Temperature and Gamma Irradiation Effects on Scoparone, a Citrus Phytoalexin Conferring Resistance to *Phytophthora citrophthora*

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ABSTRACT

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The effects of gamma irradiation and temperature of incubation on accumulation of the phytoalexin scoparone were investigated in citrus inoculated with the fungus *Phytophthora citrophthora.* In all the tested citrus species, scoparone concentration in the branches was higher and lesion length was shorter when the incubation temperature after inoculation with *P. citrophthora* was 28 rather than 20 C. The temperature effect was pronounced, especially in inoculated branches of rough lemon, which after 4 days at 28 C had a scoparone concentration of 290 μg/g fresh weight, compared with 42 μg/g fresh weight at 20 C. The comparable lesion length in this species was 5.2 mm at 28 C compared with 11.0 mm at 20 C. Gamma irradiation affected citrus resistance only at 300–400 Krad. Maximum scoparone concentration in inoculated branches accumulated after irradiation with 400 Krad and reached 970 and 530 μg/g fresh weight in macrophylla and sour orange, respectively, and 100 and 82 μg/g fresh weight in rough lemon and shamouti, respectively. In the fruit, a maximum scoparone concentration of 94.7 μg/g fresh weight accumulated in inoculated grapefruit after irradiation with 400 Krad.

The phytoalexin scoparone (6, 7-dimethoxycoumarin) is involved in defense mechanisms of citrus against several pathogens such as *Phytophthora citrophthora* (Smith & Smith) Leonian (1,4,19), *Guignardia citricarpa* Kiely (8), *Penicillium digitatum* Sacc. (13), and *Diaporthe citri* (Faw.) (5,6). Scoparone inhibited growth of various phytopathogenic fungi in vitro (1,4).

Low concentrations of scoparone exist naturally in healthy citrus bark and fruit peels (1,20). Scoparone concentrations in

citrus tissue increased rapidly following inoculation with pathogens (1,6,8,19), as well as after treatment with gamma irradiation or ultraviolet (UV) light (9,13,15,17). However, high soil salinity decreased the accumulation of scoparone and increased susceptibility of three citrus rootstocks tested to *P. citrophthora* (18). Scoparone accumulated in lemon fruit after inoculation with *P. digitatum,* but much greater increases were found in the inoculated fruits after heat treatment at 36 C for 3 days (13). After inoculation of citrus bark with *P. citrophthora,* higher scoparone accumulated in the tissue when incubated at 28 than at 20 C (2). Treatments with chemicals such as fosetyl-Al and phosphorous acid increased scoparone concentration in citrus bark inoculated with *P. citrophthora* much more than in nontreated citrus (3). The purpose of this study was to determine the effect of gamma irradiation and high temperature on scoparone concentration in citrus bark and peel, and on citrus resistance against *P. citrophthora.*

MATERIALS AND METHODS

Fungal and plant material. The fungus *P. citrophthora* (isolate C-16) was isolated from infected fruit grown in Lerrer Grove, Rehovot, Israel, in January, 1983. The fungus was cultured on potato-dextrose agar (PDA) at 25 C to serve as inoculum. The following 3-yr-old citrus seedlings from Kibutz Nezer Sereni Nursery, Israel, were grown outdoors and at 22–26 C in the greenhouse: *Citrus sinensis* (L.) Osbeck (shamouti); *C. aurantium* L. (sour orange); *C. jambhiri* Lush. (rough lemon); *Poncirus trifoliata* Raf. (trifoliate orange); *C. reticulata* Blanco × *C. sinensis* (niva); and *C. macrophylla* Webster (macrophylla). The following citrus fruits were picked from a 12-yr-old orchard located at the ARO Experiment Station, The Volcani Center, Bet Dagan, Israel: *C. paradisi* Macfadden (grapefruit); shamouti; sour orange, and *C. sinensis* (L.) Osbeck (valencia).

