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Causal Factors of "Kohansho" Disorder in 'Kiyomi' Tangor Fruit

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Summary

The causes of the occurrence of "Kohansho", a physiological disorder of 'Kiyomi' tangor ['Miyagawa wase' (*Citrus unshiu* Marc.) × 'Trovita' orange (*Citrus sinensis* Osbeck)] fruit were investigated to establish effective preventive measures. The disorder appeared mainly on the fruit surface exposed to sunlight that was over 10 ℃ warmer than the shaded side which was slightly affected. On a clear day, the transpiration rate of sun-exposed fruit was three-fold faster and at night it was only 25% as rapid as that of shaded fruit. Hence, greater water loss of sun-exposed parts is closely associated with the disorder. No significant differences were found in the mineral contents between the sun-exposed and shaded parts, although starch and total sugars were slightly higher in the flavedo of sun-exposed parts. The affected parts had a little greater a* value and more carotenoids than had the normal rind. Shaded fruit had an uneven surface under which the oil-glands protruded, whereas sun-exposed surface had smooth part between round shaped oil-glands.

Key Words: 'Kiyomi' tangor, "Kohansho" disorder, rind roughness, sun-exposed fruit, transpiration rate.

Introduction

'Kiyomi' tangor bears attractive, high quality fruit that mature in late season (Araki et al., 1989). Its monoembryonic characteristic makes breeding of citrus more efficient. It is a hybrid of 'Miyagawa wase' (*Citrus unshiu* Marc. var. praecox Tanaka) and 'Trovita' orange (*Citrus sinensis* Osbeck forma Trovita). The hybrid was selected in 1949 at Okitsu Branch, Fruit Tree Research Station in Japan and registered in 1979 (Nishiura et al., 1983). As the cultivation area increased rapidly, more trees grew to bearing age; a rind disorder became apparent. The affected spots are called "Kohansho" based on the developmental features of this disorder ("Kohan" means tiger's stripes and "Sho", symptom). It initially appears as pitting on the fruit surface and develops into large brown pitted areas, a serious defect for marketing. However, the causal factors are not well understood.

Studies of the disorder with 'Kiyomi' tangor fruit have been mostly focused on storage conditions related to the symptom development (Chikaizumi and Matsumoto, 1991; Deng et al., 1990; Hasegawa and Yano, 1990; Makita and Okada, 1991). We previously reported that fruits of 'Ootani iyo' (*Citrus iyo* hort. *ex* Tanaka, var. Ootani) that had been exposed to the solar radiation on the tree developed the rind disorder during storage (Chikaizumi et al., 1987). Thus, whether or not the similar phenomena are associated with the disorder in 'Kiyomi' tangor fruit was determined.

Materials and Methods

The following series of experiments were conducted on 'Kiyomi' tangor growing in Misaki and Matsuyama, Ehime Prefecture.

Experiment 1. Occurrence of "Kohansho" disorder on the sun-exposed fruit

(a) 1990-1991. Fruit from 6-year-old trees of 'Kiyomi' tangor top-grafted onto 'Kawano Natsudaidai' (*Citrus natsudaidai* Hayata) at a commercial grove in Misaki were collected from two zones on the tree canopy. The first group of fruit was harvested from the exterior canopies at heights 1 to 2 m above the ground where their rind surfaces were exposed to sunlight. The second group of fruit was collected from the interior of canopies, located near the windbreak hedges. Fruit were harvested on December 28, 1990 and stored at ambient air temperatures. From another plot, fruit were randomly harvested and evaluated for "Kohansho" on April 5, 1991.

(b) 1994-1995. Sun-exposed fruit of uniform size were selected as above and their exposed side was circled with a marking pen on October 10, 1994. Twenty fruit were harvested on December 20, 1994 and stored at 20 ℃. The degree of "Kohansho" on the sun-exposed and shaded side of the same fruit was rated on January 20, 1995.

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Experiment 2. Some physiological, chemical and physical aspects of the sun-exposed and shaded fruit

(a) Rind surface temperatures and rate of transpiration

Surface temperatures on 50 sun-exposed and shaded sides of fruit were monitored with an emission-thermometer 505 (MINOLTA K.K.) on October 20, 1990. In another experiment, surface temperatures of the exposed and shaded sides of fruit and air temperatures were monitored with a thermocouple on November 15, 1991.

