Resistance in Broccoli to Bacterial Soft Rot Caused by *Pseudomonas marginalis* and Fluorescent *Pseudomonas* Species

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ABSTRACT

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Significant differences in susceptibility of broccoli (*Brassica oleracea* var. *italica*) genotypes to bacterial soft rot (head rot), caused by *Pseudomonas marginalis* (*P. fluorescens* biovar II) and other fluorescent *Pseudomonas* spp., were observed in four field studies from 1986 to 1989. Cultivars Green Defender and Shogun consistently appeared highly resistant to bacterial soft rot. High disease resistance was observed in 1989 in experimental line NVH 521. Cultivar Green Valiant was rated moderately resistant. Both disease incidence and severity were inversely correlated with the number of days from transplanting until harvest. Because resistance was observed even when heads of susceptible and resistant genotypes matured simultaneously under disease-conducive conditions, factors conferring resistance were only indirectly associated with the length of time required for genotypes to reach harvest maturity.

Bacterial soft rot of broccoli (*Brassica oleracea* L. var. *italica* Plenck), also known as head rot, is a destructive disease of the inflorescence, or broccoli head, and occurs when head maturation coincides with periods of prolonged wet weather. Development is more rapid with warmer temperatures, although the disease is common during both warm and cool periods (25,26). The disease appears as water-soaked florets that become malodorous, necrotic, and soft-rotted if wet conditions persist. Bacterial soft rot is reportedly a problem in broccoli production areas of Australia, Canada, Ireland, and the United Kingdom (14,25) and in the United States in Connecticut (15), Oregon (19), and Tennessee (3). It also occurs in Georgia, Illinois, New York, North Carolina, South Carolina, and Maine (R. Gitaitas, D. MacKenzie, T. Konsler, T. Garrett, W. Erhardt, and D. Thompson, *personal communication*). Disease losses may be severe and can average from 30 to 100% of the crop in some fields (14). Additional losses may occur in cold storage and in transit (1,7,18).

The etiology of bacterial soft rot is apparently complex and is further complicated by the continuing uncertainty about the taxonomic status of some phytopathogenic fluorescent pseudomonads (10,14,20,22,24). In a recent collation of crucifer diseases (12), two bacteria, *Pseudomonas marginalis* pv. *marginalis* (Brown) Stevens and *Erwinia carotovora* (Jones) Bergey et al, were listed as the causal agents for bacterial soft rot. Wimalajeewa et al (26) reported that *E. carotovora* was not the primary pathogen because it could not cause soft rot unless wounds were present. The primary pathogen was a highly pectolytic strain of *P. marginalis* that initiated disease in unwounded heads (25,26). Hildebrand (13) also considered bacterial strains resembling *P. marginalis* to be primary pathogens. Hildebrand (14) subsequently identified four distinct groups and 11 subgroups of fluorescent pseudomonads associated with diseased broccoli heads. Virulent strains were identified as *P. fluorescens* Migula biovar II (21), the *P. marginalis* of Lelliott et al (17), or *P. fluorescens* biovar IV and could cause extensive water-soaking and spreading rot on unwounded broccoli heads. In addition to the production of pectolytic enzymes, the highly virulent fluorescent pseudomonads produced a surfactantlike substance that apparently played a major role in successful pathogenesis. Avirulent strains could produce a surfactant or pectolytic enzymes but not both. Such strains resembled *P. fluorescens* biovar V or *P. viridiflava* (Burkholder) Dowson, respectively, and were capable of causing bacterial soft rot on unwounded broccoli when coinoculated or when the surfactant-positive strains were coinoculated with strains of *E. carotovora*. The etiology of bacterial soft rot may involve

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several different strains of fluorescent pseudomonads acting alone or in concert with each other or with *E. carotovora*.

In 1984, researchers at the University of Tennessee initiated a 3-yr study on commercial broccoli production in Tennessee. Bacterial soft rot frequently plagued commercial fields and research plots whenever extended periods of rainy, foggy weather occurred when broccoli heads were developing. Fungicides and copper bactericides failed to offer significant control (3).

