

PATHOGENICITY OF THE ISOLATED BACTERIAL CULTURE ON NATURAL HOST PLANTS

BY SPRAY INOCULATION METHOD

In case of spot, blight, and canker diseases where the pathogens usually enter the tissue through stomata, hydathodes, or lenticels, this method of inoculation is the most natural. Keep the plants in a humid chamber 24 hours prior to inoculation to allow the stomata to open and to create high intercellular humidity in the tissues around the natural openings. Spray the bacterial suspension with a hand atomizer or plastic sprayer to cover the plant surface thoroughly. Put the plant in a humid chamber for 3 days and, later on, in natural environmental conditions. Spray with water thrice a day for a week or until symptoms develop. The spray inoculation method is generally used for determining the pathogenicity of bacterial blossom blight, wild fire of apple and pear, and inflorescence diseases of bacterial pathogen or bacterial fruit spot diseases.

BY ROOT DIP INOCULATION METHOD

The method is probably the most natural way of inoculating bacterial pathogens that produce vascular wilts. Up root the young seedlings, wash the roots in water, clip the root tips, and immerse in the bacterial suspension for 10 minutes. Transplant the seedlings in soil and water the transplanted seedlings appropriately. Observe for the wilt symptoms' development. This method is generally used for determining the pathogenicity of bacterial wilt pathogen (*Ralstonia*) or in case of soilborne bacterial diseases.

BY VACUUM INFILTRATION METHOD

In this method, the bacterial suspension is forced through the stomata into intercellular spaces. It reduces the incubation period for symptom development and is best applicable for stomata invaders. A very diluted bacterial suspension should be used to obtain the right type of symptoms. If a concentrated suspension is used atypical symptoms may result. The method consists of applying the inoculums with an atomizer at 1.5 kg/cm² pressure on the lower surface of the leaf. The leaf is held with the hand in position and the nozzle of atomizer kept at about a 2-inch distance. The pressure is obtained by connecting the atomizer to the exhaust outlet of an electric motor pump. The vacuum infiltration method is most appropriate where the plant leaf surface is smooth

and waxy (e.g., banana leaf) where the inoculums are not retained on the leaf surface when applied with other inoculation methods.

BY PIN PRICK INOCULATION METHOD

This method is good for bacteria causing wilts, blights, soft rots, and galls. Prepare a pin bundle by fixing four to six fine insect pins on a piece of cork. Only the tips of the pins should project out of the cork piece. If a piece of cork is not available, one may use the disc of a carrot to make the pin prick bundle. Injure the plant part to be inoculated with the pin bundle and apply the bacterial suspension on the injured site. For blights, the leaf is inoculated in the center, avoiding the mid rib. For wilts, inoculate the stem at a point between the cotyledon and first leaf. This method does not work well in many of the leaf spot diseases. The pin prick method is most appropriate to test the pathogenicity of bacterial stalk rot, collar rot, or rhizome rot pathogen.

Observations The inoculated plants are examined for symptom development for at least for 1 month. In leaf spot or streak disease, which is parenchymatous, symptoms usually develop within 4–5 days. However, certain leaf spots, for example, mango canker, may take as long as 10–15 days. Wilts and blights usually take 7–10 days, while galls caused by *Agrobacterium tumefaciens* may take more than a month to develop.

While taking observations on symptoms, it is important to record and describe different developmental stages of the symptoms rather than describing the final stage. The inoculated bacterium is considered the true pathogen if it produces similar types of symptoms as seen on the original specimen from which it was isolated. In typical symptoms exactly the same alternations occur in an artificially inoculated plant as in nature and the development is slow. The susceptible cultivar of the plant produces typical symptoms while the resistant cultivar of the same plant produces atypical symptoms. In atypical symptoms, rapid (within 24 hours) necrosis of the tissue occurs at the site of inoculation without water soaking at any stage. These atypical symptoms are produced due to plant resistance, high inoculum concentration, or inappropriate methods of bacterial inoculation, even though the bacterium may be pathogenic