

# Quantification of *Botrytis cinerea* Growth in *Arabidopsis thaliana*

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## Abstract

Yield losses attributed to plant pathogens pose a serious threat to plant productivity and food security. *Botrytis cinerea* is one of the most devastating plant pathogens, infecting a wide array of plant species; it has also been established as a model organism to study plant–pathogen interactions. In this context, development of different assays to follow the relative success of *B. cinerea* infections is required. Here, we describe two methods to quantify *B. cinerea* development in *Arabidopsis thaliana* genotypes through measurements of lesion development and quantification of fungal genomic DNA in infected tissues. This provides two independent techniques that are useful in assessing the susceptibility or tolerance of different *Arabidopsis* genotypes to *B. cinerea*.

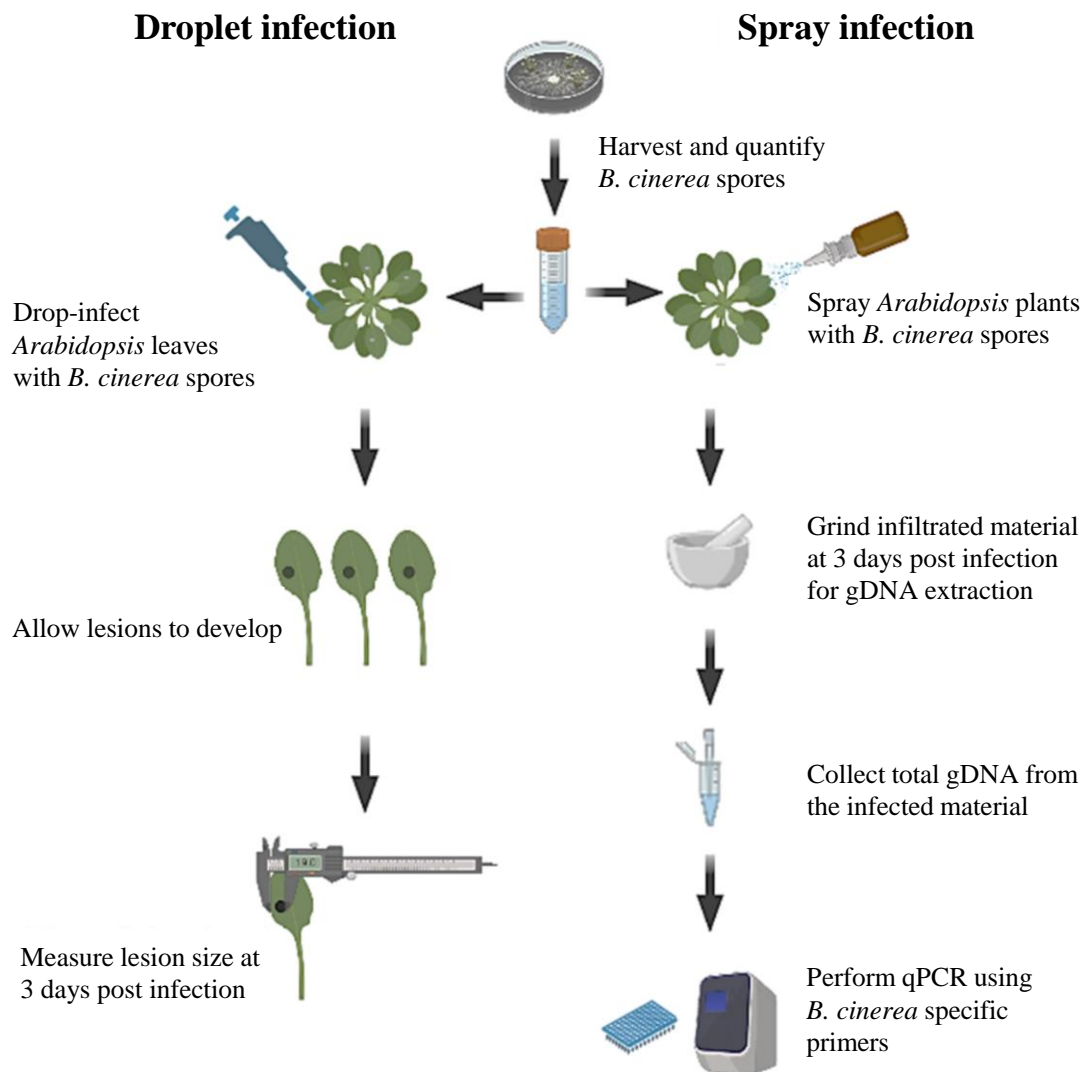
## Key features

- Protocol for the propagation of the necrotrophic plant pathogen fungus *Botrytis cinerea* and spore production.
- Two methods of *Arabidopsis thaliana* infection with the pathogen using droplet and spray inoculation.
- Two readouts, either by measuring lesion size or by the quantification of fungal DNA using quantitative PCR.
- The two methods are applicable across plant species susceptible the *B. cinerea*.

