Greenhouse Protocols for Handling and Testing Stripe Rust

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Handling samples

Collecting stripe rust samples

When collecting stripe rust samples from the field, collect 5-7 sporulating leaves for each sample from a single field or single variety if possible. Place leaf samples in a rust survey bag or glycene bag (bags used to cover crossed wheat heads are also suitable for collecting samples). Do not use plastic bags because they will cause leaves to rot quickly.

Receiving stripe rust samples

When a stripe rust sample arrives, number the sample and input the sample information into a computer file.

If plants are not ready for inoculation, keep rust samples in a refrigerator at 4^{o}C.

For stripe rust samples arriving from other countries, open the package in a bio-safety cabinet. Inoculation of plants with foreign samples should be conducted separately from U.S. samples.

Preparation of potting soil for wheat and barley

Potting Mixture	
Peat moss	6 gal
Perlite	2 gal
Sand	3 gal
Commercial potting soil	3 gal
Vermiculite	4 gal
Osmocote (14-14-14)	1 cup (at 3.3g/L)
Water	2 gal

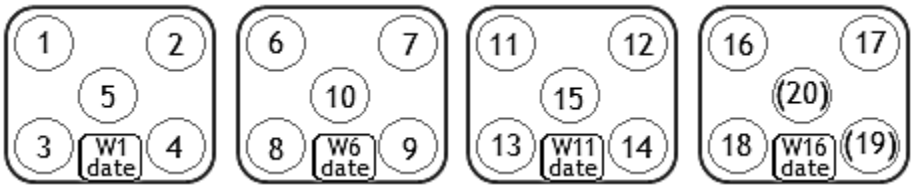
- □ Mix 30 minutes for uniformity of texture.
- □ Make sure to add in the fertilizer.
- □ Clean the mixer and mixing area after use.
- □ Take all safety precautions when operating the mixer.

Planting for spore increase and race identification

- □ Determine the number of pots needed and lay out all pots on the bench.
- □ Fill pots with potting mixture and gently firm the soil.
- □ Seeding: for planting individual varieties, use 7-15 seeds.
- □ Insert a label in each pot (or insert labels after seeding and covering).

Wheat

For planting the wheat differential set (18 varieties), use 5 seeds for each variety according to the following diagram:



An optional two additional varieties can be added to the last pot such as a susceptible check and a variety containing a particular gene of interest.

Barley

The barley differential set (12 varieties) is planted following the same protocol for wheat, but with four differentials planted per pot.

- □ Cover seeds with the prepared potting mixture, place pots in a metal tray, and bottom water by filling the tray. Do not water from the top of the pots.
- □ Clean the bench and the planting area.

Increasing spores from samples

Inoculation

Depending on the quality and quantity of the sample, transfer rust spores onto a susceptible variety (use Nugaines for wheat samples, and Steptoe for barley samples), the variety that the sample was collected from, and some differential varieties that may be susceptible.

If a sample is from a grass species, transfer spores onto both Nugaines and Steptoe to determine if the sample came from wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*), barley stripe rust (*Puccinia striiformis* f. sp. *hordei*), or other *Puccinia* species.

A rust sample is considered an original isolate. To obtain enough spores for an isolate to be used in race identification and other studies, rust spores from sample leaves should be used to inoculate seedlings of a susceptible variety as instructed above (this process is also called “spore transfer”). Plants used for inoculation should be in the two-leaf stage, and can be gently rubbed parallel to the leaf veins with fingers prior to inoculation. The following inoculation methods can be used (with the effectiveness of each method depending on the quality and quantity of a sample):

- Rub plants with sample leaves. This method is effective and efficient when samples have a reasonably good amount of spores.
- Use a fine brush. If a sample is fresh and has a limited amount of rust pustules, but is sporulating, use a fine paint brush to transfer spores onto the leaves of susceptible plants.
- Use a spatula. If sample leaves are dry, and rust pustules have not yet erupted, use a spatula to scratch open the pustules and transfer exposed spores onto leaves.
- Petri dish method. When samples are old, dry, and have a limited number of rust pustules—or rust pustules are mostly necrotic spots with few uredia—place sample leaves on a moist 3M filter paper (or paper towel) in a petri dish. Place the petri dish in a 4^{o}C refrigerator at for 10 to 24 hours depending on the dryness of the sample. Spores may be produced, and can be used for inoculation using a fine brush.

Incubation

Inoculated plants are kept in a dew chamber at 10^{o}C for 24 hours (minimum 12 hours). Before removing plants from the chamber, make sure that there is sufficient dew formation on the plants. If not, check to see if the dew chamber is functioning properly, and incubate plants until there is sufficient dew formation. Total incubation time should not exceed 48 hours.

Foreign samples

When increasing foreign stripe rust isolates, the inoculated plants should be kept in separate growth chambers from plants inoculated with U.S. isolates.

Note:

- Before inoculation, make sure the dew chamber is functioning properly, and wash dew chamber thoroughly with water.
- Before and after transferring each sample, wash hands, the inoculation box, and the air of the greenhouse section to reduce the possibility of contamination.
- Conduct inoculation in the inoculation container. Keep the section doors closed to reduce air movement.
- Save the original sample leaves and keep them in a refrigerator until successful infection of the inoculated plants is determined. After a successful infection is observed, destroy all sample leaves in an autoclave.

