THESIS

INACTIVATION AND TRANSMISSION STUDIES OF THE CARNATION VIRUSES MOSAIC AND STREAK

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY GAIL E. HUMLEY

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Chapter I

INTRODUCTION

The prevalence of viruses in carnations has increased in commercial greenhouses throughout the United States to such an extent that it is virtually impossible to find plants free from infection. The incidence of mosaic is general; streak is becoming more common.

Factors controlling the development of these diseases have been studied by various investigators (3, 4, 9) but further information concerning the transmission and inactivation of these viruses is necessary in our efforts to control these diseases that are so costly to the carnation industry of Denver. This investigation was made to determine how carnation mosaic and streak viruses may be transmitted, and to attempt to inactivate the viruses by treating carnation cuttings and plants with heat and chemicals.

Chapter II

INACTIVATION STUDIES

Thermal inactivation studies in vitro.-- Jones (9) reported that the thermal inactivation point of the carnation mosaic virus in vitro was near 60° C. but did not give a description of his method nor the length of time that the virus was subjected to the various temperatures.

In the present study an experiment was set up to determine the thermal inactivation point of the carnation mosaic virus in vitro. Knowledge of this type would give a better understanding of the virus and, perhaps, yield information indicating a method of inactivating it in vivo.

A plant of D. barbatus infected with mosaic was ground up in a meat grinder. The sap was expressed through cheese cloth and centrifuged for 10 minutes at 1700 r.p.m. Three ml. of the sap was then placed in each of 6 test tubes.

Five of the tubes were subjected to one of the following temperatures: 40° C., 50° C., 60° C., 70° C., and 80° C. for 10 minutes in a water bath. One tube was not treated as it was to serve as inoculum for the check.

Sap from each treatment was used to inoculate 5 healthy plants of D. barbatus by rubbing the leaves with inoculum and using 200 mesh carborundum as an abrasive. The incidence of visible symptoms of mosaic are shown in Fig. 1.

The virulence of the virus declined gradually from 40° C. to 60° C. above which temperature the virus became completely inactivated, no infection occurring when inoculum from tubes subjected to higher temperatures was used. The wide range of temperatures over which the virus slowly lost its virulence indicated that the virus has a low temperature coefficient. In this respect it resembles the tomato bushy stunt or tobacco mosaic viruses (1). A virus of this type may be inactivated by exposure to high temperatures for a short time or by exposure to lower temperatures for a longer period of time. The possibility of effective therapeutic treatments by heat, chemicals or irradiation may have practical applications due to the obvious instability of this virus.

