Occurrence and diversity of endophytic colonization of *Taraxacum officinale* by *Botrytis* species: A preliminary study

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Abstract

The genus *Botrytis* (sexual form *Botryotinia* Whetzel) contains renowned plant pathogenic species with a necrotrophic lifestyle in which kill and feed on its hosts. Recent reports of symptomless endophytic colonization by *Botrytis cinerea* in cultivated plants of lettuce (*Lactuca sativa*) and *Botrytis* spp. in the wild plants dandelion (*Taraxacum agg*) and spotted knapweed (*Centaura stoebe*) reveal a different “side” of *Botrytis* spp. These findings, expose additional strategies of *Botrytis* species to disseminate and grow in wild plants and also highlight the possibility that there exist (novel?) *Botrytis* species lacking virulence factors or with novel adaptations which could include some benefits to its hosts. In this work, the occurrence of endophytic colonization by *Botrytis* spp. in dandelion plants was investigated. The results, confirmed that symptomless dandelion plants were infected with *Botrytis* spp.. From these plants, 23 Botrytis isolates were obtained. By sequencing based on the genes *HSP60* (Heat-shock Protein 60 ), *G3PDH* (Glyceraldehyde-3-Phosphate Dehydrogenase) and *RPB2* (DNA-dependent RNA polymerase subunit II), 6 different genotypes were identified 3 of them showed the strongest phylogenetic association with *B. cinerea* whereas the other 3 were associated with *B. pseudocinerea*. Importantly, a pathogenicity test showed the capability of these isolates to cause disease lesions in tomato and *Nicotiana benthamiana*. In this report, the implications of the ecology of *Botrytis* species are discussed.
The genus *Botrytis* (sexual form *Botryotinia* Whetzel) contains renowned plant pathogenic species with worldwide distribution which includes both host generalists and specialists. *Botrytis cinerea*, a generalist species, affects more than 200 eudicots whereas the others are specialized species which affect a single or closely related hosts (Staats et al., 2005). *Botrytis* species are important pathogens in fruits, vegetables, bulbous monocotyledons (members of the families Liliaceae, Amaryllidaceae, and Iridaceae) and green house crops (Jarvis, 1977).

Commonly, *Botrytis* species are known as pathogens with a necrotrophic lifestyle which kill and then feed on the dead cells of plants using in this process a variety of toxic molecules and lytic enzymes (van Kan et al., 2014). For example, in *Botrytis cinerea* host penetration is attributed more to the secretion of H$_2$O$_2$ and the degrading enzymes such as lipases, cutinases and oxidases rather than the physical pressure by the pathogen (van Kan et al., 2014). Then, for successful infection, cell dead is induced using toxins that are effective against a large spectrum of plants in the case of *Botrytis cinerea* (Choquer et al., 2007). In this species, the toxins botrydial and botcinic acid have been identified as virulence factors (Choquer et al., 2007). In addition, secretion of H$_2$O$_2$ by both, the pathogen and the host (hypersensitive response), in response to the pathogen attack, contribute to the pathogen growth (van Kan, 2006; Choquer et al., 2007).

Recent reports of symptomless endophytic colonization by *Botrytis cinerea* in cultivated plants of lettuce (*Lactuca sativa*) (Sowley et al., 2010) and *Botrytis* spp. in wild plants such as dandelion (*Taraxacum agg*) (Shafia, 2009) and spotted knapweed (*Centaura stoebe*) (Shipunov et al, 2008) provide evidence of the flexibility of *Botrytis* species to establish unnoticeable associations with plants. Although, this finding exposes the additional strategies of pathogenic *Botrytis* species to disseminate and grow in wild plants also highlights the possibility that there exist (novel?) *Botrytis* species lacking virulence factors or with novel adaptations which could include some benefits to its hosts.

In this work, the occurrence of endophytic colonization by *Botrytis* spp. in dandelion plants was investigated and we tried to answer the following research questions:

- How many different *Botrytis* genotypes are detected?
- To which species do these fungi belong?
- Are they novel species or isolates of a known species?
- Are these isolates capable of causing disease?
The plants from which the isolates are sampled are not diseased, but are these isolates capable of causing disease?

2. Materials and methods

2.1 Plant sampling
Dandelion plants were sampled from 4 sites in Wageningen (The Netherlands) (Figure 1, Appendix 1) in May 2014. Sampling sites differed in soil composition and the surrounding vegetation. From each site 22-25 symptomless plants were collected and in total 97 plants were sampled.

