Bacterial Blight of Carnation Caused by *Pseudomonas woodsii* and Susceptibility of Carnation Cultivars

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ABSTRACT

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Pseudomonas woodsii was shown to be the cause of a blight of carnation on 10 Maui farms during 1989 and 1990. Small, water-soaked, yellow specks that appeared 6 days after inoculation were the initial symptoms on potted plants. When plants were inoculated with 2.9×10^6 cfu/ml, the specks appeared in 6 days and enlarged to 2- to 3-mm-diameter discrete spots 4 days after initial symptoms were visible; at 4.5×10^8 cfu/ml, however, lesions coalesced, causing extensive blight. Plants inoculated with 10^6 to 10^8 cfu/ml produced comparable numbers of lesions when incubated for 24–48 hr in a moist chamber or outdoors at ambient relative humidities. The lowest inoculum level to initiate disease was 2.9×10^3 cfu/ml. Streptomycin sulfate at 250 and 500 ppm a.i., oxytetracycline at 204 and 407 ppm a.i., and fosetyl Al at 4,800 and 9,600 ppm a.i. significantly reduced the number of leaf spots, but treatments did not economically control the disease. Of 66 cultivars evaluated in the field, Cal Red and Cal Improved White showed high disease resistance.

In Hawaii, approximately $1.3 million worth of carnations are produced annually on fewer than 25 farms (7). Most farms are located at 900–1,200 m elevation in Kula, Maui, the major production area for lei carnations (14). Kula is ideally suited for field-grown carnations, with temperatures of 10–30 C, an annual rainfall of 85 cm, and silty loam soils (7).

Bacterial blight of carnation, caused by *Pseudomonas woodsii* (Smith) Stevens, was first recorded in Hawaii in 1950 on Oahu (8) and in 1962 on Kauai (15). The disease occurs sporadically in Kula, e.g., from 1987 to mid-1989, all carnation farms were apparently free of bacterial blight. In late 1989, however, a serious outbreak of the disease occurred, providing an opportunity to study the biology of this pathogen under Maui conditions.

MATERIALS AND METHODS

Disease surveys, isolation, and pathogenicity. Disease surveys of Kula carnation farms were made monthly from October 1989 to June 1990. Isolations were made from leaves, stems, and flowers of symptomatic plants. Diseased tissue was washed, and the advanced margins of lesions were excised and disinfested in 0.5% aqueous NaOCl + 0.1% detergent or 95% ethanol for 30 sec, rinsed in sterile water, triturated, and streaked on yeast extract-dextrose-$CaCO_3$ medium (YDC) (11). Isolation plates were incubated at 27 C for 3–5 days. Single, white, mucoid colonies (3) were restreaked several times on YDC for purity and stored at room temperature (19–28 C) in sterile distilled water.

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Correlation between spectrophotometer readings and colony-forming units in dilution plate counts. Colonies of the most virulent strains of *P. woodsii*, i.e., C11 and C18, grown on YDC at 27 C for 24–48 hr were used to prepare bacterial suspensions. Concentration of the suspensions was determined with a spectrophotometer (Bausch & Lomb Spectronic 20) at 520 nm and adjusted to 0.5 absorbance. Colony counts were made by plating 0.1 ml of serial, 10-fold bacterial dilutions on 0.8% Difco nutrient broth with 2% agar and incubating the plates at 27 C for 2–3 days. The same procedure was used to prepare bacterial suspensions for inoculations.

Pathogenicity tests. Rooted cuttings of Peterson's Red Sim carnation (supplied by Yoder Brothers, Barberton, OH) were grown in 9-cm square pots filled with equal parts of soil and Fison's Sunshine Mix 3 (Fison Horticulture Inc., Vancouver, BC, Canada). Granular 16-16-16 fertilizer was applied at planting, followed by monthly drenches of 16-16-16 fertilizer at 5 ml/4 L of water. At 1 mo of age, plants were pinched above the third leaf node to stimulate axillary shoot development. Plants were kept in an outdoor nursery with 15 min of daily overhead irrigation until they were 20–30 cm tall.

