

>KC620375.1 *Botrytis pseudocinerea* culture-collection ICMP:19669 glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, exons 2, 3 and partial cds

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TAAGTTTCCGCTATCGGACTTCCCGCAGATATTACGGACCCGAGCTAATTTATATTTTGCACAGGCATACATGTTGAAGT
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AGTTCTACACCGAGAGAGACCCAGCCAACATCCCATGGGCTGAGTCTGAGGCATACTACGTCGTCGAGTCCACCGGTG
TTTTACCACCACCGAGAAGGCTAAGGCACATTTGAAGGGTGGTGCCAAGAAGGTTGTTATCTCTGCTCCTTCTGCCGA
CGCCCAATGTACGTTATGGGTGTCAACAACGAGACCTACACTGGTGATGTTGATGTTATCTCCAACGCCTCTTGACA
ACCAACTGCTTGGCTCCTCTCGCCAAGGTCATCAACGATGAGTTCACCATCATTGAAGGTTTGATGACCACCATCCACTC
CTACACCGCTACCCAAAAGACCGTTGACGGTCCATCCGCTAAGGATTGGCGTGGAGGACGTACCGCTGCTCAAACAT
CATCCCATCGAGCACCGGTGCTGCCAAGGCTGTCGAAAGGTCATCCAGTGCTTAACGGCAAACCTACCGGAATGTC
CATGCGTGTTCAACTGCCAACGTCTCAGTTGTTGACTTGACTGTCCGCATTGAGAAGGGTGCTTCTTATGATGAGATTA
AGGCCGTATCAAGAAGGCTGCTGATGGTCTCTCAAGGGTAGGTTACTCCATCACTCTTCTTTGGCTCTAATTTACTA
ATCATAATACAGGCATATTGGCTTACTGAGGACGATGTTGTCTCCACTGACATGAACGGTGACAACCACTCCTCCAT
CTTCGATGCCAAGGCCG
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Figure S1. Position of the specific primer for *Botrytis cinerea*. The positioning of the forward and reverse primer for drawing primer of *Botrytis cinerea* within the nucleotide sequence of the DNA glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of *Botrytis pseudocinerea* (GenBank accession n. KC620375).

>KU821407.1_Colletotrichum_coccodes_isolate_VIC9F_glyceraldehydes-3-phosphate_dehydrogenase_(gpdh)_gene_partial_cds

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GCCGTCAACGACCCCTTCATTGAGACCAAGTACGCTGTGAGTATCATTCCACCTTACCCCTCCGGCATCATCATGATATC
ATTCTCTCACGGCACGGCCCTTCTCTGCCCCCTATTCCGTCAGCGTGCTTGAGATGGGCTGCTGCCGACATCAGCCCT
CGGATCCTATGTCTCTGAGGCATCGTGTCTGAATAGCACGATGTGATTGATATCACGAGCATCAGAAGCCAGTAAATAT
GAACA-TTACTGACGCGCCATCTCCCAGGCCTA CATGCTCAAGTACGACTCCACCC
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Figure S2. Position of the specific primer for *Colletotrichum* sp.. The positioning of the forward and reverse primer for drawing primer of *Colletotrichum* sp. within the nucleotide sequence of the DNA glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of *Colletotrichum coccodes* (GenBank accession n. KU821407).

>MF326807.1_Phytophthora_balyanboodja_strain_VHS25675R3_beta-tubulin_(TUB)_gene_partial_cds

TACCACGGCGACTCGGACCTGCAGCTGGAGCGCATCAATGTTTACTACAACGAGGCCACCGGCGGCCGATACTGCCCC
CGCGCCATCCTCATGGACCTTGAGCCCCGGCACGATGGACTCGGTGCGCGCCGGCCCGTACGGCCAGCTCTTCCGCCCCG
GACAACTTCGTGTTTCGGCCAGACCGGCGCCGGTAACAACCTGGGCCAAGGGACTACACCGAAGGCGCCGAGCTCAT
TGACTCGGTGCTTGACGTGTCGCAAGGAGGCTGAAAGCTGTGACTGTCTGCAGGGGTTCCAGATCACGCACTCGCT
TGGTGGTGGTACCGGCTCCGGTATGGGCACGCTTCTTATTTGAAGATCCGTGAAGAGTACCCGGACCGTATCATGTG
CACGTATTCGGTGTGCCCGTCGCCAAGGTGTCGGACACCGTCTGGAGCCGTACAACGCTACACTGTCGGTGCACCA
GCTGGTCGAGAACGCCGACGAGGTCATGTGCTTGGATAACGAGGCCCTGTACGACATTTGCTTCCGTACGTTGAAGCT
CACGACCCCCACCTACGGCGACCTGAACCACCTGGTATGCGCCGCCATGTCGGGTATCACCACGTGCCTGCGTTTCCCC
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CGCCCCGCTGACGTGCGGTGGCTCGCAGCAGTACCGGGCCCTGACGGTGCCTGAGCTGACGCAGCAGCAGTITGACG
CCAAGAACATGATGTGCGCCGCGYACCCGCGCCACGGCCGCTATTTAACTGCCGCGTGTATGTTCCGCGGACGTATGA
GCACGAAGGAGGTGGATGAGCAAATGCTGAATGTGCAGAACAAGAAGTCTGCATACCTCGTCGAGTGGATCCCCAACA
ACATCAAGGCCAGCGTGTGCGACATCCCGCCCAAGGGACTCAAGATGAGCACCAGTTCATCGGTAAGTCCGACCGCCA
TCCAGGAGATGTTTAAGCGCGTGTCCGAGCAGTTCACGGCAATGTTCCGCCGTAAGGCTTTCTTGCCTGGTACTGCTG
TGAGGGTATGGATGAGATGGAGTTCCTACTGGA

Figure S3. Position of the specific primer for *Phytophthora* sp.. The positioning of the forward and reverse primer for drawing primer of *Phytophthora* sp. within the nucleotide sequence of the DNA β -tubulin (TUB) of *Phytophthora balyanboodja* (GenBank accession n. MF326807).

>HQ206959.2_Verticillium_longisporum_strain_PD591_haplotype_d1_actin_gene_partial_cds

GAGGGTAGGTTATCCGTCATGATGCCCCAAAGTTTGTCCCATCGAGATGCTGTCCATATCATCTCCGAAGGACTCGATG
CTCAAGCAGTACACATTGCTAACAACATCTTCCCGT **TACAGAAGAAGTTGCTGCCCT**CGTCATTGACAATGGGTTGCTCT
AATCCCCCCTTTTTTCGCCCGCTGCTGTGCGCCGGCTTCTCCGCCTTTGATGAGGGACCCGCCGCGATCCTTCATCACT
CGACAACCACATACTGGCACAATGTCTCAACACATGGAGGGCCATGATAAGCAGAGGCAAGAAAAGAAACACGGGAGC
TGACTTGACT **GTAGTTCGGGTATGTGCAAGG**CCGGTTTCGCCGGTGACGATGCGCCCCGTGCTGTCTTCGGTAAGTTCC
CCCACCCAATTTCCCTAGACCGCAACTTTGGGTGCTGCGGGCAAAGCTCATCATGATCTTTGATGCTGACCATTTCAAGC
TTCCATTGTCGGTCGCCCGTCACTATGGTATCATGATTGGTATGGGCCAGAA

Figure S4. Position of the specific primer for *Verticillium* sp.. The positioning of the forward and reverse primer for drawing primer of *Verticillium* sp. within the nucleotide sequence of the DNA actin (ACT) of *Verticillium longisporum* (GenBank accession n. HQ206959).

>PP085963.1 *Colletotrichum scovillei* strain Cs23-5.3 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, partial cds

TGATATCACGTCTGCCACGATAACACCAGCTTCGTTCGATATCCACGGGAAAAGAGTCGGAGCTAGCACTCTCGA
TTCTTTGCCCAAGGTTTCGATTGGGCTTGTTGTAACGACAACGACGTGACCCAATCATGCAGAACAGCCGAG
ACAAAATTTGCTGACAGACAATCCATCACAGGCCTACATGCTCAAGTACGACTCCACCC

Figure S5. Position of the specific primer for *Colletotrichum acutatum*. The positioning of the forward and reverse primer for drawing primer of *C. acutatum* within the nucleotide sequence of the DNA glyceraldehyde 3-phosphate dehydrogenase (GAPDH) of *Colletotrichum scovillei* (GenBank accession n. PP0859631) because the *C. scovillei* belongs to the *Colletotrichum acutatum* species complex (Damm *et al.*, 2012).

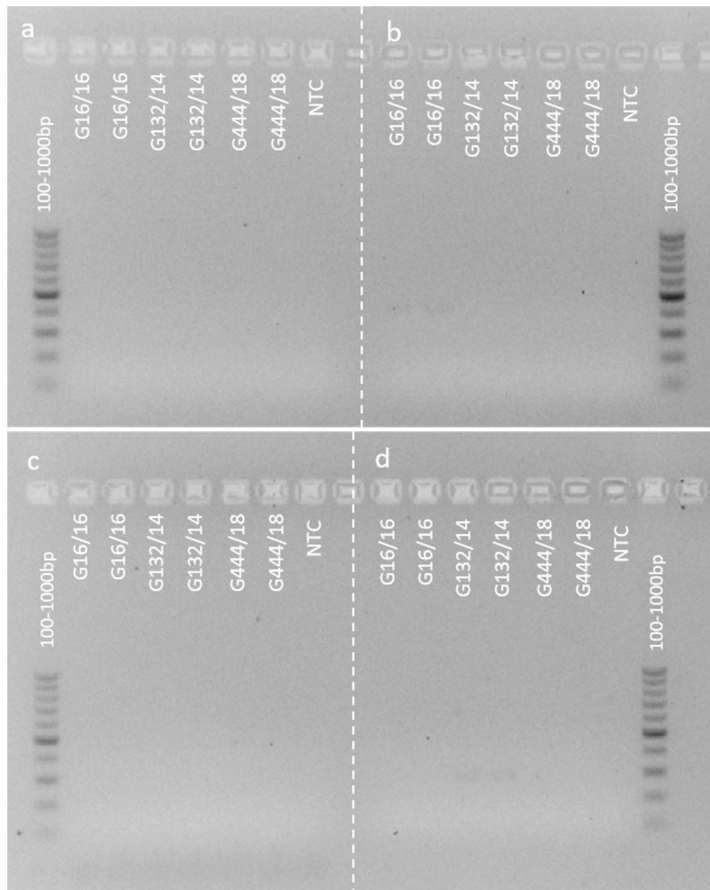


Figure S6. Specificity of the primer on pure fungal culture. PCR amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers versus different fungi, as *Petriella setifera* (G16/16), *Neosartorya fischeri* (G132/14), *Gnomonia radicicola* (G444/18). Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control.