Growth conditions

After removing inoculated plants from the dew chamber, place each pot in a plastic booth in a growth chamber at a diurnal temperature cycle gradually changing from 4^{o}C at 2am to 20^{o}C at 2pm with a 16 hour photoperiod. Water plants properly. New spores can be harvested about 16-20 days after inoculation.

Spore Collection

Spore collection should be done in the hood or within a plastic booth with a spore collector connected to a vacuum pump. Wash the hood and hose with detergent after collecting each sample. The sample should be clearly labeled. If rust infections have not occurred within 18 days after inoculation, the plants should be discarded to avoid aphid infestation and/or mildew occurrence.

Spores from foreign isolates and U.S. isolates should be collected at separate times to avoid contamination.

Differential testing and race identification

- □ Use correct seed of differential varieties for race identification. Plant seed exactly as the differential plant scheme shown above.
- □ Grow and use healthy plants at the 2-leaf stage for inoculation.
- □ Use fresh spores, or dry spores that have been stored in a refrigerator for less than one month, for race identification.
- □ The following inoculation methods can be used (with the effectiveness of each method depending on the quality and quantity of a sample):
    - Mix spores with talcum powder and dust the spores over the plants.
    - Spray plants with a spore suspension either in water with Tween 20, or in mineral oil using an atomizer or sprayer.
    - Shake infected plants directly above differential hosts.
- □ After incubation in the dew chamber for 24 hours, place inoculated plants of the whole differential set into a square plastic booth, and grow the plants in the growth chamber at the diurnal temperature cycle gradually change from 4^{o}C at 2am to 20^{o}C at 2pm with A 16 hour photoperiod. Do not let plants dry and do not over water plants.
- □ 20 days after inoculation, record infection type for each variety using the 0 to 9 scale.
- □ Determine the race of the isolate based on the avirulent/virulent pattern, and refer to previously identified races.
- □ If an isolate appears to be a mixture, obtain sub-isolates by growing spores on key differential varieties, and repeat the test.
- □ If an isolate appears to be a new race, obtain single-pustule or single-spore isolates, and test the isolates again on the whole set of differentials.
- □ If an isolate is confirmed to be a new race, obtain single-pustule or single-spore isolates, and test isolates on the supplementary set of differentials, and collect two small vials and two large vials of the spores and store in liquid nitrogen.

Storing spores

Different methods can be used for different purposes of storage. For any purpose, spores should be dry. Spores can be dried in a desiccator for two to three days. For short-term storage (less than 3 months), keep spores at 4^{o}C. For long-term storage, store spores in liquid nitrogen, strictly following the procedures below:

- □ Dry spores in a desiccator for 2 to 5 days.
- □ Put spores in a glass vial or food grade Mylar bag.
- □ Place a label with isolate number and date into the vial.
- □ Seal the vial using a gas flame (only a trained person can do this).
- □ Place sealed vials in alcohol for one to two days to check if the vials sealed perfectly. Throw away failed ones and repeat the process.
- □ Put the vials in a liquid nitrogen tank and clearly record the tank positions of the samples in a liquid nitrogen storage file.
- □ If a vial of spores in the liquid nitrogen is used, it should be replaced with spores of the same isolate as soon as possible.
- □ Depending on the type and quality of the liquid nitrogen tanks, tanks should be refilled with liquid nitrogen every 10 to 14 days to maintain adequate liquid nitrogen levels.

Two small vials of spores from the original isolates should be stored in liquid nitrogen. Spores can be stored in a -70^{o}C or -80^{o}C deep freezer for several months to a year. If small Mylar bags are used for storing spores in liquid nitrogen or a freezer, the bags should be sealed completely. Spores stored in a 4^{o}C refrigerator can be used directly for inoculation. Spores taken from liquid nitrogen, or a -70^{o}C or -80^{o}C freezer, should be set out on a bench for several minutes and then heat shocked for 2 minutes by submerging vials in warm water (about 50^{o}C), do not exceed 2 minutes. You may use fingers to adjust water temperature (water should feel warm, but not too hot). The easiest way is to hold a beaker containing the spore vials or bags under warm tap water for exactly 2 minutes.

Foreign and U.S. isolates should be stored in separate boxes.

Disposal of infected plants and disinfection of equipment

Once rust data are recorded and spores are collected, infected plants should be removed from the growth chamber as soon as possible to reduce contamination.

Infected plants should be separated from regular garbage. Use thick plastic bags for disposing infected plants. For plants infected with local isolates, leave bags of plants in greenhouse until plants have completely rotted or dried out (make sure stripe rust spores are also dead). Then the bags can be disposed into a garbage tank. For plants infected with isolates from other regions (e.g. western Washington and other states), plants should be placed in autoclavable plastic bags and autoclaved before disposing.

Collectors, dusters, plastic booths, trays, and other things used for handling rust spores should be soaked in detergent water with bleach for 15 minutes to disinfect spores, the wash thoroughly and dry completely in heating room for re-use.

