Morphology of Leaf Surfaces of Tomato Cultivars in Relation to Possible Invasion into the Leaf by *Pseudomonas syringae* pv. *tomato*

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ABSTRACT

The role of morphological characteristics of tomato leaves in the infection process of the pathogen *Pseudomonas syringae* pv. *tomato* was studied in 15 cultivars of varying susceptibility. Natural openings such as stomata, broken trichomes and cuticular cracks were counted. It was found that susceptibility increased in proportion to the number of these potential penetration sites. However, their role is probably limited because even in the highly resistant cultivars there are enough natural openings to enable successful bacterial penetration.

Key words: Bacterial speck of tomato, *Lycopersicon esculentum*, phytopathogenic bacteria, plant morphology, *Pseudomonas syringae* pv. *tomato*, tomato.

INTRODUCTION

The role of mechanical barriers in prevention of bacterial invasion into leaf tissue has been the subject of controversy (Crafts and Foy, 1962; Goodman, Kiraly and Zaitlin, 1967; Baker, 1971; Van den Ende and Linskens, 1974). It is generally agree that natural openings of the plant such as stomata, nectaries, hydathodes, lenticels and broken glandular hairs are the main sites of entry for many species of phytopathogenic bacteria (Rolfs, 1915; Lewis and Goodman, 1965; Goodman *et al.*, 1967; Burki, 1972; Goodman, 1976). Although the role of morphological barriers in resistance to infection was reported as early as 1921 (MacLean, 1921; Hildebrand, 1937), studies dealing with resistance in diseased plants have focused on physiological events such as phenol oxidation and accumulation of phytoalexins (Frič, 1976; Kuć, 1976).

Pseudomonas syringae pv. *tomato* (PST), is the causal agent of bacterial speck in leaves of tomato (Wilkie and Dye, 1974). The pathogen has been identified in several countries as causing heavy economic losses (Schneider, Hall and Grogan, 1975; Yunis *et al.*, 1980). Schneider and Grogan (1977) suggested that the tomato leaf trichomes are the source of resident inoculum, and serve as sites for penetration by PST. Bashan *et al.* (1981) confirmed these findings and located the primary sites of PST penetration in open stomata and trichome bases. Open stomata as a result of high relative humidity were implicated as a factor affecting disease severity in tomato plants infected with PST (Bashan, Okon and Henis, 1978).

The purpose of this study was to measure some morphological features of the leaf surfaces of 15 tomato cultivars of varying susceptibility to bacterial speck disease, and to evaluate their role in the invasion of PST.

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MATERIALS AND METHODS

Organisms and growth conditions

Pseudomonas syringae pv. *tomato* (WT-1) isolated from infected tomato plants (*Lycopersicon esculentum* Mill) was used. The tomato cultivars used were: Ontario 7710 (Canada); Rehovot-13; Extra Marmande; Saladette; Step-535; Hosen VF-228; Rutgers nor, acc. 364; Manalucy; Red-sherry; Kewalo; VFN-70T-81-1 (UCD); Tropic-VF; VF-198; No. 97-3 (UCD) and F_3 (Ontario 7710 × Rehovot 13). Seeds were obtained from the collection of the Department of Field and Vegetable Crops, Faculty of Agriculture, Rehovot, Israel.

Plants were grown during the summer in shed glasshouses under conditions of controlled temperature (25 ± 3 °C) and natural illumination (150 W m^{-2} at table surface). They were grown with drip irrigation in plastic pots containing 600 g of volcanic dust supplemented twice a week with Hoagland's solution.

Inoculum preparation, inoculation procedures and mist chamber conditions were described previously (Bashan *et al.*, 1978).

Disease Index (*Fallik* et al., *1983*)

Disease severity was estimated using an index of: 0 = no symptoms; 1 = 1–5 specks either in a cluster or spread over the leaf; 2 = 6–10 specks; 3 = more than 11 specks. The index was determined on the third, fourth and fifth leaves from the top of each plant. Numbers of specks per leaf were counted and the mean of three leaves served as the disease index of the plant in each experiment. All plants were examined and their disease index determined.

Cuticle and wax extraction (*Holloway and Baker, 1968; Silva Fernandez, Baker and Martin, 1964*)

Cuticle and wax were extracted separately from a total of 400 cm^2 of leaf area of uniform age from each of the 15 cultivars tested. Measurements were repeated three times. The leaves were placed in a solution of 60 per cent $ZnCl_2$ dissolved in 10 N HCl for 5 d at room temperature (4·5 ml solution per cm^2 leaf area). After separation of cuticles, they were washed more than 20 times over the course of 10 d to remove traces of acid. The extracted cuticles were lyophilized and maintained at −20 °C until used.

