Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2015 Vol. 4 (4) October-December, pp.10-21/Yahaya
Research Article

SYSTEMIC INFECTION OF *BOTRYTIS CINEREA* IN MODEL PLANT *ARABIDOPSIS THALIANA*

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ABSTRACT
Experiment is presented, which shows that a necrotic pathogen *Botrytis cinerea* grows systemically and endophyticaly in *Arabidopsis thaliana* plant. The pathogen was detected on *Botrytis* selective media from sterilized sections of symptomless *A. thaliana* plants which were inoculated from three different ways (1) plant inoculated at two leaf stage (2) plant inoculated at two leaf stage and immediately after inoculation the upper plant part removed (3) plant grown in inoculated soil. In all the three inoculation methods the plants were removed and section into roots, stems and leaves two weeks after inoculation. Re-isolation of the isolate B1.1 used in the inoculation of the plants from different section of the plant confirmed the systemic and endophytic transmission of *Botrytis cinerea* isolates used in the inoculation. The result also showed that apart from the inoculating isolates other isolates were present. This confirmed that *A. thaliana* can have multiple infections. The systemic and endophytic spread of *Botrytis cinerea* in model plant *A.thaliana* will therefore be very important aspect of the population within a species and will have a serious impact on the epidemiology and control of diseases.

Keywords: *Arabidopsis Thaliana, Botrytis Cinerea, Endophytic Infection, Systemic Infection*

INTRODUCTION
Systemic infection is an infection in which a single genotype of a pathogen is distributed throughout the body of an infected organism rather than concentrated in one area and does not show visible symptoms (Arnold *et al.,* 2000; Johnston *et al.,* 2006). Many studies support hypothesis that systemic infection can result from endophytes (Phitita *et al.,* 2001; Neubert *et al.,* 2006; Wei *et al.,* 2007; Tajesvi *et al.,* 2007). This is based on the recovery of endophytes from sections of infected plant tissues and determination of DNA of the endophyte with probes which are specific for that endophyte. Endophytes are fungi or bacteria which colonize healthy plant tissues inter- and/ or intra-cellularly, persisting for the whole or part of their life cycle without causing disease symptoms in the host plant (Bacon and White, 2000; Marquez *et al.,* 2007; Hyde and Soytong, 2008). The endophytic fungi are usually symptomless, but may produce effects ranging from beneficial to pathogenic (Phitita *et al.,* 2001;Stone *et al.,* 2004; Neubert et al., 2006; Gonthier *et al.,* 2006; Wei *et al.,* 2007). Systemic and endophytic infection of economically important plants by necrotrophic fungal pathogens has been commonly recognised in higher plants. *Botrytis cinerea* can attack all parts of the plant and remain symptomless until the tissue senesces. The pathogens may be either vertically transmitted via seed or by propagule production from distinct phases of the host plant life cycle (Tintjer *et al.,* 2006; Oses *et al.,* 2008; Rodriguez et al.2008). Schulz *et al.,* (2005) and Omacini *et al.,* (2001) reported that endophytes alter plant susceptibility to fungal and viral infections and can also alter plant mychorrhizal status.

Botrytis cinerea is a member of the phylum Ascomycetes, family Sclerotiniaceae and is regarded as a model necrotrophic pathogen (Williamson *et al.,* 2007), responsible for considerable pre and post-harvest losses of crops especially in temperate climates (Elad *et al.,* 2004). The fungus is designated by its anamorphic form (asexual form) name, because the perfect sexual stage (teleomorph) known as *Botryotinia fuckeliana* is rarely observed (Beever and Weeds, 2004). *Botrytis cinerea* has a fairly simple life cycle, it produces copious clear or grey conidia on long branched conidiophores (Agrios, 2005), which infects directly to cause necrotrophic lesions. In many crops *B. cinerea* infection may become quiescent. During quiescence, further fungal growth and colonization are halted, and signs of the pathogen are not visible (Cadle-Davidson, 2008). The spread of infection may be activated at the onset of

senescence or stress of the host plant tissues (Coertze and Holtz, 2002; Keller *et al.,* 2003). However, Elias *et al.,* (2010) reported that *B. cinerea* could grow systemically and endophytically without showing symptoms in lettuce plants. The infection is caused by one isolate which grows throughout the body of healthy plant without showing symptoms, suggesting that *B. cinerea* is able to grow endophytically by avoiding plant defences through not producing toxic compounds or by producing such toxic compounds at a level which allows it to continue to grow without being detected (Elias *et al.,* 2010).