**Keywords:** *Arabidopsis thaliana*, *Botrytis cinerea*, Fungal pathogen, Pathogen assay, Quantitative PCR

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## Graphical overview



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A simplified overview of the droplet and spray infection methods used for the determination of *B. cinerea* growth in different *Arabidopsis* genotypes

## Background

Agricultural productivity is constantly under threat from plant pathogens including bacteria, fungi, oomycetes, and viruses, contributing to global losses of 10%–28% in crop production to pests (Savary et al., 2019; IPPC Secretariat, 2021). Among the pathogens, *Botrytis cinerea* is a necrotrophic fungal phytopathogen distributed worldwide that causes great damage to a large diversity of plants, including various crops, leading to its classification among the top 10 worst fungal pathogens (Dean et al., 2012; Caseys et al., 2021). *B. cinerea* is considered a generalist necrotroph that kills the host tissues and takes up nutrients from the dead tissue. Unlike other plant pathogens, it has

a very broad host range, affecting more than 1,000 plant species (Elad et al., 2016; Caseys et al., 2021; Bi et al., 2022).

The strong economic impact with regard to yield loss and the broad range of plant hosts make *B. cinerea* an interesting model organism for plant pathogen research, especially as it also infects the model plant species *Arabidopsis thaliana*. This allowed, for example, extensive transcriptomic analysis of the necrotroph infection (Windram et al., 2012) or studies on how the genetic variation of *Arabidopsis* or *B. cinerea* influences disease outcomes (Kliebenstein et al., 2005; Rowe and Kliebenstein, 2008; Soltis et al., 2020). Furthermore, *B. cinerea* infection assays can be used to observe different aspects of the plant immune responses in *Arabidopsis* (Genenncher et al., 2016; Guzha et al., 2022). Here, we show two straightforward methods that can be used in parallel to assess the disease tolerance of different *Arabidopsis* genotypes. One method involves drop inoculation of *B. cinerea* spores and measuring the average lesion development on the infected leaves (Genenncher et al., 2016; Guzha et al., 2022). The other approach involves the quantification of *B. cinerea* genomic DNA (gDNA) in infected *Arabidopsis* tissues (Ettenauer et al., 2014; Guzha et al., 2022). These two methods provided are versatile and can be applied to different plant species susceptible to the pathogen with minimal modifications.

## Materials and reagents

### Biological materials

1. *Arabidopsis* seeds
2. *B. cinerea* strain B05-10

### Reagents

1. Vogel buffer
2. Potato dextrose agar (PDA) (Merck, catalog number: 70139)
3. Potato dextrose broth (PDB) (Merck, catalog number: P6685)
4. Glycerol (Thermo Scientific, catalog number: 17904)
5. Plant/Fungi DNA Isolation kit (Norgen Biotek Corporation, catalog number: 26200)
6. Takyon No Rox SYBR master mix dTTP Blue (Eurogentec UF-NSMT-B0701)
7. *B. cinerea* *ACTIN* forward primer 5'-TGGAGATGAAGCGCAATCCA-3' (Genewiz™)
8. *B. cinerea* *ACTIN* reverse primer 5'-AAGCGTAAAGGGAGAGGACG-3' (Genewiz™)
9. *B. cinerea* *TUBULIN* forward primer 5'-CCGTCATGTCCGGTGTAC-3' (Genewiz™)
10. *B. cinerea* *TUBULIN* reverse primer 5'-CGACCGTTACGGAAATCGG-3' (Genewiz™)

### Solutions

1. Potato dextrose agar (PDA) and potato dextrose broth (PDB) (see Recipes)
2. Sterile Vogel buffer (see Recipes)
3. 70% ethanol (see Recipes)
4. 15% glycerol (see Recipes)

### Recipes

#### 1. Potato dextrose agar (PDA) and potato dextrose broth (PDB)

Potato dextrose agar (PDA)

For 1 L of PDA, weigh 39 g of commercial PDA powder (contains 20 g of dextrose, 15 g of agar, and 4 g of potato extract), and suspend in 1 L of deionised water. Autoclave the solution at 121 °C for 20 min.

