Taxonomic Reassessment of the Causal Fungus of Peach Fusicoccum Canker in Japan*

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Abstract

The causal fungus of peach Fusicoccum canker in Japan had been identified as a species in the genus *Fusicoccum*. However, isolates from the canker of peach in Niigata had phialidic conidiogenous cells and produced beta conidia *in vitro*, which are typical in the genus *Phomopsis*. Comparative studies of the Niigata isolates and an French isolate of *Phomopsis amygdali* (synonym: *Fusicoccum amygdali*) in morphology of colony and conidia, pathogenicity to peach shoots revealed that these isolates are identical and belong to the same species. W- and G-type isolates from peach Phomopsis rot in Japan had alpha conidia similar to the Niigata isolates, but isolates of neither type caused symptoms of peach Fusicoccum canker in inoculation tests. It was concluded that the causal fungus of peach Fusicoccum canker is not a *Fusicoccum* but *Phomopsis amygdali* (Delacroix) Tuset et Portilla.

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Key words : peach Fusicoccum canker, causal fungus, *Phomopsis amygdali*, taxonomy.

INTRODUCTION

Peach Fusicoccum canker was first reported in Japan by Harada and Kagiwata¹⁰⁾ in 1951. The typical symptom of the disease was necrotic cankers at bases of peach shoots. Serious damage to the infected peach trees had been observed in Kanagawa around 1950. In Shizuoka, considerable damage to peach trees has been caused by this disease since 1977²⁰⁾. Since 1991, this disease has also been found in Niigata. In 1994, it completely destroyed the harvest in 10% of the peach fields in Niigata.

The causal fungus of the disease was identified as a species of the genus *Fusicoccum* Corda by Togashi *et al.* (1952), based on its pycnidia in erumpent stroma, small and oblong conidia but absence of beta conidia, which are typical of the genus *Phomopsis* (Saccardo) Bubáček^{10,22)}. However, taxonomic status of the fungus has not been investigated in detail so far, because the taxonomy of *Fusicoccum* is complicated⁹⁾. The taxonomic status of the genus *Fusicoccum* and its type

species has been a source of confusion in the past because the original material of the type species, *F. aesculi* Corda⁵⁾ has not been reexamined²¹⁾. Though the status of *Fusicoccum* is in contention, *Fusicoccum* is now often regarded as a conidial state of *Botryosphaeria* Ces. & de Not.^{3,11,15-17)}.

Peach Fusicoccum canker fungus in Niigata clearly differed from *Botryosphaeria* isolates in colony and conidial morphology, and we found that the peach Fusicoccum canker fungus was rather similar to members of the genus *Phomopsis*. The typical characters of the genus *Phomopsis* are (1) phialidic conidial formation, (2) both alpha and beta conidia production and (3) a *Diaporthe teleomorph*²⁵⁾. So far, beta conidia and the ascogenous stage of the causal fungus of peach Fusicoccum canker have not been observed in Japan^{9,10,20)}.

Canker and withering of young twigs in peach and almond trees have been reported in France^{7,12)}, Italy²⁾, Spain²³⁾ and the United States of America^{4,6,8)}. Recently, the causal fungus of this disease has been transferred from *Fusicoccum* to *Phomopsis* and named *P. amygdali* (Delacroix) Tuset et Portilla, because of the presence of

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beta conidia and phialidic conidiogenous cells²³⁾.

In Japan, still another disease by a *Phomopsis* sp., peach *Phomopsis* rot occurs. The pathogen also causes bud canker on peach twig¹⁾ as well as Japanese pear canker and apple canker¹³⁾. Isolates of the peach *Phomopsis* rot fungus consist of two groups of colony morphology, the W- and G-types. Alpha conidia of *P. amygdali*²³⁾, a *Phomopsis* sp. isolated from peach *Phomopsis* rot^{1,13,18,19)} and isolates from peach *Fusicoccum* canker in Japan¹⁰⁾ were morphologically similar.

To determine the taxonomic status of the causal fungus of peach *Fusicoccum* canker in Niigata, we studied its morphological and pathological characters and compared them with those of *P. amygdali* and the W- and G-type isolates of *Phomopsis* sp. from peach and Japanese pear.