In the spring of 1986, we observed some apparent differences in disease susceptibility among eight cultivars grown in a field test. Moderate bacterial soft rot first appeared in the research plots in late May after daily rain and/or fog for 5 days. The disease gradually became more severe as the inclement weather continued for 16 more days, with only brief interruptions, until 12 June. More than 90% of the heads from five cultivars harvested 6–11 June had bacterial soft rot. However, the disease appeared on 49 and 59% of the heads of Green Defender and Corvet, respectively. Moreover, the soft rot lesions on these cultivars were significantly smaller than those on the other entries. Unfortunately, the heads harvested during this period were from different planting dates and no data were collected on head maturation before harvest. Therefore, it was not possible to determine whether the observed disease differences represented actual variation in cultivar susceptibility to bacterial soft rot or merely reflected maturity differences that led to head maturation during periods when weather varied from more to slightly less conducive for disease development. This report describes the studies undertaken to resolve this issue and expands preliminary reports (4–6).

MATERIALS AND METHODS

Field studies. Four field studies to evaluate differences in the susceptibility of broccoli cultivars and lines to bacterial soft rot were conducted from 1986 through 1989 at the University of Tennessee's West Tennessee Experiment Station at Jackson. Six cultivars were evaluated in the fall of 1986, 10 cultivars in spring of 1987, 25 genotypes in 1988, and 30 genotypes in 1989. Field-grown transplants were used in 1986 and greenhouse-grown transplants were seeded in 128-cell Todd planter flats (Speedling, Sun City, FL) in the other seasons. Transplants were set by hand in single-row plots on raised beds 1 m apart with plants spaced 30 cm apart within rows. Entries were planted in four replicated blocks with 25–50 plants per entry per block. Three to four rows of a cultivar susceptible to soft rot, generally Packman, were planted along the borders of each field test to minimize edge effects. Standard management practices for soil fertility and insect and weed control were followed. To avoid any chemical injury to the developing florets, no chemical pesticides were applied during head maturation.

To achieve simultaneous maturity of all cultivars and lines, the transplanting dates of entries in each test were staggered. Transplanting dates were in the order of the estimated number of days necessary for genotypes to reach harvest maturity, with those requiring the most time being seeded and subsequently transplanted first. To increase the likelihood of obtaining some period when most genotypes would be maturing simultaneously, transplants of each entry were set on two to four different dates, 2–14 days apart, within each block in rows of 12–35 plants. In the 1986 study, for example, transplants of Shogun and Green Defender were set four times within each block from 31 July to 8 August, with 12 plants per cultivar per setting. Similarly, Corvet, Prominence, and Premium Crop transplants were set four times 6–15 August, and Packman transplants were set four times 15–22 August. In spring studies, transplanting began 1–9 March with Green Defender and Shogun and continued through March and into April with entries needing less time to reach harvest maturity.

To create disease-conducive conditions, plots were briefly irrigated with overhead sprinklers three to five times daily, 5–10 min each time, from the appearance of the first "button stage" heads (inflorescence approximately 2 cm in diameter) through the final harvest. The initial irrigation was generally applied in midmorning to coincide with dew disappearance. Irrigations were repeated every 3–5 hr, depending on the relative humidity, to maintain some water on the heads. The final irrigation usually coincided with sunset, and water from this irrigation maintained plant wetness until the formation of natural dew during the night.

Head maturation and harvest data. In the 1986 and 1987 studies, maturation data were collected to verify simultaneous head maturity. Head diameters were recorded every 4–10 days for each plant from button stage until harvest. In 1988, mean head diameters of entry rows were calculated from 12 observed head diameters per row. For all studies, heads were harvested when they attained a diameter suitable for commercial processing. Harvested heads were weighed, diameters were recorded, and each head was carefully examined for the presence of bacterial soft rot. Disease severity ratings were recorded for heads with soft rot and the percentages of heads showing any evidence of soft rot were calculated. Replication means were subjected to analysis of variance procedures (ANOVA) for a randomized complete block design with four replications. Arcsine-transformed percentages were used for analysis of variance and mean separation tests of disease incidence data.