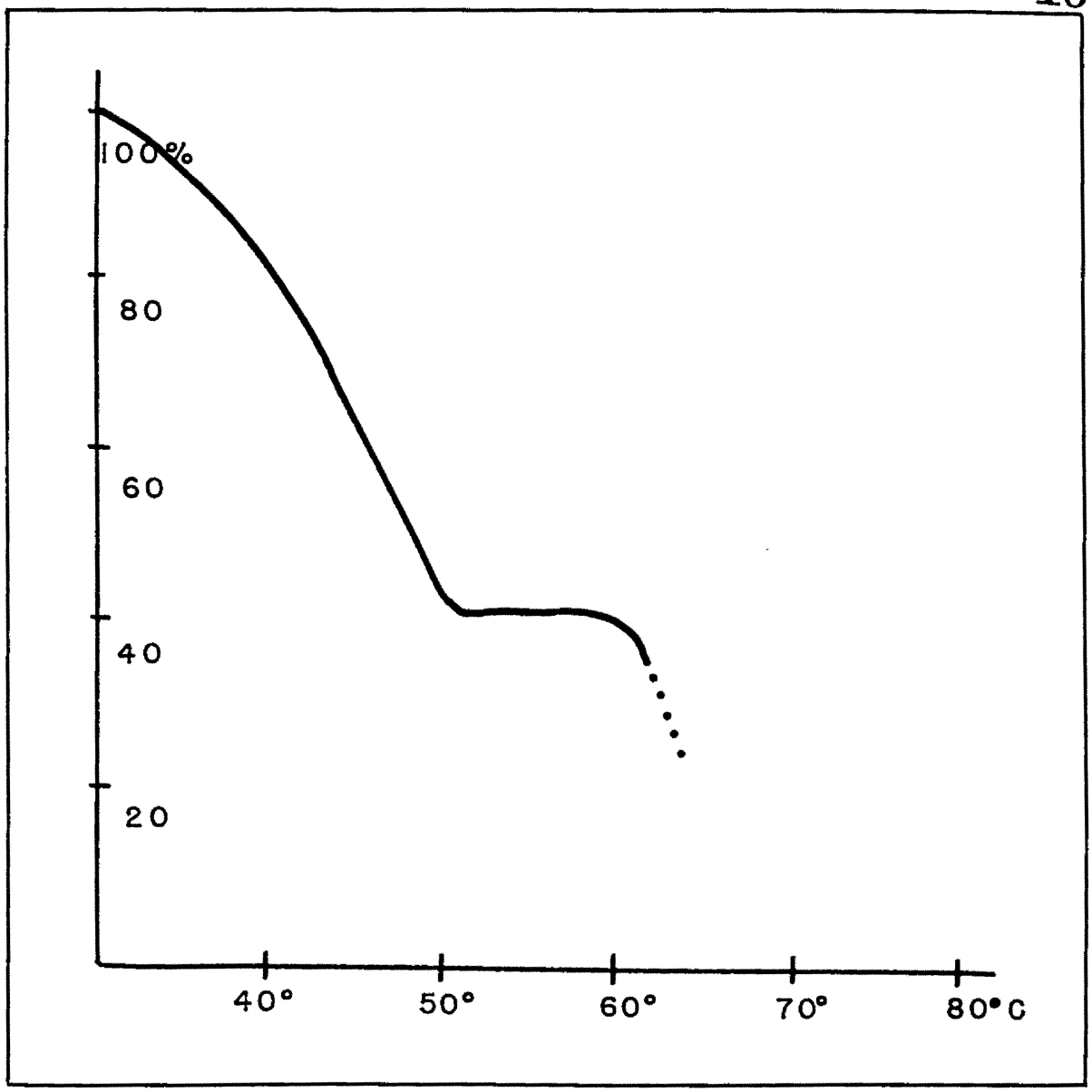

Fig. 1.--Illustration of the effect of temperature on inactivation of carnation mosaic in vitro

Thermal inactivation studies in vivo.

Dry heat inactivation.--Kunkel (10, 11, 12, 13) was able to cure yellows and mosaic of peach, aster yellows and cranberry false blossom by the use of dry heat. By exposing the plants to heat the peach yellows virus was inactivated in 8 days at 34.4° C. to 42° C. Best (2) found that 97 percent of the tomato spotted wilt virus could be inactivated in vivo by exposing an infected plant to 40° C. for 24 hours.

In the previous experiment it was found that the carnation mosaic virus was inactivated slowly by increasing the temperature. This indicated that the virus can be inactivated in vitro at lower temperatures by exposing it for a longer period of time. In view of the success of other workers (2, 10, 11, 12, 13) in using dry heat to inactivate viruses in vivo, it was decided to attempt heat inactivation of the carnation mosaic virus in vivo using the same principles. If this treatment were to be successful it would be especially practical, as commercially valuable plants could be cured of the virus by the heat treatment and then used as foundation stock for growing virus-free carnations.

A heat chamber was constructed by fastening 5 window sash together and fitting it with a solid bottom. Sixty feet of hotbed cable was fastened to the bottom to serve as a source of heat. The cable was wired to a thermostat which controlled the temperature of the chamber to within $3.5^{\circ} \pm$ C. of the desired temperature for the test. A heating cycle was completed every 24 minutes.

Potted carnations were placed in the box in 2 tin pans. The plants were watered once daily leaving a little water in the pans to maintain a high humidity in the heat chamber to prevent, as much as possible, drying of the plants.

Eighteen plants were used in the first experiment. These were not very desirable, having been taken from a year-old bench that was badly infested with red spider which, along with crowding of the plants and virus infection, resulted in stunted and hardened plants. The most desirable plants are young, vigorous and well rooted (11). In the chamber the plants were exposed to an average temperature of 43.5° C. for 18 days. One plant was removed each day.

All of the plants exposed to the heat treatment died. From the data of other investigators (12, 13), survival of the plants exposed to the heat for 10 to 12 days could be expected. The weakened condition of the plants probably allowed them to be killed more easily by the heat than was expected. It is apparent that vigorous plants are required to stand a temperature high enough to inactivate a virus.

Healthy D. barbatus plants were employed in the second experiment using the same procedure. Twelve vigorous, well-rooted plants were selected and placed in the heat chamber at an average temperature of 38.5° C. Natural light was supplemented by a 300-watt incandescent light placed 4 feet above the chamber from 5 a.m. to 5 p.m. Two plants were removed every other day so that the last 2 plants had been in the chamber 12 days.

At the end of the experiment all plants were alive and seemed to be in good growing condition. After 2 weeks 1 plant each from the 10- and 12-day treatments died. The rest continued to make good growth.

These results indicated that vigorous D. barbatus plants can withstand an average temperature of 38.5° C. for 8 days without ill effect and 10 to 12 days with an indeterminate loss.

Because of the reaction of D. barbatus to heat, the original test with carnation (D. caryophyllus) was repeated using 9 well-rooted, vigorous plants infected with mosaic. The use of healthy carnation plants would have been much more desirable for comparison with the reaction of healthy D. barbatus, but healthy carnations were unavailable at the time.

These plants were treated as in previous experiments. They were removed in groups of three at the end of 8, 10 and 12 days. Light was supplemented as before.

The plants treated 8 and 10 days were still green when taken from the heat chamber but dried rapidly when taken to the greenhouse. Those treated 12 days had dry, brittle foliage before being taken from the heat chamber. After 2 weeks in the greenhouse, none of the plants broke or showed other indications of life.

Apparently the temperatures at which healthy D. barbatus would live were detrimental to mosaic-infected D. caryophyllus. The presence of a virus may be the factor which determines how much heat either D. barbatus or D. caryophyllus will stand. The studies of Selman (15) and Esau (8) have shown the changes caused by

a virus in the metabolism and leaf structure. The presence of a virus might also account for the inability of the carnation to withstand heat treatment.

