T H E S I S

THE NATURE OF THE WILT OF CARNATIONS CAUSED BY PSEUDOMONAS CARYOPHYLLI

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY OLIVER V. HOLTZMANN

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Oliver V. Holtzmann has a ready reading knowledge of German, fully adequate for his present needs.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

Chapter I

INTRODUCTION

Carnations (Dianthus caryophyllus L.) today rank second to roses in cut flower production. The first carnations were single flowers with five petals, weak stems, and bloomed only in the spring. Today's carnations have large double flowers, strong flower stems, and the plants bloom continuously. Carnation culture has developed from a hobby to a large industry in the past 100 years. In the Denver area alone, it is a multi-million dollar business.

Although there is some outdoor production in California and elsewhere, carnations are best produced as a glasshouse crop where nights are cool and light intensity is high. The bulk of production is in New England, some Middle Atlantic and Midwestern states, Colorado, and northern California. As with other glasshouse plants grown in an artificial environment, carnations have many cultural and disease problems. One of the more important pathogenic limitations to the production of carnations is bacterial wilt caused by Pseudomonas caryophylli (Burkh.).

The most striking symptom of this disease is a sudden wilting of one or more branches. These wilted branches have a gray-green color and become dry. In the

advanced stage of wilt the branches turn tan or brown. A vascular discoloration may be found extending from the roots to the upper apical portion of the plant.

Roots of infected plants gradually disintegrate and become soft, often sloughing off when the plants are lifted from the soil. The outer basal portion of the plant may be soft with decay evident. When the bark of this area is torn away, the exposed tissue is sticky to the touch and is yellow to brown in color.

To date there is no satisfactory means of eliminating the organism, once it has entered the plant. Present controls recommended are the use of clean cuttings and soil pasteurization. In the light of recent advances in chemotherapy for the control of plant diseases, it may be possible to control bacterial wilt by such means. In order to approach properly the control of this disease by chemotherapy, it is necessary to understand the nature of the wilt.

The problem

What is the nature of the wilt of carnation caused by Pseudomonas caryophylli?

Problem analysis.--Before answering the major question, it is necessary to answer the following:

1. Does P. caryophylli plug the vessels of the host plant to produce wilt?

2. Does P. caryophylli produce a wilt-inducing toxin?

3. If a toxin is produced, what is it?

Delimitations.--1. Cultures used in this study were single-cell cultures of P. caryophylli obtained at a dilution of 1:10,000 from William Sim and Virginia Hercules varieties of carnation.

2. All work was limited to the laboratory and greenhouse.

Definition of terms.--1. Bacterial suspension, as used here, is a suspension of bacteria scraped from the surface of potato-dextrose agar in sterile distilled water.

2. A toxin is a substance produced by an organism that will cause wilting in a plant.

3. Carrier shall refer to a plant infected with P. caryophylli but not showing symptoms of wilt.

Chapter II

REVIEW OF LITERATURE

As early as 1939, in Midwestern United States, carnations were reported to wilt suddenly from causes not previously described. In 1940 Jones (13) observed carnation plants afflicted with a sudden wilting that caused the subsequent death of the plants in a glasshouse in Spokane, Washington. Isolation cultures of the organism and inoculation tests showed the disease to be caused by a bacterium that appeared to be different from any previously described bacterial plant pathogen. The disease was named bacterial wilt. Diseased plants were sent to W. H. Burkholder at Cornell University for determinative studies on the causal organism.

Burkholder (3) proposed the name Phytomonas caryophylli n. sp., as such characteristics as curved rods with polar flagella, the action on milk and asparagine, the use of formates, and the lack of H_2S production and of starch hydrolysis, placed the bacterium in the Pseudomonas group. Since then the genus Phytomonas has been regrouped and the causal organism is now known as Pseudomonas caryophylli (Burkh) Bergey, et al.

Until recently most of the research on bacterial wilt of carnations has been pursued in light of direct commercial application for control. Previous recommended controls, for the most part, have been proved unsatisfactory. Carnation cuttings were dipped for 10 minutes in a solution of one ounce of potassium permanganate dissolved in seven and one-half gallons of water (7). In the use of liquid disinfectants, danger is encountered when bactericidal ingredients become neutralized or depleted, thereby allowing the liquid to become a source of infection. Consequently, cutting dips have not been satisfactory. Steam sterilized soil, along with planting of disease-free stock, is the only present method of control.

In an effort to determine varietal resistance of carnations to bacterial wilt, Tilford, at Ohio State University in 1946, inoculated 45 varieties of carnations with the causal bacteria. Of those tested, only one third of the varieties were in that range which showed a 20 per cent incidence of infection by bacterial wilt, and of these only two, John Briery and Olivette, are currently being grown commercially in the Denver area.

Recently Lewis et al. (14) demonstrated that bacterial wilt develops rapidly at temperatures 85° F. or above. Rooted carnation plants inoculated at the time of planting showed a 66 per cent incidence of infection, whereas those inoculated one and two weeks after planting

showed four per cent and two per cent infection, respectively. Also, cuttings rooted in vermiculite did not become infected if inoculated in the rooting media without disturbing the roots. This demonstrated that carnation roots may be able to resist ingression by the bacteria providing the roots are uninjured or sufficient time is allowed for periderm to form over the wounded area.

To approach the problem of control of this disease by chemotherapy, or other means, it is necessary to understand how this organism reacts with the plant when causing wilt. In the case of Stewart's disease of corn, caused by Bacterium stewarti, there is a definite plugging of the water-conducting system which is the primary cause of the wilting (9). Pseudomonas tabaci, the causal agent for "wildfire" disease of tobacco, has been found to produce a toxin responsible for the haloes on the leaves (2). However, non-wilt inciting phytopathogenic bacteria (6,10), Fusaria (8,17), and other fungal organisms (5) have been found to produce wilt-inducing toxins.

In a preliminary report, Holtzmann and Thomas (11) tested unrooted carnation cuttings in bacterial suspensions and filtrates of bacterial suspensions of P. caryophylli. All cuttings in these treatments wilted. It was suspected that a toxin may be the primary wilt-inducing agent of the carnations.

Chapter III

METHODS AND MATERIALS

Histological studies

In order first to establish whether or not mechanical plugging occurred in the vascular elements, it was necessary to undertake histological studies of the carnation stem.

Stems of carnation plants that appeared to be free from infection, and others showing symptoms of wilting, were sectioned at various levels and placed in a killing solution of formal-acetic-alcohol saturated with $HgCl_2$. Other sections of these same stems were cultured on potato-dextrose agar to determine the presence of *F. caryophylli*. Sections resulting in positive isolations were then passed through an alcohol-xylol series, embedded in paraffin, sectioned and subsequently stained with the safranin-fast green stain (16).

Toxin studies

It has been established by Gottlieb (8) that the mechanism of Fusarium wilt of tomato is incited by a toxin, lycomarasmin. In view of this, the possibility of the production of a toxin by *F. caryophylli* seemed to be a factor not to be overlooked.

