

Change in susceptibility of satsuma mandarin fruit to sour rot pathogen (*Geotrichum candidum* citrus race) with relation to biochemical changes during maturation and storage

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Accepted for publication 2 May 1996

Change in susceptibility of satsuma mandarin fruit (*Citrus unshiu*) cultivar “Miyagawawase” to sour rot pathogen was studied with relation to biochemical changes during maturation and storage. The susceptibility of the fruit decreased with the advancement of maturity and was relatively constant during storage at 25°C for 4 wk. The young, green fruit that contained less total soluble solid, sugars and polyphenol, but more citric acid and water contents than mature, yellow fruit was more susceptible to sour rot pathogen. The susceptibility was correlated with total soluble solid, citric acid, sugars and water contents, but not with polyphenol. The results suggested that the difference in susceptibility during maturation was influenced to some extent by several constituents of fruit, although they may not be the only factors involved in susceptibility.

Key Words—biochemical changes; *Citrus unshiu*; maturity; sour rot pathogen; susceptibility.

Susceptibility of citrus fruits to sour rot incited by the citrus race of *Geotrichum candidum* Link has been shown to vary with the citrus species (Suprpta et al., 1996), ripeness of the fruit and storage period (Boudin and Eckert, 1982). A significant variability in susceptibility of lemon fruits, even within a fruit lot of uniform color harvested from a single row of trees, was observed by Boudin and Eckert (1982). The important factors influencing the susceptibility of lemon to sour rot were physiological age as measured by color change and storage time, and water content of the fruit. Yellow lemons were more susceptible to infection by *G. candidum* than green fruit harvested from the same groves at the same time, and susceptibility increased with storage time at 25°C.

Several changes occur during the maturation process of citrus fruits. The primary changes during maturation of orange were concentrations of total soluble solid and titratable acidity as citric acid (Kimball, 1984). In navel orange, the concentration of total soluble solid (sugars and organic acids) increased, while the concentration of citric acid decreased with maturity (Kimball, 1984). Several studies have investigated the changes of biochemical constituents of citrus fruits due to infection by pathogens (Babu and Reddy, 1989; Ramanjuku and Reddy, 1989; Babu and Reddy, 1990), but little study has been done on the correlation between the change in susceptibility of satsuma mandarin fruits to sour rot pathogen and changes in biochemical con-

stituents of fruit, especially sugars and citric acid during maturation and storage.

It has been shown in many experiments that a correlation may exist between the degree of resistance to pathogens and phenol content in plants (Goodman et al., 1967; Kiraly and Farkas, 1962; Lee and Tourneau, 1958; Martin et al., 1957; Snyder and Nicholson, 1990). However, little study has been done regarding the correlation between susceptibility of satsuma mandarin fruit to sour rot pathogen and polyphenol content in the fruit peel. This study was conducted to examine the change in susceptibility of satsuma mandarin fruit to sour rot pathogen, with relation to the changes in biochemical constituents of fruit during maturation and storage time.

Materials and Methods

Fruit Fruits of satsuma mandarin (*Citrus unshiu* Marc.) cv. “Miyagawawase” were obtained from Kagoshima Prefectural Fruits Experimental Station, Tarumizu, Kagoshima. Fruits were sampled at five stages of maturity from September to November 1995 at 2-wk intervals:

1. Maturity stage 1 (8 wk before harvest: 8WH)
2. Maturity stage 2 (6 wk before harvest: 6WH)
3. Maturity stage 3 (4 wk before harvest: 4WH)
4. Maturity stage 4 (2 wk before harvest: 2WH)
5. Maturity stage 5 (Harvest: H)

Sixty fruits were collected from two trees at each maturity stage and put in an ice box during transportation from field to laboratory. Collected fruits were washed with tap water and a commercial-grade detergent, and rinsed in distilled water before use for inoculation and chemical analysis. Inoculation and extraction of fruit components were done within 24 h after collecting the fruits. At maturity stage 5 (the stage at which fruits are commonly harvested), about 300 fruits were collected, 60 of them were immediately used, and the rest were packed in a carton and stored at 25°C (optimal temperature for development of sour rot), and periodically used for inoculation and chemical analysis at 1-wk intervals:

1. One wk storage (1WS)
2. Two wk storage (2WS)
3. Three wk storage (3WS)
4. Four wk storage (4WS).

For inoculation, fruits were lightly wiped with 95% ethanol after rinsing in distilled water.

