

***Pseudomonas caryophylli* in Carnation. IV. Unidentified Bacteria Isolated from Carnation**

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ABSTRACT

Standard indexing procedures were used to isolate the unidentified bacteria from healthy carnation seed, leaves, and stems. The unidentified bacteria were isolated more frequently from stems than from seeds or leaves, and were isolated more frequently from older leaves than from younger upper leaves. The bacterial contaminants became associated with the basal stem tissues during rooting of cuttings, and subsequent development of the bacteria was related to plant growth. The distribution of bacteria was continuous in the portion of the stem immediately above the roots, and was random and discontinuous in the remainder of the plant. The bacteria occurred with almost equal frequency in nodal and internodal stem areas, and were isolated from the epidermis, cortical, vascular, and pith tissues of the stems. The occurrence and distribution of the unidentified bacteria in carnation stems were not affected by soil moisture.

Culture-indexing alone detected only about 50% of the cuttings that contained unidentified bacteria. Subirrigation of stock plants and indexing cuttings immediately after they were removed from stock plants resulted in fewer cuttings contaminated with bacteria, but the height at which the cuttings were removed from the stock plant had no effect.

None of a selected group of isolates of unidentified bacteria caused any discernible symptoms when inoculated into side shoots of carnations. However, one of these isolates caused a more rapid development of symptoms when shoots were inoculated simultaneously with the isolate and the pathogen, *Pseudomonas caryophylli*, than with the pathogen alone. This isolate had been isolated from a wilted carnation plant infected by *P. caryophylli*. Phytopathology 60:647-653.

Nelson & Dickey (9) reported that unidentified bacteria were isolated from the third, fifth, or seventh internode of 64% of wilted plants that had been previously inoculated with *Pseudomonas caryophylli* (Burkh.) Starr & Burkh.; unidentified bacteria were isolated much less frequently from comparable internodes of nonwilted plants. In addition, unidentified bacteria were isolated from 91% of the nonwilted control plants when stem sections from all internodes were cultured. They were present in roots and stems of rooted cuttings, but were not isolated from the base of the cuttings when tested prior to being stuck in the rooting medium. Over 98% of the unidentified bacteria were gram-negative rods; some formed spores. When culture-indexing carnation cuttings, Nelson et al. (11) found that 10-90% of the cuttings in individual lots of 300-500 were contaminated with bacteria. The occurrence of contaminants in cuttings was a direct result of the manner in which the cuttings were handled before being cultured. Losses from contamination were smallest when cuttings were taken from plants and cultured immediately. Certain handling or storage procedures resulted in excessive losses, e.g., 90% of one group was lost when unrooted cuttings were shipped in moist sphagnum moss from Colorado to New York. Jenkins (7) also urged immediate culturing of cuttings, because the incidence of bacterial contaminants was often greater when cuttings were stored in polyethylene bags in which condensation occurred and humidity was high. Hellmers (6) found that the percentage of contamination

averaged 10-20% in carnation cuttings culture-indexed by a commercial firm in Denmark. Occasionally, losses from bacterial contaminants were 80-100%. In these instances, the cuttings had been in contact with water prior to culture-indexing. He observed that a high percentage of bacterial contamination occurred when cuttings were placed in boxes containing moist paper, were stored in polyethylene bags, or when the tops of newly rooted cuttings were culture-indexed. He attributed this to contact and absorption of water contaminated with bacteria.

Hellmers (6) suggested that some unidentified bacteria occur in the deep stomatal pits in the carnation stem epidermis and that an aqueous solution of mercuric chloride, used as a surface sterilant, does not penetrate into the pits. The bacteria are not killed and they later develop, therefore, in the broth used in the culture-indexing procedure. Ebben (5) reported that only about 5-10% of cuttings from young plants grown hygienically were normally contaminated. Ebben agreed with Hellmers' suggestion (6) that bacterial contaminants escape the action of a surface sterilant in the stomatal pits.

