting and the procedure parallels the one described elsewhere in this series by Brierley and Olson for detection of chrysanthemum viruses. The only modification is that a nodal portion of the stem of stock and scion is selected for the graft union. Expression of symptoms usually occurs in 60 days.

Brierley and Smith (2) suggested the following program for obtaining disease-free stock:

a. Selective roguing during the spring months when symptoms are most marked.
b. The selection of streak-free cuttings and maintaining such blocks under glasshouse cultivation.
c. Maintenance of effective insect control by fumigation, sprays, or dust insecticides.
d. Reselection in foundation blocks.
e. Renewal of foundation blocks with cuttings from streak-free plants.

Maintaining the Disease-free Stock. -- Since the streak virus is not mechanically transmitted, the main control measure is adequate insect control. The present-day insecticides, applied as aerosols, systemic sprays, or dusts, have effectively controlled the potential vectors. There is essentially no increase of streak in greenhouses with efficient insect control. Isolation of foundation blocks also effectively prevents the introduction of the mosaic virus into the planting.

Degree of Control Achieved. -- Such a program maintained at Yoder Brothers has proved to be very effective in the production of streak-free varieties.

3. Carnation Yellows

Carnation yellows, caused by a combination of mosaic and streak viruses, is the most destructive of the carnation virus diseases. The disease produces loss of plants in addition to the decreased productivity and lowered quality of blooms.

Detection of Infected Plants. -- Yellows-affected plants show mottling and flecking of leaves and stems, distortion, and color breaking of the flowers. Young leaves show light and dark green mottle, and older leaves show whitish, sunken, elongated flecks or streaks which may become reddish, purplish, or brown. Severely spotted foliage may die. Stems also frequently show white or light colored streaks similar to those on leaves. The color breaking of flowers, caused by carnation mosaic, is intensified in the presence of the streak virus. The effects are not visible on white varieties but all varieties often have distorted flowers of poor quality.

Obtaining Disease-free Plant Material. -- New seedlings are free from this virus complex. The procedures outlined for mosaic and streak apply to indexing carnations for yellows. Separation of mosaic from the yellows complex may be achieved by the abrasion technique referred to under mosaic.

Yellows-free stocks are quite generally available from propagators who practice continued roguing and insect control.

Maintaining Disease-free Stock. -- Procedures outlined for mosaic and streak are satisfactory.

Effectiveness of the Program Outlined

The procedures outlined for eliminating mosaic have not yet been completely applied on a large commercial scale. The techniques for obtaining and maintaining virus-free carnation plants have, however, been satisfactorily demonstrated under commercial conditions. The conclusion is warranted that the procedures can be expanded to commercial volume when it becomes so desirable economically that growers will exercise the necessary care in isolating and handling the plants. Bioassay indexing for mosaic, using Dianthus species, has been the most reliable technique used commercially in the United States. A careful analysis of the cost of such an operation will determine the future status of this procedure.

Streak indexing is successfully done today by commercial propagators. Selective roguing and effective insect control by commercial propagators have reduced this disease to a minimum.

Roguing, reselection, insect control, and discontinuance of susceptible varieties by growers have made yellows a relatively uncommon disease.

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VII. DEVELOPMENT AND PRODUCTION OF PATHOGEN-FREE GLADIOLUS CORMELS

J. G. Bald[1]

Unlike the cuttings of chrysanthemum, carnation, and geranium, gladiolus corms do not lend themselves readily to the culture technique for selecting pathogen-free stock. Progress toward obtaining such stock has, therefore, depended on developing means for freeing propagative material of pathogens. This has been accomplished by hot-water treatment of cormels at an unusually high temperature. The method is given below. It is based on investigations by the Department of Plant Pathology, University of California, Los Angeles. Results have appeared in abstract or summary form (2, 3, 6, 7), have been mimeographed (1), or prepared for publication (3, 8, 9). Tests of the method have been made in other areas (6). Hot-water treatment has successfully eliminated Fusarium oxysporum f. gladioli (Massey) Snyder & Hans. Botrytis gladiolorum Timmerm., Stromatinia gladioli (Drayt.) Whet., Rhizoctonia solani Kuehn, and several species of nematodes and mites from cormel planting stock. Curvularia lunata (Wakk.) Boed. and Septoria gladioli Pass. have not been tested because of the unimportance of these diseases in California. The virus diseases and Pseudomonas marginata (McCulloch) Stapp have not been eliminated by heat treatment, but both are of relatively minor importance, and can be adequately controlled by other means.

Detection of Infected or Infested Propagative Material

The disease may be detected in the parent crop, in the corms, and in cormels that are peeled or cut for examination. On cormels, obvious necrotic lesions or discoloration of husks may indicate the presence of pathogens, but the absence of such lesions does not necessarily indicate their absence. The presence of pathogens in a given lot of material of uncertain health may be determined by culturing methods. The original stock used in a program of the sort outlined should be from vigorous plants free from the more serious virus infections, and as nearly free from pathogenic fungi and bacteria as possible. The objective is to develop pathogen-free stock in the most certain and the cheapest possible manner, not to provide a means of utilizing worthless planting material.

Obtaining Pathogen-free Propagative Material

Only cormels can withstand the high temperatures necessary for the reduction or elimination of Fusarium, the cause of the principal corm-borne disease of gladiolus. Corms are too heat-sensitive to withstand the treatment and too large for the heat to penetrate effectively.