Disease severity scale. Disease severity on individual heads was estimated on a 0–5 scale where 0 = no disease, 1 = 1, 2 = 10, 3 = 30, 4 = 60, and 5 = 100% of the head soft rotted. Schematic diagrams depicting various lesion combinations equal to 1, 10, 30, and 60% of the surface area of heads were developed and studied before recording severity ratings (Fig. 1). Intermediate infections equal to approximately 5, 20, 45, and 80% of the surface area were given intermediate ratings of 1.5, 2.5, 3.5, and 4.5, respectively. Heads exhibiting small areas of discolored and/or water-soaked florets with the characteristic scent of bacterial soft rot but without any apparent softening of floret tissues were presumed to be diseased and were given a rating of 0.5.

Inoculation of plots. Natural inoculum was sufficient to produce disease if broccoli heads were kept wet much of the day during development. However, natural inoculum was supplemented by inoculating the stems of previously harvested plants (1986 study) or plants in border rows (1987–1989 studies). Bacterial strains collected in the spring of 1986 from diseased broccoli heads were used for these inoculations and consisted of two types. The first type of strains were aerobic, gram-negative rods that were fluorescent on King's medium B agar (KBA), produced oxidase, produced arginine dihydrolase, produced levan on sucrose-amended medium, hydrolyzed urea, and caused soft rot of potato tubers. These strains were tentatively identified as *P. marginalis* (9,10,23). The second type of strains were facultative anaerobes, gram-negative rods that were not fluorescent on KBA and were oxidase negative, arginine dihydrolase negative, and capable of causing soft rot of potato tubers but not capable of producing levan or hydrolyzing urea. These strains were tentatively identified as *E. carotovora* (10,16).

For plant inoculations, strains were grown in either Difco nutrient broth to which 2.5 g of glucose per liter was added or in neutralized sucrose-broccoli broth. The latter was prepared from 10 g of broccoli florets that had been comminuted in 250 ml of water for 1 min at high speed in a Waring blender. The macerated tissue was added to a 1-L flask, water was added to 500 ml, and 0.5 g of sucrose plus 0.5 g of $CaCO_3$ was added. The flask was swirled briefly to suspend the $CaCO_3$ and bits of broccoli florets as 50-ml aliquots were dispensed into 250-ml flasks for sterilization. Inoculated flasks of sterilized broth were incubated for 20–48 hr at laboratory temperature. Broth cultures were then

diluted with distilled water to obtain a final inoculum concentration of approximately 1×10^7 colony-forming units (cfu) per milliliter. A "well method" was used to inoculate plants. Broccoli inflorescences were excised with a horizontal cut and small depressions, or wells, 1–2 cm deep were made in the ends of the stems. Two milliliters of the inoculum was then pipetted into the freshly made wells. Wells continued to collect water from the frequent irrigations, and the rotting stems provided abundant inoculum for spread by insects, rain splash, and aerosols generated by rainfall and irrigation (11).

RESULTS

Initial evaluation of six cultivars, fall 1986. Despite staggered transplanting dates, cultivar maturity was not simultaneous. Hot, dry weather from 25 July to 8 August led to moderate drought injury in the earliest transplanted cultivars, which delayed their maturation. In addition, the days to maturity for Corvet had been underestimated. Thus, Packman, Premium Crop, and Prominence reached harvest maturity first. Heads of the latter three cultivars were harvested from 23 September until 7 November, although the majority of heads (74%) were harvested 29 September to 14 October. The remaining three cultivars were harvested from 14 October until 7 November, with most heads (79%) being harvested 23 October to 3 November.

More than 1,100 broccoli heads (164–214 heads per cultivar) were harvested in 22 cuttings and examined for bacterial soft rot. Bacterial soft rot lesions were first observed on both maturing heads (4–10 cm in diameter) and harvested heads (14–16 cm in diameter) on 26 September. Of 34 heads harvested at this time, nearly 15% had bacterial soft rot. The incidence of disease on harvested heads of susceptible cultivars increased with successive harvests. The mean disease incidence for Packman, Premium Crop, and Prominence increased from 69% on 26 September to 100% by 27–29 October. Disease severity also increased, from 0.9 on 26 September to 2.5 by the end of October. For the entire harvest period, disease incidence and soft rot severity in Green Defender and Shogun were significantly ($P = 0.05$) lower than most other cultivars (Table 1).