Wax was extracted from detached leaves incubated at 30 °C for 24 h. Stomata were closed during incubation and the leaves wilted in order to eliminate or to reduce to the minimum the extraction of other substances obtained from the inner parts of the leaves beside wax. Each leaflet was washed four times for 20 s in purified chloroform (~ 100 per cent), solutions were combined and the chloroform was evaporated at 70 °C.

Cuticle and Pectin stain (*Baker and Martin, 1963*)

Thin segments from various cultivars were slices with a microtome, stained for 30 min with either Sudan Black III (saturated in boiled 60 per cent ethanol) or Ruthenium Red (1 mg ml^{-1} in distilled water, double filtered) and observed under a light microscope.

Scanning electron microscopy (SEM)

Four to six true leaves of uniform age (the second leaf from the plant tip) were taken from four healthy and four inoculation plants. Five samples, each of 0·5 cm^2 were taken

from the centres of the leaves of each plant and prepared for SEM observation as previously described (Bashan *et al.*, 1978). Measurements were made from the SEM micrographs (three photos per sample, randomly photographed).

Statistical analysis

All experiments were repeated 2–3 times in 10 replicates using 20 plants per replicate. Correlations were done by linear regression at $P \leqslant 0{\cdot}001$ significance.

RESULTS

Relationship between number and size of leaf stomata and resistance to bacterial speck

Fifteen tomato cultivars were tested for resistance, and the number of stomata per unit leaf area was determined. Because the number of stomata varies with leaf age, counts were restricted to the second leaf from the top of the plant. This leaf is also known to be the most susceptible to bacterial speck (Yunis *et al.*, 1980).

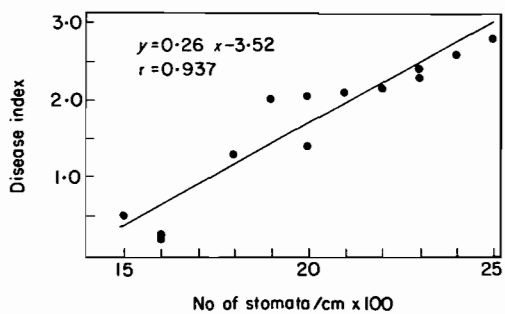

FIG. 1. Correlation between number of stomata and resistance to bacterial speck of tomato. The line is significant at $P \leqslant 0{\cdot}001$.

Direct positive correlation ($r = 0{\cdot}937$, $P \leqslant 0{\cdot}001$; Fig. 1) was found between the number of stomata per unit area and resistance to the disease. Stomatal opening in 15 tomato cultivars varied between 15 and to 39 μm with no correlation with resistance.

Relationship between number of leaf trichomes and resistance

A preliminary observation showed that a susceptible cultivar (VF-198) had more trichomes per leaf area than a resistant one (Rehovot 13) (Fig. 2A, B). Examination of 15 tomato cultivars showed a correlation between the number of leaf trichomes per unit area and susceptibility to bacterial speck ($r = 0{\cdot}927$, $P \leqslant 0{\cdot}001$; Fig. 3). In contrast, three wild tomato species examined: *Lycopersicon pimpinellifolium* PI 126927 and P.I. 126932 and *L. esculentum* var. *cerasiforme*, the leaves either lacked trichomes or were covered with thousands of degenerative trichomes (2500 cm^{-2} leaf area). In these cases, there was no correlation between trichome numbers and resistance. Basal trichome size varied from 20 to 100 μm in diameter and was randomly distributed among the cultivars with no correlation to resistance.