The rates of transpiration from the exposed and shaded surfaces were measured according to Matsui et al. (1980) with slight modifications. The flow rate of air was 1.5 liter $\cdot$ min^{-1}.

(b) The mineral, starch, and sugar contents in the rind

A portion of rind tissues from each fruit was separated into albedo and flavedo and dried. These rind tissues were pulverized and their nitrogen content measured by the Kjeldahl method. The phosphorus content was measured colorimetrically by an electrophotometer; potassium, calcium and magnesium contents were measured by atomic absorption spectrophotometer. Starch was analyzed by the technique of Carter and Neubert (1954).

Sugar composition was determined by using a gas chromatograph (Shimadzu GC-14A) equipped with a flame ionization detector and a glass column (3.2 mm i.d. $\times$ 2 m, SE 52, 5% coated on chromosorb WAW DMCS) after silylating the ethanolic extract with a trimethylsilylating reagent (TMSI-H, GL Sciences Co.).

(c) Rind color and roughness

Rind color was determined with a chroma meter (CR-200, MINOLTA K. K.). The carotenoid of flavedo tissue was extracted and the extracts analyzed by the method of Minguez-Mosquera and Hornero-Mendez (1993) with slight modifications and also by high performance liquid chromatograph (HPLC) on a reversed-phase C_{18} column (Inertsil ODS-2, 4.6 mm i.d. $\times$ 250 mm) protected with a guard column (4 mm i.d. $\times$ 50 mm). A 5-μl aliquot of extract was injected into the chromatograph that was monitored with Shimadzu SPD-6AV-visible detector at 450 nm.

Fruit harvested from commercial groves in Misaki on December 20, 1994, were evaluated for rind roughness and the degree of disorder. Rind roughness was visually classified into two types: 1) the rough surface having an irregular projection of oil glands and 2) a smooth surface that has no convex protrusions of oil glands.

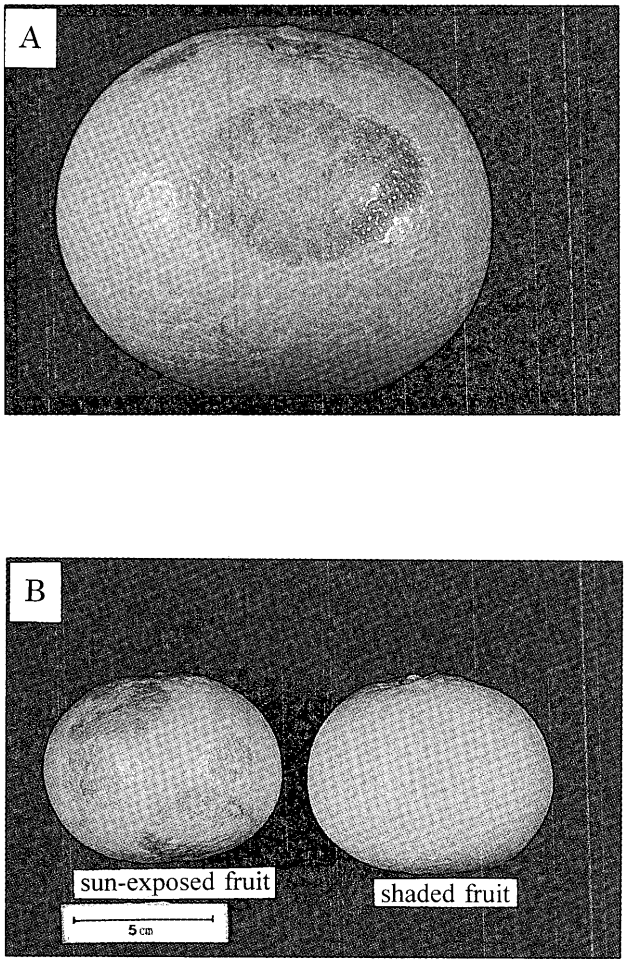

Fig. 1. External appearances of "Kohansho" of 'Kiyomi' tangor fruit. A, Typical symptom of "Kohansho" formed on the rind of 'Kiyomi' fruit; B, The sun-exposed fruit with smooth surface (left) and shaded fruit with rough surface (right).