Although represented by only 10–18 plants per cultivar, heads harvested 16–21 October appeared to come from plants that were maturing simultaneously. Only the mean head diameter of Packman was significantly different from the head diameters of other cultivars at the button stage on 29 September (Table 1). By 15 October, there was no significant difference in the head diameters of these plants, nor did they differ significantly at harvest. Within this group, the mean soft rot incidences and severity ratings in Corvet, Green Defender, and Shogun were significantly lower than those in Packman and Premium Crop. Disease incidence and severity were inversely correlated with number of days from transplanting until harvest ($R = -0.91$, $P = 0.01$ and $R = -0.97$, $P = 0.01$, respectively), whereas disease was not correlated with head diameters on 29 September, 6 and 15 October, or at harvest, evidence that differences in soft rot were not attributable solely to differences in plant maturation.

Evaluation of 10 cultivars, spring 1987. More than 1,760 broccoli heads (155–194 heads per cultivar) were harvested from 18 May through 12 June in 14 cuttings. Bacterial soft rot was first observed on harvested heads 13–15 cm in diameter on 20 May and on maturing heads 8–11

Table 1. Head maturation, soft rot incidence, and soft rot severity rating of six broccoli cultivars evaluated in the fall of 1986

	Entire harvest[w]		Head diameter (cm)		Plants harvested 16–21 October		
Cultivar	Soft rot incidence[x] (%)	Soft rot severity rating[y]	29 Sept.	15 Oct.	Harvest diameter (cm)	Soft rot incidence[x] (%)	Soft rot severity rating[y]
Corvet	77 a[z]	0.6 c	2.7 a	13 a	16 a	25 c	0.2 c
Green Defender	48 d	0.3 d	2.6 a	14 a	16 a	44 bc	0.3 c
Packman	79 a	1.7 a	1.5 b	13 a	17 a	94 a	2.3 a
Premium Crop	68 ab	1.1 b	2.5 a	14 a	16 a	88 a	1.6 b
Prominence	62 bc	0.9 b	3.1 a	14 a	16 a	88 ab	0.9 b
Shogun	49 cd	0.3 d	2.5 a	14 a	16 a	20 c	0.1 c

[w] 23 September–7 November.
[x] Arcsine-transformed percentages were used for analysis of variance and mean separation tests.
[y] Disease severity ratings were on a 0–5 scale where 0 = no soft rot, 0.5 = water-soaking and soft rot odor evident, 1 = 1, 2 = 10, 3 = 30, 4 = 60, and 5 = 100% of the head rotted.
[z] Column means followed by a common letter do not differ significantly according to Fisher's Least Significant Difference ($P = 0.05$). Values are the means of four replications.

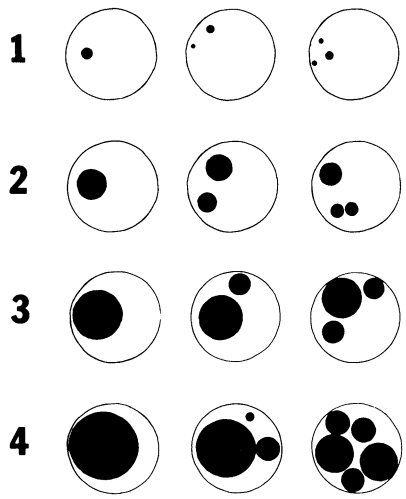

Fig. 1. Schematic representation of the rating scale used to evaluate bacterial soft rot severity. Darkened circles represent diseased tissue, and white portions represent healthy tissue. The rating scale: 0 = no soft rot (not shown), 1 = 1, 2 = 10, 3 = 30, 4 = 60, and 5 = 100% of the head soft rotted (not shown).

