Journal of
Plant Science & Research

Open Science Publications

Volume 2, Issue 2 - 2015

www.opensciencepublications.com

# Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.)

## Research article

**Yahaya SM* and Fagwalawa LD**

*Department of Biology, Kano University of Sciences and Technology, Wudil -Kano State P.M.B. 3244, Nigeria*

***Corresponding author:** Yahaya SM, Department of Biology, Kano University of Sciences and Technology, Wudil -Kano State P.M.B. 3244, Nigeria; E-mail: sanimyahya@yahoo.com



**Article Information:** **Submission:** 27/10/2015; **Accepted:** 30/11/2015; **Published:** 05/12/2015

**Abstract**

Studying the epidemiology of the wide spread of *necrotrophic* pathogen *Botrytis cinerea* is of economic importance in the production and seed export of lettuce and other horticultural crops. This study showed that seed is the prime source of systemic *B. cinerea* in lettuce plants. The presence of the pathogen in plants grown from fungicide treated seeds suggests that the pathogen is systemic. Infection arises from both internal and external seed infection. This conclusion is based on detection of seed infection from plants which were grown in controlled conditions from infected seed collected from flower inoculated plants. Fragment analysis found that 62.5% of seed isolates detected from plants which were grown from non-fungicide treated seeds was identical to the inoculation isolate. In contrast, all seed isolates detected from plants which were grown from fungicide treated seed have different genotypes to the inoculating isolate. The results showed that with time as the plant grew, the infection from the seed spread to root, stems and leaves systemically without exhibiting symptoms. Thus seed infection spread to other parts of the plants is age dependent. This confirmed the systemic spread of the seed isolate, but showed that other *B. cinerea* isolates were present and that single plants can host multiple isolates. This shows that the *endophytic* phase is an important component of the population dynamics of *B. cinerea*.

**Keywords:** *Botrytis cinerea*; Endophyte; *Lactuca sativa*; Systemic infection

## Introduction

*Botrytis cinerea* an Ascomycetes, family Sclerotiniaceae is as a model necrotic pathogen which grows endophytically without showing symptoms in greenhouse and outdoor crops, resulting in great economic losses [1-3]. Elad et al. reported that *B. cinerea* can enter into the host body and remain dormant or quiescent for a varying periods resulting in a symptomless endophytic infection [4]. However, during quiescence, *B. cinerea* is able to grow endophytically by avoiding the induction of plant defences by not producing toxic compounds or by producing toxic compounds at a level which allows it to continue to grow in the presence of host plant responses without being detected [3,5,6].

Fully endophytic *B. cinerea,* like other endophytic fungi, are only disseminated by seed; being highly and efficiently dispersed by their host seed and so they need not have spores as a means of dissemination [7,8]. With the start of seed production, the endophyte grows upward in the plant and infects the outer layers of the seed; these transfer endophytes from the plant batch to the seed, [9,1,4,6). However, seed infection is only important in circumstances where the pathogen can spread into the plant [9]. Elias et al. reported that highest rate of infection of *B. cinerea* occurred in the roots of plants grown from infected seeds, but as the plants grew the infection moved upwards into newly produced tissues [6]. The general trend is that infections progress from the roots, through the stems to the leaves and finally into the seed without causing symptoms [10,11,6]. Generally, internal infection may depend on which part of the flower is infected thus affecting the likelihood of the pathogen transferring into the seed.

Although endophytes are usually symptomless they can produce


ISSN: 2349-2805

effects ranging from beneficial to pathogenic [12-15]. However, the benefits conferred by these fungi is dependent on host species, host genotype and environmental conditions [16,17]. One of the benefits to the host is the increase in host shoot and/ or root biomass, usually due to the induction of plant hormones by the fungus. Other benefits include endophyte-induced changes in morphology and physiology leading to an increase in vigour, survival and resistance to drought tolerance. The increased growth seen in endophyte infected plants could be due to the production of growth hormones such as indole-3-acetic acid (IAA) and cytokinins or the enhancement of host nutrient uptake ability, especially of phosphorus and nitrogen. Some systemic endophytic fungi are able to produce physiologically active alkaloids in host plant tissues, providing a defence against herbivorous [18,17].