Results

Morphology

The typical external appearances of "Kohansho" disorder shows the affected parts as circles, concentric circles, pittings and irregular shapes (Fig. 1A).

Occurrence of the disorder on the fruit from exterior and interior canopies

About 78% of fruit from exterior canopies were affected with "Kohansho", whereas only 6.7% of the fruit from interior canopies displayed its symptoms (Fig. 2); 63% of the randomly harvested fruit exhibited the disorder.

When fruit without visible symptoms at harvest were stored at 20 ℃, 15 out of 20 sun-exposed sides of fruit exhibited rind disorder. Only two out of 20 shaded sides of the same fruit were abnormal (Fig. 3).

Some physiological, chemical and physical aspects of the sun-exposed and shaded fruit

(a) Rind surface temperatures and rate of transpiration

The average maximum rind surface temperature was 36.4 $\pm$ 2.4 ℃ on October 20, 1990, while that of the shaded surface on the same fruit was only 20.0 $\pm$ 0.5 ℃. On November 15, 1991 the mean maximum temperatures of sun-exposed and shaded sides were 34 ℃ and

15 ℃, respectively (Fig. 4). The rind temperatures of the shaded side synchronously fluctuated with the ambient air temperatures within 0.5 ℃.

On a clear day, the transpiration rate from 10:00 to 15:00 was 3.3 g water per 100 g fruit in sun-exposed fruit, whereas it was 1.0 g in the shaded ones (Table 1). On a clear and later cloudy day, the rate decreased to 1.9 g and 0.9 g in sun-exposed and shaded fruit, respectively. At night or on a cloudy day, the transpiration rate did not differ between sun-exposed and shaded fruit.

(b) The mineral, starch, and sugar contents in the rind

No significant differences were found in the levels of mineral elements in the rind from the sun-exposed and shaded parts (Table 2). Starch content of the rind was higher in the exposed part than that of the shaded part in the flavedo tissues; no starch was detected in the albedo

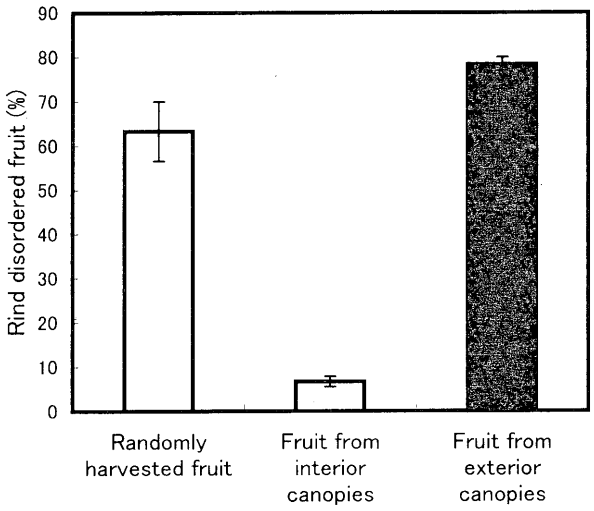

Fig. 2. The occurrence of "Kohansho" disorder on fruit from exterior and interior canopies. Two replications were conducted with 20 fruit per treatment. Vertical bars represent SE. Fruit were harvested on December 28, 1990, then stored at ambient air temperatures. The rind disorder was examined on April 5, 1991.

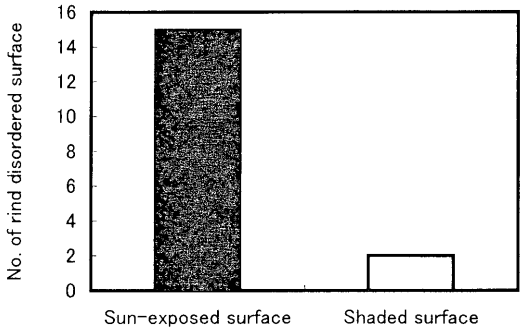

Fig. 3. The occurrence of "Kohansho" disorder on sun-exposed and shaded side of the same fruit. Twenty fruit were harvested on December 20, 1994, then stored at 20 ℃. The rind disorder was examined on January 20, 1995.

