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Effects of volatile compounds on arthrospore germination and mycelial growth of *Geotrichum candidum* citrus race

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Three groups of volatile compounds, i.e., alcohols, aldehydes and esters, were tested for their effects on arthrospore germination and mycelial growth of *Geotrichum candidum* citrus race, the causal agent of citrus sour rot. Alcohols (heptanol, octanol, nonanol, decanol, geraniol, citronellol) at a concentration of 1.0 μl/ml showed 60% or more inhibitory effects on both germination and mycelial growth of this pathogen. Among aldehydes tested, only citral had an inhibitory effect of more than 50%, while esters had no effect. The chain length of straight-chain (C6–C12) alcohols correlated with inhibitory effect, and nonanol (C9) was most active. Treatment with alcohols or citral prior to inoculation reduced colonization and maceration of lemon peel by this pathogen by 70% or more. Results suggested that alcohols or citral can probably be used to prevent the development of citrus sour rot.

Key Words——arthrospore germination; citrus sour rot; volatile compound.

Citrus sour rot caused by the citrus race of *Geotrichum candidum* Link has been reported as an important postharvest disease of citrus fruit from most areas of the world (Brown and Eckert, 1988; Butler et al., 1965; Eckert, 1978; El-Tobshy and Sinclair, 1965; Hershenhorn et al., 1992; Horn et al., 1958; Kuramoto, 1981; Smith, 1917). In Japan, the disease has been found mainly in imported citrus fruits, especially lemons from USA (Kitagawa and Kawada, 1984).

According to Eckert (1978), sour rot could not efficiently be controlled by any known postharvest treatment (other than low temperature) and the fungus is resistant to organic fungicides developed for the control of *Penicillium*. Treatment with biphenyl and sodium *O*-phenylphenate (SOPP) has been proved to be effective to control sour rot (Wild et al., 1976); but in Japan these chemicals have not been used, because biphenyl was found not to be effective and SOPP often injured the peel of satsuma mandarin (Kuramoto and Yamada, 1976).

Efforts to find new compounds to control the postharvest diseases have made by several workers. Citral (aldehyde) extracted from the peel of citrus fruit was reported to inhibit spore germination of *Penicillium digitatum* (Rers.: Fr.) Sacc. (Asthana et al., 1988; Rodov et al., 1995). Other compounds, such as 2-deoxy-D-glucose (Atkin et al., 1964) and chitosan (Ghaout et al., 1992), were also reported to have fungicidal activities on several pathogenic fungi. Davis and Smoot (1972) reported that aldehydes (C5–C9) inhibited 50% or more of the spore germination of *P. digitatum*, while alcohols, esters and terpenes did not.

In this study, the effect of some volatiles, i.e., alcohols, aldehydes and esters on spore germination and mycelial growth of *G. candidum* citrus race was studied to select compounds which might be used to control citrus sour rot.

Materials and Methods

Fungus Four isolates of *G. candidum* citrus race, isolated from soils of a citrus grove (S31), a tomato field (Tm2), a vineyard (Gr3), and a potato field (Pt3) in Japan, were used (Suprapta et al., 1995). Small mycelial pieces were transferred to potato-dextrose agar (PDA) slants and incubated at 25°C for 5 d in the dark. Arthrospores from 5-d-old cultures were harvested and put into sterile distilled water, and the resultant suspension was filtered through sterile glass wool to eliminate hyphal fragments.
Volatile compounds Ten alcohols, i.e., hexanol, heptanol, octanol, nonanol, decanol, undecanol, dodecanol (the C6–C12 straight chain alcohols), citronellol, geraniol and linalool; five aldehydes (n-butyraldehyde, n-heptaldehyde, n-octaldehyde, n-decyl aldehyde and citral); and four esters (citroneelyl acetate, terpinyl acetate, methyl iso-valerate and ethyl n-butyrate) were used in this study. All of these compounds (except n-butyraldehyde) are known to be volatile constituents of mature citrus fruit. They were purchased from Wako Pure Chemical, Osaka. They were dissolved in 25% ethanol, added to sterile molten PDA at 50°C to give concentrations of 0.25, 0.50, 0.75 or 1.0 μl/ml and poured into Petri dishes.