The harmful effects of endophytes in plants include preventing the flowering of their plant host. For example with *Botrytis anthophila* attacking red clover, its spores may replace the pollen grains on the anther of the host plant and giving a characteristic ash-grey colour to the pollen. The spores are transmitted by bees and germinate with pollen grains on the stigmas of healthy plants, usually leading to the development of an intraseminal mycelium from which the systemic infection of the adult plant is derived. The diseases have been found to be widely distributed and have adverse effects on the fertility of red clover [19].

However, the endophytic colonisation of plants by *B. cinerea* lead to, systemic infection of the host [18]. Therefore the endophytic growth of *B. cinerea* has become of great concerned for the food production industry as it results in serious unexpected post-harvest problems due to the decay of the produce without any prior warning. Therefore in order to increase availability and to reduce the cost of agricultural produce it is important to study the systemic endophytic spread of *B. cinerea* which will provide information in designing of control strategies. Thus to understand the epidemiology, we tested three hypotheses. First if seed borne endophytic *B. cinerea*results in the establishment of internal infection in lettuce plants. Second infection from root spread to the upper parts of the plants in lettuce. Third plant age in important in the spread of internal infection.

## Material and Methods

### *Botrytis cinerea* culture

*B. cinerea* isolate, B1.1 (isolated at Reading in 2005) was maintained on *Botrytis* selective medium (BSM) before being transferred to 3% malt extract agar (MEA CM0059, Oxoid, Basingstoke). After the culture covered approximately 1/3 of each plate, they were exposed to continuous UV-light to encourage sporulation. *Botrytis* selective media was used in the detection and isolation of the fungal pathogen from plant tissues.

### Seed stock

The seed used in this experiment was collected from flower inoculated lettuce plants which were initially grown from lettuce seed (Tom Thumb variety, Fothergills Seeds, Newmarket), which were harvested in 2009. The seed was sterilized by soaking in 100ml of (0.1g/l) the systemic fungicide Shirlan (active ingredient 500g/l Fluazinam, Sygenta Crop Protection UK limited) for 2h and drying over night before sowing.

**Figure 1:** Seedlings left for 24h in a black polythene bag

| Plants | Weeks After Inoculations | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Week | 3 Week 6 Week 8Week April/May | | | | | | | | | 1Week | | | 3Week 6Week 8Week June/July | | | | | | | | | | | | |
| R S L | R | S | L | R | S | L | R | S | L | R | S | L | R | S | L | R | S | L | R | S | L | Total | | | |
| -I/-F | 7 | 3 | 0 | 7 | 4 | 3 | 9 | 4 | 5 | 10 | 0 | 0 | 10 | 0 | 0 | 8 | 5 | 0 | 10 | 6 | 6 | 97 | | | |
| -I/+F | 4 | 0 | 0 | 5 | 3 | 0 | 8 | 4 | 2 | 8 | 4 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 31 | | | |
| +I/+F | 5 | 1 | 1 | 6 | 4 | 4 | 6 | 1 | 5 | 1 | 0 | 0 | 3 | 0 | 0 | 5 | 4 | 1 | 5 | 5 | 4 | 61 | | | |
| +I/-F | 4 | 1 | 2 | 5 | 4 | 3 | 6 | 4 | 6 | 8 | 0 | 0 | 10 | 0 | 0 | 10 | 4 | 2 | 10 | 6 | 6 | 91 | | | |
| Total | 20 | 5 | 3 | 23 | 15 | 10 | 29 | 13 | 20 | 19 | 0 | 0 | 23 | 0 | 0 | 25 | 13 | 3 | 27 | 18 | 16 | 280 | | | |

+I/+F = inoculated seedling/ fungicide treatment. +I/-F = non – inoculated seedling/ non-fungicide treatment
-I/-F = non inoculated seedling/ fungicide treated seedling, -I/+ F inoculated seedling/fungicide treated seedling

### Plant growth and inoculation

Infected seed from flower inoculated plants were divided into two treatments one was treated with the fungicide Fluazinam following the same method used to sterilize the initial seed stock. Fungicide and non-fungicide treated seeds were separately grown each in twenty, 15cm pots, each with four seedlings per pot. Pots were arranged in two blocks, each consisting of 20 pots (10 pots each for fungicide and non- fungicide treated seeds). At the two leaf stage, Block 1 was selected and inoculated with fungal spores by gently tapping the back of Petri plates containing a 14-day-old sporulating culture of *B. cinerea* B1.1. The seedlings were left for 10 min to allow spores to settle, then transferred into black polyethylene bags (Figure 1) and stored in the laboratory under ambient conditions (to prevent overheating and condensation) for 24h before removing the polyethylene bag. Seedlings were reared in a controlled environmental room (22 ˚C +/-, 2 ˚C, RH 66).