Inoculation. Incisions 3 mm long by 0.2–0.5 mm deep were cut with a sterile scalpel in the mature bark of 3-mo-old woody citrus branches (seedlings were pruned 3 mo before inoculation). Branches were 25–30 cm long and 7–10 mm thick, and four or five incisions were made on each branch. A 3-mm-diameter disk cut from an actively growing PDA culture of *P. citrophthora* was placed over the incisions, fungal side downward, and the inoculated branch sections were incubated in a humid chamber (RH ~98%) at 20 or 28 C in the dark. Mature fruits were inoculated by removing pieces of the flavedo with a 3-mm-diameter cork borer to a depth of 0.2–0.5 mm at five sites around the equatorial plane of the fruit. A 3-mm-diameter disk cut from an actively growing PDA culture of *P. citrophthora* was placed on the fruit wounds, fungal side downward, and the inoculated fruits were incubated in a humid chamber (RH ~98%) at 20 C in the dark.

Temperature effects. The production of scoparone and lesion length in the bark of 3-mo-old citrus branches, resistant (macrophylla, trifoliate, and sour orange), and susceptible (rough lemon, shamouti, and niva) to *P. citrophthora,* were recorded daily during incubation at 20 or 28 C, for 8 days following inoculation with the pathogen. Temperatures × species interaction effects on scoparone concentration and lesion length were measured on the fourth day after the inoculation because that is when scoparone concentration peaked (Table 1). Repeated measurements of scoparone concentrations and lesion length during the time of incubation were done in different branches.

Gamma irradiation. Gamma-ionizing irradiation was applied with Cobalt Radiant (^{60}Co). Branches and fruits were irradiated with doses of 0, 100, 200, 300, or 400 Krad and were inoculated with *P. citrophthora.* The temperature of incubation for both branches and fruit was 20 C. Regressions of scoparone concentration or lesion length or lesion area on irradiation in noninoculated or inoculated branches or fruits were calculated. Repeated measurements of scoparone concentrations and lesion length and area during the time of incubation were done in different branches and fruits.

Extraction, purification, and identification of scoparone. Slices

TABLE 1. Scoparone concentration ($\mu g\ g^{-1}$ fr wt) in noninoculated or inoculated citrus bark of species resistant (macrophylla, trifoliate orange, and sour orange) or susceptible (rough lemon, shamouti, and niva) to *Phytophthora citrophthora* at two temperatures of incubation

Incubation duration	Macrophylla		Trifoliate orange		Sour orange		Rough lemon		Shamouti		Niva	
(days)	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C
0	17 ± 1.9[a]	18 ± 2.0	16 ± 1.4	17 ± 2.2	15 ± 1.7	15 ± 1.9	14 ± 1.0	13 ± 2.1	12 ± 0.8	13 ± 0.7	12 ± 1.7	12 ± 1.2
1	45 ± 5.0	60 ± 7.4	32 ± 3.8	47 ± 5.3	29 ± 3.5	33 ± 4.0	14 ± 0.8	28 ± 3.3	13 ± 1.8	18 ± 1.1	12 ± 1.8	14 ± 1.2
2	148 ± 15.9	173 ± 20.7	170 ± 25.5	143 ± 13.2	90 ± 12.6	94 ± 11.7	25 ± 1.4	50 ± 8.0	22 ± 1.4	26 ± 3.4	20 ± 3.7	20 ± 2.3
3	300 ± 55.0	370 ± 44.2	256 ± 33.8	313 ± 46.3	170 ± 26.0	225 ± 31.3	32 ± 4.3	120 ± 21.1	27 ± 3.6	34 ± 2.7	24 ± 2.0	27 ± 3.5
4	440 ± 39.7	587 ± 71.2	415 ± 55.7	515 ± 88.1	250 ± 27.2	326 ± 26.7	42 ± 4.3	290 ± 42.0	31 ± 5.6	53 ± 5.4	28 ± 2.0	40 ± 6.3
5	405 ± 51.6	560 ± 48.0	390 ± 45.9	506 ± 53.6	235 ± 32.3	300 ± 24.2	40 ± 6.0	266 ± 15.1	30 ± 2.3	48 ± 3.5	25 ± 1.7	37 ± 7.7
6	370 ± 54.1	513 ± 47.8	347 ± 42.0	473 ± 32.7	218 ± 18.7	267 ± 37.4	38 ± 2.1	233 ± 35.4	27 ± 3.2	45 ± 5.3	23 ± 3.9	35 ± 2.4
7	341 ± 49.2	475 ± 38.1	300 ± 39.6	445 ± 38.0	200 ± 29.3	250 ± 26.5	35 ± 4.0	216 ± 16.7	22 ± 2.9	40 ± 3.6	19 ± 2.3	32 ± 2.9
8	305 ± 62.2	460 ± 73.6	270 ± 18.0	416 ± 71.1	176 ± 25.5	212 ± 14.8	29 ± 2.2	170 ± 31.3	18 ± 3.6	33 ± 2.3	16 ± 0.8	26 ± 1.7