MATERIALS AND METHODS

Isolates The Peach *Fusicoccum* canker fungus and *Phomopsis* isolates used in this study are listed in Table 1. All these isolates were obtained from a single conidium or ascospore. Gift isolates were reisolated from a single conidium prior to use. The fungal isolates were kept for long storage by freezing the conidia at -80°C in 10% glycerol solution. For routine maintenance of cultures, potato dextrose agar (PDA) (Difco) was used. To stimulate sporulation of the fungus, the agar leaf disk method¹⁴⁾ was utilized at intervals, i.e. the fungus was grown on 1.5% agar plate overlaid with boiled (for 45 sec) young leaves of peach or Japanese pear.

Morphological characters To observe conidial morphology, two culture methods were utilized: 1) each isolate was cultured on PDA plates at 25°C or 30°C under fluorescent lamps (12 hr cycle); and 2) each isolate was first grown on PDA plates at 25°C in the dark for about 5 days, when autoclaved one-year-old twigs (about 5 cm long) of peach or Japanese pear were placed on the plates and incubated at the same conditions for 5 more days. Then the twigs covered with mycelia were transferred to sterile test tubes (18 mm in diameter and 180 mm in long) containing 2 ml of sterile distilled water and incubated at 25°C or 30°C under fluorescent lamps (12 hr cycle) for conidial production. Conidiomata produced on inoculated twigs were sectioned using a freezing microtome (Leica, CM1100) to observe conidiomata and conidiophores. Conidia were mounted in water and observed under a light microscope. The size of 30 to 100 alpha, 30 to 100 beta conidia and 30 conidiophores were measured for each isolate.

To compare colony morphology, each isolate was grown on 9-cm petri dishes containing 15 ml PDA (Difco) and incubated for 14 days at 25°C under fluorescent lamps (12 hr cycle) at about 4500 Lux.

Pathogenicity at bases of young peach shoots Approximately 5 to 6 weeks after the full bloom stage, bases of current season's shoots of potted peach trees

[*Prunus persica* (L.) Batsch var. *vulgaris* Maximowicz cv. Akatsuki] in the greenhouse or trees (cv. Fukushima Hakutou) in the orchard at the NIFTS in Tsukuba were sprayed with spore suspensions in 0.1% sucrose solution (2×10^6 alpha conidia/ml) with small atomizers. As a control, 0.1% sucrose solution was used. In the greenhouse tests, inoculated peach trees were incubated for 3 days under 95–100% R.H. at 23°C and then transferred to a greenhouse. In the field test, inoculated shoots were loosely wrapped with plastic bags and further covered with newspaper for 2 days to avoid direct sunshine. The diseased twigs were counted after 2 months in greenhouse tests and 3 months in field test.

Pathogenicity on peach, Japanese pear and apple twigs with wound Two-year-old twigs of peach (cv. Akatsuki) and apple (*Malus pumila* Miller var. *domestica* Schneider cv. Fuji) and 4-year-old branches of Japanese pear (*Pyrus pyrifolia* Nakai var. *culta* Nakai cv. Kousui) were inoculated in the orchard. In May, each twig was wounded with a knife by peeling off about 0.5 cm^2 epidermal tissue, then mycelial discs, 12 mm in diameter, were placed on the wound and the disc on the wound area was wrapped with parafilm. As a control, a drop of distilled water was used instead of mycelial discs. The radius of the subsequent lesion was measured one month after inoculation. The experiment was carried out once with four to eight replications.

Pathogenicity on fruits Peach fruits (cv. Akatsuki) were covered with paper bags about 7 weeks after the full-bloom stage to protect them from fungicide sprays and/or natural infection. Approximately 11 to 13 weeks after the full-bloom stage, the bags were removed and about 5 ml of a spore suspension (4×10^6 alpha conidia/ml in 0.1% sucrose solution) was sprayed on the fruits with small atomizers. In control treatments, 0.1% sucrose solution was used instead of the spore suspension.

