Plant Pathol. J. 25(2) : 128-135 (2009)

The Plant Pathology Journal
©The Korean Society of Plant Pathology

Effects of Temperature and Moisture on the Survival of *Colletotrichum acutatum*, the Causal Agent of Pepper Anthracnose in Soil and Pepper Fruit Debris

Beum Kwan Kang[1], Joohyeong Kim[1], Kyeong Hee Lee[2], Sang Cheol Lim[2], Jae Jun Ji[2], Jong Won Lee[2] and Heung Tae Kim[1]*

[1]*Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Vegetable Research Institute Chungbuk ARES, Eumsung Chungbuk 369-824, Korea*

(Received on April 17, 2009; Accepted on May 22, 2009)

The survival of *Colletotrichum acutatum* was investigated in soil, infected fruits, and infected fruit debris incorporated into soil at several temperatures with different soil moisture levels. Samples were examined at 2-week intervals for 18 weeks to determine the survival of the pathogen based on the number of colony forming unit (CFU) of *C. acutatum* recovered on a semi-selective medium. *C. acutatum* conidia survived in both sterile and non-sterile soil at 4 and 10°C for 18 weeks. If infected pepper fruits were completely dried, *C. acutatum* survived for 18 weeks at temperature from 4 to 20°C. Soil temperature and moisture affected the survival of *C. acutatum* in infected fruit debris incorporated into soil after air-drying. The effect of soil moisture on survival was weaker at low temperatures than at high temperatures. For up to 16 weeks, conidia were recovered from fruit debris in soil that had been kept at 4 to 20°C and below 6% soil moisture. Conidia were recovered from fields until approximately 6 months after pepper fruits were harvested. Using PCR with species-specific primers and a pathogenicity test, we identified conidia recovered from soil and infected fruit from both the laboratory and field as *C. acutatum* and as the primary inoculum causing pepper anthracnose.

Keywords : *Colletotrichum acutatum*, pepper anthracnose, inoculum source, primary inoculum

Pepper anthracnose is one of the major factors limiting red pepper production, and pepper fruits exhibiting anthracnose have little market value. In Korea, the annual damage resulting from this disease is estimated at more than US $100 million, corresponding to approximately 10% of total annual pepper production. Anthracnose creates lesions mainly on immature green and mature red fruits. Initial symptoms of anthracnose appear on fruit as light brown flecks, and as the disease progresses, the lesions grow bigger and become round and sunken. From these lesions, masses of salmon-colored spores are produced and dispersed by rain. Several *Colletotrichum* species, including *C. gloeosporioides*, *Glomerella cingulata*, *C. dematium*, *C. cocodes*, and *C. acutatum*, may be implicated in anthracnose (Park & Kim, 1992). Among these, *C. gloeosporioides* is a dominant pathogen isolated from more than 90% of diseased samples collected in 1992. Recently, however, Kim et al. (2008) identified most *Colletotrichum* isolates from pepper as *C. acutatum* based on mycological characteristics, responses to carbendazim in benzimidazole fungicide, and nucleotide sequence analysis of the internal transcribed spacer (ITS) region.

Occasionally, symptoms initially develop on pepper fruits near the ground from late June to mid-July and then progress to the higher fruits and are dispersed to fruits of healthy plants horizontally through rainfall. Based on these observations, the infection process might start by a primary inoculum overwintering in soils and infected fruit debris. Overwinter survival of other anthracnose fungi, such as *C. acutatum*, *C. coccodes*, *C. lindemuthianum*, *C. truncatum*, and *C. dematium*, which infect strawberry (Wilson et al., 1992), tomato (Dillard & Cobb, 1998), bean (Tu, 1983), lentil (Buchwaldt et al., 1996), and mulberry (Yoshida & Shirata, 1999), respectively, has been reported. Yoshida and Shirata (1999) found that *C. dematium* could overwinter in infected or latently infected leaves, and that these leaves could be a source of primary inoculum the following year. In contrast, conidia in the soil were not a significant form of overwintering inoculum. Fungus in infected leaves maintained under laboratory conditions survived for at least 90 days at 25 and 35°C and for 600 days at 0°C. Conidia of *C. acutatum* survive for up to 1 year in autoclaved soil, although viability declines rapidly in untreated soils at 22% soil moisture, with a 95% reduction in the population recorded within 4 days (Freeman et al., 2002). At 11% soil moisture, the time required for a 95% population reduction of *C. acutatum* conidia is about 73.5 days. Furthermore, from 15 to 30% of the pathogen has been recovered from artificially inoculated mummified fruit after 5 months of burial. Freeman et al. (2002) demonstrated that the potential

*Corresponding author.
Phone) +82-43-261-2556, FAX) +82-43-271-4414
E-mail) htkim@cbnu.ac.kr

contribution of both conidia and mummified fruits to disease epidemics should be considered.

