Peroxidase, polyphenoloxidase, and phenols in relation to resistance against *Pseudomonas syringae* pv. *tomato* in tomato plants

Y. BASHAN,[1] Y. OKON, AND Y. HENIS

Department of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel

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Peroxidase, polyphenoloxidase, and phenols were evaluated for their involvement in the resistance mechanism of tomato plants against *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie. Healthy and infected leaf extracts of susceptible and resistant tomato cultivars were examined for their phenol content and activity of peroxidase and polyphenoloxidase. Peroxidase activity increased to a similar extent in infected mature and young leaves and in resistant and susceptible tomato cultivars. A positive correlation was found between the number of bacteria in the tissue and peroxidase activity in susceptible plants. Four different peroxidase isozymes were found in extracts from diseased plants, compared with only one in healthy plants. These facts indicated that peroxidase increases are not directly involved in the resistance mechanism of tomato plants. Significant positive correlations were found among resistance, polyphenoloxidase activity, and phenol accumulation in 20 different infected tomato cultivars, ranging from highly susceptible to highly resistant. Polyphenoloxidase activity and phenol accumulation were higher in inoculated, resistant cultivars than in inoculated susceptible ones. Inoculated resistant mature leaves showed both a higher polyphenoloxidase activity and a higher phenol content than younger susceptible leaves. A direct correlation was found between disease index, polyphenoloxidase activity, and phenol content in the tissue. Eight different polyphenoloxidase isozymes were found in susceptible diseased plants. Phenols extracted from inoculated resistant tomato plants inhibited pathogen growth. Our data suggest that the oxidative phenol system of tomato plants contributes to resistance to *P. syringae* pv. *tomato*.

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Les auteurs ont évalué l'implication de la peroxydase, de la polyphénol oxydase et des phénols dans le mécanisme de résistance de la tomate vis-à-vis le *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie. Ils ont mesuré les contenus en phénols, en peroxydase et en polyphénol oxydase dans des feuilles saines et dans des feuilles infectées de cultivars de tomates susceptibles et résistants. L'activité de la peroxydase augmente de la même façon dans les jeunes feuilles et les feuilles adultes, aussi bien chez les cultivars susceptibles que résistants. Il y a une corrélation positive entre le nombre de bactéries dans les tissus et l'activité de la peroxydase chez les plantes susceptibles. On retrouve quatre isoenzymes de la peroxydase chez les plantes malades comparativement à une seule chez les plantes saines. Ceci indique que les augmentations en peroxydase ne sont pas directement impliquées dans le mécanisme de résistance de la tomate. Des corrélations positives significatives apparaissent entre la résistance, l'activité polyphénol oxydase et l'accumulation de phénols chez 20 cultivars de tomate infectés allant de hautement susceptibles à hautement résistants. L'activité des polyphénol oxydases et l'accumulation de phénols sont plus élevées chez les cultivars résistants inoculés que chez les cultivars susceptibles également inoculés. Comparativement aux jeunes feuilles, les feuilles adultes de plants résistants inoculés montrent une plus forte activité polyphénol oxydase et un contenu en phénols plus élevé. Il y a une corrélation directe entre l'index de maladie, l'activité polyphénol oxydase et les contenus en phénols dans les tissus. On retrouve huit isoenzymes de la polyphénol oxydase différentes chez les plantes susceptibles malades. Les phénols extraits de plantes résistants de tomates inoculées inhibent la croissance du pathogène. Les résultats suggèrent que le système oxydatif des phénols contribue à la résistance des tomates vis-à-vis le *P. syringae* pv. *tomato*.

[Traduit par la revue]

Introduction

One of the most widely accepted explanations of how the phenol system and related oxidative enzymes operate in diseased plants is that, following infection by pathogens, the plant releases various phenolic compounds whose oxidation products, such as quinones, are toxic to the invading microorganisms (Goodman *et al.* 1967; Sequeria 1983).

Phenol accumulation is usually higher in infected resistant plants and in incompatible relationships than in infected susceptible plants. However, the involvement of phenols varies according to the pathogenic relationship (Fričˇ 1976). Phytopathogenic bacteria are usually less susceptible to oxidized phenols than are pathogenic fungi. It is also possible that pathogenic bacteria multiply at a much faster rate than fungi, thus avoiding inhibition by phenols (Farkas and Király 1962; Kosuge 1969). Many reports, on a wide range of pathogens, have suggested that increased peroxidase (PO) activity, following the host–pathogen interaction, results in nonspecific induction of plant resistance (Tomiyama 1963; Kuć 1966; Fehrmann and Diamond 1967; Lovrekovich *et al.* 1967; Novacky and Hampton 1968). However, two reports, one on stem rust of wheat (Seevers and Daly 1970) and the other on *Pseudomonas solanacearum* (Smith) infection of tobacco (Nadolny and Sequeira 1980) show that increases in peroxidase activity after infection are not directly involved in induced resistance.