### Isolation of *Botrytis cinerea* from letce seedlings

The experiment was performed twice (April/May 2011 and June/July, 2011). In each case, one week after inoculation one seedling was collected from each of the 40 pots in the two blocks and divided into one cm-long sections of secondary root, 1 cm diameter leaf discs and hand-cut 1 mm sections of stems. These were sterilized and individually plated in BSM plates. This was repeated at intervals of three, six and eight weeks after inoculation. The growth of *B. cinerea* from the seedling sections after each plating test was counted and recorded.


**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.

### Genetic characterization of *B. cinereaisolate* (microsatellite)

Extract of DNA from sections of roots, stems and leaves isolated eight weeks after inoculation. Mycelium (100mg) was scratched from Petri plates and manually ground in a pestle and mortar in the presence of liquid nitrogen. The resulting fine powder was transferred into a 200 ml effendorf tube and DNA was extracted using the DN easy Plant Mini Kit (Qiagen, West Sussex, UK), stored at -20 ˚C and the concentration of the DNA as adjusted to 10ng/µl using a Nanodrop ND 1000 Spectrophotometer (Applied Biosystems UK). The *B. cinerea* DNA isolates were characterised using nine microsatellite markers designed by Fournier et al. [20] which were labelled with FAM (Blue), HEX (Green) and NED (Yellow). Each reaction mixture contained 25 µl of Biomix (Bioline, UK), 20 µl of water, 1.5 µl of each forward and reverse primer and 2 µl of DNA template. The PCR of the SSR was repeated several times for successful amplification. The products from successful amplifications were multiplexed in three combinations; (BC1, BC4, BC9), (BC3, BC6, BC10), (BC2, BC5, BC7). These groups were chosen to avoid overlapping the allele size ranges of the primers in each mix. The PCR was run with an initial denaturing step of two minutes at 94 ˚C for BC1, BC2, BC3, BC5, BC6 and BC9 followed by 35 cycles of one minute at 94 ˚C, one minute annealing at 53 ˚C, 30 seconds at 72 ˚C and 5 minutes at 72 ˚C. While BC4, BC7 and BC10 an annealing temperature of 59 ˚C was used. The products were submitted for fragment analysis by Source Bioscience, UK. The results of the fragment analysis were scored by using Gene mapper software (Applied Biosystem).

### Experimental design

Factorial block design was used with three main factors (a) seed fungicide treatment (b) seedling inoculation and (c) time. One block of seedlings was dry inoculated two weeks after sowing with fungal isolate B1.1. Although the density of the spore deposition was not measured, the technique was similar to the one used for the initial commercially purchased lettuce seeds in 2009. One week after inoculation one seedling was collected from each of the 40 pots, washed under running tap water and sterilized by spraying with 70% ethanol and then allowed to air dry in a sterilized lamina flow. Seedlings were divided into roots, stems and leaves and plated in *Botrytis* selective media (BSM) at 18 ˚C for one week. The remaining seedlings were sampled and plated at interval of three, six and eight weeks after inoculation. At eight week after inoculation, the 61 colonies isolated from roots, stems and leaves were subcultured and fragment analysis was performed following the previously described protocols.

**Table 1:** Incidence of *B. cinerea* in the root (R), stem (S) and leaves (L) of lettuce at eight weeks after inoculation, N = 10 per treatment

| Fungicide treatment | Plant part | | | |
|---|---|---|---|---|
| | Root | Stem | Leaves | Total |
| Treated | 52 | 23 | 17 | 92 |
| Untreated | 120 | 41 | 33 | 194 |

**Table 2:** Effect of seed fungicide treatment on the spread of endophytic *B. cinerea* in the root (R), stem (S) and leaves (L) of lettuce grown June/July at eight weeks after inoculation.

| Inoculation | Fungicide treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | | | Untreated | | | |
| | R | S | L | Total | R | S | L | Total |
| Inoculated | 31 | 15 | 15 | 61 | 59 | 19 | 19 | 97 |
| Uninoculated | 21 | 8 | 2 | 31 | 61 | 22 | 14 | 97 |