Information about the ecology of *C. acutatum* as an inoculum source is of importance in developing effective control strategies for pepper anthracnose. Inoculum of a plant pathogen can originate from several sources, such as soil, infected plant debris, and other host plants, which may also serve as potential sources for outbreaks of the disease. In spite of its economic impact, few studies have been conducted on the epidemiology of pepper anthracnose. An improved understanding of its overwintering, infection processes, and epidemiology may lead to the development of more efficient control and management strategies.

Thus, the purpose of these experiments was to examine the possibility of soil and infected fruit debris as inoculum sources of *C. acutatum* conidia and their potential as a primary inoculum under laboratory and field conditions.

Materials and Methods

Collection and maintenance of isolates. *C. acutatum* isolate JC24 obtained from a diseased pepper fruit. Pieces of diseased fruit were surface-sterilized with 2% sodium hypochlorite for 1 min and washed twice with sterile distilled water (SDW), then placed on potato dextrose agar medium (PDA; Difco Laboratories, Detroit, MI) amended with 300 μg/ml streptomycin sulfate. Plates were incubated at 25°C for 5 days to induce fungal growth. Following incubation, spores were harvested with SDW and washed twice with SDW. The spore concentration was adjusted to 1×10^6 conidia/ml, and 50 μl of the spore suspension was spread on PDA with 300 μg/ml streptomycin sulfate. After 3 days of incubation at 25°C, mycelial discs at the margin of a small colony were removed and sub-cultured. Isolate JC24 was grown on PDA at 25°C and maintained on PDA slants at 4°C until used.

Survival of *C. acutatum* in the soil and on the pepper fruit debris. Survival of *C. acutatum* in sterile and non-sterile soil was investigated with a spore suspension of *C. acutatum* JC24. Soils were collected from pepper fields at Chungbuk National University that were not infected with *C. acutatum*. They were then air-dried and passed through a soil screen (2.0-mm opening) to remove large organic particles. Half of the soil sample was sterilized twice at 121°C for 15 min by autoclaving, and the other was not. Air-dried soil (1 kg) was placed in a plastic container ($30 \times 20 \times 12$ cm) with a plastic lid. Conidia of *C. acutatum* JC24 were harvested from 7-day-old PDA cultures and suspended in SDW. The concentration was adjusted to 1×10^7 conidia/ml with a hemacytometer under a microscope. Spore suspension (100 ml) was added to a plastic container containing 1 kg of soil and mixed by hand. Containers were placed in an incubator at 4, 10, 20, or 30°C. Each treatment was replicated three times. For the assay, 50 g soil subsamples in each container were removed at 2 week intervals for 18 weeks, then after 36 weeks. Each subsample was suspended in 250 ml SDW, shaken at 150 rpm for 30 min, and diluted in SDW as appropriate. Then 100 μl of the dilution was spread on plates of semi-selective media devised by Kang et al. (2005). To prepare the semi-selective medium, we added 100 μg/ml ampicillin and tetracycline (to inhibit bacterial growth) and 100 μg/ml of the fungicidal mixture of carbendazim plus diethofencarb (a.i. 50%, WP) to PDA. To determine the number of colony forming units (CFUs) on a plate of semi-selective medium, we counted orange colonies after 7 days of incubation at 25°C under dark conditions (conditions were the same for all experiments).

To examine survival of the fungus on fruit debris, we inoculated a spore suspension of *C. acutatum* JC24 uniformly on pepper fruits (cv. Nokkwang) harvested in a greenhouse using the non-wound inoculation method reported by Kim et al. (2008). Half of the infected fruits were dried completely at room temperature before being placed in a container, and the other half were used as they were. Plastic containers were kept at 4, 10, 20, or 30°C. Three identical containers were used for each temperature treatment, in which fruits were either maintained dried or hydrated. Subsamples (5 g of dried pepper fruit or 50 g of hydrated pepper fruits) were macerated in 50 ml SDW, finely ground in a blender, and passed through four sheets of cheesecloth. After diluting the filtrate 1/20 in SDW, we spread 50 μl diluted filtrate onto semi-selective medium. Each treatment was replicated three times.

To assess the effect of soil moisture on survival in fruit debris, we harvested pepper fruits infected naturally by *C. acutatum* in the field, and dried them at room temperature. They were ground finely and passed them through a screen (2.0-mm opening) to remove large particles. Sieved debris (400 g) was mixed by hand with air-dried soil (40 kg). We examined five soil moisture levels (0, 3, 6, 12, and 24%) simulating field conditions. Soil incorporated with sieved infected fruit debris was hydrated to the indicated moisture content based on dry weight (vol/wt). The containers were sealed and kept at 4, 10, 20, or 30°C. A subsample (50 g) was suspended in 100 ml SDW and shaken at 150 rpm for 30 min. The soil suspension was ground in a blender for 3 min and passed through four sheets of cheesecloth; 100 μl of the filtrate was spread on plates of semi-selective media.