Although cormels should be taken from relatively healthy parent crops, severely infested material has been successfully treated. Cormels that have withstood treatment undamaged have so far been from plants grown in a warm dry climate during summer, and matured and harvested before the onset of cold weather. Cormels grown in cooler climates, or grown and harvested during the cooler season in a warm climate, have not yet survived the required temperature. Tolerance to high temperatures seems to arise from the initiation of full dormancy by warm growing conditions. Cormels maturing under cool conditions become only partially dormant.

During the early stages of the investigations on hot-water treatment, the tetrazolium method of estimating viability of seeds was adapted to give a quantitative estimate of dormancy, resistance to heat, and germinability of gladiolus cormels (7, 8). The tetrazolium method was invaluable throughout the experimental work, but is not yet well adapted to routine estimates of the suitability of cormels for hot-water treatment, or their germinability after treatment.

The most favorable time for treatment of cormels is about 2 to 4 months after digging. Cool storage, like cool growing conditions, reduces and may eliminate resistance of cormels to heat. Conversely, dormancy may sometimes be deepened by warm temperature curing at 95°F (35°C) for 2-4 weeks after digging, or before hot-water treatment.

To accomplish this form of curing, cormels, or parent corms carrying cormels, are placed on trays in a well-ventilated heated room held at 95°F. There must be free circulation of air around and across the trays, and a forced draft to prevent humidity rising too far above the optimum (80 percent saturation). Fresh air may be drawn into the room if the humidity

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[1]Professor, Department of Plant Pathology, University of California, Los Angeles 24.

rises too high. Cormels carried on the corms are separated at cleaning, which occurs after 6-8 days, and cured at 95°F for 1-3 weeks longer. After curing, cormels are kept at room temperature, not in cool storage, until they are fully dormant and ready for treatment.

If gladiolus cormels are fully dormant when treated, germinability may be improved and the crop of corms increased in size and quality. If the cormels are already germinable, the hot-water treatment may reduce or eliminate their capacity for germination, or depress the yield.

The treatment, apart from any previous natural or artificial conditioning of the cormels, consists of (a) presoak, (b) immersion in hot water at 135°F for 30 minutes, (c) cooling, and (d) drying and storage.

(a) Two types of preconditioning have been used successfully. The original recommendation was to soak in water at room temperature 24 hours, drain, and then immerse in the hot water. The second type of preconditioning was designed for lots of gladiolus cormels containing high disease inoculum and mummified small corms or cormels, but it is now given as the standard recommendation. The presoak is extended to 2 days. The cormels are drained, and subsequently soaked 3-4 hours in a 1 in 200 formalin solution before immersion in hot water. A third method, which has not yet been tested on a commercial scale, is to presoak overnight in 5% ethyl alcohol and to add 5% alcohol to the hot-water bath.

(b) Containers for immersion are open mesh sacks, or preferably, metal mesh containers on a wooden or metal frame, with a door on one side. The containers should never be filled to more than 2/3 capacity. The cormels should be quickly surrounded by hot water by submerging or rolling the containers, and should be totally immersed during treatment.

The tank of water should be maintained within 1 degree on either side of 135°F. The water should be thoroughly stirred, mechanically or manually. Thermometers must be standardized against accurately calibrated thermometers at the 135°F point before they are used.

(c) The cormels are removed after 30 minutes immersion, drained quickly, and cooled by plunging them into clean cold water, or spraying with a hose. They are spread thinly on trays to dry in the sun or in a blast of warm air. If they are dried in the original containers they must be periodically turned to expose all the cormels.

All benches, floors, trays, etc., with which the cormels make contact after treatment should be free from contamination. Sterilizing methods that have been successfully used on equipment and storage areas are: spraying with, or immersion in, 1 in 50 commercial (40%) formaldehyde, or 1 in 20 commercial sodium hypochlorite solutions; or fumigation in a room or under a plastic sheet with 4 pounds methyl bromide per 100 cubic feet.

As a post-treatment protectant for the cormels, Spergon dust is recommended. It is moderately effective against surface contamination by disease-producing fungi, and is efficient against the miscellaneous molds that may invade gladiolus cormels if they dry too slowly.

Maintaining the Pathogen-free Status of Propagative Material

To get full benefit from the hot-water treatment, cormels must be planted in clean soil. Clean soil is (a) soil never previously planted to gladioli or related species such as freesia, or (b) soil sterilized by fumigation with methyl bromide, chloropicrin, or steam. Less stringent methods may be applied, but are not recommended since the corms produced in such soil will not be pathogen-free. For example, if neither clean nor treated soil is available, soil managed on an efficient rotation, that has never grown a crop of gladiolus obviously infected with Sclerotinia disease or Fusarium, may be used. In addition, treatment of the soil with calcium cyanamide or Vapam may help to reduce infestation, particularly by Sclerotinia.

A mother-block system to maintain stocks for production of clean cormels is also helpful. The selection methods used in the foundation and maintenance of a mother block may also be used to eliminate serious virus infection and bacterial scab, and to maintain superior clones of a variety. The usual features of the mother-block system are: (a) Plant only from selected hot-water-treated stock. (b) Plant only in uninfested soil, sufficiently isolated from commercial crops to prevent carry-over disease. (c) Inspect and rogue out any diseased or off-type plants. (d) Cultivate, harvest, handle, and store corms and cormels from the mother block apart from commercial crops. (e) Treat cormels from the mother blocks with hot water every year, or as often as is needed to keep the stock pathogen-free. (f) Plant successive mother blocks with corms or cormels only from previous mother blocks. (g) Never introduce new stocks, or return stocks from commercial plantings, into the mother blocks unless they have

been hot-water treated and have maintained their vigor and freedom from disease elsewhere for at least 1 year.