Figure 1. Sampling sites of *Taraxacum officinale* for *Botrytis* isolation in Wageningen

2.2 Botrytis isolation
*Botrytis* spp. were isolated from stems, leaves and flowers tissues after surface sterilization. Plant tissues were treated 30 s in a bleach solution (2%), 30 s in an ethanol solution (48%) and finally rinsed with water; 4 to 5 pieces from leaves, stems and flowers were plated separately in selective media for *Botrytis* (SBM, supplemented basal medium) (Kerssies, 1990). The media consisted of the following components (g/L distilled water): NaNO₃, 1.0; K₂HPO₄, 1.2; MgSO₄.7H₂O, 0.2; KCl, 0.15; glucose, 20.0 and agar, 25.0. The medium was sterilized, cooled
to 65 °C and supplemented with the following ingredients (g/1 distilled water): terrachlor (PCNB, pentachlorobenzene 75%WP), 15x10^{-3}; tetracycline, 2x10^{-2}; chloramphenicol (antibiotic), 5 x 10^{-2}; CuSO4, 1.0; tannic acid, 5.0. The pH of the supplemented basal medium (SBM) was adjusted to 4.5 with 5.0 N NaOH.

A total of 291 SBM plates were employed for isolation of *Botrytis* sp. from the different plant tissues. Fungal cultures appeared after 7-10 days of plating and they were screened for the production of dark pigments in the medium due to the degradation of tannic acid, a characteristic property of *Botrytis* spp. Cultures were purified by transferring hyphae from the primary culture with minimal agar onto a fresh sterile plate with the selective media (SBM). Pure cultures were grown on MEA (Malt Extract Agar, DIFCO) plates over a cellophane membrane (1 cm²) which facilitated the sampling of mycelium without agar.

An immunoassay test, using a kit QuickStix strips for *Botrytis* (Envirologix, catalog Number AS 049 GP 25) was employed to identify *Botrytis* colonies among the cultures. For the test, a small sample of mycelium (around 0.5 mm²) was immersed in a eppendorf tube containing 300 µl of a buffer provided with the kit. The mycelium was mixed with the buffer by vortex and then the QuickStix strip for *Botrytis* was placed into the prepared suspension. Positive detection for *Botrytis* sp. was indicated by strips displaying two blue lines (control line and test line).

### 2.3 Species identification

#### 2.3.1 DNA extraction and sequencing

Mycelial tissue (10-20 mg) from the pure cultures grown over the cellophane membrane was harvested, lyophilized and finely ground to extract DNA, using a modified protocol of Puregene DNA Purification Kit (Gentra Systems Inc./Biozym systems, Landgraaf, The Netherlands). The ground mycelium was placed in a 1.5 ml eppendorf tube (kept in ice) containing 600 µl of cell lysis solution and 3 µl of proteinase K solution (20mg/ml) and mixed by vortexing. Then, the cell lysate was incubated at 55°C for 1.5 h and vortexed periodically every 20 min. Tubes containing the cell lysate were cooled to room temperature and then 200µl of a protein precipitation solution were added and mixed thoroughly. Tubes were incubated on ice for 10-15 min and then centrifuged at 16000 x g for 3 min. 500µl of the supernatant were poured into a new 1.5 ml eppendorf tube containing 500µl of 100% isopropanol. The solution was mixed gently by inverting the tube. The mixture was centrifuged at 16000 x g for 1 min to obtain a DNA pellet, which was then, washed twice
using 600 µl of 70% ethanol. The ethanol was discarded after centrifugation at 16000 x g for 1 min. The DNA was dried in an oven at 37°C around 1 min and then rehydrated and dissolved in 60µl of sterile ultrapure water.