The purpose of this study was to follow changes in oxidative activities of the phenolic system during bacterial speck infection in tomato plants to determine whether such activities could be involved in resistance against *P. syringae* pv. *tomato*.

Materials and methods

Organisms, growth conditions, and experiment planning

The following organisms were used in all experiments: *Pseudomonas syringae* pv. *tomato* (WT-1) isolated from infected tomato

[1]Present address: Department of Plant Genetics, Weizmann Institute of Science, Rehovot 76100, Israel.

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plants (*Lycopersicon esculentum* Mill) cultivars 'VF-198' (highly susceptible) and 'Rehovot-13' (resistant). Plants with four to six true leaves were used in all experiments. In certain experiments (demonstrated in Figs. 6*b*, 6*c*) the lines no. 97-3 (UCD) and no. 339944-3 (UCD), two wild tomato species, *L. pimpinellifolium* Mill. P.I. 126927 and *L. esculentum* var. *cerrasiforme* (Juse) Mill, and the following tomato cultivars were added: 'Rutgers nor', accession no. 364; 'Rutgers rin', accession no. 365; 'Red Cherry'; 'Kewalo'; 'Ontario 7710'; 'VFN-70T-81-1' (UCD); 'Italian winter'; 'Hosen VF-228'; 'Step-535'; 'Saladette'; 'Extra marmande'; 'Manalucy'; 'Tropic-VF'; 'F_1' ('Ontario 7710' × 'Rehovot 13'). Seeds were obtained from the Department of Field Crops and Vegetables, Faculty of Agriculture, Rehovot.

Bacteria were grown in yeast−peptone broth for 24 h in a rotary shaker (200 rpm). The cultures were harvested by centrifugation, washed twice in sterile saline (0.85% NaCl). Bacterial number was adjusted to 10^9 colony-forming units/mL and 10^6 CFU/mL was used as inoculum.

Tomato seeds were sown in a mixture containing vermiculite and vulcanic dust (0.1- to 8-mm particle size) (1:1, v/v) in 1-L plastic pots irrigated once a week with Hoagland's nutrient solution. Plants were grown throughout the experiments in an air-conditioned greenhouse at 25±3°C under natural illumination. Plants were inoculated after the development of the third leaf. Before inoculation, plants were incubated under partial mist conditions (which kept the leaves wet) at 25±2°C for 24 h. Plants were inoculated by spraying with the bacterial suspension until runoff. After inoculation the plants were kept in the mist chamber for an additional 96 h (time of beginning of symptom formation) or as indicated in the text (Bashan *et al.* 1978).

Disease severity was estimated using an index of 0, no symptoms; 1, 1−5 specks/leaflet, either clustered or spread over the whole leaflet; 2, 6−10 specks/leaflet; and 3, more than 10 specks/leaflet, indicating heavy infection. Number of specks per leaflet were counted separately. Lesion number was determined for the third, fourth, and fifth upper leaves of each plant and the mean of the three leaves was considered as the disease index (DI) of the plant (Yunis *et al.* 1980).

When testing for PO and polyphenoloxidase (PPO) activities at different leaf ages, in plants with four to six true leaves, the first two true leaves and the leaves below the growing tips were used separately. Leaves were injured by brushing with carborundum (300 grit) powder, using a soft brush. When carborundum injury and inoculation were combined, leaves were injured 48 h before inoculation. Sites on leaves for determining peroxidase location were the necrotic area (including 1−2 mm leaf tissue surrounding necrosis); leaf areas 1−2 cm from the 2-mm necrotic lesions; apparently healthy leaflets on diseased plants and leaflets from healthy plants.

Plants (susceptible and resistant) were separated into groups showing the same DI (approximately 20 plants per DI unit; half disease index unit was obtained by mixing the same weight of leaves from two following levels of disease index 8 days after inoculation). Resistant plants produced disease at the level of only 0, 0.5, and 1.0 DI units (details given in Fig. 5). In another experiment, 20 tomato cultivars were tested for their natural resistant to *P. syringae* pv. *tomato* (20 plants per cultivar, mean DI of each cultivar was measured).

Bacteria that developed inside the plant tissue were counted by the method described by Sharon *et al.* (1982).

Enzyme extraction

Leaves (20−25 leaflets per sample) were homogenized in 0.06 *M* potassium phosphate buffer, pH 7.0, at 0°C, first with an Ultra-turrax cell disrupter (Janke and Kunkel), then an Omnimixer (Sorvall), again with an Ultra-turrax, and finally with a fine homogenizer (Elda).

The homogenate obtained was centrifuged at 12 000 × *g* for 10 min and the supernatant was subjected to two further centrifugations at 30 000 × *g* for 30 min at 4°C. All samples were partially purified by gel filtration first on a G-25 and then on a G-200 Sephadex column (61 × 2.5 cm, elution rate 0.15−0.2 mL/min, 0.06 *M* potassium phosphate buffer, pH 7.0). Fractions showing activity were lyophilized and redissolved in the same buffer before testing.

