as determined by analysis of root extracts may have been too low to support colonization of the fungus. It was proposed recently that the supply of nutrients within the root affects mycorrhizal development rather than the amount of nutrients leaked into the rhizosphere (11). It is difficult, however, to explain the lack of mycorrhizal infection in these plants by the influence of sugar content in exudates or extracts alone. In contrast, mycorrhizal infection was relatively high in girdled plants grown in soil not amended with P and was associated with a concomitant increase in plant dry weight compared to the girdled plants grown in soil amended with P. The amount of sugars in exudates, but not in extracts, from roots of these plants was considerably greater than that from plants grown in soil amended with P. In this instance, the high levels of carbohydrates in the exudates may have been sufficiently high to support growth of the fungus, or other factors may have stimulated root colonization by *G. fasciculatum*, such as an effect by other soil-borne microorganisms that use root exudates. However, mycorrhizal development appears to be dependent both on levels of photosynthates supplied to the roots and factors that lead to increased permeability of root cell membranes.

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Sources of Resistance to Bacterial Spot in Tomato

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Abstract. **Two hundred eight-four *Lycopersicon spp.* genotypes reported to have some resistance to bacterial pathogens of tomato (*L. esculentum* Mill.) were inoculated in the field with *Xanthomonas campestris* pv. *vesicatoria* (XCV), the incitant of bacterial spot, and rated for disease severity in summer 1982 and/or summer 1983. One line tested in 1983, Hawaii 7998, had no definite XCV lesions and later was determined to be resistant to XCV in the laboratory. Genotypes with the highest levels of resistance during 2 years of testing were: Ohio 4013-3, Ohio 4014-4, Heinz 1568-F_3, [(Subarctic Delite x MH1) x H603] F_5, L556, 'Campbell-28', PI 127813, Heinz 603-F_{11}, PI 224573, 'Monense', 'Heinz 2990', and PI 324708. Genotypes with highest levels of resistance in one year of testing were PI 379032 and 'Burgess Crack Proof'. In 1982, PI 270248-'Sugar' had a high level of resistance to XCV on fruit, but foliage was susceptible.**

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (XCV), is the most serious disease of tomato (*Lycopersicon esculentum*) in Florida and causes extensive damage in many other tomato-growing regions. The disease affects leaves, stems, and fruit and is most prevalent when temperatures are high and rainfall is frequent (13, 17). In Florida, the most serious losses are due to leaf infections that cause defoliation, reducing both yield and grade of fruit due to cracking, sunscald, or black shoulder. Cultural control measures are often ineffective in controlling bacterial spot (2). Breeding for resistance, using the few available genetic sources, has been difficult (5, 17), as is evident by the lack of resistant or partially resistant cultivars in Florida (16). Several researchers have reported genotypes carrying some level of resistance to bacterial spot (3–5, 9, 17; W.L. Summers, personal communication). Often these studies tested either a limited number of genotypes or used screening procedures in the laboratory or greenhouse that might not reflect resistance under heavy disease pressure in the field.

The objective of the present work was to determine the best bacterial spot-resistant genotypes to use for breeding purposes. To do this, the bacterial spot-resistant (or tolerant) genotypes reported previously were compared to several partially resistant Florida breeding lines (unpublished data) and to genotypes with reported resistance to other bacterial pathogens, including bacterial speck (*Pseudomonas syringae* pv. *tomato*) (10–12, 18), bacterial canker (*Corynebacterium michiganense*) (1, 8), and bacterial wilt (*Pseudomonas solanacearum*) (8, 16). This paper reports the best sources of bacterial spot resistance from these field evaluations. A compilation of data for all genotypes tested is available from the authors.

On 11 June 1982, 227 tomato genotypes were seeded in a greenhouse. On 15 July, the seedlings were transplanted into raised 86-cm-wide beds of Eau Gallie fine sand that were covered with white plastic mulch. The beds were previously fumigated with a mixture of methyl bromide and chloropicrin. Plants were set 46 cm apart within plots, with 91 cm between plots, and rows were 137 cm apart. Plants were staked and normal insecticide practices were used. Chlorothalonil (2,4,5,6-tetrachloro-1, 3-benzenedicarbonitrile) was used to control fungal diseases as it does not inhibit XCV. Genotypes were arranged in a completely randomized design with 2 replications of 5 plant plots. Two weeks after transplanting, a bacterial suspension was misted onto the plants early in the morning when heavy dew was present. Inoculum was prepared from a culture of XCV that had been grown on nutrient yeast dextrose agar (7) for 48 hr at 28°C.