Amplification of regions of the three nuclear genes HSP60 (Heat-shock Protein 60 ), G3PDH (Glyceraldehyde-3-Phosphate Dehydrogenase) and RPB2 (DNA-dependent RNA polymerase subunit II) described by Staats et al. (2005) was done using the primers described in the Table 1.
Table 1. Primers employed for PCR amplification of regions of the genes *HSP60*, *G3PDH* and *RPB2* in *Botrytis* spp.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Target region</th>
<th>Primer Sequence (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3PDHfor+</td>
<td>G3PDH</td>
<td>GTTTTCCCAGTCACGACATTGACACATCGTCGCTGT</td>
</tr>
<tr>
<td>G3PDHrev+</td>
<td></td>
<td>CAGGAAACAGTCATGACACCCACATCGTTGTCG</td>
</tr>
<tr>
<td>HSP60for+</td>
<td>HSP60</td>
<td>GTTTCCCCAGTCACGACCAACAATTGAGATTTCACCCAAG</td>
</tr>
<tr>
<td>HSP60rev+</td>
<td></td>
<td>CAGGAAACAGCTATGACGTGATGTTGACACGTT</td>
</tr>
<tr>
<td>RPB2for+</td>
<td>RPB2</td>
<td>GTTTCCCAGTCAGGACGTGATCGTGATCATTT</td>
</tr>
<tr>
<td>RPB2rev+</td>
<td></td>
<td>CAGGAAACAGTCATGACCACATAGCTTACC</td>
</tr>
</tbody>
</table>

PCR amplification was carried out in a 25 µl reaction mixture containing 30-50 ng of DNA, 5X Colorless GoTaq Reaction Buffer (Promega, The Netherlands), 200µM each deoxynucleoside triphosphate (Promega, The Netherlands), 0.2 pmol of each primer (Amersham Pharmacia Biotech), and 1.0 U of GoTaq G2 DNA polymerase (Promega, The Netherlands). PCR conditions for *HSP60* and *RPB2* gene fragment amplification were as follows: 94°C for 5 min (1 cycle); 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s (35 cycles), and then 72°C for 10 min (1 cycle). The same program with an annealing temperature of 64°C was used for *G3PDH* gene. The purified amplified fragments were sequenced by Macrogen Inc. (The Netherlands).

### 2.3.2 Phylogenetic analysis

From the collection of isolates obtained, initially 12 were sequenced based on *HSP60* gene. The sequence data obtained were employed to perform multiple alignments and sequence comparisons across isolates to select a set representing probably different genotypes or species. Then, the selected isolates were sequenced based on *G3PDH* and *RPB2* genes.

Sequence data of fragments of the genes *HSP60*, *G3PDH* and *RPB2* were analysed, assembled and consensus sequences were obtained using BioEdit software (Biological sequence alignment editor). Multiple sequences alignments and comparisons across isolates were performed by using Clustalw2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

*HSP60*, *G3PDH* and *RPB2* sequences of 26 *Botrytis* species were downloaded from TreeBASE (http://treebase.org/treebase-...
and phylogenetic trees based on \textit{HSP60}, \textit{RPB2}, \textit{G3PDH} and all three genes were reconstructed by Maximum-likelihood with MEGA 6 (Tamura et al., 2013). \textit{Sclerotinia sclerotiorum} and/or \textit{Monilinia fructigena} were included in the phylogenetic analysis as out groups.

2.4 Pathogenicity assay
Pathogenicity tests with five of the \textit{Botrytis} sp. isolates (V18F, V17S, B1LA, A7LA, B18F, A25SB) were carried out on detached leaves of tomato (\textit{Solanum lycopersicum}, cv. Moneymaker) and \textit{Nicotiana benthamiana} plants as described by Zhang and van Kan (2012).

In order to test each selected isolate, two composite tomato leaves and two \textit{N. benthamiana} plants 5-week old were employed. Leaves were cut from tomato plants and stacked in horizontal position with their backsides down in strips of soak oasis placed in petri dishes containing a layer of water. The detached leaves were maintained in plastic trays with a lid to create a humid chamber. Similar moisture conditions were provided for \textit{N. benthamiana} plants in pots.

For leaf inoculation, sporulating \textit{Botrytis} sp. isolates grown for 8-10 days on malt extract agar plates at 18-20°C were flooded with 20 ml of sterile distilled water; the spores were gently removed using a Drigalski spatula and filtered through a glass fibre to eliminate fungi debris. The spore suspension obtained was adjusted to \(10^6\) spores /ml using a haemocytometer and a diluted (1:2) potato dextrose solution. Three 2-µl drops of the spore suspension were inoculated on the front side of tomato leaflets and \textit{N. benthamiana} leaves.