Potato dextrose broth (PDB)

For 1 L of PDB, weigh 24 g of commercial PDB powder (contains 20 g of dextrose, 4 g of potato extract) and dissolve in 1 L of deionised water. Autoclave at 121 °C for 20 min.

## 2. Sterile Vogel buffer

- For 1 L of Vogel buffer, weigh the compounds as shown in Table 1 and fill up to 900 mL with distilled water. Mix until all compounds are dissolved.

**Table 1. Preparation of Vogel buffer**

Constituent	Amount
Sucrose	15 g
Trisodium citrate·2H <sub>2</sub> O	2.5 g
K <sub>2</sub> HPO <sub>4</sub>	5 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1 g
NH <sub>4</sub> NO <sub>3</sub>	2 g

- Adjust to pH 6.0 and fill up the volume to 1 L. Sterilise the buffer by autoclaving.

## 3. 70% ethanol

Reagent	Final concentration	Amount
Ethanol (absolute)	70%	700 mL
H <sub>2</sub> O	n/a	300 mL
Total	n/a	1,000 mL

## 4. 15% glycerol

Reagent	Final concentration	Amount
Glycerol	15%	150 mL
H <sub>2</sub> O	n/a	850 mL
Total	n/a	1,000 mL

Sterilize by autoclaving.

## Laboratory supplies

- 10 cm plant pots
- 25 cm × 51 cm × 6 cm propagation trays
- 25 cm × 51 cm × 18 cm high standard dome
- Arabidopsis* soil: Fruhstofer Erde
- Chemical-resistant gloves
- Miracloth (Merck, catalog number: 475855)
- Kimwipes® (Kimtech Science, catalog number: 34120)
- 2 mL microcentrifuge tube (Sarstedt, catalog number: 72.695.500)
- 1.5 mL microcentrifuge tube (Sarstedt, catalog number: 72.690.001)
- Flat cap PCR tubes (Sarstedt 72.985.002 (tubes) and 65.989.002 (lids))
- 60 mL glass spray bottle (Uline, catalog number: S24562A)

## Equipment

1. Growth chamber with light and temperature controls (Percival Scientific Inc.)
2. Oven for sterilisation of soil
3. Microcentrifuge
4. Micropipettes
5. Scalpel
6. Mortar and pestle
7. MarCal 16ER digital caliper (Mahr, Göttingen, Germany)
8. Bio-Rad iQ5 Multicolor Real-Time PCR Detection System
9. NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific)
10. Fuchs-Rosenthal counting chamber (Paul Marienfeld, Lauda-Königshofen, Germany)

## Software and datasets

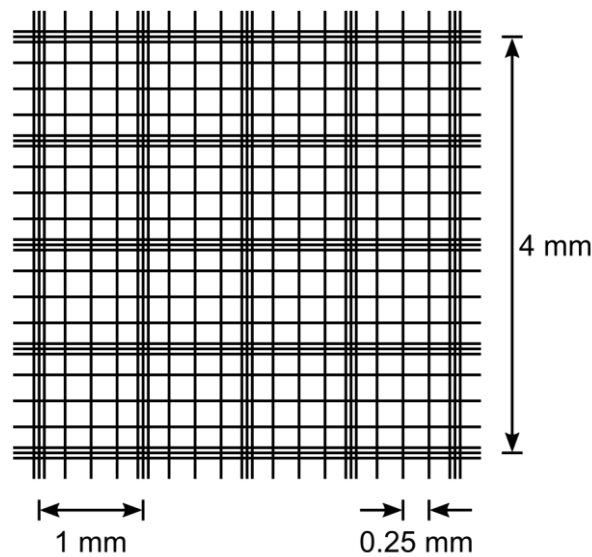
1. MarCom Standard Software for MarCal digital caliper
2. iQ5 Optical System Software (Bio-Rad Laboratories GmbH-Munich, Germany)
3. Microsoft Excel

## Procedure

### A. Preparation of fungal spores

When working with spores of *B. cinerea*, take care to avoid any infection of *Arabidopsis* plants. Keep working areas for *B. cinerea* and *Arabidopsis* plants distant from each other and avoid working with plants after you prepared *B. cinerea* spores (see Notes).