Under good growing conditions a mother block planted with cormels will provide a high proportion of corms large enough for flowering stock, a proportion of corms needing one year's increase before they attain flowering size, and enough cormels to plant the next year's mother block. If the cormels obtained are insufficient for this purpose, take the best corms from the mother block, and use them without hot-water treatment to plant a second-year mother block for the production of cormels, which will be hot-water treated and planted the following year. It may be possible to hot-water treat the smallest corms (fives and sixes) and replant for the production of cormels. These produce very high yields of cormels.

From mother blocks, corms for increase and introduction into commercial planting should be planted in soil as clean as possible.

Success so far Attained

From at least one badly diseased commercial lot of cormels of the variety Spotlight, the diseases susceptible to treatment have been almost completely eliminated by a single hot-water treatment. In other badly diseased commercial stocks a single treatment has reduced infection in the crop to a fraction of 1 percent. Even applied after the optimum period for treatment, and without proper care or safe-guards against recontamination, the method has given good commercial control of Fusarium disease, and has practically eliminated the other diseases susceptible to treatment.

The effect on germinability has varied. Germination around 70 to 80 percent has often been obtained after treatment of dormant cormels. Unless treatment is very poorly controlled or is done much too late, 30 to 70 percent germination is to be expected. A range of 50 to 70 percent is considered satisfactory. If lower germination is anticipated it may be compensated by heavy planting of cormels. Some crops showing reduced germination, smaller foliage growth, and reduced total yields of corms as compared with untreated stocks, have still out-yielded the untreated stocks in number of corms available for planting the following season. This reversal in favor of the treated stock has been due to its relative freedom from decay and loss in storage. When the crop from treated cormels equalled or exceeded the untreated checks in percentage germination and vigor, the yields of corms available for planting the following season were far in excess of the checks. There were also relatively enormous increases in cormels from treated stock.

The system has been adopted by several California growers with marked success. Commercially desirable varieties which had been deleted because of disease have been returned to highly profitable cultivation. Quality and quantity of flower and corm production have been improved, resulting in greater profits. The previous uncertainty of production has been largely eliminated. A final indication of the success of the procedure is the fact that it is now being tried and adopted in other States.

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VIII. CULTURE-INDEXING OF BUDWOOD TO PROVIDE VERTICILLIUM-FREE GREENHOUSE ROSES

Stephen Wilhelm and Robert D. Raabe[1]

The Verticillium disease incited by Verticillium albo-atrum Reinke & Berth., a semi-ubiquitous vascular pathogen of many suscepts, is at times particularly damaging to the greenhouse rose crop. Dimock (1, 2) has given poignant reasons for recent outbreaks of the disease in Eastern rose ranges, citing Pacific Coast-grown roses on the Manetti (Verticillium-resistant) rootstock as the major source of infected plants. He further reported the significant fact of bud transmission of the Verticillium disease, i. e., that buds carrying the Verticillium fungus commonly "take" and develop into diseased plants or inoculate the understock even if they do not "take". These are presumed to account for the majority of Verticillium-infected plants in the nursery.

A common practice among Pacific Coast nurserymen has been to depend somewhat upon commercial rose ranges for sources of buds of flowering varieties. The authors have found that certain lots of commercial bud sticks may be infected with Verticillium to the extent of 0.25 to 1 percent, and experiments here have corroborated Dimock's finding (1) that infected buds may develop into infected (i.e., Verticillium-carrying) plants. From the time of budding to digging in California (May 15 to November 15) infected plants, particularly on the Manetti rootstock, show no distinct symptoms of the Verticillium disease, though in experiments by the authors they grew much more slowly than did non-infected plants.

Though it was described as susceptible in Canada by Madden (4), 3 years of tests by the senior author supported by field observatoins have indicated that the Manetti rootstock is resistant here to natural root infection by a commonly prevailing strain of Verticillium. So far as is known, this resistance does not appear to be of the nature of tolerance, in the sense of tolerance of an infected plant to symptom expression. In tests here, involving numerous cultures, Manetti did not become infected naturally through the roots. If the Verticillium pathogen is artificially introduced into Manetti, as through roots cut to expose vascular bundles, or into the wood of shoots, the stock may become infected and may manifest symptoms typical of the disease, but such artificially infected plants usually recover rapidly and new shoots do not contain the pathogen. This unusual Verticillium resistance of Manetti is in sharp contrast to the susceptibility of the Rosa multiflora Thunb. (Thorny, Burr, and Grifferaie forms), R. odorata Sweet, Dr. Huey, and Ragged Robin (Gloire de Rosamanes) rootstocks. Since the recent report of resistance in Manetti (6) a Verticillium strain has been isolated from Manetti rootstocks showing symptoms suggestive of Verticillium wilt. Since original Manetti stocks of this single isolated planting were from several sources, it appears that there now is a Manetti-attacking strain in California.