**Table 3:** Effect of seed fungicide treatment and seedling inoculation on the incidence of internal *B. cinerea in* lettuce grown June/July at eight weeks after inoculation.

| Inoculation | Plant Part | | | |
|---|---|---|---|---|
| | Root | Stem | Leaves | Total |
| Inoculation | 90 | 34 | 34 | 158 |
| Uninoculated | 82 | 30 | 16 | 16 |

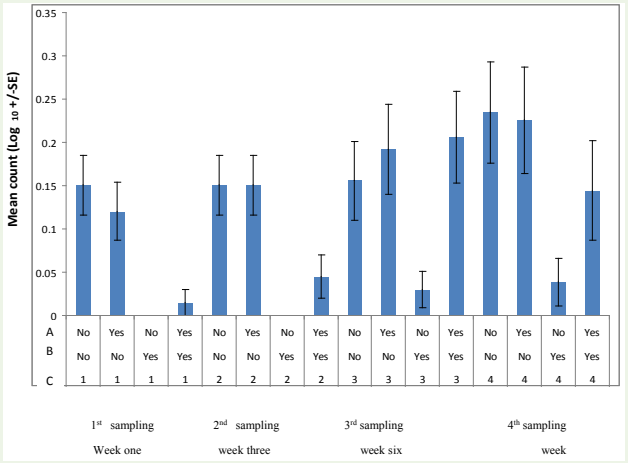


**Figure 2:** Effect of seed fungicide treatment, seedling inoculation and time on the spread of internal infection in lettuce plant. A -fungicide seed treatment B -seedling inoculation C- time (in weeks after inoculation June/ July)

### Statistical analysis

*B. cinerea* colony counted at the end of the experiment (eight week after inoculation was compared between the four different experimental treatments using a generalised linear model approach (GLM) in Mini tab software.

## Results

### Systemic and endophytic infection of *B. cinerea*

*B. cinerea* was recovered from the surface-sterilized sections of healthy leaves, stems and roots for all treatments in April/May 2011 and June/July 2011, with the exception of a single sample plated one week after inoculation in April/May 2011, where all samples became


**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.

**Table 4:** Effect of Inoculation on the spread of *B. cinerea* in the root (R), stem (S) and leaves (L) of lettuce grown June/July at eight weeks after inoculation.

| Inoculation | Plant Part | | | |
|---|---|---|---|---|
| | Root | Stem | Leaves | Total |
| Inoculation | 90 | 34 | 34 | 158 |
| Uninoculated | 82 | 30 | 16 | 128 |

contaminated (Table 1). Although growth of *B. cinerea* was recorded from the second, third and fourth sampling of April/May 2011, the difference between the treatments was very close to significant (P= 0.06). In June/July 2011 experiments, infection was initially most common in the roots with clean stems and leaves. However, as the plants grew the infection spread into stems and then into leaves. In the last plating test eight weeks after inoculation colony count from the roots stems and leaves was relatively one. (Table 1, Figure 2, time on the spread of infection $P<0.002$).

### Seed fungicide treatment

Fungicide treatment of the seed before sowing cleared most of the seed infections and greatly reduced the incidence of infections in all the plant tissues plated at first and second plating test. At three weeks after inoculation (Table 1, Figure 2) plants grown from fungicide treated seed in June/July had no stem and leaf infection but moderate levels in their roots; the stems and leaves were significantly different from the ones grown from the untreated seed ($F_{1,319} = 32.65$, $P = 0.001$, Table 2).

At the fourth plating test, eight weeks after inoculation leaves had less infection but the roots and stems were not affected by the seed fungicide treatment. The effect of seed fungicide treatment interacted significantly with the effect of seedling inoculation ($F_{1,319} = 4.76$, $P= 0.030$, Table 3).

However, there was no significant interaction between seed fungicide treatment, seedling inoculation and time on the spread of infection ($F_{3, 19} = 0.38$, $P= 0.771$).

### Seedling inoculation

Seedling inoculation in both the April/May and June/July experiments did not affect infection levels at the 1st and 3rd weeks after inoculation, but altered infection frequency at 6th and 8th weeks after inoculation. However, there was no significant interaction between seedling inoculation and time on the spread of internal infection (Table 4, $F_{3,319} = 1.50$, $P= 0.215$).

