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Resistance to Bacterial Spot Fruit Infection in Tomato

J.W. Scott[1], G. Cameron Somodi[2], and J.B. Jones[3]
Gulf Coast Research and Education Center, 5007 60th Street East, Bradenton, FL 34203

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Abstract. **Tomato (*Lycopersicon esculentum* Mill.) accession PI 270248 ('Sugar') had high levels of resistance to bacterial spot [incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye] on fruit, but foliage was susceptible. Hawaii 7998 (H7998) was highly resistant to foliar infection, but was intermediate in resistance to fruit infection. Fruit spot on hybrids between 'Sugar' and H7998 was usually intermediate to the parents. Occasionally, disease incidence of hybrids was not statistically different from one or both parents, but tended to resemble 'Sugar' more closely than H7998. There were no significant differences between reciprocal hybrids, indicating a lack of cytoplasmic inheritance. Under low disease incidence, hybrids between 'Sugar' and 'Walter' (susceptible to bacterial spot on fruit and foliage) had fruit spot incidence similar to 'Sugar' and significantly less than 'Walter'. Thus, there was a high level of dominance for resistance to bacterial spot on fruit.**

Bacterial spot of tomato incited by *Xanthomonas campestris* pv. *vesicatoria* affects all above-ground plant parts. Pohronezny and Volin (1983) reported yield and fruit grade losses to be caused by defoliation and fruit infection. A high level of resistance to the foliar phase of bacterial spot in tomato accession Hawaii 7998 (H7998) was reported recently (Jones and Scott, 1986; Scott and Jones, 1986). It was also reported that PI 270248 ('Sugar') had a high level of resistance to the fruit phase of bacterial spot in the field (Scott and Jones, 1986). More recently, we reported on the inheritance of foliar resistance and general considerations in working with bacterial spot resistance (Scott and Jones, 1989; Scott et al., 1989). In this paper, the results of further work on resistance to fruit spot are presented.

Greenhouse experiments. Five greenhouse experiments were conducted from Spring 1984 through Spring 1987. Seeds of 'Walter' (foliage and fruit susceptible), H7998 (foliage resistant but not fruit), 'Sugar' (foliage susceptible and fruit resistant), and several of the F_1s of these parents were sown in an inert medium and covered with a layer of vermiculite. Ten days after planting, seedlings were transplanted to 257-cm^3 pots and then transplanted to 1141-cm^3 pots several weeks later. The pots contained a 2 soil : 1 peat : 1 sand mixture (by volume). There were six to eight single-plant replicates per experiment grown in completely randomized designs. Plants were fertilized weekly to maintain growth once fruit began to set. With the exception of the Fall 1987 experiment, individual flowers were labeled at anthesis with dated tags. All flowers were mechanically pollinated with a vibrator. When the oldest tagged fruit were >30 days old, plants were inoculated by misting fruit and flowers with a suspension of *X. c.* pv. *vesicatoria*. Inoculum was prepared by incubating several strains of *X. c.* pv. *vesicatoria* for 48 hr on nutrient–yeast–dextrose agar plates at 28C (Jones et al., 1981). Bacterial growth was suspended in 0.01 M $MgSO_4 \cdot 7H_2O$ and adjusted to $\approx 10^8$ cfu/ml with a turbidimetric procedure using a spectrophotometer. After inoculation, plants were incubated in a growth chamber for 72 hr at 27C and 90% RH with 12-hr days. Plants were returned to the greenhouse and, 3 three weeks later, the number of infected and noninfected fruit and the number of spots per fruit were recorded.

To determine if there were differences in fruit spot susceptibility due to the age of the fruit, tagged fruit of 'Walter' and H7998 were grouped into 5-day postanthesis time intervals and disease incidence was calculated for each interval per genotype. A combined analysis of variance over all of the experiments was conducted for each genotype (Gomez and Gomez, 1984).

Field experiments. Experiments were conducted in Summer 1985 and late Summer 1987. Seeds for these experiments were sown in an inert medium and, 10 to 14 days later, transplanted to Todd planter flats (Speedling) with 2.9-cm^3 cells. After 1 month, transplants were set in a field of EauGallie fine sand on beds 15 cm high × 75 cm wide. The beds had been fumigated 2 weeks earlier with methyl bromide : chloropicrin (67% : 33%) at the rate of 392 kg·ha^{-1} and covered with white polyethylene mulch. Beds were 137 cm from center to center with plants spaced 61 cm apart in the rows. Fertilizer rates recommended for the area were used. 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile (chlorothalonil) was applied for control of fungal pathogens, and insecticides were used as needed to control insect pests. In Summer 1985, plants were irrigated solely by the seepage method. In 1987, seepage irrigation was supplemented by overhead irrigation six times per day for 10-min intervals in an attempt to encourage the development of bacterial spot. However, overhead irrigation did not enhance bacterial spot infection in our experiments (unpublished data). Inoculum of *X. c.* pv. *vesicatoria* was prepared as described previously. Plants were inoculated by spraying a bacterial suspension on them in the early morning a few weeks after transplanting. During these experiments, the weather was hot and humid with frequent, often heavy, rainfall. In 1985, day maxima ranged from 29 to 34C (mean = 32.7C) and night minima from 20 to 24C (mean = 22.6C). In 1987, day maxima ranged from 17 to 36C (mean = 28.9C) and night minima from 5 to 24C (mean = 17.8C).

