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INCIDENCES OF LEAF SPOTS, BLIGHTS AND FRUIT ROTS OF KIWIFRUIT (*ACTINIDIA DELICIOSA*) IN GUILAN PROVINCE, IRAN*

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Abstract

Kiwifruit is an economically important fruit crop in the northern part of Iran. Identify the Kiwifruit fungal pathogens in Guilan province, 65 kiwifruit orchards were visited in 2008–2010. Leaves and fruits with disease symptoms were sampled and their fungal pathogens were isolated and purified on PDA and WA. Fungal species were determined morphologically according to the relevant literature. Fungi associated with leaf spots, blights and fruit rot symptoms with their prevalence rate were identified and determined in this study as follows: Alternaria alternata (45%), Diaporthe cf. actinidiae (25%), Colletotrichum gloeosporioides (12%), Botrytis cinerea (11%) and Pestalotiopsis longiseta (7%). The pathogenicity of all isolated fungi was proved on kiwifruit leaves (in vivo) and fruits (in vitro). The leaf spots caused by P. longiseta and D. cf. actinidiae were more severe followed by blight and curling of leaf margins. In all of the species, the disease was more severe in wounded than in unwounded leaves. Effect of leaf age was different. D. cf. actinidiae at stem end and B. cinerea at both stem and distal ends showed the highest level of fruit rot developments at 25±1°C and 0–1°C, respectively. Based on the results of this study, kiwifruit is reported as new host of C. gloeosporioides and D. cf. actinidiae in Iran and infections caused by all the species can be important on injured host tissues.

Keywords: Kiwifruit, *Colletotrichum gloeosporioides*, *Diaporthe* cf. *actinidiae*, fungal pathogen, pathogenicity, north of Iran

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Introduction

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.Sliang et A.R.Ferguson) is a member of the *Actinidiaceae* family indigenous to Southeast Asia. It was relatively unknown for several decades, but it has now become an important fruit crop in many countries worldwide (Michailides & Elmer 2000). Northern region of Iran is a suitable natural growing habitat for kiwifruit cultivation. It is currently cultivated in about 6,000 ha of cultivated lands in Iran. Kiwifruit has been regarded as a "disease-free" crop in New Zealand (Hawthorne *et al.* 1982) and California (Sommer *et al.* 1994), but several diseases have been reported to occur in kiwifruit as its cultivation acreages have increased steadily during the past two decades (Jeong *et al.* 2008). Various kinds of leaf spot and blight symptoms have been reported on the leaves of kiwifruit growing in the farmers orchards in other countries. Species of *Alternaria, Colletotrichum, Diaporthe* and *Phoma* frequently have been isolated from infected leaves and flowers. The most frequently isolated fungus from both sound and rotting fruits have been *Diaporthe* species (Hawthorne *et al.* 1982). Other researchers have found *Alternaria sp., A. alternata*, *Colletotrichum acutatum, C. gloeosporioides* (*Glomerella cingulata), Fusarium acuminatum, Cryptosporiopsis* sp., *Phoma exigua*, *Phomopsis* sp., *Diaporthe* spp., *D. actinidiae, Sclerotinia sclerotiorum, Botryosphaeria sp., B. dothidea*, *Cylindrocarpon* cf. *candidum* and *Botrytis cinerea* in New Zealand, Japan, Italy, Greece and Korea (Hawthorne & Otto 1986, Kinogawa & Sasaki 1990, Ushiyama *et al.* 1996, Hoyte 1997, Corazza *et al.* 1999, Tsahouridou & Thanassoulopoulos 2000, Lee *et al.* 2001, Maning *et al.* 2003, Jeong *et al.* 2008). Pestalotia diseases caused by *Pestalotiopsis* sp., *P. longiseta* and *P. neglecta* were also reported from Japan, Turkey and Korea (Kinogawa & Sasaki 1990, Ushiyama *et al.* 1996, Karakaya 2001, Jeong *et al.* 2008).