Arabidopsis thaliana, a member of the family Brassicaceae, a widely used model plant, is an annual plant which has a life cycle of approximately six weeks (Gonzalez *et al.,* 2006). It is relatively small in size, rarely exceeding 12cm; and it is mostly found in disturbed habitats (Gonzalez *et al.,* 2006). *Arabidopsis* has one of the smallest genomes among plants (*Arabidopsis* genome initiative 2000). The small sizes genome makes *A. thaliana* very useful for genetic mapping and sequencing. *Arabidopsis* is fully sequenced and the genome well-annotated with 157 million base pairs and five chromosomes (Gonzalez *et al.,* 2006; Weigel, 2012). *Arabidopsis thaliana* is infected by a number of pathogenic organisms and may sometimes grow systemically without showing symptoms (Shafia, 2009). The notable one is the necrotrophic and endophytic fungal pathogen *B. cinerea* which causes grey mould diseases (Muckenschnabel *et al.,* 2002; Ferrari *et al.,* 2003; Shafia, 2009).

Like many other plants *A. thaliana* is exposed to wide range of microbial pathogens which employ various strategies to attack the plant at various stages of their development but most importantly at the early stages (Muckenschnabel *et al.,* 2002; Shafia, 2009). Some pathogens are biotrophic and do not kill the plant cell, instead they subvert the host metabolism to favour their development (Ferrari *et al.,* 2003). However, necrotrophic pathogens like *B. cinerea* have to kill the host cell in order to metabolize their contents (Ferrari *et al.,* 2003). Various responses are shown by *A. thaliana* aimed at counteracting the effect of various necrotrophic pathogens. Simone *et al.,* (2007) reported Salicylic acid as a very important regulator of plant defence responses shown by *A. thaliana* against the effect of pathogenic fungi and bacteria.

However, in order to assess the evolutionary potential of the pathogen in causing disease and overcoming control measures and also its survival in a new environment, knowledge of the genetic structure of the pathogen is important. This is because pathogenicity and genotype may vary in a population (Isenegger *et al.,* 2008). My preliminary studies showed that in wild *A. thaliana*, *Botrytis cinerea* could be isolated from different sections of healthy plants. The question arose as to whether *B. cinerea* might grow systemically in the internal parts of *A. thaliana*. I tested two hypotheses. First, if *B. cinerea* is systemic and endophytic in *A. thaliana*. Second, if *B. cinerea* isolated from one plant is the same throughout the plant and whether isolates vary among different plants.

MATERIALS AND METHODS

Botrytis Cinerea Culture and Fungal Isolation

Botrytis cinerea isolate B1.1 (isolated at Reading, 2005) was maintained on *Botrytis* selective media (BSM) before being transferred to 3% malt extract agar (MEA. CM0059, Oxoid, Basingstoke), and sporulation was encouraged once the culture had covered 1/3 of each plate by continuous exposure to UV light. All detection and isolation of the fungus from plant tissues was done with *Botrytis* selective media (Sowley, 2006; Rajaguru, 2008; Shafia, 2009).

Seed Stocks and Sterilization

Seed of *A. thaliana* 1092 variety purchased from the Nottingham *Arabidopsis* Centre was used. The seed was sterilized by soaking in 10% Milton solution (Laboratoire Rivadis France) for 20 minutes, then rinsed with autoclaved water and dried before sowing.

Plant Growth in Isolation Propagator

Plants were grown in an isolation propagator made with sixteen, 15cm pots, and eight per tray (Figure 3.1). Pots were filled with vermiculite growing medium and covered with colourless transparent cups with a small hole at their tops covered by cotton wool. The pots were connected to an air pump machine by a rubber tube. Seed of *A. thaliana* was sown in each of the 16 pots and wetted by capillary matting.