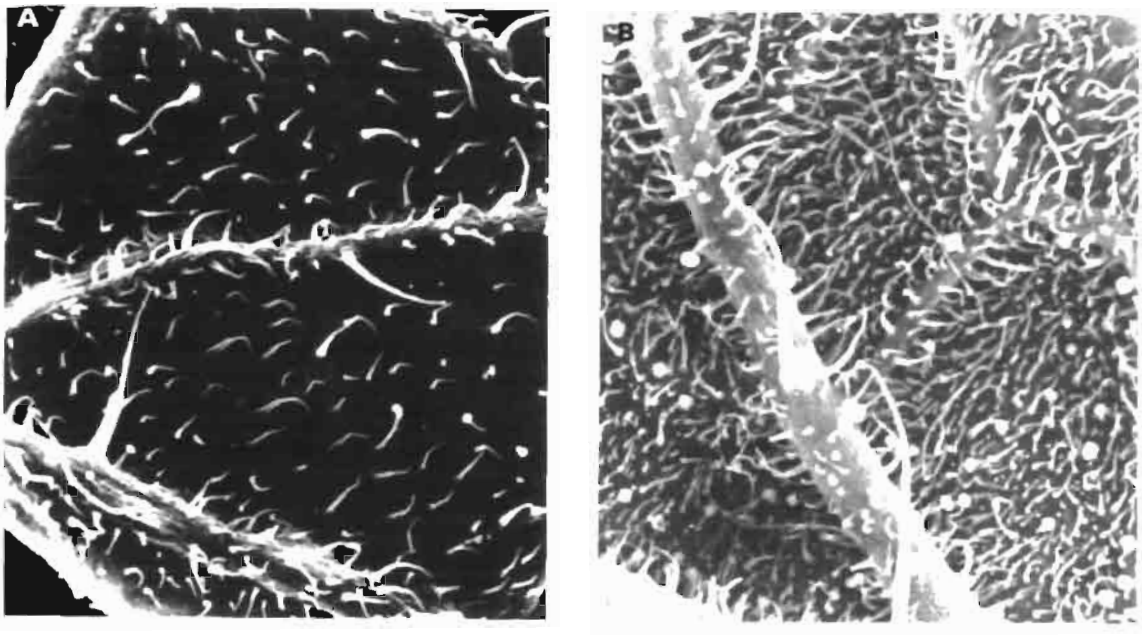

FIG. 2. Scanning electron micrographs of tomato leaf surface. A, resistant Rehovot 13: B, susceptible VF-198 (× 20).

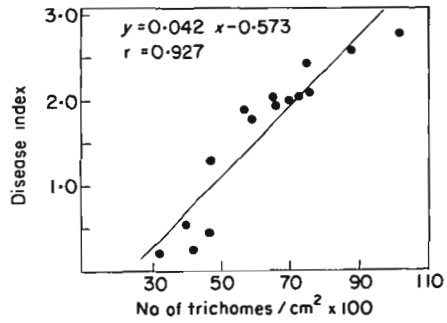

FIG. 3. Correlation between number of trichomes and resistance to bacterial speck of tomato. The line is significant at $P \leqslant 0{\cdot}001$.

Observations on the thickness and morphology of cutin and pectin in tomato cuticle

Thin sections of healthy tomato leaves from two susceptible and two resistance cultivars were stained with stains specific for cutin and pectin. It was observed (Fig. 4A–D) that the layer of cutin and pectin is thicker in susceptible than in resistance cultivars. Furthermore, the leaf surface of the susceptible cultivars was covered with indentations (arrows) while that of the resistant cultivars were smooth.

Relation between the amount of cuticle and wax and resistance

Correlations were found between amount of cuticle ($r = 0{\cdot}884$, $P \leqslant 0{\cdot}001$) and amount of wax ($r = 0{\cdot}954$, $P \leqslant 0{\cdot}001$) (separately) and susceptibility to the disease (Fig. 5A, B).

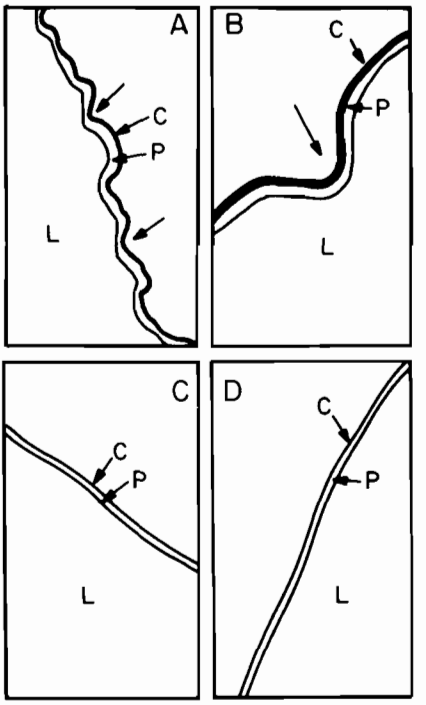

FIG. 4. Drawing of thin sections of tomato leaf. A, susceptible VF-198; B, susceptible Tropic-VF; C, resistant Rehovot 13; D, resistant Ontario 7710 (× 300). P, pectin layer; C, cutin layer; L, leaf interior.