Kiwifruit can be maintained for more than four months when stored at 0±1°C (Schroeder & Fletcher 1967). However, fruit rot diseases may cause severe losses of kiwifruits during storage, transportation, marketing and consumption after harvest. More than seven fungi have been reported to be associated with post-harvest fruit rots of kiwifruit (Hawthorne *et al.* 1982, Pennycook 1984) which among them, *Phomopsis* sp. and *Botryosphaeria* sp. were reported to be the major causal organisms of post-harvest fruit rots (Lee *et al.* 1998, Sommer & Beraha 1975).

Although leaf spots by *Alternaria* sp. and *Pestalotiopsis longiseta*, fruit gray mold by *Botrytis cinerea* and several fruit diseases by *Sclerotinia sclerotiorum, A. alternata, Penicillium digitatum, P. italicum*, and *Mucor* sp. have been reported from kiwifruit in Iran (Binesh & Pour Abdollah 1990, Golmohammadi & Rahimian 2004, Mousakhah *et al.* 2008, Taheri *et al.* 2004, Mahdavian & Javadi 2004, Taheri *et al.* 2006) but most of them have been limited to short reports in congress proceedings. However, the incidences of the leaf spot, blight and fruit rot diseases and their associated pathogens have not been adequately studied in Iran until now. The present work was undertaken to determine what potentially pathogenic fungi are associated with leaf and fruit diseases of kiwifruit plants in orchard condition of Guilan province, to determine their relative pathogenicity on leaves and fruits and to describe and illustrate the associated fungi.

Materials and Methods

Sample collection

Infected leaves, twigs and fruits samples were collected from 65 kiwifruit orchards in major regions of kiwifruit cultivation in Guilan province (including Astaneh Ashrafiyeh, Roudsar, Talesh, Bandar Anzali, Rasht, Fouman and Sowmehesara townships) during the period of 2008–2010. Samples were immediately placed in sterile plastic bags avoiding dryness and placed in refrigerator (5±1˚C) for up to 24 hours before laboratory assay. Samples were grouped based on superficial characteristics, color of spots and presence of fungus fruiting bodies. After sterilization with 0.5% sodium hypochlorite for one min and washing with sterile water (three times), infected leaf tissues were cut into 4–6 mm pieces, placed on potato dextrose agar (PDA) plates and incubated at 25±1°C in darkness for 48 h. Finally, the isolates were purified using the single spore or hyphal tip culture methods, transferred to PDA tubes and storaged at 5±1°C for subsequent studies.

Identification of the isolates

Anamorphic and/or teleomorphic characteristics of the isolated fungi were examined using a light microscope. Fungal species were determined morphologically according to the relevant literature (Barnett & Hunter 1998, Kiffer & Morelet 2000, Guba 1961, Sutton 1980, Mordue 1971, Simmons 2007).

Pathogenicity test on leaves

Two year old Hayward cultivar of kiwifruit was used for pathogenicity test. The fungal isolates were grown on PDA and incubated at 25±1°C with 12 h dark/ 12 h light regime. The leaves were wounded using scalpel (two young and two old leaves) or sandpaper (two young and two old leaves) for mechanical scratching. A wound of approximately one mm depth was made in the middle of each half leaf (Jeong *et al.* 2008, Hawthorn & Otto 1986). Each plant was inoculated with one isolate. Inoculation was made by spraying the conidial suspensions of 10^6 conidia per ml of 0.1% water agar or by using a 5-mm diameter disk from the outer edge of 4–5 days-old colony. The mycelium bearing side of the disks was placed on treatment places and the inoculated sites were sealed with parafilm (Tsahouridou & Thanassoulopoulos 2000). For evaluating germination of the conidia, the suspension of conidia was sprayed on WA plate and incubated at 20±1°C for 24 h. The sterile 0.1% WA or PDA discs were used in control treatments. Each plant was covered with a large plastic bag, placed in incubator at 25±1°C, 70% RH and 12 h dark/ 12 h light regime. The bags removed after three days (Karakaya 2001). The plants were transferred outside of incubator at 27±1°C and 65% RH, seven days after inoculation. The disease severity and leaf spots caused by each isolates were grouped as sever, mild and no symptoms 30 days after inoculation (Jeong *et al.*, 2008),