With the reaction of diseased carnations to heat in view, it was advisable to repeat the experiment with D. barbatus infected with mosaic. Ten vigorous D. barbatus plants were treated in the heat chamber as before. Two plants were removed after 8, 10, 12, 14, and 16 days to the greenhouse. In order to avoid physiological complications in symptoms expressed, the plants were repotted in fresh soil and after 30 days were grafted to healthy D. barbatus plants by approach grafts to determine if they still contained infective mosaic virus. The results shown in Table 1 were obtained.

All plants in the 8- and 12-day treatments died while one each lived in the 10-, 14- and 16-day treatments. It cannot be explained why the plants in the shorter treatments died. Perhaps the physiological differences between the plants make some less resistant to the heat even though they appear to be similar in every respect.

The 10-day treatment did not result in an absolute inactivation of the virus. Symptoms shown by

Table 1.--RESULTS OF THE HEAT INACTIVATION EXPERIMENT USING MOSAIC-INFECTED *D. BARBATUS*

No. days in heat chamber	No. plants alive after 2 weeks	No. plants transmitting mosaic
8	0	-
10	1	1
12	0	-
14	1	0
16	1	0

grafting the treated plants onto healthy D. barbatus were not typical but appeared to be very mild, suggesting attenuation of the virus by heat. The 14- and 16-day treatments resulted in no transmission of mosaic.

The above experiment indicates that carnation mosaic virus can be inactivated in D. barbatus by subjecting it to a temperature of 38.5° C. for 14 days. Due to the small number of plants available for use in this experiment, it would be advisable to repeat it with a large number of plants to confirm these results.

Hot water inactivation.--Kunkel (12) was able to inactivate the aster yellows virus in periwinkle (Vinca rosea) by immersing infected plants in water at 40° C. for 24 hours or at 45° C. for 2.5 hours. He reported inactivation of peach yellows and rosette by dipping infected plants for 2-minute intervals from 2 to 12 minutes at 50° C. (11). Edgerton (7) cured chlorotic streak in sugarcane by immersing cuttings in water at 52° C. for 20 to 30 minutes. It seemed possible that carnation cuttings could be treated the same way to free them of the deleterious effects of the carnation mosaic virus.

A 12-liter container was fitted with a heating element and a thermostat which was sensitive to within 0.5° C. of the desired temperature. An agitator was used to distribute the heat evenly throughout the water in the container during the experiment.

After the water had been adjusted to the desired temperature, mosaic-infected carnation cuttings were wrapped loosely in cheesecloth and submerged in the water. At regular time intervals a number of the cuttings were removed from the water. In this way the ability of the carnation cuttings to withstand a certain temperature for different lengths of time could be determined.

After treatment, the cuttings were placed in a flat containing vermiculite where they were allowed to remain for 3 weeks to root. Then they were potted and, at the proper time, grafted to D. barbatus to determine if the virus in the cutting was still active.

In the first experiment cuttings were selected from diseased plants rogued from foundation stock benches by ultra-violet radiation (19). The water temperature was set at 45° C. and 10 cuttings were removed from the water at intervals of 1 hour for 8 hours. The check was not immersed in water.

When the cuttings were placed in the flat they all looked normal except those treated for 7 and 8 hours. These treatments looked wilted. Within a week all of the cuttings had become white and died except for the check which rooted and grew normally.

The results of this experiment indicated that the time intervals were too long, including the lowest, for the temperature used. The time interval and temperature at which the cuttings would live was not found. In the subsequent experiment, another attempt was made to find the thermal death point of the carnation cutting.

The material for the second experiment was obtained from a commercial greenhouse in Denver, Colorado. Terminal cuttings were selected at random from established plants in benches. An interval of three days elapsed from the time of selection until the cuttings were used in the experiment, during which time they were kept in a cool place covered with damp sacks. A temperature of 50° C. was used for this experiment. Lots of 10 cuttings each were removed from the water after treating 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 minutes. The method used was the same as that used in the previous hot water treatment.

When placed in vermiculite, all cuttings including the check became white and died after approximately 1 week. In a short time a watery black soft rot appeared on the cuttings where they entered the vermiculite. The cuttings appeared to have been attacked by an unidentified damping-off organism. The rooting end of the cuttings softened and turned black. The organism did not immediately penetrate to the inner portions of the growing point, but eventually this took place and the cutting died.

The failure of this experiment cannot be explained due to the fact that more than 1 factor could have killed the cuttings or weakened them for attack by secondary invaders. The cuttings were not fresh, and this combined with the hot water treatment may have injured the tissue and opened the way for a weak pathogen which ordinarily would cause no trouble. The check also died. The check in the previous experiment was normal. This indicated that the cuttings were not in good condition and probably would not have lived even if they hadn't been treated.

The results of the hot water inactivation studies were of a negative nature; however it is believed that further experimentation might disclose a temperature and immersion time combination in which the carnation mosaic virus would be inactivated without damaging the cuttings. It would be well to try rooted plants which could be better able to withstand the heat without injury.

Chemical inactivation.--The literature contains numerous references to the inactivation of viruses in vitro and in vivo by chemicals. Takahashi (18) found that the tobacco mosaic virus could be inactivated in vitro by malachite green. Recently Stoddard (16, 17) reported the inactivation of the peach X disease virus by soaking diseased peach buds in aqueous solutions of various chemicals without injury to the plant tissue. The trees could be immunized or cured by injections or watering with these solutions.