### Effect of time on the spread of internal infection

The initial seed source was infected seed. All the plants which were grown without seed fungicide treatment initially had a high rate of root infection, but stems and leaves were free of infection in tests conducted after inoculation (Table 4). However, as the plant grew the infection moved into stems and then leaves. There was a significant interaction between time and location of infection (Table 4, $F_{3,319} = 4.92$, $P= 0.002$).

### Genotypic characterization of the isolates recovered

Isolates recovered from the roots, stems, and leaves of experimental plating in the June/July experiment eight weeks after inoculation were genotyped at nine microsatellite loci. The isolate detected from the initial seed used to grow the plants (haplotype coded A), was recovered from 62.5% of the plants which were not inoculated at seedling stage and in some cases the isolate was recovered throughout each plant. All isolates recovered from inoculated plants had a different genotypic identity from the isolate recovered from the source seed.

## Discussion

The study establishes that seed is the source of systemic *B. cinerea* infection in lettuce. Infection is initially confined to the root in plants grown from infected seed, and as the plant grows infection moves into developing tissues. In April/May, 2011, when the plants were grown from fungicide and non-fungicide treated seed, although no colony count was recorded at one week after inoculation due to the contamination of the samples; subsequent plating tests show that roots carried more infection of *B. cinerea.* When the experiment was repeated in June/July 2011, the distribution of *B. cinerea* in the parts of lettuce plant was somewhat different (Table 1). From the plating test at one and three weeks after inoculation the infection was confined to the roots while the stems, and leaves were infection free in plant grown from non-fungicide treated seeds. However, the plating test at one and three weeks after inoculation shows that roots, stems and leaves were clean in plants grown from fungicide treated seed. At six weeks after inoculation both treatments show that roots have the highest infection rate, but there was also a movement of infection from the roots to the upper parts of the plant. The plating test at eight weeks after inoculation shows a balance of infection between the plant tissues. In a related experiment, Elias et al. [6] found that healthy looking lettuce plants without any visible disease symptoms may harbour live *B. cinerea* in their roots, stems and leaves. Barnes and Shaw [9] found that *Primula* x *Polyantha* plants grown from infected commercial seeds had a high level of root infection. Since the plants were grown in isolation propagator, they concluded that the infection in the roots was likely to have spread into the plant.

In this study it is established that as plants grow, *B. cinerea* infection moves to newly produced tissues. It is possible that *B. cinerea* goes into a quiescent phase before spreading to the stems and leaves Barnes and Shaw [9]. Seed fungicide treatment was effective in removing surface contamination. The presence of the pathogen in plants grown from fungicide treated seeds suggests that the pathogen is systemic. Infection arises from both internal and external seed infection (Table 3). Sowley found that seed fungicide treatments significantly lower seedling infection rate therefore most of the infection comes from the seed coat [10]. Surface sterilization or seed washing confirmed that infection was largely due to surface contamination [21,17]. Kabeere et al. reported that infection of maize seeds by *Fusarium subglutinans* was reduced from 96% to 8% after surface sterilization [22]. They showed that the majority of seed inoculum was on the seed surface because there was a substantial reduction in recovery of *F. subglutinans* when seeds were surface sterilized.

Seedlings grown from non fungicide treated seeds tended to have a higher spread of fungal infection as shown in Table 3. In both April/May 2011 and June/July2011, there was a general increase in