Pathogenicity test on fruits

Ripening healthy Hayward variety of kiwifruits were provided from a garden of Astane Ashrafiyeh township. The fruits were surface sterilized with70% ethanol for two min, rinsed with sterile water and air-dried before inoculation (Lee *et al.* 2001). Each fruit was inoculated with one of the isolates. A 5–mm diam. cork borer wound, about 3 mm deep, was made on the flesh of each fruit, at both distal and stem ends, and inoculated with a 5-mm diameter mycelial plug of 6-days old colony, placed mycelium-side down into the wound (Biggs 1994, Hawthorne *et al.* 1982). Inoculated sites were sealed with parafilm. The inoculated fruits were placed on moist filter paper in a clear plastic box (five fruits in each box). The boxes were closed and placed in an incubator at 25±1 ºC (Lee *et al.* 2001) or 0–1 ºC (Hawthorne *et al.* 1982), separately. Lesion diameter and its characteristics were measured 7 and 10 days after inoculation (Biggs 1994, Hawthorne *et al.* 1982). The experiments were factorial on the basis of completely randomized design in which there were two factors, the isolate factor with six levels and the inoculation site factor with two levels, with five replicates at two different temperatures, 25±1°C and 0–1°C. Fruit lesion development data were transformed by square root transformation for 0–1 °C treatments. Analyses were made using the SAS GLM procedure (Version 9.00, SAS Institute 2002) with LSD test (P<0.05).

Results and Discussion

A) Description of the fungi

Five fungal species that consistently isolated from the leaf spot, blight and fruit rot symptoms of kiwifruit are described as follows:

***Alternaria alternata* (Fr.) Keissl. (1912)**. Characteristics of this species (Fig. 1 A and B) resemble *Alternaria alternata* (Ellis 1971, Simmons 2007, Jeong *et al*. 2008). This species was found in all kiwifruit growing regions of Guilan province. It was the most frequently isolated fungus from kiwifruit leaf spots and rotten fruits. The species was previously reported as leaf spot and fruit rot of kiwitfruit from Mazandaran (Binesh & Pour Abdollah 1990, Golmohammadi & Rahimian 2004, Taheri *et al*. 2006).

***Botrytis cinerea* Pers, (1794).** Our isolates (Fig. 1 C and D) were similar to *Botrytis cinerea* described by Ellis (1971), Mordue (1971) and Khodaparast & Hajaroud (1997). This species was isolated from leaves with brown spots and brown, watery small fruits from various kiwifruit orchards in Guilan province. Fruit gray mold by *B. cinerea* has been previously reported from Iran by Taheri