Figure 1: *Arabidopsis thaliana* growing in an Isolation propagator in a controlled environmental room

Artificial Inoculation of the Seedling

To inoculate the plants, 10mg dry spores of *Botrytis cinerea* was harvested from 14 days old culture in Petri dishes and diluted with 90mg of talc. The mixture was serially diluted with talc to produce seven concentrations. The lowest concentration was used for the inoculation in respect of each plant. A total of eight pots were inoculated with the remaining eight pots to serve as controls. Before inoculation the air pump machine was switched off and left for 24h to allow build up of high relative humidity for the germination of spores on the leaves (Figure 3.2).

Figure 2: Inoculation of *A. thaliana* plants with dry *B. cinerea* spores in a controlled environment Room

Another eight *A. thaliana* plants were grown in an isolation propagator. At two leaf stage the plants were inoculated as previously described. However, immediately after inoculation the stems and leaves of the

eight plants were removed. The root was allowed to grow for three weeks before sampling. Another eight plants were grown on the inoculated soil. Vermiculite soil in eight pots was inoculated with dry *B. cinerea* spores as before. Then a thin layer of soil was placed over the spores before sowing the seed. Plants were harvested and samples collected three weeks after germination (Figure 3.3).

Figure 3: *Arabidopsis thaliana* plants growing after inoculation in an isolation propagator in the Controlled Environment Room

Isolation of B. Cinerea from Plant Tissues

Complete root measuring about 1cm long, 3 leaves and 1 mm sections of stem from each plant were washed with autoclaved water and sterilized by spraying with 70% ethanol and allowed to dry. All the sections were plated in BSM and incubated at 18^{o}C for one week. Plates were observed macro and microscopically for the presence of *B. cinerea*. Colonies isolated were subcultured into MEA plates and incubated at 18^{O}C under alternating UV light for two weeks for sporulation.

Genotypic Characterisation of the Isolates

Mycelium of the isolates grown in malt extract agar (MEA) plates was harvested and 100mg was ground in a pestle and mortar in the presence of liquid nitrogen. The fine powder was transferred into 200ml Eppendorf tube and DNA was extracted using a DNeasy plant mini kit (Qiagen, West Sussex, UK), standardized by using a Nanodrop ND 1000 Spectrophotometer (Applied Biosystem) and stored at -20^{o}C until needed.

To confirm whether all the extracted isolates were *B. cinerea,* all the DNA templates were tested in polymerase chain reactions with scar primers designed by Suarez *et al.,* (2005), which are specific for *B. cinerea* and do not respond to DNA from other *Botrytis* species.

The DNA templates that gave a positive test with *B. cinerea* scar primers were characterized by using nine published microsatellite markers (Fournier *et al.*, 2002, Table 1), which were labelled with (Table 1) FAM (Blue), HEX (Green), and NED (Yellow). Each reaction contained 25µl of Biomix, 1.5 µl of forward and reverse primers, 20 µl of water, and 2 µl of DNA template. The simple sequence repeat (PCR of SSR) was done several times for successful amplifications. The products from successful amplifications were multiplexed in 3 combinations; (BC1, BC4, and BC9), (BC3, BC6, BC10), (BC2, BC5, BC7) groups were chosen to avoid overlapping the allele size ranges of the primers products in each mix. The SSR was run at an initial denaturing step of 2 minutes at 94^{0}C for BC1, BC2, BC3, BC5, BC6 and BC9, followed by 35 cycles of 1 minute at 94^{O}C, 1 minutes annealing at 53^{0}C 30 seconds at 72^{O}C and with a final extension of five minutes at 72^{O}C. BC4, BC7 and BC10 had an annealing temperature of 59^{O}C instead. The products were submitted for fragment analysis by Source Bioscience, UK. The results of the fragment analysis were scored by using the Genemapper software version 4 (Applied Biosystems).