Detection of Infected or Infested Propagative Material

Since it is a vascular pathogen, and because symptom incitation by it depends upon rather specific conditions of external environment and host maturity, V. albo-atrum can lurk unsuspected in some plants for long periods. Laboratory culture-indexing provides the only way for reliable detection. Natural straw-agar media, particularly barley and pea straws, sterilized by propylene oxide (3, 7) are particularly suitable for isolation of the Verticillium fungus. Microsclerotia of V. albo-atrum, which render the identification of the fungus easy, form abundantly in these straws (8) and contaminants produce only restricted growth. Five bud sticks containing an aggregate of at least 15 buds may be cultured in a single Petri dish, provided complete cross sections of the vascular cylinders are cut from the basal ends of the bud sticks. Sharp stout blades such as Bard-Parker Series 20 are ideal for cutting this rather hard material.

Obtaining Pathogen-free Propagative Material

A large percentage of the greenhouse roses are grown on the Manetti rootstocks. Established mother blocks of culture-indexed Verticillium-free greenhouse rose varieties for use as bud sources would go far toward the elimination of the Verticillium disease from greenhouse

[1]Associate Professor and Assistant Professor, respectively, Department of Plant Pathology, University of California, Berkeley 4.

roses grown on these rootstocks.

Susceptible rootstocks, such as R. odorata, R. multiflora, Shafter, Dr. Huey, and Ragged Robin, because they may carry the Verticillium fungus, would also have to be culture-indexed before being "struck" in the ground. Because Manetti may succumb to some Verticillium strains, it should also be cultured. Susceptible understocks would have to be maintained on land known to be free of the Verticillium fungus, as on land without a previous history of susceptible plants, particularly tomato, potato, cotton, or the nightshade weed, Solanum sarachoides Sendt., or on land fumigated to eradicate the fungus. Fumigation is at present costly and the results have not always been dependable. Once Verticillium-free root-stock blocks have been established, they could serve as a source of clean propagative material or could be budded over to greenhouse flowering varieties and serve as propagative material for these. Budwood of greenhouse flowering varieties should be culture-indexed and used only if found free of the pathogen.

The feasibility of using hot-water therapy to rid budwood of Verticillium infection was rejected in studies by Nelson and Wilhelm (5).

Maintaining the Pathogen-free Status of Propagative Material

Because of the resistance of Manetti to most of the common strains of Verticillium, Manetti would be the best stock on which to maintain and to increase culture-indexed flowering varieties. Precautions should be taken to prevent rooting of the flowering variety from above the bud union. Outbreaks of Verticillium wilt in greenhouse roses on the Manetti rootstock in the San Francisco Bay area definitely have been attributed to such rooting. An additional precaution, though of questionable necessity, would be to sterilize pruning and budding tools before using them in the mother blocks. The possibility of spread of Verticillium by pruning, which has been reported as an important factor in the Verticillium disease of raspberry (9), is under study now.

On the basis of present knowledge it would be advisable to maintain mother block bud sources of greenhouse flowering varieties on the Manetti root-stock. Should it be desirable, because of compatibility or other relationships, to maintain mother blocks on susceptible rootstocks, any introduction of the Verticillium fungus could be detected by symptoms of the disease produced in the affected plants. Careful observation and roguing of such plants should be practiced, especially during the period of best symptom expression. Symptoms in flowering varieties are more severe on susceptible rootstocks, and in central California nurseries they usually occur in severe form during the summer months. Affected plants may recover somewhat in the fall.

Success Attained in Practice

One large rose nursery, by (a) moving to land without a previous history of Verticillium-susceptible crops, (b) establishment on this land of a culture-indexed mother block of one greenhouse variety on Manetti rootstock, and (c) obtaining propagative material both of rootstocks and buds of flowering varieties from an area determined by survey and numerous cultures to be free from the disease, has attained remarkable success in producing healthy, Verticillium-free plants.

Other nurseries, established on Verticillium-free land as far as is known, undoubtedly have greatly lessened the possibility of producing Verticillium-carrying plants by avoiding buds from commercial rose ranges.

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IX. DEVELOPMENT AND PRODUCTION OF PATHOGEN-FREE PROPAGATIVE MATERIAL OF FOLIAGE AND SUCCULENT PLANTS

Kenneth F. Baker and Philip A. Chandler[1]

In recent years interest in foliage plants and succulents has greatly increased, and this has focused attention on the frequent large disease losses in the commercial production of these crops. Growers have tended to accept such losses as part of the risks of the business, and have learned to reduce disease somewhat through cultural modifications. Usually, however, these changed practices produce undesirable side effects. Thus, minimal irrigation may reduce the severity of water-mold root rots in foliage plants and succulents, but it also reduces growth rate and the size attained, and requires careful hand watering. The over-all effect is to increase production time and cost over that of a disease-free crop. Because these crops are grown in containers, it is entirely practicable to use treated soil and pathogen-free stock, and to produce disease-free plants for sale. Besides the saving to the grower, this increases the satisfactory life of the plant after sale, benefitting the customer and expanding the market for such materials.

Following the commercial demonstration of the efficacy of the program of eliminating rather than continuing to fight disease in these crops, there has been increasing interest in the procurement of pathogen-free stock (2). The results with this approach for several of these crops are presented here, illustrating several methods of obtaining such stock.

1. Dieffenbachia picta Schott

Much of the cane used for propagation of this plant is grown under field conditions in Puerto Rico and Florida. When received by the grower, the canes often have lesions of bacterial soft rot, probably caused by Erwinia carotovora (Jones) Holland, and Phytophthora stem rot caused by P. palmivora Butl. (5). A high percentage of the cut stem pieces usually decay in the propagating bench, and plants produced may later succumb to stem rot (5) or develop bacterial leaf spot caused by Xanthomonas dieffenbachiae (McCull. & Pirone) Dowson (4).