Eight suspected *P. woodsii* strains obtained from different farms were tested for pathogenicity. Each strain was suspended in water at approximately 10^8 cfu/ml and sprayed to runoff on five potted Peterson's Red Sim plants. Five plants sprayed with water served as controls. Test plants were incubated for 48 hr in sealed plastic bags in the laboratory, then were removed from the bags and placed in an outdoor nursery. Disease severity was recorded 13 days after inoculation, and reisolations were made on YDC.

Effects of inoculum concentration and incubation RH on disease incidence. Inoculum of strains at 10-fold increments from 2.9×10^6 to 2.9×10^8 cfu/ml for C11 and 4.5×10^6 to 4.5×10^8 cfu/ml for C18 was applied to four Peterson's Red Sim plants for each inoculum level. Plants sprayed with water served as controls. Peterson's Red Sim plants were inoculated outdoors in a nursery and in a laboratory. In the nursery, the plants were inoculated at 9:00–11:00 a.m. and incubated at ambient relative humidity (50–90%) and temperature (25–15 C). In the laboratory, plants were incubated at high RH in sealed plastic bags for 24 or 48 hr, then were removed from the bags and incubated in the outdoor nursery until symptoms developed. Fourteen days after inoculation, spots were counted on four randomly selected infected leaves from each test plant. Only lesions measuring >2 mm in diameter were counted to minimize errors due to smaller spots possibly caused by secondary infections. Strain C11 was used to repeat inoculations in the outdoor nursery at 10-fold increments from 3 to 2.9×10^8 cfu/ml. Three plants were used per inoculum level, and spots from four leaves per plant were recorded as previously described.

Chemical control. Twenty chemical treatments were tested for efficacy in controlling bacterial leaf blight in an outdoor nursery. These were: cupric hydroxide (Champ 23F at 382 and 764 ppm a.i., Champion 77WP and Kocide 101 77WP at 922 and 1,845 ppm a.i., and Kocide 61 0.4% DF at 736 and 1,471 ppm a.i.); copper ammonium carbonate (Copper-Count-N 8% at 538 and 1,075 ppm a.i.); streptomycin sulfate (Agri-Strep 21.2WP at 250 and 500 ppm a.i.);

oxytetracycline (Mycoshield 17WP at 204 and 407 ppm a.i.); fosetyl Al (Aliette 80WP at 4,800 and 9,600 ppm a.i.); wax emulsion (Folicote at 1:20 and 1:10 dilution); and acrylic copolymers (Stressguard at 1:40 and 1:20 dilution). A spreader-sticker, Triton B-1956, was added at 625 μl/ml to all treatments.

Five carnation plants were sprayed per treatment in the outdoor nursery and five untreated plants served as controls. Seven days after treatment, plants were inoculated with strain C11 at 10^6 cfu/ml and incubated for 2 wk in the nursery. After incubation, lesions from four leaves from each plant were counted and analyzed statistically.

Cultivar resistance. Sixty-six carnation cultivars obtained from Yoder Brothers and California Florida Plant Co. (Salinas, CA) were evaluated for resistance to *P. woodsii*. Four blocks of six rooted cuttings per cultivar were planted at random in a plot with four raised beds 20 m long × 60 cm wide using three rows of carnations per bed. Plants were supported at three heights by horizontally laid nylon trellis netting and were side-dressed once with granular 16-16-16 fertilizer (217 kg/ha), borax (19.6 kg/ha), and Epsom salts (696 kg/ha). Weekly sprays included mancozeb (Dithane F-45) or oxycarboxin (Plantvax) and insecticides and miticides as needed to control diseases and insects not under study. Plots were watered twice weekly by drip irrigation, and 1.4 kg each of $Ca(NO_3)_2$ and KNO_3 was applied weekly through the irrigation system.

At 3 mo of age, flowering plants were inoculated by lightly misting approximately 5.5 L of strain C11 at 10^6 cfu/ml with a 50-psi CO_2 backpack sprayer using a LF flat fan tip nozzle. Three overhead impact sprinklers, 3 m tall, were positioned on one side of the field to provide moisture to the plants and promote disease development. Sprinklers were turned on daily at 7 a.m. and 7 p.m. for 5 min with a battery-operated timer.