Survival of *C. acutatum* in the field. An experiment to evaluate *C. acutatum* survival in naturally infected fruits and in the field soil was conducted in two pepper fields at

Chungbuk National University. Pepper anthracnose did not develop in field A, whereas in field B more than 50% of the total fruits per pepper plant were infected. On October 7, 2005, infected plants showing more than 50% fruit infection were harvested, dried thoroughly at room temperature, and scattered in field A. The assessment of *C. acutatum* survival began on January 3, 2006, and terminated on April 26, 2006. Samples (10 g of fruit debris scattered in the field) were macerated in 100 ml SDW, finely ground in a blender for 2 min, and filtered through four sheets of cheesecloth. After appropriately diluting the filtrate in SDW, we spread 50 μl diluted filtrate on semi-selective medium. Each treatment was replicated three times. To assess the survival of *C. acutatum* in soil, we also collected soil samples from field B from January 3, 2006, to April 26, 2006. Soil samples were obtained from five locations in field B. At each sampling point, 100 g of soil was obtained and suspended in 200 ml SDW. The soil suspension was shaken at 150 rpm for 1 h and filtered through four sheets of cheesecloth. The soil suspension (50 μl) was spread onto plates of semi-selective media.

Pathogenicity test. To confirm whether *C. acutatum* conidia recovered from soil and fruit debris were pathogenic on pepper fruits, after 36 weeks of incubation we randomly selected colonies from semi-selective media inoculated with conidia from subsamples of soil and fruit debris which kept at each temperature and sampled in the fields on April 26, 2006. In the laboratory, detached fruits (cv. Nokkwang) harvested from pepper plants grown in the greenhouse were used to determine the pathogenicity of each isolate. To prepare the inoculum suspension, we cultured each isolate of *C. acutatum* recovered from the semi-selective medium on PDA for 7 days in the dark at 25°C. (10 ml) SDW was poured into the culture and filtered through four layers of cheesecloth to remove mycelial debris. The conidial suspension was washed twice with SDW by decanting the supernatant after centrifuging the conidial suspension at 3,000×*g* for 15 min and was adjusted to 1×10^6 conidia/ml using a hemocytometer. Fruits were inoculated by dropping with a 5 μl of the conidial suspension. Fruits were placed in plastic containers (30×20×10 cm) with three sheets of paper towel soaked with 100 ml distilled water to maintain high humidity (>95%, RH). Lesion length at the inoculation site was measured 10 days after inoculation.

PCR with species-specific primers. Total DNA was extracted from mycelia obtained from PDA culture at 25°C for 7 days. Aerial mycelia were harvested from the culture plates using a sterile transfer needle and placed in a sterile 1.5-ml microcentrifuge tube containing 300 μl extraction buffer (0.2 M Tris-HCl, 0.25 M NaCl, 25 mM EDTA, and 2% sodium dodecyl sulfate, pH 8.5). Uncapped tubes were placed in a boiling water bath for 5 min and then cooled to 25°C. Phenol (200 μl) that equilibrated with extraction buffer (v/v) and 200 μl chloroform were added. The tubes were vortexed for 4 min and then centrifuged at 13,000×*g* for 5 min. The supernatant was transferred to a new sterile 1.5-ml tube, 200 μl chloroform was added, and the mixture was vortexed for 30 s and then centrifuged at 13,000×*g* for 15 min. The supernatant was extracted with 200 μl isopropanol and centrifuged at 13,000×*g* for 15 min. After being washed with 70% ethanol and air-dried for 15 min, the nucleic acid pellet was resuspended in 50 μl TE buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.5). Finally, DNA was treated with ribonuclease A.

For PCR with species-specific primers, Ca1-1 (5'-CAG GGG AAG CCT CTC GCG GGC CT-3') was designed based on the sequence similarity of the ITS1 region of *C. acutatum*. The primer was used specifically to amplify the ITS1 region of *C. acutatum*. Primer Ca1-1, specific to *C. acutatum*, was used along with the backward primer ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') that is conserved in *C. acutatum*. The PCR reaction (30 μl) contained 50 ng DNA, 1 M of each primer, and 15 ml of PCR Master mix (Promega, Madison, WI, USA). Amplifications were conducted as follows: 1 cycle of 4 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 65°C, and 1 min at 72°C; 7 min at 72°C. PCR products were separated on a 0.7% agarose gel. PCR amplification was repeated at least three times.

Results

Survival of *C. acutatum* in sterile and non-sterile soil. In sterile field soil, the number of CFUs of *C. acutatum* JC24 on semi-selective medium increased approximately 10-fold after 2 weeks of incubation at 10 and 20°C, and was sustained for 18 weeks (Fig. 1A). The number of CFUs decreased gradually after increasing during the first 2 weeks of incubation at 30°C, whereas it decreased gradually from the start of the experiment at 4°C. After 18 weeks, survival of *C. acutatum* JC24 at 4°C could not be confirmed. Different population growth patterns were seen in non-sterile soil. Whereas the number of CFUs did not change over time at 10 and 20°C, it decreased after 6 weeks of incubation at both 4 and 30°C (Fig. 1B). After 12 weeks of incubation, the number of CFUs was lower at 30°C than at 4°C.