Pathogen Ten isolates of *G. candidum* citrus race, i.e. S7, S31, S152, S161, S175, S181 (isolated from soils of citrus groves), Pt3, R16, Te2 and Tm2 (isolated from noncitrus fields), were used in this study (Suprapta et al., 1995). Small mycelial pieces from colonies on potato-dextrose agar (PDA) slants were transferred to new PDA slants and incubated for 5 d in the dark at 25°C. Arthrospores from 5-d-old cultures were harvested and put into sterile distilled water, and the suspension was filtered through sterile glass wool to eliminate hyphal fragments. The density of arthrospores was adjusted to 10⁷/ml prior to inoculation.

Inoculation and disease evaluation Inoculation was conducted within 24 h after collection of fruits at each maturity stage according to the method described previously (Suprapta et al., 1995). Fruits were washed with water and a commercial-grade detergent, washed with potable water, rinsed with distilled water and wiped lightly with 95% ethanol. One wound (3 mm diam and 5 mm depth) was made in the fruit peel using a sterile cork borer. Twenty µl of arthrospores suspension (10⁷ arthrospores/ml) was pipetted into the wound. Five fruits were inoculated with each isolate, and the inoculated fruits were incubated for 5 d at 25°C in the dark. Ten isolates were used for the inoculation test.

To determine the degree of susceptibility of fruit to infection with *G. candidum*, means of lesion diameter and percentage of rotted tissues of 50 fruits were evaluated 5 d after inoculation.

Determination of color value Six fruits for each maturity stage and storage time were tested for color value by the use of Color and Color Difference Meter TC-3600U (Tokyo Denshoku Co., Ltd.) with Optical Unit C-5120 (Tokyo Denshoku Co., Ltd.) within 8 h after collection. Standard color was white with value for brightness (L)=90, index for red and green colors intensity (a)=-0.2 and index for yellow and blue colors intensity (b)=2.4.

Determination of chemical constituents Fruits were separated into exocarp (peel) and endocarp (juicy tissues) within 10–24 h after collection. Juice was collected using a juicer, and total soluble solid (Brix) was determined using Hand Refractometer type N1 (Atago Co., Ltd.). Titratable acidity (as % citric acid) was determined by titration with 0.156 N NaOH, using 1.0% (w/v) phenolphthalein ethanol solution as indicator.

Sugar analysis was done for the peel and juicy parts of the fruit. Fresh samples of the peel (approx. 5 g) were squeezed by use of a mechanical press to obtain the sap, followed by filtration on a column of ion exchange resin Dowex 1-X2 200–400 mesh Cl⁻ form and 50 W-X8 200–400 mesh H⁺ form (Muromachi Chemical, Tokyo) and filtration with chromatic filter 13A (Shimadzu Co., Tokyo). This sample was stored at -20°C prior to analysis. The juicy part of fruit was treated by the same procedure as sap from peel. Sugars (sucrose, glucose and fructose) were separated by high pressure liquid chromatography (HPLC): Shimadzu LC-6A (Shimadzu Co., Tokyo), with water as the carrier at a flow rate of 1 ml per min, and then quantified using a refractive index detector Shimadzu RID 6A (Shimadzu Co., Tokyo).

For polyphenol quantification, the peel of fruit was cut out with a 10-mm diameter cork borer from the fruit equatorial zone. A 5-g sample of peel was then cut into small pieces with scissors, and ground in a mortar with quartz sand, to which 15 ml of 80% methanol was added for polyphenol extraction. The extract was separated from residue by centrifugation at 3,000 rpm for 10 min, followed by filtration with Whatman filter paper No. 41. The residue was re-extracted two additional times with 80% methanol to give a final volume of filtrate of 50 ml. The filtrate was then concentrated with a vacuum rotary evaporator to make the volume of 10 ml. Polyphenol content was determined according to Folin-Ciocalteu's method (Waterman and Mole, 1994). The absorbance was recorded at 760 nm using a UV-Visible Recording Spectrophotometer (UV-160A, Shimadzu Co., Tokyo). Catechin at a concentration of 10–100 µg/ml was used as standard compound.