Enzyme assays

Peroxidase activity was measured according to Lee (1973). The reaction mixture contained 0.5 mL of 0.06 *M* potassium phosphate buffer, pH 5.8; 0.4 mL of solution prepared from 0.2 mL of 1% guaiacol dissolved in absolute ethanol and 0.2 mL of 15 m*M* H_2O_2 dissolved in distilled water; and 0.1 mL enzyme preparation. This mixture was incubated 20 s at 25°C in the spectrophotometer (Ultrospec 4050, LKB) before absorbance at 470 nm was recorded. Mixtures without enzyme, without substrate, with boiled enzyme, or with a commercial enzyme preparation were used as controls. Enzyme units were defined as increase in 0.1 absorbance units · s^{-1} · mg $protein^{-1}$.

Polyphenoloxidase was measured according to Leonard (1971). The reaction mixture contained 0.5 mL 0.03 *M* potassium phosphate buffer, pH 6.5; 0.4 mL of 5 m*M* 3,4-dihydroxyphenylalanine (DOPA) dissolved in the same buffer; and 0.1 mL enzyme preparation. The mixture was incubated for 2 min at 30°C and the absorbance at 475 nm recorded. Controls were as described for peroxidase measurement, including commercial phenolase. Enzyme units were defined as increase in 0.1 absorbance units · min^{-1} · mg $protein^{-1}$.

Electrophoresis and isozyme staining

PO and PPO isozyme were analyzed using Davis' (1964) method of gel electrophoresis. After a prerun (1 mA per sample site), 20-μL samples containing 10 μg protein, were subjected to electrophoresis on 7% polyacrylamide gels at 150 V, for 3 h. Peroxidase and polyphenoloxidase isozymes were visualized by the benzidine−H_2O_2 (Brewer and Sing 1970) and DOPA staining methods (Stafford and Galston 1970), respectively. Stained gels were immediately scanned on a spectrophotometer (Varian 635).

Phenol extraction, absorption, and supplementation to bacterial cultures

Phenol samples, extracted by the ethanol−hexane−water method (Walter and Purcell 1979), were absorbed to polyvinylpyrrolidone (PVP) by adding them to methanol−water (1:4) mixture and setting the pH at 3.5 with 1 *M* HCl. Ten millilitres of the mixture was combined with 0.25 g PVP and vigorously shaken for 1 h. After phenol absorption by PVP and removal of the pellet by centrifugation at 30 000 × *g* for 10 min, the methanol was evaporated under reduced pressure at 35°C. The final solution was neutralized with 0.1 *M* NaOH before testing. The reducing compound dithioerythritol (DTT) (15 mg/L) and ethylenediaminetetraacetic acid (EDTA) (10^{-3} *M*), which neutralizes PPO, were added to other phenol extractions.

Analytical methods

Total protein content of extracts was analyzed by the Coomassie-blue method (Sedmak and Grossberg 1977) and phenols by the $FeCl_3$−$K_3Fe(CN)_6$ method (Kritzman and Chet 1980).

Statistical analysis

All experiments were repeated two or three times, with five replicates, using 20 plants or 20−25 leaves or three enzymatic reactions per replicate. Results given are from a representative experiment. Correlation was done by linear regression. Significance is given by $P \leq 0.05$ in Duncan's multiple-range test.

Results

Changes in PO and PPO activity during disease development

Significant increases in PO specific activity were found in both resistant and susceptible inoculated plants 24 h after inoculation with no significant differences between the two types of plants. PO activity reached its maximum 72 h after inoculation. Healthy plants, resistant or susceptible, showed constant low activity throughout the experiment (Fig. 1*a*). PPO activity also increased in inoculated plants but was significantly higher in resistant plants, reaching the highest level 48 h after inoculation (Fig. 1*b*). At this time no visible symptoms were observed in the tested tissue. PPO activity in infected sus-

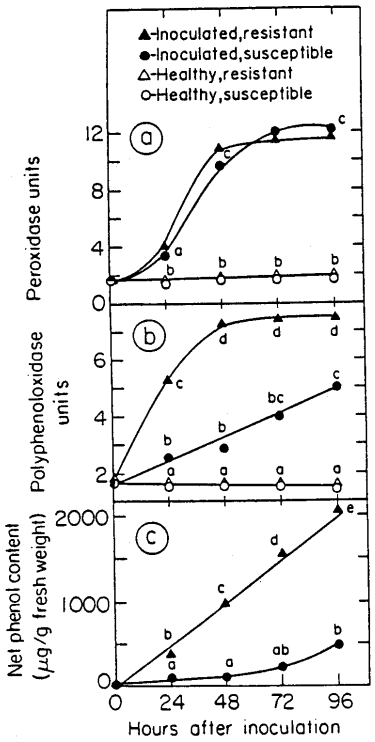

FIG. 1. Peroxidase (*a*) and polyphenoloxidase (*b*) activity and net phenol accumulation (*c*) in resistant and susceptible tomato plants inoculated with *P. syringae* pv. *tomato*. Experiments described in Figs. 1, 2, 3, and 4 were repeated three times with five replicates, using 20 leaflets as a replicate. Points on the graphs (in each subfigure, separately) followed by different letters differ significantly at $P \leq 0.5$.