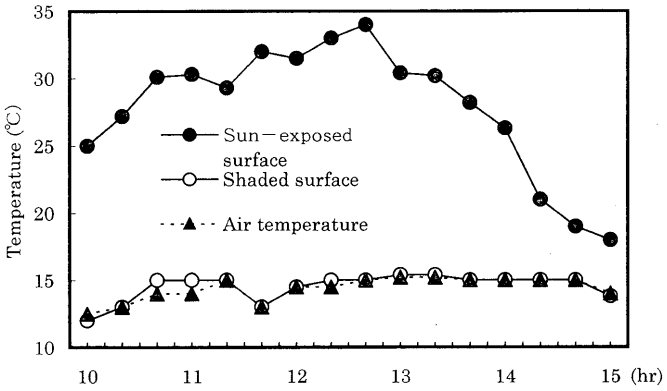

Fig. 4. Temperatures of sun-exposed and shaded side of a fruit on Nov. 15, 1991.

Table 1. The effect of the weather conditions on transpiration rates (g · 100 g^{-1} fruit) of 'Kiyomi' tangor fruit.

Fruit	Day			Night
	Clear day	Clear day, cloudy later	Cloudy day, rainy later	Sun-exposed
Sum-exposed	3.3 (330)	1.9 (211)	0.8 (100)	1.5 (125)
Shaded	1.0 (100)	0.9 (100)	0.8 (100)	1.2 (100)

Data taken on September 11-13, 1991; 10:00-15:00 (day), 18:00-6:00 (night).
Values in parentheses indicate the percentages of shaded fruit.

Table 2. Mineral content (%DW, ± SE) in the flavedo and albedo tissues of sun-exposed and shaded part of the same frurt.

Mineral element	Sun-exposed surface		Shaded surface	
	Flavedo	Albedo	Flavedo	Albedo
Nitrogen	0.90 ± 0.07	0.63 ± 0.03	0.95 ± 0.14	0.62 ± 0.13
Calcium	0.80 ± 0.08	0.51 ± 0.02	0.79 ± 0.04	0.50 ± 0.04
Phosphorus	0.13 ± 0.02	0.12 ± 0.08	0.11 ± 0.03	0.07 ± 0.01
Magnesium	0.16 ± 0.03	0.06 ± 0.02	0.15 ± 0.03	0.06 ± 0.03
Potassium	0.68 ± 0.11	0.30 ± 0.08	0.83 ± 0.11	0.35 ± 0.07

tissues (Fig. 5). Little or no difference in sugar content of the flavedo tissues existed between the sun-exposed and shaded rind tissues (Fig. 6). In the albedo tissues, the fructose and sucrose contents in the sun-exposed part were lower than that of the shaded part, whereas glucose level in the former was higher than that of the latter (Fig. 6).

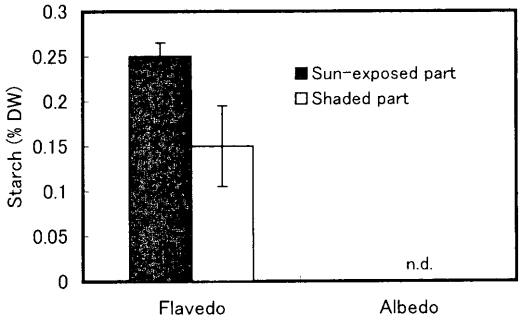

Fig. 5. Starch content in the flavedo and albedo tissues of sun-exposed and shaded parts. Vertical bars represent SE. n.d. = not detected.

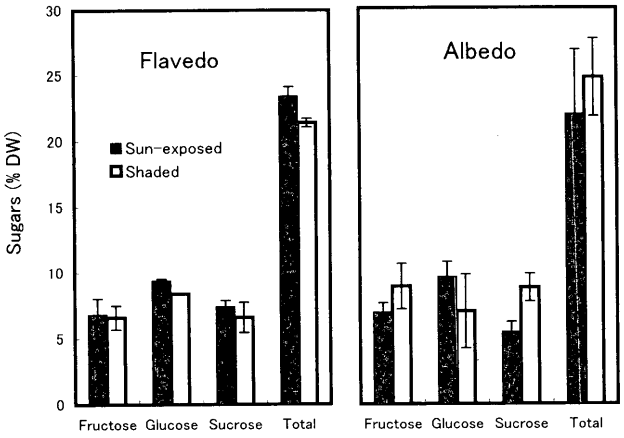

Fig. 6. Sugar content in the flavedo and albedo tissues of sun-exposed and shaded parts. Vertical bars represent SE.