In preliminary studies (11) unrooted carnation cuttings were found to wilt when placed in a filtrate of a suspension of P. caryophylli. A Seitz "Hercules" filter was used to obtain the bacteria-free filtrate. The filtrates were determined to be bacteria-free by streaking agar plates. The filtrate was filtered into sterilized test tubes. All cuttings were surface-sterilized by dipping them into a 1:1000 sodium hypochlorite solution for two minutes and then rinsing in three successive changes of sterile distilled water. Cuttings were then suspended in the desired treatments of filtrates (as further described below) or sterile water by wrapping cotton around each cutting about six centimeters from the base and then placing it in the test tube so that the base of the cutting was submersed two to three centimeters. All carnation cuttings used in these tests were of the susceptible White Patrician variety. In subsequent tests with rooted cuttings, the same methods were used.

Wilt induction by artificially cultured bacteria

Single-cell cultures of P. caryophylli were cultured on potato dextrose agar. The colonies were then scraped off the surface of the agar into 10 cc of sterile distilled water. Filtrates were obtained by passing this bacterial suspension through a bacterial filter. The filtrate was adjusted to pH 6.8, which was the same pH as the

sterile distilled water and the bacterial suspension.

Rooted carnations were treated in unfiltered bacterial suspensions, filtrates of bacterial suspensions, filtered water used to flood the surface of sterile agar plates, and sterile distilled water. These four treatments were replicated 10 times and were repeated in five separate tests. Duration of the treatments was from two to seven days.

The tubes containing the tests were placed at random in a rack and a buffer row of carnations in tubes of water was placed around the entire test in order to reduce transpiration due to air currents.

In an effort to determine whether or not the wilt-inducing agent was contained in the soluble fraction, the filtrate was treated with ethanol, 5.33 ml for each milliliter of filtrate. This was heated to facilitate the precipitation of the insoluble fraction, after which it was filtered through a Buchner funnel. This filtrate was then evaporated to one fourth the volume of the original filtrate in order to remove the ethanol. The remainder was restored to original volume by the addition of sterile distilled water. Rooted cuttings were then tested for wilt-induction in this liquid, using the previous method of testing.

Wilt-induction by extracts from diseased plants

Two hundred grams each of diseased and healthy stems were macerated separately in a Waring blender, using 200 ml of sterile water for each sample. The juice from each of these was first passed through cotton and then centrifuged. Each sample of juice was divided into two portions, one of which was passed through a bacterial filter and adjusted to pH 6.8. The other was left unfiltered and unadjusted.

Treatments of rooted carnations in filtered and unfiltered extract from diseased plants, filtered and unfiltered extract from healthy plants, and sterile distilled water were used. These five treatments were replicated five times and repeated in five separate tests.

Expressed juice from diseased and healthy carnations was treated with the alcohol extraction treatment and this purified juice was tested for toxicity in the manner previously described.

The same rack was used as before with a buffer border of carnations to reduce transpiration by the carnations in the treatments.

Transpiration studies

Rooted and unrooted carnation cuttings of equal stem diameter and leaf area were placed in unfiltered

bacterial suspensions and sterile distilled water. Readings in loss of liquid by weight were taken at 24-hour intervals. These readings were used as transpiration data. This test was conducted at room temperature 21°±3°C. and relative humidity 20 per cent ± 3 per cent. A block of 10 tubes for each treatment was replicated three times. The readings were averaged for each day and plotted as points on a transpiration curve.

Respiration observations

Respiration observations were made in each experiment. A record of amounts of gases given off from the roots was made for each of the wilt-induction experiments. This was accomplished by making an arbitrary evaluation of gas evolution by the roots.

Paper-partition chromatography

A special experiment was designed to determine the differences, if any in the amino acid and sugar content in diseased and healthy carnations. In order to obtain plant extracts that were of the same composition, diseased and healthy plants of the variety White Sim were selected from the same greenhouse bench. Twenty ml of plant extract were expressed from frozen diseased and healthy plant material by means of a hydraulic press. One hundred six ml of 95 per cent ethanol were used for

each 20 ml aliquot of plant extract in order to precipitate proteins. The proteins were filtered off, the alcohol evaporated, and the plant aliquot reduced to one fifth volume by evaporation over a hot water bath (15). To determine amino acid concentration in the diseased and healthy plant extract, three, five, six, and 10 λ aliquots of these resulting liquids were alternated at three cm intervals on sheets of Whatman No. 1 filter paper. These spots were placed two cm from the bottom of the sheet.

After the spots were dry, the filter papers were rolled into the shape of a cylinder, the edges fastened together with cellophane tape, and were placed in jars containing 80 per cent phenol. After the solvent fronts had risen to the desired height, the papers were removed, air-dried, and sprayed with ethanol containing ninhydrin regent (4).[1/] The one-dimensional spots of concentrated amino acids were measured by a Welch densichron using a white filter. A statistical analysis of the difference in the amino acid concentrations was made.

For qualitative determination of amino acids in healthy and diseased plant extracts, a 10 λ aliquot was spotted at a distance of two cm from the bottom and two cm from the edge of the paper. After the spot was dry, the paper was rolled and fastened as described before.

1/ Ninhydrin reagent--15 g of ninhydrin per 50 ml of 95 per cent alcohol plus one drop of glacial acetic acid.

and placed in a jar containing 80 per cent phenol. After the solvent front had risen to the desired height, the paper was removed and allowed to dry overnight. The paper was then turned 90°, shaped into a cylinder, and again fastened with cellophane tape. The cylinders were then placed in jars containing water-saturated lutidine so that the new solvent front would be at right angles to the phenol solvent front. After the solvent fronts had reached the desired height, the chromatograms were removed, air-dried, and sprayed with ninhydrin reagent (4).

Quantitative sugar determination followed the same methods that were used in one-dimensional paper-partition chromatography for amino acids. In addition to the diseased and healthy extracts, spots of glucose and fructose were made. Materials differed in that 77 per cent ethanol was used as the solvent and 3,5-dinitrosalicylic acid[2/] was used for the spray after drying. After the spray had air-dried, the paper chromatograms were placed in an oven at 110° C. for development of the spots. As the spots developed a record was made as to the time required for development and color (12).

2/ Aqueous solution containing 0.5 per cent of 3,5-dinitrosalicyclic acid and four per cent of sodium hydroxide.

Chapter IV

EXPERIMENTAL RESULTS

Histological studies

Upon examination of the sections made of healthy carnation tissue (Figs. 1 and 2) it was found that the vascular tissue appeared normal in that the xylem vessels retained the safranin stain and the phloem sieve-tubes and other parenchyma cells stained normally with the fast-green stain. There was no apparent obstruction in or distortion to the vascular tissue.

Microscopic investigations of cross-sections of diseased carnation stems (Figs. 3, 4, 5, 6, and 7) showed that one (Fig. 3) or several of the water conduction vessels were plugged by bacterial masses (Fig. 4). Further explorations revealed proliferation of parenchyma cells in the immediate area of infection (Fig. 5), and the walling-off of a bacterial mass by initiation of a periderm tissue. In more advanced stages of infection there appeared to be a lysis of xylem tissue (Fig. 6). There was no apparent evidence of bacteria in the parenchymatous tissue.