**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.

**Table 5:** Allele sizes of isolates screen against nine microsatellite markers

| Seed | BC1 | BC2 | BC3 | BC4 | BC5 | BC6 | BC7 | BC9 | BC10 | HHAPL |
|---|---|---|---|---|---|---|---|---|---|---|
| VSeed | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | A A |
| -N/-F1R | 160 | 160 | 165 | 96 | 164 | 90 | 85 | 150 | 180 | BB |
| -N/-F2R | 160 | 160 | 165 | 96 | 164 | 90 | 85 | 150 | 180 | BB |
| -N-F3R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F4R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F5R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F6R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F7R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F8R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F9R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F10R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | A |
| --N/-F1S | 1160 | 1160 | 1164 | 996 | 1164 | 960 | 885 | 1150 | 1 180 | AA |
| - -N/-F2S | 1160 | 1160 | 1164 | 996 | 1164 | 960 | 885 | 1150 | 1180 | AA |
| --N/-F3S | 1160 | 1162 | 1164 | 996 | 1158 | 1121 | 885 | 1150 | 1180 | DD |
| --N/-F4S | 1160 | 1162 | 1164 | 996 | 1164 | 990 | 885 | 1150 | 1180 | AA |
| --N/-F5S | 1160 | 1160 | 1164 | 996 | 1164 | 990 | 885 | 1150 | 1180 | AA |
| --N/-F6S | 1160 | 1160 | NNA | 996 | 1164 | 990 | 885 | 1150 | 1180 | EE |
| --N/-F1L | 1160 | 1160 | 1164 | 996 | 1164 | 990 | 885 | 1150 | 1180 | AA |
| --N/-F2L | 1160 | 1162 | 1164 | 996 | 1163 | 1121 | 1117 | 1150 | 1180 | DD |
| --N/-F3L | 1160 | 1162 | 1164 | 996 | 1164 | 1121 | 1117 | 1150 | 1180 | DD |
| --N/-F4L | 1160 | 1160 | 1164 | 996 | 1164 | 990 | 885 | 1150 | 1180 | DD |
| -N/-F5L | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/-F6L | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/+F1S | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| -N/+F1R | 160 | 160 | 164 | 96 | 164 | 90 | 100 | 150 | 180 | FF |
| -N/+F2R | 160 | 160 | 164 | 96 | 164 | 90 | 85 | 150 | 180 | AA |
| +N/-F1R | 160 | 160 | 156 | 93 | 164 | 94 | 85 | 150 | 180 | HH |
| +N/-F2R | 160 | 160 | 164 | 96 | 164 | 160 | 85 | 150 | 180 | II |
| +N/-F3R | 160 | 160 | 164 | 103 | 164 | 90 | 85 | 150 | 179 | JJ |
| +N/-F4R | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| +N/-F5R | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| +N/-F6R | 160 | 160 | 164 | 96 | 156 | 96 | 85 | 150 | 163 | L |
| -+N/-F7R | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1 163 | GG |
| +N/-F8R | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | GG |



**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **-+N/-F9R** | 1160 | 1160 | 1164 | 996 | 1158 | 196 | 885 | 1150 | 1163 | **G**G |
| **-+N/-F10R** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/-F1S** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N-F2S** | 1160 | 1160 | N164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/-F3S** | 1160 | 1158 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1164 | **M**M |
| **-+N/-F4S** | 1160 | 1160 | 1164 | 996 | 1163 | 196 | 185 | 1150 | 1163 | **G**G |
| **+N/-F5S** | 1160 | 1160 | 1164 | 996 | 1164 | 196 | 185 | 1150 | 1163 | **G**G |
| **-+N/-F6S** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **+N/-F1L** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | **G**G |
| **+N/-F2L** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| **+N/-F3L** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| **+N/-F4L** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| **+N/-F5L** | 160 | 160 | 164 | 96 | 156 | 96 | 85 | 150 | 163 | NN |
| **N/-F5R** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | GG |
| **+N/-F6L** | 160 | 160 | 164 | 96 | 164 | 94 | 100 | 150 | 163 | OO |
| **+N/+F1R** | 160 | 160 | 164 | 97 | 164 | 96 | 85 | 150 | 164 | GG |
| **+N/+F2R** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | PP |
| **+N/+F3R** | 160 | 160 | NA | 96 | 164 | 96 | 85 | 150 | 160 | GG |
| **+N/+F4R** | 160 | 160 | 164 | 96 | 164 | 96 | 85 | 150 | 163 | G |
| **+N/+F5R** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1 163 | **G**G |
| **+N/+F1S** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/+F2S** | 1160 | 1160 | 1164 | 996 | 1158 | 196 | 885 | 1150 | 1163 | **G**G |
| **-+N/+F3S** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/+F4S** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/+F5S** | 1160 | 1160 | N163 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N/+1L** | 1160 | 1160 | 1164 | 996 | 1164 | 996 | 885 | 1150 | 1163 | **G**G |
| **-+N+F2L** | 1160 | 1160 | 1164 | 996 | 1164 | 196 | 185 | 1150 | 1163 | **G**G |
| **-+N/+F3L** | 1160 | 1160 | 1164 | 996 | 1164 | 196 | 185 | 1150 | 1163 | **G**G |
| **-+N/+F4L** | 1160 | 1160 | 1164 | 996 | 1164 | 990 | 885 | 1150 | 1163 | **G**G |

