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Original Article

Is it a commensalism that non-plant pathogenic *Fusarium* spp. were frequently isolated from the canker on pumpkin (*Cucurbitae moschata*)?

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Abstract

Dry rot of pumpkins caused by *F. solani* f. sp. *cucurbitae* race 2 is a well known post harvest disease. In Malaysia, crust-like lesions are often observed on the surface of pumpkins in local markets. These symptoms look like the scab caused by *Cladosporium cucumerinum* or the Fusarium rot caused by *Fusarium* spp. Most of the symptomatic crusts did not soften or develop further in our observations. As these crusts are merely considered as the mechanical injuries in the local markets, they do not reduce their marketability of pumpkin. However, *F. solani* was frequently isolated from these symptoms in our preliminary study. Thus these crust symptoms were distinguished from the scab, the Fusarium rot, the preharvest dry, hard rot (type 1) and the dry rot caused by other *Fusarium* spp. as reported previously. Histological observation of naturally occurring raised pimples or crust symptoms showed periderm formation that apparently healed the loss of palisade layer. Therefore, hereafter, the term 'canker' is used to explain the crust symptoms and 'scab' for the raised pimples. A majority of the fungal isolates from the canker was identified as *F. solani* (41.6%). The pathogenicity test with 12 isolates of *F. solani* on mature pumpkins did not efficiently reproduce canker. The wound on immature fruit, 1 to 5 weeks-old, successfully produced canker regardless of the inoculum, while the unwounded part did not show any symptom with inoculation. Canker symptom of pumpkin is considered like the fruit defense mechanisms after mechanical injury.

Keywords: canker, *Fusarium solani*, pumpkin, wound healing

1. Introduction

Crust lesions are often observed on the surface of pumpkins in local markets in Malaysia. Lesions appear as crusts or raised pimples. These symptoms look like the scab caused by *Cladosporium cucumerinum* Ellis & Arth. (Zitter, 1996) and the Fusarium rot caused by *F. acuminatum* Ellis & Everh, *F. avenaceum* (Fr.:Fr.) Sacc., and *F. graminearum* Schwabe among the infectious diseases of pumpkin (Bruton and Duthie, 1996). Elmer (1996) found a preharvest dry, hard rot (type 1) and a postharvest soft, sunken rot (type 2) on cultivars of *Cucurbita pepo* L. in USA. Type 1 caused dry, hard, circular to over lesions with depressed dry, corky centers (0.2 to 0.5 cm deep) accompanying frequently orange sporodochia. *Fusarium acuminatum, F. equiseti* (Corda) Sacc., and *F. graminearum* were commonly recovered and showed pathogenicity, whereas *F. solani* (Mart.) Appel et Wr. emend Snyd. et Hans. was rarely recovered and did not show the pathogenicity. Fruit rots of *Cucurbita maxima* Duch. and *C. moschata* (Duch.) Duch. were surveyed in New Zealand (Hawthorne, 1988). Fourteen fungi including *F. culmorum* (W. G. Smith) Sacc. and *F. solani* were identified as the major pathogens and *F. acuminatum, F. equiseti* (Corda) Sacc., *F. lateritium* Nees and *F. sulphureum* Sch. as the minor ones. Symptoms of the fruit rots on *Cucurbita* spp. were softened or water-soaked tissue. Dry rot of pumpkins caused by *F. solani* f. sp. *cucurbitae* race 2 was firm, dry type rot and became apparent after fruit rind began to harden (Tousson and Snyder, 1961).

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On the contrary, most of the symptomatic crusts did not soften or develop further in our observations. These crusts were merely considered as the recovery from the mechanical injuries during harvest when the pumpkins were in the local markets. Therefore, these crusts did not reduce their marketability. However, *Fusarium* spp. were frequently isolated from these symptoms in our preliminary study. *Fusarium solani* was the most common species among the isolates. Thus, these crust symptoms were distinguished from the scab, the Fusarium rot, the preharvest dry, hard rot (type 1) and the dry rot as described above.

Fusarium spp. are known as producers of the three major classes of mycotoxin: trichothecenes, fumonisins, and zearalenones, and also as the producers of minor mycotoxins and metabolites (beuverisin, and enniatins, equisetin, fusarins) (Desjardins and Proctor, 2007). It is important to investigate whether *Fusarium* isolates from pumpkins have potential to produce mycotoxin and also whether the pumpkin flesh surrounding the crusts contains the mycotoxin.

In this study, we characterized the histology of crust for the determination of the symptom development and conducted inoculation tests with the isolated *F. solani*.