Table 1: Characteristics of nine microsatellite primers (Fournier *et al.*, 2002)

Locus	Primer Sequences	Repeat motif	T	Size (bp)	Fluorescent Dye
Bc1	F: AGGGAGGGTATGAGTGTGTA R: TTGAGGAGGTGGAAGTTGTA	CTTT) 10-CA)2CG(CA)10	50	245-281	FAM
Bc2	F: CATACACGTATTTCTTCCAA R: TTTACGAGTGTTTTGTTAG	(AC)12 AT(AC)4	53	161-205	FAM
Bc3	F: GGATGAATCATTGTTTGTG R: CACCTAGGTATTTCCTGGTA	(GA)10	50	197-229	NED
Bc4	F: CATCTTCTGGGAACGCACAT R: ATCCACCCCCAAACGATTGT	(TTC)6 TTATC(TTC)2	59	98-125	NED
Bc5	F: CGTTTTCCAGCATTTCAAGT R: CATCTCATATTCGTTCCTCCA	(AT)12	53	143-163	NED
Bc6	F: ACTAGATTCGAGATTCAGTT R: AAGGTGGTATGAGCGGTTTA	(CA)10	50	88-158	FAM
Bc7	F: CCAGTTTCGAGGAGGTCCAC R: GCCTTAGCGGATGTGAGGTA	(TA)9	59	113-131	HEX
Bc9	F: CTCGTCATAACCACGCAGAT R: GCAAGGTCTCGATGTCGATC	(CT)11	50	154-194	HEX
Bc10	F: TCCTCTTCCCTCCCATCAAC R: GGATCTGCGTGGTTATGACG	(AC) 13	59	158-189	HEX

Experimental Design

The experiment used a randomised block design with seedling inoculation as the factor. Seedlings were dry inoculated at the two-leaf stage seven days after sowing, with spores of isolate B1.1 (10mg spore + 90mg talc) serially diluted to eight different concentrations. Eight plants were inoculated each with the lowest concentrations, while eight plants were not inoculated. No infection or yellowing of leaves was recorded throughout the duration of the experiment.

Two other sets of control experiments were set up. *Arabidopsis thaliana* was grown in eight pots. At the two-leaf stage, seven days after germination, all the plants were inoculated with fungal spores B1.1 diluted with talc as previously done. Immediately after inoculation the upper plant parts were removed and roots were allowed to grow for three weeks before sample collections.

Another control was set up with eight *Arabidopsis thaliana* plants. However, before sowing the soil in the pot was inoculated with fungal spores B1.1 diluted with talc and another thin layer of soil was placed on top of the inoculated soil before sowing the seed.

All plants grown were sampled three weeks after inoculation without any sign of infection, washed with autoclaved water and sterilized by spraying with 70% ethanol and allowed to dry. Sections of root about 1cm long, 3 leaves and 1 mm sections of stem from all the plants were plated on *Botrytis* selective media at alternating UV light. The resulting colonies were genetically characterized and sent for fragment analysis at Source Bioscience, UK. The results of the analysis were interpreted using Genemapper V4 software and the peak height was converted into scores of one and zero.

The highest peaks, of the microsatellite data were converted into score of one and zeros, which represent the presence or absence of bands of a giving size, respectively. The similarity matrix formed by the table of ones and zeros was analysed by ANOVA Genstat version 13.

RESULTS AND DISCUSSION

Results

Systemic Infection

No visible symptoms of *B. cinerea* were detected on the *A. thaliana* plants throughout the duration of the experiments. *Botrytis cinerea* was commonly recovered from surface-sterilised sections of leaves, stems and roots of all inoculated plants and from some of the two controls; but no infection was detected from the sterilised sections of uninoculated plants (Table 2). While the nine microsatellite markers used to

screen the isolates produced a total of twelve different haplotypes from both the inoculated plants and the control (i) plant where the upper part was removed immediately after inoculation, (ii) plant grown on inoculated soil). The size of the alleles generated by the individual loci varies between 96-249 bp (Figure 4). One haplotype A was found to occur frequently in the inoculated plants (Table 3) and was subsequently isolated from most of the roots of plants where the upper part was removed after inoculation (Table 4), and also from most parts of the plants grown in inoculated soil (Table, 3.5).