Obtaining Pathogen-free Propagative Material. -- If cane is obtained from field plantings in humid areas it has been found best to treat it routinely in hot water before planting. Alternatives are for the grower to produce his own cane from healthy plants which may sometimes be found, or to buy cane or started plants from a specialist propagator who maintains pathogen-stock.

The growth-status of the cane is important in determining the extent of injury from the treatment. If mature hardened canes are used they tolerate the necessary 125°F, if they are soft (as is much of the field-grown material) only 120°F may safely be used. Cane may be hardened by growing with minimal quantities of water and nitrogen for several months prior to cutting. Young leafy tips should be removed, as they will not survive treatment. The canes are cut into pieces about 2 feet long and treated in hot water at 125°F for 30 minutes. They are cooled at once by either dipping in clean cold water for several minutes, or by flooding with tap water. The canes are held in benches of steamed sphagnum moss until roots or buds start, and then cut into pieces, each with a single bud. These are planted immediately in a perlite and peat mixture, and transplanted to treated soil when the top is well started.

Stock obtained in this way may be grown in a glasshouse without any overhead watering, and tip cuttings taken when the plants are 12 inches or more tall. Such cuttings will be pathogen-free, even when the initial stock may have been treated only at 120°F. In some cases it may even be possible to obtain the initial healthy stock from this measure alone.

Maintaining the Pathogen-free Status of Propagative Material. -- The healthy cuttings obtained must be so handled that recontamination is avoided. The cane or cuttings must not be placed in untreated containers or on untreated soil. Only steamed sphagnum and treated perlite-peat or soil should be used in treated pots or benches. There is evidence that soft-rot bacteria may be spread through the propagating bed by larvae and adult fungus flies. Spraying the soil surface with Dieldrin or Malathion (wettable powders, 1 ounce per 7 1/2 gallons of water) has given promise

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[1] Professor and Principal Laboratory Technician, respectively, Department of Plant Pathology, University of California, Los Angeles 24.

in controlling these insects. If the pathogen-free stock obtained is to be used for subsequent propagation it must be isolated from the commercial production area and carefully handled as a nucleus block. This will further protect the stock from contamination by fungus flies.

Success Attained in Practice. -- One commercial grower, who has been using these methods for 5 years, has largely eliminated disease losses in production. It has also been found by him and by us that the percentage of buds which break dormancy is increased by the hot-water treatment.

2. Fittonia verschaffeltii Coem. var. argyroneura Nichols and Pellionia pulchra N. E. Br.

These plants sustain heavy losses from stem rot and leaf decay caused by Rhizoctonia solani Kuehn and by water fungi.

Obtaining Pathogen-free Propagative Material. -- If a few healthy plants are available, or can be obtained from a specialist propagator, a grower may build up his own pathogen-free stock. Usually, however, it is necessary for the grower to develop his own nucleus stock. Plants may be grown up on a wire frame; by removal of lateral shoots the stems may reach 12 inches from the soil. If overhead watering is avoided, the tips of such shoots may provide a source of pathogen-free stock. It has been found commercially possible to obtain clean stock by planting such tip cuttings in treated soil, and repeating the procedure several times. They may, however, be more easily and rapidly obtained by hot-water treatment.

Hardened plants are best used for the treatment in hot water at 124°F for 30 minutes. Unhardened plants may survive only 120°F. The plants may be hardened as explained for Dieffenbachia. Clean the plant of dead leaves and soil before treatment. Cool rapidly in clean cold water following treatment. Make cuttings and divisions, removing any damaged leaves, and plant at once in a mixture of perlite and peat, where roots may form in less than a week. The leaves are very sensitive to the treatment and may be killed. Such dead leaves should be promptly removed to reduce to reduce invasion by Botrytis cinerea Fr. ex Pers. and bacterial soft rot. The stems are quite heat tolerant, and new shoots will develop from them.

Maintaining the Pathogen-free Status of Propagative Material. -- The healthy status of the nucleus stock may be maintained by growing in treated soil and containers in an isolated glasshouse area. It would be advisable to grow the plants on wire frames up off of the soil and to take tip cuttings for planting in the increase block. Wetting the foliage should not be permitted in watering, and careful sanitation should be routinely followed.

Success Attained in Practice. -- One commercial grower has utilized the program with outstanding success. The Fittonia plants are hot-water treated and planted in individual pots of steamed soil. When these have grown to a height of 6 inches under conditions of general sanitation without ever wetting the foliage, tip cuttings are taken. These are rooted in steamed sand and planted in pots. Completely pathogen-free nucleus blocks were established by the time this procedure had been followed through 3 generations. At first the cuttings were rooted in sand and planted in small pots, taking 8 to 10 weeks to produce saleable size. Now the cuttings are planted directly into small pots of a University of California type soil mix (50% peat, 50% fine sand). Automatic misting is practiced, without loss from Rhizoctonia or water molds. Production now requires only 5 weeks. Over a quarter million young plants are raised annually by this grower in scheduled production by this method.

3. Syngonium auritum (L.) Schott

A black cane rot, caused by a specialized form of Ceratocystis fimbriata Ell. & Halst., is sometimes destructive on this crop in commercial nurseries (3).