2. Material and Methods

2.1 Definition of symptom of pumpkins

Pumpkins with raised pimples or crusts were collected from local markets at different areas such as Johor, Terengganu, Sungai Petani, Guar Chempedak in Malaysia (Table 1). Symptom development and size were determined for the collected pumpkins. The raised pimples or crusts were prepared for histological observations (Jensen, 1962). Paraffin was removed from the sections by placing the slides in Histosolve 1 (Thermos Scientific) for 5 minutes and in the Histosolve 2 (Thermos Scientific) for 3 minutes. The sections were partially hydrated by passing through a series of alcohols of decreasing concentration: absolute, 95%, 70%, and 50% for 2 minutes each. Then, slides were stained by Safranine – Fast Green (Jensen, 1962) and observed under light microscope. Terminology of fruit tissue followed that of Whitaker and Davis (1962).

2.2 Isolation of fungi from the raised pimples or crusts

Prior to the isolation of fungi, the collected pumpkins were washed thoroughly in running tap water. The lesion was quarried and cut into pieces using a sterilized scalpel. Each piece was subjected to surface sterilization by dipping in 70% alcohol for 1 minute, 10% sodium hypochlorite solution for 4 minutes and sterilized distilled water for 1 minute for 3 times. The disinfested pieces were air-dried and then incubated on acidified water agar for 3 days. Grown colonies were transferred to Potato sucrose agar (PSA), proceeded to single spore isolation and were stocked in PSA slants. Colony from the single spore isolation was used for the morphological identification on Carnation leaves agar (CLA) and cultural characteristics on Potato Dextrose Agar (PDA) (Leslie and Summerell, 2006); 50-100 macro- or micro-conidia were randomly measured.

2.3 Pathogenicity on matured pumpkin fruit

Mature pumpkin fruits were used to test the pathogenicity of 12 isolates of *F. solani*. The isolates were deposited in NBRC (Biological Resource Center, National Institute of Technology and Evaluation, Japan). All fruits were cleaned and surface sterilized with 70% alcohol. The surface of the fruits was stripped in 1cm × 1cm pieces at the upper and lower parts with a sterilized scalpel, pasted with mycelium colonized on PSA, covered with adhesive tape and incubated for 3-4 weeks in plastic bags. Symptom appearance was examined. Re-isolation was conducted to confirm the pathogen.

2.4 Pathogenicity on growing pumpkin fruits

Six isolates were inoculated on 1 week-old pumpkin and 9 isolates on 2, 3 and 5 weeks-old pumpkins with 2 replicates at each age. Wounded and unwounded parts were set on each fruit. The surface of the pumpkin was scratched with sand paper around 1cm × 1cm size for wounded parts. Isolates of *F. solani* were cultured on PSA for seven days as the source of inoculum. A mass of cultured mycelium was inoculated into the wounded and unwounded parts with a sterilized scalpel. Wounded and unwounded parts without inoculum were set as controls to compare the symptom development. Treated pumpkins were covered with plastic bag for the night to prevent the inoculum from being washed away by the rain. Inoculated fruits were observed daily to record symptom development.

2.5 Pathogenicity on seedlings, fruits, and tubers

Nine isolates were tested on the seedlings of 5 known hosts of *F. solani.* The hosts used were pumpkin (*Cucurbita moschata),* soybean (*Glycine max*), green pepper (*Capsicum annum* L.) French bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), and cowpea (*Vigna unguiculata* (L.) Walp.). Seeds were surface sterilized in 5% NaOCl in 5 minutes and rinsed overnight to induce germination. Sterilized compost mixed soil was potted, 1kg per bag, for planting. Soil was inoculated with 10^7 spore suspension/g of soil. Ten seedlings were used for each isolate and control. Symptom development on the seedlings was examined 21 days after inoculation.

The disease severity of seedlings after being inoculated with isolates of *F. solani* was used to compare between the inoculated seedlings by referring to the condition of the control of each host. The roots were classified based on the severity of the symptoms and disease index was calculated

Table 1. Frequency of fungal isolates from canker symptom on pumpkin .