Results

Color change and susceptibility to sour rot pathogen

Color of fruit changed from dark green at maturity stage 1 (8 wk before harvest) to yellow at maturity stage 5 (harvest) (Fig. 1). An increase of color value (L, a, b) was observed during maturation, after which the color was relatively constant during storage at 25°C for 4 wk (Table 1). Susceptibility of satsuma mandarin fruit to sour rot pathogen (expressed by lesion diameter and percentage of rotted tissues) differed significantly according to maturity stage (Fig. 1, Table 2). The young, green fruit with lower color value was more susceptible than the mature, yellow fruit with higher color value. The fruit at maturity stage 2 (6 wk before harvest), was most susceptible, with the mean lesion diameter and percentage of rotted tissues of 39.3 mm and 9.5%, respectively (Fig. 1B, Table 2), then the susceptibility decreased with

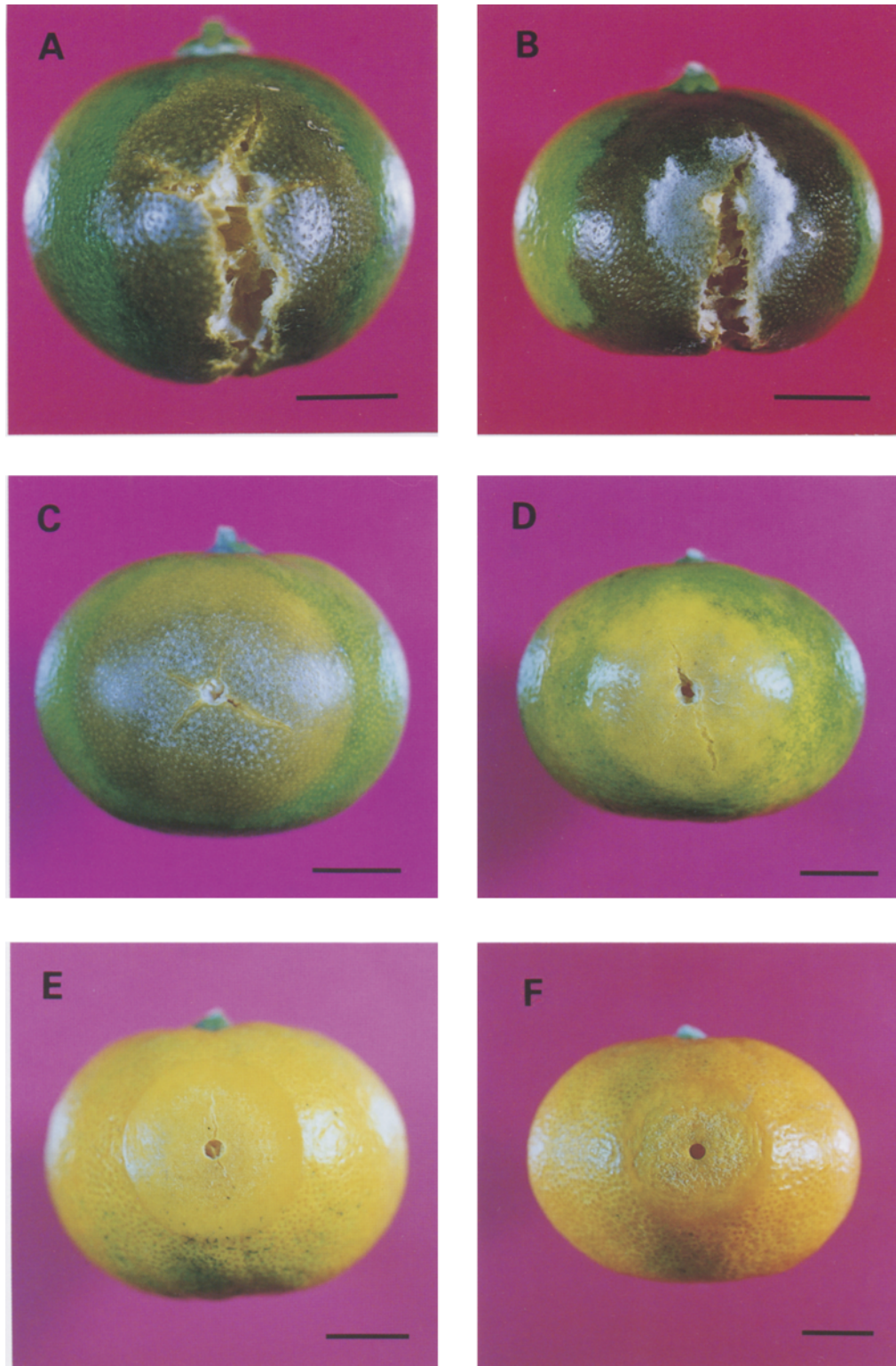


Fig. 1. Symptoms of sour rot on satsuma mandarin fruits at five stages of maturation and a 4-wk storage, 5 d after inoculation with *G. candidum* isolate S31.

A. Maturity stage 1; B. Maturity stage 2; C. Maturity stage 3; D. Maturity stage 4; E. Maturity stage 5; F. Four-wk storage. Bars represent 15 mm.

the advancement of maturation. As shown in Figs. 1A and 1B, on young, green fruit showing severe cracking of

the peel, the rot developed rapidly on both peel and juice sacs in response to infection by *G. candidum* citrus race.

Table 1. Changes in appearance (color, diameter and weight) of satsuma mandarin fruit during maturation and storage at 25°C.

Maturity/ storage time	Color value ^{a)}			Diam (mm)	Fresh weight (g)
	L	a	b		
Maturity					
Stage 1	31.7 ^{b)}	-11.1	22.1	54.8 ^{a^{c)}}	73.0 ^a
Stage 2	37.6	-10.6	25.1	60.3 ^b	96.0 ^b
Stage 3	44.2	-13.5	31.4	64.4 ^c	116.1 ^{cd}
Stage 4	51.2	-7.7	33.9	66.8 ^c	122.9 ^d