It is with particular interest that Stoddard's reports (16, 17) of chemical inactivation of the X-virus of peach in vivo were studied with regard to the possibility of using the same technique to inactivate the

carnation mosaic virus. He obtained complete control of X-virus disease of peach with several organic and inorganic chemicals.

An experiment was therefore set up in an attempt to inactivate one or both of the carnation viruses using several of the most successful chemicals and a modification of Stoddard's technique.

The method used in the experiment differs from Stoddard's method in that he used peach seedlings rooted in soil before treatment with chemicals, and in this experiment carnation cuttings were placed in vermiculite and treated with the chemicals immediately before being allowed to root. White enameled pans 10 inches in diameter and 4 inches deep were two-thirds filled with fine vermiculite to serve as a rooting medium. For the first watering of the cuttings 250 ml. of chemical solution was used and in subsequent waterings tap water was used. Twenty cuttings were placed in each pan and the 12 best were selected for potting after 4 weeks. The plants were allowed to grow for 2 months before being tested for the presence of active virus by ultra-violet radiation (19). The chemicals and the concentrations used in the experiment are shown in Table 2. The results are tabulated in Table 3.

Table 2.--CONCENTRATIONS OF CHEMICALS USED IN CHEMICAL INACTIVATION EXPERIMENT

No. of treatment	Chemical	% concentration by weight	Gms. chemical in 250 gms. solution
1	8-hydroxyquinoline sulfate	0.025	0.0625
2	Calcium chloride	0.2	0.5
3	Hydroquinone	0.0125	0.0312
4	Zinc sulfate	0.025	0.0625
5	Sulfathalidine	0.0166	0.0415
6	Sulfathiosole	do.	do.
7	Sulfamerizine	do.	do.
8	Sulfaguanidine	do.	do.
9	Sulfasuxidine	do.	do.
10	Check[1]		

[1] Tap water alone used to water plants

Table 3.--RESULTS OF CHEMICAL INACTIVATION EXPERIMENT

No. of treatment	No.* of plants	Ultra-violet radiation			Mechanical transmission		
		Healthy	Streak	Mosaic	Healthy	Streak	Mosaic
1	12	0	1	11	1	0	11
2	12	6	2	4	6	0	6
3	1	1	0	0	1	0	0
4	12	6	0	6	8	0	4
5#	0						
6#	0						
7#	0						
8#	0						
9#	0						
Ck.	12	0	0	12	0	0	12

*Number of plants indicates the number removed from the pans and potted in soil. Twenty cuttings were stuck in each pan and the twelve best used.

#In these pans the cuttings were all killed by the chemical.

All of the cuttings that were treated with the various sulfa compounds failed to root and died. 8-hydroxyquinoline sulfate yielded only 1 healthy plant when tested for active virus by mechanical transmission to healthy D. barbatus and when tested by ultra-violet radiation no healthy plants were found. This can be explained by the fact that the carnation streak virus cannot be transmitted mechanically but can be detected by the ultra-violet radiation. This plant probably was infected with streak virus which failed to be transmitted by mechanical inoculation, consequently resulting in a healthy reading. However, when the plant was tested by ultra-violet radiation the virus was detected and the plant gave a positive test.

Calcium chloride gave better results. Half of the plants were found to be free of active virus by both methods of testing. Only 1 cutting survived in the pan treated with hydroquinone, and it was found to be free of active virus. Six of the plants tested free of active virus by the ultra-violet radiation and eight by mechanical transmission out of 12 cuttings treated with zinc sulphate.

The results of the chemical inactivation experiment indicated that there are possibilities for controlling the carnation mosaic virus by the use of chemicals. Perhaps the other methods used by Stoddard (16), such as treating rooted plants or allowing a mature plant to take up the chemical through a cut stem, might yield more satisfactory results.

Chapter III

TRANSMISSION STUDIES

Mechanical transmission studies of carnation mosaic.--The literature contains conflicting reports as to the actual means of transmission of carnation mosaic. Creager (4) was unable to obtain transmission of carnation virus by mechanical means. He did not differentiate between mosaic and streak viruses. Jones (9) reported transmission of carnation mosaic by mechanical means. Due to this evidence, it was desirable to determine the various methods of transmission and their relative efficiencies in an attempt to clarify some of the conflicting ideas concerning the mode of transmission of the carnation viruses.

The experimental work on mechanical transmission consisted of 4 experiments. In the first the object was to test various techniques for their efficiencies and speed for use in scientific investigation. The second experiment was set up to test the percentage of infection in mass transmission. The object of the third was to determine the incidence of infection resulting from the use of the cutting knife and the fourth to test infection resulting from contact.

Because of the difficulty of reading virus symptoms on the narrow, thick leaves of the carnation, the method suggested by Brierly (3) was adopted. In this method the virus is transmitted from Dianthus caryophyllus (carnation) to D. barbatus, in which the virus symptoms are more easily read. D. barbatus was used as a test plant throughout this investigation.

Transmission by mechanical means.--There is a question as to whether some carnations are infected with mosaic or streak or yellows which is a streak-mosaic complex. If a virus can be transmitted mechanically from a plant the presence of mosaic would be indicated since the streak virus, if it was present, would not transmit. Streak cannot be transmitted mechanically. If further experiments indicate that streak is also present, the presence of yellows is indicated. By mechanical transmission the mosaic virus can be detected and the plant discarded. The purpose of this experiment was to determine the best means of mechanical transmission to be used in routine investigations.