Obtaining Pathogen-free Propagative Material. -- Since there is still a good deal of stock available that is free from this fungus, there is little difficulty in obtaining healthy stock. A heat treatment is a more certain and faster method. Plants should be hardened as described under Dieffenbachia to increase the heat tolerance. Whole plants are bare-rooted and treated in hot water at 120°F for 30 minutes. They are then promptly cooled and planted in soil. There is some leaf injury, but plants quickly recover. If stems are badly cankered it would be well to cut

them above the injured area; new roots will form quickly and the plant will grow faster without the stricture.

Maintaining the Pathogen-free Status of Propagative Material. -- See Fittonia and Pellionia.

Success Attained in Practice. -- There has been no commercial attempt as yet to place this program in practice, but the ease of its accomplishment in experimental glasshouses gives strong assurance of its practicability should it later be needed.

4. Syngonium podophyllum Schott var. Emerald Gem

This plant frequently is injured by root rot caused by water fungi in commercial nurseries.

Obtaining Pathogen-free Propagative Material. -- The use of tip cuttings from plants grown up 12 inches or more above the soil under conditions where the foliage has not been wetted will often give healthy stock. A heat treatment is coming into use because of its effectiveness both against pathogens and in breaking dormancy of cane buds. Cane is treated in pieces about 2 feet long and is relatively tolerant of the hot-water treatment at 120°F for 30 minutes. Material hardened as for Dieffenbachia will survive 125°F for 30 minutes. Because of injury this temperature should be used only for treatment of nucleus stock. The canes are cooled rapidly in water, and then held in a humidity cabinet at 70°F for 2-3 weeks before being cut into pieces and planted in perlite and peat.

Maintaining the Pathogen-free Status of Propagative Material. -- See Fittonia and Pellionia.

Success Attained in Practice. - Used in one commercial nursery with marked success. The heat treatment probably will be largely used for its increase in the number of buds breaking dormancy, the riddance of disease organisms being a gratuitous accompaniment.

5. Aloe variegata L. and Haworthia attenuata Haw.

Young seedlings of these plants frequently are discarded because the roots have been rotted by Pythium ultimum Trow. Since 2 to 3 years are required to produce such plants, the economic loss may be considerable. Usually the decay does not progress from the roots into the stem, and the plants therefore can be salvaged by a hot-water treatment.

The disease can easily be prevented in seedlings by (a) using seed produced on stalks kept up off of the ground in semi-arid localities, (b) planting only in treated soil and containers, and (c) practicing reasonable sanitation. The hot-water treatment is to be viewed purely as a salvage operation of either seedlings improperly grown or of large, old, field-grown seed plants whose roots have been decayed. The method has shown such merit as to warrant its description here.

The seedlings or adult plants are cleaned of dead basal leaves, soil, and dead roots before immersion in hot water at 115°F. The period of treatment varies from 20 minutes for small plants to 40 minutes for large ones. The plants are cooled promptly in cool clean water, and planted at once in treated soil and containers. With care to avoid recontamination the treatment has given complete elimination of the disease. Plant injury is so slight as to be inconsequential (1).

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X. DEVELOPMENT OF ROSE PROPAGATIVE MATERIAL FREE FROM BLACK SPOT

Eldon W. Lyle[1]

Black spot, caused by the fungus Diplocarpon rosae Wolf, is the most widespread and destructive disease of roses. It occurs in nearly every State of this country, being naturally restricted only in semi-arid areas, and even there the disease may become prevalent if overhead watering is practiced.

Damage from black spot is mainly from its defoliation effect, which weakens the bushes, reduces flower production, and increases susceptibility to cane die-back and winter-killing. Severe infection decreases cane size of the bushes (4), diminishes number and size of blooms, lightens flower color, and decreases fragrance, besides impairing the chances of transplanting survival. Also objectionable are the unsightly blotches of the fungus infections.

The disease is serious in commercial fields as well as in home gardens. Sanitary measures and treatments to delay or prevent primary infections are of great importance in the control of black spot.

Detection of Infected or Infested Propagative Material

While the leaf symptoms of black spot are important in detection of the disease, it is the cane lesions which probably provide the spores most effective in spreading the disease during rose propagation by budding. Inspection of rose fields or gardens from which scions are to be taken offers the best means for detecting infected propagative material. The usual inconspicuous cane lesions of black spot ordinarily are accompanied sometime during the year by numerous and noticeable leaf infections. Absence of leaf infections at one time of year would not necessarily mean lack of cane lesions. However, periodic examinations of a rose planting through its complete season of growth without the finding of foliage symptoms would be acceptable proof of the freedom from black spot of the branches to be used as scions.

Obtaining Pathogen-free Propagative Material

Control of black spot and complete prevention of its spread has been accomplished under greenhouse conditions by keeping water off the foliage during irrigation, and by elimination of syringing as a means of insect control. Infected plants have been maintained in close proximity to healthy ones without spread of the fungus, as long as spattering of water on the foliage did not occur. Studies on the epiphytology of the black spot disease (1) showed that the conidia are disseminated mainly by splashing water. The conidia remain stuck together above the acervuli until contacted by particulate water. Once loosened, the spores are rapidly spread by spattering water or by windblown rain. After being wetted, the spores are capable of germination, but require continued contact with water or relative humidity of 92 percent or more for at least 6 hours to germinate. Both the upper and lower surfaces of the leaves may be infected.

Besides careful production in greenhouses another source of scions free from the pathogen would be regions that are so dry and deficient in rainfall that extensive ditch irrigation is necessary for bush production (e.g., in the commercial rose fields of California and Arizona). Black spot has not been observed by the writer under such conditions except as noted in some home gardens where overhead sprinkling is practiced.

