

INFECTING TOMATO LEAVES WITH *P. INFESTANS*

1. Growing the plants

Planting: Fill a 4 inch pot with UC4 mix (which gives better drainage than the standard UC mix). Pat the soil down gently. Dip the pot into a tub filled with water to wet the soil. Make 4 holes in the soil, about 0.5 cm deep, and place one seed in each hole. Fill in the holes. Label the pot using a stake, labelled with your initials, the plant genotype, and date. Cover the soil with a wet paper towel; the towel should be removed after about 5 days (about a day after the seedlings emerge). Place the pot in the growth chamber (25C).

Watering: Water with the watering can, taking care to avoid contacting the leaves with water. The watering schedule will vary depending on the age of the plants. In general, water every three days for plants up to 3 weeks old; every day for plants 3 to 5 weeks old; twice a day for plants older than 6 weeks. Once a week water using a fertilizer solution (MiracleGrow).

2. Infection assays

Harvesting the leaves: Use healthy, well-watered plants that are 5-8 weeks old. Using a sharp forceps, pluck off leaflets and place in a 100 mm petri dish containing 0.6% water agar. Place the top of the leaves up. Avoid very small or very large leaves; try to pick leaves 3-4 cm in length. You should be able to fit about 5 leaves per dish. Pick a representative sample of leaves for each assay; always place leaves from at least two plants in each plate. Put the lids on the plates, but do not seal in a bag yet (to allow ethylene evolution).

Harvesting sporangia: Scrape off mycelia from a plate using bent-tip forceps and place in water (about 1 ml per cm² of mycelia). Avoid using plates older than 3 weeks, as spore viability will be reduced. Vortex 5 seconds. Reach through the mycelia with a pipet tip and withdraw an aliquot. Count using a hemacytometer. Adjust the concentration to 2-4 x 10⁴/ml. [Alternate method: flood a plate with 5-10 ml water, rub off sporangia using a glass rod, and then decant into a tube]. The yield of sporangia varies greatly between strains; some will give >10⁶ per cm², others less than 10⁴.

Inoculation: Place 15 µl droplets of sporangia along the midline of each leaflet. Place a drop every cm; therefore most leaves will have 2-3 drops. From now on, be very careful when handling the plates to avoid knocking the drops off the leaflets! Label the plates with the isolate and date, and seal in a ziplock bag.

Zoosporeogenesis: Incubate the plates at 10C for 4 hr.

Incubation: Incubate at 16C with 12 hr of day and night.

Scoring: Sporulation will begin at about 5 d. On day 7 score as:

- 0 no lesion
- 1 necrosis at the site of inoculation
- 2 spreading necrotic lesion of maximum 1 cm diameter
- 3 spreading necrotic lesion of >1 cm diameter, with little or no sporulation
- 4 entire leaf covered with sporulation, little or no necrosis evident
- 4N entire leaf covered with sporulation, with major necrosis (never seen?)