Location	Label of fruit	Number of cankers examined	FS*	FO*	FC*	FSM*	FD*	FP*	FN*	FCL*	FSB*	P*	UND*	Total number of isolates
Johor	J1	6	1	1	2	1	-	-	-	1	-	4	-	**10**
	J2	8	7	-	-	-	-	-	-	-	-	-	5	**12**
Kedah	GC1	3	6**	2	-	-	-	-	1	-	1	-	-	**10**
- Guar Chempedak														
Kedah	G1	1	1	1	-	-	-	-	-	-	-	-	-	**2**
- Gurun	G2	3	6**	-	-	-	-	-	-	-	-	-	-	**6**
Kedah	SP1	3	2	1	-	-	-	1	-	-	-	-	-	**4**
- Sungai Petani	SP2	3	1	1	-	-	-	-	-	-	-	-	-	**2**
Kelantan	K1	6	3	-	-	-	-	-	1	-	-	3	-	**7**
Pulau Pinang	KB1	1	1	-	-	2	-	-	-	-	-	-	-	**3**
- Kepala Batas	KB2	2	-	1	1	-	-	1	-	-	-	-	-	**3**
Terengganu	T1	10	1	6	2	1	1	-	-	-	-	-	-	**11**
	T2	6	3	1	-	-	1	-	-	-	-	-	-	**5**
	T3	8	5	-	-	-	-	-	-	-	-	1	8	**14**
Total	13	60	37	14	5	4	2	2	2	1	1	8	13	**89**
	Percentage of isolation		**41.57**	**15.73**	**5.62**	**4.49**	**2.25**	**2.25**	**2.25**	**1.12**	**1.12**	**8.99**	**14.61**	**100.00**

* Fungal species were abbreviated as below; FS(*Fusarium solani*), FO (*Fusarium oxysporum*), FC (*Fusarium camptoceras*), FSM (*Fusarium semitectum*), FD (*Fusarium dimerum*), FP (*Fusarium poae*), FN (*Fusarium nelsonii*), FCL (*Fusarium culmorum*), FSB (*Fusarium subglutinans*), P (*Penicilium* spp.), and UND (Unidentified species).

** Different appearance colonies of *F. solani* were considered as the independent origins and some of them ranged in different septal numbers on macroconidia (Table 2).

by using the formula noted below (Nagao *et al.*, 1994).

$$DI = (0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4) + (5 \times n_5)/N$$

N indicates the number of plants examined and n_0 to n_5 are the number of plants corresponding to each disease severity of stage 0 to stage 5.

Mature fruits of green peppers and tubers of potato (*Solanum tuberosum* L.) were used to test the pathogenicity of 12 isolates of *F. solani*.

3. Results

3.1 Definition of symptom

Crust symptom shows as a corky, hardened lesion that is slightly raised on the surface like wound healed appearance due to callus growth beneath the wounded surface and did not affect the pumpkin flesh physically. The crusts (Figure 1A, B) or raised pimples (Figure 1B, arrowed) symptoms on pumpkins were commonly seen in markets. The size was variable, 2-15 mm.

Four clearly differentiated layers were observed in the healthy tissue of pumpkin (Figure 2A). The epicarp was composed of polygonal and elongated cells and formed the palisade layer. The hypodermis was the layer of isodiametric cells. The outer mesocarp was composed of thickened isodiametric cells with cellulose or schlerenchymaties pitted walls. The middle mesocarp was composed of isodiametric, large cells with thin walls which contained small amount of starch. The crusts or raised pimples became the raised part of the surface of fruit (Figure 2B). Comparison with the ordinary tissue, the palisade layer had disappeared and was replaced by the periderm composed of rectangular-shaped cells and arranged in columnar rows (Figure 2C). In the middle part of the crust, the cell wall became thick. Brilliant red staining by safranine indicated the existence of lignification in the thickened cell walls (Figure 2D, arrowed). The shapes of the cells were obviously uneven and irregularly arranged. These cells became larger and distorted.

From these observations, the raised pimples or crusts symptoms were composed of the periderm that apparently healed the loss of the palisade layer. Therefore, the term 'canker' was used to explain the crusts symptoms and 'scab' for the raised pimples hereafter.

3.2 Isolation of fungi

The majority of the fungal isolates from the canker were identified as *F. solani* (41.6%); 37 isolates of *F. solani* was obtained from 13 fruits of symptom pumpkins (Table 1). Seven other *Fusarium* spp. were found in 34.9%. *Fusarium oxysporum* Schlecht. emend Snyd. et Hans. was isolated in 15.7% of the cankers, while *F. camptoceras, F. culmorum, F. dimerum, F. nelsonii, F. poae* (Peck) Wollenw., and *F. semitectum* were less frequent. Macroconidia of *F. solani* were distinguished into 2 types, either short-stout shape with 3 septa (Figure 3A) or long-slender shape with 3-5 septa (Figure 3B). Some isolates had both type macroconidia (Table 2). Microconidia were oval, kidney shaped with 0-1 septa (Figure 3C) on long monophialide (Figure 3D). Chlamydospores abundantly formed in the medium. However, the pigmentation on colonies and patterns of mycelial growth on PDA varied. The pigmentation varied from white (Figure 3E), light yellow to light brown color (Figure 3F). The growth of some of the colonies was very extensive while those of others very thin. *Penicillium* spp. and unidentified species were obtained in 23.6% of the cankers.