1. Inoculate 5  $\mu$ L of spore suspension onto the centre of PDA plates at room temperature under sterile conditions. Incubate the closed plate with spores at room temperature. Do not seal plates until mycelium on the agar is covered with grey spores (4–5 days).
2. Propagate the spores by transferring clean mycelium grown on the PDA plate to new PDA plates. To that end, cut out a small piece of mycelium-covered agar (1 cm  $\times$  1 cm) using a sterile scalpel and transfer it upside down on the new plate. Take care to transfer only areas with a clean layer of *B. cinerea* mycelium that does not contain any contaminations. An example of a clean *B. cinerea* culture can be found in Vasquez-Montano et al. (2020). For long-term storage of spores, harvest the spores from *B. cinerea* growing for 10 days on PDB plates without contaminations. In case of contaminations, cut out a section in the media with clean hyphae and grow on fresh PDA plates until a clean culture is obtained as described in step A2. Scrape spores from plates using a sterile scalpel before resuspending them with 20 mL of 15% sterile glycerol and filter through Miracloth.
3. Estimate the harvested spore counts using a Fuchs-Rosenthal counting chamber.
  - a. Clean the counting chamber and the coverslip with 70% ethanol. Put the glass cover on the counting chamber. Vortex the spore suspensions and pipette at least 10  $\mu$ L aliquot slowly against the edge of the coverslip so that the counting chamber is filled by capillary force.
  - b. The Fuchs-Rosenthal counting chamber has a grid pattern of 16 squares of 1 mm  $\times$  1 mm that are subdivided further into 16 squares of 0.25 mm  $\times$  0.25 mm (see Figure 1) and a depth of 0.2 mm. Count the spore numbers in the 1 mm  $\times$  1 mm square areas. For one aliquot, count 16 square millimetre areas and calculate the average spore number.



**Figure 1. Grid of a Fuchs-Rosenthal counting chamber.** Sixteen areas of 1 mm<sup>2</sup> are delimited by triple lines and further subdivided into 0.25 mm × 0.25 mm squares by single lines. The counting chambers depth is 0.2 mm, so a 1 mm<sup>2</sup> area corresponds to 0.2 μL or 0.0002 mL.

- c. Count three independent aliquots of 10 μL from the spore suspension in 15% glycerol and calculate the average spore number per square millimetre area. Vortex the spore suspension before each aliquot to obtain a homogeneous suspension. Calculate the spore concentration as follows:

$$\text{spore concentration (spores/mL)} = \frac{\text{number spores in square millimeter area}}{0.0002 \text{ mL}}$$

4. Adjust the concentration to  $2 \times 10^6$  spores/mL in 15% glycerol. If the spore concentration is too low, centrifuge the spore suspension and remove some of the supernatant liquid. Determine the spore concentration again (as described in step A3) and then adjust to a final concentration of  $2 \times 10^6$  spores/mL.
5. Prepare 1 mL aliquots from this spore suspension for storage. While preparing the aliquots, take care to keep the spores homogeneously suspended by regularly vortexing. Flash-freeze the aliquots in liquid nitrogen and store at -80 °C.

## B. Plant growth

To facilitate the accurate comparison of *Arabidopsis* genotypes, the plants must be grown under identical conditions and preferably in the same trays, which are rotated at least once every week. As an example, the procedure used in Guzha et al. (2022) and Jacobs et al. (2017) that allow comparisons of *Arabidopsis* genotypes' susceptibility to plant pathogens is outlined below. In addition, check the Notes section for comments on biological replicates.

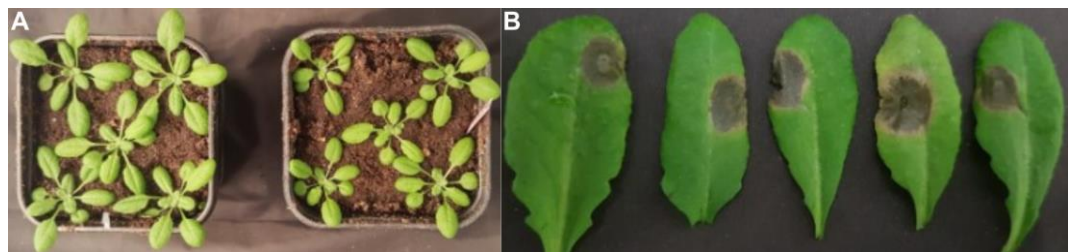
1. Prepare semi-sterile soil sufficient for the desired number of pots by heating it in an oven at 80 °C for 8 h.
2. Transfer enough soil to fill square 10 cm pots and water the pots by placing water in the trays until the soil reaches saturation, before draining excess water. Place 6–10 *Arabidopsis* seeds in the four corners and the centre of the pots, which will be thinned out later to leave one plant per station (Figure 2A). Cover with plastic dome.
3. After stratification at 4 °C, transfer to growth cabinet with short day conditions (8 h light and 16 h darkness) at 22 °C, a relative humidity of 65%, and a light intensity of 120–140 μmol m<sup>-2</sup>·s<sup>-1</sup>. Remove the plastic dome after germination of the seeds.
4. Grow plants for 7–10 days; then, thin out excess seedlings to leave one plant per position (Figure 2A).

