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Distribution of *Geotrichum candidum* citrus race in citrus groves and non-citrus fields in Japan

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The presence of *Geotrichum candidum* citrus race, the citrus sour rot pathogen, was examined in soils of citrus groves and non-citrus fields of Japan. Soil samples were collected from 223 sites (118 sites in citrus groves, and 105 sites in fields cultivated with 33 species of non-citrus plants and in evergreen broad-leaved forest) in 11 main citrus-growing prefectures, and Hokkaido, a non-citrus-growing area. Of 236 soil samples from citrus groves, 95.76% contained *G. candidum* citrus race and 0.42% contained the non-citrus race; while of 210 samples from non-citrus fields, 62.85% and 4.76% contained the citrus race and the non-citrus race respectively. All of the citrus race isolates obtained either from citrus groves or non-citrus fields were pathogenic on lemon (*Citrus limon*) and satsuma mandarin (*Citrus unshiu*), but some of these isolates failed to infect orange (*Citrus sinensis*). The non-citrus races were pathogenic on ripe tomato fruit (*Lycopersicon esculentum*) and ripe muskmelon fruit (*Cucumis melo* var. *reticulatus*). Results indicated that citrus sour rot pathogen is widely distributed in citrus groves and non-citrus fields of diverse plant species in Japan.

Key Words——citrus grove; citrus sour rot; *Geotrichum candidum* citrus race; non-citrus field.

Geotrichum candidum Link, frequently encountered in dairy products and respiratory and digestive tracts of man and other warm-blooded animals (Tubaki, 1978; van Uden and Carmo-Sausa, 1959), is ubiquitous in soil and other substrata (Carmichael, 1957). This fungus was also reported to be an important pathogen of ripe tomato fruit, ripe squash and watermelon in California (Butler, 1960). The plant isolates of *G. candidum* from either citrus fruit or soil were pathogenic to tomato fruit, orange, lemon, grapefruit, cucumber and carrot root (Aoki, 1992; El-Tobshy and Sinclair, 1965; Kitajima, 1989). Butler et al. (1965) designated *G. candidum* as the causal agent of sour rot of citrus fruit and they proposed the term “citrus race” to designate this pathogen.

Sour rot of citrus fruit was first described by Smith (1917) and the pathogen was designated as *Oospora citri-aurantii* (Ferr.) Sacc. et Syd. Since then, several different names have been used to designate the pathogen. *G. candidum* Link var. *citri-aurantii* (Ferr.) R. Ciferri. et F. Ciferri n. comb. was proposed by Ciferri in 1955 (Butler et al., 1965). *Geotrichum candidum* Link ex Pers. was proposed by Butler et al.(1965), and the term “citrus race” was introduced. Butler and Peterson (1972) proposed *G. candidum* Link ex Pers. (asexual form) and *Endomyces geotrichum* Butler et Peterson (sexual form) to indicate the citrus sour rot pathogen. Several years later, Butler et al. (1988) designated the cause of citrus sour rot as *G. citri-aurantii* (Ferr.) Butler (asexual form) and *Galactomyces citri-aurantii* Butler (sexual form). In Japan, the citrus sour rot pathogen is known as *G. candidum* var. *citri-aurantii* R. Ciferri et F. Ciferri (Aoki, 1992; Kuramoto and Yamada, 1975), *G. candidum* Link ex Pers. (Kitajima, 1989), *E. geotrichum* Butler et Petersen and *G. candidum* Link ex Pers. (Tubaki, 1978). In recent works on citrus sour rot, *G. candidum* Link ex Pers. citrus race is commonly cited to indicate the citrus sour rot pathogen (Barash et al., 1984; Baudoin and Eckert, 1985; Chalutz and Wilson, 1990; Mor et al., 1984; Morris, 1982; Wild, 1992), especially as the perfect state of the pathogen has not yet been demonstrated. Development of the perfect state by Japanese isolates of *G. candidum* has not yet been demonstrated; therefore, the pathogenic isolates on citrus fruit will be referred to as *G. candidum* citrus race and non-pathogenic isolates as *G. candidum* non-citrus race.

Sour rot of citrus fruit is an important cause of postharvest losses of citrus fruit and has been reported from most areas of the world where citrus is grown (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; Kuramoto and Yamada, 1975; Smith, 1917). The causal fungus occurs commonly in soil (Brown and Eckert, 1988) and is highly adapted to the citrus environment (Butler et al., 1965; Hershenhorn et al., 1992). There is no evidence that the citrus race of *G. candidum* is prevalent in non-citrus sites (Butler et al., 1965). The purpose of present study was to detect the presence of *G. candidum* in soils of citrus groves and non-citrus fields in Japan and to examine whether the citrus race of this fungus (the cause of citrus sour rot) is restricted to the citrus environment.