Inoculated fruits were covered again with paper bags containing a wet paper to keep the humidity high. One week before the ordinary harvesting stage (about 100 days after full bloom), the inoculated fruits were harvested and stored at 20°C . The number of rotted fruits in each group of twelve fruits inoculated with a fungal isolate was counted until 13 days after harvesting. The experiment was conducted twice.

RESULTS

Conidial morphology of peach *Fusicoccum* canker fungus

Conidiophores are hyaline, cylindrical, sparingly branched, measuring $11.3\text{--}22.5 \times 1.5\text{--}2.5\text{ }\mu\text{m}$ (Niigata 2) (Fig. 1A). Conidiogenous cells are enteroblastic and phialidic. Both alpha and beta conidia were produced on autoclaved twigs incubated at 30°C (Fig. 1B): alpha conidia are hyaline, fusiform to ellipsoidal, unicellular, usually biguttulate, measuring $4.6\text{--}8.6 \times 2.3\text{--}3.5\text{ }\mu\text{m}$; beta conidia are hyaline, filiform, hamate or straight, measuring $9.3\text{--}23.1 \times 0.7\text{--}2.0\text{ }\mu\text{m}$ (Niigata 74-1). Production of

Table 1. Fungal isolates used in the present study with their pathogenicity on base of peach shoots

Isolate		Origin	Locality	Collector	Greenhouse ^{a)}		Field test ^{a)}
					test I	test II	
From peach Fusicoccum canker							
1	Niigata 74-1	Peach twig, conidia	Niigata	Y. Yokoyama		6/14 ^{b)}	11/20
2	Niigata 2	Peach twig, conidia	Niigata	Y. Yokoyama	8/10	12/15	13/32
<i>Phomopsis amygdali</i>							
3	ATCC42412 ^{c)}	Peach twig, conidia	France	F. Jailloux		15/16	
<i>Phomopsis</i> sp. W-type							
4	P-Pt-16	Peach twig, ascospore	Ibaraki	S. Kanematsu			0/26
5	P-Pt-43	Peach twig, conidia	Nagano	Y. Kawai	0/5	0/7	
6	P-JP-18	Japanese pear twig, ascospore	Ibaraki	S. Kanematsu		1/11	0/18
<i>Phomopsis</i> sp. G-type							
7	930811-14	Peach fruit, conidia	Ibaraki	A. Kudo	0/5	0/11	0/19
8	P-JP-24	Japanese pear twig, conidia	Ibaraki	S. Kanematsu		0/13	0/21

a) Base of current season's shoots was sprayed with spore suspensions of 2×10^6 alpha conidia/ml 0.1% sucrose solution. Canker development was assessed about 2 and 3 months after inoculation in greenhouse and field, respectively.

b) Number of diseased shoots/number of inoculated shoots.

c) ATCC, American Type Culture Collection, Rockville, MD., U.S.A.

Fig. 1. Morphology of peach Fusicoccum canker fungus. A: Conidiophores (Niigata 2). B: Alpha and beta conidia (Niigata 74-1) formed on autoclaved twigs. Bar represents 10 μ m.

Fig. 2. Conidia of peach *Phomopsis* rot fungus formed on autoclaved twigs. A: W-type isolate (P-Pt-16). B: G-type isolate (930811-14). Bar represents 10 μ m.

beta conidia on autoclaved twigs was rare at 25°C. On the contrary, beta conidia were not observed in cultures on PDA plates incubated at 25°C or 30°C. Beta conidia did not germinate on 1.5% water agar and on PDA after incubation for 24 hr at 25°C.

Colony morphology

Colonies of the Niigata isolates from peach Fusicoccum canker were white and had a characteristic rosulate form. Pycnidial stromata with globose or applanate locules were observed on the lobes of rosulate mycelial growth (Plate I-A). Shizuoka isolates (Fu11 and Fu12) from peach Fusicoccum canker showed the same colony morphology (data not shown). On the other hand, colonies of *P. amygdali* from France (ATCC42412) were white but developed in a lobular form (Plate I-B). A *P. amygdali* isolate from almond in Italy (ATCC 42610) had the same colony morphology as the French isolate (data not shown).