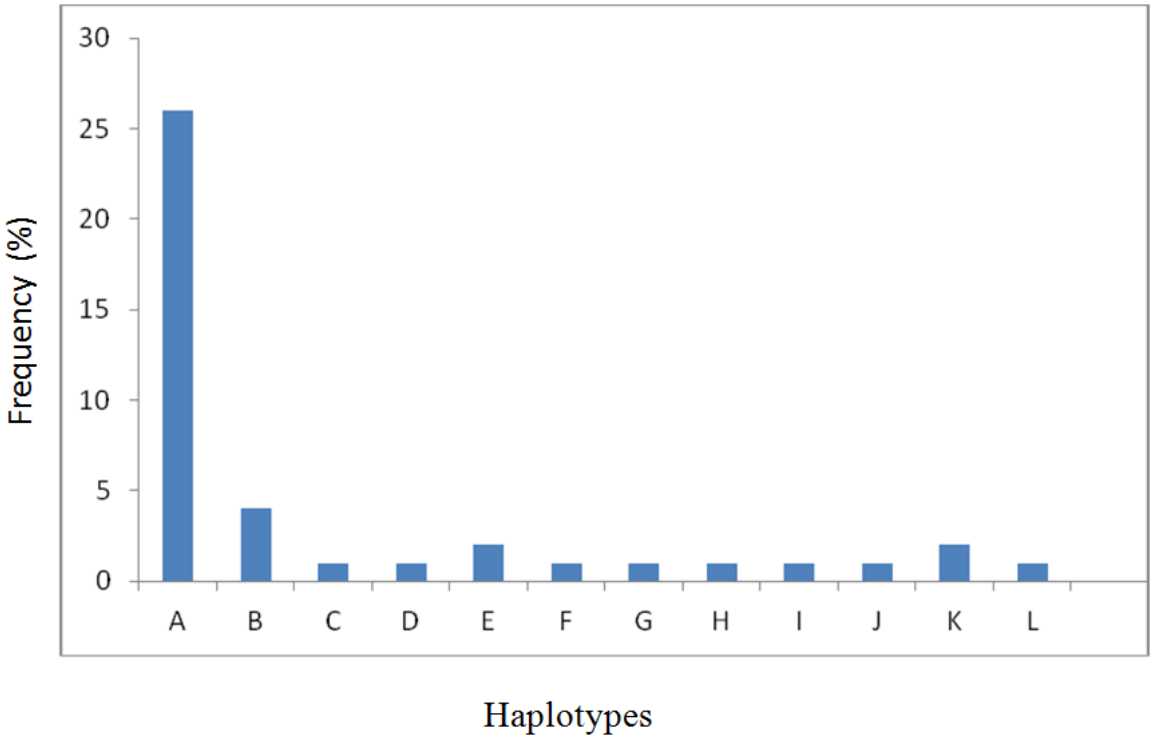

Figure 3.4: Genotype frequency among 42 *B. cinerea* isolates characterised by nine microsatellite markers. Letters A- L indicates isolates grouped into haplotypes. Detailed list of haplotypes is provided in Table 1, 2, 3 & 4

Inoculated Plants

Eighty seven percent of the haplotypes in the inoculated plants were similar to the isolate B1.1 used in the inoculation. Stem isolates were isolated from five inoculated plants (24%) and all the isolates were genetically similar to the isolate B1.1 used in the inoculation. Isolates were detected from the root and leaves of all the inoculated plants (38%). Out of these isolates, four plants had isolates in the root and leaves which were genetically similar to the inoculating isolates B1.1. Four plants had haplotypes which had a different genotypic identity from the inoculating isolate. Out of the isolates detected in inoculated plants (Table 3), four different haplotypes were present (A, B, C, & D).

Roots Where the Upper Plant Part was Removed Immediately after Inoculation

Isolates were detected in all of the roots of plants where the upper plant part was removed immediately after inoculation, Out of the isolates detected root of five plants (62%) were found to be similar genotypically with the inoculating isolate B1.1. Isolates from the roots of three plants have different genotypic identification with the inoculating isolate (Table 4). A total of three haplotypes were detected from the roots of plants where the upper part was removed immediately after inoculation (A, E, &F).