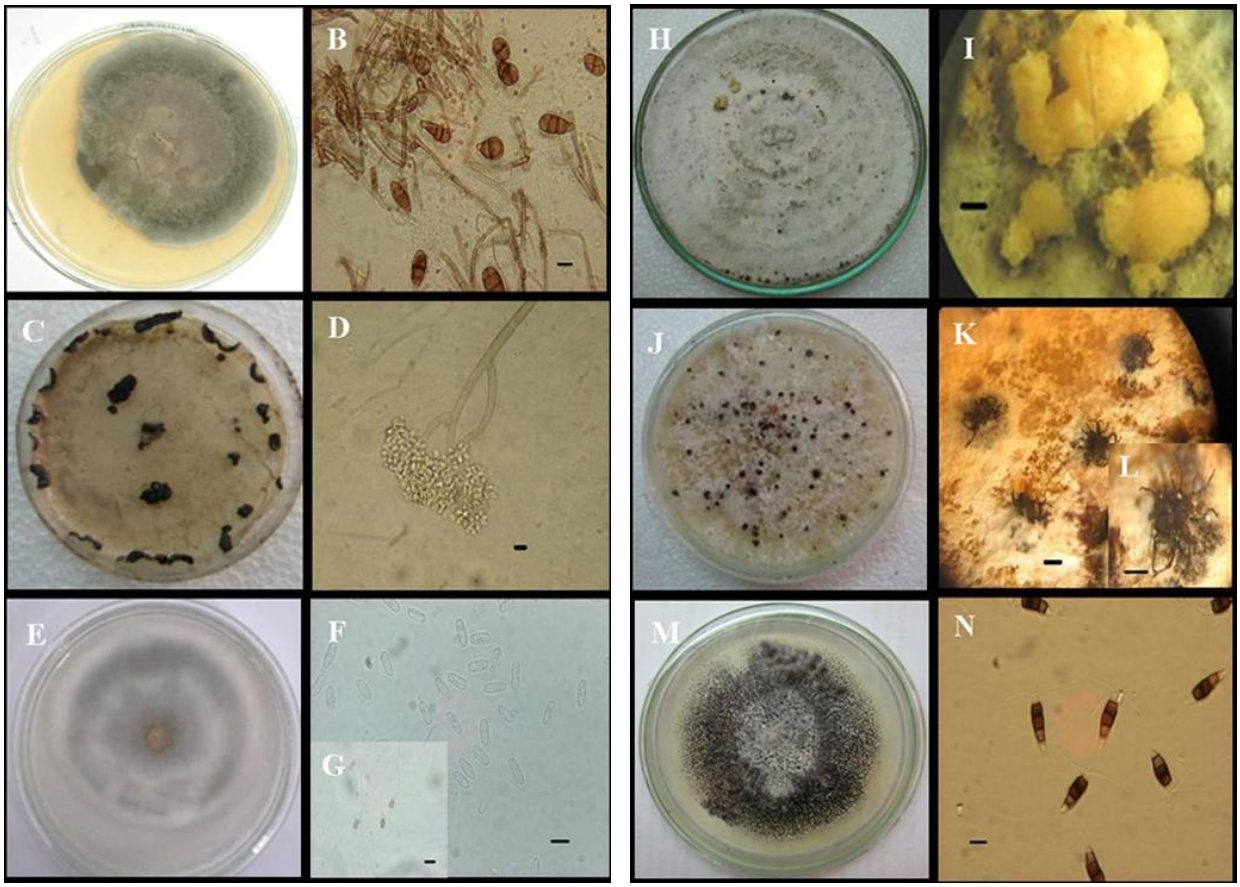

Fig. 1. Cultural and morphological characteristics of fungi isolated from kiwifruits with leaf spot, blight and fruit rot symptoms. A and B, *Alternaria alternata* colony and conidia; C and D, *Botrytis cinerea* colony with sclerotia and conidia and conidiophore; E, F, and G, *Colletotrichum gloeosporioides* colony, conidia, and appressorium, respectively; H and I, *Diaporthe* cf. *actinidiae* 7–day-old culture and conidiomata (picnidia); J, K, and L, *D*. cf. *actinidiae* 45–day-old culture showing abundant perithecia, perithecia with sinuous and filiform necks, respectively; M and N, *Pestalotiopsis longiseta* culture and conidia. Scale bars = 10 µm for B, D, F, G, N and 500 µm for I, K, L.

et al. (2004, 2006).

***Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (1884)**. Characteristics of our studied isolates (Fig. 1 E-G) were in accordance with *Colletotrichum gloeosporioides* described by Mordue (1971), Sutton (1980), Jeong *et al.* (2008), Cano *et al*. (2004) and Khodaparast & Hajaroud (1997). The fungus was mostly isolated from leaf margin blight and clear grayish brown ring spot as the typical symptom of anthracnose in many regions of Guilan province. It is the first report of this species on kiwifruit in Iran.

***Diaporthe* cf. *actinidiae* N.F. Sommer & Beraha (1975)**. Colony color varied from white to brown and reached 7–9 cm diam. on PDA after 7 days. Center of the colony was sometimes depressed and dark whereas the rest of the colony was raised; aerial mycelium was cottony, white to tan or reddish brown. Reverse side of the colony was yellowish to brown at first, then black stromata formed extensively in concentric rings; sometimes with a reddish-brown pigment. Pycnidia solitary, partly immersed in agar, globose, 500–1500 µm diam, exuded masses of white-salmon pink spores at the centre. On PDA, Alpha-conidia elliptical, 5–8 × 2 µm; beta-conidia produced on milky conidial masses, hyaline, unicellular, filiform to hamate, 18–27 × 1 µm. Perithecia abundant, in irregular clusters, formed in PDA after two month incubation in 5–6 °C, usually embedded in distinct, black, elevated stromata. Perithecia black, globose with necks sinuous, filiform, 600–1000 × 70–140 µm. Asci clavate, sessile, 22–29 × 5–8 µm. Ascospores biseriate, hyaline, 2–celled, constricted

Table 1. Pathogenicity of isolates on old and young leaves of kiwifruit in different methods of artificial inoculation, 30 days after inoculation.