The first experiment was set up in which the following techniques were used: transmission by rubbing

the leaves between the thumb and forefinger with and without the use of carborundum dust as an abrasive; transmission by piercing the leaves with a fine needle; transmission using a cutting knife.

Inoculum was obtained from 3 sources. A random selection of infected leaves from experimental carnation fertility plots was used as one source. The symptoms were pronounced with light green streaks and spots on the broad leaves of the carnation variety White Patrician. According to the descriptions of Creager (4, 5) and Jones (9) the plants appeared to be infected with mosaic. Leaves were selected from a number of plants for each experiment in which they were used. Inoculum from this source was designated #3. The second and third sources of inoculum were 2 plants that appeared to be infected with yellows. One of the carnation plants was of the Pink Spectrum Supreme variety and was designated #1; the other plant was of the White Patrician variety and was designated #2. Both plants were maintained in the greenhouse in 12-inch pots as known sources of inoculum. Many experiments could be carried out using the 2 plants and the results correlated because of the unchanging source of inoculum.

Several methods were tried for extracting sap from the plant material used to determine the effect on the virulence of the inoculum. Different methods were used in applying the inoculum and treating the plant after inoculation to determine the relative efficiencies of these techniques. The source and designation of the various inocula are given above. The methods used in the various experiments are given in Table 4, together with the results.

Results were determined after 45 days. The first symptoms appeared in about 20 days. Clearing of the veins in the young leaves followed by a distinct light and dark green mottling were the symptoms manifested by D. barbatus. After 3 months the mottling was still apparent and the leaves had become crinkled and brittle. An example of this is shown in Fig. 2.

The results of the experiments shown in Table 4 indicate that the carnation mosaic virus can be transmitted with a high degree of infectivity by the various methods and materials used. Washing the leaves after inoculation was the only variable in treatments 1 and 2. Treatment 3, in which the leaves were washed after inocu-

Table 4.--RESULTS OF MECHANICAL INACTIVATION EXPERIMENT

No. of experiment	No. plants inoculated	Source of inoculum	Grade of carborundum	Method of expressing juice[1]	Method of inoculating[2]	Washed after inoculating[3]	No. plants infected	Percent infection
1	24	#3	200 mesh	Hand	Fingers	No	14	58
2	12	#3	do.	do.	do.	Yes	9	75
3	12	#1	200 mesh	Grinder	Hand	No	9	75
4	12	#2	do.	do.	do.	Yes	10	83
5	12	#1	90 mesh	Grinder	Hand	No	6	50
6	12	#2	do.	do.	do.	Yes	7	58
7	12	#1		Grinder	Needle	No	10	83
8	12	#2		do.	do.	Yes	9	75
9	12	#3		Superimposed leaf	Needle	No	10	83
10	12	#1	200 mesh	Freezing	Hand	No	11	91
11	12	#1	90 mesh	do.	do.	No	6	50
12	12	#2		Freezing	Needle	Yes	8	66

[1] Explanation of terms used in this column as follows: Hand--Macerating a leaf between fingers to express sap; Grinder--Grinding diseased tissue in meat grinder and expressing sap through cheesecloth; Superimposed leaf--Placing diseased leaf over healthy one; Freezing--Plant material frozen at -20° C. for 1 hour and allowed to thaw out before macerating the tissue and expressing sap through cheesecloth.

[2] Explanation of terms in this column as follows: Fingers--Inoculum may be applied to fingers or leaf. A leaf is drawn between fingers with slight pressure. Abrasive may or may not be used. Needle--Diseased leaf is superimposed on healthy one and a fine needle used to pierce both leaves.

[3] Washing after inoculating refers to practice of washing off surplus inoculum after inoculating a leaf.

Fig. 2.--Symptoms of carnation mosaic expressed by D. barbatus. Mechanical transmission from D. caryophyllus to D. barbatus.

lation, resulted in an infection of 75 percent while treatment 1, in which the leaves were not washed after inoculation, yielded only 58 percent infection. These results are in accord with the observations of investigators (14) who recommend washing surplus inoculum off in order to get a greater incidence of infection.

Another factor which appeared to affect the amount of infection was the grade of carborundum used. Treatments 5 and 6, in which 90 mesh carborundum was used as an abrasive, resulted in 50 and 58 percent infection, respectively. In treatments 3 and 4, which were identical with treatments 5 and 6 except that 200 mesh carborundum was used, yielded 75 and 83 percent infection respectively. This indicated that the finer abrasive was more desirable, supporting the work of Rawlins (14). Similar observations were made with treatments 10 and 11.

Treatments 7 and 8 resulted in 83 and 75 percent infection respectively. These results are contrary to those of treatments 3 and 4. Apparently washing the leaves after inoculation with expressed sap and piercing with a needle does not have the same effect as it does when the leaves are inoculated by use of an abrasive.

Treatment 9 gave 83 percent infection. This was not the highest degree of infection obtained from the various treatments, but the minimum of time and effort required to prepare for inoculation and the speed with which the operation could be accomplished made it a desirable technique.