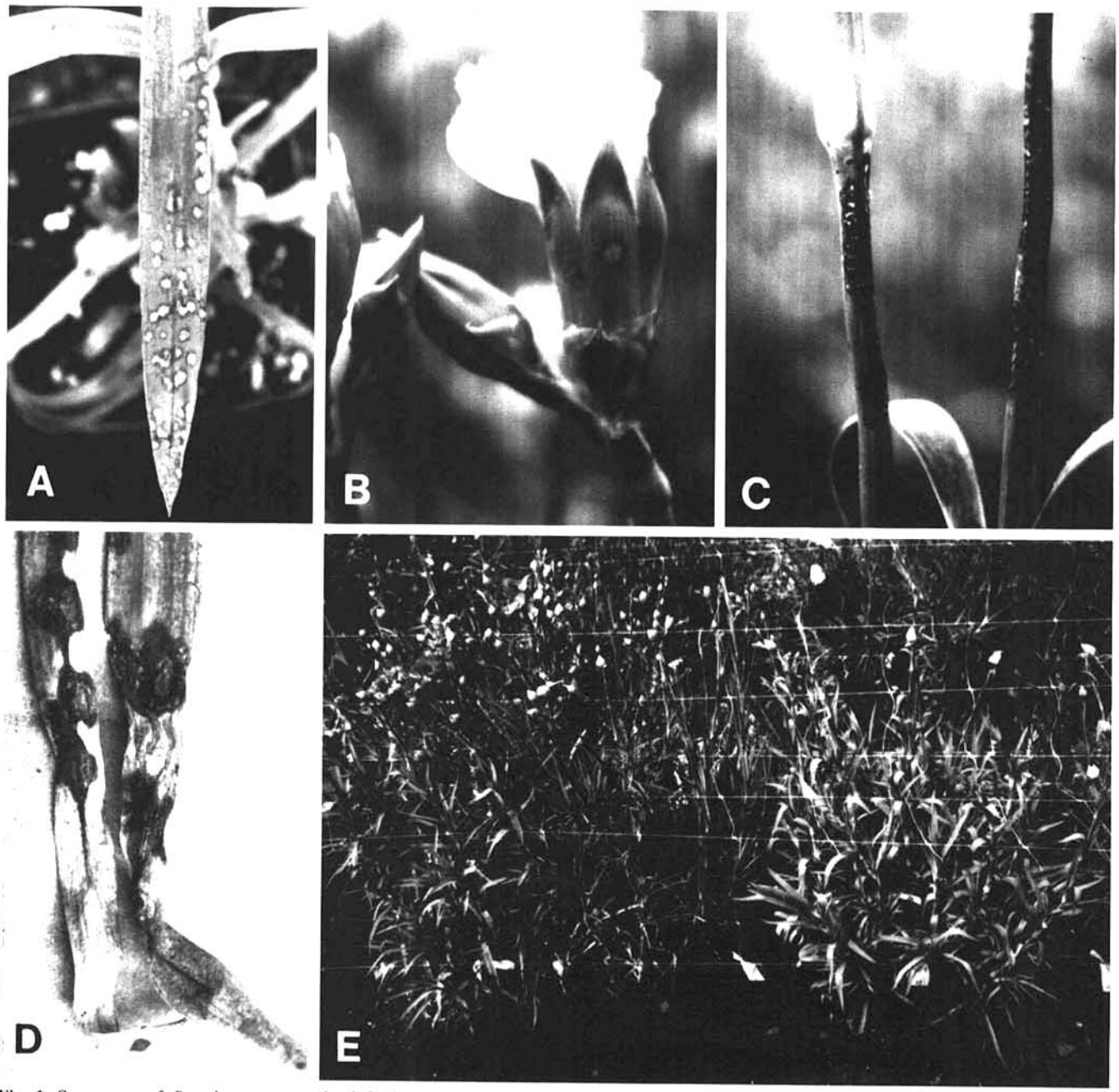

Fig. 1. Symptoms of *Pseudomonas woodsii* infection on spray-inoculated carnation cultivars: **(A)** Necrotic, sunken spots 2 mm in diameter on leaves of susceptible cv. Peterson's Red Sim 14 days after inoculation; **(B)** slightly sunken, purple-tan spots with hydrotic borders on calyx of highly susceptible cv. Ocean Spray; **(C)** lesions on stems of susceptible cv. Sparkle 4 wk after inoculation; **(D)** leaf blight of susceptible cv. Peterson's Red Sim 26 days after inoculation; and **(E)** symptomatic highly susceptible cv. Lavender Lace (left) compared with nearly asymptomatic partially resistant cv. Cal Red (right).

Six weeks after inoculation, three plants from each block were evaluated for disease. Disease severity ratings for leaves were: 0 = no disease, 1 = 1–10% of leaf area with discrete spots, 2 = 11–25% of leaf area infected with coalescing spots, 3 = 26–50% of leaf blighted, and 4 = >50% of leaf area blighted. The ratings for stems were: 0 = no disease, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, and 4 = >50% of stem area infected. The ratings for calyxes were: 0 = no disease, 1 = 1–3, 2 = 4–10, 3 = 11–25, and 4 = >25 spots. Cultivars were grouped into disease categories according to their combined severity ratings: resistant = no disease, partially resistant = severity rating of 0.01–1.50, susceptible = severity rating of 1.51–2.50, and highly susceptible = severity rating of 2.51–4.00

RESULTS AND DISCUSSION

Disease outbreak and isolation. Surveys of Kula carnation farms in October 1989 after several weeks of rainy weather disclosed a serious outbreak of *P. woodsii* at a low-elevation farm. The cultivar Peterson's Red Sim was severely affected, with >50% leaf blight. Other cultivars had various degrees of disease. Two months later, the disease appeared on other farms at higher elevations located several miles downwind of the initial outbreak. During October 1989–June 1990, serious outbreaks occurred on 10 of more than 20 carnation farms surveyed. A winter storm that lasted for several days contributed to the widespread distribution of the pathogen from the original center. Windblown rain and thunderstorms have been implicated in the dissemination of bacterial pathogens (5,6,9,10), and it is well documented that phytopathogenic bacteria exist in the air (10), rain (13), and irrigation water (4).