Analysis of variance[b]

Source	df	MS	*F* value
Species (A)	5	428,865.1	283.8*[c]
Temperature (B)	1	153,217.1	101.4*
A × B	5	19,321.9	12.8*
Error	48	1,511.3	

Mean separation for scoparone concentration ($\mu g\ g^{-1}$ fr wt) on the fourth day after the inoculation

Species	20 C	28 C
Macrophylla	440 Ab	587 Aa[d]
Trifoliate orange	415 Ab	515 Ba
Sour orange	250 Bb	326 Ca
Rough lemon	42 Cb	290 Ca
Shamouti	31 Ca	53 Da
Niva	28 Ca	40 Da

[a] Mean of five replicates ± standard error.
[b] Analysis of variance was conducted for the fourth day after the inoculation.
[c] *F* test significant at $P < 0.01$*.
[d] Means followed by different uppercase letters within a column are significantly different among species, and means followed by different lowercase letters within a row are significantly different between temperatures, according to Fisher's protected least significant difference ($P = 0.05$).

(approximately 1.5 g per branch) of inoculated, necrotic (lesion) bark cut from the margins of the wounds were extracted with distilled water at 10 ml/g fresh weight tissue for 2 h at 40 C. Following partition with ethyl acetate (EtOAc), the predominant antifungal component (bioassays with *P. citrophthora* were done at every stage of purification) extracted from the inoculated bark was then concentrated by evaporating the solvent at 40 C in a Rotovac evaporator (Buchi Ltd., Switzerland). The crude concentrate was chromatographed on a silica gel H-column, 20 × 150 mm (70–230 mesh, ASTM Art. 7734, Kieselgel 60, E. Merck, Darmstadt, Germany), with increasing concentrations of EtOAc in petroleum ether (PE). The active ingredient was eluted with EtOAc/PE 1:1 (v/v), partitioned with $CHCl_3$ and then crystallized upon evaporation as colorless needles with a melting point of 146–147 C. The substance was analyzed by thin-layer chromatography (TLC) (0.5 mm, Art. 7730, Kieselgel 60 GF 254, E. Merck, Darmstadt, Germany). Ascending TLC was developed in a mixture of toluene/EtOAc 1:1 (v/v). The developed chromatogram was dried and then illuminated under UV light (350 nm), which revealed one spot at $R_f = 0.6$. Infrared analysis, ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra indicated that the active antifungal agent was 6,7-dimethoxycoumarin (scoparone) (1,4).