Survival of *C. acutatum* in the infected fruits. In hydrated pepper fruits, the number of CFUs of *C. acutatum* JC24 condidia from infected fruits declined rapidly at 20 and 30°C over 4 weeks of incubation (Fig. 2A). After incubation for 10 weeks at 10°C, the number of CFUs decreased

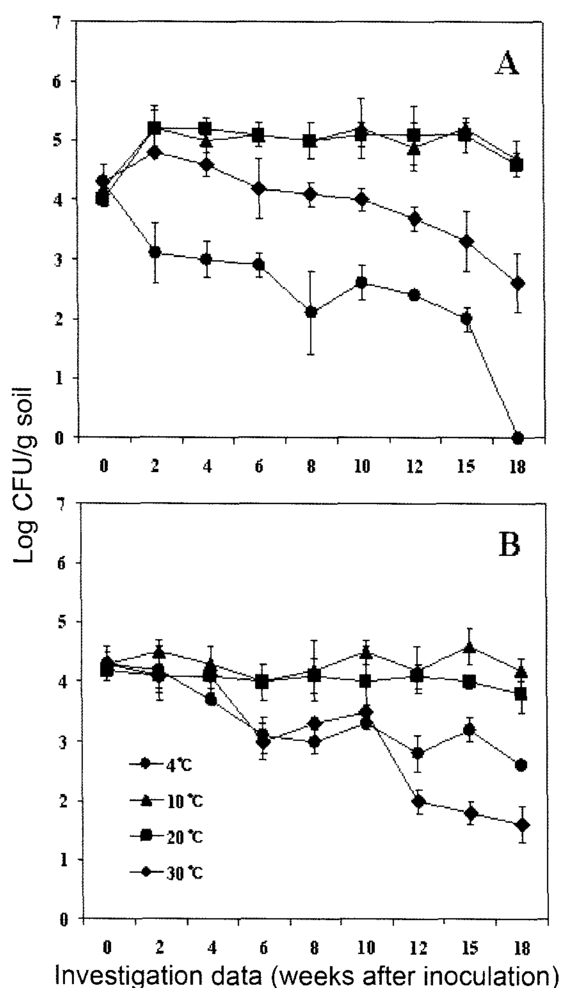

Fig. 1. The number of colony forming units (CFUs) of viable *Colletotrichum acutatum* JC24 conidia recovered from sterile (A) and non-sterile (B) soil

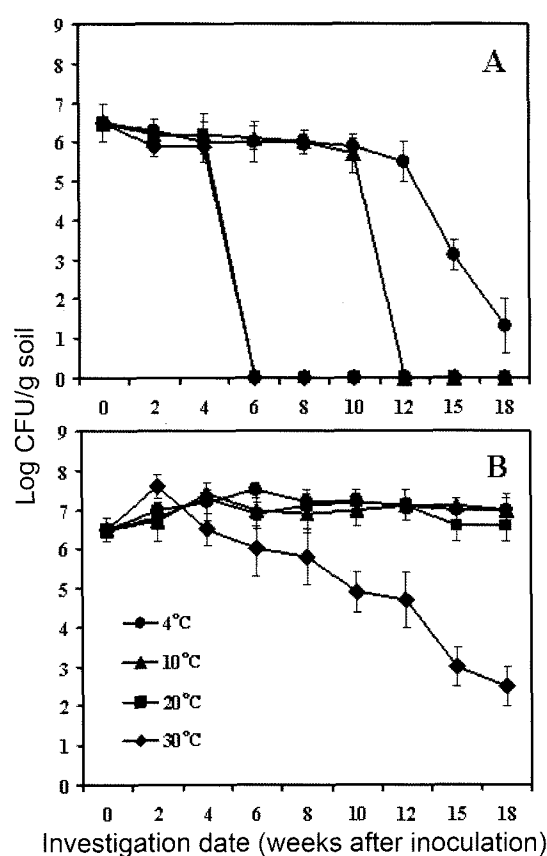

Fig. 2. The number of CFUs of viable *C. acutatum* JC24 conidia recovered from hydrated (A) and air-dried (B) pepper fruits infected with *C. acutatum* JC24.

from approximately 5.01×10^5 to 0 CFU/g fruit. Although the number of CFUs at 4°C was constant during the first 12 weeks of incubation, 1.25×10^3 and 2.0×10 CFU/g fruit were recovered at 15 and 18 weeks, respectively. In contrast, the survival of *C. acutatum* JC24 on dried fruits was very different. The number of CFUs at 4, 10, and 20°C ranged from 3.98×10^6 to 1.0×10^7 CFU/g fruit after 18 weeks of incubation, and survival at 30°C decreased from 3.98×10^7 CFU/g fruit at 2 weeks to 3.16×10^2 CFU/g fruit at 18 weeks (Fig. 2B).