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Table 1. Bacterial spot leaf ratings in 1983 on the most resistant *Lycopersicon* genotypes selected from 227 tested in 1982 at Bradenton, Fla.

Genotype[z]	Disease rating[y]	Rank in 1982[x]
Ohio 4013-3*	2.00 o	1
Ohio 4014-4*	2.50 no	2
Heinz 1568-F_3*	3.13 mn	3
[(SubArctic Delite X MH1) × H603] F_5*	3.50 lm	5
L556*	3.63 klm	20
Campbell-28*	3.88 jkl	12
PI 127813	3.88 jkl	14
Heinz 603-F_{11}*	4.25 ijk	12
PI 224573	4.33 hij	20
Monense	4.38 hij	14
Heinz 2990	4.38 hij	7
PI 324708*	4.50 ghij	10
Heinz 1568-F_6*	4.63 ghi	10
PI 117899	4.75 fghi	17
[(6204) × Cl 11d] F_7*	4.88 efghi	6
Texas 204-B-5*	5.00 defgh	9
PI 270217	5.00 defgh	18
Heinz 820*	5.13 defg	4
PI 159199	5.13 defg	18
PI 203232*	5.37 cdef	20
Early Red Rock	5.38 cdef	- - -[w]
(420) F_8	5.50 bcde	20
PI 309666	5.63 bcd	14
L2024	6.00 bc	20
PI 270248–Sugar	6.00 bc	218
Walter	6.00 bc	- - -[w]
Fla 1339*	6.13 b	20
Lyconorma	7.00 a	- - -[w]

[z]Genotypes with * were selected from previous trials in the Univ. of Florida Breeding Program. All genotypes are *L. esculentum* except for PI 324708, which is a *L. pimpinellifolium*.

[y]Means in columns separated by Duncan's multiple range test, 5% level. Data are Horsfall–Barratt numbers, where low numbers indicate less disease.

[x]Low rank number indicates lower Horsfall–Barratt ratings. There was no statistical difference (LSD at the 5% level) between lines ranked 4 through 20.

[w]Not tested in 1982.

The bacteria were washed from the plates, suspended in 0.01M $MgSO_4$, and adjusted to 10^8 colony forming units/ml.

The plants were rated 3 times for bacterial spot severity on 4 Aug., 20 Aug., and 14 Sept. 1982. Infection on the top and bottom halves of the plants were rated using the Horsfall–Barratt (HB) scale (6). Data also were recorded on incidence of fruit spot; however, many genotypes did not set much fruit and could not be evaluated accurately for fruit susceptibility.

In 1983, 59 accessions that were not tested in 1982 and 'Walter' (susceptible control) were seeded on 29 June and transplanted to the field on 9 Aug. The field design, all growing and inoculating procedures, and the rating system were the same as in 1982. Disease ratings were made on 22 Sept.

In a 2nd experiment in 1983, the 25 genotypes with lowest disease incidence from 1982 (except PI 414172, which did not germinate), plus 'Early Red Rock', PI 270248-'Sugar' (low fruit spot in 1982), 'Walter' (susceptible control), and 'Lyconorma' (highly susceptible control) were seeded on 3 July, transplanted to the field on 12 Aug., and rated for disease on 23 Sept. A randomized block design with 4 blocks of 5 plant plots was used. The test was conducted as previously described. Only data from the bottom half of the plant is presented since infection was minimal in the top half.

The bacterial spot ratings in 1983 of the most resistant genotypes from 1982 are presented in Table 1. Of the 24 genotypes that had lowest disease incidence in 1982 and were retested in 1983 (Table 1), only 11 were statistically similar to or better than 'Campbell-28' ('C-28'), which has been one of the most resistant genotypes in previous breeding trials (5; unpublished data). These 12 genotypes appear to have useful levels of resistance for breeding purposes. Seven other genotypes had significantly less disease than 'Walter' and may have some breeding value (Table 1). Of the new entries tested in 1983, only 3—Hawaii 7998, PI 379032, and 'Burgess Crack Proof'—had statistically less disease than 'Walter' and thus could be considered to have some resistance.