The association of unidentified bacteria with carnation plants was studied to determine (i) the distribution of unidentified bacteria in carnation plants; (ii) methods of eliminating or reducing the occurrence of unidentified bacteria to reduce losses in culture-indexing and to produce plant material free from unidentified bacteria for a study of pathogenesis; and (iii) the effect

of unidentified bacteria on development of symptoms of carnation plants inoculated with *P. caryophylli*. A preliminary report of these studies has been made (3).

MATERIALS AND METHODS, RESULTS.—*Disinfestation of carnation plant surfaces.*—Several methods of surface-disinfesting carnation (*Dianthus caryophyllus* L.) stems for the isolation of unidentified bacteria were investigated before a standard method was chosen. Carnation stem pieces were selected from 22 plants (cultivars Improved White Sim and Durango) and treated. The replicated treatments were (i) 70% ethyl alcohol (5 min), sterile distilled water rinse (5 min), 1% mercuric chloride solution (5 min), sterile distilled water rinse (5 min), 20% solution of commercial Clorox (5.25% sodium hypochlorite) containing 1 drop of Triton X100 (alkyl aryl polyethoxy ethanol)/100 ml of solution as a wetting agent (5 min), and sterile distilled water rinse (5 min); (ii) 0.1% mercuric chloride in 70% ethyl alcohol (10 min) and 2 successive 5-min rinses in sterile distilled water; (iii) 20% commercial Clorox solution plus wetting agent (5 min) and sterile distilled water rinse (5 min); and (iv) sterile distilled water rinse (5 min). Eight to ten 1-mm sections were cut from each treated stem piece for a total of 100 sections for each treatment; each section was placed in a tube containing nutrient broth plus 1.5% dextrose (NBD), and incubated at 30 C for 5 days. Unidentified bacteria were isolated from 71, 66, 65, and 97% of the sections for treatments i, ii, iii, and iv, respectively. The occurrence of bacterial contaminants was reduced only about 30% by the surface sterilants.

In addition, cells from 48-hr cultures of either *P. caryophylli* or one of several common unidentified bacteria were added to a 20% solution of commercial Clorox. A loopful of the resulting suspension then was removed after 1, 3, 5, 7, 9, 11, 13, 15, and 20 min and added to a tube of NBD. The tubes were kept at 27 C for 10 days and observed for bacterial growth. Bacterial growth was not observed in the tubes at the end of the incubation period. The Clorox solution plus wetting agent also was tested after it had been used to surface-disinfest as many as 20 leaves or stem pieces. Bacterial growth did not occur in any of the tubes containing NBD to which approximately 0.1 ml samples of the Clorox solution had been added. In contrast, bacterial growth always occurred in NBD to which was added a sample of sterile distilled water used to rinse for 5 min plant parts not surface-disinfested.

Young expanding leaves of mature carnation plants were used to determine the effectiveness of 20% Clorox solution plus wetting agent for disinfesting the surface of carnation tissue. The leaves were soaked for 1 hr in aqueous suspensions of two types of unidentified bacteria previously isolated from carnation leaves. The leaves were dried and either were surface-disinfested in the Clorox solution and rinsed in sterile distilled water or were not surface-disinfested. The leaves were sectioned and cultured as previously described. Broth from tubes with bacterial growth was streaked on nutrient agar and examined for colony growth after incubation at 27 C for 3 days. The unidentified bacteria were iso-

lated from 90% of the leaf sections not disinfested; bacteria were not isolated from any of the disinfested leaf sections.

An aqueous suspension of unidentified bacteria containing approximately 10^6 cells/ml was injected by a 26 gauge hypodermic needle into the intercellular spaces of leaf tissue. The leaves were held at room temperature until they no longer appeared water-soaked (2 hr). The leaves either were surface-disinfested or were rinsed in sterile distilled water for 5 min. Sections were cut from areas that had been water-soaked, and these were cultured. Bacteria were isolated from each section. These results indicate that the Clorox solution apparently does not inhibit recovery of the bacteria from interior portions of the carnation leaves.

On the basis of the above tests, a 5-min soak in a 20% solution of Clorox plus wetting agent followed by a 5-min rinse in sterile distilled water was selected as an effective method to disinfest the surfaces of carnation plant parts and was used throughout these investigations. These results agree with those reported by Jenkins (7).