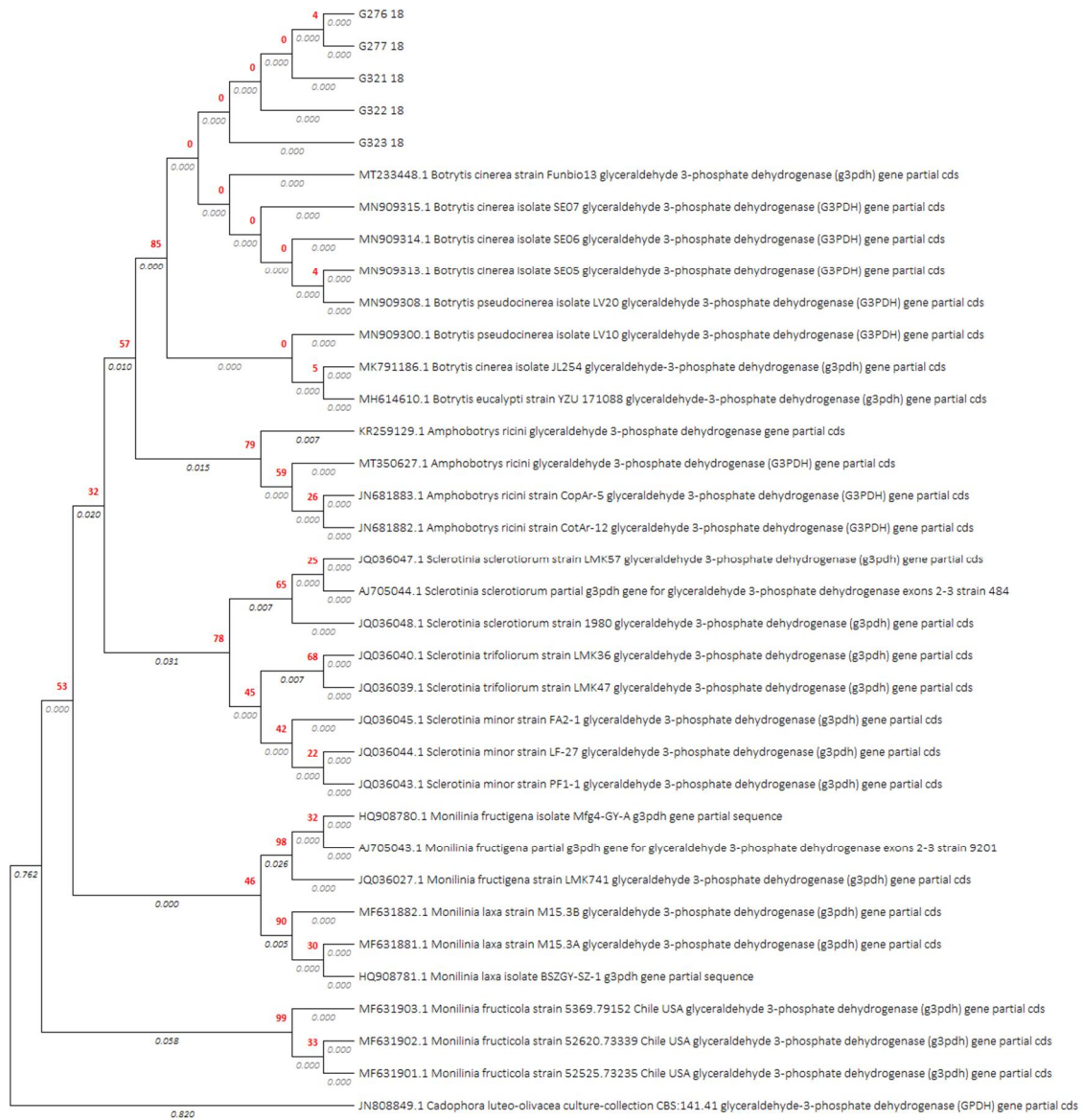


Figure S7. Phylogenetic analysis of *Botrytis cinerea* sequences based on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Phylogenetic tree using the sequences obtained from tested pure strains of *Botrytis cinerea* (G276 18; G277 18; G321 18; G322 18; G323 18) and from environmental samples originating from National Center for Biotechnology Information (NCBI). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model with rate gamma distributed plus invariant sites. For this analysis was used the Bootstrap method (4000 number of Bootstrap replications). The percentage of trees in which the associated taxa clustered together is shown next to the branches in red bold, whereas in italic is written the branch lengths.

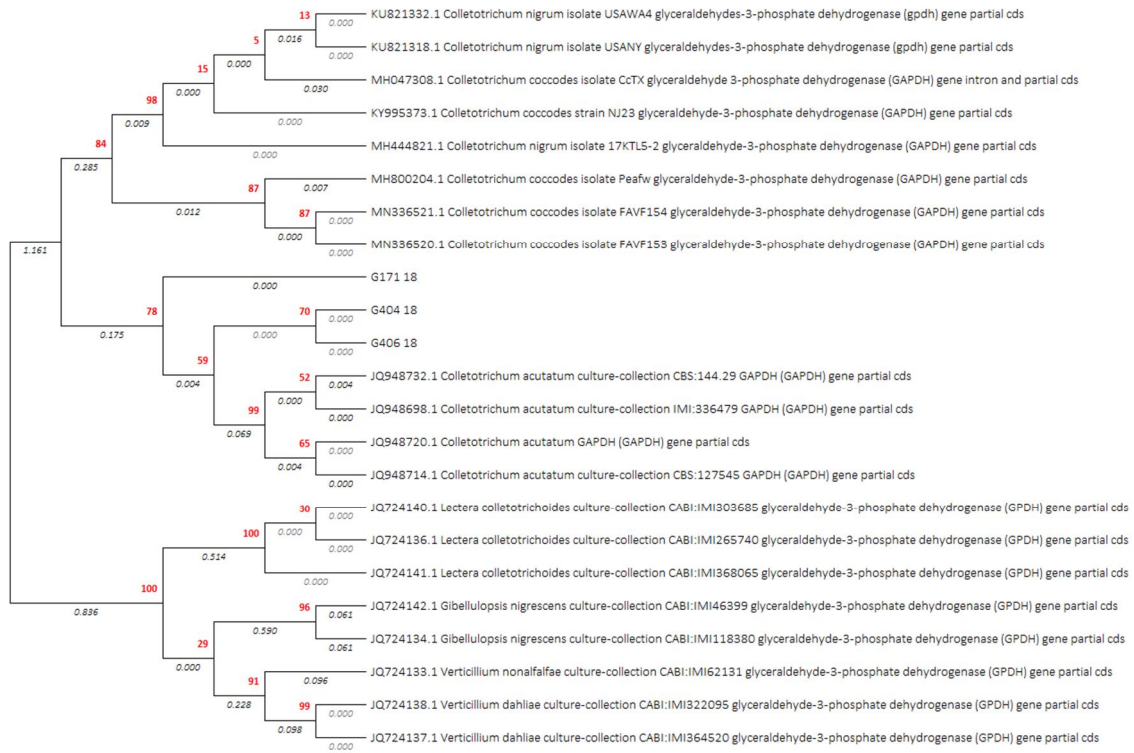


Figure S8. Phylogenetic analysis of *Colletotrichum* sp. sequences based on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Phylogenetic tree using the sequences obtained from tested pure strains of *Colletotrichum* sp. (G171 18; G404 18; G406 18) and from environmental samples originating from National Center for Biotechnology Information (NCBI). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model with rate gamma distributed. For this analysis was used the Bootstrap method (4000 number of Bootstrap replications). The percentage of trees in which the associated taxa clustered together is shown next to the branches in red bold, whereas in italic is written the branch lengths.

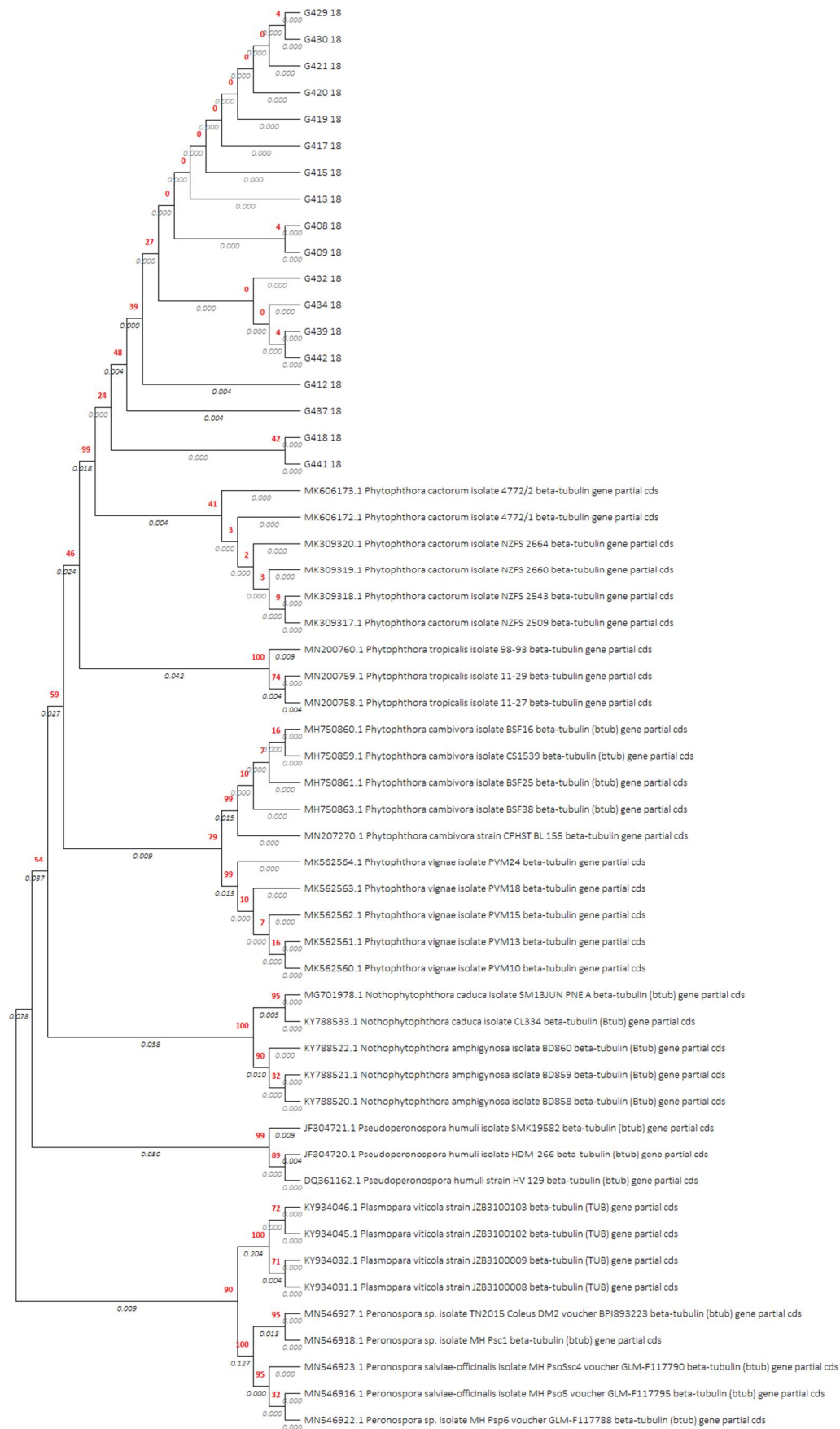


Figure S9. Phylogenetic analysis of *Phytophthora* sp. sequences based on β -tubulin (TUB) gene. Phylogenetic tree using all the sequences obtained from tested pure strains (G429 18; G430 18; G421 18; G420 18; G419 18; G417 18; G415 18; G413 18; G408 18; G409 18; G432 18; G434 18; G439 18; G442 18; G412 18; G437 18; G418 18; G441 18) and from environmental samples originating from National Center for Biotechnology Information (NCBI). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model with invariant sites. For this analysis was used the Bootstrap method (4000 number of Bootstrap replications). The percentage of trees in which the associated taxa clustered together is shown next to the branches in red bold, whereas in italic is written the branch lengths.