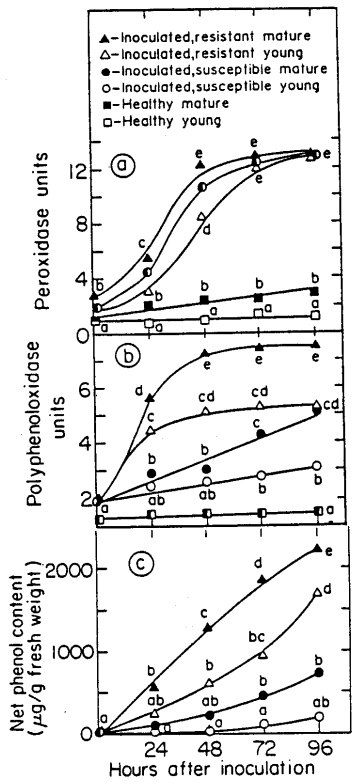

FIG. 2. Effect of leaf age on peroxidase (*a*) and polyphenoloxidase (*b*) activity and on net phenol accumulation (*c*). Points on the graphs (in each subfigure, separately) followed by different letters differ significantly at $P \leq 0.05$.

ceptible plants increased constantly until symptom appearance about 96 h after inoculation (Fig. 1*b*). Phenol content of both cultivars increased during the symptomless phase of disease development and was significantly higher in the resistant plants throughout the experiment (Fig. 1*c*). The phenol content of healthy plants was very low and constant throughout the experiment.

PO activity increased more rapidly in inoculated resistant mature leaves, compared with young ones, reaching its maximum 48 h after inoculation. After 72 h, this activity reached the same level in both mature and young leaves. No difference in PO activity was detected in inoculated susceptible mature and young leaves. Activity in healthy mature leaves was higher than in younger ones. Nevertheless, it was low in healthy leaves throughout the experiment (Fig. 2*a*).

Generally, the highest PPO activity was found in mature leaves of both resistant and susceptible cultivars. However, the infected susceptible cultivar exhibited a significantly lower activity than the infected resistant cultivar (Fig. 2*b*). A similar pattern was observed in phenol accumulation (Fig. 2*c*). The differences in disease index between young and mature leaves from both resistant and susceptible cultivars towards *P. syringae* pv. *tomato* inoculation at the time of symptom formation were as follows: inoculated susceptible young plants exhibited a DI of 2.87 and inoculated susceptible mature plants of 2.15, whereas resistant young plants exhibited a DI of 0.51 and inoculated resistant mature plants of 0.18. Healthy uninoculated mature or young plants showed no bacterial speck symptoms.

PO activity was high only in areas very close to the necrotic spot formed 100–120 h after inoculation, both in infected susceptible and resistant plants, decreasing markedly 2 cm away from it; activity was negligible in the apparently healthy leaflet on diseased plant (Table 1). Similarly, highest PPO activity was also found close to the necrotic spot. PPO activity and phenol content were higher in infected resistant plants than in uninoculated healthy plants; it was detected far from necrosis and even in apparently healthy leaflets. On the other hand, in inoculated susceptible plants, PPO activity and phenol content were relatively high only very close to the necrotic spot, decreasing in areas far from necrosis (Table 1).

Effect of leaf injury on PO and PPO activity and phenol content

Preliminary observations have shown that artificial leaf

TABLE 1. Location of peroxidase and polyphenoloxidase activity and phenol content in tomato leaf tissue

Leaf site	Plant type	PO activity (units)	PPO activity (units)	Phenol content (μg/g fresh weight)	DI of the leaf* (0–3)
Average activity of all plant leaves from diseased plants	S†	3.86*b**‡	4.96*b*	480*b*	2.61*a*
	R†	3.61*b*	7.60*ab*	2020*a*	0.52*b*
Area surrounding the necrotic spot§	S	11.4*a*	4.18*b*	540*b*	2.85*a*
	R	10.8*a*	9.02*a*	2100*a*	0.75*b*
2 cm in diameter away from the necrotic spot‖	S	3.2*b*	2.48*c*	510*b*	2.74*a*
	R	3.4*b*	6.17*ab*	1740*a*	0.85*b*
Apparently healthy leaflet on inoculated plant	S	1.95*c*	2.68*c*	370*b*	0*c*
	R	2.06*c*	5.71*b*	1630*a*	0*c*
Leaflet from healthy plant	S	1.80*c*	1.21*c*	40*c*	0*c*
	R	1.78*c*	1.32*c*	30*c*	0*c*

