

Leaf Rust / Brown Rust (*Puccinia triticina*) Inoculation Protocol

1. Plant 5-10 seeds / plantlets diagonally across a 4in. (10.16cm) diameter pot and grow to the two leaf stage.
2. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O*. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for leaf rust is 15-16°C this can be achieved with dew formation by setting the water to 28°C and the wall to 11°C. To ensure the chamber is preconditioned set it up 1hour in advance.

* diH₂O is used to reduce salt precipitation on the submerged heating elements and to reduce trace minerals which inhibit the germination and growth of rust spores.

3. Prepare the inoculum immediately prior to use by suspending urediospores in isoparaffin oil (Soltrol170)* or in a solution of diH₂O and ~1ppm surfactant (Tween20). When using a water-surfactant / spore suspension prepare ~0.6mL per plant at concentration of ~6x10⁵ spores / mL. When using an oil / spore suspension prepare ~0.06mL per plant at concentration of ~6x10⁶ spores / mL.

* Isoparaffin suspensions allow for dense and consistent inoculum distribution but the oil is phytotoxic to wheat and can be lethal to barley.

4. Attach the inoculum bottle to the propellant can. The spores are viable in this suspension for a day or so.
5. Spray the plants on front and back from ~6in. away with one hand behind the plants to catch most of the inoculum on the plant.
6. Incubate in the dew chamber for 18-48hours, ideally 24hours.
7. Transfer plants to greenhouse and grow under 16hour photoperiod at ~25°C until disease development (6-10days).

Rust Spore Germination Test

This can be done while plant inoculations are being conducted.

1. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O*. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for leaf rust is 15-16°C this can be achieved with dew formation by setting the water to 28°C and the wall to 11°C. To ensure the chamber is preconditioned set it up 1hour in advance.
2. Wet a microscope slide with diH₂O and shake off most of the water. Sprinkle the spores on to the slide and spread around with finger.
3. Mark the side of the slide with the spores with a sharpie.
4. Stand the slide up in a Styrofoam holder in the dew chamber so that the spores are facing a wall and the slide is parallel to the wall.
5. Incubate in the dew chamber for 18-48hours, ideally 24 hours.
6. Observe the spores under a microscope and estimate the percentage of germinated spores. Good germination is 80% however; infection can be achieved even when germination is 20%.

Rust Spore Count

To determine the concentration of your inoculum, position the coverslip and load each side of the hemocytometer with ~20µL of your inoculum.

Every spore seen on each total grid = 105 spores / mL.

Leaf rust spore are spherical in shape and orange in color. If the spores appear pale in color or deformed it is an indication of damage and may not be viable.

Dry leaf rust spores can be stored at 4°C for up to 6 months. For long term storage dehydrate spores for 48hours, flash-freeze in liquid nitrogen, and store at -80°C.

Stem Rust / Black Rust (*Puccinia graminis*) Inoculation Protocol

1. Plant 5-10 seeds / plantlets diagonally across a 4in. (10.16cm) diameter pot and grow to the two leaf stage.
2. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O*. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for stem rust is 20-22°C this can be achieved with dew formation by setting the water to 35°C and the wall to 15°C. To ensure the chamber is preconditioned set it up 1hour in advance.

* diH₂O is used to reduce salt precipitation on the submerged heating elements and to reduce trace minerals which inhibit the germination and growth of rust spores.

3. Prepare the inoculum immediately prior to use by suspending urediospores in isoparaffin oil (Soltrol170)* or in a solution of diH₂O and ~1ppm surfactant (Tween20). When using a water-surfactant / spore suspension prepare ~0.6mL per plant at concentration of ~6x10⁵ spores / mL. When using an oil / spore suspension prepare ~0.06mL per plant at concentration of ~6x10⁶ spores / mL.

* Isoparaffin suspensions allow for dense and consistent inoculum distribution but the oil is phytotoxic to wheat and can be lethal to barley.

4. Attach the inoculum bottle to the propellant can. The spores are viable in this suspension for a day or so.
5. Spray the plants on front and back from ~6 inch away with one hand behind the plants to catch most of the inoculum on the plant.
6. Incubate in the dew chamber for 18-48hours, ideally 24hours.
7. Transfer plants to greenhouse and grow under 16hour photoperiod at ~25°C until disease development (10-14days).

Rust Spore Germination Test

This can be done while plant inoculations are being conducted.

1. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for stem rust is 20-22°C this can be achieved with dew formation by setting the water to 35°C and the wall to 15°C. To ensure the chamber is preconditioned set it up 1hour in advance.
2. Wet a microscope slide with diH₂O and shake off most of the water. Sprinkle the spores on to the slide and spread around with finger.
3. Mark the side of the slide with the spores with a sharpie.
4. Stand the slide up in a Styrofoam holder in the dew chamber so that the spores are facing a wall and the slide is parallel to the wall.
5. Incubate in the dew chamber for 18-48hours, ideally 24hours.
6. Observe the spores under a microscope and estimate the percentage of germinated spores. Good germination is 80% however; infection can be achieved even when germination is 20%.

Rust Spore Count

To determine the concentration of your inoculum, position the coverslip and load each side of a hemocytometer with ~20µL of your inoculum.

Every spore seen on each total grid = 105 spores / mL.

Stem rust spore are slightly elongated (oblong) and red in color. If the spores appear pale in color or deformed it is an indication of damage and may not be viable.

Dry stem rust spores can be stored at 4°C for up to six months. For long term storage dehydrate spores for 48hours, flash-freeze in liquid nitrogen, and store at -80°C.

Stripe Rust/Yellow Rust (*Puccinia striiformis*) Inoculation Protocol

1. Plant 5-10 seeds / plantlets diagonally across a 4in. (10.16cm) diameter pot and grow to the two leaf stage.
2. Cold treat plants at night temperatures of 4°C and day temperatures of below 25°C for 10-21 days.
3. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O*. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for stripe rust is 13-15°C this can be achieved with dew formation by setting the water to 20°C and the wall to 5°C. To ensure the chamber is preconditioned set it up 1 hour in advance.

* diH₂O is used to reduce salt precipitation on the submerged heating elements and to reduce trace minerals which inhibit the germination and growth of rust spores.

4. Prepare the inoculum immediately prior to use by suspending urediospores in isoparaffin oil (Soltrol170)* or in a solution of diH₂O and ~1ppm surfactant (Tween20). When using a water-surfactant / spore suspension prepare ~0.6mL per plant at concentration of ~6x10⁵ spores / mL. When using an oil / spore suspension prepare ~0.06mL per plant at concentration of ~6x10⁶ spores / mL.

* Isoparaffin suspensions allow for dense and consistent inoculum distribution but the oil is phytotoxic to wheat and can be lethal to barley.

5. Attach the inoculum bottle to the propellant can. The spores are viable in this suspension for a day or so.
6. Spray the plants on front and back from ~6 inch away with one hand behind the plants to catch most of the inoculum on the plant.
7. Incubate in the dew chamber for 18-48 hours, ideally 36 hours.
8. Transfer plants to greenhouse and grow under 16 hour photoperiod at night temperatures of 4°C and day temperatures below 25°C until disease development (15-21 days).

Rust Spore Germination Test

This can be done while plant inoculations are being conducted.

1. Prepare the dew chamber by first checking that the water level in the holding tray is at the fill line if needed add diH₂O*. Turn the dew chamber on and set the water and wall temperatures by adjusting the corresponding dials. The optimal air temperature for stripe rust is 13-15°C this can be achieved with dew formation by setting the water to 20°C and the wall to 5°C. To ensure the chamber is preconditioned set it up 1 hour in advance.
2. Wet a microscope slide with diH₂O and shake off most of the water. Sprinkle the spores on to the slide and spread around with finger.
3. Mark the side of the slide with the spores with a sharpie.
4. Stand the slide up in a Styrofoam holder in the dew chamber so that the spores are facing a wall and the slide is parallel to the wall.
5. Incubate in the dew chamber for 18-48 hours, ideally 36 hours.
6. Observe the spores under a microscope and estimate the percentage of germinated spores. Good germination is 80% however; infection can be achieved even when germination is 20%.

Rust Spore Count

To determine the concentration of your inoculum, position the coverslip and load each side of the hemocytometer with ~20µL of your inoculum.

Every spore seen on each total grid = 105 spores / mL.

Stripe rust spore are spherical in shape and yellow in color. If the spores appear pale in color or deformed it is an indication of damage and may not be viable.

Dry stripe rust spores can be stored at 4°C for up to six months. For long term storage dehydrate spores for 48 hours, flash-freeze in liquid nitrogen, and store at -80°C.