Materials and Methods

Sampling Sampling was carried out in 11 main citrus-growing prefectures in Japan, and in Hokkaido, a non-citrus-growing area. According to Kozaki (1981), 23 prefectures in Japan produce some citrus fruit, but about 83% of the total citrus fruit is produced by 12 major prefectures, namely, Ehime, Wakayama, Shizuoka, Kumamoto, Saga, Nagasaki, Oita, Fukuoka, Hiroshima, Kagoshima, Miyazaki and Tokushima. Sampling was conducted in all major prefectures except Tokushima (Table 1). Soil samples were collected from citrus groves with nine species of citrus and from non-citrus fields with 33 different cultivated plants and evergreen broad-leaved forest (Table 2). Samples were taken at 118 sites of citrus groves and 105 sites of non-citrus fields (9-17 sites of citrus groves and 6-13 sites of non-citrus fields in each prefecture) with a distance at 1-2 km between sites. Two soil samples were taken each site at 20-25 m distance. A soil sample of about 30 g was obtained by combining soils taken at 3 different points under a tree canopy at a depth of about 3-5 cm.

Isolation *Geotrichum candidum* was isolated by use of a slight modification of the shake-culture technique, a sensitive method for isolation of *G. candidum* from soil developed by Butler and Eckert (1962) and Butler et al. (1965). Three grams of fresh soil was put in a 100-ml Erlenmeyer flask containing 25 ml of potato broth pH 5.8 with 100 μg/ml novobiocin and shaken for 24 h. After shaking, 2 ml of soil-potato broth solution was transferred into Petri dish containing 15 ml of autoclaved lemon juice (pH 2.7) and incubated for 24 h in darkness at 25°C. A half milliliter of lemon juice-soil solution was spread onto PDA (potato-dextrose agar) pH 5.8 in a Petri dish containing 100 μg/ml novobiocin and 100 μg/ml benomyl and incubated in darkness for 24 h at 25°C. Pure culture was obtained by streaking the spores on plates of PDA. Three replicates were made for each soil sample. The fungus was identified based on the morphological criteria described by Carmichael (1957), Butler (1960) and Butler et al. (1965), such as color of colony on PDA, shape of arthrospores, arthrospore chains and hyphal branching at the periphery of a colony. Hyphal dichotomous branching was considered to be a characteristic of *G. candidum* (Carmichael, 1957). To verify that the isolate is the citrus sour rot pathogen, pathogenecity test was conducted on lemon (*Citrus limon*), satsuma mandarin (*C. unshiu*) and orange (*C. sinensis*) fruits. Since *G. candidum* has also been reported as soft rot pathogen of tomato (*Lycopersicon esculentum*) and muskmelon (*Cucumis melo* var. *reticulatus*) fruits (Butler, 1960), isolates that non-pathogenic on citrus fruit were inoculated into ripe tomato and ripe muskmelon fruits.

Inoculation Lemon, satsuma mandarin, orange, tomato and muskmelon fruits obtained from market were washed with water and a commercial grade detergent; washed with potable water; rinsed with distilled water and wiped slightly with 95% ethanol. One conical wound (3 mm diam and 5 mm depth) was made in the fruit peel using sterile cork borer. Twenty microliter of a conidial suspension (10^7conidia/ml) was pipetted into the wound. Inoculated fruits were incubated in plastic boxes with a wet sterile paper towel for 5 days at 25°C. Twelve fruits (four lemon, four satsuma mandarin and four orange fruits) were inoculated with each isolate. The isolates that were non-pathogenic on citrus fruit were then inoculated into tomato and muskmelon fruits. The diameter of lesion and amount of rotted tissue were determined after incubation.

Table 1. Distribution of *Geotrichum candidum* in 11 main citrus-growing prefectures, and Hokkaido.

No.	Prefecture	No. isolates[a]/no. soil samples	
		Citrus groves	Non-citrus fields
1.	Kagoshima	34/34	18/26
2.	Miyazaki	17/17	8/20
3.	Kumamoto	16/20	14/18
4.	Fukuoka	15/20	9/12
5.	Saga	20/20	23/24
6.	Nagasaki	20/20	7/12
7.	Oita	20/20	9/18
8.	Ehime	19/19	13/16
9.	Hiroshima	24/24	10/18
10.	Wakayama	18/18	10/14
11.	Shizuoka	24/24	15/22
12.	Hokkaido[b]	—	6/10
	Total	277/236	142/210

a: One isolate was selected from each soil sample that contained *G. candidum*. b: A non-citrus-growing area.