5. Grow until desired stage.

### C. Droplet infection and measurements of lesion diameters

Take care not to infect other plants that are not part of the infection experiment. Keep infected plants separate from the normal plant growth chambers and avoid working with other plants after carrying out infection assays.

1. Prepare spores by gently thawing the frozen spores on ice. Dilute the thawed spore suspension to a final concentration of  $5 \times 10^4$  spores/mL in sterile Vogel buffer.
2. Let the spores germinate (40 mL in a Falcon tube) for at least 4 h at 25 °C (shaking is not necessary). *B. cinerea* spores germinate readily in Vogel buffer. Germination of spores can be checked under a light microscope.
3. Use 6-week-old plants grown under short day conditions as described above (section B). For each leaf, place a single 6 µL droplet of the germinated spore suspension onto the adaxial side of the leaf, in the centre next to the middle vein (Figure 2B). Inoculate at least 30 fully expanded leaves of each plant genotype (at least six leaves from each plant in a pot, as shown in Figure 2A). Between application of spore suspension droplets, regularly vortex the spore suspensions to keep a homogeneous spore distribution (every 2–3 droplets). Spore suspension droplets will stay on the leaves due to surface tension; however, take care not to shake the pots to ensure that droplets do not fall off the leaf.



**Figure 2.** *Arabidopsis* plants for infection with *Botrytis cinerea*. A. For each genotype, five plants are grown per pot for use in the infection assays. B. At least 30 leaves per pot are inoculated with 6 µL droplets of a *B. cinerea* spore suspension. Three days post infection, lesions develop at the infected area.

4. Add 0.5 L of water to the tray containing the plants and cover them with a lid to maintain high humidity conditions. Return plants to short-day growth conditions (distinct from normal growth chambers) until determination of lesion size.
5. Analyse lesion size 3 days post infection. To that end, use the MarCal 16ER digital calliper to measure lesion diameters.
  - a. Before first use: install the MarCom Standard Software according to manufacturer's instructions on the notebook you will subsequently use for measurements. Connect your digital calliper to the notebook and install the required software drivers. Use a notebook with Windows operating system that has MS Excel installed (see Notes).
  - b. For measurements, connect the digital calliper to the notebook with the installed MarCom software, open the software, and verify that the calliper has been recognised. Detach infected leaves from *Arabidopsis* plants and measure the diameter of the developed lesion with the calliper. Press the measurement button to transfer the data point to an Excel file, which will open after the first measurement.
  - c. Measure leaves from one plant genotype at a time and mark different genotypes in the Excel file with the measured data.



## D. Spray infection and isolation of fungal gDNA

Another way to assess susceptibility of *Arabidopsis* genotypes to *B. cinerea* infection is by spray-infection and subsequent quantification of fungal gDNA in plant tissues. When using the spray infection protocol, take care not to infect other plants that are not part of the infection experiment through aerosols generated. Keep infected plants separate from the normal plant growth chambers and avoid working with other plants after carrying out infection assays.