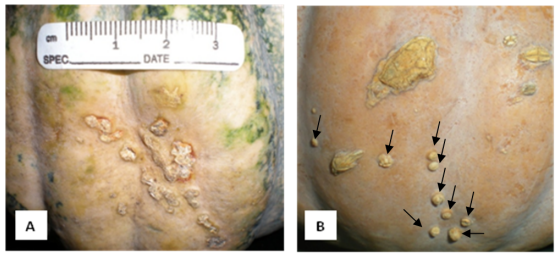

Figure 1. Crusts or raised pimples symptoms on pumpkins collected from different areas of Peninsular Malaysia. A. Crusts symptom on the pumpkin collected in Sungai Petani, B. Crusts or raised pimples (arrowed) symptoms on the pumpkin from Terengganu.

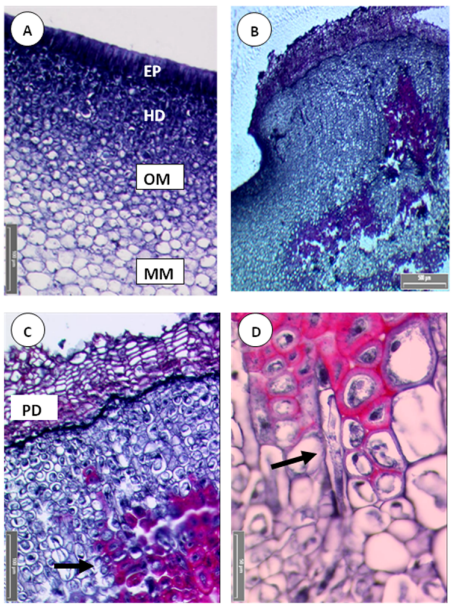

Figure 2. Histology of crusts symptom on pumpkins observed under compound microscope. A. Structure of healthy pumpkin tissue as control. EC = epicarp, HD = hypoderm, OM = outer mesocarp, MM = middle mesocarp. B. Structure of raised part of canker. Periderm layer (PE) formed on surface of canker tissue. C. Structure of canker symptom. Palisade layer was replaced with periderm. D. Lignification in the thickened cell wall (arrowed).

Table 2. Origin of isolates for the inoculation tests.

Isolate	Locations	Symptom	Characteristic on PDA		Macroconidia	Species
			Pigment in the medium	Diameter (cm)	No. of septum	
C112	Guar Cempedak, Kedah	Canker	ND*	ND	3	*F. solani*
GC111	Guar Cempedak, Kedah	Canker	Pale bown to brown	3.12	3-5	*F. solani*
GC113	Guar Cempedak, Kedah	Canker	Pale brown	3.58	ND	*F. oxysporum*
GC118	Guar Cempedak, Kedah	Canker	ND	ND	3-5	*F. solani*
GC135	Guar Cempedak, Kedah	Canker	White to beige	4.3	3-4	*F. solani*
GC137	Guar Cempedak, Kedah	Canker	Pale brown	3.75	3-4	*F. solani*
GC138	Guar Cempedak, Kedah	Canker	White	3.77	3	*F. solani*
G413	Gurun, Kedah	Limited infection, canker	Purple to brown	4.6	ND	*F. subglutinans*
G414	Gurun, Kedah	Limited infection, canker	Brown	3.55	3-4	*F. solani*
G522	Gurun, Kedah	Limited infection, canker	White	2.9	3-4	*F. solani*
G531	Gurun, Kedah	Limited infection, canker	White	3.42	4-5	*F. solani*
G532	Gurun, Kedah	Limited infection, canker	White	3	3	*F. solani*
G533	Gurun, Kedah	Limited infection, canker	Pale brown	3.5	3-6	*F. solani*
G534	Gurun, Kedah	Limited infection, canker	ND	ND	3-5	*F. solani*
G535	Gurun, Kedah	Limited infection, canker	White to dark brown	3.3	4-7	*F. solani*
KB221	Kepala Batas, Pulau Pinang	Large infection, canker	Reddish	3.7	ND	*F. poae*
SP111	Sungai Petani, Kedah	Limited infection, canker, yellowish	Cream	3.55	3-4	*F. solani*
SP115	Sungai Petani, Kedah	Limited infection, canker, yellowish	White	4	3-4	*F. solani*
SP121	Sungai Petani, Kedah	Limited infection, canker, yellowish	Cream	3.8	3-4	*F. solani*

*ND Not determined

3.3 Pathogenicity test of *F. solani* to mature pumpkin fruits

Twelve isolates of *F. solani* were inoculated to mature pumpkin (Table 3). Rot symptom occurred with 8 isolates in the wounded condition and with 2 isolates in the unwounded condition. Re-isolations were successful from the rot symptom induced except G535. Three isolates, KB221, G532, and GC111, did not show any symptom in ether condition. The pathogenicity test on mature pumpkins did not efficiently reproduce canker. *Fusarium oxysporum* was unexpectedly re-isolated from this rot symptom instead of the inocula, *F. solani* G535. Re-isolations from the canker were also successful except G531. In this inoculation test, *F. oxysporum* was also re-isolated from the canker instead of the inocula, *F. solani* G531.