NOTE: Experiment was carried out twice in five replicates each. Results are from a representative experiment.
*Mean disease index of leaves from which samples were taken.
†S, susceptible plants, cv. VF-198; R, resistant plants, cv. Rehovot 13.
‡Numbers followed by different letters in each column differ significantly at $P \leq 0.05$ using Duncan's multiple-range test.
§Leaf discs (6 mm in diameter including a necrotic spot in the middle).
‖Leaf discs obtained from the same leaflet by cork borer.

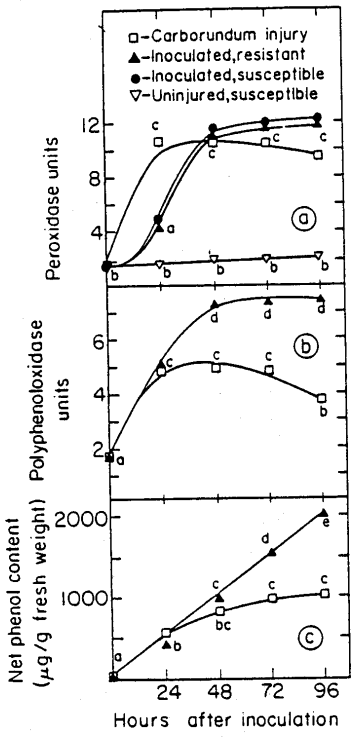

FIG. 3. Effect of leaf injuries without inoculation, or inoculation with *P. syringae* pv. *tomato* on peroxidase (*a*) and polyphenoloxidase (*b*) activity and on net phenol accumulation (*c*) in susceptible and resistant tomato plants. Points on the graphs (in each subfigure, separately) followed by different letters differ significantly at $P \leq 0.05$. Carborundum injury refers only to resistant plants.

injuries can stimulate activity of the phenol-oxidizing system in tomato plants (Y. Bashan, Y. Okon, and Y. Henis, unpublished data).

In both the resistant and susceptible cultivars, PO activity markedly increased as a result of injuries alone. After 24 h, PO activity in these injured leaves was significantly higher when compared with PO activity in leaves inoculated with *P. syringae* pv. *tomato* or in healthy leaves. However, 48 h after inoculation, PO specific activity reached the same level in both injured and inoculated plants (Fig. 3*a*).

In the uninoculated resistant cultivar, PPO activity (Fig. 3*b*) and phenol content (Fig. 3*c*) markedly increased during the first 24 h after injury, thereafter remaining stable or even slightly decreasing. In inoculated resistant plants PPO activity in the diseased tissues increased with time, reaching its maximum 48 h after inoculation (Fig. 3*b*).

Susceptible and resistant plants which exhibited high PO and PPO activities and phenol content, 48 h after injury, were inoculated with *P. syringae* pv. *tomato*. PO activity remained high in both resistant and susceptible plants and inoculation did not increase its activity (compare Figs. 3*a* and 4*a*). Throughout the experiment, PPO activity and phenol content in injured resistant inoculated plants was greater than that in injured resistant noninoculated plants (Figs. 4*b*, 4*c*). On resistant plants in which PPO activity was previously induced by injury, the DI after 8 days was significantly lower than in inoculated resistant plants only (DI of 0.06 compared with 0.43, respectively). Also there was a decrease in DI (from 2.83 to 1.91 units) when susceptible plants were injured before inoculation. Injured only or uninjured plants exhibited no symptoms. The same trends were obtained in susceptible plants but with lower PPO specific activity and phenol content (Figs. 4*b*, 4*c*).

Relationship among disease severity, number of P. syringae *pv.* tomato *cells, and PO and PPO activity and phenol content in* P. syringae *pv.* tomato *infected resistant and susceptible tomato plants*

Plants (susceptible and resistant) showing all ranges of visible DI (0.5–3.0) 8 days after inoculation, had similar PO

