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Review:

Chilli anthracnose disease caused by *Colletotrichum* species

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Abstract: Anthracnose disease is one of the major economic constraints to chilli production worldwide, especially in tropical and subtropical regions. Accurate taxonomic information is necessary for effective disease control management. In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host. Little information is known concerning the interactions of the species associated with the chilli anthracnose although several *Colletotrichum* species have been reported as causal agents of chilli anthracnose disease worldwide. The ambiguous taxonomic status of *Colletotrichum* species has resulted in inaccurate identification which may cause practical problems in plant breeding and disease management. Although the management and control of anthracnose disease are still being extensively researched, commercial cultivars of *Capsicum annuum* that are resistant to the pathogens that cause chilli anthracnose have not yet been developed. This paper reviews the causal agents of chilli anthracnose, the disease cycle, conventional methods in identification of the pathogen and molecular approaches that have been used for the identification of *Colletotrichum* species. Pathogenetic variation and population structure of the causal agents of chilli anthracnose along with the current taxonomic status of *Colletotrichum* species are discussed. Future developments leading to the disease management strategies are suggested.

Key words: *Capsicum annuum*, Disease management, Identification, Taxonomy, Pathogenicity
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INTRODUCTION

Colletotrichum is one of the most important plant pathogens worldwide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits (Bailey and Jeger, 1992). Among these hosts, chilli (*Capsicum* spp.), an important economic crop worldwide (Poulos, 1992), is severely infected by anthracnose which may cause yield losses of up to 50% (Pakdeevaraporn *et al*., 2005). Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability (Manandhar *et al*., 1995).

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al*., 1998; Cannon *et al*., 2000). Anthracnose of chilli has been shown to be caused by more than one *Colletotrichum* species including *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz.) Penz. and Sacc., and *C. coccodes* (Wallr.) S. Hughes (Simmonds, 1965;

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Johnston and Jones, 1997; Kim *et al*., 1999; Nirenberg *et al*., 2002; Voorrips *et al*., 2004; Sharma *et al*., 2005; Pakdeevaraporn *et al*., 2005; Than *et al*., 2008).

There is little information concerning the interactions between the complexes of species involved in chilli anthracnose (Than *et al*., 2008). This information is necessary for plant breeding purposes and disease management. Some *Colletotrichum* species respond differently to various control measures, e.g., *C. acutatum* was found to be moderately susceptible to the fungicide, benzimidazole, while *C. gloeosporioides* was highly susceptible (Peres *et al*., 2004). Correct and accurate identification will thus ultimately lead to more effective disease control and management, e.g., selecting appropriate fungicides, or long lasting resistant cultivars (Whitelaw-Weckert *et al*., 2007).

The current taxonomic status of *Colletotrichum* species is unclear (Sreenivasaprasad and Talhinhas, 2005). The broad host range of many species has caused problems for plant pathologists who need to identify specific plant pathogens to control disease. Molecular techniques have provided additional data to aid the naming of fungi, and have been applied to taxonomically difficult genera including *Fusarium* (O′Donnell *et al*., 1998; Maxwell *et al*., 2005), *Pestalotiopsis* (Jeewon *et al*., 2002; 2003; 2004; Lui *et al*., 2007), *Mycosphaerella* and its anamorphs (Crous *et al*., 2000; Mancini *et al*., 2006), but to a lesser extent to *Colletotrichum* (Sreenivasaprasad *et al*., 1996; Moriwaki *et al*., 2002; Photita *et al*., 2005; Du *et al*., 2005). The management and control of anthracnose diseases are still being extensively researched. This paper reviews the host 'chilli' as well as the causal agents of the chilli anthracnose, their pathogenic variability and approaches leading to the disease management.

HOST: CHILLI

The genus *Capsicum* was originated in the American tropics and has been propagated throughout the world including the tropics, subtropics, and also temperate regions (Pickersgill, 1997). The fruit of *Capsicum* has a variety of names, such as 'chilli', 'chilli pepper' or 'pepper' depending on place (i.e., differences between the English-speaking countries) and type of fruits. The term "chilli" in most of the world refers exclusively to the smaller, hot types of *Capsicum* (Wikipedia, 2007).

Capsicum contains approximately 20~27 species, 5 of which are domesticated: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, and are cultivated in different parts of the world. Among the five species of cultivated *Capsicum*, *C. annuum* is one of the most common cultivated crops worldwide (Tong and Bosland, 1999) followed by *C. frutescens* (Bosland and Votava, 2003).

Chilli has many culinary advantages. It comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements (Bosland and Votava, 2003). Many chilli constituents are important for nutritional value, flavor, aroma, texture and color. Chillies are low in sodium and cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E. Fresh green chilli peppers contain more vitamin C than citrus fruits and fresh red chilli has more vitamin A than carrots (Osuna-García *et al*., 1998; Marin *et al*., 2004). Two chemical groups produced by chilli are capsaicinoids and carotenoids. The capsaicinoids are alkaloids that make hot chilli pungent. A large number of carotenoids provide high nutritional value and the color to chilli (Britton and Hornero-Méndez, 1997; Hornero-Méndez *et al*., 2002; Pérez-Gálvez *et al*., 2003).

CONSTRAINTS TO CHILLI PRODUCTION

Chilli is considered to be one of the most important crops in the tropics. The area cultivated with chilli worldwide is about 1 700 000 ha for producing fresh chilli, and around 1 800 000 ha for producing dried chilli; a total area of 3 729 900 ha with a total production of 20 000 000 t (FAO, 2003). The most important producers and exporters of chilli include China, India, Mexico, Morocco, Pakistan, Thailand and Turkey. Diseases caused by fungi, bacteria and viruses are the major constraints to chilli production. Anthracnose disease caused by *Colletotrichum* species, bacterial wilt caused by *Pseudomonas solanacearum*, and mosaic disease caused by chilli veinal mottle virus (CVMV) or cucumber mosaic virus (CMV) are the

most serious destructive diseases of chilli (Isaac, 1992).

Anthracnose disease caused by *Colletotrichum* species is one of the most economically important diseases reducing marketable yield from 10% to 80% of the crop production in some developing countries, particularly in Thailand (Poonpolgul and Kumphai, 2007). Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre- and post-harvest fruit decay (Hadden and Black, 1989; Bosland and Votava, 2003).

ANTHRACNOSE DISEASE

Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 1992). Generally, anthracnose disease is caused by *Colletotrichum* species which belongs to the Kingdom Fungi; Phylum Ascomycota, Class Sordariomycetes; Order Phyllachorales; and Family Phyllachoraceae. The anamorphs are *Glomerella* species. Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) in 1890 who described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by von Arx (1957).

Anthracnose causes extensive pre- and post-harvest damage to chilli fruits causing anthracnose lesions. Even small anthracnose lesions on chilli fruits reduce their marketable value (Manandhar *et al*., 1995). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection (Jeffries *et al*., 1990). Appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Bailey and Jeger, 1992). Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Prusky and Plumbley, 1992). Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits (Isaac, 1992). Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses (Fig.1). Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions. Many studies have concluded that disease management practices are often inadequate to eliminate the diseases. Breeding to develop the long-lasting resistant varieties has also not been successful due to involvement of multiple *Colletotrichum* species in anthracnose infection.

Fig.1 Anthracnose symptoms on chilli fruits

Causal agents of chilli anthracnose

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al*., 1998; Cannon *et al*., 2000). *Colletotrichum* species causing anthracnose of chilli have been reported from different countries and regions (Table 1). Although these species have been the subject of numerous investigations, there remain many gaps in the knowledge of the disease process and understanding of the complex relationships between the species involved. Kim *et al*.(2004) reported that different species cause diseases of different organs of the chilli plant; for example, *C. acutatum* and *C. gloeosporioides* infect chilli fruits at all developmental stages, but usually not the leaves or stems, which are mostly damaged by *C. coccodes* and *C. dematium*. Leaf anthracnose of chilli seedlings caused by *C. coccodes* was first reported in chilli growing in a field in Chungnam Province of Korea in 1988 (Hong and Hwang, 1998). Different *Colletotrichum* species may also play an important role in different diseases of mature stages of chilli fruit as well. For example, *C. capsici* is widespread in red chilli fruits, whereas *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits (Hong and Hwang, 1998; Kim *et al*., 1999).