Isolations from diseased tissues from 10 farms consistently produced 1-mm-diameter, convex, white mucoid colonies after 5 days of incubation at 27 C on YDC medium. As the culture aged, colonies enlarged to several millimeters in diameter and turned light tan. The bacterium rarely survived for more than 1 mo on YDC. Stored in sterile distilled water, however, it survived for over 18 mo.

Dilution plate counts of strains C11 and C18 contained approximately 2.9×10^8 and 4.5×10^8 cfu/ml, respectively, when standardized with a spectrophotometer at 520-nm wavelength and 0.5 absorbance. Strain C11 was more mucoid than C18.

Symptoms (Fig. 1). Symptoms of the disease appeared on both surfaces of the leaves as numerous chlorotic flecks and tan, necrotic, sunken spots (<0.5 mm in diameter) 6 days after inoculation of Peterson's Red Sim plants with the *P. woodsii* strains from eight farms at 10^8 cfu/ml. Lesions expanded and coalesced into large necrotic areas, causing blight of the entire leaf in 10 days (Fig. 1D). All eight strains were highly virulent. Initial small lesions expanded in 14 days into localized, necrotic, 2-mm-diameter spots, many with a dark purple border (Fig. 1A). All strains were recovered on YDC medium.

Stem and calyx symptoms appeared 2 wk after inoculation. Initial lesions on stems were white flecks with dark purple margins. In 4 wk, these lesions enlarged to 5–7 mm in diameter and their centers necrosed and became brown (Fig. 1C). Stem lesions apparently had no measurable effect on plant growth. Early calyx infections were water-soaked flecks that enlarged to 2–3 mm in diameter and became slightly sunken, purple-tan spots with hydrotic borders (Fig. 1B). Flower petals had no symptoms. Both the purple staining of the stem and the calyx spots were unique to this disease and were good diagnostic features resembling natural field symptoms of the disease.