Freezing the plant tissue before expressing the sap resulted in the highest incidence of infection. Treatment 10, in which 200 mesh carborundum was used as an abrasive, showed 91 percent infection. Treatment 11, in which 90 mesh carborundum was used, resulted in a 50 percent infection. Here, again, the finer abrasive was more efficient in transmitting the virus. The freezing technique allowed the sap to be expressed more easily from the plant tissue yielding a greater concentration of virus and resulting in an apparently more virulent inoculum. No explanation can be given for low incidence of infection obtained in treatment 12 except that the small sample was subject to experimental error.

Mass transmission.--The second experiment was set up to test transmission to a number of plants. One plant heavily infected with mosaic was selected from the

first experiment to provide inoculum for this experiment. The leaves were frozen for 1 hour at -20° C., allowed to thaw, macerated in a mortar and the sap expressed through cheesecloth.

The plants were inoculated by smearing inoculum over the 4 terminal leaves, sprinkling them with 200 mesh carborundum and rubbing them between thumb and forefinger.

Sixty young plants of D. barbatus were inoculated. After 45 days 47 plants showed definite mosaic symptoms. The symptoms were a little different than those in the first transmission experiment. The same mosaic pattern was expressed but the colors were lighter. The leaves became thin and flaccid with very little crinkling (Figs. 3 and 4). The incidence of infection obtained was 78 percent and in the previous experiment under similar conditions it was 96. Only 12 plants were inoculated, however, the 60 plants used in the second experiment yielded more reliable results.

These results indicated that the carnation mosaic virus is readily transmitted from plant to plant by mechanical means. It was transmitted first from diseased carnation to D. barbatus and then transmitted to other plants

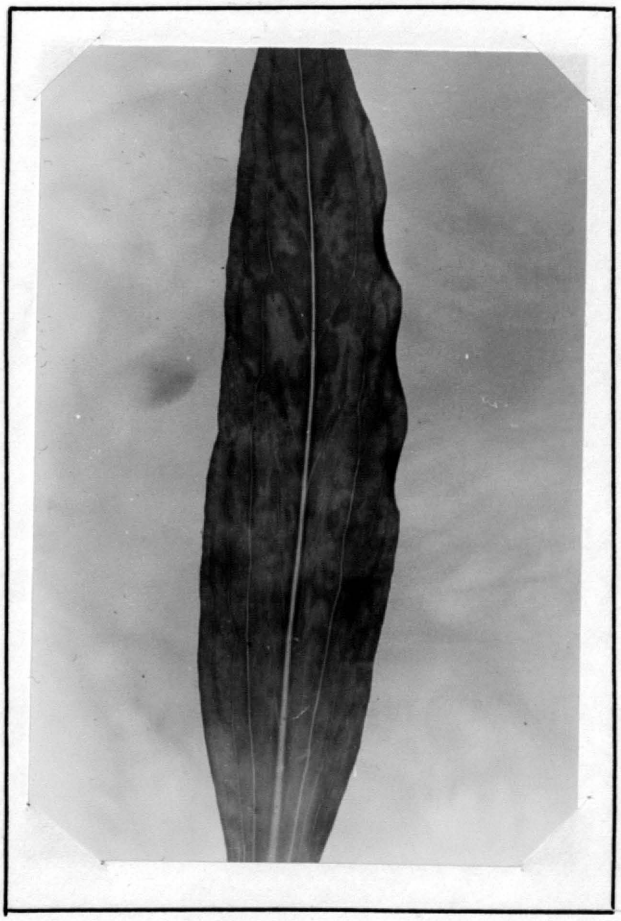

Fig. 3.--Enlarged D. barbatus leaf showing symptoms of carnation mosaic. Mechanical transmission from D. barbatus.