Stage 5	56.9	18.4	37.9	67.4 ^c	115.8 ^{cd}
Storage					
1 wk	54.5	22.3	37.7	67.0 ^c	114.3 ^{cd}
2 wk	54.4	24.4	34.1	66.4 ^c	112.9 ^c
3 wk	56.0	25.5	38.4	65.8 ^c	111.3 ^c
4 wk	53.7	26.2	37.0	66.0 ^c	110.6 ^c

a) L, Brightness; a, index for red and green intensity; b, index for yellow and blue intensity. Standard color was white (L=90, a=-0.2, b=2.4).

b) Average of 6 fruits.

c) Means followed by the same letters are not significantly different ($P=0.05$) according to Duncan's multiple range test.

No significant differences in susceptibility were observed with fruits in storage at 25°C up to 4 wk. In further discussion, data of rotted tissues per fruit will be used to ex-

Table 2. Changes in susceptibility of satsuma mandarin fruits to sour rot pathogen during maturation and storage at 25°C.

Maturity/storage	Susceptibility ^{a)}	
	Lesion diam (mm)	Rotted tissues (%)
Maturity		
Stage 1	25.58 ^{ab^{b)}}	6.59 ^e
Stage 2	39.27 ^d	9.47 ^d
Stage 3	35.32 ^{cd}	4.82 ^c
Stage 4	31.32 ^{bc}	3.48 ^{bc}
Stage 5	25.34 ^{ab}	1.44 ^a
Storage		
1 wk	20.36 ^a	1.01 ^a
2 wk	25.84 ^{ab}	1.69 ^a
3 wk	24.64 ^a	2.46 ^{ab}
4 wk	25.86 ^{ab}	2.35 ^{ab}

a) Inoculations were conducted within 24 h after collection of fruits at each maturity stage. Fruits were put in an ice box during transportation from field to laboratory. Collected fruits were then washed with tap water and detergent, washed with potable water, rinsed with distilled water and lightly wiped with 95% ethanol prior to inoculation. Lesion diameter and percentage of rotted tissues per fruit were examined 5 d after inoculation. Data are means of 50 fruits of 10 isolates (5 fruits were inoculated with each isolate).

b) Means followed by same letters are not significantly different ($P=0.05$) according to Duncan's multiple range test.

press the susceptibility of fruit to sour rot pathogen.