Securing black spot-free scions by breeding for resistance to the disease is another possibility, but not much progress has been made yet in present-day varieties. However, certain strains of Rosa multiflora Thunb. have been found immune or nearly so, and a few other species and varieties have shown resistance, if not immunity, and could be used in hybridization.

Maintaining the Pathogen-free Status of Propagative Material

Once obtained, it is only necessary to keep the foliage dry and or to use protective fungicides to prevent primary infections of black spot. Periodic inspections of the foliage would determine this need.

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[1]Plant Pathologist, Texas Rose Research Foundation, Inc., Tyler, Texas.

Success Attained in Practice

Unfortunately, the practice of starting with pathogen-free scions and using the immune Rosa multiflora understock has not entirely prevented the subsequent development of black spot under Texas field conditions. However, the appearance of the disease was delayed, and showed the value of using pathogen-free scions (2, 3). In one such trial using scions developed in a greenhouse and known to be free from black spot, the disease became evident in the field late in the first year after budding and forcing; this trial was isolated from other roses by about 550 feet. In another trial, it was early in the second year before primary infections occurred. The isolation was not sufficiently great to exclude introduction of the pathogen by some natural means.

For the commercial propagation of roses, many nurserymen now appreciate and are capitalizing on the advantage of securing budwood from locations that do not have black spot, or from fields that have been effectively treated with fungicides to control it. Thus, there is a definite carryover of benefit into the second year following the control of black spot, delaying and decreasing its incidence. Furthermore, the percentage survival of scions has been as much as 50 percent better when the budwood came from bushes free from black spot than when budwood was used from fields where the disease was prevalent.

This development of clean budwood does not represent complete exclusion of a disease, but it does provide an important control measure for the rose growers in areas where black spot might otherwise be a problem. If it becomes commercially desirable to provide blackspot-free plants, these can presently be produced under field conditions in semi-arid areas, provided all overhead sprinkling is avoided.

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XI. DEVELOPMENT AND PRODUCTION OF PATHOGEN-FREE GERANIUM PROPAGATIVE MATERIAL

Donald E. Munnecke[1]

Since geraniums (Pelargonium hortorum Bailey) are almost exclusively propagated vegetatively rather than from seeds, the production of pathogen-free cuttings is of the greatest importance. Because cuttings generally are taken from well up on the plant, only a few of the pathogens of the various diseases, including bacterial stem rot (Xanthomonas pelargonii (Brown) Starr & Burk.), virus diseases, Verticillium wilt (V. albo-atrum Reinke & Berth.), and cutting rots (Pythium sp. and Rhizoctonia solani Kuehn), are commonly transmitted by them. Of these, bacterial stem rot and virus diseases are the most important.

Methods of Detecting Infected or Infested Propagative Material

The diseases of geraniums most difficult to detect are those which either invade the vascular system (bacterial stem rot and Verticillium wilt), or are systemic (virus diseases). These diseases produce characteristic symptoms, but they may be masked and thus be difficult to diagnose accurately by visual means. Furthermore, cuttings of some varieties may harbor the causal pathogens without any external symptoms.

Symptoms are helpful in initial elimination of plants suspected of harboring disease. However, the only positive way of detecting pathogens is to culture a portion of each cutting on laboratory media. With bacterial stem rot the culturing method may be confirmed by macerating in water a portion of the cutting and inoculating a young susceptible plant (Radio Red variety) by puncturing the stem with a needle and placing the decoction in the wounds. In 2 to 4 weeks the lower leaves wilt and die, and gradually the stem rots and blackens if the decoction contains the bacterial pathogen. This latter method is most useful in detecting the bacteria in rotted tissue which cannot be cultured because of the presence of other organisms. In several cases in which we have been unable to isolate bacteria by culturing, the inoculation technic was positive. This applies especially to rotted cuttings.

Methods for Obtaining Pathogen-free Stock

Pathogen-free geraniums may be obtained commercially by the following procedures:

1. Select Apparently Disease-free Plants for Initial Cutting Source. --

a. Fungus and bacterial diseases. Use plants for source of cuttings which have been observed for at least 6 months with no symptoms of these diseases.

b. Virus diseases. The detection of virus diseases of geraniums is still based upon the visual symptoms produced. This is a serious obstacle in producing clean stock, since the virus symptoms are frequently masked in summer, and occasionally in winter as well. Another difficulty has been the uncertainty of the role of insects in transmission of the common virus diseases. Furthermore, graft transmission studies have shown that symptoms may not appear for 6 months on the stock plant on which a virus-infected scion was grafted.

Virus diseases could be avoided if plants produced directly from seed were used as stock plants. Although geranium seed is offered for sale, seed of commercial varieties, true to type, is not available. As virus diseases are becoming extensive, a breeding program may have to be undertaken to produce varieties for commercial production which come true from seed. The alternative, less certain way, is to select parent plants that have been closely observed for several years, especially in the cool seasons, and that have shown no symptoms of virus. By promptly roguing any plants with the slightest symptom of virus infection (leaf roll, mosaic, small leaves, dwarfed plants, distorted flowers) virus-free plants may be obtained. In this way under glasshouse conditions we have obtained plants presumed to be free of virus diseases.

2. Culture the Cuttings. -- The laboratory cultured-cutting technic (agar slant, as described in Papers 2 and 5 of this series) is used to be certain that the supposedly disease-free cuttings selected actually are free of pathogens. This method will permit selection of stock free from bacterial and fungus pathogens, but is inapplicable for viruses. Cuttings presumed to be free from virus should be tested for the other diseases even though the parent plants were without

[1]Assistant Professor, Department of Plant Pathology, University of California, Los Angeles 24, California.

symptoms for several years.