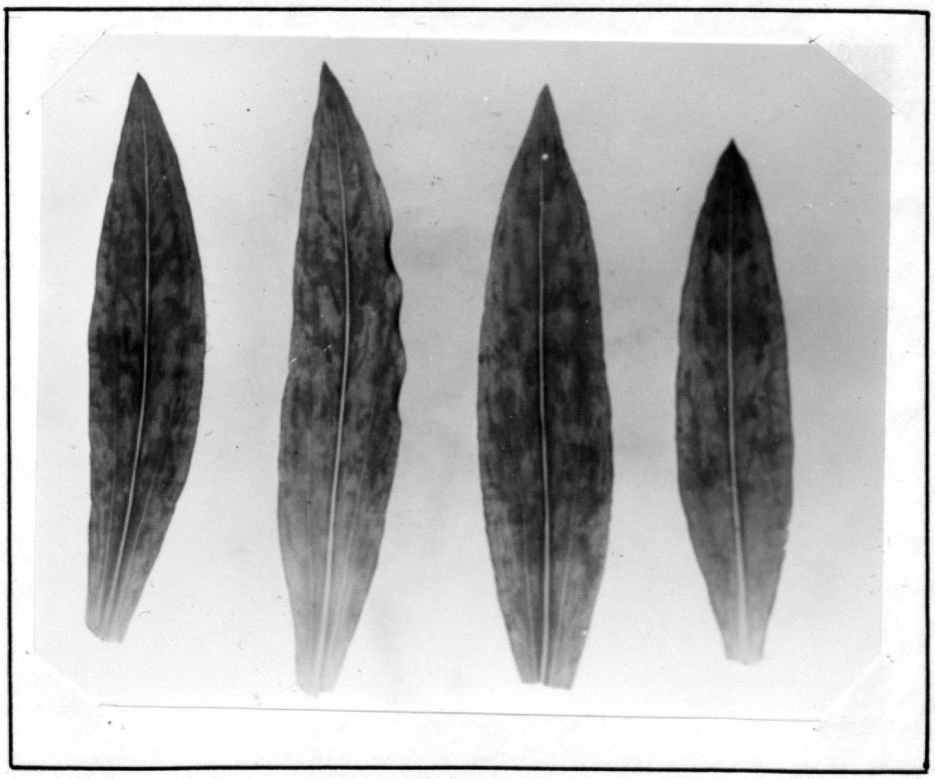

Fig. 4.--Symptoms of carnation mosaic expressed on D. barbatus. Mechanical transmission from D. barbatus to D. barbatus.

of D. barbatus. To carry the experiment out completely transmission from D. barbatus to healthy carnation plants would have been necessary, but no healthy carnation plants were available.

Knife transmission.--A common method of harvesting carnations is to use a knife to cut the stems. It seemed probable that the knife blade might be contaminated with the virus in the plant sap when an infected plant is cut. The infected blade then might inoculate the next plant that is cut by smearing the infected sap over the stub. An experiment was set up to test the effectiveness of this type of transmission.

Forty young D. barbatus plants were used in the experiment. Plants #1 and #2 were used as sources of inoculum and 20 plants were inoculated with inoculum from each.

The technique of inoculation followed as closely as possible that used in cutting carnations in commercial greenhouses. A pocket knife was used to cut off a portion of the stem from one of the infected carnations; then the tip of one of the D. barbatus plants was cut off using the same section of the blade for both cuts. After cutting a

healthy plant the infected plant was cut again to reinfect the blade before the next healthy plant was cut. Infection was determined by visible symptoms.

Incidence of infection readings were determined 30 days after inoculation. Out of 20 plants inoculated using inoculum from plant #1, 14 expressed symptoms of virus infection. The 20 plants inoculated with inoculum from plant #2 showed 13 with virus infection.

There was 67.5 percent transmission in this experiment. These results indicated that virus transmission incurred by the use of a cutting knife was high and is to be considered a factor in the transmission of carnation mosaic in commercial ranges. The presence of only a few diseased plants could lead to a gradual dissemination of the virus throughout the range. Cuttings taken from these plants would gradually increase diseased stock with a subsequent reduction of yield and flower quality (6).

Transmission by contact.--The transmission of carnation mosaic by handling the plants might be an important means of virus dissemination. Plants are handled 3 or 4 times from the time they are taken as cuttings. First they are cut, then propagated in sand and after

3 weeks they are placed in soil for 4 weeks. Then they are transplanted to the production benches. Each time they are handled there is a possibility of infecting the healthy ones by contact with hands which have previously handled diseased plants. An experiment to test the effectiveness of virus transmission by contact was set up.

Twenty-two healthy D. barbatus plants were inoculated by rubbing the leaves of an infected D. barbatus between the thumb and forefinger and then rubbing the leaves of a healthy plant with the same fingers. The results were apparent within 30 days after inoculation. Infection was determined by visible symptoms. Seven plants were used as uninoculated checks.

The experiment resulted in a high degree of infection. Out of 22 plants inoculated, 14 expressed visible mosaic symptoms with 63.6 percent infection. These results indicated that carnation mosaic can be transmitted easily by contact. The transmission incurred by handling the plants in a commercial range may not be so pronounced as these results indicate due to the fact that no effort is made to rub the leaves with the fingers, but there very well could be some incidental transmission when the plants

are handled. However slight this may be, wherever diseased plants are mixed with healthy ones there probably is a gradual spreading of the virus to healthy plants by this means.

Transmission studies of carnation viruses by grafting.--Heretofore all of the transmission experiments have concerned the mechanical transmission of the carnation mosaic virus which, the data show, can be transmitted easily. The following experiment deals with the transmission of carnation streak and mosaic by grafting. According to Jones (9) both carnation mosaic and streak viruses may be transmitted by grafting. This is accepted as a demonstration of the virus-nature of both of these diseases.

A grafting experiment was set up to confirm the results obtained by Jones (9). Seven large carnation plants, including plant #1 which was previously described in conjunction with mechanical transmission, were used as a source of inoculum. The 6 plants had yellows as indicated by ultra-violet radiation (19).

The approach graft method was used. In using this method, the plants are set side by side and a thin slice, which penetrates into the xylem, is cut from both

stems with a razor blade. The two wounds then are bound together. If some sections of the cambium layers match, the wound heals and the vascular systems merge. The sap from the infected plant then should infect the healthy one.

Seventeen healthy D. barbatus plants were grafted onto the diseased plants previously described. It was necessary to remove most of the lower leaves on the D. barbatus in order to provide space for the graft. The best binding material was found to be electician's tape. String and adhesive tape gave good results but were difficult to use.

Out of 17 grafts, 14 were successful and expressed virus symptoms. The symptoms first appeared as a white spotting mainly on the younger leaves. This later became general throughout the midrib section of the leaf with the leaf margins remaining light green as shown in Fig. 5. Later the midrib and lateral veins became brown. There was no wilting which would indicate vein necrosis. Gradually the whole plant became etiolated and died. Symptoms developed within 20 days after the plants were grafted.