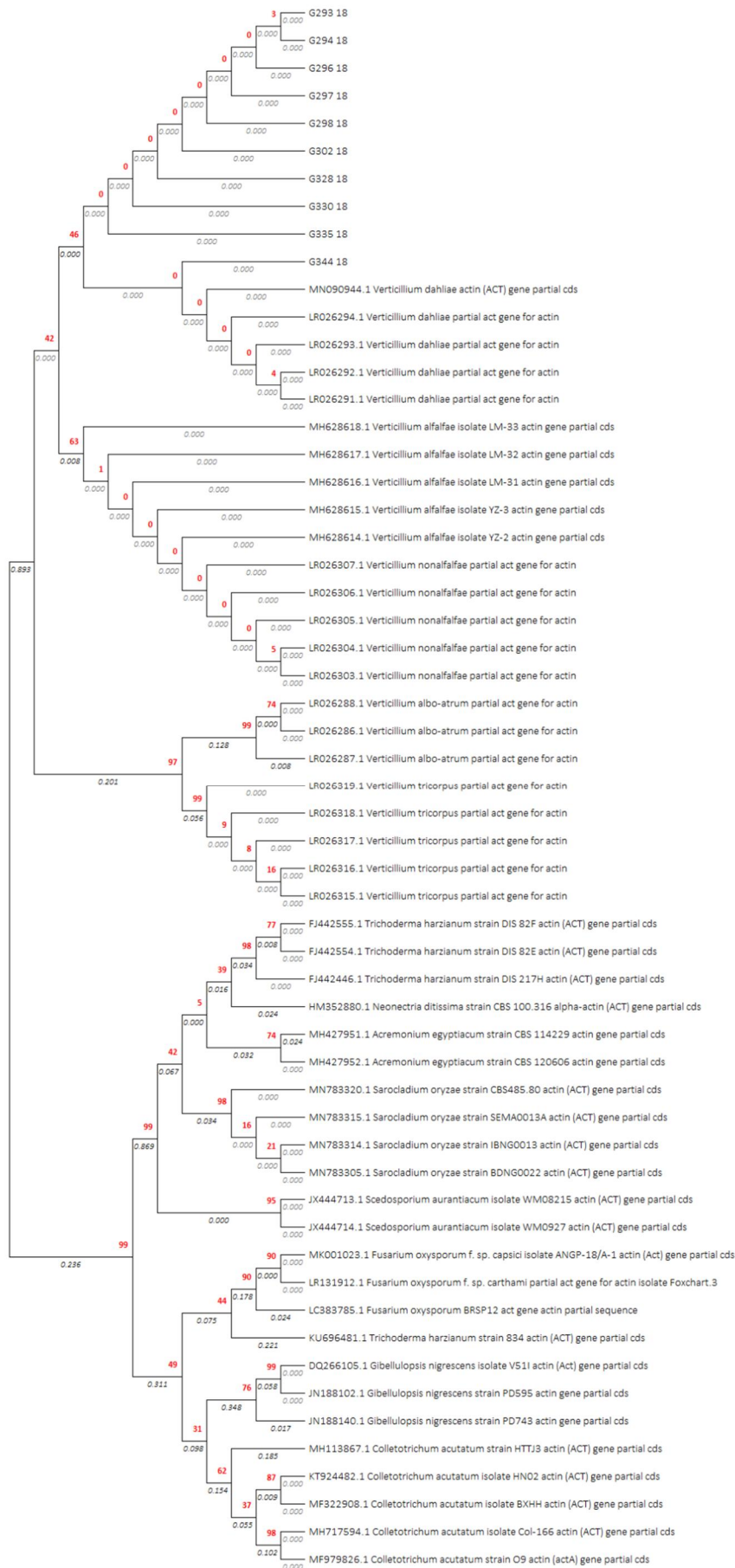


Figure S10. Phylogenetic analysis of *Verticillium* sp. sequences based on the actin (ACT) gene . Phylogenetic tree using all the sequences obtained from tested pure strains of *Verticillium* sp. (G293 18; G294 18; G296 18; G297 18; G298 18; G302 18; G328 18; G330 18; G335 18; G344 18) and from environmental samples originating from National Center for Biotechnology Information (NCBI). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model with rate gamma distributed. For this analysis was used the Bootstrap method (4000 number of Bootstrap replications). The percentage of trees in which the associated taxa clustered together is shown next to the branches in red bold, whereas in italic is written the branch lengths.

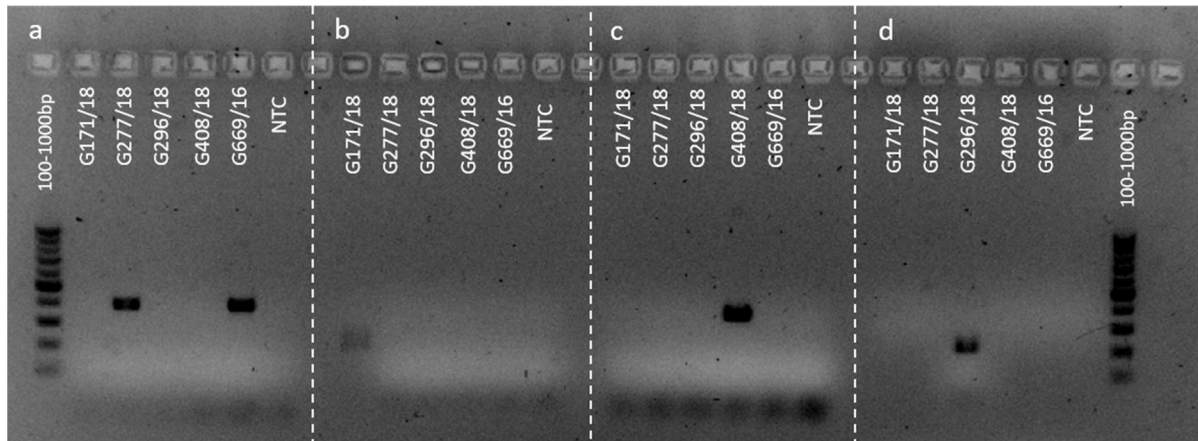


Figure S11. Specificity of the primer on pure fungal culture. PCR replication of the amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in pure cultures. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control. The strains are *Colletotrichum* sp. (G171/18), *Botrytis* sp. (G277/18, G699/16), *Verticillium* sp. (G296/18), and *Phytophthora* sp. (G406/19).

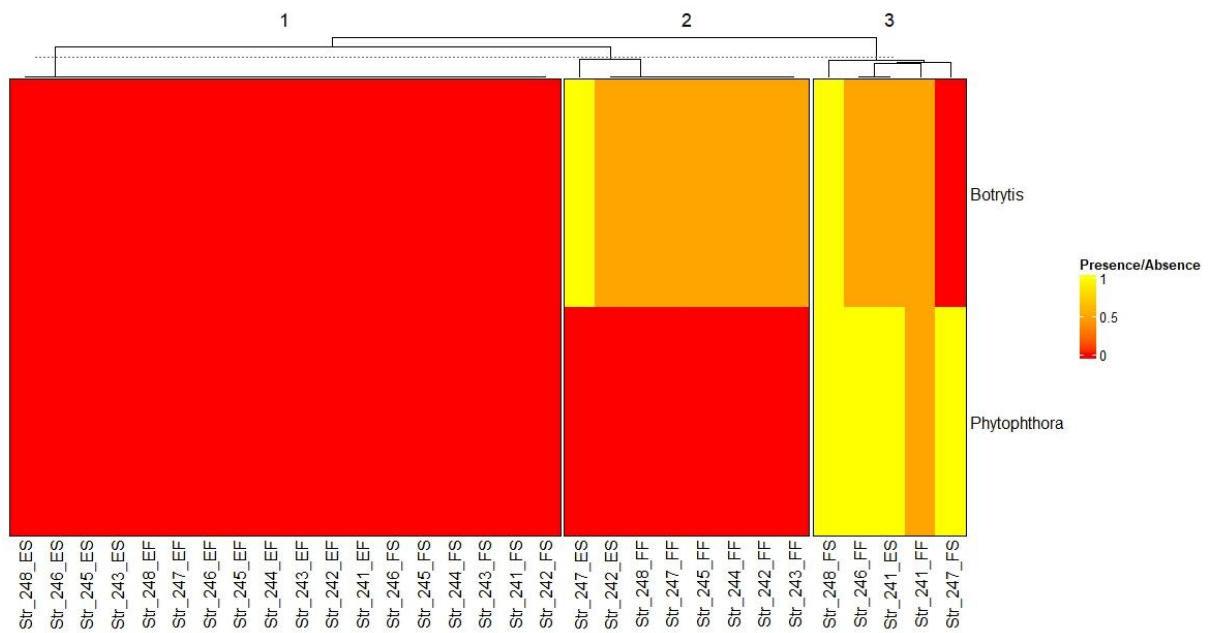


Figure S12. Specificity of the primer on naturally infected strawberry fruit. Heatmaps of the presence or absence of the phytopathogens *B. cinerea* and *Phytophthora* sp. using the specific primers in strawberry naturally infected working with 4 different kits of DNA extraction. The samples 244/19 and 245/19 came from a healthy plant; whereas 241/19-243/19 and 246/19 and 247/19 came from a plant that showed symptoms of the presence of the pathogens that we analysed.

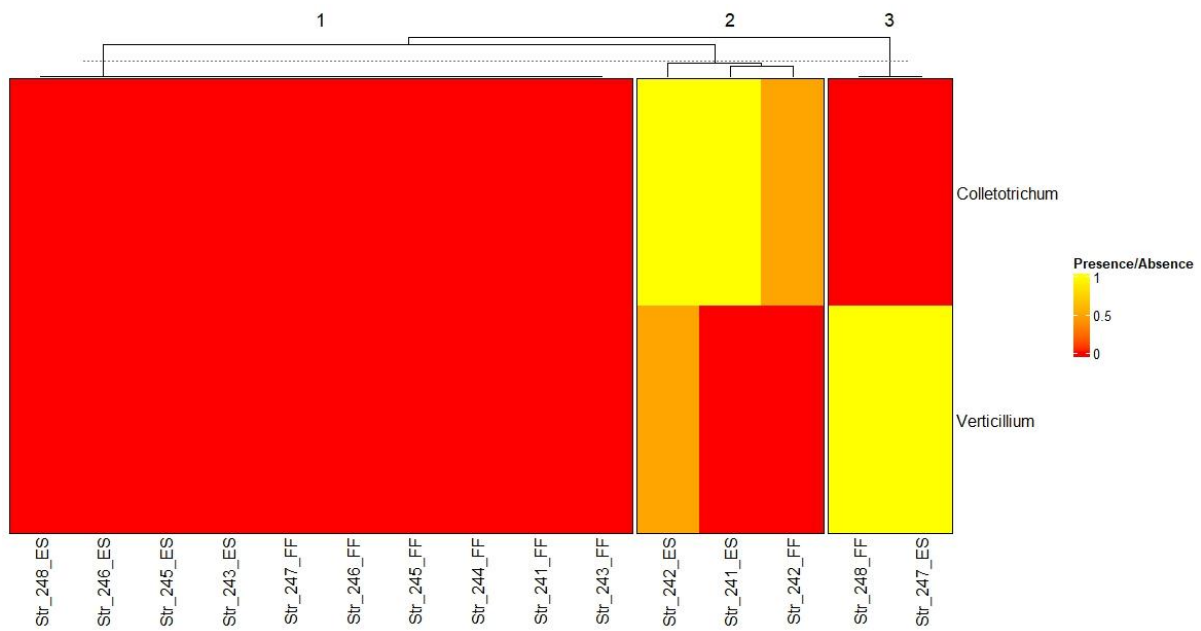


Figure S13. Specificity of the primer on naturally infected strawberry fruit. Heatmaps of the presence or absence of the phytopathogens *Colletotrichum* sp. and *Verticillium* sp. using the specific primers in strawberry naturally infected working with 2 different kits of DNA extraction. The samples 244/19 and 245/19 came from a healthy plant; whereas 241/19-243/19 and 246/19 and 247/19 came from a plant that showed symptoms of the presence of the pathogens that we analysed.