Effect of inoculum concentration and incubation RH on disease incidence. Peterson's Red Sim plants inoculated outdoors with C11 and C18 at all levels of inoculum incubated at ambient RH and temperatures developed more leaf spots than inoculated plants incubated at 100% RH for 24 or 48 hr and then placed outdoors (Table 1).

With the C11 strain, an inoculum concentration of 2.9×10^3 cfu/ml was the minimum level required for production of disease symptoms on Peterson's Red Sim plants in outdoor inoculations. Disease incidence increased directly with inoculum increase.

Outdoor inoculations with 10^6 cfu/ml were ideal for evaluating severity of this disease by leaf spot counts. Leaf spots were discrete and uniform in size and appearance. Even with daily overhead irrigation, reinfections were undetectable when data were collected 14 days after inoculations. Inoculum levels $>10^7$ cfu/ml often resulted in numerous spots that quickly coalesced and blighted the leaves.

Traditional methods of inoculating phytopathogenic bacteria by wounds or other invasive techniques and pre- or postdisposition to high humidity were not necessary for infection by *P. woodsii*. Our outdoor inoculations were done on sunny days between 9:00 and 11:00 a.m. when stomata are normally open. Infection on carnations occurred primarily on leaf blades and calyxes and rarely through hydathodes. High levels of infection on these surfaces with a light bacterial mist suggested that infection occurred through stomata, as indicated by other workers (16).

Chemical control. None of the copper compounds and antitranspirants provided adequate control for this disease. Copper-based compounds significantly increased the incidence of leaf spots. Agri-Strep and Mycoshield at the high rate and Aliette at both rates significantly reduced the severity of the disease, and Aliette was the most promising treatment (Table 2). Our results suggest that cupric hydroxide may interfere with stomatal closing at night, thus increasing incidence of infection. McInnes et al (10) found that in vitro *Xanthomonas campestris* pv. *vesicatoria* was tolerant to field rates of cupric hydroxide but sensitive to cupric hydroxide + mancozeb; however, it did not control disease in the field. Cupric hydroxide or streptomycin in combination with mancozeb have been recommended for the control of *Pseudomonas syringae* pv. *tomato* on tomato (12). We did not test these mixtures, but carnation growers who have used mancozeb with streptomycin have had poor disease control. The highest rate tested for all chemicals was twice the label for ornamental plants. Aliette showed significant reduction of the number of leaf spots at the recommended and high rate. Although control of bacterial pathogens with chemical inhibitors generally is ineffective (9), frequent applications of Aliette at <7-day intervals could provide adequate disease control.

Cultivar resistance. Sixty-six carnation cultivars inoculated outdoors developed leaf spots 11 days after inoculation.

Table 1. Effects of incubation method and inoculum levels on pathogenicity of *Pseudomonas woodsii* on potted carnation cv. Peterson's Red Sim

Strain Concentration	Leaf spots with incubation method[z]		
		Moist chamber	
	Outdoors	24 hr	48 hr
C11			
2.9×10^6	41.2	9.5	23.2
2.9×10^7	Blight	24.8	33.4
2.9×10^8	Blight	Blight	Blight
C18			
4.5×10^6	22.2	11.0	23.8
4.5×10^7	34.1	22.2	54.6
4.5×10^8	Blight	43.8	33.9
Water control	0	0	0

[z] Average number of lesions per infected leaf 14 days after inoculation. The first test included four plants per treatment, and lesions were counted on four leaves per plant. The second test included three plants per treatment, and lesions were counted on eight leaves per plant. Blight = all infected leaves were blighted, with no distinct spots.