Scoparone quantification. Solutions of scoparone in distilled water were used to prepare a standard curve for quantification of scoparone concentrations in inoculated and noninoculated citrus bark and peel. Spectrofluorometry of these solutions revealed an excitation peak at 340 nm and an emission peak at 430 nm. Samples (1 g fresh weight) of inoculated and noninoculated citrus bark and peel were first extracted in 10 ml of distilled water for 2 h at 40 C and then concentrated to a volume of 1 ml. Samples were then chromatographed on TLC plates with a mixture of toluene/EtOAc 1:1 (v/v). Developed chromatograms were dried and illuminated with UV light. The scoparone band at $R_f = 0.6$ of each sample was scraped from the TLC plates into a beaker (one sample per beaker), and the scoparone of each sample was reextracted with 10 ml of distilled water. Solutions were then analyzed spectrofluorometrically with excitation at 340 nm and emission at 430 nm. Concentrations of scoparone in the tissue were estimated by interpolation on the standard curve.

All experiments were conducted in a completely randomized design. Data were analyzed by ANOVA procedure and regression analysis with the Statistical Analysis System (SAS) package (Cary, NC). Experiments were conducted three times, and similar results were obtained (differences in each treatment were less than 15%). Each treatment was replicated five times, with a replicate consisting of 10 branches or fruits. The results of the third experiment are presented in this paper.

RESULTS

Scoparone was induced in two groups of citrus species, resistant (macrophylla, trifoliate, and sour orange) and susceptible (rough lemon, shamouti, and niva) to *P. citrophthora.* Four days after the inoculation, scoparone concentration was significantly higher in the resistant than in the susceptible species (Table 1). Lesion length in the bark 4 days after the inoculation was significantly greater in the susceptible than in the resistant species (Table 2).

Temperature effects. Species × temperature interaction effects on scoparone concentration and lesion length were significant on the fourth day after the inoculation (Tables 1 and 2). Temperature effect was especially marked in rough lemon, in which scoparone concentration after 4 days at 28 C was approximately seven times that at 20 C. Lesion length in the bark of rough lemon at 20 C 4 days after the inoculation was twice that at 28 C. Scoparone concentrations in the other species at 28 C increased by 33, 24, and 30% in macrophylla, trifoliate, and sour orange, and by 70 and 40% in shamouti and niva, respectively, as compared with respective scoparone concentrations at 20 C (Table 1). Lesion length in the bark 4 days after the inoculation at 20 C was 1.9, 1.7, and 1.4 times that at 28 C for macrophylla, trifoliate, and sour orange, respectively, and 1.5 times that in shamouti and niva 28 C (Table 2).

TABLE 2. Lesion length (mm) in noninoculated or inoculated citrus bark of species resistant (macrophylla, trifoliate orange, and sour orange) or susceptible (rough lemon, shamouti, and niva) to *Phytophthora citrophthora* at two temperatures of incubation

Incubation duration	Macrophylla		Trifoliate orange		Sour orange		Rough lemon		Shamouti		Niva	
(days)	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C	20 C	28 C
1	1.2 ± 0.09[a]	0.6 ± 0.1	1.3 ± 0.24	1.2 ± 0.20	1.4 ± 0.11	1.5 ± 0.23	2.3 ± 0.35	2.1 ± 0.18	2.2 ± 0.22	2.2 ± 0.27	2.3 ± 0.40	2.5 ± 0.23
2	2.5 ± 0.35	0.8 ± 0.05	3.0 ± 0.52	1.4 ± 0.09	3.5 ± 0.30	2.3 ± 0.30	5.0 ± 0.40	3.4 ± 0.63	6.1 ± 0.13	4.6 ± 0.45	6.5 ± 0.89	5.4 ± 0.97
3	2.5 ± 0.41	1.1 ± 0.09	3.2 ± 0.56	1.6 ± 0.21	4.5 ± 0.47	3.2 ± 0.55	8.2 ± 0.30	4.2 ± 0.66	11.3 ± 1.90	7.5 ± 0.15	12.0 ± 0.08	8.8 ± 0.10
4	2.5 ± 0.36	1.3 ± 0.20	3.2 ± 0.19	1.9 ± 0.22	5.0 ± 0.72	3.5 ± 0.48	11.0 ± 1.21	5.2 ± 0.87	15.5 ± 3.20	10.8 ± 2.60	17.0 ± 0.97	11.7 ± 2.11
5	2.5 ± 0.25	1.3 ± 0.14	3.2 ± 0.32	1.9 ± 0.12	5.0 ± 0.23	3.5 ± 0.27	14.1 ± 1.19	5.2 ± 0.45	16.7 ± 3.37	13.5 ± 1.01	17.5 ± 2.34	15.4 ± 1.01
6	2.5 ± 0.20	1.3 ± 0.16	3.2 ± 0.22	1.9 ± 0.31	5.0 ± 0.89	3.5 ± 0.81	15.4 ± 2.99	5.2 ± 0.68	19.0 ± 2.23	19.6 ± 1.17	21.0 ± 3.39	22.0 ± 2.72
7	2.5 ± 0.30	1.3 ± 0.13	3.2 ± 0.46	1.9 ± 0.08	5.0 ± 0.29	3.5 ± 0.29	17.0 ± 2.80	5.2 ± 0.53	23.5 ± 4.51	22.3 ± 1.65	24.6 ± 1.93	24.3 ± 2.55
8	2.5 ± 0.33	1.3 ± 0.20	3.2 ± 0.30	1.9 ± 0.14	5.0 ± 0.40	3.5 ± 0.40	19.6 ± 3.44	5.2 ± 0.50	25.0 ± 3.15	26.4 ± 3.17	28.6 ± 2.56	29.1 ± 5.77