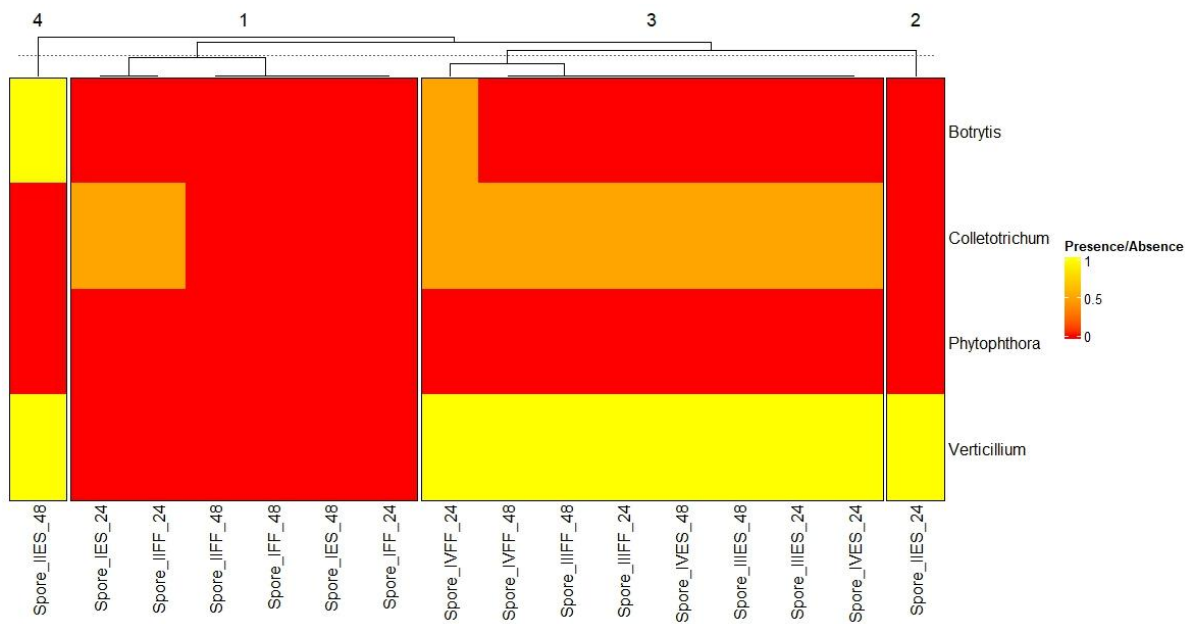


Figure S14. Specificity of the primer on soil artificially contaminated. Heatmaps of the presence or absence of *B. cinerea*, *Colletotrichum* sp., *Phytophthora* sp., and *Verticillium* sp. Using specific primers in soil artificial contaminated incubated at 24°C for 24, and 48 hours using two different kits of DNA extraction (FastDNA™ SPIN kit for Feces, FF; Soil DNA Purification kit, ES). The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 500; II – 1,000; III – 5,000; IV – 10,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil.

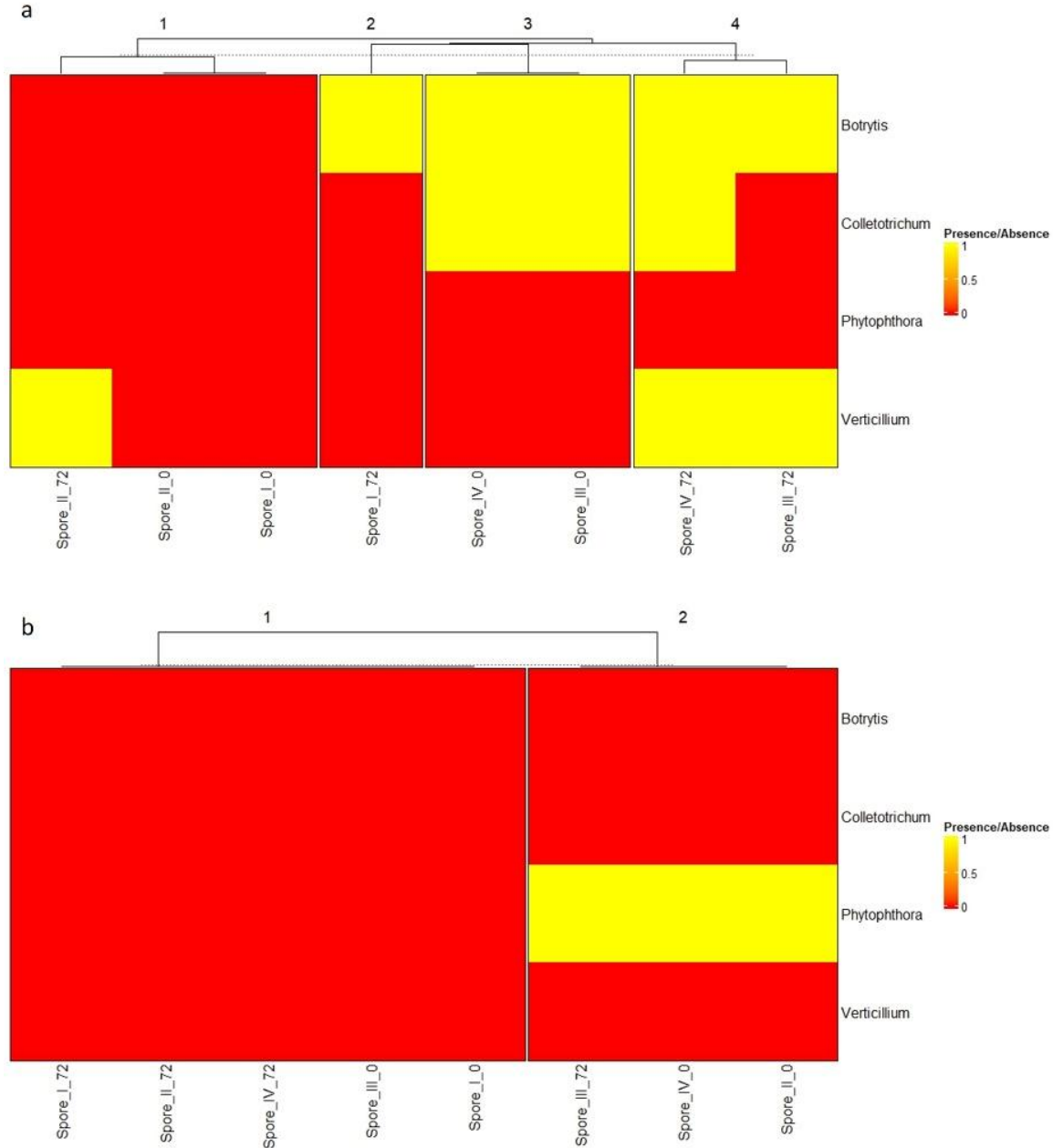


Figure S15. Specificity of the primer on soil artificially contaminated. Heatmaps of the presence or absence of *B. cinerea*, *Colletotrichum* sp., *Phytophthora* sp., and *Verticillium* sp. in soil (a) and strawberry (b) artificial contaminated incubated at 24°C for 0 and 72 hours extracted with the Soil DNA Purification kit (ES). The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 100; II – 1,000; III – 10,000; IV – 100,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil.

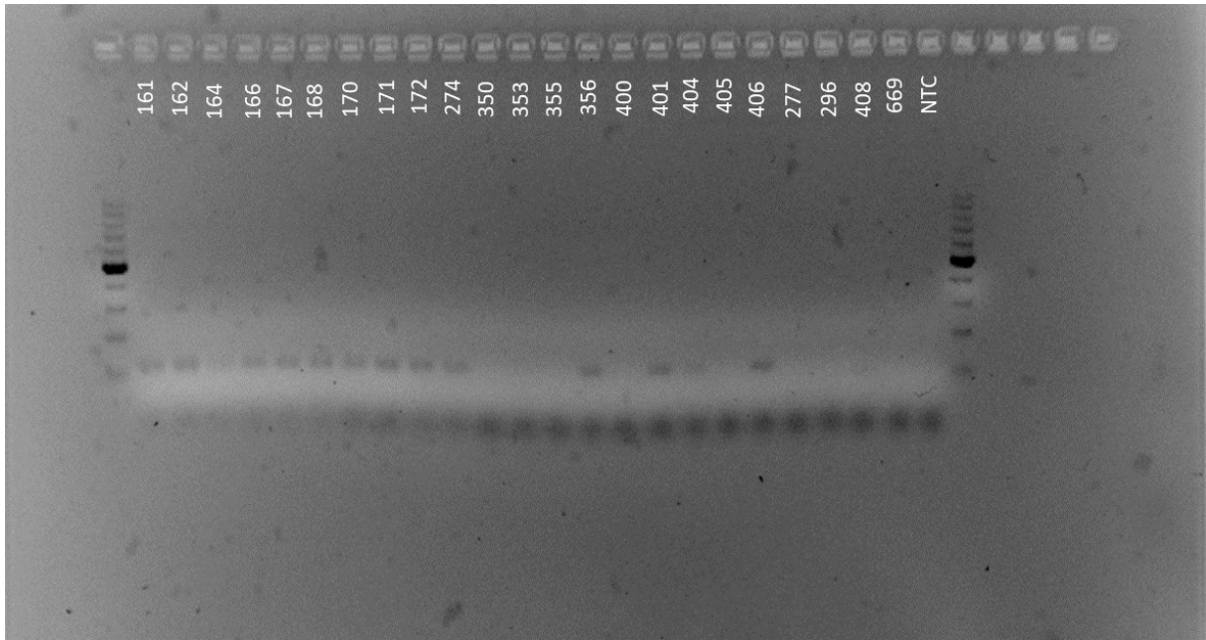


Figure S16. PCR amplification using *Colletotrichum acutatum* specific primer in pure culture. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control. The replications are presented in the Figure S10. The number means: *Colletotrichum* sp.: 161, 162, 164, 166, 168, 170, 171, 172, 274, 350, 355, 356, 400, 401, 404, 405, 406; *Botrytis* sp.: 277, 669; *Verticillium* sp.: 296; *Phytophthora* sp.: 408.