Table 2. Head maturation, soft rot incidence, and soft rot severity rating of 10 broccoli cultivars evaluated in the spring of 1987

	Entire harvest[v]		Plants harvested 25 May–2 June				
Cultivar	Soft rot incidence[w] (%)	Soft rot severity rating[x]	Head diameter 21 May (cm)	Harvest diameter (cm)	Soft rot incidence[w] (%)	Soft rot severity rating[x]	Days to maturity[y]
Emperor	51 bc[z]	0.4 cd	5.3 a	14 a	65 bc	0.5 cd	63 d
Green Comet	74 a	0.8 a	5.5 a	13 a	93 a	1.0 a	63 d
Green Defender	16 f	0.1 e	5.1 a	14 a	21 e	0.1 e	79 a
Green Duke	59 abc	0.5 b	6.1 a	13 a	83 ab	0.8 ab	63 d
Green Valiant	27 e	0.2 de	3.8 a	11 a	43 cde	0.4 de	73 b
Packman	71 a	0.7 a	4.5 a	14 a	71 abc	0.8 abc	59 e
Pirate	34 de	0.2 e	6.0 a	13 a	28 de	0.2 e	74 b
Premium Crop	64 ab	0.5 bc	4.6 a	13 a	84 ab	0.7 bcd	67 c
Prominence	47 cd	0.4 bc	5.7 a	13 a	60 bcd	0.5 cd	67 c
Shogun	13 f	0.1 e	6.5 a	13 a	19 e	0.1 e	80 a

[v] 18 May–12 June.
[w] Arcsine-transformed percentages were used for analysis of variance and mean separation tests.
[x] Disease severity ratings were on a 0–5 scale where 0 = no soft rot, 0.5 = water-soaking and soft rot odor evident, 1 = 1, 2 = 10, 3 = 30, 4 = 60, and 5 = 100% of the head rotted.
[y] Number of days from transplanting to head diameter of 14 cm.
[z] Column means followed by a common letter do not differ significantly according to Fisher's Least Significant Difference ($P = 0.05$). Values are the means of four replications.

cm in diameter on 21 May. Moderate rainfall (6 cm) from 22 May to 1 June supplemented the frequent overhead irrigations and created a period very favorable for disease development. Disease incidence in harvested heads increased from 22% on 20 May to 72% on 28 May, then fell to 27% on 4 June. Disease severity ratings were similarly affected. From 35 to 137 heads per cultivar were harvested when soft rot incidence and severity were highest (i.e., from 25 May to 2 June).

Head diameters were similar for all cultivars on 21 May, evidence for simultaneous maturation (Table 2). Most cultivars were highly susceptible to bacterial soft rot, however, Shogun, Green Defender, Pirate, and Green Valiant possessed resistance (Table 2). Once again, disease incidence and severity were inversely correlated with the number of days from transplanting to harvest ($R = -0.89$, $P < 0.001$ and $R = -0.87$, $P < 0.001$, respectively), but not with head diameters of plants on 15 May, 21 May, or at harvest. Thus, as with the fall experiment, the observed differences in soft rot on cultivars were not attributable solely to differences in plant maturation.

Evaluation of 25 cultivars and lines, spring 1988. Warmer temperatures and improved staggering of transplanting dates led to more concentrated plant maturation than in previous experiments. More than 2,550 heads were harvested from 18 to 31 May in six cuttings. As before, disease incidence and severity were low at the time of the first harvest, so the initial harvest was not used in statistics. Heads <8 or >17 cm in diameter also were not evaluated to minimize differences in head maturation. Thus, 2,148 heads harvested 24–31 May (60–110 per entry) were used in statistical analyses.

Disease incidence and severity were significantly lower on Green Defender and Shogun than on all other entries except NVH 517 and Pirate (Table 3). For the 10 cultivars common to both tests, disease incidence in 1987 was highly correlated with that in 1988 ($r^2 = 0.84$, $P < 0.001$) (Fig. 2A). There were again significant inverse correlations between disease incidence or severity and the number of days from transplanting to harvest. Disease incidence and severity were not significantly correlated with the diameters of maturing heads on 13 May or at harvest and, therefore, did not appear to be related to differences in plant maturation.