Table 1 Reported causal agents of chilli anthracnose

Countries and regions	Causal agent	Reference
Australia	*Colletotrichum acutatum*, *C. atramentarium*, *C. dematium*, *C. gloeosporioides* var. *minor*, *C. gloeosporioides* var. *gloeosporioides*	Simmonds, 1965
India	*C. capsici*	Maiti and Sen, 1979; Paul and Behl, 1990
Indonesia	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Voorrips *et al*., 2004
Korea	*C. acutatum*, *C. gloeosporioides*, *C. coccodes*, *C. dematium*	Park and Kim, 1992
Myanmar (Burma)	*Gloeosporium piperatum* E. and E., *C. nigrum* E. and Hals	Dastur, 1920
Papua New Guinea	*C. capsici*, *C. gloeosporioides*	Pearson *et al*., 1984
New Zealand	*C. coccodes*	Johnston and Jones, 1997
Taiwan	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Manandhar *et al*., 1995
Thailand	*C. acutatum*, *C. capsici*, *C. gloeosporioides*	Than *et al*., 2008
UK	*C. acutatum*, *Glomerella cingulata*	Adikaram *et al*., 1983
USA	*C. acutatum*	Roberts *et al*., 2001
Vietnam	*C. acutatum*, *C. capsici*, *C. gloeosporioides*, *C. nigrum*	Don *et al*., 2007

Anthracnose caused by *C. coccodes* does not result in severe epidemics on chilli fruits (Hong and Hwang, 1998). *C. gloeosporioides*, the predominant species on chilli in Korea, was differentiated into G and R strains by isozyme analysis of esterase, leucine amino peptidase, phosphatase and glutamine oxalocetic trasminase (Park *et al*., 1987).

Colletotrichum species can survive in and on seeds as acervuli and micro-sclerotia (Pernezny *et al*., 2003). Survival of mycelia and stomata in colonized chilli seeds had been reported (Manandhar *et al*., 1995). It has been shown that the pathogen readily colonizes the seed coat and peripheral layers of the endosperm even in moderately colonized seeds. Heavily colonized seeds had abundant inter- and intracellular mycelia and acervuli in the seed coat endosperm and embryo, showing disintegration of parenchymatous layers of the seed coat and depletion of food material in endosperm and embryo (Chitkara *et al*., 1990).

Fungi can overwinter on alternative hosts such as other solanaceous or legume crops, plant debris and rotten fruits in the field (Pring *et al*., 1995). *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions, and these micro-sclerotia can survive for many years (Pring *et al*., 1995). During warm and wet periods, conidia from acervuli and micro-sclerotia are splashed by rain or irrigation water from diseased to healthy fruit and foliage. Diseased fruit acts as a source of inoculum, allowing the disease to spread from plant to plant within the field (Roberts *et al*., 2001).

Initial infection by *Colletotrichum* species involves a series of processes including the attachment of conidia to plant surfaces, germination of conidia, production of adhesive appressoria, penetration of plant epidermis, growth and colonization of plant tissue and production of acervuli and sporulation (Bailey and Jeger, 1992; Prusky *et al*., 2000). Anthracnose is mainly a problem on mature fruits, causing both pre- and post-harvest fruit decay resulting severe economic losses (Hadden and Black, 1989; Bosland and Votava, 2003). Appressoria that formed on immature fruits may remain quiescent until the fruits mature or ripen.

Disease cycle and epidemiology of anthracnose

Environmental factors play a major role in the development of disease epidemics. The relationships among rainfall intensity, duration and crop geometry and the dispersal of inoculum possibly lead to different levels of disease severity (Dodd *et al*., 1992). The effects of temperature often interact with other factors, such as leaf surface wetness, humidity, light or competitive microbiota (Royle and Butler, 1986). The duration of the surface wetness, however, appears to have the most direct influence on the germination, infection and growth of the pathogen on the host. Generally infection occurs during warm, wet weather. Temperatures around 27 °C and high humidity (a mean of 80%) are optimum for anthracnose

disease development (Roberts *et al*., 2001).

Colletotrichum species utilize diverse strategies for invading host tissues, which vary from intracellular hemibiotrophy to subcuticular intramural necrotrophy (Bailey and Jeger, 1992). *Colletotrichum* species produce a series of specialized infection structures such as germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae (Perfect *et al*., 1999). These pathogens infect plants by either colonizing subcuticular tissues intramurally or being established intracellularly. The preinfection stages of the both are very similar, in which conidia adhere to and germinate on the plant surface, producing germ tubes that form appressoria which in turn penetrate the cuticle directly (Bailey and Jeger, 1992). Following penetration, the pathogens that colonize the intramural region beneath the cuticle invade in a necrotrophic manner and spread rapidly throughout the tissues (O′Connell *et al*., 1985). There is no detectable biotrophic stage in this form of parasitism. In contrast, most anthracnose pathogens exhibit a biotropic infection strategy initially by colonizing the plasmalemma and cell wall intracellularly. After the biotrophic state, intracellular hyphae colonize one or two cells and subsequently produce secondary necrotrophic hyphae (Bailey and Jeger, 1992). These pathogens are therefore regarded as hemibiotrophs or facultative biotrophs (Kim *et al*., 2004). For example, *C. gloeosporioides* on avocado, chilli and citrus can produce both types of colonizations: intracellular biotrophy at an early stage and intramural necrotrophy later (O′Connell *et al.*, 2000).

Although the mechanisms developed by *Colletotrichum* species appear similar in prepenetration events, there are differences between species in the later mechanisms such as spore adhesion, melanization and cutinization in penetration of the plant cuticle by the appressoria. For example, the host-pathogen interaction of *C. acutatum* appears to be more biotrophic than that of some other species such as *C. gloeosporioides* (Wharton and Diéguez-Uribeondo, 2004). Based on studies with *C. acutatum* on specific hosts, four types of interactions or infection strategies were described by Peres *et al*.(2005) as follows:

(1) Biotrophic growth of *C. acutatum* with secondary conidiation in which conidia germinate to form appressoria and quiescent infections, and secondary conidia are formed after germination of the appressoria (e.g., predominantly biotrophic disease cycle on citrus leaves).

(2) Subcuticular intramural necrotrophy with hyphal development within periclinal and anticlinal walls of epidermal host cells which are swollen and wider apart (e.g., predominantly necrotrophic disease cycle on strawberry).

(3) Hemibiotrophic interaction with infection vesicles and broad primary hyphae within host cells. Inter- and intracellular hyphal growth could be seen as the subsequent necrotrophic phase (e.g., combination of biotrophy and necrotrophy but mostly a biotrophic disease cycle on blueberry fruits).

(4) Hemibiotrophic and subcuticular, intra- and intercellular development of *C. acutatum* (e.g., combination of biotrophy and necrotrophy but mostly a necrotrophic disease cycle on almond leaves and fruits).

There are only a few detailed studies on penetration and colonization by *Colletotrichum* species on chilli. Kim *et al*.(2004) noticed that there was no biotrophic infection vesicle found during the infection process of *C. gloeosporioides* in susceptible chilli (*C. annuum* cv. *jejujaerae*). Epidermal cytoplasm became condensed and small vacuoles increased and cell destruction extended to the subepidermal cells of the plant, which are likely to be damaged by the pathogen enzymes. At later stages of infection, tissues were colonized inter- and intracellularly by the pathogen. This structural feature indicated that the infection was governed by necrotrophic fungal growth.