Alphabet A-G represents the code for the haplotypes. –I = uninoculated plant, -F= non- fungicide treated seed, +I= inoculated plant, +F= fungicide treated seed, R= root, S=stem, L=leaves. Same number in plant columnindicate isolates collected from the same plant.

the spread of infection from one week after inoculation to eight weeks after inoculation as shown in Table 1, in both fungicide and non fungicide treated seedlings. The general increase in infection could be due to *B. cinerea* spreading within plant tissues without causing symptoms. There are two alternative hypotheses for the pattern of infection described. First, each part of the plant could be independently infected. Second, the pattern in the result of systemic infection resulting from the initial source infection. These data support the later hypothesis, given the timing of expression of infection and the results from the fragment analysis.

Stewart and Franicevic [23] investigation of the spread of internal infection by *Botrytis* species, revealed transmission of *B. allii* from infected onion seed into the seedling tissue. However, the percentage of seedling infection was less than that of seed infection which suggests that not all seed infection results in seedling infection. Also no differences were found in the amount of *B. allii* infection between plants grown from water-washed and fungicide treated seeds. This suggest that *B. allii* is present within the seed rather than on the seed surface. The ability of endophytic *B. cinerea* to be disseminated by seed makes it the most widely distributed disease of vegetables, ornamentals, fruits, and field crops throughout the world [3].

The study show that recovery of infection in plants grown from


**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.

*B. cinerea* infected seed varies with time the plant age. Azevedo and Welty (1995) found that the recovery of *Acremonium coenophiilum* from infected plants varies with seedling age, root type and medium. Similarly, Kelly and Wallin found that *Aspergillus flavus* is confined to the roots of maize at the 3-leaf stage while by the 4-leaf stage it is uniformly distributed in the root, stem and leaves [24]. *Aspergillus flavus* became confined to the root and lower portions of the stem at the 5-leaf stage. In *Primular x polyantha* grown from infected commercial seeds, Barnes and Shaw [9] found the highest concentration of *B. cinerea* infection in the root which later spread to other parts of the plant endophytically with symptoms appearing three months later at flowering. *Botrytis fabae* was isolated from all parts of symptomless field bean seedlings grown from infected seeds. However, whether plants were likely to show infection was found to be temperature dependent [25].

The SSR results shows that isolates recovered from roots, stems and leaves of 62.5% of uninoculated plants was similar to the genotype of *B. cinerea* from the initial seed source. Out of that 6.4% of uninoculated plants have isolates in their roots, stems and leaves similar to the *B. cinerea* isolated from the source seed. Therefore, Isolates from the inoculated plants have different genotypic identities from that of the seed source (Table 5).

To conclude, this study has established that seed is the source of infection by *B. cinerea* in lettuce seedlings and as the plant grows infection spreads to newly produced tissues leading to a long-lived systemic endophytic infection in all parts. The finding here are similar to the results of Sowley [10] and; Shafia [11] that susceptibility of lettuce plant to infection increases with aging, the incidence of infection on matured leaves was higher than of younger and healthy leaves. Previous works have suggested that *B. cinerea* is root specific but this study suggested that while *B. cinerea* may be initially confined to the roots it may eventually spread to other parts of the plant.It is therefore recommended that a good quarantine system should be put in place to check all commercial seed stocks for planting and especially those earmarked for export and import in order to reduce the spread of systemic *B. cinerea* from one country to another.

## Acknowledgement

Thanks to Mark, Sue, Lee, Val Jasper, Jang, Anushka and Matthew for their support and assistance.