Biochemical changes During the process of maturation, total soluble solid (Brix) in juice of satsuma mandarin fruit gradually increased, while titratable acidity (as % citric acid) decreased (Fig. 2A). The total soluble solid was the lowest at maturity stage 1 (3.0%) and increased to 10.4% at maturity stage 5. In contrast, the citric acid was the highest at maturity stage 1 (1.7%) and decreased to 1.1% at maturity stage 5. During storage at 25°C, no marked changes were observed in total soluble solid and citric acid. The susceptibility of fruit to sour rot pathogen correlated with total soluble solid (r^2

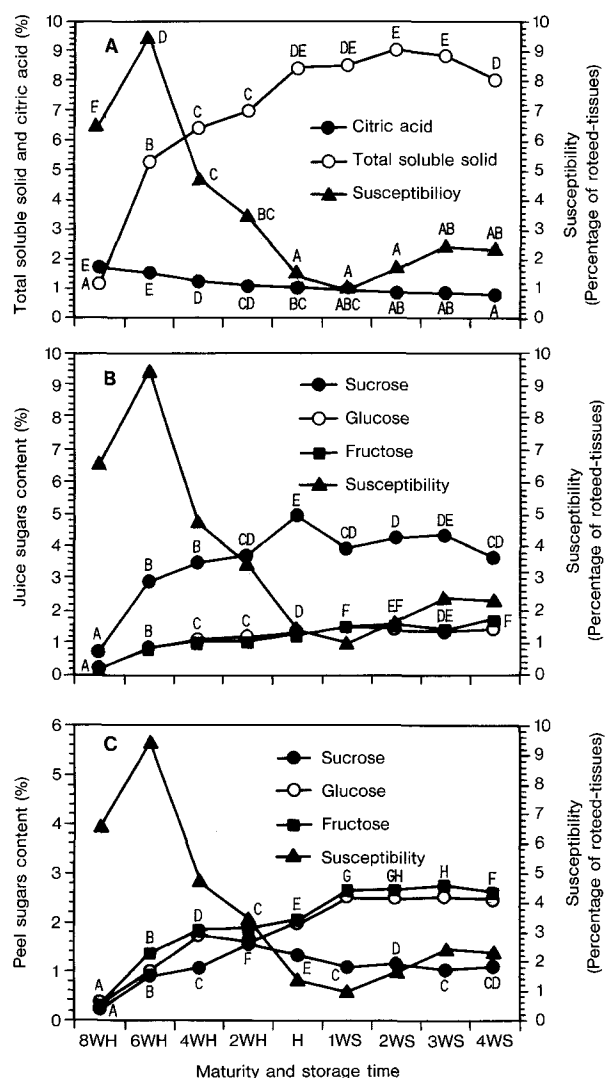


Fig. 2. Changes in citric acid and total soluble solid in juice (A), sugars content in juice (B), and sugars content in peel (C) of satsuma mandarin fruit during maturation and storage, and its susceptibility to *G. candidum*.

Susceptibility to *G. candidum* is expressed as the percentage rotted tissues of 50 fruits of 10 isolates. WH, Wk before harvest; H, Harvest; WS, Wk of storage. Points marked with the same letters are not significantly different ($P=0.05$) according to Duncan's multiple range test.

=0.54) and citric acid ($r^2=0.74$) (Table 4). Fruit which contained less total soluble solid and more citric acid was more susceptible to sour rot pathogen.