Colletotrichum species are generally able to survive in or on seeds and one of the ways that anthracnose is introduced to the chilli field is through infected transplants (Manandhar *et al*., 1995). *C. capsici* infection of chilli was shown to have two pathways: invasion through the seed coat and invasion through the openings of the testa (Jewsakun, 1978). Jewsakun (1978) mentioned that *C. capsici* caused root rot of seedlings; however, whether chilli anthracnose can be seed-transmitted, and the role of seed infection and seedling infection in pre- and post-emergence damage of chilli plants are still questionable. We have found that *C. acutatum* can infect chilli seeds either by reducing the germination rates or by causing damping off of seedlings. However, very little is known about the disease cycle of the patho-

gens that cause anthracnose in chilli. There are still several questions to be answered.

IDENTIFICATION OF *COLLETOTRICHUM* SPECIES

Conventional methods

Accurate identification of *Colletotrichum* species along with the knowledge of populations responsible for epidemics are essential for developing and implementing effective disease control strategies (Freeman *et al*., 1998). Traditionally, identification and characterization of *Colletotrichum* species have been based on morphological characters, such as size and shape of conidia and appressoria; existence of setae; the teleomorph state and cultural characters such as colony colour, growth rate and texture (von Arx, 1957; Smith and Black, 1990). These criteria alone, however, are not always adequate for species identification due to overlap in morphological characters and phenotypic variation among species under different environmental conditions. Conidial shape has been applied as a reliable means of discriminating certain species; for example, conidial shape has differentiated *Colletotrichum* species pathogenic to strawberry (Denoyes and Baudry, 1995). However, in other cases, identification can be complicated because of overlapping ranges of conidial morphology and variation in colony characteristics (Adaskaveg and Hartin, 1997). Correct taxonomic identification is important in disease management such as choosing appropriate fungicides (Whitelaw-Weckert *et al*., 2007). For instance, it could be seen in different responses of *C. acutatum* and *C. gloeosporioides* to benzimidazole-based fungicides (Peres *et al*., 2004).

Molecular genetics approach

To overcome the inadequacies of traditional morphology based identification schemes, DNA sequence analyses have been used to characterize and analyze the taxonomic complexity of *Colletotrichum* (Sreenivasaprasad *et al*., 1996; Moriwaki *et al*., 2002; Du *et al*., 2005; Photita *et al*., 2005; Than *et al*., 2008). Cannon *et al*.(2000) stated that data derived from nucleic acid analyses should provide the most reliable framework to build a classification of *Colletotrichum*, as DNA characters were not directly influenced by environmental factors. Most fungal phylogenetic studies utilized sequences from the ribosomal gene cluster, since they were present in large numbers as tandem repeats and evolved as a single unit (Mitchell *et al*., 1995). In particular, sequence analysis of the internal transcribed spacer (ITS) regions which lie between the 18S and 5.8S genes and the 5.8S and 28S genes, has proved useful in studying phylogenetic relationships of *Colletotrichum* species because of their comparative variability (Sreenivasaprasad *et al*., 1994; 1996; Moriwaki *et al*., 2002; Photita *et al*., 2005). Apart from ITS region, sequence analysis of protein coding genes such as partial β-tubulin gene, has also been applied to resolve phylogenetic relationships among *C. acutatum* species complexes (Sreenivasaprasad and Talhinhas, 2005). Sequences of introns from two genes (glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase) were also used to evaluate a diverse collection of isolates of *C. acutatum* (Guerber *et al*., 2003). *C. acutatum* isolates clustered into groups (Guerber *et al*., 2003; MacKenzie *et al*., 2008; Peres *et al*., 2008). These groups might represent phylogenetically distinct species of *C. acutatum sensu lato* (Guerber *et al*., 2003). Yun *et al*.(1999) stated that, because of the high intra-species variability and the low inter-species variability, MAT1-2 mating type sequences gave strong support for branches, allowing differentiation of closely related *Cochliobolus* spp. whose relationships were not resolved by ITS sequences alone. Consequently, Du *et al*.(2005) confirmed that MAT1-2 mating type was useful in differentiating the groups of isolates from the species complexes (*C. graminicola*, *C. gloeosporioides* and *C. acutatum*). However, there is no report concerning the use of these genes to differentiate between the *Colletotrichum* species involved in chilli anthracnose.

A combined application of molecular diagnostic tools along with traditional morphological characterization is an appropriate and reliable approach for studying *Colletotrichum* species complexes (Cannon *et al*., 2000). Than *et al*.(2008) differentiated isolates of chilli anthracnose from Thailand into three species: *C. acutatum*, *C. capsici* and *C. gloeosporioides*, based on morphological characterization, sequencing based on rDNA-ITS region and partial beta tubulin gene and pathogenicity testing. Hong and Kim (2007) reported that Korea isolates of *C. acutatum* were phyloge-

netically separated from the global groups of *C. acutatum* A1 to A8 based on the sequences in partial beta-tubulin 2 (exons 3-6). Restriction fragment length polymorphisms (RFLP) of ITS region resulting from *Alu*I, *Rsa*I and *Bam*HI digestions have also been employed to differentiate *Colletotrichum* species from chilli anthracnose in Taiwan region (Sheu *et al*., 2007). Four species of *Colletotrichum* were identified by ITS-RFLP fingerprinting and observation of undistinguishable isolates of *Colletotrichum* from their studies indicated the various inter- and intra-species variations in *Colletotrichum* species. Ratanacherdchai *et al*.(2007) distinguished *C. capsici* and *C. gloeosporioides* causing chilli anthracnose in Thailand using random amplified polymorphic DNA (RAPD) markers.

PATHOGENIC VARIABILITY OF *COLLETOTRICHUM* SPECIES

When any of the progeny exhibits a characteristic that is different from those present in the ancestral individuals or descent individuals, this individual is called a variant (Agrios, 2005). This may involve a change in any conceivable biological characteristic, such as color, shape, growth rate and reproduction rate. In the case of pathogens, changes in host range may occur, i.e., it may be able to infect a variety of the host plant or cultivar not previously infected by the ancestor, or in virulence, i.e., it may produce a milder or much more severe disease than the ancestors (Agrios, 2005). This is the way that resistance of a plant variety is 'broken down' (Agrios, 2005). The spread of a resistant genotype capable of escaping a current prevalent pathogen will be challenged by a new parasitic strain that harbors a virulent gene which is capable of overcoming that resistance (McDonald and Linde, 2002). Compatibility of plant-pathogen interactions is often governed by the gene-for-gene model in many pathosystems (Flor, 1971). This suggests a continuous co-evolutionary change in both host and parasite.

Some pathogen populations are known to be pathogenitically diverse, and the diversity seems to be due to continuous generation of novel pathogenic variations (Taylor and Ford, 2007). Information on pathogen diversity and the geographic distribution of the pathogen population is therefore a prerequisite for accurate assessment of durable resistant germplasm in breeding programs (Abang, 2003). Taylor and Ford (2007) suggested that knowledge of pathotype diversity is important when choosing the appropriate isolates to screen for resistance in plant breeding programs.

We have studied pathogenic variation among 10 isolates of *C. acutatum* against 7 cultivars including reportedly susceptible species of *Capsicum* (*Capsicum annuum* 'Bangchang') (Mongkolporn *et al*., 2004a) and resistant species such as *Capsicum chinense* 'PBC 932' (AVRDC, 1999; 2003; Mongkolporn *et al*., 2004a), *Capsicum baccatum* 'PBC 80' and 'PBC 81' in Thailand. This revealed two pathotypes based on qualitative differences in infection of a reported resistant genotype, *C. baccatum* genotype 'PBC 81'. Interestingly, of 10 isolates assayed, the genotype showed complete resistance against 5 isolates tested, whereas it showed a highly susceptible reaction to the other 5 isolates tested. Sharma *et al*.(2005) studied the pathogenic variability in *C. capsici* in India and proposed that 15 pathotypes of *C. capsici* existed among 37 isolates from different chilli growing regions from Himachal Pradesh in India. However, these pathotype differences were based on quantitative differences in host reaction, i.e., level of aggressiveness.