One of the tomato detached leaves and one of the \textit{N. benthamiana} plants was inoculated with \textit{Botrytis cinerea} strain B05.10 (control) and with one of the testing \textit{Botrytis} isolates. Each of these was inoculated on one-half of a leaflet/leaf. The other detached tomato leaf and \textit{N. benthamiana} plant was inoculated with the testing isolate alone.

Infected developing lesions were monitored after 24 h and expanding lesions were measured (mm) using a calliper after 48 h.

3. Results

3.1 \textit{Botrytis} isolates
Colonization in dandelion plants (\textit{Taraxacum officinale}) by \textit{Botrytis} sp. isolates was detected in 21\% of the plants sampled (N=97) based on the visual observation of dark pigments in the selective medium (an indication of tannic acid degradation, a property of \textit{Botrytis} spp.) and
the immunoassay test. From the 291 SBM plates containing the plant tissues sampled in the 4 sites for isolation, 28 fungal cultures were identified as *Botrytis* by the immunoassay test (Table 2). The percentage of isolates obtained from flowers, stems and leaves were 48%, 39% and 13% respectively (Figure 2). 5 fungal cultures identified as *Botrytis* by the immunoassay were not considered (Table 2, Figure 3). In these isolates, formation of sclerotia and conidiphores was absent, they presented slow growth and their morphology was quite distinct to the majority of isolates obtained and identified as *Botrytis*.

Table 2. *Botrytis* cultures identified by immunoassay test (kit QuickStix strips for *Botrytis* spp.) isolated from symptomless dandelion plants (*Taraxacum officinale*).

<table>
<thead>
<tr>
<th>#</th>
<th>Isolate name</th>
<th>Isolate name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B1LA</td>
<td>A14L</td>
</tr>
<tr>
<td>2</td>
<td>B1FA</td>
<td>A25SA</td>
</tr>
<tr>
<td>3</td>
<td>B13F</td>
<td>A25SB</td>
</tr>
<tr>
<td>4</td>
<td>B14FA</td>
<td>A12F</td>
</tr>
<tr>
<td>5</td>
<td>B18F</td>
<td>A24FA</td>
</tr>
<tr>
<td>6</td>
<td>B21F</td>
<td>H17SA</td>
</tr>
<tr>
<td>7</td>
<td>B24F</td>
<td>H23SA</td>
</tr>
<tr>
<td>8</td>
<td>B25F</td>
<td>H23SB</td>
</tr>
<tr>
<td>9</td>
<td>V8S</td>
<td>H24S</td>
</tr>
<tr>
<td>10</td>
<td>V9S</td>
<td>A1LA*</td>
</tr>
<tr>
<td>11</td>
<td>V17S</td>
<td>A11FA*</td>
</tr>
<tr>
<td>12</td>
<td>V5F</td>
<td>B2L*</td>
</tr>
<tr>
<td>13</td>
<td>V18F</td>
<td>H1L*</td>
</tr>
<tr>
<td>14</td>
<td>A7LA</td>
<td>H15S*</td>
</tr>
</tbody>
</table>

*Unlikely *Botrytis* isolates

Figure 2. Percentage of *Botrytis* isolates obtained from different tissues of symptomless *Taraxacum officinale* plants (N=23).
Figure 3. Morphology of unlikely *Botrytis* cultures (7 days old) on PDA with a positive identification by using QuickStix strips immunoassay test for *Botrytis* spp (Envirologix).

3.2 *Botrytis* species associated to *Taraxacum officinale*

The immunoassay test detected 23 isolates as *Botrytis* sp.. The phylogenetic analysis based on the *HSP60* gene of 12 of these isolates namely, B1LA, V17S, H24S, H23SA, H23SB, V5F, V9S, B24F, A24F, V18F, A14L and A25SA clustered them in the clade containing members; 3 different genotypes were found that showed the strongest phylogenetic association with *B. cinerea*; the genotype B1LA clustered closest to *B. pelargonii* strain 459.50; whereas V17S and a group of 5 strains (H24S, H23SA, H23SB, V5F, V9S) clustered as different genotypes together with *B. cinerea* strain MUCL87 (Figure 4, 5). Three other genotypes were distinguished that were associated with *B. pseudocinerea* they were B24F, A24F and a cluster of 3 isolates (V18F, A14L and A25SA) (Figure 4, 5). A set of 5 genotypes was chosen for further phylogenetic analysis.