Results and Discussion

Geotrichum candidum was detected in most soil samples from all 11 main citrus-growing prefectures of Japan, either in soils of citrus groves or non-citrus fields. The fungus was also detected in soils of apple and grape orchards of Hokkaido, which has no history of citrus cultivation. All isolates obtained from citrus groves and non-citrus fields showed similar morphological characteristics. Colonies on PDA were mostly dull white in color, and some were bright white, with a daily growth rate of 8.8-16.0 mm for 5 days at 25°C. Dichotomous branching of mycelium at the periphery of a colony, a characteristic of *G. candidum* (Butler, 1960; Butler et al., 1965; Carmichael, 1957), was observed in all isolates on PDA (Fig. 1-A). Chains of arthrospores formed from hyphal segmentation (Fig. 1-B) were also observed in all isolates. Arthrospores were mostly oval, and some were cylindrical (Fig. 1-C).

Of 236 soil samples from citrus groves, 226

Table 2. Origin of isolates of *Geotrichum candidum*.

Type of field and plant species	No. isolates*/ no. samples
A Citrus grove	
1. Tankan (*Citrus tankan* Hayata)	6/6
2. Natsu mikan (*Citrus natsudaidai* Hayata)	22/24
3. Satsuma mandarin (*Citrus unshiu* Marc.)	146/149
4. Sour Pomelo (*Citrus grandis* Osbeck)	8/8
5. Lemon (*Citrus limon* Burm.f.)	6/6
6. Hassaku (*Citrus hassaku* Hort. ex Tanaka)	18/18
7. Navel orange (*Citrus sinensis* Osbeck)	16/18
8. Iyokan (*Citrus iyo* Hort. ex Tanaka)	4/6
9. Ponkan (*Citrus reticulata* Blanco)	1/1
B. Non-citrus field	
1. Apple (*Malus pumila* Mill. var. *domestica* Schneider)	4/4
2. Asparagus (*Asparagus officinalis* L.)	0/4
3. Broadbean (*Vicia faba* L.)	1/2
4. Cabbage (*Brassica oleracea* L. var. *capitata* L.)	4/4
5. Corn (*Zea mays* L.)	0/6
6. Egg plant (*Solanum melongena* L.)	0/2
7. Grape (*Vitis vinifera* L.)	6/10
8. Igusa (*Juncus effusus* L. var. *decipiens* Buch.)	3/4
9. Kiwi (*Actinidia chinensis* Planch.)	8/8
10. Lettuce (*Lectuca sativa* L.)	2/2
11. Loquat (*Eriobotrya japonica* (Thunb. ex Marray) Lindl.)	8/10
12. Mango (*Mangifera indica* L.)	1/1
13. Okra (*Abelmoschus esculentus* Moench)	3/4
14. Onion (*Allium* cepa L.)	4/4
15. Passion fruit (*Passiflora edulis* Sims)	0/1
16. Pea (*Pisum sativum* L.)	3/8
17. Peach (*Prunus persica* Batsch)	3/6
18. Pear (*Pyrus pyrifolia* (Burm.f.)	6/6
19. Persimon (*Diospyros kaki* Thunb. ex Murray)	15/16
20. Black pine (*Pinus thunbergii* Parl.)	5/14
21. Mume (*Prunus mume* (Sieb.) Sieb. et Zucc.)	2/4
22. Potato (*Solanum tuberosum* L.)	4/6
23. Pumpkin (*Cucurbita moschata* (Duch.) Poir. var. *melonaeformis* (Carr.) Mikano)	2/6
24. Radish (*Raphanus sativus* L. var. *hortensis* Backer)	2/2
25. Rice (*Oryza sativa* L.)	26/26
26. Soybean (*Glycine max* Merr.)	2/4
27. Stone leek (*Allium fistulosum* L.)	2/2
28. Strawberry (*Fragaria chiloensis* Duch. var. *ananassa* Bailey)	1/4
29. Sweetpotato (*Ipomoea batatas* Lam.)	2/6
30. Tea (*Thea sinensis* L.)	4/4
31. Tobacco (*Nicotiana tabacum* L.)	4/8
32. Tomato (*Lycopersicon esculentum* Mill.)	4/6
33. Wheat (*Triticum aestivum* L.)	5/10
34. Evergreen broad-leaved forest	6/6

* One isolate was selected from each soil sample that contained *G. candidum*.