Taylor *et al*.(2007) defined the pathotype as "a subclass or group of isolates distinguishable from others of the same species by its virulence on a specific host (genotype), i.e., a qualitative difference in disease severity" and the aggressiveness as "the natural variation in virulence or level of disease (measured quantitatively) within the pathogen population." According to Taylor and Ford (2007), there might be confusion as to whether true pathotype differences exist, or whether the differences observed in disease severity are a measure of the natural distribution of aggressiveness within a population, ranging from low to high. However, the level of aggressiveness of isolates is also an important consideration in resistance breeding programmes and disease control management. Genotypes with partial resistance would result in lower level of infection which eventually will decrease the inoculum amount in the field to limit the potential of epidemics.

Several studies (AVRDC, 1999; Yoon *et al*.,

2004) have screened *C. acutatum*, which is the very virulent species (Than *et al*., 2008), against chilli genotypes and found that *Capsicum baccatum* genotype 'PBC 80' is a genetic resource pool for resistance to anthracnose. We have also confirmed this. This genotype is assumed to be useful for studying genetics of resistance and practical breeding in chilli pepper. However, we found that another genotype of *C. baccatum*, 'PBC 81', showed high susceptibility to some *C. acutatum* isolates. This would make it questionable as to whether *C. baccatum* species are a useful genetic resource pool for breeding for resistance to anthracnose.

In contrast to *C. baccatum*, susceptibility of the *C. annuum* cultivars has been reported in several studies (Mongkolporn *et al*., 2004b; Park, 2007). In addition, *Capsicum chinense* 'PBC 932' has been reported as a resistant variety to *Colletotrichum capsici* and hence has been introgressed with *C. annuum* 'Bangchang' to produce F_1 progeny (AVRDC, 2003). However, Yoon *et al*.(2004) and Than *et al*.(2008) found that the 'PBC 932' was highly susceptible to *C. acutatum* isolates. Nevertheless, this information about highly susceptible reaction of 'PBC 932' will help chilli breeders to be aware of the potential for breaking down its resistance to *C. acutatum*.

POPULATION STRUCTURE AND MOLECULAR MARKER TECHNIQUES

The genetic structure of plant pathogen populations, the potential for gene flow and long distance dispersal, and the relative contribution of sexual and asexual reproduction have direct inference with agricultural ecosystems (McDonald and McDermott, 1993; McDonald, 1997). Abang (2003) stated that genetic population structure refers to the amount and distribution of genetic variation within and between populations. For example, how rapidly a pathogen can evolve could be indicated by the amount of genetic variation maintained within a population and then which may eventually be used to predict how long a control measure is probable to be effective (McDonald and Linde, 2002). Pathogens with large genetic variations are assumed to adapt faster to fluctuating environments (e.g., resistant genes and fungicides).

In general, organisms reproduce by means of a sexual process. Variation in progeny is introduced primarily through segregation and recombination of genes in meiotic division of the zygote (Agrios, 2005). However, in the case of anamorphic fungi such as *Colletotrichum*, reproduction is mainly or exclusively vegetative (Katan, 2000). Parasexual reproduction, by which a system of genetic recombination can occur within fungal heterokaryon, will likely lead to variation. The new variants come into existence, which may be identical in appearance to that of the ancestral types, but behave differently as far as disease production is concerned (Agrios, 2005).

In recent decades, molecular markers have been widely used to measure the variation in a pathogen population. Random amplified polymorphic DNA (RAPD), simple sequence repeat (microsatellite), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and genomic DNA RFLP, are among the most frequently used neutral genetic markers in population genetic studies (Brown *et al*., 1996; Milgroom, 1996; McDonald, 1997).

Despite its importance, there have only been a few studies concerning the diversity of *Colletotrichum* species associated with chilli anthracnose. Sharma *et al*.(2005) found a variable population of *C. capsici* causing fruit rot/die back or anthracnose of chilli in the northwestern region of India based on differential inoculation tests and RAPD analysis. In contrast, examination of *C. acutatum* from a limited collection of isolates from chilli anthracnose epidemics in Brazil, Korea, Taiwan region and the US indicated that they belonged to a single group based on mtDNA-RFLP analysis (Correll *et al*., 2007). They also found that most isolates (36 out of 43) examined from the various countries and regions belonged to a single vegetative compatibility group (VCG). As the sexual stages of *C. acutatum* have never been found in nature (Wharton and Diéguez-Uribeondo, 2004), the asexual reproduction mode of *C. acutatum* is likely to be an important role in its population structure.

DISEASE MANAGEMENT OF CHILLI ANTHRACNOSE

Bailey (1987) and Agrios (2005) recommended integrated management techniques, as no single spe-

cific management program could eliminate chilli anthracnose. Effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic resistance (Wharton and Diéguez-Uribeondo, 2004).

Cultural practices

Pathogen-free chilli seed should be planted and weeds eliminated. Crops should be rotated every 2~3 years with crops that are not alternative hosts of *Colletotrichum*. Transplants should be kept clean by controlling weeds and solanaceous volunteers around the transplant houses. The field should have good drainage and be free from infected plant debris. If disease was previously present, crops should be rotated away from solanaceous plants for at least 2 years (Roberts *et al*., 2001). Sanitation practices in the field include control of weeds and volunteer chilli plants. Choosing cultivars that bear fruit with a shorter ripening period may allow the fruit to escape infection by the fungus. Wounds in fruit from insects or other means should be reduced to the extent possibly because wounds provide entry points for *Colletotrichum* spp. and other pathogens such as bacteria that cause soft rot. At the end of the season, infected plant debris from the field must be removed or deep ploughed to completely cover crop diseases (Agrios, 2005).

Use of resistant cultivars

The use of resistant varieties not only eliminated losses from diseases, but also eliminated chemical and mechanical expenses of disease control (Agrios, 2005). Some genetic resources resistant to anthracnose in chilli have been independently reported from different countries and regions (Kim W.G. *et al*., 1986; Kim B.S. *et al*., 1987; Park *et al*., 1987; Hong and Hwang, 1998; Pae *et al*., 1998; AVRDC, 1999; Yoon and Park, 2001). In particular, some lines of *C. baccatum* show strong resistance to the pathogen, and pathogen inoculation resulted in no or limited lesions on the chilli fruits (Yoon, 2003). However, to date, no strong resistance has been found in *Capsicum annuum*, which is the only species grown worldwide (Park, 2007). Mongkolporn *et al*.(2004a) carried out a genetic study of anthracnose resistance to *C. capsici*, which was expressed in the interspecific cross of Thai susceptible *C. annuum* cv. 'Bangchang' and anthracnose resistant *C. chinense* 'CM 021'. The genetic purity of the F1 was proven by using molecular marker analysis. Recently, Voorrips *et al*.(2004) have found one main quantitative trait locus (QTL) with high significance and large effects on resistance and three other QTLs with smaller effects on the F2 population (cross between *C. annuum* and *C. chinense*) on the traits they tested, such as infection frequency, the true lesion diameter and overall lesion diameter after inoculation with *C. gloeosporioides* in the study of resistance to anthracnose disease in Indonesia.

Use of chemicals

Chemicals are the most common and practical method to control anthracnose diseases. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991). The fungicide traditionally recommended for anthracnose management in chilli is Manganese ethylenebisdithiocarbamate (Maneb) (Smith, 2000), although it does not consistently control the severe form of anthracnose on chilli fruit. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been labeled for the control of anthracnose of chilli, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003). The disease can be controlled under normal weather conditions with a reasonable spray program. However, there are numerous reports of negative effects of using chemicals on farmers' income and health, and toxic contamination to the environment, particularly in developing countries (Voorrips *et al*., 2004).