The phylogenetic analysis based on *G3PDH* showed similar results; 4 of the genotypes clustered together with the species *B. cinerea* and 1 genotype was clustered with the species *B. pseudocinerea* (Figure 6). These results, were consistent with the phylogenetic analysis based *RPB2* and on all 3 genes (Figures 6, 7, 8). Unfortunately, for the phylogenetic analyses based on *RPB2* and all 3 genes the genotype V18F identified as *B. pseudocinerea* was excluded due to the low quality of the sequence data generated.
Figure 4. Cultures of *Botrytis* species *pseudocinerea* (A) and *cinerea* (B) on malt extract agar isolated from symptomless dandelion plants (*Taraxacum officinale*). Sclerotia in cultures of 12 days old grown in darkness (left side in the pictures) and sporulating cultures of 15 days old (right side of the pictures).
Figure 5. Phylogenetic tree based on HSP60 gene for *Botrytis* species isolated from symptomless dandelion plants (*Taraxacum officinale*)
Figure 6. Phylogenetic tree based on G3PDH gene for Botrytis species isolated from symptomless dandelion plants (Taraxacum officinale)
Figure 7. Phylogenetic tree based on RPB2 gene for *Botrytis* species isolated from symptomless dandelion plants (*Taraxacum officinale*)
Figure 8. Phylogenetic tree based on **HSP60** and **G3PDH** genes of *Botrytis* species isolated from symptomless dandelion plants (*Taraxacum officinale*).
Figure 9. Phylogenetic tree based on HSP60, G3PDH and RPB2 genes of Botrytis species isolated from symptomless dandelion plants (Taraxacum officinale)
3.3 Pathogenicity assay
Inoculation of tomato leaves and *N. benthamiana* plants demonstrated that 5 of the 6 testing *Botrytis* sp. isolates had the capacity to cause infective expanding lesions. In some of the isolates, lesions were comparable to those caused by *Botrytis cinerea* strain B05.10 employed as control (Figure 10, Table 3). Among the *Botrytis* isolates tested, inoculations with A25SB resulted in smaller lesions which developed slower compared with the other isolates inoculated.

![Figure 10](image)

**Figure 10.** Lesion development (48hpi) on (A) tomato leaflets (cv. Moneymaker) and (B) *N. benthamiana* leaves inoculated (10^6 spores/ml) with *Botrytis cinerea* B05.10 (control) (leaf left side) and *Botrytis* sp. (leaf right side) isolated from symptomless *Taraxacum officinale* plants.
Table 3. Infective lesions (mm) on tomato (S. lycopersicum) and N. benthamiana leaves, produced by *Botrytis* spp. isolated from symptomless *Taraxacum officinale* plants.

<table>
<thead>
<tr>
<th><em>Botrytis</em> isolates</th>
<th>Tomato</th>
<th>N. benthamiana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion diameter (mm)</td>
<td>Lesion diameter (mm)</td>
</tr>
<tr>
<td><em>B. cinerea</em> strain B05.10 (control)</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td><em>Botrytis pseudocinerea</em> (V18F)</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> (V17S)</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> (B1LA)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Botrytis</em> sp A7LA</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Botrytis</em> sp B18F</td>
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<td>13</td>
</tr>
<tr>
<td><em>Botrytis</em> sp A25SB</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>

4. Discussion

*Botrytis cinerea* is known as a polyphagous necrotrophic pathogen of plants which kills the host’s cells by secreting toxic compounds and degrading enzymes, and inducing a programmed cell death by the host itself (van Kan, 2006; Choquer et al., 2007; Shlezinger et al., 2011). However, recent studies show a different “side” of this pathogen, in which it behaves as an endophyte and causes symptomless systemic infections in some plants (Sowley et al., 2010; Shipunov et al., 2008). Our results confirm the flexibility of this pathogen to behave as an endophyte. Dandelion plants investigated for endophytic colonization by *Botrytis* species resulted in isolation of different strains, which were identified by sequencing as *B. cinerea* and *B. pseudocinerea*. In this study, the limited set of isolates sequenced made it difficult to elucidate if *Botrytis* species less related to *B. cinerea* were also present.

The flexible behaviour by *Botrytis* species agrees with a phylogenetic study of some fungal lineages in which it was determined that an interchangeable switch between a necrotrophic and endophytic behaviour occurred multiple times (Delaye et al., 2013). In contrast, a biotrophic condition in pathogen fungi was considered a more stable trait (Delaye et al., 2013).