1. Prepare spores by gently thawing the required number of aliquots on ice. Dilute the thawed spore suspension to a final concentration of  $2 \times 10^5$  spores/mL in sterile Vogel buffer.
2. Let the spores germinate (50 mL in a Falcon tube) for at least 4 h at 25 °C. *B. cinerea* spores germinate readily in Vogel buffer.
3. Use 6-week-old plants grown under short day conditions as described above (section B). For spray infection, transfer the germinated spores to a spray bottle and spray evenly on the plants until liquid runs off the leaves.
4. Add 0.5 L water to the tray with the plants and cover them with a lid to keep humid conditions. Return plants to short-day growth conditions (distinct from the normal growth chambers) until harvest for gDNA isolation.
5. After three or four days, flash-freeze the rosettes in liquid nitrogen and store at -80 °C or proceed to isolation of plant and fungal gDNA.
6. For isolation of gDNA, the commercially available Plant/Fungi DNA Isolation kit was used according to manufacturer's instructions. The isolation of gDNA included the following steps:
  - a. Keep the harvested plant material cooled with liquid nitrogen and grind into a powder using a mortar and pestle. Transfer  $\leq 100$  mg of the powder to a 1.5 mL microcentrifuge tube, add 500  $\mu$ L of Lysis buffer and 1  $\mu$ L of RNase A, and vortex for 20 s.  
Note down the exact mass of powdered plant material for sample normalisation.
  - b. Lyse for 10 min at 65 °C, add 100  $\mu$ L of binding buffer I, mix by inverting the tube, and incubate on ice for 5 min.
  - c. Transfer the lysate to a filter column, centrifuge at  $20,000 \times g$  for 2 min, and transfer the supernatant to a clean 1.5 mL microcentrifugation tube. Add an equal volume of 70% ethanol and vortex.
  - d. Transfer 650  $\mu$ L of the sample to a spin column and centrifuge for 1 min at  $10,000 \times g$ . Discard the flowthrough and repeat if necessary.
  - e. Wash once with 500  $\mu$ L of Solution WN, centrifuge at  $20,000 \times g$  for 1 min, and discard the flowthrough.
  - f. In a similar manner, wash twice with 500  $\mu$ L of Wash Solution A. After the final washing step, centrifuge the column for 2 min at  $20,000 \times g$ .
  - g. Transfer the spin column to a fresh elution tube, add 100  $\mu$ L of Elution Buffer B, and incubate for 1 min at room temperature. Centrifuge for 1 min at  $10,000 \times g$ . If not all buffer has been eluted, centrifuge again for an additional 1 min at  $20,000 \times g$ .
  - h. Store isolated gDNA at -20 °C. The gDNA isolated in this step will be a combination of both plant and fungal gDNA. Quantification of fungal gDNA is made possible by the use of primers specific for fungal gDNA during quantitative PCR (section F).

## E. Fungal gDNA isolation for calibration

1. To generate a calibration curve for quantification of fungal gDNA, harvest spores from *B. cinerea* mycelium that has been growing on PDB plates for 10 days and collect them in a sterile Falcon tube. Harvest the spores by scraping them off with a sterile spatula.
2. Extract the gDNA using the Plant/Fungi DNA Isolation kit as described above.
3. Measure the concentration of the genomic DNA using the NanoDrop 2000 spectrophotometer.
4. Perform a serial dilution (1/2 dilution steps) of the gDNA and measure the concentrations on the NanoDrop 2000 spectrophotometer.
5. Perform qPCR analysis as described below.



## F. Quantitative PCR for fungal gDNA fungal genomic DNA

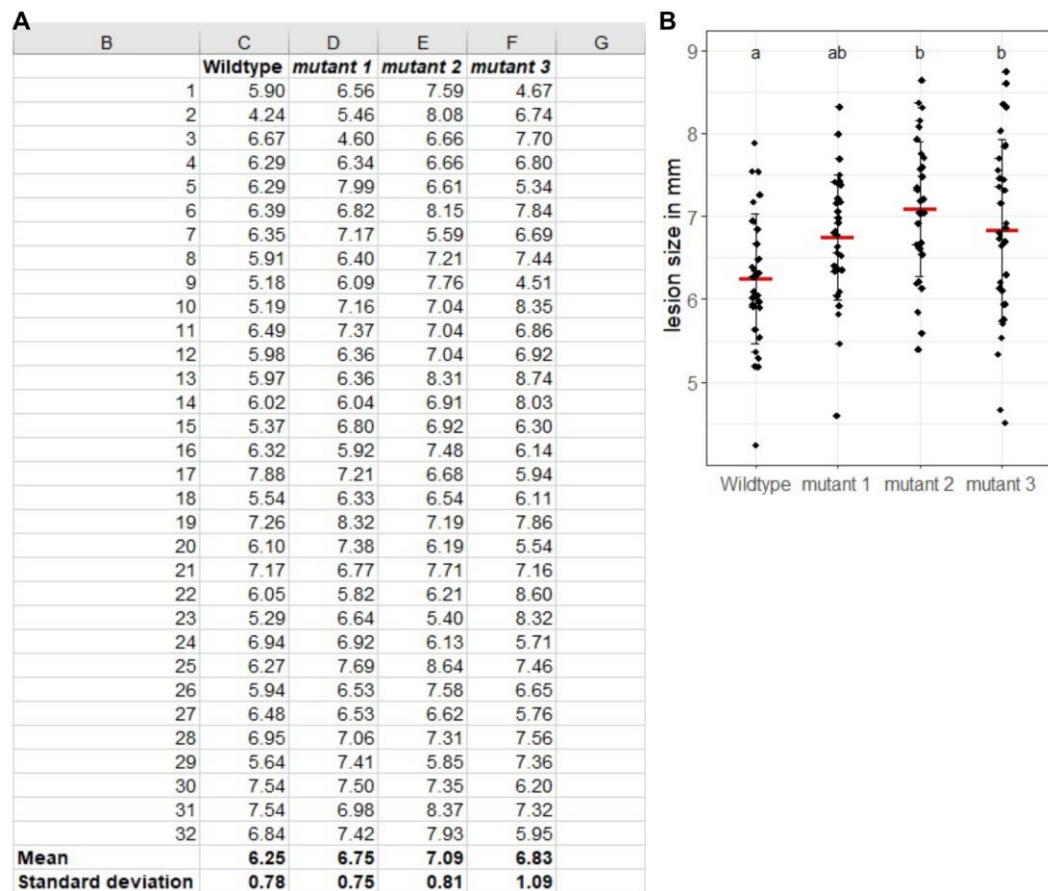
For quantification of fungal gDNA, primers for *B. cinerea* *ACTIN* or *TUBULIN* that are specific for fungal gDNA are used. Prepare three technical replicates per sample, including the calibration samples described in section E.