(c) Rind color and roughness

The a* value of affected fruit surface was 28 ± 1, while the healthy surface of the same fruit was only 22.5 ± 2. The HPLC analysis revealed that the exposed side had slightly more carotenoids than had the shaded parts (Fig. 7).

The sun-exposed fruit had a smooth surface, whereas the shaded fruit had characteristically sunken, rough surfaces, especially around the oil glands (Fig. 1B). Of 20 fruit examined, 15 fruit with smooth surface exhibited "Kohansho", whereas only two fruit with rough surface expressed the symptoms (Fig. 8).

Discussion

"Kohansho" disorder in 'Kiyomi' tangor fruit develops on the rind surface both on the tree and during postharvest storage. However, this disorder does not become apparent on the tree as much as it does during

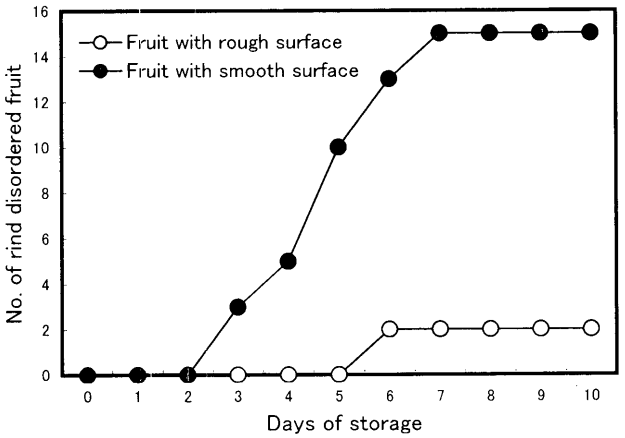

Fig. 8. Effect of rind roughness on the occurrence of "Kohansho" disorder of 'Kiyomi' tangor fruit. Fruit were harvested on December 20, 1994. Twenty fruit were allocated for each group.

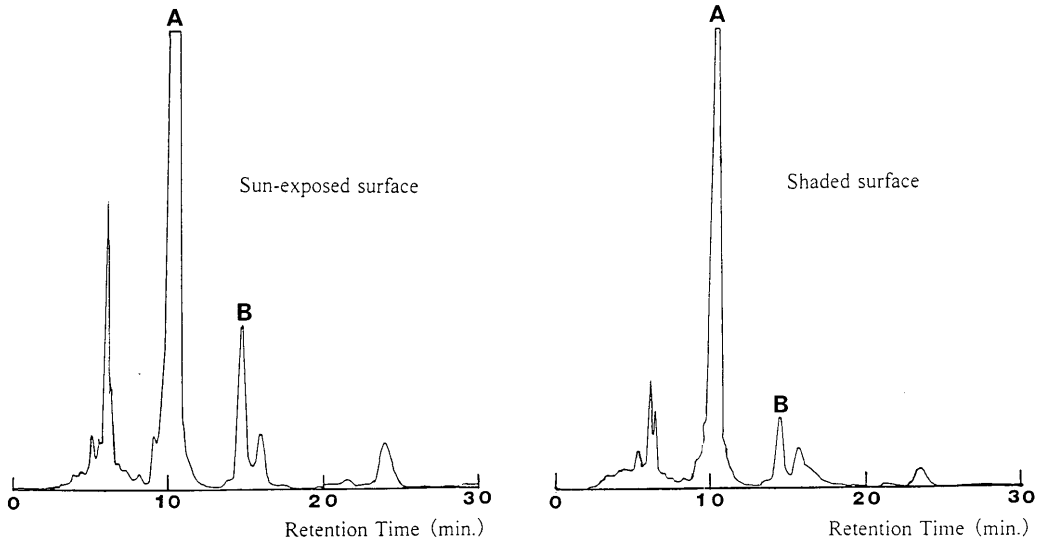

Fig. 7. Separation of carotenoids by high-performance liquid chromatography. A, violaxanthin; B, zeaxanthin; other peaks were not identified.

storage (Chikaizumi and Matsumoto, 1991). The affected portions of the fruit rind are slightly sunken as the flavedo tissue collapses and acquires various shapes that eventually darkens.