Effects on arthrospore germination A 100-ml portion of arthrospore suspension (10^5/ml) was streaked onto PDA amended with various concentrations of volatile compounds by use of a sterile glass rod. Two replicates of five plates were used for each compound and each concentration. The plates were incubated at 25°C for 24 h in the dark, then the germination rates (GR) of 200 arthrospores per plate were determined microscopically. An arthrospore was considered to have germinated when the length of the germ tube equaled or exceeded the length of the spore body.

Effects on mycelial growth PDA plates with various concentration of volatile compounds were seeded with 4-mm diam mycelial agar plugs taken from the margin of 5-d-old colonies on PDA plates. Two replicates of five plates were used for each compound and each concentration. The plates were incubated at 25°C for 5 d in the dark, then the colony diameter was measured.

Fungal colonization on and maceration of lemon peel Discs of lemon peel (20 mm in diam and 8 mm thick) were taken from the equatorial zone of lemon fruit (purchased from the market) by use of a sterile cork borer. The discs were dipped in 95% ethanol for about 5 s, then washed with sterile distilled water three times. The peel discs were then placed in volatile solution (1.0 μl/ml) for 10 min, then dipped in arthrospore suspension (10^7/ml) for about 5 s. The peel discs were placed in Petri dishes (3 peel discs/dish) that had been moistened with wet filter paper and incubated at 25°C for 72 h in the dark. Fungal colonization of peel discs was determined as follows. The peel disc was cut out into small pieces with sterile scissors and ground in a mortar with quartz sand. Then 20 ml of sterile distilled water was added, and the mixture was agitated in a mixer (Test Tube Mixer TTM-1, Sibata) for about 2 min. A series of dilutions was made by adding sterile distilled water, and 0.2 ml of each suspension was plated on PDA plates. The plates were incubated at 25°C for 48 h in the dark, then the number of colonies (colony-forming units) was examined.

The degree of tissue maceration was determined 72 h after inoculation. The peel disc was cut out into 8 pieces with sterile scissors, then put in a test tube with 10 ml of sterile distiled water and mixed in a mixer for 2 min. By this procedure, the macerated tissue was disintegrated and suspended in the distilled water. This suspension was diluted with 30 ml of distilled water. The turbidity of the suspension was determined by measuring the absorbance at 700 nm (UV-VIS Spectrophotometer, UV-1200, Shimadzu). A linear relationship was found between the percentage of macerated tissues and the absorbance, and the absorbance at 700 nm was used to express the degree of maceration of peel tissues.

Results

Effect of volatiles on arthrospore germination Of the three groups of volatiles tested, most of the alcohols markedly reduced the arthrospore germination of *G. candidum*. Of the ten alcohols, heptanol, octanol, nonanol, decanol, citronellol and geraniol at a concentration of 1.0 μl/ml reduced arthrospore germination by more than 60%, as shown in Table 1. The chain length of straight-chain alcohols correlated with inhibitory effect, as shown in Fig. 1. A relatively high inhibitory effect was obtained with the C7, C8, C9 and C10 alcohols with the peak at C9, whereas the inhibitory effect was low with C6, C11 and C12. The other alcohol (linalool) had no effect on arthrospore germination.

Among aldehydes tested, only citral showed an inhibitory effect of more than 75% on arthrospore germination. Esters had no inhibitory effect.

Table 1. Inhibitory effect of alcohols and aldehyde on arthrospore germination of four isolates of *Geotrichum candidum*.

Compounds	Inhibitory effect (%)[a]			
	Fungus isolates			
	S31	Tm2	Gr3	Pt3
Alcohols:				
Heptanol	65[b]	79	81	72
Octanol	85	92	90	87
Nonanol	100	100	100	100
Decanol	100	100	99	99
Citronellol	100	99	97	95
Geraniol	100	100	99	100
Aldehyde:				
Citral	84	79	82	78

a) Inhibitory effect $= \frac{\text{GR of control} - \text{GR of treatment}}{\text{GR of control}} \times 100\%$.

b) Data are the means of two replicates of five plates.