Longitudinal sections of diseased plants showed that infected areas of xylem tissue were continuous from the basal portion of the plant up into the leaves (Fig. 7).

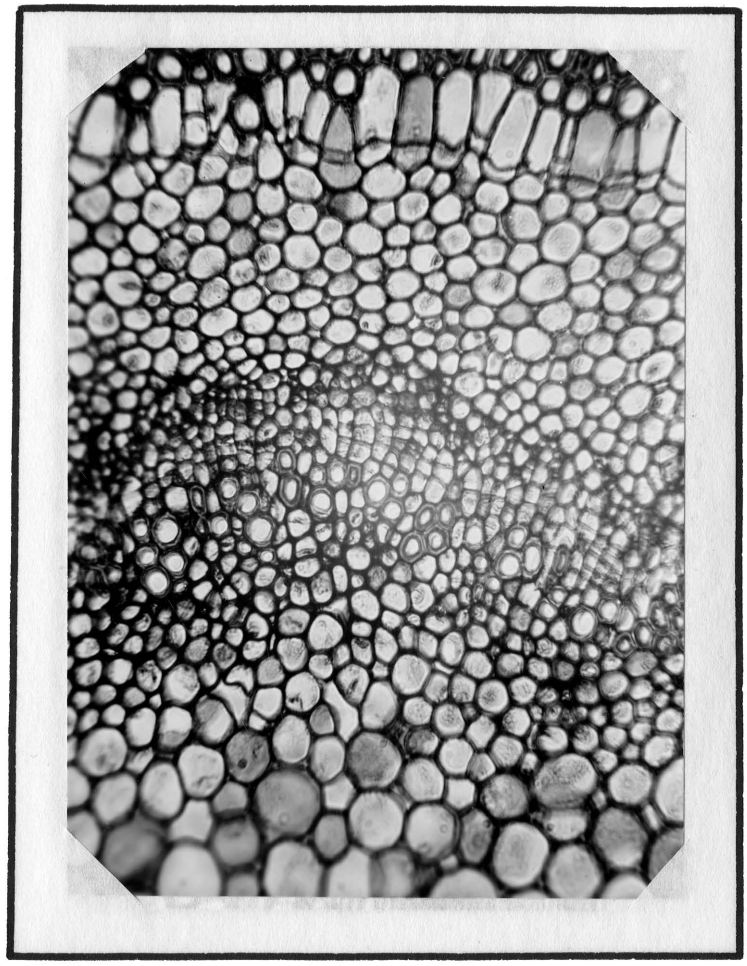

Fig. 1.--Cross section of a portion of a normal carnation stem.

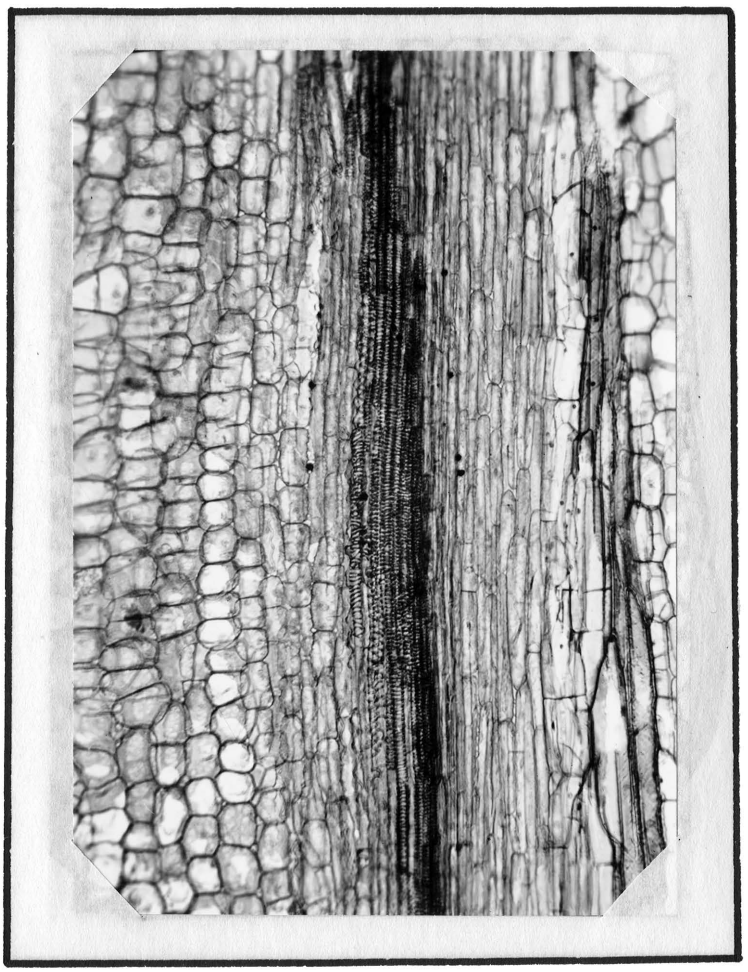

Fig. 2.--Longitudinal section of a normal carnation stem.

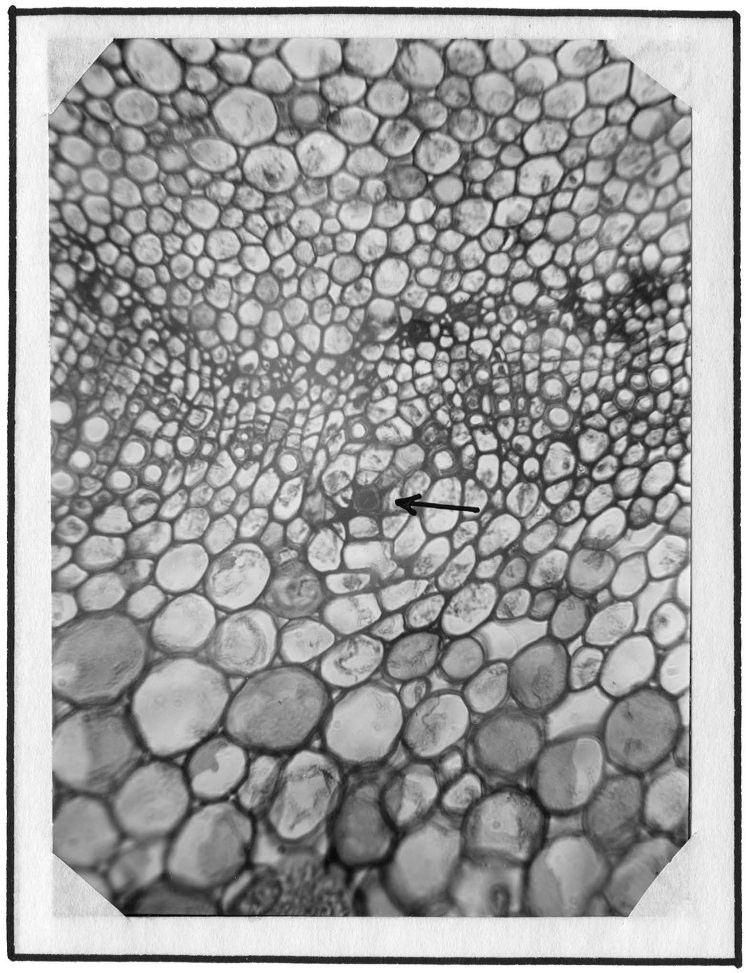

Fig. 3.--Cross section of a diseased carnation stem showing one vessel plugged.