Plants, soils, and pots used for increasing and testing foreign isolates must be autoclaved before disposing or reusing.

APPENDIX

Differential Cultivars of Wheat

Yr single-gene line differentials

Differential No.	Name	Type	*Yr* gene
1	AvSYr1NIL	Spring	*Yr1*
2	AvSYr5NIL	Spring	*Yr5*
3	AvSYr6NIL	Spring	*Yr6*
4	AvSYr7NIL	Spring	*Yr7*
5	AvSYr8NIL	Spring	*Yr8*
6	AvSYr9NIL	Spring	*Yr9*
7	AvSYr10NIL	Spring	*Yr10*
8	AvSYr15NIL	Spring	*Yr15*
9	AvSYr17NIL	Spring	*Yr17*
10	AvSYr24NIL	Spring	*Yr24*
11	AvSYr27NIL	Spring	*Yr27*
12	AvSYr32NIL	Spring	*Yr32*
13	Avs/IDO377s (F3-41-1)	Spring	*Yr43*
14	Avs/Zak (1-1-35-line1)	Spring	*Yr44*
15	AvSYrSPNIL	Spring	*YrSP*
16	AvSYrTres1NIL	Spring	*YrTr1*
17	Avs/Exp1/1-1 Line 74	Spring	*YrExp2*
18	Tyee	Winter	*YrTye*

Supplementary set

Differential No.	Name	Type	*Yr* gene
S1	Kalyansona	Spring	*Yr2*
S2	Vilmorin 23	Winter	*Yr4a, YrV23*
S3	Hybrid 46	Winter	*Yr4b, YrH46*
S4	Hugenoot	Spring	*Yr25*
S5	AvSYr28NIL	Spring	*Yr28*
S6	AvSYr31NIL	Spring	*Yr31*
S7	AvSYrANIL	Spring	*YrA*

Old U.S. differential set

Differential		Genotype		*Yr* gene(s)
No.	**Name**	**ID Number**	**Type**	
1	Lemhi	CI 011415	Spring	*Yr21*
2	Chinese 166	CI 011765	Winter	*Yr1*
3	Heines VII	PI 201195	Winter	*Yr2, YrHVII*
4	Moro	CI 013740	Winter	*Yr10, YrMor*
5	Paha	CI 014485	Winter	*YrPa1, YrPa2, YrPa3*
6	Druchamp	CI 013723	Winter	*Yr3a, YrD, YrDru*
7	AvSYr5NIL	YR 00004	Spring	*Yr5*
8	Produra	CI 017460	Spring	*YrPr1, YrPr2*
9	Yamhill	CI 014563	Winter	*Yr2, Yr4a, YrYam*
10	Stephens	CI 017596	Winter	*Yr3a, YrS, YrSte*
11	Lee	CI 012488	Spring	*Yr7, Yr22, Yr23*
12	Fielder	CI 017268	Spring	*Yr6, Yr20*
13	Tyee	CI 017773	Winter	*YrTye*
14	Tres	CI 017917	Winter	*YrTr1, YrTr2*
15	Hyak	PI 511674	Winter	*Yr17, YrTye*
16	Express	DA 984034	Spring	*YrExp1,YrExp2*
17	AvSYr8NIL	YR 000008	Spring	*Yr8*
18	AvSYr9NIL	YR 000009	Spring	*Yr9*
19	Clement	PI 518799	Winter	*Yr9, YrCle*
20	Compair	PI 325842	Spring	*Yr8, Yr19*

Differential Cultivars of Barley

Differential		Genotype	Resistance Gene(s)
No.	**Name**	**ID Number**	
1	Topper	-	-
2	Heils Franken	PI 290183	*Rps4, rpsHF*
3	Emir	CIho 13541	*rpsEm1, rpsEm2*
4	Astrix	CIho 13862	*Rps4, rpsAst*
5	Hiproly	CIho 03947	*rpsHil, rpsHi2*
6	Varunda	PI 410865	*rpsVa1, rpsVa2*
7	Abed Binder 12	PI 327961	*rps2*
8	Trumpf	PI 548762	*rpsTr1, rpsTr2*
9	Mazurka	PI 399501	*Rps1.c*
10	Bigo	CIho 11795	*Rps1.b*
11	I 5	PI 288187	*Rps3, rps15*
12	Bancroft	PI 605474	Not determined

0-9 infection type scale

Infection Type	Signs and Symptoms for Infection Types
0	No visible signs or symptoms
1	Necrotic and/ or chlorotic flecks; no sporulation
2	Necrotic and/ or chlorotic blotches or stripes; no sporulation
3	Necrotic and/ or chlorotic blotches or stripes; trace sporulation
4	Necrotic and/ or chlorotic blotches or stripes; light sporulation
5	Necrotic and/ or chlorotic blotches or stripes; intermediate sporulation
6	Necrotic and/ or chlorotic blotches or stripes; moderate sporulation
7	Necrotic and/ or chlorotic blotches or stripes; abundant sporulation
8	Chlorosis behind sporulating area; abundant sporulation
9	No necrosis or chlorosis; abundant sporulation