Evaluation of 30 cultivars and lines, spring 1989. Frequent rainfalls during May supplemented the overhead irrigations to create conditions very favorable for soft rot. More than 2,200 heads were collected from three harvests from 22 to 31 May. Disease incidence and severity were moderate at the time of the first harvest on 22 May, but several entries had not produced mature heads. Disease was very severe at the second and third harvests, and all entries had produced mature heads. During the latter two harvests, 1,392 heads 9–19 cm in diameter (17–69 per entry) were evaluated. Whereas most entries were highly susceptible to soft rot, Green Defender, Shogun, NVH 521, and Green Valiant were resistant (Table 3). Pirate, which appeared moderately resistant to soft rot

Table 3. Soft rot incidences, soft rot severity ratings, and days to maturity of 25 entries evaluated in the spring of 1988 and 30 entries evaluated in the spring of 1989

	1988			1989		
Entry	Soft rot incidence[w] (%)	Soft rot severity rating[x]	Days to maturity[y]	Soft rot incidence[w] (%)	Soft rot severity rating[x]	Days to maturity[y]
Baccus	ND[z]	ND	ND	96 a–d	1.5 b–h	63 no
Brigadier	ND	ND	ND	93 a–e	0.9 ijk	69 g–j
Cruiser	83 ab	1.6 ab	67 ef	100 a	1.8 bcd	72 efg
Dandy Early	79 a–d	1.1 a–e	66 fg	100 a	1.3 d–i	67 lm
Early Dawn	ND	ND	ND	90 a–e	1.3 d–j	57 p
Embassy	56 c–g	0.6 def	67 ef	95 a–e	1.7 b–e	67 jkl
Emperor	64 a–g	0.9 a–f	64 gh	88 b–e	0.8 jk	70 f–i
Galaxy	64 a–e	1.0 a–f	58 ij	100 a	1.9 abc	62 no
Gem	69 a–e	0.8 b–f	68 def	97 ab	1.2 g–j	71 fgh
Green Comet	82 abc	1.4 a–d	67 ef	98 ab	1.9 ab	69 hij
Green Defender	12 h	0.1 f	83 a	15 g	0.1 l	85 a
Green Duke	83 a	1.4 a–d	60 i	100 a	1.4 c–i	68 i–l
Green Lady	ND	ND	ND	96 a–d	1.9 abc	66 kl
Green Valiant	41 efg	0.7 c–f	77 b	67 f	0.5 kl	76 bc
Kwik Green	78 a–d	1.1 a–e	63 h	98 ab	1.2 e–j	67 jkl
NVH 517	37 gh	0.2 ef	64 h	ND	ND	ND
NVH 521	ND	ND	ND	27 g	0.1 l	69 hij
Packman	57 c–g	0.9 a–f	60 i	85 cde	1.5 b–h	61 o
PSR 16284	ND	ND	ND	100 a	2.0 ab	71 fgh
PSR 51286	ND	ND	ND	84 e	0.9 ijk	77 b
Pirate	39 fgh	0.7 c–f	76 b	88 b–e	1.5 b–h	74 cde
Premium Crop	66 a–g	0.8 b–f	68 def	89 b–e	1.4 c–i	75 bcd
Prominence	78 a–d	1.8 a	68 de	88 de	1.1 hij	75 bcd
Sakata 12 (Ninja)	53 d–g	1.1 a–e	69 cd	96 a–e	1.4 c–i	72 def
Sakata 19	72 a–e	1.2 a–d	64 gh	ND	ND	ND
Sakata 83-1	63 a–g	1.0 a–e	69 de	ND	ND	ND
Sakata 86-9	ND	ND	ND	91 a–e	1.2 d–j	74 cde
Sakata 90	70 a–d	1.5 abc	71 c	ND	ND	ND
Sakata 91	60 b–g	0.8 b–f	67 ef	87 b–e	0.8 jk	68 i–l
Shogun	13 h	0.1 f	83 a	15 g	0.1 l	85 a
Southern Comet	86 ab	1.0 a–e	58 j	96 abc	1.7 b–f	64 mn
Surfer	ND	ND	ND	97 ab	2.4 a	69 h–k
Symphony	76 a–d	1.4 a–d	63 h	92 b–e	1.2 f–j	67 jkl
XPH 5168	70 a–f	0.8 b–f	59 ij	98 ab	1.6 b–g	63 no