In 1985, H7998, 'Sugar', and their reciprocal hybrids were grown in a completely randomized design with three replicates and 10 plants per plot. The plants were rated for the percentage of infected foliage on a leaf-area basis. The percentage of infected fruit was determined by harvesting fruit and scoring for the presence of lesions. The genotypes 'Walter', 'Walter' x 'Sugar', 'Walter' x H7998, and the four genotypes used in the 1985 field experiment were used in Summer 1987. The experimental design was a completely randomized block design with three blocks and 10 plants per plot of each genotype. Fruit were harvested from the top half of the plants and scored for the presence of

Table 1. Bacterial spot, incited by *Xanthomonas campestris* pv. *vesicatoria*, on fruit and foliage of tomato genotypes Hawaii 7998, 'Sugar', and their reciprocal hybrids inoculated in the field at Bradenton, Fla., Summer 1985.

		Bacterial spot[z]	
Genotype	No. of fruit	Fruit infected (%)	Defoliation (%)
Hawaii 7998 (H)	1082	17 a	3 d
H x S	3980	11 ab	32 b
S x H	5485	6 b	24 c
Sugar (S)	1892	5 b	38 a

[z]Mean separation in columns by Duncan's multiple range test, $P = 0.05$; data transformed to arcsin square root for analysis.

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[1]Associate Professor of Vegetable Crops.
[2]Assistant in Plant Pathology.
[3]Associate Professor of Plant Pathology.

Table 2. Incidence of bacterial spot[z] on fruit, incited by *Xanthomonas campestris* pv. *vesicatoria*, on several tomato genotypes inoculated in the greenhouse.

Genotype	Disease reaction[y]	Spring 1984 Total no. fruit	Spring 1984 Fruit spot (%)	Fall 1984 Total no. fruit	Fall 1984 Fruit spot (%)	Fall 1985 Total no. fruit	Fall 1985 Fruit spot (%)	Spring 1986 Total no. fruit	Spring 1986 Fruit spot (%)	Spring 1987 Total no. fruit	Spring 1987 Fruit spot (%)
Walter (W)	S	124	26 a	174	21 a	133	48 a	---	---	163	24 a
Sugar (S)	R	945	0 b	---	---	653	<1 c	1330	1 c	1255	<1 c
Hawaii 7998 (H)	PR	---	---	553	13 b	236	21 b	258	31 a	339	3 bc
H x S	PR x R	---	---	---	---	477	5 c	694	6 b	818	2 bc
S x H	R x PR	---	---	---	---	---	---	725	4 b	815	3 bc

fruit spots. There was no fruit spot on the bottom half of the plants, so these fruit were not harvested.

Data on fruit spot will be presented for disease incidence only. Disease severity (i.e., the number of spots per infected fruit) was also measured in the greenhouse experiments; genotypes with greater disease incidence also had more spots per fruit (data not shown). Thus, either disease incidence or severity could be used to measure relative resistance levels.

In the 1985 field experiment, 'Sugar' had significantly fewer fruit with bacterial spot lesions, but significantly more bacterial spot on foliage than H7998 (Table 1). This relationship indicates that resistance to fruit and foliage phases of bacterial spot are under separate systems of genetic control. In this field experiment, the fruit spot infection of hybrids was not significantly different from that of the resistant parent, and the hybrid with H7998 as the seed parent was not significantly different from H7998.