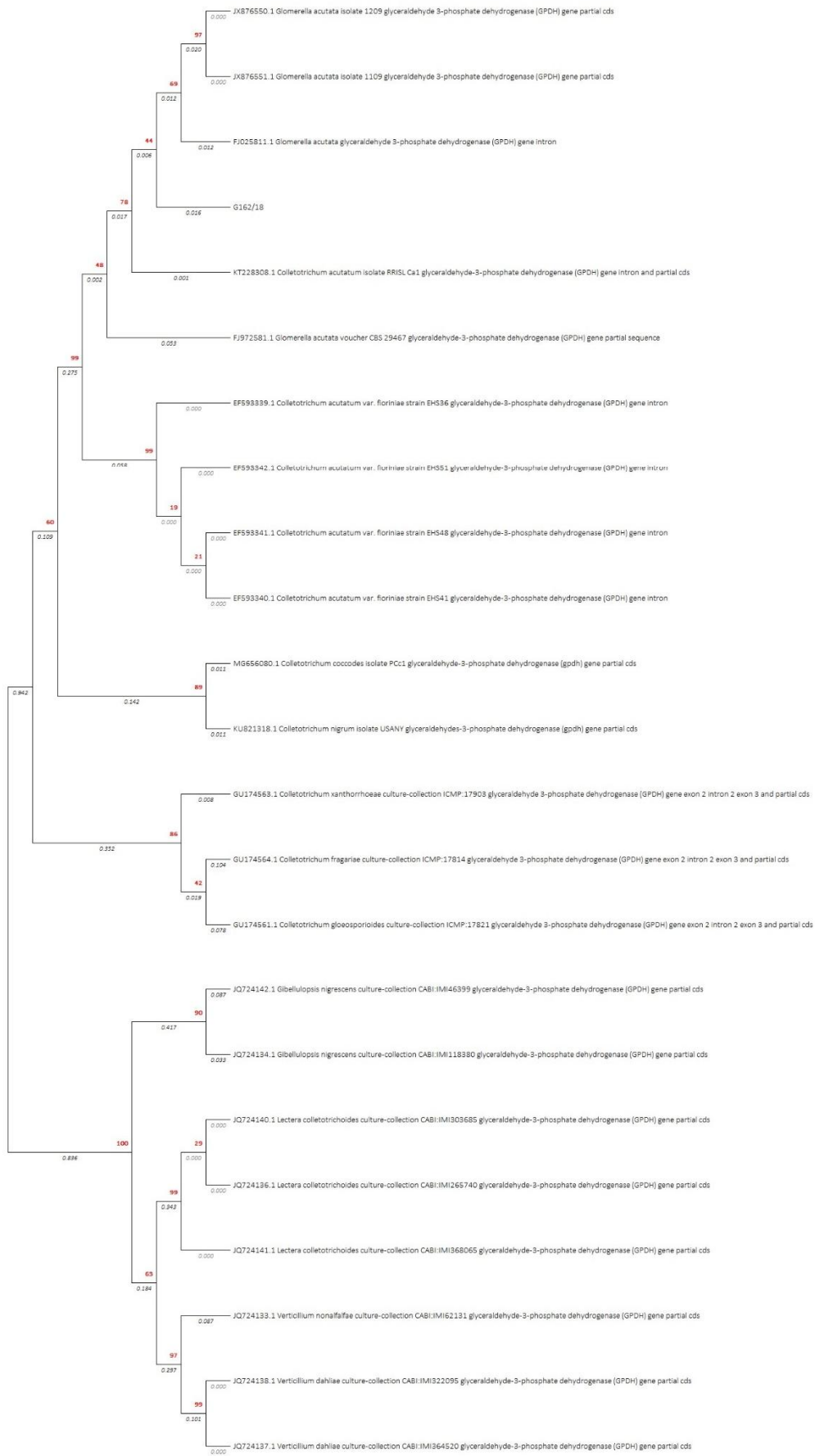


Figure S17. Phylogenetic analysis of *Colletotrichum acutatum* sequences based on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Phylogenetic tree using the sequence obtained from tested pure strain (G162 18) of *Colletotrichum acutatum* and from environmental samples originating from National Center for Biotechnology Information (NCBI). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model with rate gamma distributed. For this analysis was used the Bootstrap method (4000 number of Bootstrap replications). The percentage of trees in which the associated taxa clustered together is shown next to the branches in red bold, whereas in italic is written the branch lengths.

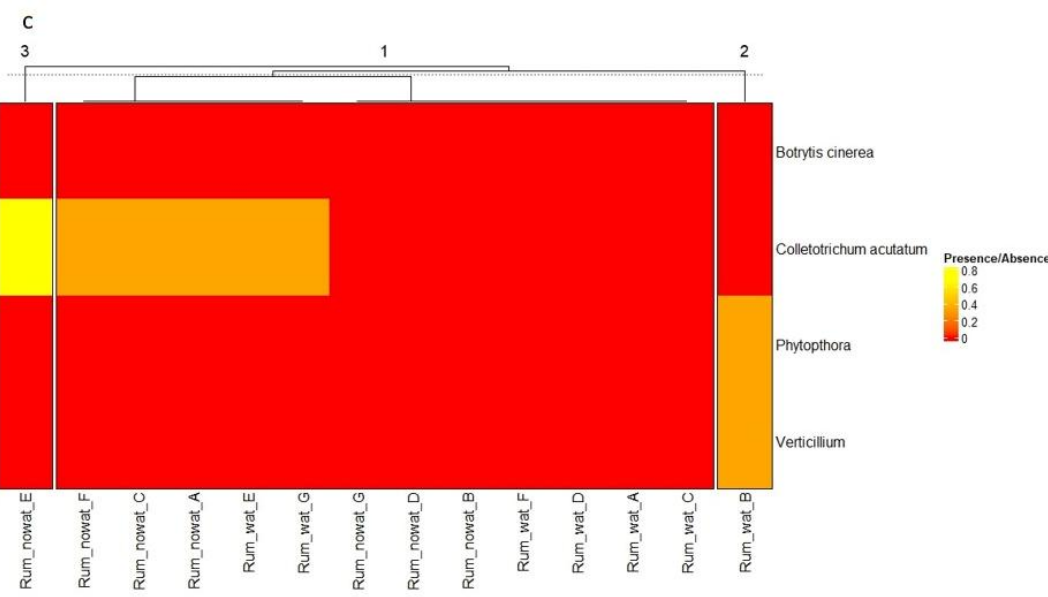
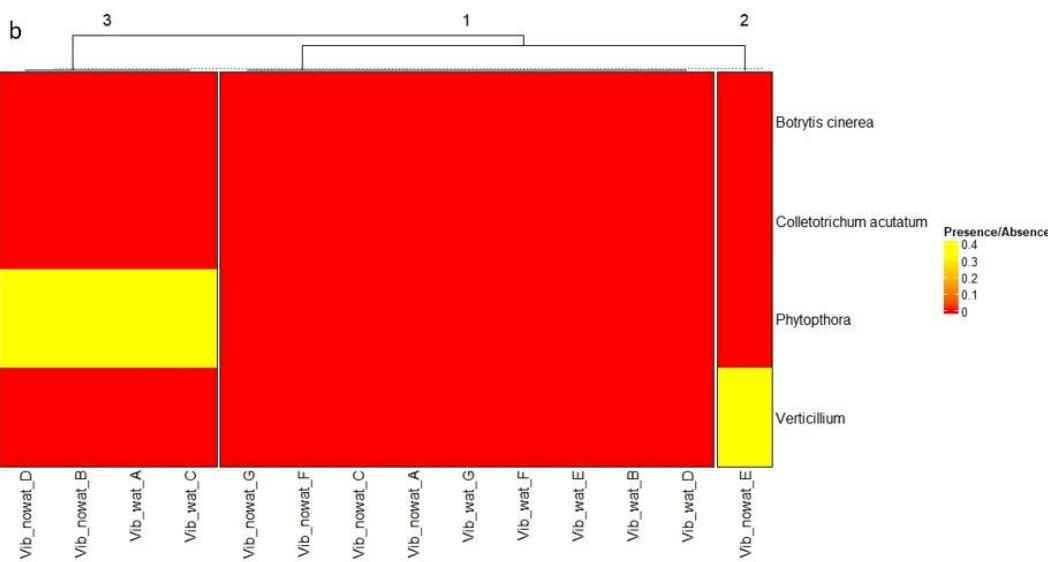
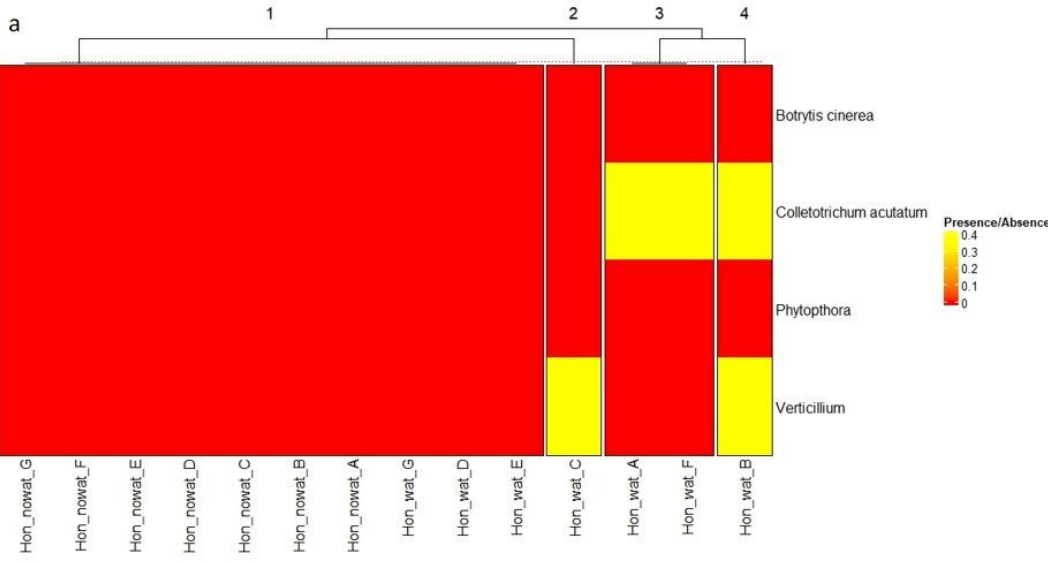


Figure S18. Heatmaps about the presence/absence of *B. cinerea*, *Colletotrichum acutatum*., *Phytophthora* sp., and *Verticillium* sp. in strawberry fruit from field experiment for Honeoye (a), Vibrant (b), and Rumba (c). Explanations: Honeoye (Hon), Vibrant (Vib), Rumba (Rum), irrigated (wat), no-irrigated (nowat), Control without carrier (A), Control with carrier (B), P3 (C), P3+P1 (D), P3+P2 (E), P3+P1+P2 (F), P1+P2 (G).

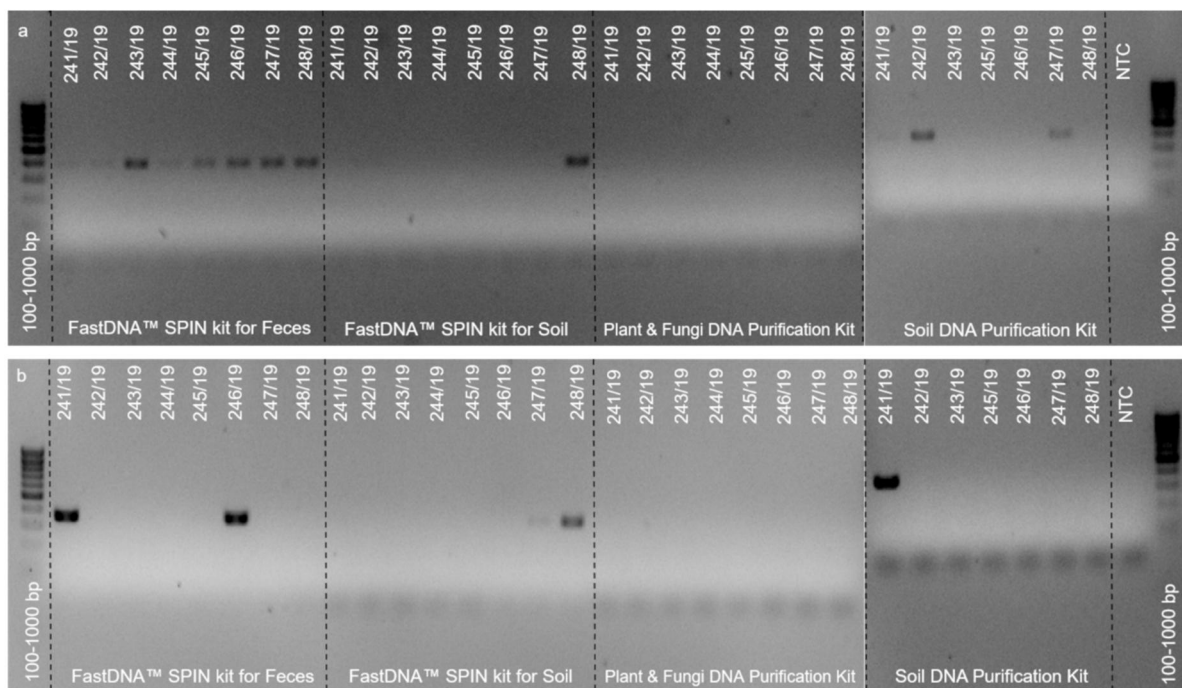


Figure S19. The specificity of the primer on naturally infected strawberry fruit. PCR amplification using *B. cinerea* (a) and *Phytophthora* sp. (b) specific primers in strawberry naturally infected using 4 different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control. The samples 244/19 and 245/19 came from a healthy plant; whereas 241/19-243/19 and 246/19 and 247/19 were collected from symptomatic plants diseased by pathogens. The replications are presented in the Figure S20.