Plants Grown on Inoculated Soil

Out of the plants grown in inoculated soil, isolates were recorded in different sections of six plants (50%). In both roots and leaves three out of the six isolates detected were similar to the haplotype used in

inoculation, while three isolates had a different genotypic identity from the inoculating isolate in both roots and leaves. While only two isolates were detected from stems of plant grown in inoculated soil, one isolate was similar to the haplotype used in inoculation while the other was different genotypically. The isolates detected from different sections of plants grown in inoculated soil (Table 3.5) have seven different haplotypes (A, G, H, 1, J, K, & M).

Overall the isolate used in the inoculation was the most commonly occurring haplotype (A) in the whole study and dominated 100% ($F_{4,11}$ =1400.42,P < 0.001) other haplotypes detected from both inoculated plants, roots where the upper part was removed immediately after inoculation and plants grown in inoculated soil. This strongly suggests that the majority of the isolates detected came through plant inoculation with fungal spores B1.1.

Table 2: Frequency of haplotype distribution among the isolates summarized in respect to the total number of *B. cinerea* isolated from inoculated plants, root with upper part removed after inoculation, and plant grown in inoculated soil

Plant part variation	Inoculated Plant	Uninoculated Plant	Root with upper part removed after inoculation	Inoc soil	Total
Root	8	-	8	6	21
Stem	5	-	-	2	7
Leaves	8	-	-	6	19
Total	21	-	8	12	41

Table 3: Detail of the allele sizes of different *B. cinerea* isolated. NA- stands for no amplification, in the haplotypes column. The letters A to M are the codes used to denote the twelve haplotypes. The letters, L- stands for leaves, R- stands for root, and S- stand for stem

Isolates	BC 1	BC 2	BC 3	BC 4	BC 5	BC 6	BC 7	BC 9	BC10	HAPLOTYPE
Stock	231	163	200	96	157	121	117	163	179	A
IR	231	163	200	96	157	121	117	163	179	A
1S	231	163	200	96	157	121	117	163	179	A
1L	231	163	200	96	157	121	117	163	179	A
2R	231	163	164	96	157	121	117	163	178	B
2L	231	163	164	96	157	121	117	163	178	B
3R	231	163	200	96	157	121	117	163	179	A
3S	231	163	200	96	157	121	117	163	179	A
3L	231	163	200	96	157	121	117	163	179	A
4R	231	163	200	96	157	121	117	163	179	A
4L	231	163	164	96	157	121	117	163	178	B
5R	231	163	200	96	157	121	117	163	179	A
5L	231	163	200	96	157	121	117	163	179	A
6R	231	163	164	96	157	121	117	163	179	B
6S	231	163	200	96	157	121	117	163	179	A
6L	231	163	200	96	157	121	117	163	179	A
7R	231	163	200	96	157	121	117	163	179	A
7S	231	163	200	96	157	121	117	163	179	A
7L	NA	163	200	96	157	121	117	163	179	C
8R	231	163	200	96	157	121	117	163	179	A
8S	231	163	200	96	157	121	117	163	179	A
8L	211	163	200	96	157	121	117	163	179	D

Table 4: Details of the allele sizes of different *B. cinerea* isolated in roots with upper part removed before inoculation. NA- stands for no amplification, in the haplotypes column. The letters A to M are the codes used to denote the twelve haplotypes. The letter R- stands for root

Isolate	BC 1	BC 2	BC 3	BC 4	BC 5	BC 6	BC 7	BC 9	BC10	HAPLOTYPE
R1	231	163	200	96	157	121	117	163	179	A
R2	231	163	200	96	157	121	117	163	179	A
R3	231	163	200	96	157	121	117	163	179	A
R4	231	163	200	96	157	121	117	163	179	A
R5	231	163	200	96	157	121	117	163	179	A
R6	211	163	200	96	157	121	117	163	179	E
R7	211	163	200	96	157	121	117	163	179	E
R8	NA	163	200	NA	157	121	117	NA	179	F