Isolation of unidentified bacteria from carnation seed and leaves and distribution of unidentified bacteria and the pathogen, Pseudomonas caryophylli, in stems.—Seed of *Dianthus caryophyllus* was obtained from W. D. Holley, Colorado State University, Fort Collins. The seeds were soaked for 5 min in 20% Clorox solution plus wetting agent and rinsed with 4 changes of sterile distilled water at 5-min intervals. Each seed was placed in a tube containing NBD. The tubes were incubated at 27 C for 10 days and observed for bacterial growth. Bacteria were isolated from 7.5% of the seed.

Five pairs of opposite leaves were removed from the bottom, middle, and top portions of carnation plants (Improved White Sim) at a mean height of 10.3, 30.8, and 52.3 cm, respectively, above the soil line. One of each pair was surface-disinfested and rinsed in sterile distilled water; the other was not treated. The leaves were sectioned at 5-mm intervals, and each section was cultured in a tube of NBD. The tubes were incubated for 6 days at 27 C, and the occurrence of bacterial growth was recorded. Bacteria were isolated from 13.1, 9.7, and 5.6% of surface-disinfested sections and 54.6, 28.1, and 28.6% of the nontreated sections of leaves collected from the bottom, middle, and top portions, respectively, of plants. In addition, unrooted cuttings were stuck in steam-treated rooting medium and placed under mist. After 17 and 50 days, bacteria were isolated from 8.5 and 19.6%, respectively, of surface-disinfested leaf sections, and from 59.9 and 85.5%, respectively, of nontreated sections. These results showed that one-third or more of the bacteria occurred interior to the epidermis.

Rooted carnation cuttings (Improved White Sim) were inoculated with *P. caryophylli* to study the distribution of the pathogen and unidentified bacteria in the stems. The roots were placed for 1 hr in 40-60 ml of an aqueous suspension of cells from three 48-hr-old potato-dextrose agar slant cultures of *P. caryophylli* grown at 33 C. After inoculation, the cuttings were

planted in 4-inch pots of steam-sterilized soil and placed on a greenhouse bench. A check plant and an inoculated plant were removed, examined, and cultured at various time intervals after inoculation. The plant stems were cultured, as previously described (4), at 5-mm intervals from the base to the apex to determine the location of the pathogen and any unidentified bacteria. The typical distribution of the pathogen and unidentified bacteria is shown in Fig. 1. There was a random, discontinuous distribution of unidentified bacteria in contrast to the uninterrupted upward distribution of *P. caryophylli* from the base of the stem. Although the unidentified bacteria were always isolated from the basal portion of the stem, they occurred only irregularly in areas of the upper portion of the stem, and apparently were not continuous with bacteria isolated from the base.

The distribution of unidentified bacteria was continuous in a portion of the basal stem of each of 70 culture-indexed rooted cuttings sectioned immediately after their removal from the rooting medium. Therefore, the effect of rooting cuttings under mist on the occurrence of unidentified bacteria in the stems was determined for 40 Northland cuttings. Although bacteria were isolated from the basal sections of only 30% of the cuttings when they were stuck for rooting, bacteria occurred in the basal sections of 70 and 100% of the cuttings after 10 and 25 days under mist, respectively (Table 1). The distance that the bacteria were continuous from the base increased as the number of days under mist increased. These results indicate that an association between bacterial contaminants and the

TABLE 1. The effect of rooting carnation cuttings, cv. Northland, under mist on the occurrence of unidentified bacteria in the stems^a

No. days under mist ^b	% Cuttings with bacteria continuous from base	Height bacteria continuous from base (cm)
0	30	1.2
10	70	2.5
25	100	5.7
41	100	7.2

^a Values are means of 10 cuttings for each number of days under mist.

^b Root development was not visible until 25 days under the mist.

basal stem tissues commences during the rooting of the cuttings.

The portion of the stem from which unidentified bacteria were isolated from successive sections, commencing at the base immediately above the roots, also was determined for three groups of stock plants. The mean height of the plants for each group was 15.7, 39.0, and 68.8 cm, and the mean distance at which bacteria were isolated from successive sections was 4.9, 6.2, and 8.3 cm, respectively. These results demonstrate a positive correlation between the increase in the distance at which successive sections containing bacterial contaminants occurred from the base of the stem and the increase of plant growth.

