Silwet L-77 Dip Inoculation Procedure for A. thaliana
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1. Sow Arabidopsis seeds in small (~3 inch) square pots at a density of 16 to 20 seedlings per pot. Cover pot with nylon mesh, (6" x 6" squares). Secure with a wide rubber band (No 16). Make sure that there is no space left between the surface of the soil and the screen; seedlings may get trapped under screen after germination. Cover plants with a clear plastic lid to prevent the soil from drying out prior to germination. Cold-treat the seed if necessary (2-3 days at 4°C).

2. Grow plants for ~ four weeks under an 8 hour photoperiod at 24°C and 70-80 % relative humidity (RH) under a mixture of fluorescent and incandescent lights at an intensity of ~200 uE/m²/sec. Conviron growth chambers work very well for this purpose.

3. Dip whole rosettes into a bacterial suspension of ~5 x 10^8 cfu/ml in 10 mM MgCl_2 containing 0.02% Silwet L-77(Union Carbide)^1. Do this by inverting the pot and submerging the leaves in the bacterial suspension (Figure 1). Swirl the plants in the bacterial suspension for about one second. The leaf surfaces should be coated with fluid and appear shiny. Place plants in a flat or tray and cover with a clear plastic lid for 24 hours^2. NOTE: If the plants remain covered (and thus at very high RH, ~ 100%) for more than 24 hours, the severity of disease will be very high. We have observed a breakdown of gene-for-gene resistance under these conditions. For optimal disease symptoms the temperature should be maintained around 25-30°C.

4. Score disease symptoms three to four days after inoculation. Pseudomonas syringae pv. tomato strain DC3000 symptoms consist of small, individual gray lesions surrounded by a halo of chlorosis (Figure 2). The lesions are usually most apparent on the inner leaves of the rosette (e.g. leaves 5-7, when counting out from center). Do not wait longer than 4 to 5 days to score the symptoms, as the individual lesions will coalesce. Diseased plants will also become stunted.

^1For other plant species you should experiment with the level of L-77 present in the inoculum. For example, Salmeron et al, 1994. Plant Cell 6:511-520 recommend using 0.05% L-77 when inoculating tomato plants using the dip method.

^2Larger plant species, such as tomato, soybeans and tobacco do not need to be placed under a plastic dome after inoculation. However, they should be placed in a relatively cool and humid environment.