Table 5: Detail of the allele sizes of different *B. cinerea* isolated in plants grown from inoculated soil. The letters A to M are the codes used to denote the twelve haplotypes. The letter L- stands for leaves, R- stands for root, and S- stands for stem

Isolates	BC 1	BC 2	BC 3	BC 4	BC 5	BC 6	BC 7	BC 9	BC10	HAPLOTYPE
Stock	231	163	200	96	157	121	117	163	179	A
SR1	231	163	200	96	157	121	117	163	179	A
SS1	231	163	200	96	157	121	117	163	179	A
SL1	211	163	200	96	157	121	117	163	179	H
SR3	243	163	200	96	157	121	117	163	179	G
SL3	211	163	200	96	157	94	117	163	179	I
SR5	245	163	200	96	157	121	117	163	179	J
SL5	231	163	200	96	157	121	117	163	179	A
SR6	231	163	200	96	157	121	117	163	179	A
SL6	231	163	200	96	157	121	117	163	179	A
SR7	211	163	200	96	157	121	117	163	179	K
SS7	211	163	200	96	157	121	117	163	179	K
SL7	249	163	200	96	157	121	117	163	179	L
SR8	231	163	200	96	157	121	117	163	179	A
SL8	231	163	200	96	157	121	117	163	179	A

Discussion

Results show that isolate B1.1 used in the inoculation was recovered from most parts of the inoculated and control plants while no infection was detected in uninoculated plants. Single isolates can grow throughout the plant body without showing symptoms, systemically and endophytically. The microsatellite markers used for the screening of *B. cinerea* isolates show little genetic diversity among the isolates detected from *A. thaliana* plants grown in the controlled environmental room. A total of 42 isolates were detected during the study which represent 12 haplotypes.

This study agrees with the results of Shafia (2009) who found the population of *B. cinerea* isolated from lettuce plants moderately variable and the result is also similar to that of Michailides (2005) who found moderate genetic variation within *B. cinerea* isolates collected from fig plants within the same location in California. The present research shows a complete absence of *B. cinerea* spores in the controlled environmental room which means that spores are either absent or are in too low a density to be frequently detected by plating methods. This shows that inoculation made a significant difference, with inoculated plants having infection while their being none from uninoculated ones.

In the present study it was found that artificial air borne spores used in the inoculation infect the plants, which gradually spread to different part of the plant body without showing symptoms. This shows that artificial inoculation from airborne spores resulted in successful endophytic establishment and systemic

spread of *B. cinerea* in the *A. thaliana* plants grown in the controlled environment room. The results suggest that *B. cinerea* can move from one part of the plant to another implying that it is not localised. Sowley (2006) recovered isolates used for airborne inoculation from both inoculated and uninoculated lettuce plants. He concluded that a successful artificial inoculation at high concentration can eliminate natural infestations and spread within plant populations. Sowley (2006) also showed that isolates from leaves, stems and roots of plants were genetically very similar and spread systemically. Similarly the present research established that in many plants isolates from leaves, stems and roots were genetically similar and spread systemically without showing symptoms.

Infection of *B. cinerea* was first detected in the roots gradually spreading to stems and leaves. This finding agrees with the results of Sowley (2006) who detected infection of *B. cinerea* first in the roots of lettuce which gradually spread to the upper plant parts as the plants grew. This shows that fungal spores can spread rapidly in the soil and infect plant roots. Jennings and Rayners (1983) used glass Petri dishes to investigate the spread of mycelia through sterile and unsterile sand of cord-forming fungi and found that in sterile sand a slow and small growth of fungal mycelium for a few millimetres around the colonised pellet was seen but without cord formation. However, cord formation in non-sterile sand was extensive with a number of cords of diameter greater than 0.25mm produced in each type of experimental arrangement increasing with the number of pellets colonised originally. In the same experiment Jennings and Rayners (1983) found that many cords that form the central colonised pellets grow directly toward those pellets which were initially not colonised. Jennings and Rayners (1983) concluded that the directional growth of cord is an indication that fungus can spread through the soil to potentially colonisable resources. Oren *et al.,* (2003) reported that most root pathogens have a preference in the site of infection in the host body. They reported that the *Fusarium verticilliodes*-maize root interaction prefers the lateral roots as its site of penetration into the host body. The specificity for site of infection is an indication that there is tissue–specific variation in factors that determine susceptibility to *Colletotrichum graminicola* (Oren *et al.,* 2003).