Contents of non-reducing sugar (sucrose) and reducing sugars (glucose, fructose) in the peel or juice of satsuma mandarin fruit increased during maturation (Figs. 2B, 2C). The sucrose content in juice increased from 0.9% at maturity stage 1 to 5.0% at maturity stage 5, and slightly decreased during storage time. In the fruit peel, sucrose content changed in a similar pattern as in the juice, but the content was much less in the peel. In the juice, the content of non-reducing sugar was higher than that of reducing sugar, but the opposite was found in the peel. Susceptibility to sour rot pathogen correlated with sucrose content in the juice ($r^2=0.49$) but did not correlate with sucrose in the peel ($r^2=0.27$). Glucose and fructose gradually increased during maturation and storage in both juice and peel (Figs. 2B, 2C), and correlated with susceptibility (Table 4). Fruit which contained less sugars was more susceptible to sour rot pathogen than the fruit with more sugars.

Peel water content decreased during maturation and storage, from 78% at maturity stage 1 to 75% at 4 wk storage (Fig. 3A) and correlated with susceptibility ($r^2=0.55$). On the other hand, polyphenol content in the peel gradually increased during maturation and storage, from 253.2 mg/100 g fresh weight at maturity stage 1 to

538 mg/100 g fresh weight at 4 wk storage (Fig. 3B). As shown in Fig. 3B, the relation between polyphenol content and susceptibility was irregular. From maturity stage 1 to maturity stage 4, polyphenol content paralleled the change in susceptibility, but from maturity stage 4 to 2 wk storage, the changes of the two were in opposite directions. To verify the effect of polyphenol extracted from the peel of satsuma mandarin on spore germination and mycelial growth of *G. candidum* citrus race, two isolates, S31 and Tm2, were grown in potato-dextrose (PD) broth containing 10% of peel extract of each maturity stage. However, the addition of peel extract into the culture did not obviously affect either spore germination or mycelial growth of this fungus (Table 3).

Discussion

The susceptibility of citrus fruit to citrus sour rot pathogen has been shown to vary with the species of citrus fruits (Suprapta et al., 1996). Baudoin and Eckert (1982) reported that the susceptibility of lemon to sour rot depended on the physiological condition of the fruit. Mature (yellow color, prolonged storage) and turgid lemons were more susceptible to sour rot pathogen than less mature (light green, recently harvested) and subturgid fruits.

In the present study we showed that satsuma mandarin (*C. unshiu*) cultivar "Miyagawawase" varied in its susceptibility to sour rot pathogen according to maturity stage. Young, dark green fruits were more susceptible to infection than mature, yellow fruits, and the susceptibility was relatively constant during storage at 25°C for 4 wk. These results were in contrast with lemon, as described by Baudoin and Eckert (1982). Several constituents of the peel and juicy tissues of fruit were analyzed in relation to susceptibility to infection. We found that sugars (sucrose, glucose, fructose) both in the peel and juicy tissues inversely correlated with susceptibility, as the susceptibility decreased with the increase of sugar concentrations. The concentration of citric acid in juice was positively correlated with susceptibility. In young fruit with higher content of citric acid but lower content of sugar, lesions developed more rapidly toward the juice sacs than in fruit with lower citric acid and higher sugar contents (Fig. 1). The differences in susceptibility between satsuma mandarin and lemon fruit are probably in part due to the differences in contents of sugars and citric acid. Titratable acidity of satsuma mandarin fruit decreased and total sugars increased during maturation. Lemon fruit contained 7.20% citric acid and 1.92% total sugars in juice (Money and Christian, 1950), but satsuma mandarin contained less citric acid (1.05%) and more sugars (7.57%). During storage, we observed that the content of citric acid in juicy tissues of satsuma mandarin was relatively constant, but Vandercook (1964) observed that citric acid increased in lemon fruit during storage. Babu and Reddy (1989) reported that sucrose concentration in lemon fruit decreased gradually with the progress of infection by *Colletotrichum gloeosporioides* and *Syncephalostrium racemosum*, with a fluctuation in