Fungal isolates	Wounding methods					
	Sandpaper		Scalpel		Unwounded	
	old	young	old	young	old	Young
Alternaria alternata	+*	++	+	+	-	-
Botrytis cinerea	×	+	×	++	×	-
Colletotrichum gloeosporioides	++	+	++	-	-	-
Pestalotiopsis longiseta	++	+	++	+	+	-
Diaporthe cf. *actinidiae*	++	++	+	++	×	+

*++: Severe symptoms, +: mild symptoms, -:no symptoms, ×: no treatment

at the septum, fusoid to ellipsoid, 6–9.5 × 2–3 µm (Fig. 1 H-L).

Phomopsis sp. was consistently isolated from angular leaf spot, silvering gray leaf blight, brown spotted leaves and fruit stem end rot where the brown skin at the area become soft and lighter in color than the adjacent firm healthy tissues. Watery exudates, bitter taste, white mycelial mats turning black, reduced fruit size and fruit drop were frequently visible. Fruit rot of kiwifruit caused by *Phomopsis* sp. was named stem-end rot and its teleomorphic state reported as *Diaporthe actinidiae* (Beraha 1970, Sommer & Beraha 1975). Yi and Lee (1998) identified *Phomopsis mali* as the species of *Phomopsis* causing fruit decay, but teleomorph state of the fungus has not been reported until now. Lengths of alpha and beta-conidia, conidiomata, perithecia, asci and ascospores belonging to our studied isolates were slightly different from those of *D. actinidiae* reported in the relevant literature. Since *D. actinidiae* is the only species of this genus reported on kiwifruit and the morphological characteristics of the teleomorph state of *Phomopsis* sp. were fairly in accordance with those of *D. actinidiae* (Sommer & Beraha 1975, Lee *et al.* 2001), this species is identified as *Diaporthe* cf. *actinidiae*. *Phomopsis* sp. is most strongly associated with distal end rot, side rot (Maning *et al.* 2003) and stem end rot of kiwifruit during storage, transportation, marketing and consumption after harvest (Beraha 1970, Koh *et al.* 2005, Lee *et al.* 2001, Sommer & Beraha 1975). It is the first report of the species on kiwifruit in Iran. Leaf and fruit samples were collected in Astaneh Ashrafiyeh (Lafout), Roudsar (Rahim Abad), Talesh (Taki Tazeh Abad), Bandar Anzali (Abkenar), Rasht (Lashtenesha) and Fouman.

***Pestalotiopsis longiseta* (Speg.) K. Dai & Ts. Kobay. (1990).** Characteristics of our studied isolates (Fig. 1 M and N) were in accordance with those of *Pestalotiopsis longiseta* described by Guba (1961), Hino (1962), Dai *et al.* (1990), Yasuda *et al.* (2003), Jeong *et al.* (2008) and Khodaparast & Hajaroud (1997). This species has been previously reported in association with kiwifruit leaf spot in Guilan proince, Iran (Mousakhah *et al.* 2008)

Among the fungi isolated in this study, *Alternaria alternata* was the most dominant (45%), followed by *Diaporthe* cf. *actinidiae* (25%), *Colletotrichum gloeosporioides* (12%), *Botrytis cinerea* (11%) and *Pestalotiopsis longiseta* (7%).

B) Pathogenicity on leaves

All inoculated isolates except *A. alternata* were able to penetrate and cause leaf spot on wounded or unwounded leaves of kiwifruit. Disease severity of leaf spots caused by each isolate is shown in Table 1. In this study *A. alternata* could only cause leaf spot on wounded leaves which is in agreement with Jeong *et al.* (2008) and Hawthorn and Otto (1986). Many species of this genus have been identified as saprophyte and it is difficult to know whether the fungus found on diseased leaves is a primary pathogen or secondary contaminant (Rotem 1994, Agrios 2005). However, *A. alternata* isolated from brown ring spots in this study caused distinct symptoms on wounded leaves of kiwifruit by artificial inoculation (Fig. 2A,B). Several diseases caused by members of this genus have been reported on different hosts including kiwifruit from Korea (Jeong *et al.* 2008). For *B. cinerea*, browning regions were developed from inoculation