Analysis of variance[b]

Source	df	MS	*F* value
Species (A)	5	289.7	139.5*[c]
Temperature (B)	1	161.4	77.7*
A × B	5	11.8	5.7*
Error	48	2.1	

Mean separation for lesion length (mm) on the fourth day after the inoculation

Species	20 C	28 C
Niva	17.0 Aa	11.7 Ab[d]
Shamouti	15.5 Aa	10.8 Ab
Rough lemon	11.0 Ba	5.2 Bb
Sour orange	5.0 Ca	3.5 BCa
Trifoliate orange	3.2 Ca	1.9 Ca
Macrophylla	2.5 Ca	1.3 Ca

[a] Mean of five replicates ± standard error.
[b] Analysis of variance was conducted for the fourth day after the inoculation.
[c] *F* test significant at $P < 0.01$*.
[d] Means followed by different uppercase letters within a column are significantly different among species, and means followed by different lowercase letters within a row are significantly different between temperatures, according to Fisher's protected least significant difference ($P = 0.05$).

In the noninoculated branches the concentration of scoparone was 12–18 μg/g fresh weight (at both temperatures) and wounding had no effect on scoparone production.

Gamma irradiation effects. In all the species, scoparone concentration in noninoculated or inoculated gamma-irradiated (300 or 400 Krad) branches was higher than in the nonirradiated branches (Table 3). In noninoculated branches, scoparone concentration after irradiation at 400 Krad was approximately seven times as large in the resistant species (macrophylla and sour orange), and two to three times as large in the susceptible species (rough lemon and shamouti) as compared with the nonirradiated control (Table 3). A significant regression of scoparone concentration on irradiation in noninoculated and inoculated branches occurred for both the resistant and the susceptible species. However, a significant regression of lesion length on irradiation in inoculated branches occurred only for rough lemon (Table 3).

The concentration of scoparone in sour orange, valencia, shamouti, and grapefruit fruits, not inoculated or inoculated with *P. citrophthora,* and gamma irradiated at 400 Krad, was approximately three to five times as large than in nonirradiated fruits. Maximum concentration (94.7 μg/g fresh weight) was achieved in inoculated grapefruit. Infected area of nonirradiated grapefruit was approximately two to three times greater as compared with grapefruit lesion area following irradiation with 300 or 400 Krad. A significant regression of scoparone concentration on irradiation in noninoculated fruits occurred only for shamouti and in inoculated fruits for grapefruit and shamouti. A significant regression of lesion area on irradiation in inoculated fruits occurred for valencia and grapefruit (Table 4).