3.4 Pathogenicity test of *F. solani* to immature, growing pumpkin fruits

Infection and canker formation did not occur on the unwounded surface.

On the wounded part of 1- and 2-weeks-old pumpkins, inoculation did not affect the appearance or the duration of canker formation compared with the non-inoculated condition (Table 4). All isolates developed the canker in both ages. For 1-week-old pumpkin, exudate was secreted during its healing process (Figure 4A). The lumping tissue could be observed after 2 days of the inoculation and the wounded surface healed up after 3 days (Figure 4B). For 2-weeks-old pumpkin, exudate was produced after 1 day of inoculation and the amount of exudate depended upon the fruit (Figure 4C). The exudate already stopped after 2 days of inoculation and the lumping tissue was observed. The wounded surface healed up after 3 days and the canker formation stopped after 5 days of inoculation on both the 1- and 2-weeks-old pumpkins. Dry and corky appearance was judged as the completion of canker formation (Figure 4D). The sizes of canker formed on 1-week-old pumpkin were between 5 to 15 mm (Figure 4B) and for 2-weeks-old pumpkin about 10 to 15 mm (Figure 4D). Variance in the size of canker was not significantly different ($P = 0.05$) in each 1-, 2-, and 5-weeks-old pumpkin (Table 4). Unexpected rot or decay did not occur on these young fruits by the wound treatment.

In the inoculation test on 3-weeks-old pumpkin, cankers successfully developed on the wounded areas and their development was dependent on the isolates. On the wounded part without inoculum, the wound began to heal after 2 days of inoculation. After 3 days, healed areas were slightly raised (Figure 4E). After 5 days of inoculation, wounded areas began to dry and harden. Canker formation stopped after 7 days (Figure 4F). On the wounded part with inoculum, the inoculated wound part began to heal after 3 days. Canker formation stopped after 7 days. Canker was observed with the isolates G522, GC118, and SP115, whereas

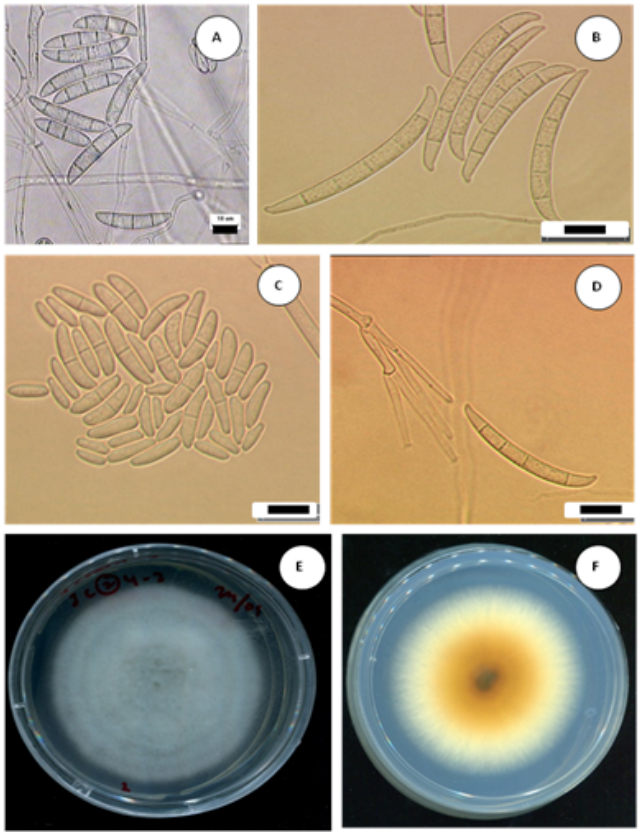

Figure 3. Characteristics of *F. solani* isolated from canker symptom of pumpkin. A. Macroconidia with 3 septa, short and stout, B. Macroconidia with 3-5 septa, long and slender, C. Microconidia 0-1 septum, D. Branched monophialide, E. Aerial hypha on the surface of colony on PDA, F. Pigmentation on the bottom of colony. Bar = 10 μm.

the isolates GC135, GC137 and C112 caused canker on one fruit and scab on the other fruit.

In the inoculation test on 5-weeks-old pumpkin, the wound surface healed after 4 days of inoculation on the wounded part without inoculum (Figure 4G) and canker formation stopped after 7 days and hardened (Figure 4H). On the wounded part with the inoculum, the inoculated wound part healed after 4 days and the canker or scab occurred after more than 8 days of inoculation. Inoculation with some isolates caused the canker while inoculation with other isolates caused the scab (Figure 4I). However, the inoculated isolates were re-isolated from these symptoms.