Many genotypes with reported levels of resistance to bacterial spot (3–5, 9, 17) did not exhibit any apparent resistance in these experiments (Table 1 and data not shown). A high proportion of those genotypes with a fair level of resistance in this experiment had been evaluated previously and/or bred in Florida (Table 1). These trends could be due to different bacterial races between locations or to the high level of disease pressure in Florida that overcame the apparent resistance evident elsewhere. Except for 'C-28' (5, 9) and 'Burgess Crack Proof' (3), no genotypes with reported bacterial spot resistance appeared to have resistance in these experiments. However, PI 127813, PI 224573, PI 117899, PI 270217, PI 159199, and PI 379032 did have some resistance in our experiments and in data kindly provided to us by W.L. Summers from screening done at Iowa State Univ. Other genotypes that had some resistance were bacterial canker-resistant lines (1) 'Heinz 2990' and 'Monense' (1) and bacterial wilt-resistant Hawaii 7998 (R.M. Sonoda, personal communication).

No typical bacterial spot symptoms were found on Hawaii 7998, but it was given a value of 1% (i.e., 2 on the HB scale) because there were a few unidentified spots on the plants. In subsequent laboratory experiments, the hypersensitive resistance of this line was confirmed. Hawaii 7998 has fruit slightly larger than cherry tomato size, and is much more advanced horticulturally than PI 379032 (*L. parvifolium*), which had an equal field rating. Furthermore, in subsequent laboratory testing, PI 379032 did not give a hypersensitive-resistant reaction.

Ohio 4014-4 (Table 1) is a frost-tolerant tomato (14) that grows slowly and wilts on sunny days. In, F_2s from crosses with susceptible lines, recovery of bacterial spot resistance was associated with wilting (J.W. Scott, unpublished data). Ohio 4013-3 is related to Ohio 4014-4, but may have more breeding value as it does not wilt as severely as Ohio 4014-4.

Other partially resistant bacterial spot genotypes probably would not provide resistance much greater than that of 'C-28' (Table 1). Several genotypes could be related to 'C-28', such as the Heinz lines or those with Heinz lines in their pedigree. Another genotype, 'Burgess Crack Proof', is in the pedigree of 'C-28'. The bacterial spot resistance of these genotypes could be derived from the same unknown ancestor, and, if so, is likely controlled by a similar genetic mechanism.

One other noteworthy genotype was PI 270248-'Sugar'. Of the genotypes with enough fruit set to evaluate, 'Sugar' had the highest level of resistance to XCV on fruit, although its foliage was quite susceptible (Table 1). Only one spot was seen on thousands of fruit of 'Sugar'.

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External Leaf Features of Tissue-cultured 'Silvan' Blackberry

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Abstract. **'Silvan' blackberry (*Rubus* sp.) has 3 types of leaf hairs: multiseriate stalked, multicelled head colleters; thick-walled unicellular hairs; and setose hairs (multiseriate trichomes that taper from a stout base). In culture, 'Silvan' blackberry leaves were unifoliolate, smaller, and thinner, with less cuticle and a decreased number of trichomes compared to mature leaves of greenhouse-grown plants, which were tri- or pentafoliolate. Cultured leaves had permanently open stomata, raised guard cells, and an altered stomatal and trichome distribution compared to greenhouse-grown plant leaves. Stomatal index was unaffected, but leaf size in vitro was only 1% to 2% of greenhouse control leaf area. Leaves of shoots in multiplication medium were half as large as those of plantlets in rooting medium.**

Investigations of foliar anatomy and morphology of plantlets and/or transplants have revealed structural differences in culture that lower tolerance to water stress. Ex vitro transplant shock has been ascribed to reduced stomatal control (1, 2, 5, 18, 19), poor epicuticular and cuticular wax formation (5, 8, 9, 18), reduced trichome number, altered trichome and stomatal distribution (6), and other foliar anatomical effects related primarily to development under conditions of elevated relative humidity and low light intensity in culture. Tissue culture propagation of several blackberry cultivars has been described (3, 10, 17, 20) and summarized by Caldwell (4).