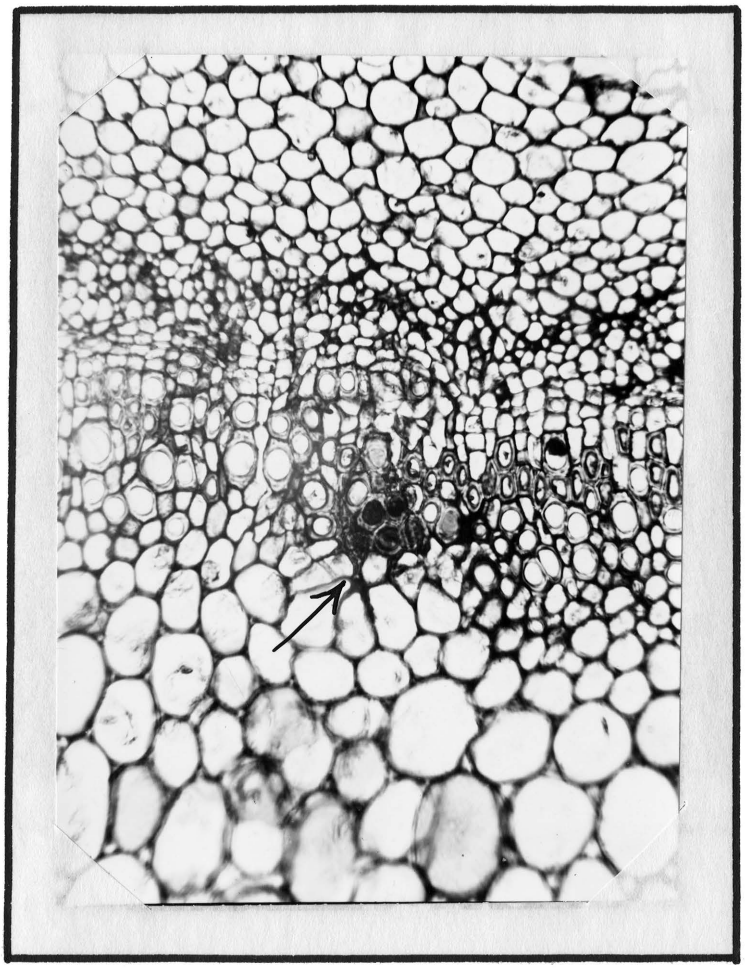

Fig. 4.--Cross section of a diseased carnation stem showing a group of vessels plugged.

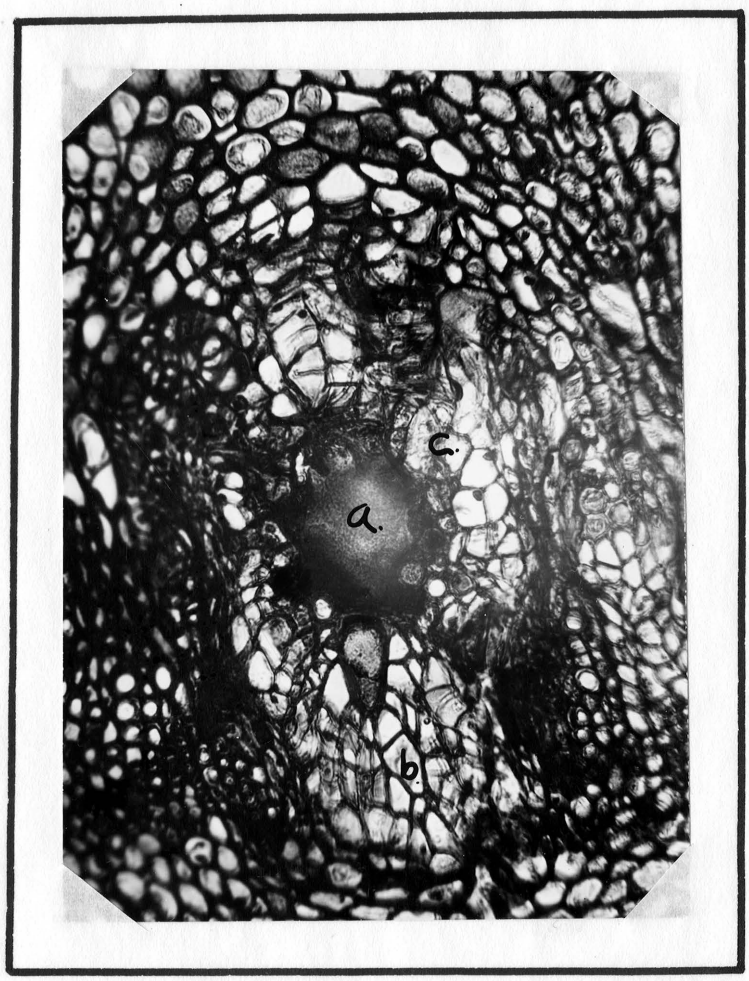

Fig. 5.--Cross section of diseased carnation stem showing (a) bacterial mass walled-off by (b) a proliferation of cells and (c) the initiation of periderm tissue.

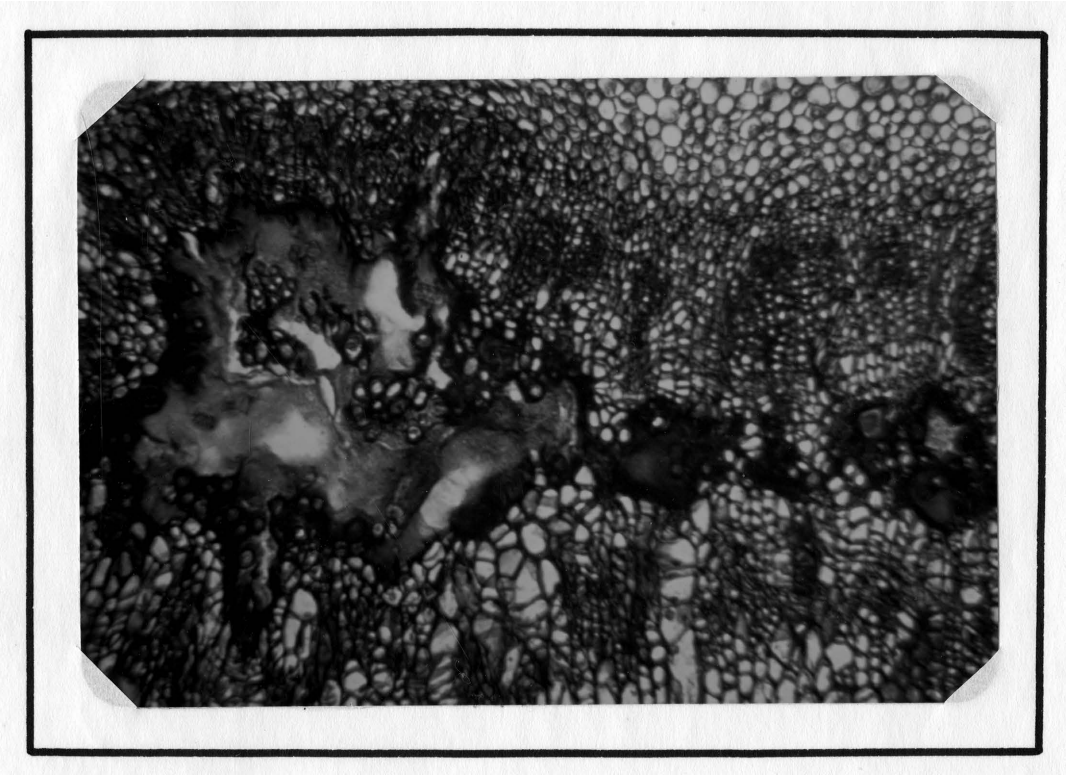

Fig. 6.--Cross section in color of a diseased carnation stem showing a lysis of xylem tissue and some proliferation of cells.