3.5 Pathogenicity on seedling and tuber of other host plants

Disease indices on pumpkin, soybean, green pepper, French bean, pea, and cowpea were generally low (Table 5). All isolates except G533 were considered to be non-pathogenic to these host plants. Isolate of G533 showed disease index of 4 on pea *(P. sativum)* seedlings (Table 5).

Pathogenicity test was also conducted to 2 other mature fruits to see if isolates of *F. solani* could cause any infection (Table 3). Six out of 12 isolates on wound and 3 on unwounded parts of potato showed rot, browning, yellowing or water soaking. Inoculation on wounded green pepper caused softening and yellowing except KB221. Seven isolates caused softening, browning and yellowing on unwounded green pepper. Inoculated *F. solani* were re-isolated from the symptoms that developed.

4. Discussion

Cankers on the trees show necrotic lesion on periderm, cortex, phloem, and vascular cambium tissues (Biggs, 1992). Walling-out response is known in woody plants as a non-specific resistance mechanism. Either mechanical injury or the actions by insects and fungal pathogens is responsible for this response (Merrill, 1992). Development of corky tissue along vein tracts was observed in cantaloupe infected by *F. roseum* 'semitectum' (Carter, 1979). Corky dry rot extended internally and turned the flesh dark brown, dry and mealy. As we described the histological characteristics of canker, this symptom was histologically different from Fusarium rots. From the histological observation, canker tissue contained lignified thick cells beneath the periderm (Figures 2C, D). Biggs (1992) described the necrophylactic periderm, including wound periderm, as protecting living tissues from the adverse effects of cell death. He recognized the formation of a ligno-suberized boundary as a prerequisite to wound periderm in response to pathogens. Even if hyphal distribution was obscure around the boundary of wound periderm in our observations, detection of lignified cells beneath the periderm suggested the effect of fungal infection. Hawthorne and Sutherland (1991) also examined the wound healing process in growing fruits of *C. maxima* hybrid 'Delica' and confirmed a two-stage process of wound repair; i.e. the very rapid exudation and a wound periderm formation accompanied with callus tissue production. Our observations were identical with their results in non-inoculated condition.

Inoculation test on mature pumpkin failed to efficiently reproduce canker without regard to wound or not (Table 3). The wound on younger fruit successfully produced canker regardless of the inoculum while the inoculation on the unwounded part did not show any symptom. This showed that the canker was induced when the rind of pumpkin was naturally or artificially wounded in the growing stage. Inoculation did not affect the appearance (Figure 4B, D, F, H. I) or size (Table 4) of cankers. However, inoculation of *F. solani* on the 5-weeks-old pumpkin fruits delayed the healing process. As the pumpkin fruit were close to mature stage during these experiments, secondary morphogenesis such as healing of epicarp and hypodermis seemed be slowed down. Inoculation of *F. solani* subsequently prolonged the canker formation.

Eight *Fusarium* spp. were isolated from canker. *Fusarium solani* was most abundant and the other *Fusarium* spp. less so (Table 1). These isolated *Fusarium* spp., except *F. solani,* were different from those causing Fusarium rot

Table 3. Results of inoculation test on wounded and unwounded parts of mature pumpkin, green pepper and potato.

Isolate	Fruits												
	Pumpkin					Potato				Green Pepper			
	Replicate	Wounded	RI[1)]	Unwounded	RI[1)]	Wounded	RI[1)]	Unwounded	RI[1)]	Wounded	RI[1)]	Unwounded	RI[1)]
Control	1	–		–		–		–		–		–	
	2	–		–		–		–		–		–	
GC111	1	–		–		–		–		Yellowing	(+)	–	
	2	–		–		–		–		Yellowing	(+)	–	
GC113	1	Rot	(+)	–		–		–		Yellowing, Brrowning, Softening	(+)	Brrowning, Softening	(+)
	2	–		–		Brrowning, rot	(+)	–		Brrowning, Softening	(+)	Brrowning, Softening	(+)
G413	1	Canker	(+)	–		–		–		Yellowing	(+)	–	
	2	Rot	(+)	–		–		–		Brrowning, Softening	(+)	–	
G414	1	Rot	(+)	–		Brrowning, Softening	(+)	–		Brrowning, Softening	(+)	–	
	2	–		–		Brrowning, Softening		–		Brrowning, Softening	(+)	–	
G522	1	Rot	(+)	Canker	(+)	Brrowning, Softening	(+)	–		Brrowning, Softening	(+)	Brrowning	(+)
	2	Rot	(+)	–		Brrowning, Softening	(+)	Brrowning	(+)	Brrowning, Softening	(+)	–	
G531	1	–		Rot	(+)	–		–		Yellowing, Softening	(+)	Yellowing	(+)
	2	Softening	(+)	Canker* [2)]	(+)	–		–		Yellowing, Softening	(+)	Yellowing	(+)