The location on the stem above the basal portion of the plants from which each cultured section was cut and the isolation of unidentified bacteria were recorded throughout all investigations. These data were used to determine the frequency of isolation of bacteria from nodal and internodal areas. There was no significant difference because bacteria were isolated from 24.1% of 5,616 internodal sections and from 29.4% of 1,717 nodal sections.

The effect of soil moisture on the occurrence and distribution of unidentified bacteria in carnation stems.

—Rooted cuttings of Improved White Sim were planted in Hagerstown sandy loam soil (moisture equivalent = 23.4%) in gal plastic containers. The soil was maintained at soil moisture regimes of either 0.5 field capacity (FC), FC, or 1.5 FC, as previously described (4). The water loss was determined twice a day, and the soil watered as necessary. The soil moisture regimes were continued for 69-80 days before the plants were sectioned at 5-mm intervals and cultured. The soil temperature for the first 50 days was approximately 21.1 C, and for the next 19-30 days was 25.9-26.7 C. The height of the plants when sectioned was 40.6 to 80.3 cm. The per cent of all sections containing bacteria in successive sections commencing at the base immediately above the roots was 13.3, 13.3, and 14.2 for soil moisture regimes of 0.5 FC, FC, and 1.5 FC, respectively; bacteria were isolated from 14.2, 17.8, and 16.6% of the sections cut from the upper portion of the stems where the distribution of bacteria was random and discontinuous (Fig. 1). The soil moisture regimes did not appreciably affect the occurrence or distribution of unidentified bacteria.

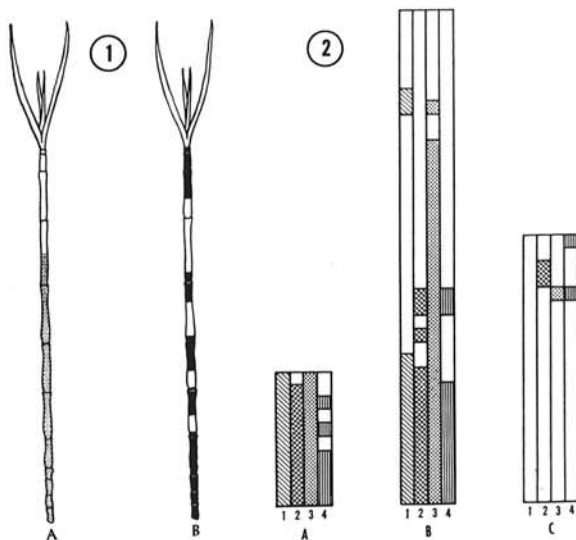


Fig. 1-2. 1) Distribution of *Pseudomonas caryophylli* (A, stippled area) and unidentified bacteria (B, dark areas) in carnation, cv. Improved White Sim, 35 days after root-inoculation with the pathogen. 2) Location of unidentified bacteria (shaded areas) isolated from tissues of stems removed from the A) basal (cv. Northland), B) lower (cv. Improved White Sim), and C) upper (cv. Improved White Sim) portions of healthy carnation plants. 1 = Epidermis plus adhering cortical tissue; 2 = cortex, fibers, and phloem; 3 = xylem; and 4 = pith.

Location of unidentified bacteria in carnation stems.

—Stem pieces were removed from the basal portion and side shoots of stock plants. The pieces were surface-disinfested, rinsed in sterile distilled water, and cut into consecutive 5-mm sections. Each section was aseptically separated into the following parts: 1) epidermis plus adhering cortical tissue; 2) cortex, fibers, and phloem; 3) xylem; and 4) pith (10). Each part was placed in a tube containing NBD and incubated for 6 days at 27 C. Bacteria were isolated from all parts (Fig. 2). They usually were continuous for a longer distance in the xylem tissue than in other parts of the lower portions of stems (Fig. 2-B). Bacteria were not consistently isolated from any part of the upper portions (Fig. 2-C).