In four greenhouse–growth chamber experiments, 'Sugar' fruit had ≤1% disease incidence (Table 2). 'Sugar' had significantly less fruit spot incidence than 'Walter' in all three experiments where they were compared. The disease incidence of 'Sugar' was less than that of H7998 in Fall 1985 and Spring 1986, but not in Spring 1987, when disease incidence was low (Table 2). H7998 had less fruit spot incidence than 'Walter' in Fall 1984, Fall 1985, and Spring 1987, indicating that H7998 has intermediate resistance to *X. c.* pv. *vesicatoria* on fruit. The incidence of bacterial spot on fruit of hybrid H7998 x 'Sugar' was similar to that for 'Sugar' and significantly less than that for H7998 in Fall 1985 (Table 2). In Spring 1986, the reciprocal hybrids of 'Sugar' x H7998 had fewer diseased fruit than H7998, but more diseased fruit than 'Sugar'. In Spring 1987, disease incidence was lower than in Spring 1986, and there were no significant differences among 'Sugar', H7998, and the reciprocal hybrids, all of which were significantly less affected than 'Walter'. Overall, the incidence of fruit spot in hybrids between partially resistant and resistant parents was more similar to the resistant parent. This indicated a high level of dominance for fruit spot resistance. The lack of differences among reciprocal crosses indicated no cytoplasmic effect on bacterial fruit spot resistance.

In Fall 1987, a resistant x susceptible hybrid was also tested, but the disease incidence was low (Table 3). In both greenhouse and field experiments, 'Walter' had significantly more diseased fruit than H7998, 'Sugar', and all hybrids, except 'Walter' x H7998 in the greenhouse experiment. Although the disease incidence was low, the resistant x susceptible hybrid was more similar to the resistant parent than the susceptible parent, which indicated a high level of dominance. Further work with greater disease incidence would better clarify the resistant x susceptible hybrid resistance level.

Field foliar infection was greater on 'Sugar' than on H7998, whereas hybrids between the two were intermediate in 1985 (Table 1). In other experiments, H7998 had lower populations of *X. c.* pv. *vesicatoria* on foliage than susceptible genotypes (R.G.M. and J.B.J., unpublished data). Thus, the bacterial populations in the vicinity of 'Sugar' fruit in the 1985 field experiment were likely greater than the bacterial populations in the vicinity of H7998 fruit. This may have accounted for the greater fruit infection for 'Sugar' in the 1985 field experiment than in the greenhouse–growth chamber experiments (Tables 1 and 2). The relatively low fruit infection of 'Sugar' in the field further substantiates its value as a source of fruit spot resistance.

Incidence of bacterial spot in H7998 and 'Walter' fruit of different ages from the greenhouse–growth chamber experiments is shown in Fig. 1. Although there were significant differences in incidence between the time intervals after anthesis, these are not presented due to a significant interaction between the time interval and the experiments. This interaction was due, in part, to small sample sizes for some intervals in some of the experiments, which may have resulted in sampling errors. Nevertheless, both genotypes followed the same pattern, with the highest peaks between 5 and 19 days after anthesis and no infection after 30 days. The breaker stage would be ≈40 days after anthesis for H7998 and 45 to 50 days for 'Walter'. Thus, it appears that young fruit are more susceptible than more mature fruit. This finding is similar to results of Getz et al. (1983b) for bacterial speck (*Pseudomonas syringae* pv. *tomato*); 'Pik-Red' fruit up to 3 cm in diameter were more susceptible than larger fruit. Trichomes have been postulated as infection sites for both bacterial canker and bacterial speck (Bryan, 1930; Getz et al., 1983a). Observations of 'Sugar' fruit indicates that trichomes are abundant; thus, it is not a lack of trichomes that prevents infection to bacterial spot.

Losses in marketable yield due to bacterial spot have been caused by both the foliar and fruit phases of the disease (Pohronezny and Volin, 1983). The best assurance against such losses would be to combine both foliar and

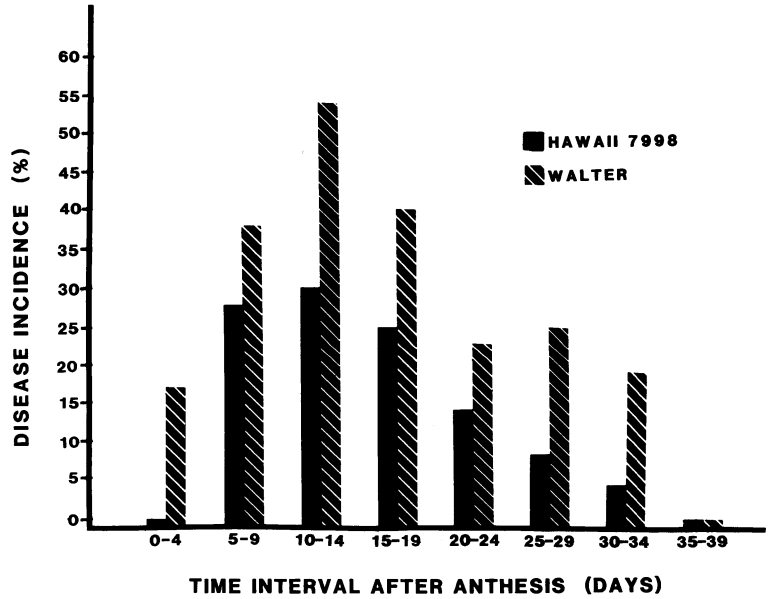

Fig. 1. Incidence of bacterial fruit spot on 'Walter' and Hawaii 7998 fruit of different ages at inoculation.