[w] Arcsine-tranformed percentages were used for analysis of variance and mean separation tests.
[x] Disease severity ratings were on a 0–5 scale where 0 = no soft rot, 0.5 = water-soaking and soft rot odor evident, 1 = 1, 2 = 10, 3 = 30, 4 = 60, and 5 = 100% of the head rotted.
[y] Number of days from transplanting to head diameter of 14 cm.
[z] Column means followed by a common letter do not differ significantly according to Fisher's Least Significant Difference ($P = 0.05$). ND = no data; entry not tested this year. Values are the means of four replications.

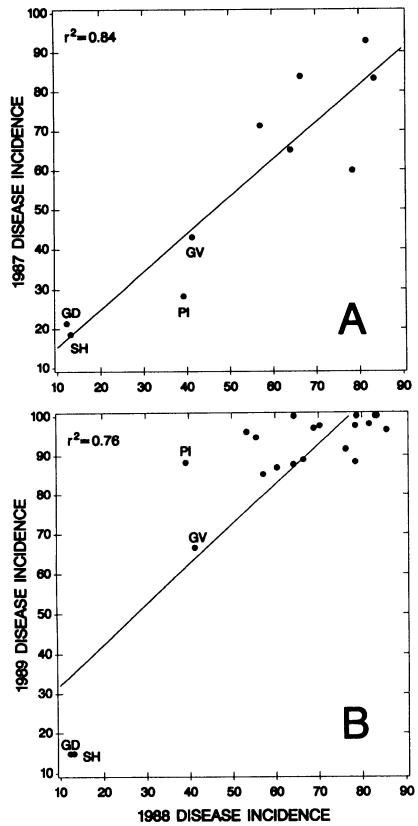

Fig. 2. Comparison of the disease incidences of entries in 1988 with those of the same entries in **(A)** 1987 and **(B)** 1989. GD = Green Defender, GV = Green Valiant, PI = Pirate, and SH = Shogun. Unlabeled dots are other entries common to both **(A)** 1987 and 1988 or **(B)** 1988 and 1989.

in the 1987 and 1988 tests, was not different from the highly susceptible entries when disease was severe. Although mean head diameters of entries at harvest (11–16 cm) were significantly different, harvest diameters were not significantly correlated with either soft rot incidence or severity. Disease incidence and severity were inversely correlated with the number of days from transplanting to harvest ($R = -0.63$, $P < 0.001$ and $R = -0.56$, $P = 0.001$, respectively). The disease incidence on 21 entries common to both the 1988 and 1989 studies was significantly correlated ($r^2 = 0.76$, $P < 0.001$) (Fig. 2B).

DISCUSSION

In the tests reported here, certain genotypes were consistently resistant to the bacterial soft rot caused by *P. marginalis* and other fluorescent *Pseudomonas* spp. Whereas most cultivars and lines appeared moderately to highly susceptible to the disease, Shogun, Green Defender, and experimental line NVH 521 were much less susceptible and apparently possess high levels of resistance to bacterial soft rot. Green Valiant consistently appeared less susceptible to bacterial soft rot than most other genotypes, but generally not to the same degree as Green Defender, Shogun, and NVH 521.

The general experimental protocol used in these studies (i.e., staggering the transplanting dates of entries and liberal use of overhead irrigation during head maturation) permitted direct comparison under disease-conducive conditions of cultivars and lines with widely different days to maturity. This protocol led to very reproducible results, as indicated by the significant correlations between the disease incidence on entries in 1988 and the disease incidences in 1987 and 1989 (Fig. 2). Although the maturation of all entries was not perfectly synchronous, analysis of subpopulations that were maturing simultaneously in the 1986 and 1987 studies provided evidence that differences in susceptibility are not attributable to differences in maturation per se.