Effect of soil moisture on *C. acutatum* survival. As shown in Fig. 3, soil moisture affected the survival of *C. acutatum* JC24 on fruit debris incorporated into soil. Survival on fruit debris decreased as soil moisture increased at all temperatures. At 4°C, *C. acutatum* JC24 was recovered at the starting density even after 36 weeks of incubation in soil adjusted to 0 and 3% soil moisture. No colonies were recovered after incubation at 4°C for 36 weeks at more than 6% soil moisture (Fig. 3A). At 6% soil moisture, *C. acutatum* JC24 conidia incubated at 10°C survived on infected fruit debris for 36 weeks, although no conidia could be found at 4°C (Fig. 3B). At 20°C, the effect of soil moisture on *C. acutatum* JC24 survival was much higher. As shown in Fig. 3C, no colonies were recovered at 12 and 24% soil moisture even after 2 weeks of incubation. At 30°C and 3% soil moisture, no colonies were recovered after 10 weeks of incubation. At 30°C and 6% soil moisture, no colonies were recovered 2 weeks after inoculation (Fig. 3D).

Survival of *C. acutatum* in the fields. From 3 months after harvest, survival of *C. acutatum* in the fields was investigated. As shown in Table 1, conidia were recovered more on infected fruit debris rather than in soil. Whereas conidia of *C. acutatum* were recovered up to 2.6×10^3 CFU/g fruit at 26 April, just 1.0×10 CFU/g soil were recovered from soil. As passing the winter, survival of *C. acutatum* in soil decreased due to the snow and the fluctuation of soil temperature and moisture.

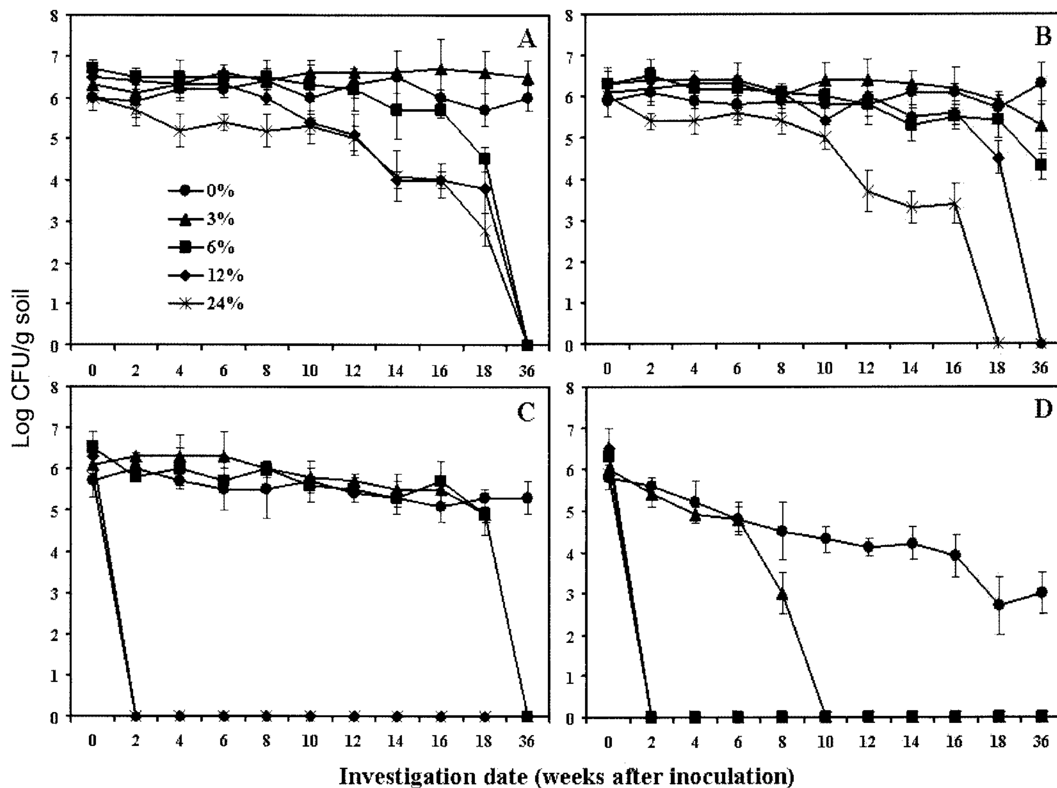

Fig. 3. Number of viable *C. acutatum* JC24 conidia recovered from the debris of infected pepper fruits incorporated into soil that was maintained in the laboratory at 4 (A), 10 (B), 20 (C), or 30°C (D).