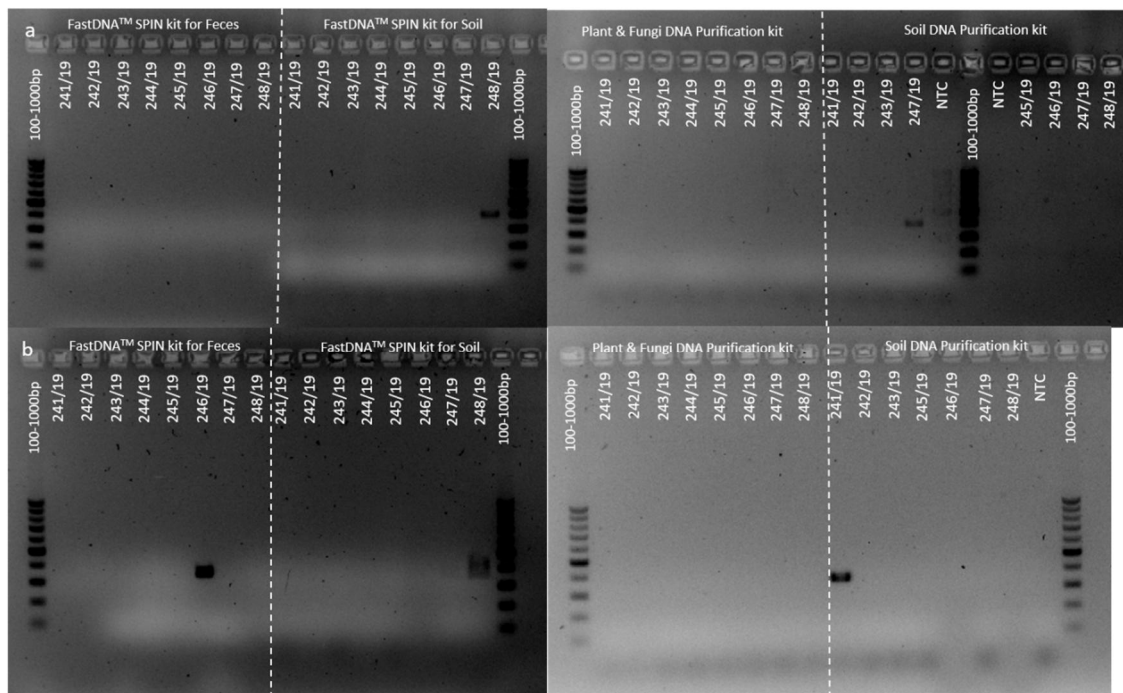


Figure S20. The specificity of the primer on naturally infected strawberry fruit. PCR replication of the amplification using *B. cinerea* (a) and *Phytophthora* sp. (b) specific primers in strawberry naturally infected using 4 different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control. The samples 244/19 and 245/19 came from a healthy plant; whereas 241/19-243/19 and 246/19 and 247/19 were collected from symptomatic plants diseased by pathogens.



Figure S21. The specificity of the primer on naturally infected strawberry fruit. PCR amplification using *B. cinerea* (a), *Colletotrichum sp.* (b), *Phytophthora sp.* (c), and *Verticillium sp.* (d) specific primers in strawberry fruit (241/19; 245/19; 246/19) naturally infected incubated at 24°C for 24, 48, and 72 hours using 2 different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control, and PTC is positive-template control (*B. cinerea* – G277/18; *Colletotrichum sp.* – G171/18; *Phytophthora sp.* – G408/18; *Verticillium sp.* – G296/18). The sample 245/19 came from a healthy plant; whereas 241/19 and 246/19 were collected from symptomatic plants diseased by pathogens. The replications are presented in the Figure S22.

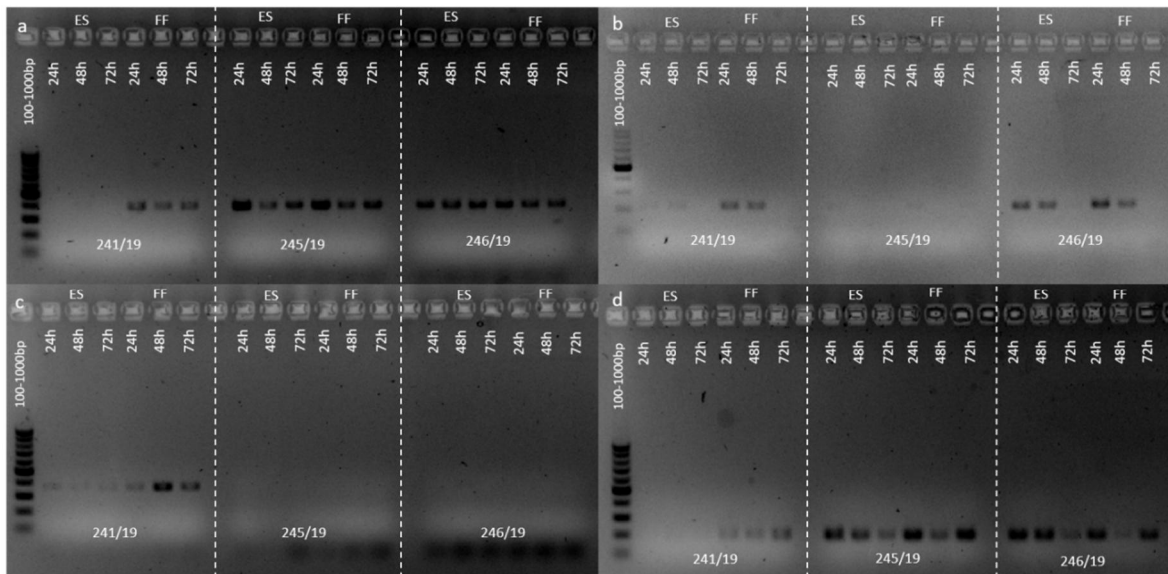


Figure S22. The specificity of the primer on naturally infected strawberry fruit. PCR replication of the amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in strawberry fruit naturally infected incubated at 24°C for 24, 48, and 72 hours using 2 different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control, PTC is positive-template control, ES is Soil DNA purification kit, and FF is FastDNA™ SPIN kit for Feces. The sample 245/19 came from a healthy plant; whereas 241/19 and 246/19 were collected from symptomatic plants diseased by pathogens.

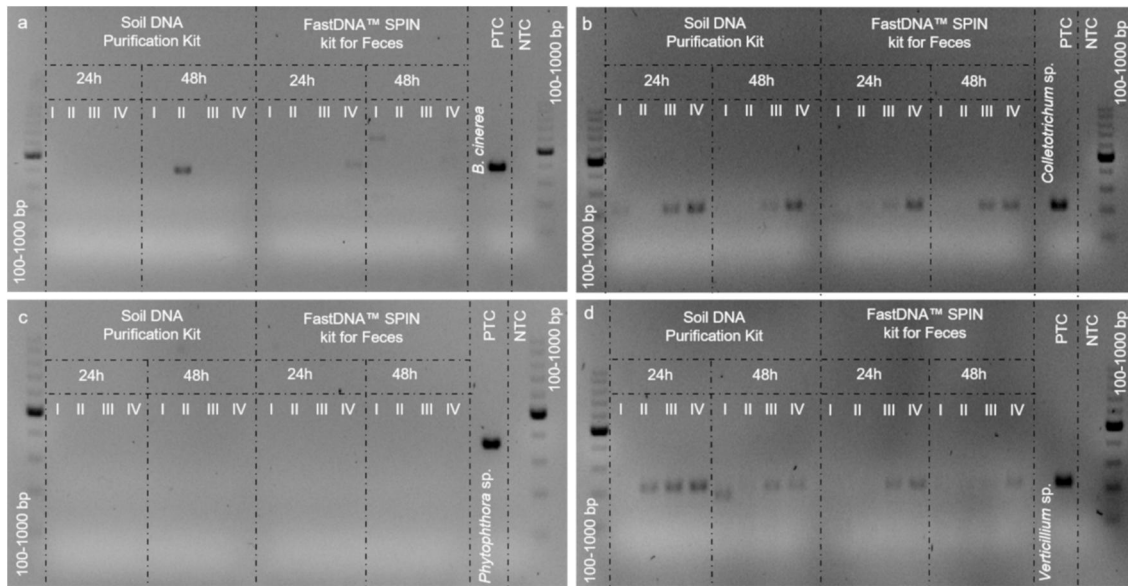


Figure S23. The specificity of the primer on artificially contaminated soil. PCR amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in soil artificially contaminated and incubated at 24°C for 24, and 48 hours using two different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC refers to non-template control, and PTC denotes positive-template control. The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 500; II – 1,000; III – 5,000; IV – 10,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil. The replications are presented in Figure S24.

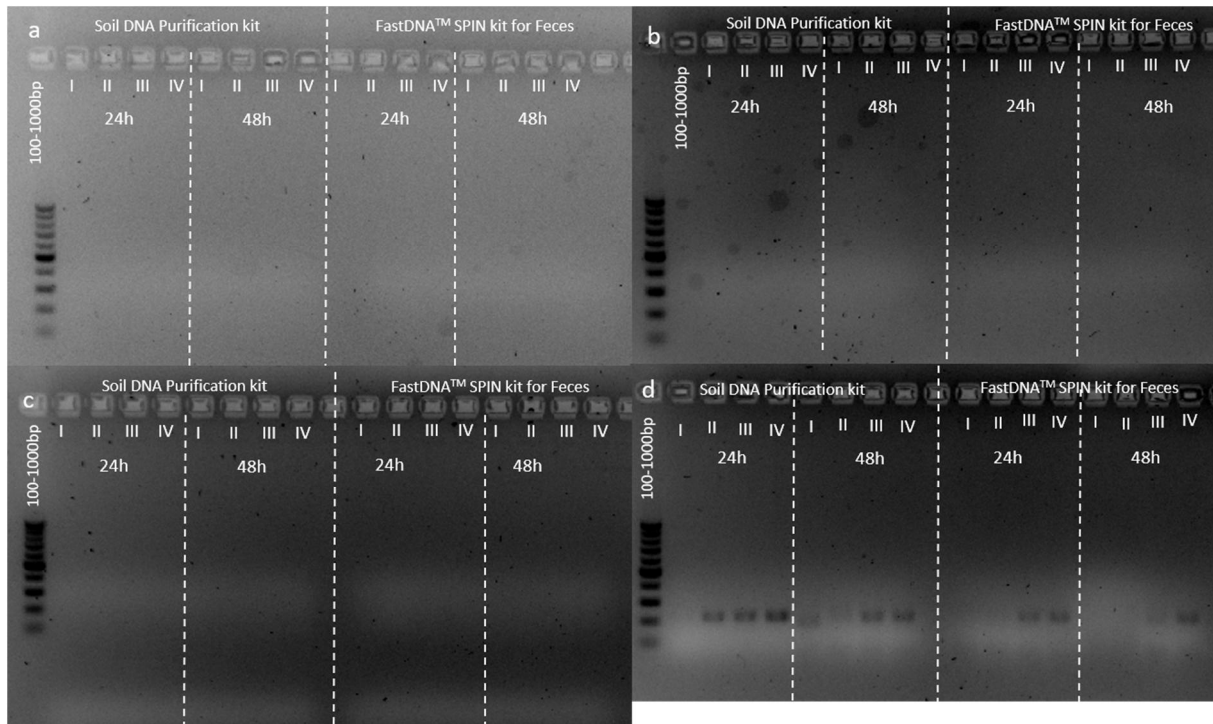


Figure S24. The specificity of the primer on artificially contaminated soil. PCR replication of the amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in soil artificial contaminated incubated at 24°C for 24, and 48 hours using two different kits of DNA extraction. Gel agarose 2% with 100-1,000 bp ladder. NTC means non-template control, and PTC is positive-template control. The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 500; II – 1,000; III – 5,000; IV – 10,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil.