Histological studies of basal stem portions of healthy stock plants also were made. The stem sections were fixed, dehydrated, embedded, sectioned, and stained with Harris' hematoxylin and orange G as previously described (10). Scattered xylem vessel elements in stem sections of the first internode were plugged, and small clumps of bacteria could be seen in the material plugging the cell lumens. Bacteria were not found in other tissues. These results indicate that the population of unidentified bacteria probably is relatively small and scattered.

Isolation of unidentified bacteria from carnation cuttings and the influence of some factors on the occurrence of the bacteria.—A total of 1,003 cuttings was used to determine whether the normal culture-indexing procedure of culturing sections from the base of the cuttings accurately reveals all cuttings which contain unidentified bacteria. Six to 10 one-mm stem sections were cut at 5-mm intervals from the base of the cutting to the shoot apex. Bacteria were isolated from the basal sections of 27.0% of the cuttings, and from sections, other than basal sections, of an additional 27.8% of the cuttings. These results show that in only half of the cuttings yielding bacteria were the bacteria present in the basal section of the cutting.

Cuttings were taken from various locations on stock plants (cultivars Durango, Northland, Starlite, Tangerine Sim, Elegance, and Dark Pink Virginia) to determine relation of height of cutting on stock plants to occurrence of bacteria in the cutting. The distance between the growing point of the cutting and the soil line was recorded. Eight 1-mm sections from each cutting were cultured for the presence of bacteria. The first section was cut from the stem immediately below the apex; subsequent sections were cut at 5-mm intervals. There was no consistent relationship between the height of the cuttings on the stock plant and the occurrence of bacteria in the cuttings of two lots (Table 2). This apparently is due to the random distribution of the bacterial contaminants in the stem (Fig. 1).

The effect of the method of watering stock plants on the occurrence of bacteria in cuttings was investigated. Improved White Sim cuttings were planted in 4- or 5-inch pots and irrigated for 33 days by applying water from a flask to the soil so that foliage and stems were not wetted. The plants were pinched at approxi-

TABLE 2. Isolation of unidentified bacteria from carnation cuttings taken at different heights on stock plants

Height of cutting growing point from soil (cm)	% Cuttings yielding unidentified bacteria		Total no. cuttings
	Lot No. 1	Lot No. 2	
11.5-20	37.9	0.0	30
21-30	12.5	42.9	55
31-40	47.4	13.3	34
41-50	12.5	0.0	16
51-75	0.0	33.3	9

mately 8 inches above the soil, and 1 week later they were separated into three groups. One group was placed under intermittent mist, the second group was placed on a greenhouse bench and received regular overhead watering, and the third group was placed on a greenhouse bench and watered only by subirrigation. A total of 89 to 134 cuttings were removed thereafter from each group at irregular intervals and cultured. Eight 1-mm stem sections were cultured separately for each cutting; the first section was cut from the stem area immediately behind the shoot apex, the subsequent sections at 7-mm intervals. Bacteria were isolated from 58.9, 52.2, and 40.4% of cuttings from plants watered by intermittent mist, overhead watering, and subirrigation, respectively. It was found that any method of watering stock plant foliage and stems results in a higher per cent of cuttings yielding unidentified bacteria than methods which keep water off the foliage and stems. The results also indicate that a more frequent application of water to foliage and stems (mist treatment) causes an increase in the occurrence of bacteria.

Several lots of cuttings were taken from carnation plants growing in Ithaca and Farmingdale, New York, and were used to determine the effect of method of handling cuttings before culturing on the occurrence of bacteria. The carnation cultivars Improved White Sim, Red Sim, Tangerine Sim, Improved Sidney Littlefield, and Durango were used in this test. Each lot of cuttings was divided into four groups and subjected to different treatments. After each treatment, 8 to 10 1-mm stem sections from each cutting were cultured for the detection of bacteria. Treatments and results are shown in Table 3. The smallest percentage of cuttings containing bacteria occurred when the cuttings were cultured the same day they were removed from the stock plants. Storing cuttings at 3.5 C in closed plastic bags caused the least increase in the occurrence of the bacteria. Rooting of cuttings under mist and storing cuttings at 3.5 C in closed plastic bags containing either moist peat moss or sphagnum moss caused the greatest increases in the occurrence of bacterial contaminants. There was an increase not only in percentage of cuttings but also in the average number of sections per cutting from which unidentified bacteria were isolated. Conditions providing increased moisture apparently favor the spread, development, or entry of the bacterial contaminants.