In the present study the detection of inoculating isolates from roots where the upper plant part was removed immediately after inoculation was satisfactory. The inoculating isolate was detected in 62.5% of the plants which showed that soil did not prevent plant infection by the air-borne *B. cinerea* spores. Lagopodi *et al.,* (2002) found that tips of susceptible root species are more resistant than other root tissues to pathogenic infection under diverse conditions, however, the underlying principles for this phenomenon is not clear. Gunawardena and Hawes (2002) reported root tip resistance to infection by pathogenic fungi, where the pathogenic fungi is prevented from forming intimate contact with the root surface, but there is formation of a mantle of germinating hyphae and border cells that detaches to leave the root cap uninvaded.

In their study of association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches,* Mark *et al.,* (2011) found that root cap and border cells of *Pisum sativum* were uninvaded at the early stages of the infection by the pathogenic oomycete *Aphanomyces euteiches.* They found that in response to various challenges by pathogenic infections the border cells change their morphology by producing a significant amount of chemicals with antimicrobial activity such as pisatin, the major isoflavonoid phytoalexin synthesised by pea. However, Wu and Van Etten (2004) observed that in *Pisum sativum* high amounts of pisatin were detected in the root maturation zone showing the presence of mycelium on the surface, but no internal infection of the tissue was recorded and the root border cells and root cap cells from the pathogen at an early stage of infection. However, the root elongation zones do not produce pisatin or do not produce it in enough quantity to protect the root against infection by pathogens such as *A. euteiches* (Wu and Van Etten, 2004). .

In this study, detection of inoculating isolates from different sections of *A. thaliana* plants grown in inoculated soil was successful in some plants. This result agrees with the finding of Serenella *et al.,* (2007) who reported that *Colletotrichum graminicola* can infect roots of maize plants and the root infections result in the colonization and infection of the above ground plant tissues. In the present study isolate B1.2, used to inoculate the plants grown on inoculated soil, was recovered from the roots and

leaves of two plants, the root and stem of one plant and the leaf of one plant. This shows that the recovery of the inoculating isolate in the .*A. thaliana* grown in inoculated soil was only partly successful. The results support the finding of Barnes and Shaw (2003) and Sowley (2006) that soil is not an important source of inoculum for *B. cinerea* infection.

The results of the present research has shown that *A. thaliana* can be dry inoculated with spores of *B. cinerea* and the pathogen transmitted to the different parts of the plant body without showing symptoms. This was shown by the *A. thaliana* plants grown from clean seed in an isolation propagator and dry inoculated at the two leaf stage.

The recovery of the inoculating isolates in roots of plants where the upper plant parts were removed immediately after inoculation showed that *B. cinerea* could be transmitted into the root. This was also evident by the detection of inoculating isolates from different sections of clean *Arabidopsis thaliana* plants grown in inoculated soil in a controlled environment without showing symptoms. This finding confirmed that *B. cinerea* is endophytic and systemic.

Conclusion

This study has provided the answer to the unexpected post-harvest problems where agricultural produce decay occurs without any prior warning. It is clear those losses occur due to the ability of *B. cinerea* to grow systemically without showing symptoms on apparently healthy parts of the plants. Confirmation of the systemic growth of *B. cinerea* will therefore affect disease management strategies which can lead to improved disease control. Otherwise any control of *B. cinerea* designed without considering its ability to grow systemically in healthy plants will end in failure.

ACKNOWLEDGEMENT

Thanks to Mark Fellowes, for comment on earlier version of this paper. Sue Ramsey and Y. Lee for their technical assistance.

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