Table 2. Chemical control of Pseudomonas leaf blight on potted carnation cv. Peterson's Red Sim inoculated with isolates C11[w]

Treatment[x]	Rate (ppm a.i.)	Lesions per plant[y] Low rate	High rate
Bactericides			
Champ	382	92.4 b[z]	...
	764	...	142.6 a
Champion	922	62.4 b–e	...
	1,845	...	62.0 b–e
Kocide 101	922	63.0 b–e	...
	1,845	...	66.6 bcd
Kocide DF	736	58.8 b–f	...
	1,471	...	93.4 b
Copper-Count-N	538	54.8 b–f	...
	1,075	...	64.4 bcd
Agri-Strep	250	51.4 c–f	...
	500	...	34.0 d–g
Mycoshield	204	49.0 c–f	...
	407	...	28.2 d–g
Fungicide			
Aliette	4,800	23.0 efg	...
	9,600	...	21.3 fg
Antitranspirants			
Folicote	50,000	75.4 bc	...
	100,000	...	53.6 b–f
Stressguard	25,000	56.8 b–f	...
	50,000	...	45.6 c–f
Untreated controls			
Inoculated		65.0 bcd	
Water		0.0 g	

[w] Plants were inoculated outdoors with C11 at 10^6 cfu/ml 7 days after treatment.
[x] Treatments contained 625 ppm of Triton B-1956.
[y] Means derived from lesion counts on five plants per treatment 14 days after inoculation.
[z] Values in the same column followed by different letters are significantly different ($P < 0.05$).

In 16 days, symptoms on susceptible cultivars became quite severe, with spots coalescing and causing early leaf blight. Resistance to *P. woodsii* as measured by estimating intensity of leaf blight and counting stem and calyx spots grouped the 66 cultivars into partially resistant, susceptible, and highly susceptible. The partially resistant cultivars (disease indices from 1.00 to 1.50) were Blaze, Danilo, Univ. Conn. Sim 1, Cal Red (Fig. 1E), Improved New Pink Sim, Crowley Pink, Dusty, and Cal Improved White, with Cal Red and Cal Improved White the most resistant. The susceptible cultivars (disease indices from 1.75 to 2.50) were Chianti, Biancochinera 6161, Atlantis, Barlo II Nora 3406, S. Arthur Sim, Lolita, Mamon, Pink Ice, Ember Sim, Etna Red, Shiro, Leks Paquita P., Leks Capello 5395, Baranna Soana 3534, Improved White Sim, Portrait, Media Peach, Samonie, Corona, Sandra, Epomeo, Scania 3C, Peterson's Red Sim, Starlight 5917, Moonlight, Lydia Lonwadec, Salmon Ministar P., Barbi, Colorado Red Sim, Sparkle, Orchid Beauty, Raggio di Sole, Tanga 5435, Prunelle Lonsimox, Exquisite Select, Orange Citronella, Koreno, Maj. Britt, Light Pink Barbi, and Leks Canasta. The highly susceptible cultivars (disease indices from 2.75 to 4.00) were Gigi, Hellas, Ramon, Tornado, Georgia Anne 4099, Elsy Londonie 5439, Sweetheart, Dark Orange Ministar, Ocean Spray 3299, Pallas, Candy, Vanessa, Elegance, Peachy, Sorentino, Riantino, Pamir, and Lavender Lace (Fig. 1E).

Overhead irrigation was suggested as a possible control method for certain bacterial diseases by decreasing epiphytic bacteria and aerosols through a "washing out" effect (10). Carnation fields are watered by drip irrigation, and during dry weather the disease always subsides. Carnations are fertilized heavily, and this excessive feeding could make them more susceptible to infections (2,6).

Some plants reduce the spread of bacterial diseases by defoliation (5,6), but infected and dead carnation leaves remain attached to the stems, thereby providing a constant source of inoculum. Arbelaéz (1) reported that bacterial blight of carnation was eradicated in Colombia by eliminating carnation cultivation in that region. This is not feasible for our growers because of successive carnation plantings and because carnation is grown as a perennial crop.

We have established the disease reaction of many carnation cultivars available to Hawaiian growers. Carnations that are grown for their individual flowers were less affected by bacterial blight then those harvested for their flowering stems. Other resistant cultivars have been identified for the United States by Burkholder and Guterman (3).

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