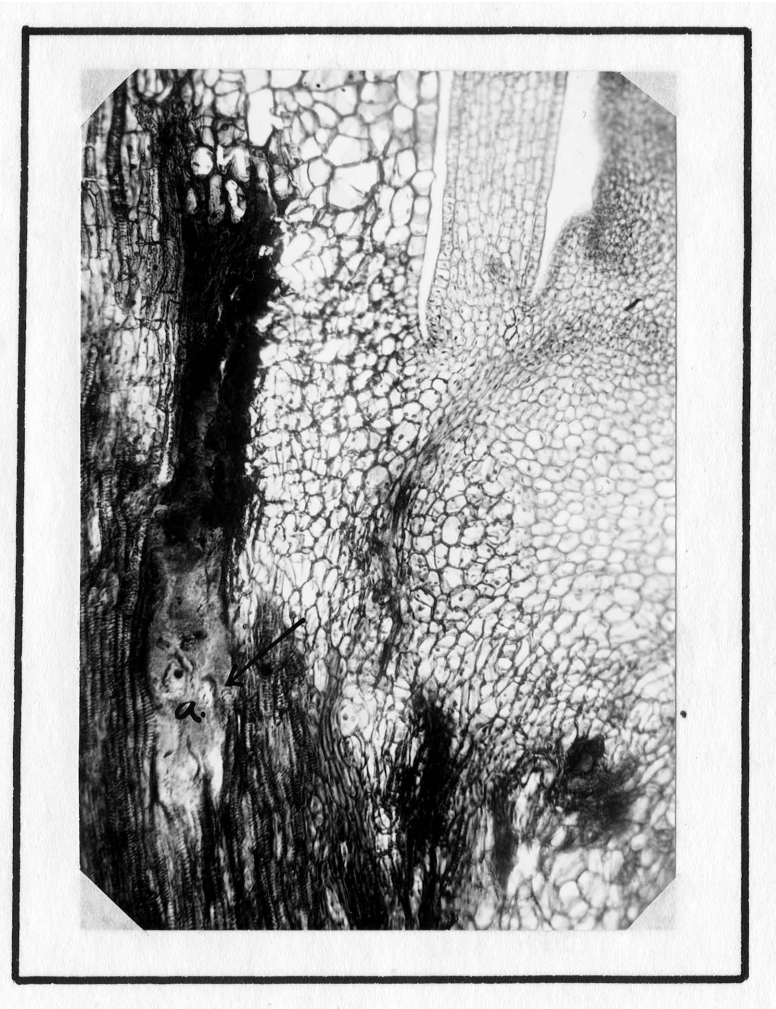

Fig. 7.--Longitudinal section of a diseased carnation stem at a node showing lysis and infection by bacterial mass (a) in the base of a leaf.

Where the infection was restricted to a small group of the vessels, the infectious mass of bacteria was confined to that area throughout the length of the stem.

Wilt-induction by artificially cultured bacteria

All rooted carnation cuttings placed in treatments of bacterial suspensions and filtrates of bacterial suspensions wilted in two to three days. Rooted cuttings in sterile water which had been used to flood the surface of sterile potato dextrose agar plates showed signs of wilting in four to five days. All rooted cuttings placed in sterile distilled water were still turgid at the end of a week.

Alcohol precipitation of the filtrate of a bacterial suspension of P. caryophylli resulted in the removal of proteins and pectins. Rooted carnation cuttings placed in the filtrate of the soluble fraction (as described in Chapter III) did not wilt, whereas checks of rooted cuttings in the unprecipitated bacterial filtrate did wilt.

Wilt-induction by extracts from diseased plants

Filtered and unfiltered extracts (obtained as described in Chapter III) from diseased carnation plants induced wilt in rooted carnation cuttings within two to

three days, while filtered and unfiltered extracts of healthy carnation plants showed a slight amount of wilt after five days. Those rooted cuttings in the soluble fraction of the extract of diseased and healthy plants failed to induce wilt within the period of seven days.

Transpiration studies

Transpiration curves for rooted and unrooted carnation cuttings placed in sterile water showed the same tendency to take up a large amount of water during the first two days and then decrease on succeeding days (Fig. 8). The sloping decline of the curves of the rooted and unrooted cuttings in bacterial suspensions of P. caryophylli are correlated in that they show an inability to take up as much water as similar cuttings in sterile water during the first two days.

Respiration observations

Observations made of rooted carnations in various treatments were based on the comparative amount of gas given off by the roots. These observations showed that carnations in treatments of bacterial suspension, filtered bacterial suspension, diseased plant extract, and filtered diseased plant extract gave off large amounts of gas. Those cuttings in healthy plant extract and soluble fraction of healthy plant extract gave off a moderate