1. Prepare the reagents for the qPCR in the following proportions:  
 10 µL of 2× Takyon master mix  
 2.5 µL of DNA  
 2 µL of 4 µM forward primer  
 2 µL of 4 µM reverse primer  
 3.5 µL of H<sub>2</sub>O  
 Final volume: 20 µL
2. Prepare a master mix with 2× Takyon master mix, primers, and H<sub>2</sub>O in an autoclaved 1.5 mL tube (all reactions should be performed on ice). Perform each reaction in triplicate.
3. Load 17.5 µL of the master mix to PCR tubes with flat caps. When loading samples, take note of the arrangement of the samples.
4. Load 2.5 µL of the genomic DNA to the PCR tubes and carefully seal the flat caps.
5. Briefly vortex the samples before spinning them down. Ensure that there are no bubbles in the samples as they may interfere with signal detection.
6. Load samples into the Bio-Rad iQ5 qPCR cycler and wipe the tops of the PCR tubes with a Kimwipe to remove any dust or debris that may interfere with signal detection.
7. Reaction cycle: 95 °C for 1 min 20 s; (95 °C 20 s, 62 °C 20 s, 72 °C 40 s) × 40; 72 °C 4 min.  
 Melt curve analysis: 95 °C for 1 min; 60 °C for 1 min; increase to 95 °C in 0.5° increments, hold each temperature for 10 s.
8. After completion of the qPCR run, export the data to an Excel file to compute the quantifications.

## Data analysis

### A. Analysis of drop infections

With the digital calliper, data has already been transferred to an Excel spreadsheet. For calculation of mean, standard deviation, and standard error as well as graphical representation, Excel functions can be used. For statistical analysis beyond Student's *t*-test, export the data into software for statistical analysis of your choice. Figure 3 shows exemplary results derived from the drop infection method.



**Figure 3. Exemplary results of *B. cinerea* droplet infection assay.** At least 30 leaves were measured per genotype. The respective mean lesion diameter and the standard deviation were calculated in Microsoft Excel and are shown in the bottom two lines. (A) For visualisation and statistical analysis, R-Studio was used in this example. (B) Individual data points are shown as black dots in addition to the mean value (red crossbar) and the standard deviation. Different letters indicate significant differences with  $p < 0.05$  as calculated by ANOVA with post-hoc Tukey-test.

## B. Analysis of spray infections

1. Calibration of fungal gDNA
  - a. Calculate the used volumes of fungal gDNA in nanograms from the concentrations of your different samples from the serial dilution and the used volume per qPCR reaction. Determine the natural logarithm of the gDNA volume and plot the  $\log_{10}$  values of gDNA volume vs. respective mean Ct-values. Perform a linear regression analysis on the plot.  $R^2$  must be as close as possible to 1.
  - b. Determine the parameters  $m$  and  $b$  of the linear correlation between log-values of gDNA volume and mean Ct-values, for example with the “LINEST” function in Excel.