DISCUSSION

The phytoalexin scoparone is associated with citrus resistance against *P. citrophthora* (1,19). Furthermore, two compounds, fosetyl-Al and phosphorous acid, induced resistance to this pathogen in association with an increase in scoparone accumulation (3). Soil salinity, however, decreased concentration of scoparone and increased susceptibility of citrus to *P. citrophthora* (18). In a preliminary study (2), elevated temperature induced scoparone production in citrus compared with low temperature. In the present study, additional treatments were tested which influence the amount of scoparone in citrus. Results of this research show that treatments such as gamma irradiation and incubation at 28 C induced citrus resistance against *P. citrophthora* in association

TABLE 3. Scoparone concentration and lesion length in noninoculated (noninoc) or inoculated (inoc) citrus bark of species resistant (macrophylla and sour orange) or susceptible (rough lemon and shamouti) to *Phytophthora citrophthora* (branches gamma irradiated and measurements taken 4 days after inoculation at 20 C)

Dose (Krad)	Scoparone ($\mu g\ g^{-1}$ fr wt)		Lesion length (mm)
	Noninoc	Inoc	Inoc
Macrophylla			
0	18	451	2.6
100	17	443	2.7
200	20	467	2.4
300	80	630	1.0
400	127	970	0.8
r^{2a}	0.97*[b]	0.98**	0.88 NS
Regression model	$18 - 0.16X + 0.001X^2$	$466 - 1.16X + 0.006X^2$	$2.72 - 0.00044X - 0.00001X^2$
Sour orange			
0	17	242	5.3
100	16	257	4.5
200	18	253	5.0
300	83	380	2.5
400	115	530	2.0
r^{2a}	0.94*	0.98**	0.86 NS
Regression model	$15.6 - 0.11X + 0.0009X^2$	$249 - 0.45X + 0.0029X^2$	$5.2 - 0.0017X - 0.000017X^2$
Rough lemon			
0	17	38	10
100	16	42	11
200	17	45	11
300	35	73	6
400	57	100	4
r^{2a}	0.99**	0.98**	0.93*
Regression model	$17.6 - 0.081X + 0.00045X^2$	$38.7 - 0.048X + 0.0005X^2$	$10.2 + 0.014X - 0.000078X^2$
Shamouti			
0	14	32	13
100	15	28	12
200	15	35	14
300	24	50	9
400	34	82	6
r^{2a}	0.98**	0.99**	0.88 NS
Regression model	$14.5 - 0.028X + 0.00019X^2$	$32.4 - 0.11X + 0.00057X^2$	$12.6 + 0.014X\ 0.000079X^2$

[a] Coefficient of determination.
[b] * Significant at $P < 0.05$; ** significant at $P < 0.01$; NS, nonsignificant.

with an increase in scoparone in the citrus tissue. This study also indicates that scoparone concentration increases when noninoculated branches and fruits are irradiated. Similarly, ionizing radiation stimulated the accumulation of scoparone in the flavedo of grapefruit 7 days after irradiation with 300 Krad, but no scoparone was detected in nonirradiated grapefruit (15). Results of this research show that a small amount of scoparone exists naturally in the branches and fruits of various citrus species.

In another study, gamma irradiation increased the activity of phenylalanine ammonia-lyase (PAL) (16), a key enzyme in biosynthesis of coumarins and phenols in plants (10,12,14). This theory is supported by evidence showing that aminooxyacetic acid, a competitive inhibitor of PAL, suppressed scoparone production in citrus and was accompanied by nullification of resistance to *P. citrophthora* (1).

Gamma irradiation (300 and 400 Krad) increased scoparone concentration in both branches and fruits. In inoculated branches the increase in scoparone concentration was accompanied by a suppression of lesion elongation. However, in fruits, maximum decrease in lesion area was observed in grapefruit. A probable explanation for this is that in the inoculated and irradiated grapefruit fruit was the level of scoparone sufficient to inhibit the growth of the fungus in vivo.