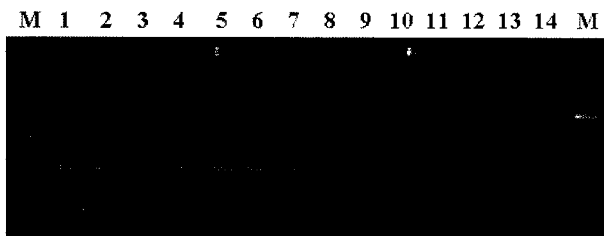

Fig. 4. Amplification of a species-specific fragment from *Colletotrichum* isolates recovered from soil (S) and plant debris (D) Lanes 1 and 2, 4-S and 4-D; lanes 3 and 4, 10-S and 10-D; lanes 5 and 6, 20-S and 20-D; lanes 7 and 8, 30-S and 30-D. Isolates of lanes 9 and 10 (FS-1 and FS-2) were recovered from field soil sampled on April 26, 2006. Lanes 11 and 12 represented isolates (FD-1 and FD-2) recovered from infected pepper fruit in the field on April 26, 2006. Lane 13 indicates the representative isolate JC24 of *C. acutatum*. Lane 14 is sterile distilled water.

Identification with species-specific primers and the pathogenicity of *Colletotrichum* isolates recovered from soil and pepper debris. Using PCR with species-specific primers for *C. acutatum*, we amplified a 496-bp PCR product from all isolates recovered from all soil and infected fruit debris samples, regardless of temperature, that had been incubated for 36 weeks, and from field samples taken on April 26. All isolates were similar to the representative isolate of *C. acutatum* JC24 (Fig. 4). Also, no isolates showed virulence against pepper fruits that was significantly different from that of JC24 (Fig. 5).

Table 1. Survival[a] of *Colletotrichum acutatum* recovered from fruit debris and soil in the field.

Inoculum source	Fungal population (CFUs)[b]			
	January 3	February 3	March 1	April 26
Fruit debris	$(5.1\pm0.4)\times10^5$	$(2.4\pm0.7)\times10^5$	$(6.1\pm0.9)\times10^4$	$(2.6\pm0.5)\times10^3$
Soil	$(2.9\pm1.0)\times10$	$(2.5\pm0.9)\times10$	$(2.7\pm0.5)\times10$	$(1.0\pm0.6)\times10$

[a]Survival was measured as the number of colony forming units (CFUs) growing on a semi-selective medium that had been flooded with a suspension of conidia obtained from soil or infected fruit debris. Semi-selective media were incubated at 25°C for 7 days under dark conditions.

[b]The number of CFUs on semi-selective medium was measured from January 3 to April 26 at 1-month intervals.

Discussion

We have demonstrated that the anthracnose pathogen can overwinter in the soil and on dried fruits infected by the pathogen that remain in the field; these dormant pathogens play a role as a primary inoculum and attack new fruits near the ground the following year. For more than 18 weeks, *C.*

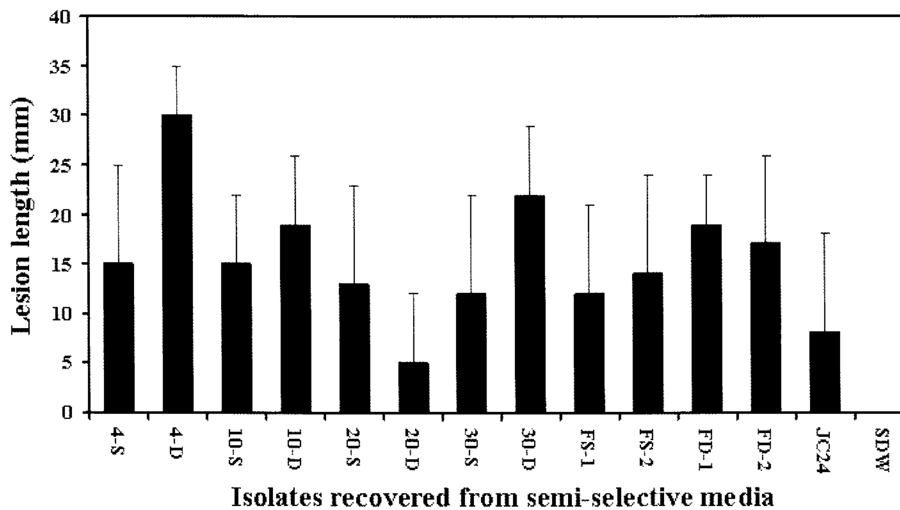

Fig. 5. Pathogenicity of *C. acutatum* isolates recovered from soil (S) and plant debris (D) in the laboratory and field.

acutatum was able to survive in soil and plant debris in the laboratory, and in plant debris incorporated into soil, at temperatures from 4 to 20°C and at soil moisture levels below 6%. The survival of *C. acutatum* conidia was also confirmed in fields until 7 months after the pepper harvest. Furthermore, *C. acutatum* conidia recovered from inoculum sources such as soil and plant debris in the laboratory and field showed strong pathogenicity on pepper fruits, indicating that overwintering of *C. acutatum* conidia serve as a primary inoculum by which pepper fruits are infected.