Effect of volatiles on mycelial growth Only volatiles

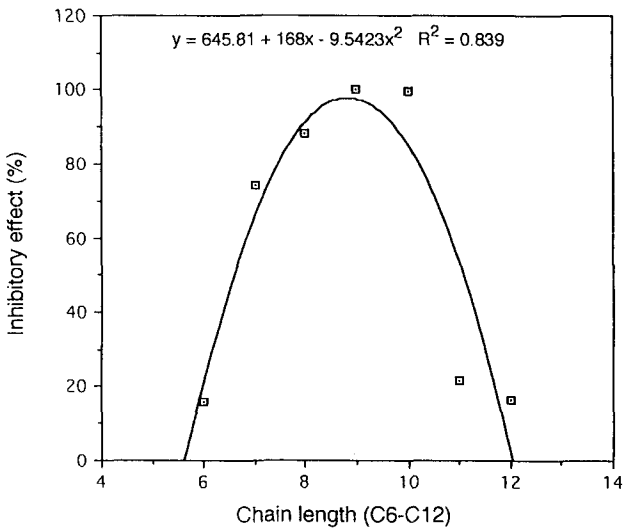

Fig. 1. Relationship between chain length (C6–C12) and inhibitory effect of straight-chain alcohols on arthrospore germination of *Geotrichum candidum* (data points indicate mean values of four isolates).

C6, hexanol; C7, heptanol; C8, octanol; C9, nonanol; C10, decanol; C11, undecanol; C12, dodecanol.

with inhibitory effects of 50% or more on arthrospore germination were tested. Results revealed that the compounds which inhibited arthrospore germination also inhibited the mycelial growth of *G. candidum* (Table 2). The inhibitory effects were found to vary between 50 and 78%.

Fungal colonization on and maceration of lemon peel

The volatile compounds with inhibitory effects of 50% or more on arthrospore germination and mycelial growth were tested for their ability to prevent the colonization on and maceration of lemon peel by the citrus race of *G. candidum*. The number of colonies (colony-forming units) recovered from the peel discs treated with alcohols (heptanol, octanol, nonanol, decanol, citronellol, geraniol) at a concentration of 1.0 μl/ml for 10 min revealed inhibitory effects of more than 65%, as shown in Table 3. The inhibitory effect on the fungal colonization well correlated with the inhibitory effect on tissue maceration. The turbidity (absorbance at 700 nm) of the peel disc suspension revealed that the alcohols could prevent the maceration of tissue by more than 80%. The same result was also observed when the peel disc was treated with citral, as shown in Table 4.

Table 2. Inhibitory effect of alcohols and citral on radial growth of four isolates of *Geotrichum candidum*.

Compounds	Inhibitory effect (%)[a]			
	Fungus isolates			
	S31	Tm2	Gr3	Pt3
Heptanol	50[b]	50	50	531
Octanol	53	52	51	55
Nonanol	76	78	75	75
Decanol	71	71	72	70
Citronellol	66	70	69	67
Geraniol	70	70	67	71
Citral	63	62	59	56

a) Inhibitory effect $= \frac{\text{Colony diam of control} - \text{Colony diam of treatment}}{\text{Colony diam of control}} \times 100\%$.

b) Data are the means of two replicates of five plates.

Discussion

Arthrospore germination of *G. candidum* on PDA was inhibited by six types of alcohol. Complete inhibition on arthrospore germination was observed with nonanol (Table 1). Mycelial growth was also inhibited by about 50–78% by these alcohols (Table 2). The results suggested that the alcohols probably act as germination and growth inhibitors of this fungus. Citral was the only aldehyde which had an obvious inhibitory effect on arthrospore germination and mycelial growth.