Endophytes have commonly been defined as organisms which live inside plants without causing symptoms of disease (Carroll, 1988). However, this definition should be considered as a temporary status which includes a variety of microorganisms with different life history strategies (Schulz and Boyle, 2006). Endophytes can display variable associations which range from mutualism, commensalism, latent pathogenicity, and exploitation (Saikkonen et al.,...
1998; Schulz and Boyle, 2006). The flexibility of these associations depends on the genetic dispositions of the two partners, their developmental stage and nutritional status as well as environmental factors (Schulz and Boyle 2006). For example, van Kan et al. (2014) suggest that a reduced genetic diversity in cultivated Hemerocallis hybrids could be a significant factor in the abrupt occurrence of a foliar disease called “spring sickness” that emerged from a switch of B. deweyae from endophytic to pathogenic infections in these plants. In grape, kiwi, strawberry and other hosts, B. cinerea may produce dormant infections which are then activated by the onset of senescence or stress of the host tissue (Sowley et al., 2010).

In the present study, the pathogenicity tests carried out on tomato and N. benthamiana with 5 of the 6 strains isolated from symptomless dandelion plants revealed that these strains were able to degrade plant tissues and cause the typical expanding disease lesions observed in aggressive infections by Botrytis pathogens. These results were similar to those reported by Sowley et al. 2010 in which Botrytis cinerea isolated from symptomless lettuce plants and later drop-inoculated on detached lettuce leaf pieces developed typical disease lesions. Endophytes and pathogens can have many of the same virulence factors which are required to infect and colonize the host (Schulz and Boyle, 2006). However, if the fungal virulence and the defensive response (which could include defensive metabolites and a general defence) by the plant are balanced, this interaction is asymptomatic (Schulz and Boyle, 2006). The conditions under which endophytic symptomless colonization by Botrytis species occurs have not yet been elucidated (Sowley et al. 2010; van Kan et al., 2014). Sowley et. al. (2010) suggest that during endophytic colonization by B. cinerea in lettuce, toxic compounds are not produced or are maintained at low levels to minimize host plant defence responses, and also mentions that active and aggressive infections by B. cinerea are only found in ripened fruits and wounded plant tissues. Van Kan et al. (2014) suggest that the switch from one lifestyle to another as described in Botrytis sp. is controlled by a mechanism which prevents or induce the expression of toxic compounds and degrading enzymes.

Overall this work we consider some information from some of the isolates is still lacking. Among the cultures identified as Botrytis by the immunoassay 5 isolates were considered unlikely members of the Botrytis genus due to their distinct morphology. However, the immunoassay test employed in this work is thought to be highly specific, therefore, obtaining information of these isolates by sequencing and a morphological description could confirm our assumption. Similarly, information by sequencing of the isolate A25SB could contribute
to explain if its reduced aggression to produce disease lesions is due to its adaptation as an endophyte or due to species particularities.

This study supports previous research in which the flexibility of *Botrytis* species to behave as necrotrophic pathogens or endophytes was established (Shipunov et al., 2008; Sowley et al. 2010), and highlights the necessity to generate new knowledge about the complex interactions which *Botrytis* species display with plants as well as the need to elucidate what could be the key factors capable of modulating such contrasting behaviours in these fungal species.

5. Bibliography


van Kan, J., Shaw, M., Grant-Downton, R. (2014). *Botrytis* species: relentless necrotrophic thugs or endophytes gone rogue? *Molecular Plant Pathology*.


6. Appendices

Appendix 1.

Table 1. Sampling sites of *Taraxacum officinale* for *Botrytis* isolation in Wageningen

<table>
<thead>
<tr>
<th>Sites</th>
<th>Latitude</th>
<th>Longitude</th>
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<td>5.653710</td>
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<td>51.985182</td>
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<td>3 Arboretum de Driegen 6703 BL Wageningen</td>
<td>51.967068</td>
<td>5.677716</td>
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<td>4 Veerdam 6703 PA, Wageningen</td>
<td>51.963223</td>
<td>5.688082</td>
</tr>
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</table>
Appendix 2.