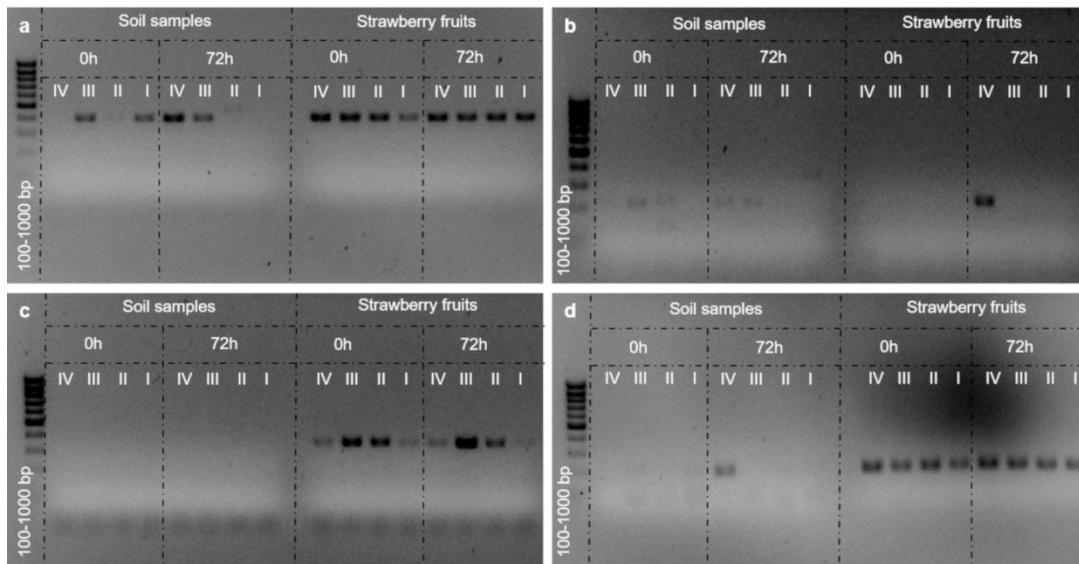


Figure S25. The specificity of the primer on artificially contaminated soil and strawberry fruit. PCR amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in artificially contaminated soil and strawberry, incubated at 24°C for 0 and 72 hours extracted with the FastDNA™ SPIN kit for Feces. Gel agarose 2% with 100-1,000 bp ladder. The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 100; II – 1,000; III – 10,000; IV – 100,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil. The replications are presented in the Figure S26.

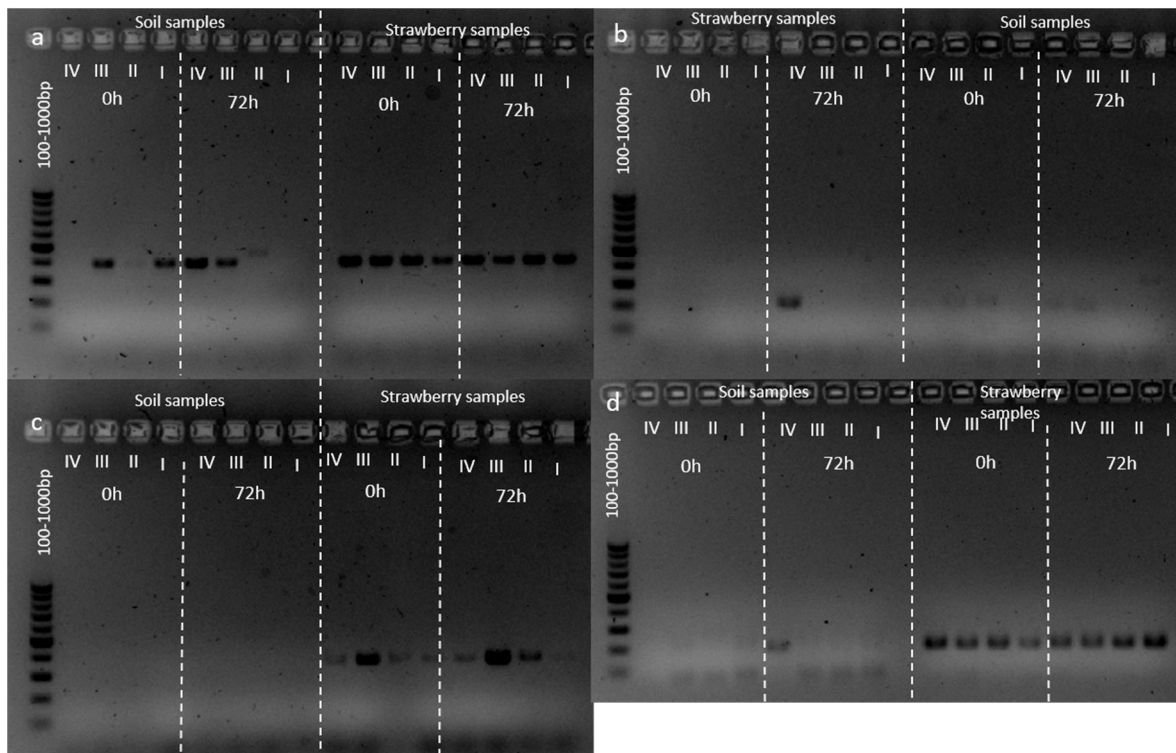


Figure S26. The specificity of the primer on artificially contaminated soil and strawberry fruit. PCR replication of the amplification using *B. cinerea* (a), *Colletotrichum* sp. (b), *Phytophthora* sp. (c), and *Verticillium* sp. (d) specific primers in artificially contaminated soil and strawberry, incubated at 24°C for 0 and 72 hours extracted with the FastDNA™ SPIN kit for Feces. Gel agarose 2% with 100-1,000 bp ladder. The roman numerals indicate the concentration of pathogen spores per gram of soil (I – 100; II – 1,000; III – 10,000; IV – 100,000 spores g⁻¹). The increase in roman numerals indicates the increase in spores of each pathogen inside the soil.

Table S1. List of the sequence obtained using the specific-primer designed on the functional genes.

| Organism | Strain | Source | D2 LSU identification | Functional gene identification |
|-----------------------------|---------------|------------------|----------------------------------|---|
| <i>Botrytis cinerea</i> | G276/18 | Root | MT154303.1 | MW826561 |
| | G277/18 | Root | MT154304.1 | MW826562 |
| | G321/18 | Root | MT154305.1 | MW826563 |
| | G322/18 | Root | MT154306.1 | MW826564 |
| | G323/18 | Root | MT154307.1 | MW826565 |
| <i>Colletotrichum</i> | G171/18 | Fruit | MT126802.1 | MW829155 |
| | G404/18 | Strawberry plant | MT126797.1 | MW829156 |
| | G406/18 | Strawberry plant | MT126795.1 | MW829157 |
| <i>Phytophthora</i> | G408/18 | Strawberry plant | MT126670.1 | MW829168 |
| | G409/18 | Strawberry plant | MT126671.1 | MW829169 |
| | G412/18 | Strawberry plant | MT126672.1 | MW829170 |
| | G413/18 | Strawberry plant | MT126673.1 | MW829171 |
| | G415/18 | Strawberry plant | MT126674.1 | MW829172 |
| | G417/18 | Strawberry plant | MT126676.1 | MW829173 |
| | G418/18 | Strawberry plant | MT126677.1 | MW829174 |
| | G419/18 | Strawberry plant | MT126678.1 | MW829175 |
| | G420/18 | Strawberry plant | MT126679.1 | MW829176 |
| | G421/18 | Strawberry plant | MT126680.1 | MW829177 |
| | G429/18 | Strawberry plant | MT126681.1 | MW829178 |
| | G430/18 | Strawberry plant | MT126682.1 | MW829179 |
| | G432/18 | Strawberry plant | MT126684.1 | MW829180 |
| | G434/18 | Strawberry plant | MT126685.1 | MW829181 |
| | G437/18 | Strawberry plant | MT126686.1 | MW829182 |

| | | | | |
|------------------------------------|---------|------------------|------------|----------|
| | G439/18 | Strawberry plant | MT126687.1 | MW829183 |
| | G441/18 | Strawberry plant | MT126689.1 | MW829184 |
| | G442/18 | Strawberry plant | MT126690.1 | MW829185 |
| <i>Verticillium</i> | G293/18 | Root | MT133324.1 | MW829158 |
| | G294/18 | Root | MT133317.1 | MW829159 |
| | G296/18 | Root | MT133320.1 | MW829160 |
| | G297/18 | Root | MT133316.1 | MW829161 |
| | G298/18 | Root | MT133318.1 | MW829162 |
| | G302/18 | Root | MT133314.1 | MW829163 |
| | G328/18 | Root | MT133326.1 | MW829164 |
| | G330/18 | Root | MT133315.1 | MW829165 |
| | G335/18 | Root | MT133322.1 | MW829166 |
| | G344/18 | Root | MT133321.1 | MW829167 |
| <i>Colletotrichum acutatum</i> | G162/18 | Strawberry fruit | - | PV013547 |

Table S2. Description of the primer's concentration and PCR thermoprofile for detection in naturally and artificially contaminated environmental samples.

| Fungal strain | Final concentration primer | DNA | Thermoprofile |
|---|-----------------------------------|----------------------------------|--|
| <i>Botrytis cinerea</i> (G669/16; G277/18) | 1 μ M | 3 μ l of diluted eDNA (1:10) | 94°C for 3 min; 35 cycles at 94°C for 15 sec, 50°C for 30 sec, 72°C for 45 sec; followed by 72°C for 10 min |
| <i>Colletotrichum sp.</i> (G171/18) | 1 μ M | 4 μ l diluted eDNA (1:10) | 94°C for 3 min; 5 cycles at 94°C for 15 sec, 54°C (touchdown -1°C) for 15 sec, 72°C for 15 sec; followed by 35 cycles at 94°C for 15 sec, 48°C for 15 sec, 72°C for 15 sec; followed by 72°C for 7 min |
| <i>Phytophthora sp.</i> (G408/18) | 1 μ M | 2 μ l diluted eDNA (1:10) | 94°C for 3 min; 35 cycles at 94°C for 15 sec, 60°C for 40 sec, 72°C for 45 sec; followed by 72°C for 10 min |
| <i>Verticillium sp.</i> (G296/18) | 1 μ M | 4 μ l diluted eDNA (1:10) | 94°C for 3 min; 5 cycles at 94°C for 15 sec, 51°C (touchdown -1°C) for 15 sec, 72°C for 15 sec; followed by 40 cycles at 94°C for 15 sec, 46°C for 15 sec, 72°C for 15 sec; followed by 72°C for 7 min |