Although several aldehydes, alcohols and esters have been reported to show antifungal activity against *P. digitatum*, the causal agent of citrus green mold (Davis and Smoot, 1972), little attention has been paid to their toxicity against *G. candidum*. Davis and Smoot (1972) reported that aldehydes (butyraldehyde, heptanal, octanal, nonanal, decanal, citral and citronellal) inhibited the conidia germination of *P. digitatum*, while alcohols (nerol, hexanol, octanol, nonanol, decanol, geraniol and citronellol) and esters (ethyl butyrate, citronellyl acetate, methyl isovalerate and terpinyl acetate) did not affect the germination of this fungus. This finding, in part was contrary to our results. We found that alcohols obviously inhibited arthrospore germination and mycelial growth of *G. candidum*, whereas aldehydes (except citral) and

Table 3. Effect of alcohols and citral on the colonization of peel disc of lemon fruit inoculated with four isolates of *Geotrichum candidum*.

Compounds	Fungus isolates							
	S31		Tm2		Gr3		Pt3	
	CFU[a]/ disc	IE (%)[b]	CFU/ disc	IE (%)	CFU/ disc	IE (%)	CFU/ disc	IE (%)
Heptanol	9.81[c]	76	11.20	74	13.12	66	7.92	80
Octanol	4.62	89	5.11	88	4.16	89	5.08	87
Nonanol	1.83	96	2.31	95	2.82	93	2.42	94
Decanol	2.52	94	2.68	94	3.03	92	2.94	92
Citronellol	5.35	87	6.42	85	4.72	88	5.10	87
Geraniol	2.48	94	3.37	92	3.48	91	2.64	93
Citral	10.18	75	9.88	77	12.42	68	7.81	80
Control	41.07		43.58		38.78		39.07	

a) Colony-forming units ($\times 10^6$).

b) Inhibitory effect $= \frac{\text{CFU of control} - \text{CFU of treatment}}{\text{CFU of control}} \times 100\%$.

c) Data are the means of two replicates of five peel discs.

Table 4. Effect of alcohols and citral on maceration of lemon peel inoculated with four isolates of *Geotrichum candidum*.

Compounds	Fungus isolates							
	S31		Tm2		Gr3		Pt3	
	Abs[a]	IE (%)[b]	Abs	IE (%)	Abs	IE (%)	Abs	IE (%)
Heptanol	0.282[c]	82	0.209	85	0.198	87	0.223	85
Octanol	0.214	87	0.197	86	0.188	87	0.208	86
Nonanol	0.098	94	0.103	93	0.134	91	0.117	92
Decanol	0.094	94	0.113	92	0.124	92	0.118	92
Citronellol	0.166	90	0.178	88	0.182	94	0. 190	92
Geraniol	0.078	95	0.107	93	0.094	88	0.112	87
Citral	0.227	86	0.198	86	0.252	83	0.168	88
Control	1.587		1.438		1.468		1.442	

a) Absorbance at 700 nm.

b) Inhibitory effect $= \frac{\text{Abs. of control} - \text{Abs. of treatment}}{\text{Abs. of control}} \times 100\%$.

c) Data are the means of two replicates of five peel discs.

esters did not.

French et al. (1978) demonstrated that nonanal in 1% water agar stimulated germination and swelling of conidia of *P. digitatum* and *Penicillium italicum* Wehmer, the pathogens of citrus green mold and blue mold respectively. Nonanal was found to be the most active of the C6–C12 aldehydes in stimulating conidia germination of *Penicillium*. The activity of aldehydes in general was found to increase with the increase of chain length from C6 to C9 (French et al., 1978). A similar result was shown in our experiment, where the inhibitory action of alcohols increased with the increase of chain length from C6 to C9. The alcohols of shorter (C6) or longer chain length (C11 or C12) had only small inhibitory effects. This phenomenon is probably related to the volatility and solubility of the compounds, as suggested by Davis and Smoot (1972).

The effects of aldehydes (especially citral) on germination of *Penicillium* conidia have been shown to be stimulatory (French et al., 1978) or inhibitory (Davis and Smoot, 1972; Asthana et al., 1988; Onawunmi, 1989; Ben-Yehoshua et al., 1992). Nonanol and geraniol were reported to have stimulatory effects on germination of *Penicillium* conidia (French et al., 1978). This finding is in contrast with our results. We found that nonanol and geraniol clearly inhibited both arthrospore germination and mycelial growth of *G. candidum*. The difference in response of *Penicillium* and *G. candidum* to volatiles illustrates the difficulties encountered in controlling citrus fruit rot, especially when both pathogens are present in the fruit.