Use of biofungicides

The control of chilli anthracnose fruit rot has, for many years, relied on chemicals and resulted in many undesirable problems. There is a need to incorporate alternative control components that are effective in field. Biological control of fruit rot and dieback of chilli with plant products tested in many laboratories and field trials showed that the crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem

(*Azadirachia indica*) oil could restrict growth of the anthracnose fungus (Jeyalakshmi and Seetharaman, 1998; Korpraditskul *et al*., 1999). Among the biofungicides used against the fungus *Colletotrichum* spp. on chilli fruit, Charigkapakorn (2000) found that the most effective control was sweetflag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage.

Biological control

So far, biological control methods for chilli anthracnose disease have not received much attention. The potential for biological control of *Colletotrichum* species had been suggested as early as in 1976 by Lenné and Parbery (1976). Jeger and Jeffries (1988) also stressed the possibilities of biological control of post-harvest fruit diseases by using *Pseudomonas fluorescens*. Antagonistic bacterial strains (DGg13 and BB133) were found to effectively control *C. capsici*, the major anthracnose pathogen in Thailand (Intanoo and Chamswarng, 2007). It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Jeffries and Koomen, 1992; Maymon *et al*., 2004). *Trichoderma* species have been applied to control *Colletotrichum* species in chilli (Boonratkwang *et al*., 2007), strawberries (Freeman *et al*., 2001), and citrus in Belize (Moretto *et al*., 2001) with concomitant disease reduction. Other biological control agents that have been tested for efficacy against *C. acutatum* include *Bacillus subtilis* and *Candida oleophila* (Wharton and Diéguez-Uribeondo, 2004).

ASPECTS TO CURRENT STATUS OF CHILLI ANTHRACNOSE DISEASE MANAGEMENT

Management and control of the anthracnose disease are still under extensive research (Yoon *et al*., 2004). Among disease control management, the use of resistant cultivars is the cheapest, easiest, safest and most effective means of controlling the disease. This is not only to eliminate losses from the disease but also decrease the cost of chemical and mechanical control, as well as reduce contamination of the environment from the use of toxic chemicals. However, management of disease through breeding of pathogen-resistant cultivars has only had limited success due to frequent breakdown of resistance under field conditions. Commercial cultivars of *Capsicum annuum* resistant to the pathogens that cause anthracnose have not yet been developed (Park, 2007). Nevertheless, high levels of resistance to the *Colletotrichum* species that infect chilli have been found in some species of *Capsicum*, for instance, *C. baccatum*. Current research is focusing on introgression of this resistance into susceptible commercial cultivars of *C. annuum* (AVRDC, 2003; Pakdeevaraporn *et al*., 2005). Recently in Thailand, Mongkolporn *et al*.(2004a) have studied the inheritance of resistance to anthracnose specifically caused by *Colletotrichum capsici*, in a *Capsicum annuum* population established from a cross between accession '83-168' and cv. 'KKU-Cluster', and their progenies. They observed a promising dominant gene responsible for the resistance to *C. capsici*. Voorrips *et al*.(2004) found one main QTL with high significance and strong resistance against *C. gloeosporioides* associated with chilli anthracnose disease in Indonesia.

Although there are currently extensive research on disease control management including breeding programs for resistant cultivars to anthracnose, the current status of the chilli anthracnose disease still requires improvement. There remain many questions to be answered concerning characterization of *Colletotrichum* species associated with anthracnose; in particular species present in different countries and regions; pathogenetic or genetic diversity of *Colletotrichum* species worldwide; infection processes and the disease cycle of *Colletotrichum* species leading to effective disease control and resistant plant breeding.

References

Abang, M.M., 2003. Genetic diversity of *Colletotrichum gloeosporioides* Penz. causing anthracnose disease of yam (*Dioscorea* spp.) in Nigeria. *Bibliotheca Mycologia*, **197**:20-33.

Adaskaveg, J.E., Hartin, R.J., 1997. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopathology*, **87**(9): 979-987. [doi:10.1094/PHYTO.1997.87.9.979]

Adikaram, N.K.B., Brown, A., Swinburne, T.R., 1983. Observations on infection of *Capsicum annuum* fruit by *Glomerella cingulata* and *Colletotrichum capsici*. *Transactions of the British Mycological Society*, **80**: 395-401.

Agrios, G.N., 2005. Plant Pathology, 5th Ed. Academic Press, San Diego, p.922.

Alexander, S.A., Waldenmaier, C.M., 2002. Management of Anthracnose in Bell Pepper. Fungicide and Nematicide Tests [Online]. New Fungicide and Nematicide Data Committee of the American Phytopathological Society. Vol. 58, p.49. Available from http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS.2004.88.11.1198 (Accessed 25/12/2007). [doi:10.1094/FN58]

AVRDC (Asian Vegetable Research and Development Centre), 1999. Off-Season Tomato, Pepper, and Eggplant. Progress Report for 1998. Asian Vegetable Research and Development Centre, Taiwan, China.

AVRDC (Asian Vegetable Research and Development Centre), 2003. AVRDC Progress Report for 2002. Asian Vegetable Research and Development Centre, Taiwan, China.

Bailey, J.A., 1987. Phytoalexins: A Genetic View of Their Significance. *In*: Day, P.R., Jellis, G.J. (Eds.), Genetics and Plant Pathogenesis. Blackwell, Oxford, p.13-26.

Bailey, J.A., Jeger, M.J. (Eds.), 1992. *Colletotrichum*: Biology, Pathology and Control. Commonwealth Mycological Institute, Wallingford, p.388.

Boonratkwang, C., Chamswarng, C., Intanoo, W., Juntharasri, V., 2007. Effect of Secondary Metabolites from *Trichoderma Harzianum* Strain Pm9 on Growth Inhibition of *Colletotrichum Gloeosporioides* and Chilli Anthracnose Control. Proceeding of the 8th National Plant Protection Conference. Naresuan University, Phisanulok, Thailand, p.323-336.

Bosland, P.W., Votava, E.J., 2003. Peppers: Vegetable and Spice Capsicums. CAB International, England, p.233.

Britton, G., Hornero-Méndez, D., 1997. Carotenoids and Colour in Fruits and Vegetables. *In*: Tomás-Barberán, F.A., Robins, R.J. (Eds.), Photochemistry of Fruits and Vegetables. Clarendon Press, Oxford, England, p.11-28.

Brown, A.E., Sreenivasaprasad, S., Timmer, L.W., 1996. Molecular characterization of slow-growing orange and key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. *Phytopathology*, **86**(5):523-527. [doi:10.1094/Phyto-86-523]

Cannon, P.F., Bridge, P.D., Monte, E., 2000. Linking the Past, Present, and Future of *Colletotrichum* Systematics. *In*: Prusky, D., Freeman, S., Dickman, M. (Eds.), *Colletotrichum*: Host specificity, Pathology, and Host-pathogen Interaction. APS Press, St. Paul, Minnesota, p.1-20.

Charigkapakorn, N., 2000. Control of Chilli Anthracnose by Different Biofungicides. Thailand. Available from http://www.arc-avrdc.org/pdf_files/029-Charigkapakorn_18th.pdf (Accessed 24/06/2008).

Chitkara, S., Singh, T., Singh, D., 1990. Histopathology of *Colletotrichum dematium* infected chilli seeds. *Acta Botanica Indica*, **18**:226-230.

Correll, C.J., Cornelius, K., Feng, C., Ware, S.B., Gabor, B., Harp, T.L., 2007. Overview of the Phylogenetics Species Concept in *Colletotrichum* as it Relates to Chilli Anthracnose. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.20.

Crous, P.W., Aptroot, A., Kang, J.C., Braun, U., Wingfield, M.J., 2000. The genus *Mycoshaerella* and its anamorphs. *Studies in Mycology*, **45**:107-121.