In the laboratory, it was possible to isolate and count *C. acutatum* directly from soils and infected pepper fruits on a semi-selective medium. Use of this medium allowed us to easily distinguish *C. acutatum* colonies from those of other fungi. In this study, we used the commercial fungicidal mixture of carbendazim plus diethofencarb. Carbendazim was used to prepare the semi-selective medium because it is included in benzimidazole fungicides such as benomyl, which has been included in semi-selective media developed in other studies isolating *Colletotrichum* spp. from soil (Eastburn and Gubler, 1990; Farley, 1972). Diethofencarb inhibits the growth of isolates of *Colletotrichum* spp. resistant to benzimidazole fungicides because it shows a negative cross-resistance (Elad et al., 1988; Kato et al., 1984).

Colletotrichum acutatum was able to survive for 18 weeks after inoculation in both sterile and non-sterile soil. Whereas *C. acutatum* survival in sterile soils at 10, 20, and 30°C was sustained or decreased slowly after increasing by approximately 10 folds within 2 weeks of inoculation into soil, conidia of *C. acutatum* recovered from non-sterile soil were sustained without increasing tendency or gradually decreased until 18 weeks. The number of days needed to recover 50% of the original population of *C. acutatum* isolated from pepper was up to 126 days at 20°C, whereas that of *C. acutatum* causing strawberry anthracnose ranged from 3.3 to 3.7 days (Freeman et al., 2002). This might indicate that *C. acutatum* from pepper is better able than *C. acutatum* from strawberry to survive in soil. We confirmed the survival of *C. acutatum* in soils in the field 7 months after the pepper harvest. Vizvary and Warren (1982) found that soil-induced sporulation was not uncommon among soil fungi and that *C. graminocola* conidia produced under those conditions extended the viability of the pathogen to about 60 days despite total mycelial lysis after 11 days at or above 16°C. Thus, conidia or mycelia free in the soil might not be considered effective primary sources of inoculum, although conidia derived from mycelia in decomposing residue have been considered a source of inoculum for root and stalk infection (Vizvary and Warren, 1982). Ekefan et al. (2000), Freeman et al. (2002), and Ripoche et al. (2008) found that soil was not a potential source of *C. gloeosporioides* inoculum and that survival of the pathogen was dependent on colonization of plant residue before its incorporation into soil. However, Eastburn and Gubler (1990) detected *C. acutatum* conidia in soil from a fallow nursery plot 9 months after strawberry plants had been tilled before harvest because of high levels of anthracnose. This supports our finding that *C. acutatum* can survive in soil for an extended period of time. Based on our results, soil is a potential inoculum source, and conidia recovered from soil play the role of a primary inoculum that infects pepper fruits, as shown in Fig. 5.

Moreover, there is a high potential for survival of *C. acutatum* in infected pepper fruits. Survival was higher on dried fruits than on hydrated fruits at all temperatures (Fig. 2). It was not possible to detect *C. acutatum* in hydrated infected fruits because unknown fungi overwhelmed the pepper fruits after 6 weeks of incubation, except at 4 and

10°C. This result indicates that *C. acutatum* survival on infected fruits depends on moisture. Furthermore, the survival of *C. acutatum* on pieces of infected fruit debris was also fluctuated with soil moisture when pieces were incorporated into the soil (Fig. 3). Eastburn and Gubler (1992) monitored the survival of *C. acutatum* in strawberry tissue over a 7-week period at three temperature (10, 25, and 40°C) and at three soil moisture levels (air-dried, moist, and flooded). Recovery of *C. acutatum* from infected tissue declined rapidly under flooded soil conditions above 25°C, whereas recovery from tissue samples in air-dried soil was 100% after 7 weeks at both 10 and 25°C. Norman and Strandberg (1997) measured the survival of conidia free in soil and conidia in infected leaf debris in soil maintained at five soil moisture levels. Survival of conidia declined rapidly under moist conditions (≥12% moisture, vol/wt), but under dry conditions viable conidia could be detected up to 12 months after incorporation into soil. Similar results were obtained in our study. Occasionally, the increased moisture available to a crop tends to inhibit disease development (Bailey and Duczek, 1996; Bockus and Shroyer, 1998).

Detecting the survival of conidia in fields is important for developing strategies aimed at eliminating this inoculum source, which may be necessary to control the disease. Because soil fumigants, such as methyl bromide and chloropicrin, are not available for use in many countries, control depends on cultivating alternative crop plants or developing alternative soil fumigants (Martin, 2003). Other control methods, such as removing plant debris, fallowing pepper fields for more than 1 year, or rotating crops, may also reduce the inoculum (Feil et al., 2003). Understanding the effects of temperature, soil moisture, and soil treatments on survival could provide a basis for developing management strategies for reducing inoculum in the field. The solar heating of soil is a disinfection method (Katan, 1981) that aims to eradicate or reduce, prior to planting and using drastic means, any inoculum existing in the soil. Thus, during winters, it may be important to increase soil temperature and moisture using methods such as soil solarization and sprinkler irrigation to reduce the inoculum of *C. acutatum* in the soil. However, outbreaks of pepper anthracnose can result from *C. acutatum* conidia that have colonized other host plants, because soil and infected plant debris are not the only sources of *C. acutatum* inoculum causing pepper anthracnose. Further studies should identify these other host plants of *C. acutatum* and measure the survival of *C. acutatum* on them to help reduce the primary inoculum and manage pepper anthracnose in the field.