The data show that the rind disorder mainly appears during storage on fruit previously exposed to sunlight; it seldom occurs on shaded fruit. Similar conditions are prevalent on the same fruit, indicating that 'Kiyomi' tangor fruit are resistant to solar radiation while attached to the tree; they are subject to "Kohansho" disorder during postharvest storage. That "Kohansho" disorder occurs mainly on the heated surface of the fruit indicates that the prime factor is the intense solar radiation. Purvis (1980, 1984) found similar phenomena with chilling injury of grapefruit. Grapefruit from the sun-exposed exterior canopies are more susceptible to chilling injury than those from the shaded interior canopies, and, likewise, on the same fruit. McDonald et al. (1993) also reported that the sun-exposed surface of fruit with larger wax platelets from the exterior canopy suffered significantly more chilling injury than the shaded surface of the same fruit. However, no observation was conducted on wax platelets in this work. During harvest, the tangor fruit on the trees were exposed daily to about 5 ℃, but this factor, with respect to "Kohansho", was not pursued in this work.

The average maximum temperature of the fruit surface was 36.4 ℃ in the sun, but 20.0 ℃ in the shaded surface in late October so that a differential of 8 to 19 ℃ can exists between the two sides of the same fruit (Fig. 4). Such a temperature differential may undoubtedly alter metabolism of the rind. Langridge (1963) suggested that if imbalance occurs at extreme temperatures, there may be an accumulation of toxic products, followed by metabolic disturbances, and consequently, growth inhibition.

The transpiration rate in sun-exposed fruit during a sunny day was three-fold greater than that of shaded fruit; this may have altered the membrane integrity as it does in banana and papaya fruit during their ripening processes. However, the role of transpiration in the development of "Kohansho" remains to be investigated.

No significant differences were found in the mineral contents between sun-exposed and shaded parts, indicating that these factors have nothing to do with the disorder. The starch content in the rind was higher in the sun-exposed part than in the shaded part of the flavedo tissues, but undetectable in the albedo tissues. Total sugar and carotenoid contents and a* value in the flavedo tissues was also slightly higher in sun-exposed parts of the healthy rind. Thus, coloration was promoted in the affected parts, similar to citrus rind damaged mechanically or by insects.

These aspects of "Kohansho" and their relationship to membrane integrity need further study.

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タンゴール'清見'果実のこはん症の発生原因

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摘　要

'清見'果実のこはん症の発生に及ぼす原因を明らかにすると共にその防止対策を確立する目的で本研究を行った．'清見'の貯蔵中の果実に発生するこはん症は，果実が樹上で受けた日射並びに日照が原因の一つであることを初めて明らかにした．すなわち，収穫時には肉眼的にみて健全な果実であるが，果実が樹上で受けた果面の陽光部に，貯蔵中にこはん症が発生し，日射を受けていない日陰の果実ではその発生がほとんど認められなかった．陽光部の果面温度は同じ果実の日陰部のそれより10℃以上高かった．快晴の日における果実からの蒸散量は陽光部で日陰部の3倍，夜間でも25%も高かった．このことから陽光部の蒸散量とこはん症の発生は密接な関係があるものと思われた．果皮の陽光部と日陰部における無機成分含量には有為な差は認められなかったが，デンプン含量と全糖含量はわずかであるが陽光部のフラベドで高かった．果皮のa*値およびカロチノイド組成については，こはん症の発生した陽光部でわずかながら高かった．観察の結果，陽光部の果皮表面は滑らかであるが日陰部の果面は油胞と油胞の間が陥没した粗い果面であり，果面の滑らかな部分に主にこはん症が発生し，果面の粗い果実にはその発生が少なかった．