Our results revealed that six types of alcohol and citral could reduce fungal colonization on lemon peel and maceration of the peel by *G. candidum* (Tables 3, 4), which suggested that these compounds could probably be used to control the development of citrus sour rot.

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Literature cited

Asthana, A., Dixit, K. and Kishore, N. 1988. Toxicity of citral and eugenol against some storage fungi. Arch. Phytopathol. Pflanzenschutz **24**: 417–421.

Atkin, R. K., Spencer, D. M. and Wain, R. L. 1964. Investigation on fungicides: The antifungal activity of 2-deoxy-d-glucose. Ann. App. Biol. **53**: 437–443.

Ben-Yehoshua, S., Rodov, V., Kim, J. J. and Carmeli, S. 1992. Preformed and induced antifungal materials of citrus fruit in relation to enhancement of decay resistance by heat and UV treatment. J. Agric. Food Chem. **40**: 1217–1221.

Brown, G. E. and Eckert, J. W. 1988. Sour rot. In: Compediu m of citrus diseases, (ed. by Whiteside, J. O., Gransey, S. M. and Timmer, L. W.), pp. 37–38. The American Phytopathological Society, St. Paul, Minnesota, USA.

Butler, E. E., Webster, R. K. and Eckert, J. W. 1965. Taxonomy, pathogenecity and physiological properties of the fungus causing sour rot of citrus. Phytopathology **55**: 1262–1268.

Davis, P.I. and Smoot, J.J. 1972. Germination of *Penicillum digitatum* as affected by solutions of volatile components of citrus fruits. Phytopathology **62**: 488–489.

Eckert, J. W. 1978. Postharvest disease of citrus fruit. Outlook Agric. **9**: 225–259.

El-Tobshy, Z. M. and Sinclair, J. B. 1965. *Geotrichum candidum*: Plant and animal isolates pathogenic to certain plant tissues. Phytopathology **55**: 1210–1212.

French, R. C., Long, R. K., Latterell, F. M., Graham, C. L., Smoot, J. J. and Shaw, P. E. 1978. Effect of nonanal, citral and citrus oils on germination of conidia of *Penicillium*

digitatum and *Penicillium italicum*. Phytopathology **68**: 877–882.

Ghaout, A. E., Arul, J., Grenier, J. and Asselin, A. 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology **82**: 398–402.

Hershenhorn, J., Dori, S. and Barash, I. 1992. Association of *Geotrichum citri-aurantii* with citrus groves in Israel. Phytoparasitica **20**: 31–36.

Horn, N. L., Sinclair, J. B. and Brown, R. T. 1958. The occurrence of *Oospora* rot of citrus in Louisiana. Plant Dis. Reptr. **42**: 262–265.

Kitagawa, H. and Kawada, K. 1984. Effect of sorbic acid and potassium sorbate on the control of sour rot of citrus fruit. Proc. Fla. State Hort. Soc. **97**: 133–135.

Kuramoto, T. 1981. Postharvest disease. In: Citrus diseases in Japan, (ed. by Miyakawa, T. and Yamaguchi, A.), pp. 27–32. N.Y.P., Tokyo.

Kuramoto, T. and Yamada, S. 1976. Df-125, a new experimental fungicide for control of satsuma manderin postharvest decays. Plant Dis. Reptr. **60**: 809–812.

Rodov, V., Ben-Yehoshua, S., Fang, D. Q., Kim, J. J. and Ashkenazi, R. 1995. Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. J. Agric. Food Chem. **43**: 1057–1061.

Smith, C. O. 1917. Sour rot of lemon in California. Phytopathology **7**: 37–41.

Suprapta, D. N., Arai, K. and Iwai, H. 1995. Distribution of *Geotrichum candidum* citrus race in soils of citrus groves and noncitrus fields in Japan. Mycoscience **36**: 277–282.

Wild, B. I., Rippon, L. E. and Nicholls, P. J. 1976. Effect of decay between inoculation and SOPP treatment on the development of *Geotrichum candidum* in oranges. Plant Dis. Reptr. **60**: 488–489.