Fig. 5.--Symptoms expressed by grafting *D. barbatus* onto *D. caryophyllus*

Eighty-two percent of the plants grafted to the diseased carnations became infected. These results indicated that the grafting method of transmitting the virus is efficient and yielded excellent results. These facts suggested that another virus was transmitted. Due to the variation in symptoms, the streak virus and probably the mosaic virus were transmitted by the graft. Mechanical transmission of mosaic from the D. barbatus infected by grafting to healthy D. barbatus may confirm this.

Chapter IV

DISCUSSION

The dry heat inactivation of carnation mosaic was successful when infected D. barbatus were used. Mosaic-infected carnation plants apparently were more sensitive to the heat and died. This may have been prevented to some extent by placing the plants in a humidity chamber after the heat treatment to prevent their rapid drying out. A young, vigorously growing plant was apparently best able to withstand the treatment. Further experimentation with various temperatures and periods of treatment may result in a procedure by which the carnation mosaic virus can be inactivated in a large number of foundation plants from which virus-free carnation plants can be propagated. This method may have an additional advantage in that plants infected with vascular fungus diseases, which are not apparent at the time, will be killed by the increased activity of the fungus.

Hot water treatment of carnation cuttings was not successful. The cuttings are too easily injured by the heat and either dried up or were attacked by patho-

genic fungi. Rooted plants immersed in the hot water may have been able to withstand the ill effects of the heat.

The chemical inactivation of the carnation viruses was partially successful. Here again the cuttings were unable to root in the presence of certain chemicals. Further experimentation with the methods used by Stoddard (17) such as treating young plants by introducing the chemical into the soil or allowing mature plants to take the chemical up through cut stems may result in a more successful technique.

All methods for inactivating the carnation viruses *in vivo* used in this thesis have possibilities for practical use in obtaining virus-free stock from which mass production of commercial cuttings may be obtained.

Virus-free plants must be handled with care to avoid contamination from infected plants as was shown by the experimental transmission of carnation mosaic by the cutting knife and by contact. The carnation mosaic virus is easily transmitted by mechanical means, and the presence of a few infected plants in a bench may lead to rapid infection of the healthy plants and plants propagated from cuttings taken from infected plants.

The variation in symptoms expressed by D. barbatus was observed with interest. In the first mechanical transmission experiment the mosaic virus was transmitted from D. caryophyllus to D. barbatus. The mosaic pattern manifested itself in a dark green-light green pattern as shown by Fig. 2. The leaves became thick and brittle with a definite crinkling of the leaf margins. In the second mechanical transmission experiment in which the virus was transmitted from D. barbatus to D. barbatus the mosaic pattern was expressed by a light green with pale green blotches developing in the areas between the veins giving the appearance of vein banding as shown in Figs. 3 and 4. Later the light areas grew larger until the leaf surface was nearly covered. The leaves were thin and flaccid.

Any explanation of the variability in virus symptoms as described above would be conjecture, but it seems that there are 2 important possibilities. The virus increased in virulence as it was passed through D. barbatus or that there are metabolic or physiologic differences in the individual plants. Since carnation varieties usually originate from a single clone the genetic variation between individual plants would be

negligible. Therefore, it would appear that changes of some nature in the virus would cause the variability in symptomatology. On the other hand, very little is known about the effect of soil fertility, pH, soil moisture, temperature, light intensity and light period on the expression of virus symptoms. Casual observation has indicated that reduced light intensity is more effective in producing virus symptoms than high temperatures.

The carnation plants used in the grafting experiment were infected with both mosaic and streak (yellows). The symptoms expressed by the D. barbatus were different from those expressed when mechanical transmission was used. This would indicate that a different virus was influencing the symptoms. The streak virus was evidently transmitted, and probably the mosaic virus also, since it is so easily transmitted. Mechanical and insect transmission tests which are selective for mosaic and streak respectively on D. barbatus infected by grafting would determine this.

Chapter V

SUMMARY

The thermal inactivation point of the carnation mosaic virus *in vitro* was found to be between 60° C. and 70° C.

Dry heat inactivation of mosaic *in vivo* in infected carnation plants (*Dianthus caryophyllus*) at average temperatures of 38.5° C. and 43.5° C. was unsuccessful in that the plants were killed by the heat. Mosaic-infected *D. barbatus* plants under similar conditions survived, and tests indicated that the virus was inactivated after 14 days at the above temperature.

Hot water inactivation of mosaic in carnation cuttings at 45° C. and 50° C. was unsuccessful because the cuttings were killed.

Chemical inactivation of mosaic in carnation cuttings was partially successful. Nine chemicals, including 5 sulfa compounds, at low concentrations were used. Calcium chloride, hydroquinone and zinc sulphate yielded the best results.

The best technique in mechanical transmission of carnation mosaic was found to be that of applying

expressed sap, from frozen diseased tissue, with the fingers using 200 mesh carborundum as an abrasive. Twelve different combinations of inoculum extraction methods and inoculation techniques were used.

Tests of the transmission of carnation mosaic on D. barbatus incurred by harvesting and handling indicate that the virus can be transmitted by these operations and may be an important factor in natural transmission of the virus.

Transmission by grafting was successful with the streak virus, and preliminary tests to date indicate that the mosaic virus is probably transmitted in the same manner.

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