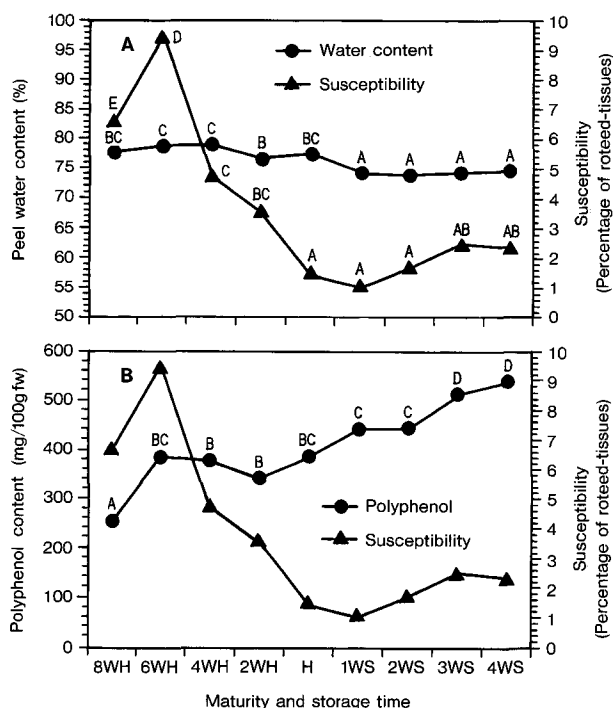


Fig. 3. Changes in water content (A) and polyphenol content (B) in peel of satsuma mandarin during maturation and storage, and its susceptibility to *G. candidum* citrus race. Susceptibility is expressed by means of percentage rotted tissues of 50 fruits of 10 isolates. WH, Wk before harvest; H, Harvest; WS, Wk of storage. Points marked with same the letters are not significantly different ($P=0.05$) according to Duncan's multiple test.

Table 3. Effect of peel extracts of satsuma mandarin fruit from varying maturity stages and storage on spore germination rate and mycelial growth of *Geotrichum candidum* citrus race in PD broth.

Maturity and storage time	Spore germination rate (%) ^{a, b)}				Mycelial dry weight (mg) ^{a, c)}			
	Isolate S31		Isolate Tm2		Isolate S31		Isolate Tm2	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Maturity								
Stage 1	79.7 ^{d)}	78.5	79.4	81.2	99.3	102.3	102.3	98.7
Stage 2	80.8	81.3	81.2	80.9	121.4	120.3	114.1	112.4
Stage 3	78.7	77.9	79.0	78.3	112.9	118.5	105.4	108.4
Stage 4	76.3	82.7	82.4	81.4	102.3	98.7	99.8	105.4
Stage 5	79.3	82.9	79.8	83.9	118.2	123.4	92.5	96.7
Storage								
1 wk	83.5	87.5	78.2	80.7	114.8	116.3	109.3	117.4
2 wk	85.2	90.3	83.7	82.1	101.7	99.3	98.5	100.9
3 wk	83.4	88.5	74.5	79.4	126.2	125.7	109.3	113.4
4 wk	81.2	79.4	82.8	81.3	121.4	123.8	112.8	109.4

a) Forty-five ml of PD broth was dispensed into a 200-ml Erlenmeyer flask and autoclaved at 121°C for 20 min, then 5 ml of peel extract (10%) from satsuma mandarins of varying maturities and storage times was added to the flask. A suspension of 100 µl of arthrospores of *Geotrichum candidum* (10⁷ arthrospores/ml) was inoculated into each flask and the flask was then incubated at 25°C in the dark. Tests were conducted separately at each maturity stage and storage time after extraction of fruit.

b) Germination rate was observed 24 h after incubation under a light microscope. Five areas of 100 arthrospores were examined for germination.

c) Dry weight was determined 5 d after incubation by filtering the culture with Whatman microfibre glass filters GF/C and drying at 80°C for about 5 d to constant weight.

d) Data are means of 8 flasks.

glucose and fructose concentration. The concentration of citric acid in healthy fruit decreased during storage, but that in infected fruit fluctuated. The decrease in titratable acidity (% citric acid) during storage was more

evident in infected fruit than healthy fruit (Ramanjuku and Reddy, 1989). These results showed that the pathogen utilized the citric acid for growth during infection.

Table 4. Correlation between susceptibility of satsuma mandarin fruit to sour rot pathogen and chemical constituents of juice and peel.