Pathogenicity

Only the isolates from peach Fusicoccum canker (Niigata 74-1 and Niigata 2) and *P. amygdali* (ATCC 42412) incited a canker at the bases of current season's peach shoots in both greenhouse and field tests (Table 1). Symptoms caused by these fungi were very similar (Plate I-C, D). Cankers at the bases of twigs were slightly sunken, became brown to dark brown, and finally girdled the twig and produced longitudinal cracks on them.

The radius of the canker that developed on peach, Japanese pear and apple twigs with wounds varied with the fungi, Niigata isolates from peach Fusicoccum canker developed the biggest canker on peach twigs in particular as well as on Japanese pear and apple twigs (Fig. 3).

Incidence of peach fruit rot was compared after inoculations with isolates of the W- and G-types of *Phomopsis* and of the Niigata isolates from peach Fusicoccum canker (Table 2). The G-type isolate of *Phomopsis* caused a higher disease incidence than the W-type and Niigata isolates. Symptoms of fruit rot caused by iso-

Fig. 3. Lesion development after field inoculations with peach *Fusicoccum* canker fungus and *Phomopsis* W- and G-types of isolates in artificially wounded 2-, 4- and 2-year-old twigs and branches of peach, Japanese pear and apple, respectively. Isolate numbers are the same as listed in Table 1. The radius of the canker lesions was measured one month after inoculation. The experiment was carried out once with four to eight replications. Columns indicate the average radius of the canker lesions with standard errors.

Table 2. Incidence of fruit rot on peach in inoculation tests

Isolate		Test I ^{a)}	Test II ^{a)}
Peach <i>Fusicoccum</i> canker fungus	Niigata 2	50% ^{b)}	27%
<i>Phomopsis</i> sp. W-type	P-Pt-16	40	33
<i>Phomopsis</i> sp. G-type	930811-14	82	89
Control		0	0

a) Peach fruits were sprayed with 5 ml of aqueous suspensions of 4×10^6 alpha conidia/ml of 0.1% sucrose. Control was treated only with 0.1% sucrose solution.

b) Diseased fruits were counted until 13 days after harvest. Disease incidence was shown with percentage of diseased fruits.

lates of the W- and G-types *Phomopsis* and by the Niigata isolates were all similar and indistinguishable.

DISCUSSION

Despite the unsatisfactory state of our knowledge of fungi given the name *Fusicoccum*, its use for anamorphs of some species of *Botryosphaeria* seems to be justified^{3,11,15-17)}. On the other hand, the generic name *Phomopsis* had not been correctly typified for a long time because of the absence of the lectotype for the genus²¹⁾. Later, an amended proposal that *Phomopsis* (Saccardo) Bubák was to be conserved, with *Phomopsis lactucae* (Saccardo) Bubák as the lectotype, was accepted at the International Botanical Congress in 1987²⁵⁾.

The Niigata isolates from peach *Fusicoccum* canker were distinctly different from *Botryosphaeria* in colony and conidial morphology. As the isolates had phialidic conidiogenous cells and bore beta conidia typical of the genus *Phomopsis* *in vitro* (Fig. 1), we judged that the Niigata isolates do not belong to the genus *Fusicoccum* but to the genus *Phomopsis*, although we could not find the ascogenous stage of the fungi in the orchards.

We compared the morphology of alpha conidia, colony characters and disease symptoms of the Niigata isolates with those of the Kanagawa isolates described by Harada and Kagiwata (1951), as well as those of the Shizuoka isolates (Full and 12), kindly given by Dr. Serizawa. We concluded that the causal agents of peach *Fusicoccum* canker in Japan are identical and belong to the same species.