(95.76%) samples yielded the *G. candidum* citrus race (citrus sour rot pathogen) and 1 (0.42%) sample yielded the non-citrus race (non-pathogenic on citrus fruit); while of 210 samples from non-citrus fields, 132 (62.85%) and 10 (4.76%) samples yielded citrus race and non-citrus race, respectively (Fig. 2). Citrus fruits inoculated with *G. candidum* citrus race developed a water-soaked lesion (Fig. 1-E) with abundant arthrospores on the surface of

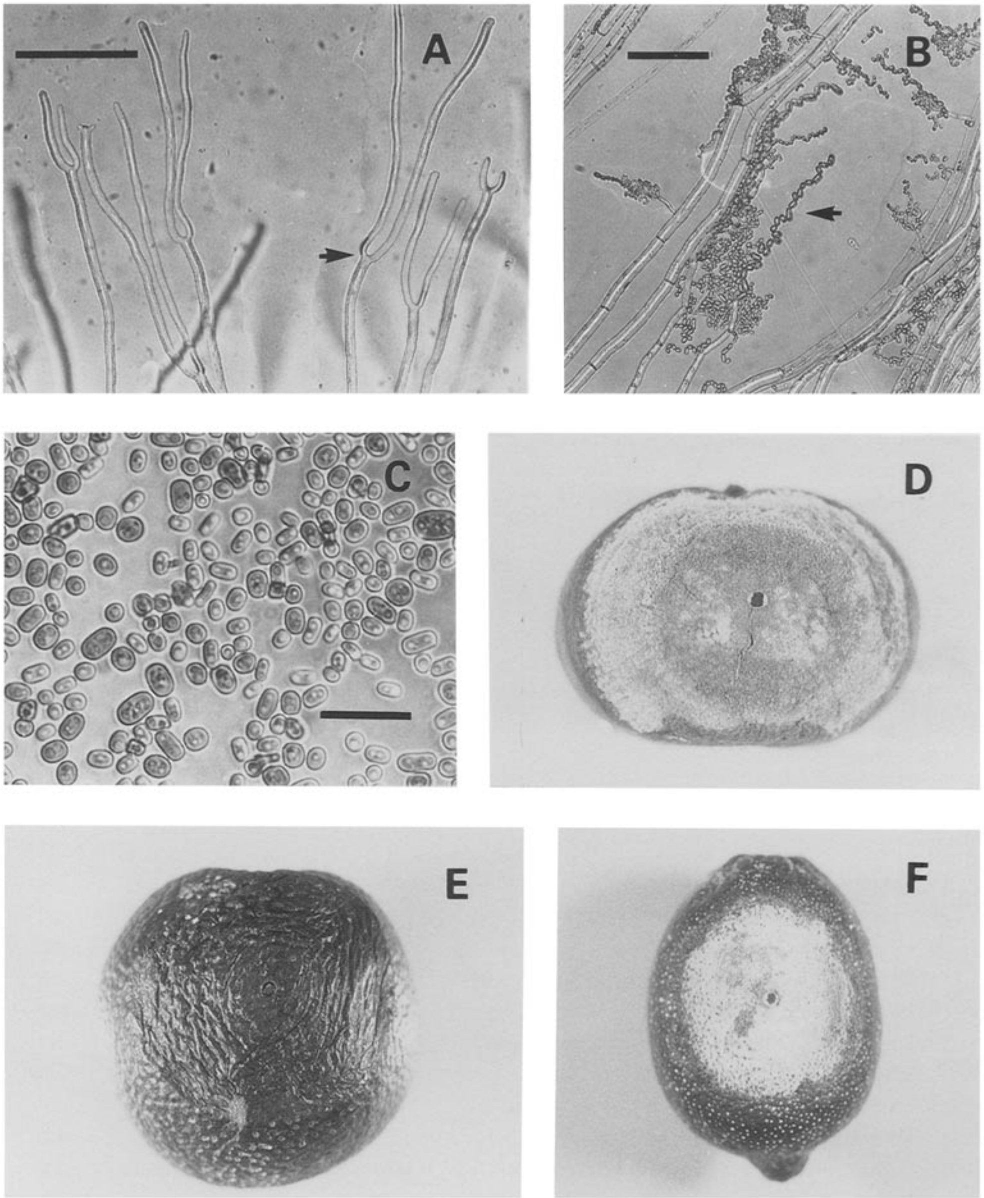

Fig. 1. Light microscopy of morphological characteristics of *Geotrichum candidum* and symptoms of citrus sour rot.
A: Dichotomous branching of mycelium at the periphery of a colony (arrow). Bar represents 30 μm.
B: The chain of arthrospores formed from segmentation of aerial hyphae (arrow). Bar represents 30 μm.
C: Arthrospores of *G. candidum*, which are oval or cylindrical. Arthrospores were removed with a needle from a 2-day culture on PDA slant medium, mounted in 5% glycerol in water, and stained with 2% crystal violet in glycerol (5% glycerol in water) before photographing. Bar represents 5 μm.
D, E and F: Symptoms of sour rot on lemon, orange and satsuma mandarin, respectively, 5 days after inoculation with isolate Tm2.