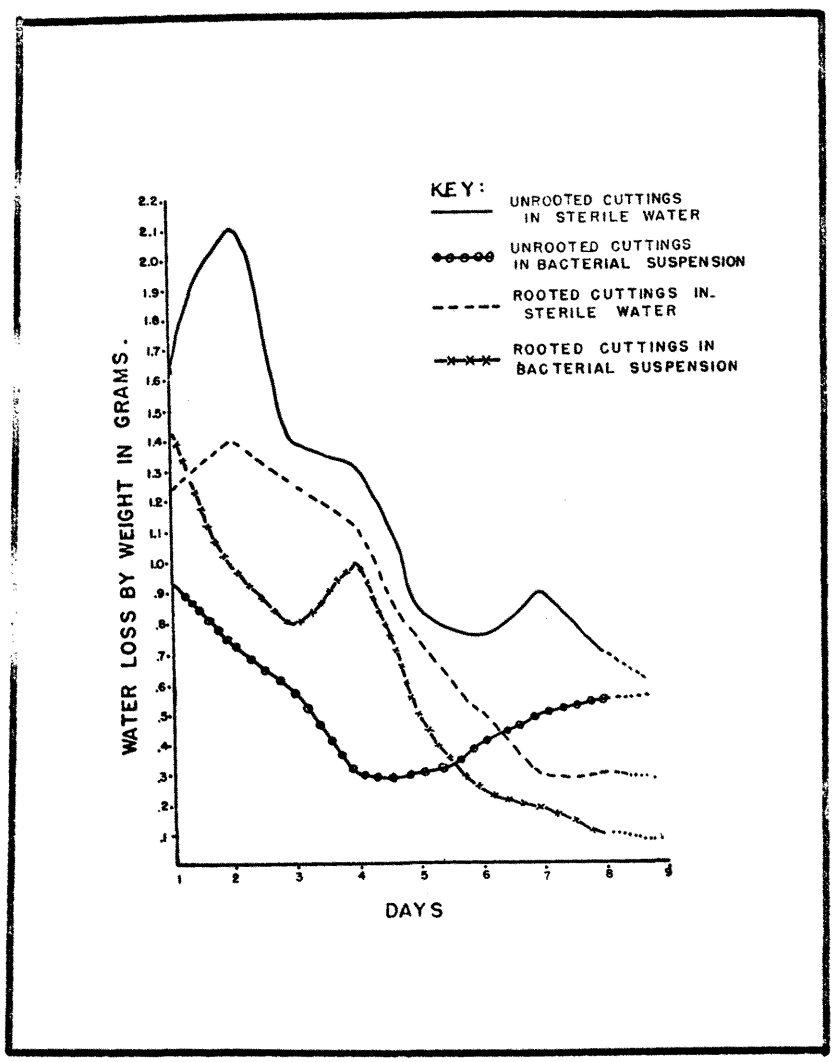

Fig. 8.--Transpiration curves for rooted and unrooted carnation cuttings in bacterial suspension and sterile water.

amount of gas. A small amount of gas was given off by roots of cuttings in filtered agar water, filtered healthy plant extract, and the soluble fraction of diseased plant extract. There was no gas given off by the roots of cuttings in sterile water, soluble fraction of bacterial suspension, soluble fraction of filtered bacterial suspension, soluble fraction of filtered healthy plant extract, and soluble fraction of filtered diseased plant extract, Table 1.

Paper-partition chromatography

Upon the development of free amino acids on one-dimensional chromatograms, characteristic spots were shown to have separated out into five distinct spots at various levels (Fig. 9). Each of these spots contained one or more amino acids. The principal amino acids measured in these spots (as described below) were:

Spot 1 - asparatic acid and cysteic acid

Spot 2 - not measured

Spot 3 - asparagine, serine, and glutamic acid

Spot 4 - Glutamine and tyrosine

Spot 5 - Gamma amino butyric acid, valine, and leucine, or isoleucine.

R_f values were measured for each spot. Since variance analyses for each three, five, six, and 10 λspots were comparable, only that for the analysis of the five λconcentration is shown in Table 2.

Table 1.--ROOT RESPIRATION OBSERVATIONS MADE IN WILT-INDUCTION STUDIES.

Treatments	Relative rates of gas evolution[1]
Artificial culture materials[2]	
Bacterial suspension	3
Bacterial suspension (filtered)	3
Agar water (filtered	1
Soluble fraction of bacterial suspension	0
Soluble fraction of filtered bacterial suspension	0
Natural culture test[3]	
Healthy plant extract	2
Healthy plant extract (filtered)	1
Soluble fraction of healthy plant extract	2
Soluble fraction of filtered healthy plant extract	0
Diseased plant extract	3
Diseased plant extract (filtered)	3
Soluble fraction of diseased plant extract	1
Soluble fraction of filtered diseased plant extract	0
Sterile water control	0

[1] Key 0 = no gas given off
1 = small amount of gas given off
2 = moderate amount of gas given off
3 = large amount of gas given off

[2] Treatments obtained from P. caryophylli grown on potato dextrose agar.

[3] Treatments obtained from healthy carnations and carnations infected with P. caryophylli macerated in a Waring blender.

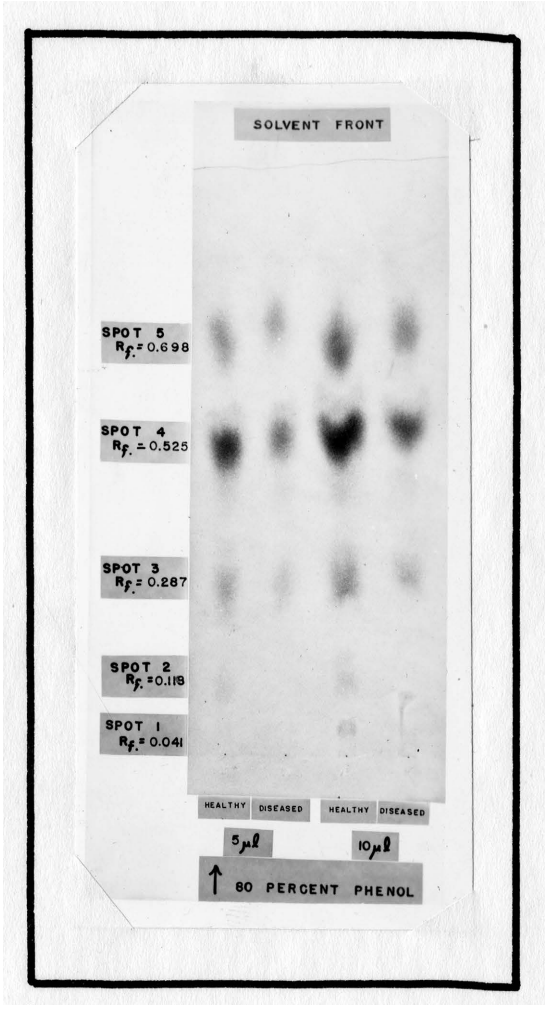

Fig. 9.--One-dimensional free amino acid chromatogram showing variance in density of spots between extracts from diseased and healthy carnation plants.

Table 2.--ANALYSIS FOR FIVE LAMBDA SPOTS OF DISEASED AND HEALTHY CARNATION PLANT EXTRACTS FOR ONE-DIMENSIONAL FREE AMINO ACID CHROMATOGRAMS.

Spot	Amino acids	Mean densichron units		Difference	Standard deviation	Standard error	Minimum difference required for significance	
		Healthy	Diseased				.05	.01
1	Aspartic acid Cysteic acid	1.18	1.10	-.08	.03	.01	.04	.06
3	Asparagine Serine Glutamic acid	1.71	1.50	-.21	.06	.02	.07	.10
4	Glutamine Tyrosine	2.92	2.30	-.62	.05	.26	.08	.12
5	G.A.B.A.* Valine Leucine or Isoleucine	1.78	1.55	-.23	.10	.04	.13	.18

* Gamma-amino butryic acid

The variance analysis of the mean densichron readings for spot 1 showed that the healthy was 1.18 while the diseased was 1.10, a difference of 0.08, which was highly significant. The low density of spot 2 in the diseased treatment did not warrant reading. The mean densichron reading of 1.71 for the healthy spot 3 as compared to 1.50 for the diseased showed a difference of 0.21, which was highly significant. Spot 4 for the healthy showed a reading of 2.92 while the diseased showed a reading of 2.30, a difference of 0.62, which was highly significant. The mean densichron reading for spot 5 was 1.78 for the healthy treatment and 1.55 for the diseased, a difference of 0.23, which was highly significant, Table 2.