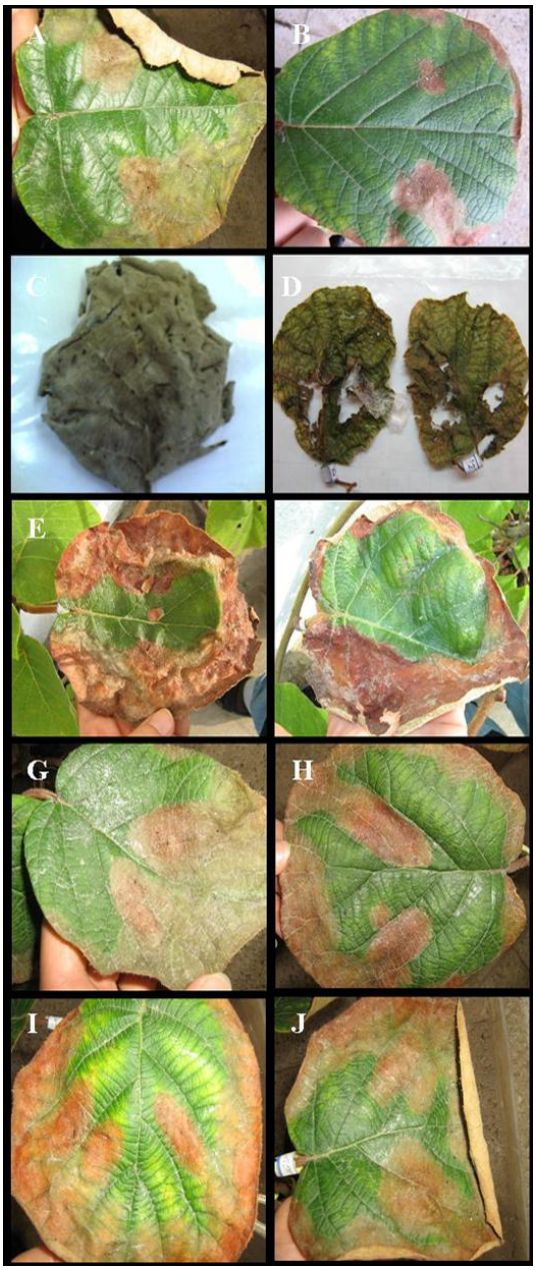

Fig. 2. Symptoms of Leaf Spot and blight on artificially infected kiwifruit cultivar Hayward. A and B, *Alternaria alternata;* C and D, *Botrytis cinerea;* E and F, *Colletotrichum gloeosporiodies;* G and H, *Pestalotiopsis longiseta,* I and J, *Diaporthe* cf. *actinidiae.*

sites and the leaves gradually became necrotic, senescent, and dead with red brown lesions. These symptoms were in accordance with four types of necrotic tissues on kiwifruit caused by *B. cinerea* described by Hoyte *et al* (in Michailides & Elmer 2000). Furthermore, some microsclerotia were seen on leaves after complete colonization by *B. cinerea* (Fig. 2C–2D). The *Colletotrichum gloeosporioides* isolate caused light brown ring spots at inoculation sites on wounded leaves and the leaf margins became necrotic. The disease was more severe in old than in young leaves (Fig. 2E–2F). Jeong *et al.* (2008) reported severe symptoms on wounded leaves and mild symptoms on unwounded leaves but in our study no symptoms were seen on unwounded leaves inoculated with *C. gloeosporioides*. Inoculation with *Pestalotiopsis longiseta* showed leaf blight on old wounded leaves while milder symptoms were seen on young wounded and old unwounded leaves (Fig. 2G,H). The pathogenicity of this species has been previously reported by Ushiama *et al.* (1996), Karakaya (2001) and Jeong *et al.* (2008). The *Diaporthe* cf. *actinidiae* caused mild to severe leaf blight with browning and rolling of leaf margins (Fig. 2I,J) similar to what reported by Hawthorn and Otto (1986) and Jeong *et al.* (2008). Each studied fungus was successfully reisolated from the edge of infected area developed on inoculated leaves.

