

Corn Stalk Inoculations (Method of Etta Nuckles)

Preparation of plant material

Grow corn seed in a suitable soil mixture with adequate watering and fertilization under 16 hours daylight or supplemental lighting until the V13 or V14 stage.

In Vitro (Stalk Piece) Inoculation:

When the plant reaches V13 or V14, it can be harvested for in vitro inoculations. We use only the 1st and 2nd internodes above the brace roots.

Remove sheath covering from the corn stalk.

Excise and discard nodes.

The internodes are cut into 1" sections and placed in a large beaker. A double layer of cheesecloth is secured to the beaker top with rubber bands. The beaker is placed under a mild stream of cold running water (enough to agitate them) for 1h. This acts as a surface sterilization. 10% bleach or 70% ethanol is rather toxic to the cut surfaces of the corn stalk.

After an hour under running water, the corn sections are drained and placed onto sterile seed germination paper or sterile paper towels to dry.

The sections are moved aseptically to a sterile foil covered 9"x11" metal pan lined with seed germination paper that has been moistened with sterile water. The paper should be saturated, but there should be no standing water.

The corn sections should be arranged into rows for inoculation with the pathogen. Include a water control and a *C. sublineolum* (non-pathogen) control. A sharpie can be used to label the stalk sections by writing directly on the rind.

Take a size 00 rubber stopper and cut it in half crosswise. There will be 2 pieces, a small end and a large end. Force a metal dissecting probe through the larger flat end of the stopper so that 2 mm of the probe extends beyond the cut surface of the stopper. Sterilize by dipping into 95 % ethanol then flaming. Use this device to pierce the corn rind in the middle of the length of each stalk section.

Pipette a 10 μ l drop of a 5×10^6 spores/ml suspension of washed *Colleotrichum graminicola* conidia onto the wound site.

The pan is covered with the sterile foil.

The inoculated corn sections are incubated 25° for up to 3 days in the dark.

Add sterile water as necessary to keep the germination paper moist but with no standing water.

To collect data: slice each corn section in half longitudinally, with the cut centered on the wound site, and measure the depth and length of necrosis and water soaking.

NOTE added by L. Vaillancourt. Although this is a useful method for screening large numbers of isolates, or for use with isolates that cannot be inoculated in the greenhouse (e.g. BL2), it is not our preferred method because the interaction can be very atypical in these cut pieces: thus, Venard in her dissertation (Appendix 1) found that resistant and susceptible corn and pathogenic and nonpathogenic mutants produced similar amounts of growth, although the non-pathogenic mutant 6-2 caused less discoloration to the tissue than the wild type. Never leave the stalk pieces longer than three days as they become very degraded after that time point.

In Vivo or In Planta (Intact Plant) Inoculation

Plants are grown in the greenhouse as above to stage V13-V14.

The plants are laid down on the bench so that stalk breakage is avoided.

The sheath is removed from the bottom of the stalk up to the 4th node above the soil line.

A sharpie can be used to label the stalk sections directly on the rind of internodes 2 or 3 above the soil line. Internode 2 is normally used unless it is very short, in which case we use the third internode. It is important to be consistent. We inoculate only a single internode per plant.

Take a size 00 rubber stopper and cut it in half crosswise. There will be 2 pieces, a small end and a large end. Force a metal dissecting probe through the larger flat end of the stopper so that 2 mm of the probe extends beyond the cut surface of the stopper. Sterilize by dipping into 95 % ethanol then flaming. Use this device to pierce the corn rind in the middle of the length of each stalk section.

Pipette a 10 μ l drop of a 5×10^6 spores/ml suspension of washed *Colleotrichum graminicola* conidia onto the wound site.

To make a miniature "humidity chamber, the lid from a 1.5 ml Eppendorf tube is placed over the inoculated site and secured with parafilm wrapped around the

stalk and lid. Try not to wound the stalk excessively, but a small amount of damage to the epidermis is difficult to avoid. This damage should be distant from the wound site, however, if an effort is made to center the tube cap over the wound.

The lid and the parafilm is removed the next morning (12-16 hours), at which time the plants are also returned to an upright position on the bench in the greenhouse.

The stalk is harvested for analysis 1-2 weeks after inoculation (before tasseling). The stalk is split longitudinally, with the cut centered on the wound site, to reveal the interior. Record the length and depth of the necrosis and water soaking. Observe the upper area to determine if the necrosis (fungus) has colonized the upper tissue.

All plant material and soil must be autoclaved prior to disposal. No more than one mature corn plant and its root ball, together with all the soil from the pot, should be put in each orange autoclave bag. Autoclave on a liquid cycle of 1h to penetrate the root ball and foliage of one corn plant. We tested these parameters using Thermo Scientific Indicators (Fisher Scientific 14-490-27) embedded in the center of the root ball to confirm efficacy. After autoclaving the orange bags can be placed into normal garbage bags and deposited with the regular trash.

NOTE added by L. Vaillancourt. In a highly susceptible corn variety, such as Jubilee sweet corn, the pathogen can move through several internodes within two weeks. We like Jubilee because it is quite happy under greenhouse conditions. We are also using some of the shorter day-length varieties of dent corn for this assay. This protocol has been used for several of our publications including Thon et al., 2000 and 2002, and Venard et al., 2007 and 2008.