Acknowledgment

This work was supported by a grant (200803A01070143) from Rural Development Administration, Republic of Korea.

References

Bailey, K. L. and Duczek, L. J. 1996. Managing cereal diseases under reduced tillage. *Can. J. Plant Pathol.* 18:159-167.

Bockus, W. W. and Shroyer J. P. 1998. The impact of reduced tillage on soilborne plant pathogens. *Annu. Rev. Phytopathol.* 36:485-500.

Buchwaldt, L., Morrall, R. A. A., Chongo, G. and Bernier, C. C. 1996. Windborne dispersal of *Colletotrichum truncatum* and survival in infected lentil debris. *Phytopathology* 86:1193-1198.

Dillard, H. R. and Cobb, A. C. 1998. Survival of *Colletotrichum coccdes* in infected tomato tissue and in soil. *Plant Dis.* 82:235-238.

Eastburn, D. M. and Gubler, W. D. 1990. Strawberry anthracnose: Detection and survival of *Colletotrichum acutatum* in soil. *Plant Dis.* 74:161-163.

Ekehan, E. J., Simons, S. A. and Nwankiti, A. O. 2000. Survival of *Colletotrichum gloeosporioides* (causal agent of yam anthracnose) in soil. *Trop. Sci.* 40:163-168.

Elad, Y., Shabi, E. and Katan, T. 1988. Negative cross resistance between benzimidazole and *N*-phenylcarbamate fungicides and contol of *Botrytis cinerea* on grapes. *Plant Pathol.* 37:141-147.

Farley, J. D. 1972. A selective medium for assay of *Colletotrichum coccodes* in soil. *Phytopathology* 62:1288-1293.

Feil, W. S., Butler, E. E., Duniway, J. M. and Gubler, W. D. 2003. The effects of moisture and temperature on the survival of *Colletotrichum acutatum* on strawberry residue in soil. *Can. J. Plant Pathol.* 25:362-370.

Freeman, S., Shalev, Z. and Katan, J. 2002. Survival in soil of *Colletotrichum acutatum* and *C. gloeosporioides* pathogenic on strawberry. *Plant Dis.* 86:965-970.

Kang, B. K., Min, J. Y., Park, S. W., Van Bach, N.,and Kim, H. T. 2005. Semi-selective medium for monitoring *Colletotrichum acutatum* causing pepper anthracnose in the field. Res. *Plant Dis.* 11:21-27.

Katan, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Ann. Rev. Phytopathol.* 19:211-236.

Kato, T., Suzuki, I., Takahashi, J. and Kamoshita, K. 1984. Negatively correlated cross-resistance between benzimidazole fungicides and methyl *N*-(3,4-dichloropheny) carbamate. *J. Pesticide Sci.* 9:489-495.

Kim, J. T., Park, S.-K, Choi, W., Lee, Y.-H. and Kim, H. T. 2008. Characterization of *Colletotrichum* isolates causing anthracnose of pepper in Korea. *Plant Pathol. J.* 24:17-23.

Martin, N. M. 2003. Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. *Annu. Rev. Phytopathol.* 41:325-350.

Norman, D. J. and Strandberg, J. O. 1997. Survival of *Colletotrichum acutatum* in soil and plant debris of leather leaf fern. *Plant Dis.* 81:1177-1180.

Park, K. S. and Kim, C. H. 1992. Identification, distribution and etiological characteristics of anthracnose fungi of red pepper

in Korea. *Korean J. Plant Pathol.* 8:61-69.

Ripoche, A., Jacqua, G., Bussiere, F., Guyader, S. and Sierra, J. 2008. Survival of *Colletotrichum gloeosporioides* (causal agent of yam anthracnose) on yam residues decomposing in soil. *Appl. Soil Ecol.* 38:270-278.

Tu, J. C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in southern Ontario: Survival of the pathogen. *Plant Dis.* 67:402-404.

Vizvary, M. A. and Warren, H. L. 1982. Survival of *Colletotrichum graminicola* in soil. *Phytopathology* 72:522-525.

Wilson, L. L., Madden, L. V. and Ellis, M. A. 1992. Overwinter survival of *Colletotrichum acutatum* in infected strawberry fruits in Ohio. *Plant Dis.* 76:948-950.

Yoshida, S. and Shirata, A. 1999. Survival of *Colletotrichum dematium* in soil and infected mulberry leaves. *Plant Dis.* 83:465-468.