In Japan, *Phomopsis* isolates from peach *Phomopsis* rot consist of two groups, W- and G-types¹³⁾. Morphology of alpha conidia of these *Phomopsis* isolates was similar to that of the Niigata isolates from peach *Fusicoccum* canker. The shape of beta conidia of Niigata isolates was similar to that of the G-type isolates (930811-14: $11-32.9 \times 1-2 \mu\text{m}$) rather than to the W-type isolates (P-Pt-16: $19.5-31.7 \times 1-2 \mu\text{m}$) (Fig. 2). Beta conidia of G-type isolates were not observed on PDA cultured at 25°C¹³⁾ but were produced on PDA or autoclaved twigs at 30°C. On the other hand, colony morphology and pathogenicity to peach shoots of the peach *Fusicoccum*

canker fungus were different from both W- and G-types of isolates. The typical symptom, canker on the base of peach shoot, was reproduced by the Niigata isolates and a French *P. amygdali* isolate but not by W- and G-type isolates of *Phomopsis* sp. (Table 1). Therefore, the fungus from peach *Fusicoccum* canker in Japan is best identified as *P. amygdali*.

Niigata isolates from peach *Fusicoccum* canker and *P. amygdali* used in this study failed to produce beta conidia on PDA plates in all culture conditions used. Tuset and Portilla (1989) found beta conidia of *P. amygdali* only on extremely dehydrated leaves but not *in vitro*²³⁾. We observed beta conidia of Niigata isolates on autoclaved twigs *in vitro* (Fig. 1B).

Peach *Phomopsis* rot fungus causes peach twig canker around buds, as the fungus generally enters through either dead buds or dead tissues around the bud^{1,18)}. In our experiment, isolates from peach *Phomopsis* rot could not develop canker lesion on the base of the current season's peach shoots, whereas Niigata isolates and *P. amygdali* caused typical symptoms of peach *Fusicoccum* canker on peach twigs without any apparent wound (Table 1). On the other hand, the Niigata isolates and the *Phomopsis* W- and G-type isolates were distinct in pathogenicity on twigs of peach, Japanese pear and apple. Niigata isolates were highly virulent on these hosts, whereas *Phomopsis* W-type isolates were weakly pathogenic. *Phomopsis* G-type isolates were moderately virulent, as already reported¹³⁾. *Phomopsis* isolates of the W- and G-types have also been obtained from Japanese pear canker and apple canker¹³⁾. From these results, the causal agent of peach *Fusicoccum* canker might be able to infect Japanese pear and apple trees in the orchard. Similar results were reported by Uddin *et al.* (1998). Isolates of *Phomopsis* sp. causing shoot blight of peach in Georgia, USA, could infect twigs of apple, plum and pear after artificial inoculation, in contrast to other *Phomopsis* isolates from plum and Japanese pear which developed significantly smaller cankers on peach²⁴⁾. To explore the genetic identity or relatedness of these groups of fungi, DNA analyses of the Niigata *P. amygdali* isolates and W- and G-types of *Phomopsis* isolates are in progress.

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和 文 摘 要

兼松聡子・横山泰裕・小林享夫・工藤 晟・大津善弘：日本産

モモ枝折病菌の再同定

モモ枝折病は従来、*Fusicoccum* 属菌による病害とされていたが、新潟県において発生した罹病樹から分離された菌株の分生子形成細胞はフィアライドであり、*in vitro* で *Phomopsis* 属菌に特徴的な β 胞子を形成したことから、本菌は *Fusicoccum* 属菌ではなく、*Phomopsis* 属菌であると考えられた。この新潟分離株と、フランスで分離されたモモに canker を引き起こす *P. amygdali* (synonym: *F. amygdali*), および本邦産でモモに果実腐敗や芽枯れ症状を引き起こす *Phomopsis* 属菌の W 型株と G 型株とを用いて、胞子形態、コロニーの形態、ならびにモモ新梢基部に対する病原性を比較した。その結果、モモ枝折病菌は、コロニー形態が白色で特徴的な隆起があること、およびモモ新梢基部に特徴的な病徴を示すこと、の2点において、W 型株や G 型株とは異なった。しかし、欧州産の *P. amygdali* (Delacroix) Tuset et Portilla には諸性質が似ており、これと同一種であると同定した。

Plate I

Plate I

A and B : Colonies of peach *Fusicoccum* canker fungus. A : Niigata 74-1 and B : ATCC42412 on PDA, grown for 2 weeks at 25°C. C and D : Cankers on the base of young peach shoot (black arrow) after inoculation with the peach *Fusicoccum* canker fungus (Niigata 2) (C) and *Phomopsis amygdali* (ATCC42412) (D).