Table 3. Incidence of bacterial fruit spot, incited by *Xanthomonas campestris* pv. *vesicatoria*, for several tomato genotypes grown in greenhouse and field experiments during Summer 1987 at Bradenton, Fla.

Genotype	Reaction[y]	Greenhouse fruit		Field fruit	
		Total	Infected[z] (%)	Total	Infected[z] (%)
Walter (W)	S	89	21 b	1259	11 a
Hawaii 7998 (H)	PR	179	5.6 c	3187	0.2 b
Sugar (S)	R	922	1.5 c	7605	0.1 b
W x S	S x R	422	1.6 c	3647	0.6 b
W x H	S x PR	78	35 a	1218	2.0 b
H x S	PR x R	427	0.6 c	3663	0.0 b
S x H	R x PR	315	0.2 c	2991	0.0 b

[z]Mean separation by Duncan's multiple range test, $P = 0.05$.
[y]S = susceptible, PR = partially resistant, R = resistant.

fruit resistances in a cultivar; however, effectively incorporating both types of resistance would be difficult. The genetics of foliar resistance is somewhat complex and requires careful selection and reselection under environments favorable to the disease (Scott and Jones, 1989; Scott et al., 1989). Furthermore, fruit spot has been reported to be erratic in expression (Scott and Jones, 1984); this is also indicated by some of our present data. Even with inoculation, high levels of fruit spot infection do not always occur (Tables 2 and 3). Other times, fruit spot can occur readily even with limited foliar symptoms. Thus, simultaneous selection for resistance to both phases of bacterial spot could prove difficult. It is not known how serious fruit spot damage would be in a monoculture with a cultivar resistant only to foliar spot, which would reduce inoculum levels. Once such cultivars are developed, the economic importance of fruit spot resistance can be assessed more adequately.

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In Vitro Flowering of Potato

Hassan Al Wareh[1], Norma L. Trolinder[2], and J.R. Goodin
Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409

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Abstract. **Apical meristem explants of three cultivars of potato (*Solanum tuberosum* L.) formed flowers directly or callused and subsequently formed flower buds when cultured in vitro. Frequency of flower formation was 45%, 59%, and 55% for 'Red LaSoda', 'Viking', and 'Norgold "M"', respectively. Pistils and stamens were reduced in size compared to sepals, and <1% of the anthers contained mature pollen.**

Formation of roots, shoots, embryos, and specialized cell types in vitro have been characterized extensively (Bojwani and Razdon, 1983; Fukuda and Komamine, 1980; Gamborg et al., 1968); however, only a few reports exist on flower formation. All morphogenic patterns can be produced in vitro from thin-layer explants of flowering tobacco shoots (Kaur-Sawhney et al., 1988; Smulders et al., 1988; Tran Thanh Van, 1980). Embryoids of ginseng produce flowers in vitro (Chang and Hsing, 1980), as do meristem-derived shoots of amaranthus (Tisserat and Galletta, 1988). It has been suggested that such systems would be valuable for the direct observation of changes in a single cell leading to different morphogenic patterns and for the study of specific aspects of flowering and the reproductive process (Bhojwani and Razdon, 1983; Tisserat and Galletta, 1988). Because of the low frequency of in vivo flowering and seed set in many potato cultivars (Bajaj and Sopory, 1986), such as 'Red LaSoda' (D. Smallwood, personal communication), this phenomenon could be important for the study of the flowering response. Thus, this research was begun in an effort to characterize the in vitro culture responses of three North American potato cultivars.

Stock plants were grown from tubers obtained from the Texas A&M Research and Extension Center, Lubbock. Plants were established in December from tuber cuttings planted in 15-liter plastic pots in the greenhouse. The stock plants were fertilized biweekly by an application of 6 g Osmocote 14N–14P–14K to each pot. Plants were maintained under natural daylength at 25 ± 5C. Terminal shoots (1 cm) from 1-month-old greenhouse plants were sterilized by a 30-sec dip in 70% ethanol, followed by 5 to 6 min in 10% Clorox containing one drop of Tween 20 per 100 ml. Shoots were rinsed in sterile distilled water three times before meristem isolation. The apical dome and two youngest leaf primordia (2 mm) comprised the explant.

One meristem each was placed in a 25 × 150-mm culture tube containing 20 ml of

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[1]Present address: Faculty of Agriculture, Univ. of Aleppo, Aleppo, Syria.
[2]Present address: USDA/ARS, Rte. 3, Box 215, Lubbock, TX 79401.