Dastur, J.F., 1920. *Glomerella cingulata* (Stoneman) Spald and its conidial form, *Gloesporium piperatum* E. and E. and *Colletotrichum nigrum* E. and Hals. on chillies and *Carica papaya*. *Annals of Applied Biology*, **6**(4):245-268. [doi:10.1111/j.1744-7348.1920.tb06472.x]

Denoyes, B., Baudry, A., 1995. Species identification an pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. *Phytopathology*, **85**(1):53-57. [doi:10.1094/Phyto-85-53]

Dodd, J.C., Estrada, A., Jeger, M.J., 1992. Epidemiology of *Colletotrichum Gloeosporioides* in the Tropics. *In*: Bailey, J.A., Jeger, M.J. (Eds.), *Colletotrichum*: Biology, Pathology and Control. CAB International, Wallingford, p.308-325.

Don, L.D., Van, T.T., Phuong Vy, T.T., Kieu, P.T.M., 2007. *Colletotrichum* spp. Attacking on Chilli Pepper Growing in Vietnam. Country Report. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.24.

Du, M., Schardl, C.L., Vaillancourt, L.J., 2005. Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexes. *Mycologia*, **97**(3):641-658. [doi:10.3852/mycologia.97.3.641]

FAO (Food and Agriculture Organization of the United Nations), 2003. FAO Production Yearbook 2001. FAO, Rome, **55**(170):333.

Flor, H.H., 1971. Current status for the gene-for-gene concept. *Annual Reviews of Phytopathology*, **9**:275-296.

Freeman, S., Katan, T., Shabi, E., 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Disease*, **82**(6):596-605. [doi:10.1094/PDIS.1998.82.6.596]

Freeman, S., Minz, D., Maymon, M., Zveibil, A., 2001. Genetic diversity within *Colletotrichum acutatum sensu* Simmonds. *Phytopathology*, **91**(6):586-592. [doi:10.1094/PHYTO.2001.91.6.586]

Guerber, J.C., Liu, B., Correll, J.C., Johnston, P.R., 2003. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs and mating compatibility. *Mycologia*, **95**(5):872-895. [doi:10.2307/3762016]

Hadden, J.F., Black, L.L., 1989. Anthracnose of Pepper Caused by *Colletotrichum* spp. Proceeding of the International Symposium on Integrated Management Practices: Tomato and Pepper Production in the Tropics. Asian Vegetable Research and Development Centre, Taiwan, p.189-199.

Halsted, B.D., 1890. A new anthracnose of pepper. *Bulletin of the Torrey Botanical. Club.*, **18**:14-15.

Hong, J.K., Hwang, B.K., 1998. Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum cocodes*. *Plant Disease*, **82**(10):1079-1083. [doi:10.1094/PDIS.1998.82.10.1079]

Hong, J.K., Kim, D.H., 2007. Taxonomic Characteristics of *Colletotrichum* spp. and their Teleomorphs Causing Anthracnose of Chilli Pepper. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.31.

Hornero-Méndez, D., Costa-García, J., Mínguez-Mosquera, M.I., 2002. Characterization of carotenoids high-producing *Capsicum annuum* cultivars selected for paprika production. *Journal of Agricultural and Food Chemistry*, **50**(20): 5711-5716. [doi:10.1021/jf0256236]

Intanoo, W., Chamswarng, C., 2007. Effect of Antagonistic Bacterial Formulations for Control of Anthracnose on Chilli Fruits. Proceeding of the 8th National Plant Protection Conference. Naresuan University, Phisanulok, Thailand, p.309-322.

Isaac, S., 1992. Fungal Plant Interaction. Chapman and Hall Press, London, p.115.

Jeewon, R., Liew, E.C.Y., Hyde, K.D., 2002. Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters. *Molecular Phylogenetics and Evolution*, **25**(3): 378-392. [doi:10.1016/S1055-7903(02)00422-0]

Jeewon, R., Liew, E.C.Y., Simpson, J.A., Hodgkiss, I.J., Hyde, K.D., 2003. Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. *Molecular Phylogenetics and Evolution*, **27**(3):372-383. [doi:10.1016/S1055-7903(03)00010-1]

Jeewon, R., Liew, E.C.Y., Hyde, K.D., 2004. Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. *Fungal Diversity*, **17**:39-55.

Jeffries, P., Koomen, I., 1992. Strategies and Prospects for Biological Control of Diseases Caused by *Colletotrichum*. *In*: Bailey, J.A., Jeger, M.J. (Eds.), *Colletotrichum*: Biology, Pathology and Control. Commonwealth Mycological Institute, Wallingford, p.337-357.

Jeffries, P., Dodd, J.C., Jegerand, M.J., Plumbley, R.A., 1990. The biology and control of *Colletotrichum* species on tropical fruit crops. *Plant Pathology*, **39**(3):343-366. [doi:10.1111/j.1365-3059.1990.tb02512.x]

Jeger, M.J., Jeffries, P., 1988. Alternative to chemical usage for disease management in the post-harvest environment. *Aspects of Applied Biology*, **17**:47-57.

Jewsakun, S., 1978. Serology, Seed Transmission on Anthracnose Disease of Pepper and Effect of Foliar Fungicides to the Causal Pathogens. MS Thesis, Kasetsart University.

Jeyalakshmi, C., Seetharaman, K., 1998. Biological control of fruit rot and die-back of chilli with plant products and antagonistic microorganisms. *Plant Disease Research*, **13**:46-48.

Johnston, P.R., Jones, D., 1997. Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycologia*, **89**(3):420-430. [doi:10.2307/3761036]

Katan, T., 2000. Vegetative Compatibility in *Colletotrichum*. *In*: Prusky, D., Freeman, S., Dickman, M. (Eds.), *Colletotrichum*: Host Specificity, Pathology, and Host-pathogen Interaction. APS Press, St. Paul, Minnesota, p.45-55.

Kim, B.S., Park, K.S., Lee, W.S., 1987. Search for resistance to two *Colletotrichum* species in pepper (*Capsicum* spp.). *Journal of Korean Society and Horticultural Science*, **28**:207-213.

Kim, K.D., Oh, B.J., Yang, J., 1999. Differential interactions of a *Colletotrichum gloeosporioides* isolate with green and red pepper fruits. *Phytoparasitica*, **27**:1-10.

Kim, K.K., Yoon, J.B., Park, H.G., Park, E.W., Kim, Y.H., 2004. Structural modifications and programmed cell death of chilli pepper fruits related to resistance responses to *Colletotrichum gloeosporioides* infection. *Genetics and Resistance*, **94**:1295-1304.

Kim, W.G., Cho, E.J., Lee, E.J., 1986. Two strains of *Colletotrichum gloeosporioides* Penz. causing anthracnose of pepper fruits. *Korean Journal of Plant Pathology*, **2**:107-113.

Korpraditskul, V., Rattanakreetakul, C., Korpraditskul, R., Pasabutra, T., 1999. Development of Plant Active Substances from Sweetflag to Control Fruit Rot of Mango for Export. *In*: Proceeding of Kasetsart University Annual Conference. Kasetsart University, Bangkok, p.34.

Lenné, J.M., Parbery, D.G., 1976. Phyllosphere antagonists and appressoria formation in *Colletotrichum gloeosporioides*. *Transactions of the British Mycological Society*, **66**:334-336.

Lewis, I.M.L., Miller, S.A., 2003. Evaluation of Fungicides and a Biocontrol Agents for the Control of Anthracnose on Green Pepper Fruit, 2002. Nematicide Test Report [Online]. New Fungicide and Nematicide Data Committee of the American Phytopathological Society. Vol. 58, p.62. Available from http://apsjournals.apsnet.org/doi/abs/10.1094/PD-90-0397?cookieSet=1andjournalCode=pdis (Accessed 25/12/2007). [doi:10.1094/FN58]

Lui, A.R., Xu, T., Guo, L.D., 2007. Molecular and morphological description of *Pestalotiopsis hainanensis* sp. nov., a new endophyte from a tropical region of China. *Fungal Diversity*, **24**:23-26.