Two-dimensional chromatograms of the concentrated juice samples were developed in phenol as before, then in lutidine at right angles to the first solvent front. Ninhydrin solution was the test reagent. Thirteen amino acids were identified by Dent's method (4) of position and substitution. The following amino acids were found in healthy plant extract: aspartic acid, cysteic acid, glutamic acid, serine, asparagine, threonine, glutamine, tyrosine, lysine, gamma-amino butyric acid (GABA), valine, leucine or isoleucine, and proline (Fig. 10).

In the two-dimensional chromatograms of the concentrated diseased plant juice (Fig. 11), it was found that cysteic acid and lysine were absent. The amino acids,

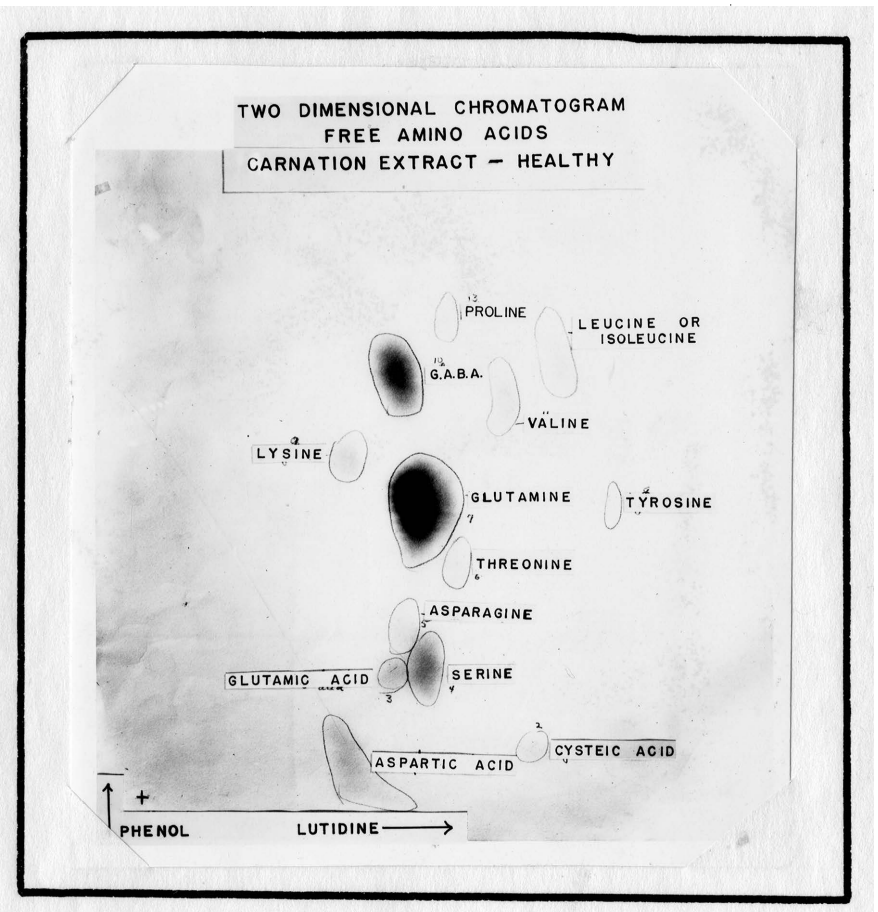

Fig. 10.--Two-dimensional free amino acid chromatogram of healthy plant extract showing 13 free amino acids.

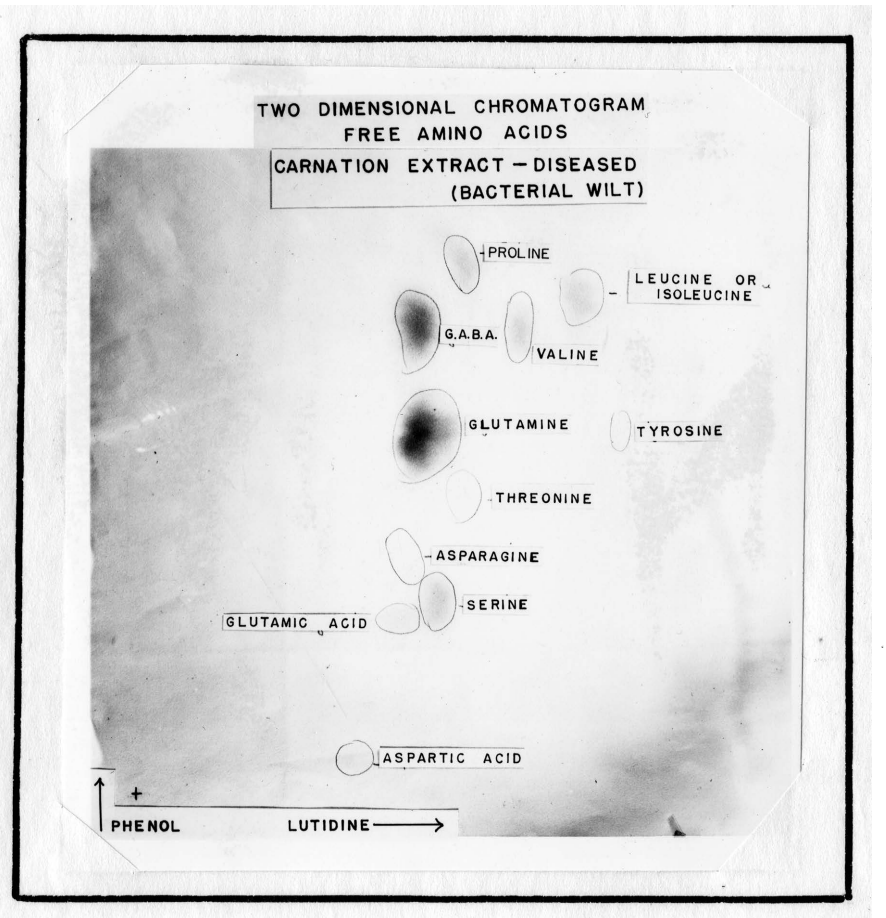

Fig. 11.--Two-dimensional free amino acid chromatogram of diseased plant extract showing the absence of cysteic acid and lysiene (Fig. 10).

aspartic acid, serine, asparagine, glutamine, and GABA were present but in a smaller concentration. However, proline was increased in the diseased plant extract and glutamic acid, threonine, tyrosine, valine, and leucine or isoleucine were apparently no different in concentration from that found in the concentrated healthy carnation plant extract.

In the quantitative one-dimensional sugar chromatograms it was found that spots were developed in the diseased and healthy treatments to correspond with glucose and fructose. There was, in addition, a spot of one or more unknown sugars.

Variance analysis of the spot of healthy and diseased plant extracts, Table 3, showed that the mean densichron reading for glucose in healthy plant juice was 1.19 and the reading for the diseased plant was 1.19, showing no difference. However, the densichron readings for fructose were 1.14 for the diseased plant extract and 1.13 for the healthy plant extract, a difference of 0.01, significant at the five per cent level. Readings for the spot of the unknown sugar gave a mean densichron reading of 1.09 for the diseased and 1.11 for the healthy, a difference of 0.02 which was significant at the five per cent level.