Table S3. List of GenBank number of the sequences were used to draw the specific primer for *Colletotrichum acutatum*

| Pathogen | Accession numbers of sequences used to design primers | Accession numbers of sequences used to check specificity of designed primers on the basis of in silico analysis |
|---|--|--|
| <i>Colletotrichum acutatum</i> | KT228308.1 <i>Colletotrichum acutatum</i> | PP737585.1 <i>Colletotrichum nymphaeae</i> |
| | JX876552.1 <i>Glomerella acutata</i> | PP729319.1 <i>Colletotrichum cairnsense</i> |
| | JX876551.1 <i>Glomerella acutata</i> | PP729309.1 <i>Colletotrichum cairnsense</i> |
| | JX876550.1 <i>Glomerella acutata</i> | LC812073.1 <i>Colletotrichum nymphaeae</i> |
| | FJ972581.1 <i>Glomerella acutata</i> | PP565773.1 <i>Colletotrichum scovillei</i> |
| | FJ972580.1 <i>Glomerella acutata</i> | OR493925.1 <i>Colletotrichum</i> sp. |
| | FJ788418.1 <i>Glomerella acutata</i> | OR493924.1 <i>Colletotrichum</i> sp. |
| | FJ025811.1 <i>Glomerella acutata</i> | PP320349.1 <i>Colletotrichum scovillei</i> |
| | MG656080.1 <i>Colletotrichum coccodes</i> | PP320348.1 <i>Colletotrichum scovillei</i> |
| | KU821407.1 <i>Colletotrichum coccodes</i> | PP085963.1 <i>Colletotrichum scovillei</i> |
| | KU821332.1 <i>Colletotrichum nigrum</i> | PP085962.1 <i>Colletotrichum scovillei</i> |
| | KU821331.1 <i>Colletotrichum coccodes</i> | PP085961.1 <i>Colletotrichum scovillei</i> |
| | KU821318.1 <i>Colletotrichum nigrum</i> | PP085960.1 <i>Colletotrichum scovillei</i> |
| | KU821304.1 <i>Colletotrichum coccodes</i> | PP085958.1 <i>Colletotrichum scovillei</i> |
| | KT228301.1 <i>Colletotrichum gigasporum</i> | PP085957.1 <i>Colletotrichum scovillei</i> |
| | KJ781335.1 <i>Colletotrichum truncatum</i> | PP085954.1 <i>Colletotrichum scovillei</i> |
| | JX870646.1 <i>Colletotrichum capsici</i> | PP085953.1 <i>Colletotrichum scovillei</i> |
| | JQ400014.1 <i>Colletotrichum hemerocallidis</i> | PP085951.1 <i>Colletotrichum scovillei</i> |
| | JQ400011.1 <i>Colletotrichum liriopes</i> | PP085950.1 <i>Colletotrichum scovillei</i> |
| | JQ400010.1 <i>Colletotrichum spaethianum</i> | PP085949.1 <i>Colletotrichum scovillei</i> |
| | JN050232.1 <i>Colletotrichum thailandicum</i> | PP048929.1 <i>Colletotrichum nymphaeae</i> |
| JN050230.1 <i>Colletotrichum tropicicola</i> | PP048922.1 <i>Colletotrichum nymphaeae</i> | |
| HM585383.1 | PP048921.1 | |

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| <i>Colletotrichum liriopes</i> | <i>Colletotrichum nymphaeae</i> |
| / | OQ511701.1 <i>Colletotrichum scovillei</i> |
| / | OQ511698.1 <i>Colletotrichum nymphaeae</i> |
| / | OR995765.1 <i>Colletotrichum nymphaeae</i> |
| / | OR767823.1 <i>Colletotrichum nymphaeae</i> |
| / | OR271561.1 <i>Colletotrichum nymphaeae</i> |
| / | OR271560.1 <i>Colletotrichum nymphaeae</i> |
| / | OR271559.1 <i>Colletotrichum nymphaeae</i> |
| / | OR251073.1 <i>Colletotrichum nymphaeae</i> |
| / | LC778045.1 <i>Colletotrichum</i> sp. |
| / | OR043479.1 <i>Colletotrichum nymphaeae</i> |
| / | OR043473.1 <i>Colletotrichum nymphaeae</i> |
| / | OR043480.1 <i>Colletotrichum fruticola</i> |
| / | OQ799892.1 <i>Colletotrichum scovillei</i> |
| / | OQ799891.1 <i>Colletotrichum scovillei</i> |
| / | OQ799890.1 <i>Colletotrichum scovillei</i> |
| / | OQ799889.1 <i>Colletotrichum scovillei</i> |
| / | OQ799888.1 <i>Colletotrichum scovillei</i> |
| / | OQ613575.1 <i>Colletotrichum scovillei</i> |
| / | OQ613574.1 <i>Colletotrichum scovillei</i> |
| / | OQ613573.1 <i>Colletotrichum scovillei</i> |
| / | OQ613572.1 <i>Colletotrichum scovillei</i> |
| / | OQ613571.1 <i>Colletotrichum scovillei</i> |
| / | OQ613570.1 <i>Colletotrichum scovillei</i> |
| / | OQ613569.1 <i>Colletotrichum scovillei</i> |
| / | OQ613568.1 <i>Colletotrichum scovillei</i> |
| / | OQ613567.1 <i>Colletotrichum scovillei</i> |

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| / | OQ613566.1 <i>Colletotrichum scovillei</i> |
| / | OQ613565.1 <i>Colletotrichum scovillei</i> |
| / | OQ613564.1 <i>Colletotrichum scovillei</i> |
| / | OP156923.1 <i>Colletotrichum scovillei</i> |
| / | OP156922.1 <i>Colletotrichum scovillei</i> |
| / | OP156921.1 <i>Colletotrichum scovillei</i> |
| / | OP156920.1 <i>Colletotrichum scovillei</i> |
| / | OP156919.1 <i>Colletotrichum scovillei</i> |
| / | OP156918.1 <i>Colletotrichum scovillei</i> |
| / | OQ874731.1 <i>Colletotrichum scovillei</i> |
| / | OQ859040.1 <i>Colletotrichum scovillei</i> |
| / | OP746004.1 <i>Colletotrichum nymphaeae</i> |
| / | OP746003.1 <i>Colletotrichum nymphaeae</i> |
| / | OP746002.1 <i>Colletotrichum nymphaeae</i> |
| / | OP746001.1 <i>Colletotrichum nymphaeae</i> |
| / | OP746000.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745999.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745998.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745997.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745996.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745995.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745994.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745993.1 <i>Colletotrichum nymphaeae</i> |
| / | OP745992.1 <i>Colletotrichum nymphaeae</i> |
| / | OQ200436.1 <i>Colletotrichum nymphaeae</i> |
| / | OQ200435.1 <i>Colletotrichum nymphaeae</i> |
| / | OQ200434.1 <i>Colletotrichum nymphaeae</i> |

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| / | OQ200433.1 <i>Colletotrichum nymphaeae</i> |
| / | OQ200432.1 <i>Colletotrichum nymphaeae</i> |
| / | OQ127268.1 <i>Colletotrichum scovillei</i> |
| / | ON457860.1 <i>Colletotrichum nymphaeae</i> |
| / | ON457859.1 <i>Colletotrichum nymphaeae</i> |
| / | LC742915.1 <i>Colletotrichum fioriniae</i> |
| / | OP781555.1 <i>Colletotrichum nymphaeae</i> |
| / | OP781547.1 <i>Colletotrichum nymphaeae</i> |
| / | OP750572.1 <i>Colletotrichum nymphaeae</i> |
| / | OP750571.1 <i>Colletotrichum nymphaeae</i> |
| / | OP750552.1 <i>Colletotrichum nymphaeae</i> |
| / | MZ999002.1 <i>Colletotrichum</i> sp. |
| / | MZ999001.1 <i>Colletotrichum</i> sp. |
| / | MZ999000.1 <i>Colletotrichum</i> sp. |
| / | MZ998999.1 <i>Colletotrichum</i> sp. |
| / | MZ998998.1 <i>Colletotrichum</i> sp. |
| / | MZ998997.1 <i>Colletotrichum</i> sp. |
| / | MZ998996.1 <i>Colletotrichum</i> sp. |
| / | MZ998995.1 <i>Colletotrichum</i> sp. |
| / | MZ998994.1 <i>Colletotrichum</i> sp. |
| / | MZ998993.1 <i>Colletotrichum</i> sp. |
| / | MZ998992.1 <i>Colletotrichum</i> sp. |
| / | MZ998991.1 <i>Colletotrichum</i> sp. |
| / | MZ998990.1 <i>Colletotrichum</i> sp. |
| / | PP760163.1 <i>Colletotrichum fioriniae</i> |
| / | PP729312.1 <i>Colletotrichum cairnsense</i> |
| / | LC812093.1 <i>Colletotrichum fioriniae</i> |

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| / | PP576667.1 <i>Colletotrichum fioriniae</i> |
| / | PP576664.1 <i>Colletotrichum fioriniae</i> |
| / | PP576662.1 <i>Colletotrichum fioriniae</i> |
| / | PP576655.1 <i>Colletotrichum fioriniae</i> |
| / | PP576654.1 <i>Colletotrichum fioriniae</i> |
| / | OR644992.1 <i>Colletotrichum</i> sp. |
| / | OR596675.1 <i>Colletotrichum fioriniae</i> |
| / | OR493928.1 <i>Colletotrichum</i> sp. |
| / | OR493927.1 <i>Colletotrichum</i> sp. |
| / | OR493926.1 <i>Colletotrichum</i> sp. |
| / | PP085959.1 <i>Colletotrichum scovillei</i> |
| / | PP085956.1 <i>Colletotrichum scovillei</i> |
| / | PP085955.1 <i>Colletotrichum scovillei</i> |
| / | PP085952.1 <i>Colletotrichum scovillei</i> |
| / | LC797741.1 <i>Colletotrichum fioriniae</i> |
| / | PP091748.1 <i>Colletotrichum cairnsense</i> |
| / | PP048931.1 <i>Colletotrichum fioriniae</i> |
| / | PP048922.1 <i>Colletotrichum nymphaeae</i> |
| / | OR140163.1 <i>Colletotrichum fioriniae</i> |
| / | OR140162.1 <i>Colletotrichum fioriniae</i> |
| / | OR140161.1 <i>Colletotrichum fioriniae</i> |
| / | OR140160.1 <i>Colletotrichum fioriniae</i> |
| / | OR140159.1 <i>Colletotrichum fioriniae</i> |
| / | OQ511700.1 <i>Colletotrichum nymphaeae</i> |
| / | OR251071.1 <i>Colletotrichum fioriniae</i> |
| / | OR251070.1 <i>Colletotrichum fioriniae</i> |
| / | OR251069.1 <i>Colletotrichum fioriniae</i> |

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| / | OR238921.1 <i>Colletotrichum fioriniae</i> |
| / | OR238920.1 <i>Colletotrichum fioriniae</i> |
| / | OR842619.1 <i>Colletotrichum fioriniae</i> |
| / | OR766557.1 <i>Colletotrichum fioriniae</i> |
| / | OR282741.1 <i>Colletotrichum fioriniae</i> |
| / | LC778049.1 <i>Colletotrichum fioriniae</i> |
| / | OQ929149.1 <i>Colletotrichum</i> sp. |
| / | OQ929148.1 <i>Colletotrichum</i> sp. |
| / | OQ929147.1 <i>Colletotrichum</i> sp. |
| / | OQ929146.1 <i>Colletotrichum</i> sp. |
| / | OQ929145.1 <i>Colletotrichum</i> sp. |
| / | OQ929144.1 <i>Colletotrichum</i> sp. |
| / | OQ929126.1 <i>Colletotrichum</i> sp. |
| / | OQ067617.1 <i>Colletotrichum fioriniae</i> |
| / | OQ613605.1 <i>Colletotrichum fioriniae</i> |
| / | OQ613604.1 <i>Colletotrichum fioriniae</i> |
| / | OQ613603.1 <i>Colletotrichum fioriniae</i> |
| / | LC722617.1 <i>Colletotrichum nymphaeae</i> |
| / | LC722616.1 <i>Colletotrichum fioriniae</i> |
| / | LC722612.1 <i>Colletotrichum fioriniae</i> |
| / | LC722611.1 <i>Colletotrichum fioriniae</i> |