MacKenzie, S.J., Peres, N.A., Barquero, M.P., Arauz, L.F., Timmer, L.W., 2008. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. *Phytopathology*, in press.

Maiti, S., Sen, C., 1979. Fungal Diseases of Betel Vine. Proceedings of the National Academy of Science of the United States of America. Vol. 25, p.150-157.

Manandhar, J.B., Hartman, G.L., Wang, T.C., 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Disease*, **79**:

380-383.

Mancini, N., Perotti, M., Ossi, C.M., Cavallero, A., Matuka, S., Paganoni, G., Burioni, R., Rama, P., Clementi, M., 2006. Rapid molecular identification of fungal pathogens in corneal samples from suspected keratomycosis cases. *Journal of Medical Microbiology*, **55**(11):1505-1509. [doi:10.1099/jmm.0.46638-0]

Marin, A., Ferreres, F., Tomás Barberán, F.A., Gil, M., 2004. Characterization and quantization of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry*, **52**(12):3861-3869. [doi:10.1021/jf0497915]

Maxwell, A., Jackson, S.L., Dell, B., Hardy, G.E., 2005. PCR-identification of *Mycospharella* species associated with leaf diseases of *Eucalyptus*. *Mycological Research*, **109**(9):992-1004. [doi:10.1017/S0953756205003539]

Maymon, M., Minz, D., Barbul, O., Zveibil, A., Elad, Y., Freeman, S., 2004. Identification to species of *Trichoderma* biocontrol isolates according to ap-PCR and ITS sequence analyses. *Phytoparasitica*, **32**:370-375.

McDonald, B.A., 1997. The population genetics of fungi: tools and techniques. *Phytopathology*, **87**(4):448-453. [doi:10.1094/PHYTO.1997.87.4.448]

McDonald, B.A., McDermott, J.M., 1993. The population genetics of plant pathogenic fungi. *Bioscience*, **43**(5):311-319. [doi:10.2307/1312063]

McDonald, B.A., Linde, C., 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica*, **124**(2):163-180. [doi:10.1023/A:1015678432355]

Milgroom, M.G., 1996. Recombination and the multiloci structure of fungal populations. *Annual Review of Phytopathology*, **34**(1):457-477. [doi:10.1146/annurev.phyto.34.1.457]

Mitchell, J.R., Roberts, P.J., Moss, S.T., 1995. Sequence or structure? A short review on the application of nucleic acid sequence information to fungal taxonomy. *Mycologist*, **9**:67-75.

Mongkolporn, O., Dokmaihom, Y., Kanchana-udomkarn, C., Pakdeevaraporn, P., 2004a. Genetic purity test of F1 hybrid *Capsicum* using molecular analysis. *Journal of Horticultural Science and Biotechnology*, **79**:449-451.

Mongkolporn, O., Thierry, J., Kanchana-udomkarn, C., Lin, Q., 2004b. Genetic analysis of resistance to pepper anthracnose caused by *Colletotrichum capsici*. *Thai Journal of Agricultural Science*, **35**:259-264.

Moretto, K.C.K., Gimenes-Fernandes, N., Dos Santos, J.M., 2001. Influence of *Trichoderma* spp. on *Colletotrichum acutatum* mycelial growth and morphology on infection of 'Tahiti' lime detached flowers. *Summa Phytopathologia*, **27**(4):357-364.

Moriwaki, J., Tsukiboshi, T., Sato, T., 2002. Grouping of *Colletotrichum* species in Japan based on rDNA sequences. *Journal of General Plant Pathology*, **68**(4):307-320. [doi:10.1007/PL00013096]

Nirenberg, H.I., Feiler, U., Hagedorn, G., 2002. Description of *Colletotrichum lupini* comb. nov. in modern terms. *Mycologia*, **94**(2):307-320. [doi:10.2307/3761809]

O′Connell, R.J., Bailey, J.A., Richmond, D.V., 1985. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. *Physiologial Plant Pathology*, **27**(1):75-98. [doi:10.1016/0048-4059(85)90058-X]

O′Connell, R.J., Perfect, S., Hughes, B., Carzaniga, R., Bailey, J.A., Green, J., 2000. Dissecting the Cell Biology of Colletotrichum Infection Processes. *In*: Bailey, J.A., Jegar, M.J. (Eds.), *Colletotrichum*: Biology, Pathology, and Control. CAB International, Wallingfords, UK, p.57-77.

O′Donnell, K., Cigilink, E., Nirenberg, H.I., 1998. Molecular systematic and phylogeography of the *Giberella fujikuroi* species complex. *Mycologia*, **90**(3):465-493. [doi:10.2307/3761407]

Osuna-García, J.A., Wall, M.W., Waddell, C.A., 1998. Endogenous levels of tocopherols and ascorbic acid during fruits ripening of New Mexican-type chilli (*Capsicum annuum* L.) cultivars. *Journal of Agricultural and Food Chemistry*, **46**(12):5093-5096. [doi:10.1021/jf980588h]

Pae, D.H., Yoon, J.Y., Lee, J.M., 1998. Screening for resistance to *Colletotrichum gloeosporioides* using detached fruits in pepper (*Capsicum annuum*) by high-pressure spray. *Journal of Korean Society and Horticultural Science*, **39**:690-692.

Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J., Mongkolporn, O., 2005. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. *Plant Breeding*, **124**(2):206-208. [doi:10.1111/j.1439-0523.2004.01065.x]

Park, H.G., 2007. Problems of Anthracnose in Pepper and Prospects for its Management. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.19.

Park, K.S., Kim, C.H., 1992. Identification, distribution, and etiological characteristics of anthracnose fungi of red pepper in Korea. *Korean Journal of Plant Pathology*, **8**:61-69.

Park, W.M., Park, S.H., Lee, Y.S., Ko, Y.H., 1987. Differentiation of *Colletotrichum* spp. causing anthracnose on *Capsicum annuum* L. by electrophoretic method. *Korean Journal of Plant Pathology*, **3**:85-92.

Paul, Y.S., Behl, M.K., 1990. Some studies on bell pepper anthracnose caused by *Colletotrichum capsici* and its control. *Seed Research*, **1**:656-659.

Pearson, M.N., Bull, P.B., Speke, H., 1984. Anthracnose of *Capsicum* in Papua, New Guinea; varietal reaction and associated fungi. *Tropical Pest Management*, **30**:230-233.

Peres, N.A.R., de Souza, N.L., Peever, T.L., Timmer, L.W., 2004. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. *Plant Disease*, **88**(2):125-130. [doi:10.1094/PDIS.2004.88.2.125]

Peres, N.A., Timmer, L.W., Adaskaveg, J.E., Correll, J.C., 2005. Life cycles of *Colletotrichum acutatum*. *Plant Disease*, **89**(8):784-796. [doi:10.1094/PD-89-0784]

Peres, N.A., MacKenzie, S.J., Peever, T.L., Timmer, L.W., 2008. Postbloom fruit drop of citrus and key lime anthracnose are caused by distinct phylogenetic lineages of *Colletotrichum acutatum*. *Phytopathology*, **98**(3):345-352. [doi:10.1094/PHYTO-98-3-0345]

Pérez-Gálvez, A., Martin, H.D., Sies, H., Stahl, W., 2003. Incorporation of carotenoids from paprika oleoresin into human chylomicron. *British Journal of Nutrition*, **89**(6): 787-793. [doi:10.1079/BJN2003842]

Perfect, S.E., Hughes, H.B., O'Connell, R.J., Green, J.R., 1999. *Colletotrichum*: a model genus for studies on pathology and fungal-plant interactions. *Fungal Genetics and Biology*, **27**(2-3):186-198. [doi:10.1006/fgbi.1999.1143]

Pernezny, K., Roberts, P.D., Murphy, J.F., Goldberg, N.P., 2003. Compendium of Pepper Diseases. The American Phytopathological Society, St. Paul, Minnedota, p.73.