3. Establish a Nucleus Block. -- Each cultured cutting should be rooted separately in sterilized sand and then planted in individual pots or in a raised bed with less than 10 plants per block in soil that has been steamed or treated with methyl bromide or chloropicrin. It is desirable to grow them in a glass or plastic house to afford protection from insects and inclement weather. Since the nucleus block is the most valuable part of the whole operation, the plants must be very carefully grown, preferably by the proprietor or the most careful grower in the organization. Great care must be taken to prevent these plants from becoming infected. Botrytis rot may be avoided by removing the flowers as they are formed, cleaning off dead leaves and debris, and pruning the plants to form an "open" type of growth so that air circulates freely in the center and around the base of the plants. A special set of tools, water hoses, etc., should be reserved for use only in this nucleus block. Implements should be disinfested before use by washing off the dirt and immersing for 1 minute in a 5% formaldehyde solution; they should then be rinsed with water. Hands should be washed with soap and water before handling the plants. Insect control should be maintained at all times by frequent spraying or dusting with malathion, DDD or DDT, or a combination of the 2 materials. When taking cuttings use 2 knives and disinfest by dipping in 1:1000 mercuric chloride solution. Use one knife to take the cuttings from a plant while the other knife is in the disinfectant and then exchange knives for taking cuttings from the next plant. Cuttings may be broken off as is done with carnations and chrysanthemums; however, this often results in excessive wounding of the stock plant and predisposes the plant to attack by Botrytis. Each plant should be numbered and the cuttings taken from them for the increase block (see below) should be numbered so that they may readily be identified with the parent plant. In the event a nucleus block plant or one of its progeny develops symptoms of stem rot, virus, Verticillium wilt, or other disesase, it and all of its progeny should be immediately destroyed. Here the advantage of growing the nucleus block plants in individual pots becomes obvious. If a plant becomes infected the spread of disease is easily checked by prompt removal of the entire plant and soil if planted in an individual pot. If planted in a bed all of the plants in that bed must be removed and the soil and bench resterilized before replanting. Thus, the advantage of better disease control obtained by the individual pot outweighs the cultural advantages obtained by the raised bed method. No plant from the increase block should ever be planted in or near the nucleus block, without prior long observation of its health status.

4. Establish an Increase Block. -- Root cuttings from the nucleus block plants in steamed or chemically-treated sand, and plant in ground beds, greenhouse beds, or benches likewise treated for disease control. Number each as outlined above. Exercise every reasonable care in handling. However, since this is a much larger planting it cannot be protected so rigidly as the nucleus block. Follow the same strict sanitary precautions and procedures in so far as possible, and allow only the best workers to handle the plants in this block.

5. Establish Production Blocks. -- This varies with the size of the operation and the number of cuttings desired. Plant rooted cuttings produced from the increase block in ground beds or in a field which has been treated with chloropicrin or methyl bromide. Cuttings taken from this field are never retained as propagative stock, but are sold. Again, sanitation and culture operations should be as nearly like those of the nucleus block as possible. Since mercuric chloride is poisonous and may be misused by field laborers, use 70% methyl alcohol as a knife disinfectant.

In the system outlined above, the movement of cuttings is from the nucleus to the increase block, and from that to the production block. Also, there is a size progression from a small, compact, one-man-operated plot to large production blocks.

Methods for Maintaining the Pathogen-free Status of the Stock

The stock may be maintained pathogen-free by continued application of knowledge of the nature of the diseases and how they are spread, and practicing of methods of preventing recontamination. Among such measures are:

Soil Treatment. -- All cuttings should be rooted in steamed or chemically treated media. Soil for the nucleus block and increase blocks must also be treated. The production block should be planted on steamed or chemically treated soil.

Disinfestation. -- Knives may be disinfested by dipping in mercuric chloride or methyl alcohol for about 1 minute before reusing. Five percent formaldehyde may be used in a similar manner to disinfest tools.

Sanitation -- Remove debris, dead plants, and plant trimmings from growing area. The tools used on the nucleus block should only be used there. Avoid contaminating beds by scuffing dirt on them, walking on them, handling the soil unnecessarily, etc.

Isolation. -- Nucleus blocks must be isolated from other geraniums by growing in a greenhouse or plastic house in a different location from the main growing area.

Culture Methods. -- A 1 year crop rotation or soil sterilization with steam or chemicals is necessary to avoid the present worst disease, bacterial stem rot. Careful watering of plants to avoid wetting the foliage is necessary to prevent the spread of disease. Ditch irrigation should be used for field operations, and hand watering using a breaker nozzle on the hose to prevent splashing should be used for the nucleus block and increase blocks. Insects should be kept down by frequent spraying or dusting with insecticides.

Success Attained in Commercial Practice

A program similar to the one outlined here has been under way in California for about 18 months in several commercial establishments. It is too early to evaluate the success it has had but the growers have been enthusiastic about their early results. It is estimated that disease-free plants in the nucleus blocks and increase blocks have produced from 2 to 3 times the number of cuttings as compared to plants grown in the usual way. Another point which has not yet been commercially proved is the fact that disease-free plants may yield so many more cuttings per plant that the extensive acreages now planted may be cut to a relatively small, highly intensified type of agriculture with much more efficient use of land, labor, and materials.

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