The effect of unidentified bacteria on development of symptoms in plants inoculated with Pseudomonas

TABLE 3. Effect of methods of handling carnation cuttings before culturing on the occurrence of unidentified bacteria

Treatment ^a	Total no. cuttings	% Cuttings yielding unidentified bacteria	Mean no. sections/cutting yielding unidentified bacteria ^b
A	90	58.9	3.5
B	89	80.9	5.0
C	77	66.2	4.0
D	45	86.7	6.2

^a A = Cuttings cultured immediately after collection. B = Cuttings rooted 56-64 days under intermittent mist before culturing. C = Cuttings stored at 3.5 C in closed plastic bags for 57-68 days before culturing. D = Cuttings stored at 3.5 C for 70-71 days in closed plastic bags containing either moist peat moss or sphagnum moss.

^b Only the total numbers of sections for cuttings yielding unidentified bacteria were used to compute the means. The mean number of sections cultured per cutting for treatment A = 9.58, B = 9.75, C = 9.30, and D = 9.56.

caryophylli.—A study of the possible association of unidentified bacteria and *P. caryophylli* requires the selection or production of carnation tissue entirely or relatively free of any bacteria. An effort was made to produce bacteria-free tissue by the use of cuttings developed from apical meristems, seedlings grown under aseptic conditions, and cuttings rooted without the use of an intermittent mist system. All these attempts were cumbersome and unsatisfactory. The following method was selected to produce plants suitable for inoculation. Rooted cuttings of Improved White Sim were potted in steam-sterilized soil and placed on a greenhouse bench. The plants were spaced on the bench, staked to keep the foliage off the soil, watered without splashing the foliage, and fertilized weekly. The terminal shoot tips were removed to provide succulent, fast-growing shoots. When samples of these shoots were cultured, unidentified bacteria usually were not isolated. Fifteen isolates of unidentified bacteria that exhibited different cultural characteristics of colonies on nutrient agar were used for the inoculations. Ten of the cultures were isolated from stems of healthy plants, and five were isolated from wilted plants infected by *P. caryophylli*. Two side shoots of uniform plants were inoculated at an internode by gently puncturing the stem with a needle containing cells from 48-hr-old slant cultures. Plants were inoculated with *P. caryophylli* alone, or with each of the 15 isolates of unidentified bacteria alone, and in combination with *P. caryophylli*.

The side shoots of plants inoculated with unidentified bacteria alone showed no adverse effects. The shoots inoculated with *P. caryophylli* alone developed the typical symptoms of bacterial wilt, and were either dead or severely wilted when the tests were terminated; other parts of the inoculated plants appeared unaffected. Symptoms that developed on shoots inoculated with *P. caryophylli* and each unidentified bacterium were similar to those produced by the pathogen alone, except for one isolate that caused an acceleration of symptom development (Fig. 3) and complete wilting of the

plants at the termination of the tests. This latter isolate had been isolated from the basal portion of wilted carnation plants which had been artificially inoculated with *P. caryophylli*.

DISCUSSION.—Culture-indexing carnation cuttings is based on the assumption that phytopathogenic fungi and bacteria spread throughout the host by progressive and continuous growth in the stem. If the pathogen is not detected in the basal portion of the indexed cutting, it is assumed that the cutting is free of the organism. *Pseudomonas caryophylli* can be successfully detected by the culture-indexing method (11), due to the continuous upward distribution and growth of the pathogen in the stem (Fig. 1-A). However, culture-indexing does not guarantee that the cuttings are free of other organisms. It was found that the indexing procedure detected about 50% of the cuttings containing unidentified bacteria due to their random distribution in the cutting (Fig. 1-B).