Temperature effects on citrus resistance to *P. citrophthora* and *Hendersonula toruloidea* Nattrass have been reviewed (11). Lemon trees susceptible to *P. citrophthora* at 20 C were resistant at 28 C. The optimal temperature for mycelium growth of this pathogen in vitro is 26–28 C (11). Disease inhibition in citrus bark at 26–28 C seems to be caused by defense mechanisms of the host tissue. Results of this study suggest that the higher temperature (28 C) may increase citrus resistance against *P. citrophthora* by stimulation of scoparone production, more than at the lower temperature (20 C). However, at both temperatures, scoparone concentration reached a peak on the fourth day after inoculation and then declined. Such a pattern of accumulation and degradation is typical for phytoalexins in plants (7). Much more scoparone accumulated in lemon peel inoculated with *Penicillium digitatum* after incubation at 36 C than at 17 C, and incubation at 36 C prevented decay development in the inoculated fruit. Incubation at 17 C did not prevent decay development (13), although the optimum temperature for growth of *P. digitatum* in vitro is 24 C. However, both the 36 C treatment and scoparone

TABLE 4. Scoparone concentration and lesion area in citrus peels of valencia, sour orange, grapefruit, and shamouti noninoculated (noninoc) or inoculated (inoc) with *Phytophthora citrophthora* (fruits gamma irradiated and measurements taken 4 days after inoculation at 20 C)

Dose (Krad)	Scoparone ($\mu g\ g^{-1}$ fr wt)		Lesion length (mm^2)
	Noninoc	Inoc	Inoc
Valencia			
0	8	15	196
100	10	16	179
200	10	17	162
300	19	31	167
400	18	30	162
r^{2a}	0.82 NS[b]	0.82 NS	0.94*
Regression model	$7.63 + 0.02X + 0.000021X^2$	$14.1 + 0.019X + 0.000064X^2$	$196 - 0.21X + 0.00033X^2$
Sour orange			
0	8	13	171
100	8	16	170
200	9	14	191
300	17	33	167
400	18	35	163
r^{2a}	0.88 NS	0.85 NS	0.47 NS
Regression model	$7.48 + 0.0033X + 0.000064X^2$	$12.7 + 0.0067X + 0.00014X^2$	$169 + 0.13X - 0.00036X^2$
Grapefruit			
0	9	25	175
100	11	28	171
200	9	27	163
300	26	77	83
400	31	94	50
r^{2a}	0.89 NS	0.92*	0.94*
Regression model	$8.97 - 0.012X + 0.00018X^2$	$24 - 0.039X + 0.00056X^2$	$177 + 0.033X - 0.00093X^2$
Shamouti			
0	9	16	183
100	9	18	186
200	10	18	160
300	17	25	154
400	19	28	150
r^{2a}	0.92*	0.94*	0.86 NS
Regression model	$8.6 - 0.00057X + 0.00007X^2$	$16 + 0.0053X + 0.000064X^2$	$187 - 0.12X + 0.000043X^2$

[a] Coefficient of determination.
[b] NS, nonsignificant; * significant at $P < 0.05$; ** significant at $P < 0.01$.

accumulation may inhibit *P. digitatum* growth in vivo.

The largest quantity of scoparone was produced in citrus melanose spots and scars at 25 C, with progressively reduced levels at lower temperature until no scoparone was detected at 10 or 5 C (5). Similarly, results of the present study indicate that high temperature has a significant effect on scoparone production in rough lemon, which is considered susceptible to *P. citrophthora* (1,19). Rough lemon branches, which had a susceptible reaction at 20 C, reacted as a resistant species at 28 C. In this case scoparone concentration in the bark was seven times higher at 28 than at 20 C, in parallel with the increase in citrus resistance against *P. citrophthora.*

An understanding of the mechanism of resistance in citrus, as affected by high temperature, can help to explain the seasonal variations in citrus susceptibility. Furthermore, approaches such as high temperature and gamma irradiation treatments to increase resistance against diseases may replace or at least reduce the use of chemicals for the control of citrus pathogens.

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