the lesions (Figs. 1-D, 1-F). The non-citrus race isolates when inoculated into ripe tomato and muskmelon fruits developed watery soft lesions surrounding inoculation sites, suggesting that these isolates might be the soft rot pathogen of these fruits as previously described by Butler (1960). Butler et al. (1965) stated that the citrus race of *G. candidum* is prevalent in connection with citrus culture, and there is no evidence that the citrus race is prevalent in non-citrus sites. A study in Israel (Hershenhorn et al., 1992) showed that 80% of soil samples in the center and margin of citrus groves in Israel contained *G. citri-aurantii* (*G. candidum* citrus race), whereas only less than 21% of the soil samples outside the groves (100 m from the margin) contained this pathogen. Our present results indicated a similar tendency, in that the citrus race of this fungus was detected in most of soil samples from citrus groves, which indicated a possible linkage between the presence of the sour rot pathogen and the citrus en-

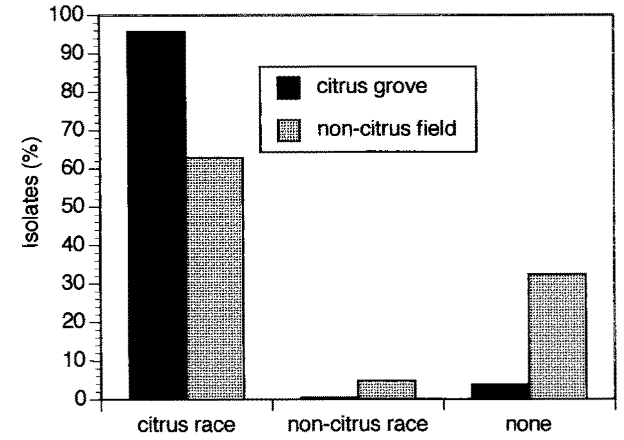

Fig. 2. Proportion of soil samples from which citrus race, non-citrus race, and no *Geotrichum candidum* were detected in soils of citrus groves and non-citrus fields in Japan.

vironment as claimed by Butler et al. (1965). On the other hand, our results also indicate that the presence of *G. candidum* citrus race is not restricted to the citrus environment. The detection of the citrus race in more than 60% of soil samples from non-citrus fields of various plant species suggests its wide distribution outside of the citrus environment. Moreover, the citrus races were detected from soils of Hokkaido and evergreen broad-leaved forest, both of which have no history of citrus cultivation. This finding supports the notion of Hershenhorn et al. (1992) that sour rot pathogen can survive in the soil containing organic matters of non-citrus dead plant and dead animal. Most saprobes obtain organic materials from the remnants of dead plants and animals in soil, fresh water or salt water (Talaro and Talaro, 1993), and *G. candidum* as one saprobe is considered to possess a high saprophytic ability in diverse habitats.

Most of the isolates of *G. candidum* were pathogenic on either lemon, satsuma mandarin or orange fruits. Two hundred twenty-six (99.55%) and 132 (92.25%) isolates obtained from citrus groves and non-citrus fields respectively were pathogenic on lemon and satsuma mandarin (Table 3). All of the citrus race isolates either from citrus groves or non-citrus fields were pathogenic on lemon and satsuma mandarin, but some of these isolates failed to infect oranges. There is no immediate explanation for this failure. In our pathogenecity test, the average weights of rotted tissue of an inoculated lemon and satsuma mandarin were 18.91 g (17.51%) and 22.81 g (19.20%) respectively, but that of orange was only 3.55 g or 2.00% of the weight of the inoculated fruit (data not shown). This result suggests different responses of these citrus species (lemon, satsuma mandarin and orange) to citrus sour rot pathogen. This is probably because of differences in the chemical constituents or physical structure of the peels of the different species.

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Table 3. Number of soil samples, presence of *Geotrichum candidum* and pathogenecity on citrus fruits.

Type of field	No. soil samples	No. isolates[a]	No. isolates pathogenic on		
			lemons	oranges	mandarins
Citrus groves	236	227 (96.18%)[b]	226 (99.55%)[c]	221 (97.35%)	226 (99.55%)
Non-citrus fields	210	142 (67.76%)	132 (92.25%)	59 (41.54%)	132 (92.25%)

a: One isolate was selected from each soil sample that contained *G. candidum*. b: Percent to soil samples, c: Percent to isolates.

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