C) Pathogenicity on fruit

There were significant differences ($P< 0.05$) among the isolates with respect to fruit lesion development at 25±1°C, 7 days after inoculation or at 0–1°C, 14 days after inoculation (Table 2). The isolates were classified in three distinct groups with respect to fruit lesion development. *D.* cf. *actinidiae* and *B. cinerea* caused more fruit lesion development at 25±1°C and 0–1°C, respectively. There were significant differences ($P< 0.05$) between fruit lesion development on distal and stem end at 25±1°C but no significant differences at 0–1°C (Table 3). The flesh of the fruits inoculated with *D.* cf. *actinidiae* became decayed drastically with a sour or fermented odor and producing drops of liquid as reported by Lee *et al.* (2001). The optimum temperature for mycelium growth of *D.* cf. *actinidiae* is 25–30°C (Kinogawa & Sasaki 1990). *D.* cf. *actinidiae* had the most fruit lesion development at 25±1°C but it could not produce severe fruit rot at 0–1°C which is in agreement with the report of Hawthorn *et al.* (1982). Lee *et al.* (2001) suggested that *D. actinidiae* can hardly infect healthy fruits and that only wounded fruits are vulnerable to the infection by the fungus. It is believed that the kiwifruits are infected by *D. actinidiae* through wounds, since

Table 2. Analysis of variances of fruit lesion development on kiwifruit by fungal isolates at 25±1°C and 0–1°C, 7 and 10 days after inoculation.

	25±1°C					0–1°C				
Sources	D.F.	SS	MS	F	*P*>*F*	Df	SS	MS	F	*P*>*F*
Isolate	5	34.58	6.91	24.51	<0.0001	5	1.54	0.30	1.67	<0.1605
Inoculation site	1	4.30	4.30	15.27	<0.0003	1	0.61	0.61	3.30	<0.0756
Isolate×Inoculation site	5	1.87	0.37	1.33	0.268	5	0.37	0.07	0.41	<0.840
Error	48	13.54	0.28			48	8.90			
			CV(%)= 26.60	r^2= 0.75				CV(%)= 86.71	r^2= 0.22	

Table 3. Means of fruit lesion development (mm) produced by different isolates at 25±1°C and 0–1°C, 7 and 10 days after inoculations on fruits of kiwifruits of Hayward variety.

	25±1°C			0–1°C		
Isolates	Stem end	Distal end	Mean	Stem end	Distal end	Mean
Alternaria alternata	23.0	20.0	21.5 ab[†]	0.0	2.0	1.0 ab
Botrytis cinerea	28.0	15.0	21.5 ab	2.0	7.0	4.5 a
Colletotrichum gloeosporioides	30.0	19.0	24.5 ab	0.0	0.0	0.0 b
Diaporthe cf. *actinidiae*	39.0	18.0	28.5 a	0.0	2.0	1.0 ab
Pestalotiopsis longiseta	27.0	11.0	19.0 b	0.0	2.0	1.0 ab
Control	0.0	0.0	0.0 c	0.0	0.0	0.0 b
Mean	**24.5 A**[†]	**13.8 B**		**0.33 A**	**2.16 A**	

[†] Values followed by the same letters are not significantly different according to LSD test (*P*<0.05).

there are numerous chances to be wounded inevitably during harvest, selection, storage, packing, transportation and marketing. Therefore, the kiwifruit should be carefully handled in order to prevent wounds on fruits. It is reported that *B. cinerea* can soften and macerate kiwifruit tissue at both high and low temperature but more severe fruit rot at low temperature, so it can cause storage decay (Opgenorth 1983, Maning *et al.* 2003). In this study, *B. cinerea* caused the most fruit lesion development at 0–1°C and significantly differed from the others, as well. *A. alternata* could cause mild fruit lesion development at 25±1°C and no severe fruit lesion development at 0–1°C which is in agreement with Opgenorth (1983). The *C. gloeosporioides* isolated from leaf and *P. longiseta* isolated from fruit were able to cause fruit rot at 25±1°C and were reisolated from the margins of fruit lesions. These results showed that these species could be important leaf and fruit pathogens of kiwifruit under optimum conditions and incorrect disease management.

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