Table 3.--ANALYSIS FOR FIVE LAMBDA SPORS OF DISEASED AND HEALTHY CARNATION PLANT EXTRACTS FOR ONE-DIMENSIONAL SUGARS.

Spot	Sugar	Mean densichron units		Difference	Standard deviation	Standard error	Minimum difference required for significance	
		Healthy	Diseased				.05	.01
1	Glucose	1.19	1.19	0	0	0	0	0
2	Fructose	1.13	1.14	+.01	.02	.006	.01	.02
3	Unknown sugar	1.11	1.09	-.02	.03	.008	.02	.03

Chapter V

DISCUSSION

Carnation cuttings from infected mother-plants may have a small amount of the causal bacterial wilt organism in them. During the course of rooting, which takes about four weeks, the temperature of the rooting media is controlled to about 65° F., a temperature which is not favorable for the rapid growth and spread of the organism. The length of time that is required to induce wilt in the plant is dependent on the temperature; the higher the temperature, the quicker the plant wilts. Wilt proceeds rapidly after air and soil temperatures rise above 80° F. (14). A period of cool temperatures allows the bacteria to be walled-off (Fig. 5). The plant contains the bacteria but shows no symptoms of infection, thus serving as a carrier. If the rooted cutting is planted into a bench where the soil temperature is higher than that of the rooting bench, the bacteria become infectious and the plant subsequently succumbs to bacterial wilt.

In the case of disease-free carnation cuttings planted into a bench infected with *P. caryophylli*, the process of ingression into the plant must occur through wounded roots. The natural methods of wounding roots are

pulling of weeds, cultivation, soil insects, and others (14). After ingression, the bacteria readily invade the vascular elements. The rate of invasion and infection of roots and stems is correlated with air and soil temperature.

During the course of this investigation it was demonstrated that plugging of the xylem vessels of the carnation may result from infection by P. caryophylli. Accompanying this plugging or blocking of the water conducting vessels, an apparent lysis occurs. The effect of the bacterial infection may be apparent from the roots up into the apical portions of the plant and out into the leaves. In the dissolution of the lignified cells various products may be liberated, such as a form of glucose, pectins, and lignin (1).

Tests of the effect of bacterial suspensions and filtrates of bacterial suspensions resulted in the wilting of both rooted and unrooted carnation cuttings. This wilting of these cuttings showed that the toxic agent was filterable. Upon treatment of filtrate of bacterial suspensions with ethanol, it was found that cuttings did not wilt. This indicated that either the toxic agent responsible for wilt of these cuttings was precipitated by the ethanol or it was inactivated. Experiments with extracts from diseased and healthy plants also bore this out.

Wilt of tomato cuttings *in vitro* may result from an excess of glucosans or polysaccharides obtained from non-wilt producing phytopathogenic bacteria (6). It is a matter of conjecture, therefore, whether or not a toxin of a nature any other than that of a polysaccharide could be the wilt-inducing agent in carnations affected by bacterial wilt.

Observations of respiration effects showed that in bacterial suspensions (filtered or unfiltered) and diseased plant extracts (filtered or unfiltered) there was greater quantity of gas liberated. This indicated that the agent responsible for bacterial wilt infection may increase the respiration rate of carnation roots.

Transpiration studies indicated a correlation between the effects of rooted and unrooted cuttings in sterile water and between rooted and unrooted cuttings in bacterial suspension. Since rooted and unrooted cuttings responded in similar manner to the bacterial suspension, it is possible to rule out mechanical blocking as the primary cause of wilt-induction in the unrooted cuttings.

The reduction of the concentration of the free amino acids, aspartic acid, serine, asparagine, glutamine, and GABA in the diseased plant extract indicated a change in normal metabolism in diseased plants probably due to the presence of the bacteria. Asparagine can be utilized by *P. caryophylli* (3). There may be direct correlations

between these changes and the evidence of wilting, but the exact mechanisms involved await further exploration.

Cellulose consists of long chains of linked glucose residues (1). Variance analysis of glucose showed no change in the concentration of glucose from the healthy to diseased plants. However, total sugars in diseased and healthy plants were not measured. Fructose was significantly increased and an unidentified sugar was significantly decreased in diseased plant extract. Due to insufficient experimentation and data, any interpretations of this would be a matter of conjecture.

Since a toxin as an exclusive agent of wilting was not substantiated, it would appear that the increased rate of invasion and infection due to high temperature, accompanied by lysis, increased respiration, and blocking off of the water supply by plugging of the xylem vessels together with the presence of a toxin could account for the sudden wilting of carnation plants infected with bacterial wilt. The wilt, however, may probably be due to combinations of these factors rather than to any one factor.

Suggestions for future research

1. Investigate the cause of proliferation of cells adjacent to a bacterial mass in a carnation stem infected with bacterial wilt.

2. Make a study of the host range of P. caryophylli.

3. Screen chemotherapeutants to be used as cutting dips or soil treatments that might inactivate the bacteria in vivo.

4. Determine whether the toxic agent is precipitated in the insoluble fraction or whether it is inactivated by the ethanol.

5. Study the chemical and physical characteristics of carnations that enhance varietal resistance.

6. Test osmotic concentration of cell sap of healthy carnations and diseased carnation infected with bacterial wilt.

7. Test total ninhydrin-reacting free amino acids in healthy and diseased carnations.

8. Test total sugars in healthy and diseased carnations.

Chapter VI

SUMMARY

In preliminary experiments carnations wilted when placed in a filtrate of a bacterial suspension of P. caryophylli. Since bacterial wilt of carnations is a vascular disease, it is possible either that a toxin or mechanical plugging or a combination of both may be the cause of the wilt.

Histological explorations revealed that the xylem vessels in part were plugged. Lysis of the xylem vessels was also observed. Longitudinal stem section showed that infection was carried to the upper portions of a branch and into the leaves. It was evident that plugging and lysis were not confined exclusively to the lower portions of the stem. Bacteria were not found in parenchyma tissue, although in some cases there was apparently a proliferation of cells in the form of a periderm around a small infected area of xylem vessels.

Proteins in the bacterial filtrate were removed by precipitation with ethanol. The excess alcohol was removed by evaporation and the residue tested for wilt-inducing properties on healthy rooted carnation cuttings. The results were negative. This suggests that the wilt-

inducing factor is either destroyed by the alcohol or may be present in the insoluble fraction.

Paper-partition chromatography of free amino acids showed significant reductions in the concentration of some of the free amino acids in the diseased as compared with the healthy carnation plants. This indicates a change in normal metabolism in the diseased plants probably due to the presence of the bacteria.

Quantitative sugar analysis resulted in an increase in fructose and a decrease in an unidentified sugar in the diseased plants, whereas glucose remained the same.

It is postulated that the wilt-induction is due to the combination of several factors such as, a) increased temperature, b) accompanied by lysis, c) increased respiration, and d) the mechanical blocking of water conduction vessels.

A P P E N D I X

Raw data for this study are on file in the Department of Botany and Plant Pathology, Colorado Agricultural and Mechanical College, Fort Collins, Colorado.

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