The actual losses sustained because cuttings are discarded when the growth of unidentified bacteria occurs in the broth are not known for commercial propagators; however, studies have shown that the losses may range from 5-100% (5, 6, 11). Although the current practice of discarding all cuttings that are not free of any type of organism is wasteful, it still is the most economical method available to the commercial propagator. Most commercial operators cannot afford the time or facilities necessary to identify each culture and thereby determine whether a cutting should be discarded. This emphasizes the extreme importance of proper handling of cuttings prior to culture-indexing and the need for further improvement of the culture-indexing method, e.g., the development of a selective medium. Our findings concerning methods of handling cuttings before culture-indexing and methods of watering stock plants to reduce the occurrence of bacterial contaminants agree with the findings of other workers (5, 6, 7, 11). In general, any method which results in the presence of free water on the leaf and stem surfaces of carnation cuttings or stock plants increases the percentage of cuttings that will yield or harbor unidentified bacteria. Neither the height at which the cuttings were removed from the stock plants nor the soil moisture maintained for the stock plants affected the occurrence of unidentified bacteria in the cuttings. The data also suggest that the more frequent occurrence of unidentified bacteria in older leaves of the plants than in younger upper leaves probably reflects in part the effect of regular watering of the lower portion of the plants during irrigation.

The association of unidentified bacteria with the carnation tissues apparently begins during rooting of the cuttings under mist (Table 1). The wounded, broken stem at the base of the cutting and wounds made by removal of leaves undoubtedly serve as areas for ingress by these bacteria. The distribution of the bacteria becomes continuous in the stem immediately above the roots and continues to extend upward even after the rooted cuttings are planted. The random and discontinuous distribution of these bacteria in the upper