Multiple sequence alignment (Clustal 2.1) of Botrytis spp. based on consensus sequences of HSP60, G3PDH, RPB2 genes

HSP60

CLUSTAL 2.1 multiple sequence alignment

V17S

B1LA

H23SA

V9S

H24S

V5F

H23SB

V18F

A24F

B24F

A14L

A25SA

---TTTAAGATACGACTGAGATATCGTGATT---------30
---__________________________---------TGGTGATT-8
---__________________________---------CCACGTGGTTAGATAGGGATATCCGGTGATT---39
---__________________________---------GCTCTAAGATACGACTGAGATATCCGGTGATT--32
---__________________________---------TCCACGTGGTTAGATAGGGATATCCGGTGATT--40
---__________________________---------ATT-3
---__________________________---------CGTGGTGATT-8
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**CLUSTAL 2.1 multiple sequence alignment**

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**RPB2**

**CLUSTAL 2.1 multiple sequence alignment**

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<th>H24S</th>
<th>B1LA</th>
<th>H23SB</th>
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<tr>
<td>H23SB</td>
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**Alignment Output**

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V17S      CCAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCAACGGTCTGAAATAT
H24S      CCAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCAACGGTCTGAAATAT
B1LA      CCAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCAACGGTCTGAAATAT
H23SB     CCAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCAACGGTCTGAAATAT

V17S      CGAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
H24S      CGAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
B1LA      CGAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
H23SB     CGAGAGTTTAATTTGACTTTGGGTGTGAAATCAACAACAATCACCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG

V17S      TCTTTGGCCACAGGTAACTGGGGTGACCAGAAGAAGGCAGCAAGTTCTACCGCCGGAGTG
H24S      TCTTTGGCCACAGGTAACTGGGGTGACCAGAAGAAGGCAGCAAGTTCTACCGCCGGAGTG
B1LA      TCTTTGGCCACAGGTAACTGGGGTGACCAGAAGAAGGCAGCAAGTTCTACCGCCGGAGTG
H23SB     TCTTTGGCCACAGGTAACTGGGGTGACCAGAAGAAGGCAGCAAGTTCTACCGCCGGAGTG

V17S      ACACCCATTGGACGTGATGGAAAGATCGCCAAACCTAGACAGCTGCATAATACCCATTGG
H24S      ACACCCATTGGACGTGATGGAAAGATCGCCAAACCTAGACAGCTGCATAATACCCATTGG
B1LA      ACACCCATTGGACGTGATGGAAAGATCGCCAAACCTAGACAGCTGCATAATACCCATTGG
H23SB     ACACCCATTGGACGTGATGGAAAGATCGCCAAACCTAGACAGCTGCATAATACCCATTGG

V17S      GGCTTGGTCTGTCCGGCAGAGACGCCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
H24S      GGCTTGGTCTGTCCGGCAGAGACGCCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
B1LA      GGCTTGGTCTGTCCGGCAGAGACGCCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG
H23SB     GGCTTGGTCTGTCCGGCAGAGACGCCCGAAGGACAAGCTTGTGGTTTGGTTAAGAATTTG

V17S      GCTCTGATGTGTTACGTTACAGTCGGTACGCCAAGTGATCCAATCGTTGAGTTCATGATT
H24S      GCTCTGATGTGTTACGTTACAGTCGGTACGCCAAGTGATCCAATCGTTGAGTTCATGATT
B1LA      GCTCTGATGTGTTACGTTACAGTCGGTACGCCAAGTGATCCAATCGTTGAGTTCATGATT
H23SB     GCTCTGATGTGTTACGTTACAGTCGGTACGCCAAGTGATCCAATCGTTGAGTTCATGATT

V17S      CAACGAAATATGGAAGTATTGGAGGAGTATGAACCACTCCGAGCCCCCAATGCAACAAAG
H24S      CAACGAAATATGGAAGTATTGGAGGAGTATGAACCACTCCGAGCCCCCAATGCAACAAAG
B1LA      CAACGAAATATGGAAGTATTGGAGGAGTATGAACCACTCCGAGCCCCCAATGCAACAAAG
H23SB     CAACGAAATATGGAAGTATTGGAGGAGTATGAACCACTCCGAGCCCCCAATGCAACAAAG

V17S      GTTTTCGTCAATGGTGTTTGGGTTGGTATTCATCGAGATCCTGCTCATTTGGTCAAATGT
H24S      GTTTTCGTCAATGGTGTTTGGGTTGGTATTCATCGAGATCCTGCTCATTTGGTCAAATGT
B1LA      GTTTTCGTCAATGGTGTTTGGGTTGGTATTCATCGAGATCCTGCTCATTTGGTCAAATGT
H23SB     GTTTTCGTCAATGGTGTTTGGGTTGGTATTCATCGAGATCCTGCTCATTTGGTCAAATGT