1) (+) indicated positive result of re-isolation.
2) * *Fusarium oxysporum* was re-isolated instead of inoculated *F. solani.*

Table 3. Continued

Isolate	Fruits											
	Pumpkin					Potato				Green Pepper			
	Replicate	Wounded	RI[1)]	Unwounded	RI[1)]	Wounded	RI[1)]	Unwounded	RI[1)]	Wounded	RI[1)]	Unwounded	RI[1)]
G532	1	–		–		–		–		Brrowning, rot, water-soaking	(+)	Brrowning, Softening	(+)
	2	–		–		Brrowning, Softening	(+)	Brrowning	(+)	Brrowning, Softening	(+)	–	
G533	1	Rot	(+)	–		Brrowning, rot,	(+)	–		Brrowning, rot, water-soaking	(+)	Brrowning	(+)
	2	Rot	(+)	–		–		Brrowning, rot, water-soaking	(+)	Brrowning, Softening	(+)	–	
G535	1	–		Rot * [2)]	(+)	–		–		Yellowing, Softening	(+)	Yellowing	(+)
	2	–		–		–		–		Yellowing, Softening	(+)	Yellowing	(+)
KB221	1	–		–		–		–		–		–	
	2	–		–		–		–		–		–	
SP111	1	Rot	(+)	–		Rot	(+)	–		Brrowning, Softening	(+)	–	
	2	–		–		–		–		Brrowning, Softening	(+)	–	
SP121	1	Rot	(+)	–		–		–		Brrowning, Softening	(+)	–	
	2	–		–		–		–		Yellowing, Brrowning, Softening	(+)	Brrowning, Softening	(+)

1) (+) indicated positive result of re-isolation.
2) * *Fusarium oxysporum* was re-isolated instead of inoculated *F. solani.*

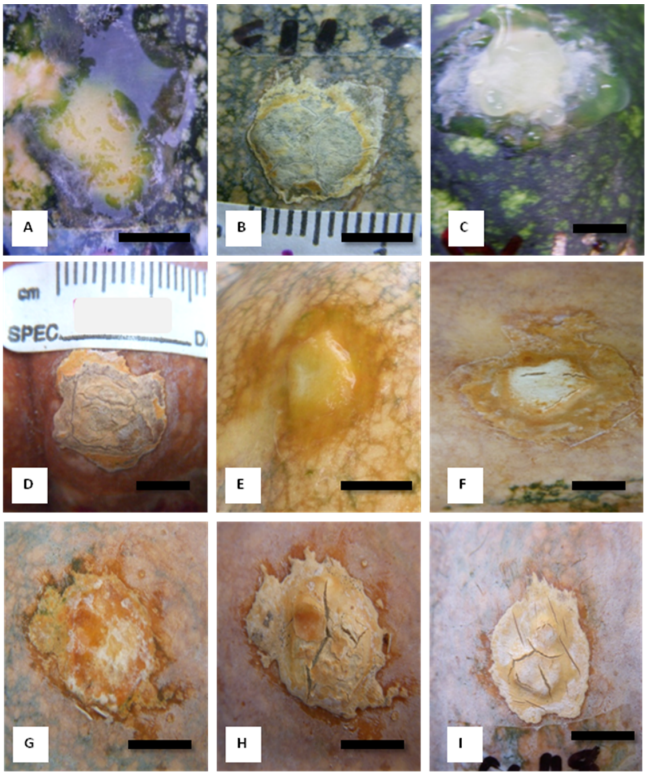

Figure 4. Canker formation by the artificial wounding on growing pumpkin fruits. A. Exudating wound on the 1-week-old pumpkin fruit inoculated with *F.solani* GC138. B. Canker formation on the 1-week-old pumpkin fruit inoculated with *F.solani* G112. C. Exudate from wound on the 2-weeks-old pumpkin fruit inoculated with *F.solani* GC138. D. Canker formation on the 2-weeks-old pumpkin fruit inoculated with *F.solani* GC138. E. Emerged canker on healing process of wounding on the 3-weeks-old pumpkin fruit without pathogen after 3 days. F. Canker formation on the 3-weeks-old pumpkin fruit without pathogen after 7 days. G. Canker formation on healing process of wounding on the 5-weeks-old pumpkin fruit without pathogen after 4 days. H. Canker formation on the 5-weeks-old pumpkin fruit without pathogen after 7 days. I. Canker formation on the 5-weeks-old pumpkin fruit inoculated with *F.solani* GC118.