The experimental protocol appears to be an improvement over the traditional cultivar trial in which all entries are set at the same time but mature at different times under different environmental conditions. While the latter protocol may be ideal for evaluating many horticultural properties, it appears inappropriate for evaluation of genotype resistance to bacterial soft rot, especially because the time to reach harvest maturity after transplanting can vary from less than 7 to more than 11 wk, depending on the cultivar. Under these circumstances, it becomes difficult to distinguish differences in disease susceptibility from simple disease escape because some entries mature under slightly drier conditions less favorable for disease. To ensure proper evaluation of differences in susceptibility to bacterial soft rot, staggered planting dates for entries as described here, or alternatively, multiple planting dates, should be used. If this is unfeasible, the incorporation into the test of multiple plantings of appropriate reference cultivars with high and low levels of disease resistance (e.g., Shogun and Green Comet, respectively) should be considered.

Although disease incidence and severity were strongly correlated, they differ in their importance. Because any evidence of bacterial soft rot can render a head unmarketable, the differences in disease incidence have obvious economic significance. Differences in disease severity have considerable epidemiological significance. Heads with large lesions provided more inoculum for spread by rain splash, irrigation, etc. than heads with small lesions. Disease losses in a moderately resistant cultivar may be greater when planted near a highly susceptible cultivar than if planted near a resistant cultivar with smaller lesions. Because the disease severity scale was based on equal increments of radial growth from a central lesion equal to approximately 1% of the area of the head, it often reflected disease spread in tissue over time. In the 1986 study, disease severity ratings taken on maturing, unharvested heads increased with successive observations over 7–10 days (C. H. Canaday, *unpublished*). In this case, disease severity ratings probably reflected differences in the time of infection.

The consistency with which bacterial soft rot can be induced when heads of maturing broccoli are kept wet with natural rainfall or overhead irrigation provided evidence that the causal bacteria were ubiquitous in the environment. Although bacterial inoculum may originate from a variety of sources, the prevalence of both *P. marginalis* and *E. carotovora* in some field soils (8,9) and the appearance of this disease in widely scattered areas of Tennessee in the fields not previously cropped to broccoli suggest a soilborne origin. Cultural practices that reduce soil splash on maturing heads, e.g., interplanting with grasses, have shown potential in reducing disease (P. D. Hildebrand, *personal communication*). Whereas the initial cultural tests performed on the 1986 strains were not sufficient to positively confirm the strains as *P. marginalis* and *E. carotovora*, these tentative identifications seem probable and support the observations of other investigators. Recently collected bacterial strains have further confirmed the report by Hildebrand (14) that fluorescent pseudomonads other than *P. marginalis* may be involved in bacterial soft rot. Of 15 pectolytic bacteria isolated on KBA in the fall of 1989 from freshly collected heads, most at early stages of infection, three were identified as *E. carotovora* subsp. *carotovora* (Jones) Bergey et al. The remaining 12 strains all produced fluorescence on KBA, tested arginine dihydrolase positive and oxidase negative, caused soft rot of potato slices but did not produce pits on crystal violet pectate medium (8), and did not grow at 41 C (M. L. Powelson, *personal communication*). Although further work with these strains is needed to verify their role in pathogenesis and to ascertain their identification, their presence supports the observations of Hildebrand that a complex of fluorescent pseudomonads may be involved in the etiology of bacterial soft rot of broccoli.

Disease incidence and severity on genotypes were consistently correlated with the amount of time needed for genotypes to reach harvest maturity. Several horticultural characteristics (head tightness, doming, floret size, and head weight/diameter ratio) were significantly correlated with the observed differences in genotype susceptibility to bacterial soft rot (2) (C. H. Canaday, *unpublished*). Some of these traits, such as doming and floret size, also appear to be associated with the number of days required for broccoli genotypes to reach harvest maturity (G. Ruttencutter, crucifer breeder, Asgrow Seed Company, *personal communication*). The association, perhaps genetic in nature, of horticultural traits influencing genotype resistance to bacterial soft rot with the length of the time necessary for entries to reach harvest maturity may explain the significant correlations observed in these studies.

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