Part of fruit/constituent	Regression equation (y)	Coef. correlation (r ²)	Probability (P)
Juice			
Total soluble, solid (Brix)	$y = -0.82x + 10.98$	0.54 ^{a)}	0.0245
Sucrose	$y = -1.6x + 9.36$	0.49 ^{a)}	0.0357
Glucose	$y = -5.22x + 9.64$	0.58 ^{a)}	0.0177
Fructose	$y = -4.48x + 8.95$	0.59 ^{a)}	0.0154
Citric acid	$y = -7.61x - 4.91$	0.74 ^{b)}	0.0029
Peel			
Sucrose	$y = -4.03x + 7.90$	0.27	0.1516
Glucose	$y = -2.9x + 9.13$	0.58 ^{a)}	0.0174
Fructose	$y = -2.69x + 9.18$	0.58 ^{a)}	0.0178
Water content	$y = -1.01x - 73.4$	0.55 ^{a)}	0.0218
Polyphenol	$y = -0.02x + 10.46$	0.27	0.1546

a) Correlation is significant ($P < 0.05$).

b) Correlation is highly significant ($P < 0.01$).

The young, green fruit contained more water in the peel and was more susceptible to sour rot pathogen than the mature, yellow fruit. Several studies have been done regarding the peel water status of lemon fruit in relation to infection of sour rot pathogen (Baudoin and Eckert, 1982; Baudoin and Eckert, 1985b; Davis and Baudoin, 1986). The turgid lemon fruit was more susceptible to infection by *G. candidum* than subturgid fruit (Baudoin and Eckert, 1982). Davis and Baudoin (1986) showed that production of extracellular polygalacturonase (PG) was strongly influenced by osmotic potential, and noted that this phenomenon might partially account for the differences in susceptibility to sour rot of lemons with different water potentials.

The content of polyphenol in the fruit peel of satsuma mandarin was significantly less in young, green fruit than mature, yellow fruit. However, the content of polyphenol did not significantly correlate with the susceptibility. The germination rate of spores and mycelial growth of *G. candidum* citrus race in culture were apparently not affected by the extract of fruit peel containing polyphenol. This result suggested that the polyphenol in the peel does not influence the susceptibility of satsuma mandarin to sour rot pathogen.

In another test, five phenolic compounds, namely, tannic acid, tea polyphenol (containing catechin), gallic acid, quercetin and *p*-cumaric acid, were tested in vitro to examine their effect on the germination rate of spores and mycelial growth of *G. candidum* citrus race. Of these, only tannic acid could suppress the growth of the fungus at 1 mg/ml, and polyphenol did so at 5 mg/ml (data not shown). It appeared that the inhibitory effect of polyphenol on *G. candidum* citrus race depends on the type of compound and its concentration. This finding probably explains the low correlation between polyphenol content in the peel of satsuma mandarin fruit and sour rot susceptibility. Baudoin and Eckert (1985a) showed that accumulation of lignin-like substances around inoculated tissues paralleled the development of resistance of lemon fruit, but the role of these compounds in determining the resistance of fruit to sour rot pathogen is still not clear. In the case of other diseases, Homma and Arimoto (1988) showed that the extract of satsuma mandarin fruit peel inhibited spore germination and germ tube elongation of *Diaporthe citri* (Faw.) Wolf (the cause of citrus melanose and stem-end rot). In addition, the extract was found to be effective in controlling such postharvest diseases of citrus fruit as black rot, gray mold, anthracnose, and phoma rot; but sour rot was not tested.

Acknowledgements—We gratefully acknowledge our colleagues at Kagoshima Prefectural Fruit Tree Experimental Station, Tarumizu, Kagoshima, for their kind corporation in supplying citrus fruits for this study. We thank Dr. Itaru Kozaki, Dr. Shigeto Tominaga and M. Sc. Mebelo Mataa, the Laboratory of Pomology, Faculty of Agriculture, Kagoshima University, for their useful suggestions and technical assistance during the course of the experiment.

This research was supported in part by Research Grant

No. 07660063 from the Ministry of Education, Science, Sports and Culture of Japan.

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