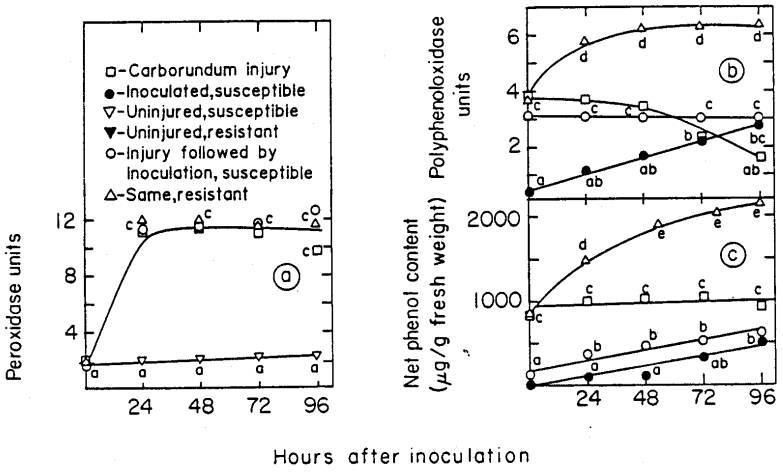

FIG. 4. Activity of PO (*a*) and PPO (*b*) and net phenol accumulation (*c*) after injuries followed by inoculation with *P. syringae* pv. *tomato* in resistant and susceptible plants. Points on the graphs (in each subfigure, separately) followed by different letters differ significantly at $P \leq 0.05$. Carborundum injury refers only to resistant plants.

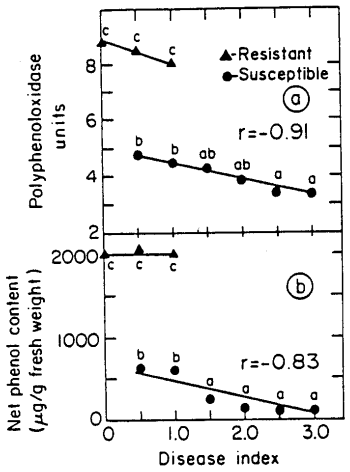

FIG. 5. Relationship between disease index and polyphenoloxidase (*a*) and net phenol content (*b*) in inoculated resistant and susceptible tomato plants. The experiment was repeated twice with five replicates using 20 plants per replicate. Points on the graphs (in each subfigure, separately) followed by different letters differ significantly at $P \leq 0.05$. All correlations are significant at $P \leq 0.05$.

activity (ranging from 11.85 to 13.85 units) and had no relation to susceptibility and resistance ($r = 0.25$). PPO activity and net phenol content decreased with the increase in DI in these plants. Additionally, PPO activity and phenol content in inoculated resistant plants were 1.8 and 4 times higher, respectively, than in inoculated susceptible plants (Figs. 5*a*, 5*b*).

A good correlation ($r = 0.96$) was found between the log number of *P. syringae* pv. *tomato* cells inside the tissue and PO activity in infected susceptible plants (Fig. 6*a*). Such a correlation was not found in inoculated resistant plants ($r = 0.25$).

A negative correlation ($r = -0.94$) was found in most cases between the degree of disease index, representing resistance, and PPO activity in 20 infected tomato cultivars (Fig. 6*b*). Similarly, the more resistant the cultivar, the higher was its total phenol content ($r = -0.85$) after infection (Fig. 6*c*). Before infection the phenol content of the 20 cultivars and PPO activity did not correlate with susceptibility and resistance ($r = -0.56$ and $r = -0.41$, respectively).

PO and PPO isozymes in healthy and diseased plants

Four different PO isozymes were detected in visibly infected (DI = 2.68) plants (Fig. 7*a*, 1) as compared with a single isozyme in uninoculated healthy ones (Fig. 7*a*, 2). No PO isozymes were found in bacterial cultures. Of the eight different PPO isozymes detected in the same diseased plants (Fig. 7*b*, 2), three (Fig. 7*b*, 2, bands 5, 7, 8) were also found in uninoculated healthy plants (Fig. 7*b*, 1), three others (Fig. 7*b*, 2, bands 4, 6, 9) were induced in the plant during disease development, and the remaining two (Fig. 7*b*, 2, bands 2, 3) were of bacterial origin (Fig. 7*b*, 3). A single isozyme (Fig. 7*b*, 3, band 1), which was produced in culture by the pathogen, did not appear in the diseased plant.

Effect of phenols, extracted from plants, on pathogen growth

The addition of plant phenols alone, extracted from inoculated resistant plants, to the growth medium did not affect pathogen growth. Adding phenols plus EDTA (a known PPO inhibitor) inhibited *P. syringae* pv. *tomato* growth. The addition of PVP (which adsorb phenols) or PVP plus EDTA did not affect bacterial growth, whereas the addition of phenols with EDTA and DTT, a treatment that maintained phenols as extracted without oxidation, did inhibit bacterial growth (Table 2).