(Bruton and Duthie, 1996), Preharvest dry, hard rot (type 1) (Elmer, 1966), and Fruit rot (Hawthorne, 1988). Hawthorne and Sutherland (1991) surveyed the incidence of fungi on the surface of 100 fruits of *C. maxima* 'Delica'. *Fusarium* spp. were dominantly isolated from the 3 locations on the surface of fruits; 36.4 % in contact with stalk, 46.0 % in contact with corolla scar, and 23.0 % in contact with soil. These data indicated that *Fusarium* species were predominantly colonized on pumpkin fruits. Even though the very rapid exudation of a compound occurs (Hawthorne and Sutherland, 1991), *Fusarium* species can overcome this barrier to enter the pumpkin fruit. Since *F. solani* differentiated into 11 formae specialis (Chung *et al.*, 2011) and also showed the particular pathogenicity for avocado (Darvas *et al.*, 1987), caladium (Goktepe *et al.*, 2007), citrus (Nemec *et al.*, 1980), passion fruit (Ploetz, 1991), and peppers (Fletcher, 1994), the inoculation tests were necessary. Pathogenicity test against the known host plants showed 2 formae specialis, G533 as *F. solani* f. sp. *pisi* and G522 as *F. solani* f.sp. *cucurbitae* race 2. Another isolate of *F. solani* G533 partially showed rot and water-soaking on unwound potato but the type of macroconidia (Table 2) was not identical with that of f. sp. *radicicola* (Matuo and Snyder, 1973).

Canker symptom of pumpkin is considered to be the fruit defense mechanisms after mechanical injury. Since canker only occurred on growing fruit, it was different from the known rot symptom on mature pumpkin. In addition, formation of canker only occurred with the presence of wound as fungal entrance and *F. solani* survived in the disrupted tissues and can be highly isolated again after inoculation. Gordon and Leveau (2010) discussed an alternative path open to plant-associated microbes other than the destructive parasitism. They introduced the paper regarding to recovery of *Fusarium verticilloides* from seed of wild teosintes (*Zea* spp.) in Mexico and Central America (Desjardins *et al.*, 2000). It indicated the commensal relationship of *F. verticilloides* with *Zea* spp. that inspired the beginning of the present association between *F. verticilloides* and corn (*Z. mays*) (Gordon and Leveau, 2010). This fungus systemically infects corn plant throughout its life cycles, reaching even kernels, often without any visible impact (Munkvold *et al.*, 1997). As many *F. solani* were absolutely isolated from the canker symptom (Table 1), these *F. solani* have a potential to break through the physiological defense action on wounded pumpkin fruit. These *F. solani* survived as endophytes but did not develop to be pathogenic. It was interpreted as the commensal relationship with pumpkin fruit.

Table 4. Diameter of canker development on different ages of pumpkin inoculated with *F.solani* isolates.

Isolate	Diameter of canker (mm)[2]		
	1 week-old	2 weeks-old	5 weeks-old
Control[1]	10.0	13.2	12.7
C112	10.0	14.0	14.5
GC118	8.5	14.0	14.5
GC135	ND[3]	12.5	12.0
GC137	9.0	15.0	14.0
GC138	10.5	14.0	13.0
G522	9.0	13.5	14.5
G534	ND	16.0	13.0
SP115	9.0	16.0	14.5
SP121	ND	12.0	ND

1) Wounded without inoculation.
2) The size of canker was not significantly different (P=0.05) in each 1, 2, and 5 weeks-old pumpkin.
3) ND: Not determined.

Table 5. Disease index on 6 different host plants inoculated with the pumpkin canker isolates.

Plant	Isolate												
	Control	GC111	GC113	G413	G414	G522	G531	G532	G533	G535	KB221	SP111	SP121
Pumpkin (*C. moschata*)	0	0	0.1	0.1	0	0	0	0.2	0.1	0	0	0.3	0.5
Soybean (*G. max*)	0	NT[1)]	0.8	0.5	0.6	0.2	NT	0.3	0.6	NT	0.4	0.6	0.5
Green pepper (*C. annuum*)	0	NT	2.3	2.9	NT	NT	0	1	NT	0	1.6	0.1	0.4
Pea (*P. sativum*)	0	1.2	0	0.3	0.3	0.1	0	0.3	4	1.9	0.4	0.8	0.2
French bean (*P. vulgaris*)	0	0	0.4	0.6	0.3	0.3	0	0.5	0.3	0	0.3	0.1	0.1
Cowpea (*V. unguiculata*)	0	NT	NT	NT	2.5	2.1	NT	2.0	2.6	NT	NT	1.8	1.9

1) NT: not tested.

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