$$\text{mean Ct - value} = m \times \ln(\text{gDNA amounts in ng}) + b$$

- c. Note down  $m$  and  $b$  for subsequent calculation of fungal gDNA in infected plant samples.
2. Calculation of fungal gDNA in infected plant samples
  - a. With the parameters  $m$  and  $b$  determined above, calculate the volume of gDNA in the infected plant samples.

$$gDNA \text{ in ng} = e^{(mean \text{ Ct-value} - b)/m}$$

- b. As only a part of the isolated gDNA was used for the qPCR reaction, multiply the determined mass with a correction factor:

$$correction \ factor = \frac{elution \ volume \ of \ gDNA \ isolation}{gDNA \ volume \ used \ in \ qPCR \ reaction}$$

- c. Normalise the gDNA volume to the mass of the plant material used for gDNA extraction. Exemplarily, quantification of fungal gDNA from a spray-infected *Arabidopsis* leaf sample is shown below.

Sample mass: 190 mg ground *Arabidopsis* leaf material

Threshold cycle (Ct) value as determined by qPCR: 15.993

- Based on the calibration curve obtained, m and b values are determined:  
m = -2.179, b = 19.621
- Calculate the gDNA in each reaction cycle:

$$gDNA \text{ in ng} = e^{(mean \text{ Ct-value} - b)/m}$$

$$gDNA \text{ in ng} = e^{\frac{(15.993 - 19.621)}{-2.1793}} = 5.285 \text{ ng}$$

- Calculate the correction factor:

$$correction \ factor = \frac{elution \ volume \ of \ gDNA \ isolation}{gDNA \ volume \ used \ in \ qPCR \ reaction} = \frac{50 \ \mu L}{2.5 \ \mu L} = 20$$

- Calculate total volume of gDNA:

$$total \ gDNA = 5.285 \text{ ng} \times 20 = 105.7 \text{ ng}$$

- Normalise amount of fungal gDNA to plant material:

$$fungal \ gDNA \ per \ 100 \ mg \ plant \ material = \frac{105.7 \text{ ng fungal gDNA}}{1.9 \times 100 \text{ mg plant material}} = 55.63 \text{ ng}$$

## Validation of protocol

This protocol or its parts have been used in the following research articles:

- Genencher et al. (2016). Nucleoporin-Regulated MAP Kinase Signaling in Immunity to a Necrotrophic Fungal Pathogen. *Plant Physiol* (Figure 1).
- Guzha et al. (2022). Cell wall-localized BETA-XYLOSIDASE4 contributes to immunity of *Arabidopsis* against *Botrytis cinerea* (Figure 2C, 2D, 2E, 3C, 3D, and 3E).

## General notes and troubleshooting

1. It is important to carry out independent infection experiments on plants grown at different times to account for variances caused by slight variations in infection or growth conditions. We recommend performing at least three independent experiments with at least 30 infected plant leaves per genotype in case of lesion size measurements, or with at least three biological replicates and at least three technical replicates in case of quantification of fungal gDNA.
2. In the described protocol, *B. cinerea* does not contain any resistance or selection markers that would allow growth on selective plates. Work under sterile conditions when preparing *B. cinerea* spores. When transferring mycelium to fresh PDA plates, make sure that you select regions of the previous PDA plate that contain only *B. cinerea* mycelium and no contaminations.
3. As *B. cinerea* infects plants via air-borne spores, it is important to separate the working area for *B. cinerea* assays from the area where you work with non-infected plants.
  - a. Keep infected plants separate from normal plants in a dedicated growth chamber.
  - b. Avoid all normal plant areas after you prepared *B. cinerea* spores or carried out an infection assay.
  - c. After infection assays, clean all tools used for infection by incubation with anti-fungal disinfectant and 70% ethanol. Similarly, clean the working area for infection after use.
4. During the configuration of your digital calliper, edit the parameters of the measuring instrument so that data will be transferred to Excel. Individual measurements will then be transferred into successive rows of an Excel sheet. Adjust the end row number so that all measurements from a single experiment will fit into the same Excel sheet.
5. Lesion size is usually not perfectly circular (Figure 2B); so, measuring the diameter becomes more difficult. Measure lesion size diameter for all leaves in a similar angle to the middle vein (e.g., always parallel to the middle vein); all leaves should be measured by the same experimenter. For generation of the standard curve for gDNA quantification, it is important to accurately measure the gDNA concentrations on a NanoDrop after the serial dilutions.

## Troubleshooting

Poor fungal growth after infection: maintain the plants under high humidity by placing a dome over the plants after infection.

## Acknowledgments

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## Competing interests

The authors have no financial or non-financial competing interests.

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