Discussion

The role of phenols and their oxidation products has been

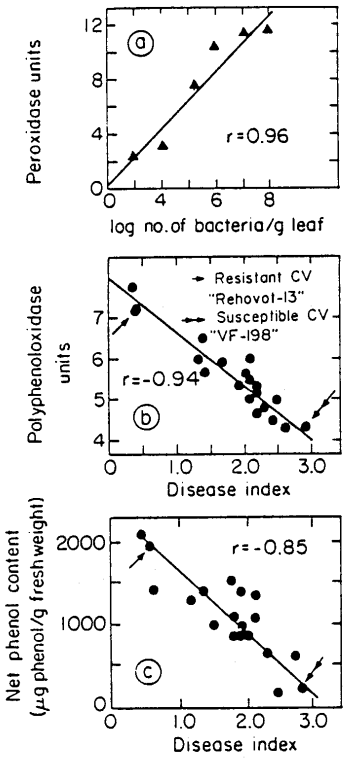

FIG. 6. (*a*) Correlation between number of *P. syringae* pv. *tomato* cells inside susceptible cv. VF-198 tissue and peroxidase activity. (*b*) Correlation between disease index of 20 tomato cultivars and polyphenoloxidase activity. (*c*) Correlation between net phenol content of 20 tomato cultivars and the disease index induced in these cultivars by *P. syringae* pv. *tomato*. The experiments were repeated twice in five replicates, using 20 plants per replicate. All correlations are significant at $P \leq 0.05$.

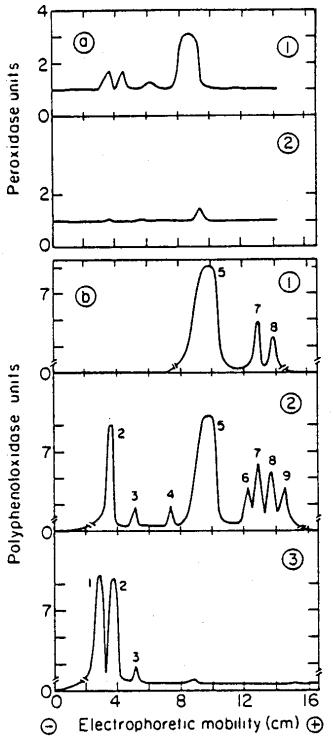

FIG. 7. (*a*) Isozymes and (or) multiple forms of peroxidase in diseased (*1*) and healthy (2) plants. (*b*) Isozymes and (or) multiple forms of polyphenoloxidase in healthy (*1*) and diseased (2) plants and in *P. syringae* pv. *tomato* (*3*). Peaks in each subfigure followed by the same numbers are identical.

extensively studied in fungal and viral diseases, but their role in bacterial diseases has received less attention. The results presented in this study reveal that, as in many other phytopathological systems, peroxidase activity increases in tomato leaves infected with *P. syringae* pv. *tomato*. However, a more detailed critical analysis of this activity and a study of the factors affecting peroxidase production show that peroxidase is apparently not directly related to the resistance mechanism of the plant. The above assumption is based on the following observations: activity increased to a similar extent in both resistant and susceptible tomato cultivars, showing different degrees of infection; similar activities were detected in both mature and young inoculated tomato leaves, which differ in their susceptibility to *P. syringae* pv. *tomato*; mechanical injuries induced higher PO activity than *P. syringae* pv. *tomato* infection; moreover, inoculating injured tomato plants did not enhance peroxidase activity, which was localized and could not be detected at a distance of 2 cm from the necrotic site. In addition, there was also a correlation between the number of bacterial cells developing in the infected tissue and PO activity. These findings of no correlation between PO activity and resistance in bacterial speck of tomato corroborate results reported by Nadolny and Sequeira (1980) for *P. solanacearum* in tobacco.

On the other hand, the possible involvement of the oxidative phenol system in resistance to bacterial speck of tomato was shown. PPO activity was higher in resistant than in susceptible cultivars. Mature leaves showed higher PPO activity and phenol content than the younger more susceptible leaves. Induction of PPO activity and phenol accumulation by mechanical injuries increased resistance. Furthermore, a direct correlation was found between disease severity and PPO activity and phenol content in the tissue, in addition to a direct correlation between resistance and PPO activity in 20 tomato cultivars ranging from the highly resistant to the highly susceptible. A supportive *in vitro* evidence was that nonoxidized phenols remaining as extracted from resistant plants inhibited pathogen growth. The resistant cultivar used in this study 'Rehovot-13' (an old commercial variety of the Marman type) is known to possess a high degree of field resistance to the disease (Yunis *et al.* 1980). This resistance is controlled by a single dominant gene in interaction with minor genes (Fallik *et al.* 1983). This type of resistance is not characterized by the hypersensitive reaction (HR). A population of this cultivar reacted towards *P. syringae* pv. *tomato* infection by producing diseased plants

TABLE 2. Effect of extracted phenols from inoculated resistant tomato plants and various inhibitors on pathogen growth*

Addition of extract (200 mg/mL)	Inhibitor			Lag phase (h)	Bacterial growth†
	PVP (3%)	DTT (15 mg/L)	EDTA ($10^{-3}M$)		
+	+	−	−	3	1.07
	−	+	+	7	0.42
	−	−	+	11	0.08
	+	−	+	3	1.05
	−	−	−	3	1.09
−	−	−	−		
	+	−	−	3	1.12
	−	+	−		
	−	−	+		

*Eight replicates.
†Measured as absorbance at 540 nm after 14 h.

exhibiting few symptoms or symptomless plants 5 days after inoculation. Multiplication of the pathogen in the inoculated plants resembled after 24 h its multiplication in susceptible 'VF-198' plants, but the number of bacterial cells per leaf is much smaller. However, the population later steadily decreased to a low level at the time of symptom formation (Bashan *et al.* 1981). Thus, it seems that the resistance mechanism demonstrated in this study is not due to a total inhibition of pathogen growth as in HR but apparently is due to continuous inhibition of disease development.