## References

1. Elad Y, Williamson B, Tudzynski P, Delen N (2004).*Botrytis* spp. and diseases they cause in Agricultural systems-An introduction. In Y.Elad (eds*). Botrytis*: Biology, Pathology and Control. 1-6 Springer, Dordrecht. The Netherland.
2. Tejesvi M, Kini KR, Prakash HS, Shatty HS (2007) Genetic diversity and antifungal activity of species of pestalotiopsis isolated as endophytes from medicinal plants. Fungal Diversity 31: 37-54.
3. Williamson B, Tudzynski B, Tudzynski P, Van Kan JA (2007) *Botrytis cinerea:* the cause of grey mould diseases. Mol Plant Pathol 8: 561-580.
4. Elad Y, Zimand G, Zaqs Y, Zuriel S, Chet I (2007) Use of *Trichoderma harzianum* in combination or alteration with fungicides to control cucumber grey mould (*Botrytis cinerea*) under commercial conditions. Plant pathology 42: 324-332.
5. Oses R, Valenzuela S, Freer J, Sanfuetes E, Rodriquez J (2008) Fungal endophytes in xylemof healthy Chilean trees and their possible role in early wood. Fungal Diversity 33: 77-86.
6. Elias SNK, Fiona MD, Shaw MW (2010) Persistent, Symptomless, systemic and seed-borne infection of lettuce by *Botrytis cinerea*. Eur J Plant Pathol 126: 61-71.
7. Saikkonen K, Ion D, Gyllenberg M (2002) The persistence of vertically transmitted fungi ingrass metapopulations. Proc Biol Sci 269: 1394-1403.
8. Stone JK, Polishook JD, White JF (2004) Endophytic fungi. Biodiversity of fungi:inventoryand monitoring methods, Burlington, MA, USA: Elsevier, 241-270.
9. Barnes SE, Shaw MW (2003) Infection of commercial hybrid seed by *Botrytis cinerea* and latent diseases spread through the plants. Phytopathology 93: 573-577.
10. Sowley ENK (2006) Epidemiology of *Botrytis cinerea*. PhD: University of Reading, UK.
11. Shafia A (2009) Latent infection of *Botrytis cinerea*. PhD thesis, School of Biological Sciences, University of Reading, UK.
12. Bacon CW, White LF jr. (2000) Microbial endophytes Markel Dekker inc., New York, Basel USA: 487.
13. Arnold AE, Maynard Z, Gilbert GS, Kursar TA (2000) Are tropical fungal endophytes hyperdiverse? Ecol Lett 3: 267-274.
14. Stone JK, Viret O, Petrini O, Chapela I (2000) Histological studies of host penetrationand colonization by endophytic fungi. In host wall Alteration by parasitic fungi. 115-128, American Phytopathological Society Press, St Paul, MN.
15. Photita W, Lumyong P, Hyde KD (2001). Endophytic fungi of wild banana (*Musa acuminate*) at Doi suthep pui National park, Thailand. Mycological Research 105: 1506-1514.
16. Faeth SH, Sullivan TJ (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. Am Nat 161: 310-325.
17. Faeth SH, GardnerDR, Hayes CJ, Jani A, Wittlinger SK, et al. (2006) Temporal and spiral variation in alkaloid levels in *Achnatherum robustrum*, a native grass infected with the endophyte *Neotyphodium.* J Chem Ecol 32: 307-324.
18. Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. National product report 18: 448-459.
19. Silow RA (2009) A systemic diseases of red clover caused by *Botrytis anthorphila* Bond. Transaction of British Mycological Society 18: 239-248.
20. Fournier E, Giraud A, Loiseau D, Vantrin A, Estoup M, et al. (2002) Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). Molecular Biology Ecology Notes 2: 253-255.
21. Burgess DR, Bretag TW, Keane PJ (1997) Seed to seedling transmission of *Botrytis cinerea* chickpea and disinfestation of seed with moist heat. Aust J Experimental Agric 37: 223-229.
22. Kabeere F, Hill MJ, Hampton JG (1997) The transmission of *Fusarium subglutinans* from maize seeds to seedlings. Aust Plant pathol 26: 126-130.
23. Stewart A, Franicevic SC (1994) Infected seed as a source of inoculum for *Botrytis cinerea* infection of onion bulb in store. Aust Plant Pathol 23: 36-41.
24. Kelly SM, Willin JR (1986) Systemic infection of maize plants by *Aspergillus flavus.* Aflatoxins in maize. El Batan. Mexico, CIMMYT, Mexico, DF Mexico.
25. Harrison JG (1978) Role of Seed-borne infection in epidemiology of *Botrytis fabae* on field beans. Transactions of the British Mycological Society 70: 35-40.



**Citation:** SM Y, LD F. Seed the Source of Systemic *Botrytis cinerea* in Lettuce (*Lactuca sativa* L.). J Plant Sci Res. 2015;2(2): 141.