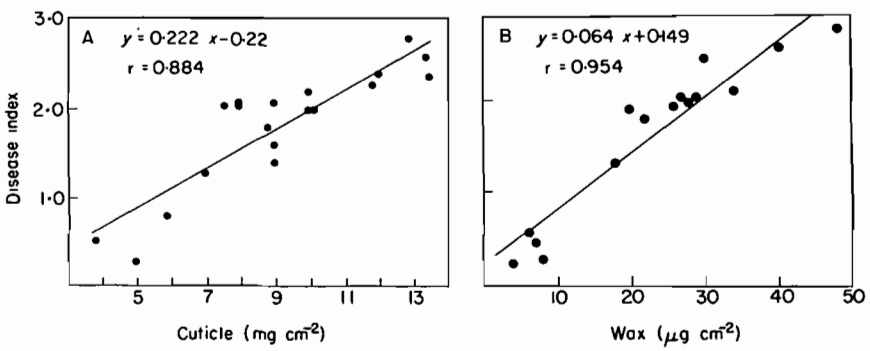

FIG. 5. Correlations between amount of cutin, A, and amount of wax, B, and susceptibility to bacterial speck of tomato. The lines are significant at $P \leqslant 0{\cdot}001$.

Relationships between number of stomata, trichomes, cuticle and wax

Every two parameters were correlated between themselves in the six possible combinations. The equations of correlations between: number of trichomes and amount of wax is $y = 1{\cdot}45x + 18{\cdot}92$ and $r = 0{\cdot}978$; Number of trichomes and amount of cuticle is $y = 6{\cdot}07 + 0{\cdot}92$ and $r = 0{\cdot}895$; Number of trichomes and number of stomata is $y = 594x - 62{\cdot}73$ and $r = 0{\cdot}933$; amount of wax and amount of cuticle is $y = 3{\cdot}95x - 10{\cdot}06$ and $r = 0{\cdot}894$; amount of wax and number of stomata

is $y = 402x - 54{\cdot}72$ and $r = 0{\cdot}951$ and amount of cuticle and number of stomata is $y = 98x - 10{\cdot}91$ and $r = 0{\cdot}942$. All r values are significant at $P \leqslant 0{\cdot}001$.

DISCUSSION

Tomato leaves, when compared to plants of other botanical families, have unique surface features (Madsen, 1976; Seithe, 1979). These features, which include large numbers of ventral stomata, trichomes of different sizes and breakage characteristics, and broken epidermis on the upper surface of the leaf, provide potential penetration sites for phytopathogenic bacteria.

The surface features may vary according to cultivar. Therefore different invasion mechanisms may operate depending on the cultivar used and on the invasion site that can be reached by the bacteria at random.

Similar studies have shown that in some cases there is a relationship between the morphology of the plant and penetration by bacteria. Examples include penetration through flowers in fireblight disease of apple (Hildebrand, 1937; Lewis and Goodman, 1965), through stomata in bacterial citrus canker (MacLean, 1921) and through trichomes in bacterial speck of tomato (Schneider and Grogan, 1977; Bashan *et al.*, 1981).

The main question addressed in this work was whether there is a general connection between tomato leaf morphology and degree of resistance to bacterial speck disease. Positive correlations at significant r values were obtained between number of stomata and leaf trichomes and resistance to the disease. In PST-infected tomato leaves examined by scanning electron microscopy, it had been observed that trichomes serve as sites of entry, particularly if they are broken by mechanism means (Schneider and Grogan, 1977; Bashan *et al.*, 1981).

It must be emphasized that the number of potential infection sites even in the most resistant cultivar is relatively high (16000 stomata per cm^2 and 800 trichomes in the resistant cultivar as compared to 25000 stomata per cm^2 and 11000 trichomes per cm^2 in the most susceptible cultivar). The relationship between cuticle and wax thickness to disease was the opposite to that expected. It may be an outcome of increase in the number of trichomes in susceptible plants, the trichomes being mainly composed from cutin and wax. In addition, it is also possible that a thicker outer layer reduces leaf flexibility and makes the leaf more susceptible to cracks through which bacteria may invade. An increase in the number of 'bays' and cracks on leaf surfaces of susceptible cultivars may increase the probability of successful infection by concentrating bacteria at these sites (Bashan *et al.*, 1978, 1981). Cutin may also serve as inducer of cutinase production by the invading bacterial pathogen (Y. Bashan, unpublished).

The morphology of the tomato leaf and especially the number of trichomes has a significant importance in breeding against various species of insects and mites, since in general the more 'hairy' the plants the more resistant they are to these pests (McKinney, 1938; Stoner *et al.*, 1968). However, such a breeding programme may enhance susceptibility of tomato plants to bacterial speck as demonstrated in our study and should be taken into consideration when making the breeding programme.

There are many other factors potentially able to control resistance to bacterial infection. For example, recent studies (Bashan, unpublished) show that oxidative metabolism of phenols may be important. The relative importance of this mechanism, versus those based on surface morphology, is as yet undetermined.

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