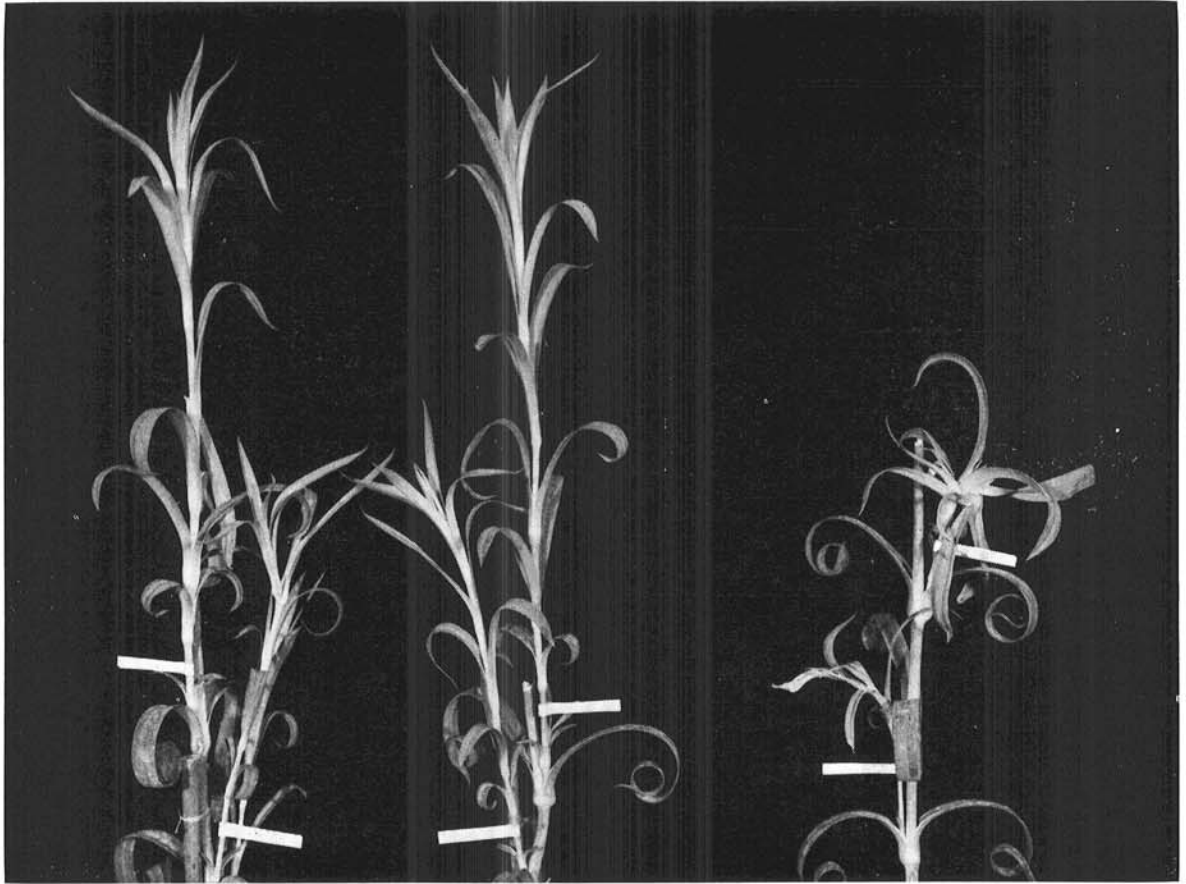


Fig. 3. Carnation plants, cv. Improved White Sim, 25 days after inoculation with unidentified bacterium (left), *Pseudomonas caryophylli* (middle), and unidentified bacterium plus *P. caryophylli* (right). Note twisting of the terminal shoot and wilting of leaves on right. White tags denote points of inoculation.

portion of the plants was not related to nodes or internodes. The reason for the limited continuous distribution of the bacteria at the base and their discontinuous distribution in the remainder of the plant was not ascertained by these studies.

Other workers have reported the presence of bacteria in apparently healthy plant tissue (8). Samish & Etinger-Tulczynska (12, 13) found that bacteria are frequently present in the tissue of normal, healthy green or ripe tomatoes, and that the bacteria are not uniformly distributed within the fruit. They also noted that fruit harvested from plants watered by overhead irrigation generally contained more bacteria than fruit from furrow-irrigated plants. Our findings and those of Hellmers (6) and Jenkins (7) indicate that unidentified bacteria are present within the plant parts of healthy carnations. Hellmers (6) reported the occurrence of bacteria in stomatal pits on the stem, and suggested that a surface-disinfectant does not penetrate sufficiently to kill the bacteria. During our earlier study of the pathological anatomy of carnation stems infected by *P. caryophylli* (10), we examined many stem sections with stomatal pits. We were unable to demonstrate in stained cross sections the presence of bacteria in any of these

stomatal pits. The histological studies of healthy carnation stems reported herein failed to detect the exact location of bacteria in the tissues, except that bacteria were observed in the lumens of vessel elements. We attempted, therefore, to isolate unidentified bacteria from other stem tissues, and were able to isolate the bacteria from all tissues (Fig. 2).

Although it is believed that nonpathogenic bacteria may aid or hinder a phytopathogenic bacterium in infection and that inhibition of a pathogen is a frequent occurrence (1), there are few reports concerning the enhancement of symptom development by unidentified bacteria. Burkholder & Guterman (2) report that both the pathogen, *Xanthomonas hederae*, and another bacterium were isolated from lesions on leaves of *Hedera helix*. Although the unidentified bacterium when tested alone on ivy was not pathogenic, it had an accelerating effect on disease development when tested in combination with the pathogen. This work was confirmed by White & McCulloch (14), who were able to isolate the pathogen and 10 associated bacteria from older lesions on ivy foliage. When each bacterial isolate was inoculated simultaneously with the pathogen, one caused a distinct increase in the size of infected area of the

leaf while nine produced lesions smaller than those produced by the pathogen alone and lacking the water-soaked margins characteristic of active invasion of tissue. A similar effect was observed by us when carnation shoots were inoculated simultaneously with 15 isolates of unidentified bacteria and *P. caryophylli*; one caused a marked acceleration in development of symptoms (Fig. 3), while the other isolates had no discernible effect. These results reinforce the possibility that symptom development may be influenced by the association of unidentified bacteria and a bacterial pathogen. Additional evidence of an interaction between unidentified bacteria and *P. caryophylli* during pathogenesis will be reported elsewhere.

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