V17S      GTCCAAGATCTTCGTAGATCACACTTGATCTCTCATGAAGTTTCACTTATTCGAGAAATT
H24S      GTCCAAGATCTTCGTAGATCACACTTGATCTCTCATGAAGTTTCACTTATTCGAGAAATT
B1LA      GTCCAAGATCTTCGTAGATCACACTTGATCTCTCATGAAGTTTCACTTATTCGAGAAATT
H23SB     GTCCAAGATCTTCGTAGATCACACTTGATCTCTCATGAAGTTTCACTTATTCGAGAAATT

V17S      CGTGATAGAGAATTCAAGATTTTCACAGATGCAGGACGAGTGTGCAGACCTCTATTGGTT
H24S      CGTGATAGAGAATTCAAGATTTTCACAGATGCAGGACGAGTGTGCAGACCTCTATTGGTT
B1LA      CGTGATAGAGAATTCAAGATTTTCACAGATGCAGGACGAGTGTGCAGACCTCTATTGGTT
H23SB     CGTGATAGAGAATTCAAGATTTTCACAGATGCAGGACGAGTGTGCAGACCTCTATTGGTT
```
**B1LA**

```
CTTGATAGGAAATTCTAGTTTTGCAGATCGGAGGACTGTCATGATTACAATGACACCTGAAGATCTGGACATCTCCCGACAG 660
```

**H23SB**

```
CTTGATAGGAAATTCTAGTTTTGCAGATCGGAGGACTGTCATGATTACAATGACACCTGAAGATCTGGACATCTCCCGACAG 660
```

**V17S**

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**H24S**

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ATTGACAATGATCCTGACAGCGCAAACAAAGGTAACTTGGTGTTGAACAAGGATACATT 715
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**B1LA**

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ATTGACAATGATCCTGACAGCGCAAACAAAGGTAACTTGGTGTTGAACAAGGATACATT 720
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**H23SB**

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ATTGACAATGATCCTGACAGCGCAAACAAAGGTAACTTGGTGTTGAACAAGGATACATT 720
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**V17S**

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**H24S**

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ACGGATACGCTATGGAATTCCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 835
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**B1LA**

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ACGGATACGCTATGGAATTCCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 840
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**H23SB**

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ACGGATACGCTATGGAATTCCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 840
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**V17S**

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GGAAGAAGGAAAGACTGTCATGATTACAATGACACCTGAAGATCTGGACATCTCCCGACAG 900
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**H24S**

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GGAAGAAGGAAAGACTGTCATGATTACAATGACACCTGAAGATCTGGACATCTCCCGACAG 900
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**B1LA**

```
GGAAGAAGGAAAGACTGTCATGATTACAATGACACCTGAAGATCTGGACATCTCCCGACAG 900
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**H23SB**

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**V17S**

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**H24S**

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GTTCAGGCTGGTTACCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 960
```

**B1LA**

```
GTTCAGGCTGGTTACCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 960
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**H23SB**

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GTTCAGGCTGGTTACCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 960
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**V17S**

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**H24S**

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**B1LA**

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TCACGGCTGGTTACCAAATTCGTCCTGACGAAAGTGGTGATTTGAACAAGCGTGTTAAG 990
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**H23SB**

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**V17S**

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**H24S**

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**B1LA**

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**H23SB**

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**V17S**

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**H24S**

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**B1LA**

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GCACCTATGACTCACTGCTAGTCTGGAGCTATTGTGAATTTCATACTTCCAAGTGATTC 1055
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**H23SB**

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**V17S**

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**H24S**

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TTGGGATATCACAGGACTATTACCATCCCCGTACGACTGAGCA------1057
```

**B1LA**

```
TTGGGATATCACAGGACTATTACCATCCCCGTACGACTGAGCA------1057
```

**H23SB**

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TTGGGATATCACAGGACTATTACCATCCCCGTACGACTGAGCA------1057
```

**V17S**

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**H24S**

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AATGAAATTATTG 1089
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**B1LA**

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```

**H23SB**

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AATGAA--------1086
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