Photita, W., Taylor, P.W.J., Ford, R., Lumyong, P., McKenzie, H.C., Hyde, K.D., 2005. Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity*, **18**:117-133.

Pickersgill, B., 1997. Genetic resources and breeding of *Capsicum* spp. *Euphytica*, **96**(1):129-133. [doi:10.1023/A:1002913228101]

Poonpolgul, S., Kumphai, S., 2007. Chilli Pepper Anthracnose in Thailand. Country Report. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.23.

Poulos, J.M., 1992. Problems and Progress of Chilli Pepper Production in the Tropics. *In*: Hock, C.B., Hong, L.W., Rejab, M., Syed, A.R. (Eds.), Proceedings of the Conference on Chilli Pepper Production in the Tropics. Kuala Lumpur, Malaysia, p.98-129.

Pring, R.J., Nash, C., Zakaria, M., Bailey, J.A., 1995. Infection process and host range of *Colletotrichum capsici*. *Physiological and Molecular Plant Pathology*, **46**(2): 137-152. [doi:10.1006/pmpp.1995.1011]

Prusky, D., Plumbley, R.A., 1992. Quiescent Infections off *Colletotrichum* in Tropical and Subtropical Fruits. *In*: Bailey, J.A., Jeger, M.J. (Eds.), *Colletotrichum*: Biology, Pathology, and Control. CAB International Wallingford, p.289-307.

Prusky, D., Koblier, I., Aridi, R., Beno-Moalem, D., Yakoby, N., Keen, N.T., 2000. Resistance Mechanisms of Subtropical Fruits to *Colletotrichum gloeosporioides*. *In*: Bailey, J.A., Jeger, M.J. (Eds.), *Colletotrichum*: Biology, Pathology, and Control. CAB International Wallingford, p.232-244.

Ratanacherdchai, K., Wang, H.K., Lin, F.C., Soytong, K., 2007. RAPD analysis of *Colletotrichum* species causing chilli anthracnose disease in Thailand. *Journal of Agricultural Technology*, **3**:211-219.

Roberts, P.D., Pernezny, K., Kucharek, T.A., 2001. Anthracnose caused by *Colletotrichum* sp. on pepper [Online]. *Journal of University of Florida/Institute of Food and Agricultural Sciences*. Available from http://edis.ifas.ufl.edu/PP104 (Accessed 25/12/2007).

Royle, D.J., Butler, D.R., 1986. Epidemiological Significance of Liquid Water in Crop Canopies and its Role in Disease Forecasting. *In*: Ayres, P.G., Boddy, L. (Eds.), Water, Fungi and Plants. Cambridge University Press, Cambridge, p.139-156.

Sharma, P.N., Kaur, M., Sharma, O.P., Sharma, P., Pathania, A., 2005. Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. *Journal of Phytopathology*, **153**(4):232-237. [doi:10.1111/j.1439-0434.2005.00959.x]

Sheu, Z., Chenand, J., Wang, T., 2007. Application of ITS-RFLP Analysis for Identifying *Colletotrichum* Species Associated with Pepper Anthracnose in Taiwan. *In*: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.32.

Simmonds, J.H., 1965. A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. *Queensland Journal Agriculture and Animal Science*, **22**:437-459.

Smith, B.J., Black, L.L., 1990. Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Plant Disease*, **74**(1):69-76. [doi:10.1094/PD-74-0069]

Smith, K.L., 2000. Peppers. *In*: Precheur, R.J. (Ed.), Ohio Vegetable Production Guide. Ohio State University Extension, Columbus, Ohio, p.166-173.

Sreenivasaprasad, S., Talhinhas, P., 2005. Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. *Molecular Plant Pathology*, **6**(4):361-378. [doi:10.1111/j.1364-3703.2005.00291.x]

Sreenivasaprasad, S., Mills, P., Brown, A., 1994. Nucleotide sequence of the rDNA spacer 1 enables identification of isolates of *Colletotrichum* as *C. acutatum*. *Mycological Research*, **98**:186-188.

Sreenivasaprasad, S., Mills, P., Meehan, B.M., Brown, A., 1996. Phylogeny and systematics of 18 *Colletotrichum* species based on ribosomal DNA spacer sequences. *Genome*, **39**(3):499-512. [doi:10.1139/g96-064]

Staub, T., 1991. Fungicide resistance: practical experience and antiresistance strategies and the role of integrated use. *Annual Review of Phytopathology*, **29**(1):421-442. [doi:10.1146/annurev.py.29.090191.002225]

Taylor, P.W.J., Ford, R., 2007. Diagnostics, genetics diversity and pathogenic variation of ascochyta blight of cool season food and feed legumes. *European Journal of Plant Pathology*, **119**(1):127-133 [doi:10.1007/s10658-007-9177-x]

Taylor, P.W.J., Mongkolporn, O., Than, P.P., Montri, P., Ranathunge, N., Kanchana-udonkarn, C., Ford, R., Pongsupasamit, S., Hyde, K.D., 2007. Pathotypes of *Colletotrichum* spp. Infecting Chilli Peppers and Mechanisms of Resistance. *In*: Oh, D.G., Kim, K.T. (Eds.), Ab-

stracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea, p.29.

Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O., Taylor, P.W.J., 2008. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*, **57**(3):562-572. [doi:10.1111/j.1365-3059.2007.01782.x]

Tong, N., Bosland, P.W., 1999. *Capsicum tovarii*, a new member of the *Capsicum* complex. *Euphytica*, **109**(2):71-72. [doi:10.1023/A:1003421217077]

von Arx, J.A., 1957. Die Arten der Gattung *Colletotrichum* Cda. *Phytopathologische Zeitschrift*, **29**:414-468.

Voorrips, R.E., Finkers, R., Sanjaya, L., Groenwold, R., 2004. QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics*, **109**(6):1275-1282. [doi:10.1007/s00122-004-1738-1]

Wharton, P.S., Diéguez-Uribeondo, J., 2004. The biology of *Colletotrichum acutatum*. *Anales del Jardin Botanico de Madrid*, **61**:3-22.

Whitelaw-Weckert, M.A., Curtin, S.J., Huang, Steel, R.C.C., Blanchard, C.L., Roffey, P.E., 2007. Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia. *Plant Pathology*, **56**(3):448-463. [doi:10.1111/j.1365-3059.2007.01569.x]

Wikipedia, 2007. Capsicum [WWW]. Wikimedia Foundation, Inc., USA. Available from http://en.wikipedia.org/wiki/Capsicum (Accessed 25/12/2007).

Yoon, J.B., 2003. Identification of Genetic Resource, Interspecific Hybridization and Inheritance Analysis for Breeding Pepper (*Capsicum annuum*) Resistant to Anthracnose. Ph.D. Thesis, Seoul National University, Korea.

Yoon, J.B., Park, H.G., 2001. Screening method for resistance to pepper fruits anthracnose: Pathogen sporulation, inoculation methods related to inoculum concentrations, post-inoculation environments. *Journal of Korean Society and Horticultural Science*, **42**:382-393.

Yoon, J.B., Yang, D.C., Lee, W.P., Ahn, S.Y., Park, H.G., 2004. Genetic resources resistant to anthracnose in the genus *Capsicum*. *Journal of Korean Society and Horticultural Science*, **45**:318-323.

Yun, S.H., Berbee, M.L., Yoder, O.C., Turgeon, B.G., 1999. Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. *Proceedings of the National Academy of Science of the United States of America*, **96**(10):5592-5597. [doi:10.1073/pnas.96.10.55 92]