Therefore, it may be suggested that resistance of tomato plants to *P. syringae* pv. *tomato* involves oxidation processes related to the phenols system.

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BASHAN, Y., Y. OKON, and Y. HENIS. 1978. Infection studies of *Pseudomonas tomato*, causal agent of bacterial speck of tomato. Phytoparasitica, **6**: 135–143.

BASHAN, Y., E. SHARON, Y. OKON, and Y. HENIS. 1981. Scanning electron and light microscopy of infection and symptom development in tomato leaves infected with *Pseudomonas tomato*. Physiol. Plant Pathol. **19**: 139–144.

BREWER, G. J., and C. F. SING. 1970. An introduction to isozyme techniques. Peroxidase. Academic Press, New York, pp. 102–104.

DAVIS, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 409–427.

FALLIK, E., Y. BASHAN, Y. OKON, A. CAHANER, and N. KEDAR. 1983. Inheritance and sources of resistance to bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato*. Ann. Appl. Biol. **102**: 365–371.

FARKAS, G. L., and Z. KIRÁLY. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathol. Z. **44**: 105–150.

FEHRMANN, H., and A. E. DIAMOND. 1967. Peroxidase activity and *Phytophthora* resistance in different organs of the potato plant. Phytopathology, **57**: 68–72.

FRIČ, F. 1976. Oxidation enzymes. Encycl. Plant Physiol. New Ser. **4**: 617–631.

GOODMAN, R. N., Z. KIRÁLY, and M. ZAITLIN. 1967. The biochemistry and physiology of infectious plant disease. Van Nostrand Reinhold Company, New York.

KOSUGE, T. 1969. The role of phenolics in host response to infection. Annu. Rev. Phytopathol. **7**: 195–222.

KRITZMAN, G., and I. CHET. 1980. The role of phenols in the pathogenicity of *Botrytis allii*. Phytoparasitica, **8**: 27–37.

KUĆ, J. 1966. Resistance of plants to infectious agents. Annu. Rev. Microbiol. **20**: 337–370.

LEE, T. T. 1973. On extraction and quantitation of plant peroxidase isozymes. Physiol. Plant. **29**: 198–203.

LEONARD, T. J. 1971. Phenoloxidase activity and fruiting body formation in *Schizophyllum commune*. J. Bacteriol. **106**: 162–167.

LOVREKOVICH, L., H. LOVREKOVICH, and M. A. STAHMANN. 1967. The importance of peroxidase in the wildfire disease. Phytopathology, **58**: 193–198.

NADOLNY, L., and L. SEQUEIRA. 1980. Increase in peroxidase activity is not directly involved in induced resistance in tobacco. Physiol. Plant Pathol. **16**: 1–8.

NOVACKY, A., and R. E. HAMPTON. 1968. Peroxidase isozymes in virus infected plants. Phytopathology, **58**: 301–305.

SEDMAK, J. J., and S. E. GROSSBERG. 1977. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. Anal. Biochem. **79**: 544–552.

SEEVERS, P. M., and J. M. DALY. 1970. Studies on wheat stem rust resistance controlled at the Sr6 locus. II. Peroxidase activity. Phytopathology, **60**: 1642–1647.

SEQUEIRA, L. 1983. Mechanisms of induced resistance in plants. Annu. Rev. Phytopathol. **37**: 51–79.

SHARON, E., Y. OKON, Y. BASHAN, and Y. HENIS. 1982. Detached leaf enrichment: a method for detecting small numbers of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in seeds and symptomless leaves of tomato and pepper. J. Appl. Bacteriol. **53**: 371–377.

STAFFORD, H. A., and A. W. GALSTON. 1970. Ontogeny and hormonal control of polyphenoloxidase isozymes in tobacco pith. Plant Physiol. **46**: 763–767.

TOMIYAMA, K. 1963. Physiology and biochemistry of disease resistance of plants. Annu. Rev. Phytopathol. **1**: 295–316.

WALTER, W. M., JR., and A. E. PURCELL. 1979. Evaluation of several methods for analysis of sweet-potato phenolics. J. Agric. Food Chem. **27**: 942–946.

YUNIS, H., Y. BASHAN, Y. OKON, and Y. HENIS. 1980. Two sources of resistance to bacterial speck of tomato caused by *Pseudomonas tomato*. Plant Dis. **64**: 851–852.