Studies of ex vitro red raspberry transplants have indicated that the culture-induced phenotype is permanent in leaves persistent from culture, that new leaves formed soon after transplant are transitional in their anatomy and physiology, and that with time in soil, newly developing organs approach the control phenotype (6, 7). In red raspberry, filiform hairs densely cover and obscure the abaxial surfaces of control leaves and are present in plantlets incubated at high light intensity (6). As 'Silvan' blackberry (13) lacks these hairs, it is more convenient than red raspberry for such investigations. The purpose of this study was to characterize features of the leaf morphology of shoots and plantlets of 'Silvan' blackberry growing in multiplication and rooting media.

Cultures were initiated from 1–5 mm shoot tips from lateral and apical leaf buds of actively growing outdoor-potted plants. Shoot tips were explanted to Murashige–Skoog (MS) (15) medium containing elevated thiamine hydrochloride (1.20 μM; 0.4 mg/liter) (12), myoinositol (550 μM; 100 mg/liter), sucrose (87.6 mM; 30 g/liter) *N*-(phenylmethyl)-1*H*-purin-6-amine (BA) (4.4 μM; 1 mg/liter) and 1*H*-indole-3-butanoic acid (IBA) (0.49 μM; 0.1 mg/liter). After 2 months, cultures had outgrown their 10-cm test tubes and were transferred to 400-ml jars containing 40 ml of medium. The shoots were inserted between the glass and filter-paper support strip that held them above the medium. At one-month intervals, proliferating shoots were separated into 3–5 leaf cuttings for subculture to new multiplication medium or for rooting. Leaf samples were taken at this time. Shoots from multiplication medium were rooted in vitro in medium with IBA increased to 2.46 μM (0.5 mg/liter) and BA omitted. Leaf samples from plantlets in rooting medium were taken one month after subculture. Cultures were exposed to temperatures of 27° to 28°C and cool-white fluorescent lighting (25 $\mu mol \cdot s^{-1} \cdot m^{-2}$; 16 hr daylength). In the greenhouse, 'Silvan' control plants were grown at ambient temperatures and natural and fluorescent lighting at 100 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

Fresh leaf samples of mature greenhouse-grown or in vitro 'Silvan' to be used for scanning electron microscopy (SEM) were fixed in 4% glutaraldehyde in cacodylate buffer (pH 7.0) for at least 2 hr, washed in distilled water, soaked for 1.5 hr in 1% osmium in 0.1 M cacodylate buffer (pH 7.0), again washed in distilled water, and dehydrated through an ethanol series. Samples were critical-point-dried, mounted on aluminum stubs with silver epoxy paste, sputter-coated with gold, and examined in a Hitachi S500.

Stomatal index (SI) and sizes (length, breadth) were calculated from photomicrographs of no fewer than 10 fields of view, on 5 leaves, from detached epidermal leaf strips or cleared leaves (6). At least 100 stomata were measured on both leaf surfaces of mature greenhouse-grown 'Silvan' leaves and those in multiplication and in rooting medium in vitro. Within each group, leaf areas were obtained by photocopying 30 leaves, cutting them out, weighing them, and calculating areas based on known-area paper weights. Student's *t* tests were used to compare SI, stomatal size measurements, and leaf areas among treatments.

Shoot multiplication in vitro was prolific (Fig. 1a). A 5- to 10-fold increase in shoot numbers per monthly cycle was established by the 3rd month after initial explantation, which was the end of the first month in large culture jars. Some rooting was seen in multiplication medium. Cuttings with 3 to 5

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Table 1. Leaf area, stomatal index (SI), stomatal length (L), breadth (B), and estimate of total number of abaxial stomata per leaf from 'Silvan' blackberry shoots and plantlets in vitro and mature leaves of greenhouse-grown plants (GH).

Specimen	Leaf		Stomata		Estimate of stomatal
	Area (mm)	SI	L (μm)	B (μm)	Number/leaf
Shoots	35 a[z]	261 a	28.28 a	23.52 b	9,150 a
Plantlets	80 b	229 a	32.55 b	28.53 c	18,170 b
GH controls	7735 c	227 a	31.83 b	18.41 a	1,752,155 c

[z]Means within a column followed by different letters are significantly different at the 5% level according to *t* tests.