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BGRI Wheat Rust Survey Protocols

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Wheat Rust Survey Protocol

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1. Overview of field survey procedure

1. Ensure that the survey team has all items on the check-list before departure
2. Travel to the 1st survey location
3. Switch on the GPS at the survey location
4. Wait until the GPS receives satellite signals – Status equals "Ready to Navigate"
5. Record the location with the GPS – "Mark a Waypoint"
6. Fill in the location (latitude and longitude) and elevation details on the *Global Cereal Rust Survey Form*
7. Switch off the GPS
8. Carry out survey for rust and enter the observations on the *Global Cereal Rust Survey Form*
9. If possible, collect a live rust sample(s), place in a paper or glycine survey envelope and record the key data (date, location, lat/long, variety, crop and disease) on the envelope
10. If possible, collect a single isolated pustule, for a dead DNA sample. Place the excised pustule in a cryovial with 70% ethanol. Label the tube with a unique number code. Record any sample codes on the survey form.
11. Move to the next survey location – within about 20 km from the 1st location
12. Repeat steps 2 to 11
12. At the end of each survey day, the survey team leader should check that the *Global Cereal Rust Survey Forms* have been properly completed and any samples were collected as necessary.
13. Within 7 days of completing the survey, the survey team leader should make a copy of the *Global Cereal Rust Survey Forms* and sample data envelopes, and mail or deliver the original forms and samples to National Focal Point who will forward the survey data to Dr. Dave Hodson the International Focal Point at CIMMYT-Ethiopia by email (d.hodson@cgiar.org).

2. Field survey checklist

Prior to starting field survey work, field survey teams should have following:

- Pre-printed field survey forms (sufficient number plus spares)
- Sample collection envelopes, paper or glycine (sufficient number plus spares)
- Pencils (at least 3 per team member)
- GPS unit (1 per team) – With standard settings for units
- Spare AA batteries (at least 4 per team)
- Small Cryovials and 70% Ethanol / absolute alcohol
- Permanent marker pen
- Scissors

3. Instructions for filling Survey Form

A one-page standard survey form, the *Global Cereal Rust Survey Form*, should be completed at each unique survey location visited. [NB: The survey form works best for farmer field surveys. If used at a trial site / trap plots, the following is advised. If you have separated plots then fill out a separate form at each. If you have e.g., a trap plot with a mix of cultivars in same place, note down the susceptible check data and any interesting cultivar reactions that you sample and sample codes on same sheet.]

A few key points should be noted when completing the form:

- GPS coordinates should be in decimal degree format (hdddd.ddddd) and not in other formats such as degree, decimal minute or degrees, minutes, seconds
- **Bold** words and numbers can be circled for faster completion of the form.

If multiple samples are collected from the same location, please indicate this on the survey form by adding corresponding multiple ID numbers for samples.

4. Garmin Etrex H GPS Unit- Overview

NB: The Garmin Etrex H unit is commonly used. Other handheld GPS units have near identical functionality, although screen layouts and key buttons differ between units.

5. Notes on basic GPS use and functions

What can you use a GPS for?

- Measuring location (latitude, longitude – elevation is also included) – this is termed **Waypoints** and is the most important function

Can I use my GPS anywhere?

- GPS units can work anywhere in the world, in any weather, 24 hours a day
- The basic units described here will give up to about 4-5 meters horizontal accuracy and 15-20 meters vertical accuracy
- GPS units will **not** work inside buildings
- If you use a GPS next tall buildings or in dense forest, you may get problems in receiving the signal
- Moving to a new location hundreds of kilometers away will usually result in an initial delay in getting a fix on satellite signals. A few minutes wait may be required in new locations.

What is the best way to use a GPS?

- Make sure the unit has two AA batteries installed – Alkaline are best
- Always write down location coordinates, elevation and waypoint number – even if you plan to download data
- Always use a GPS to record location data!

Is there anything else I should remember?

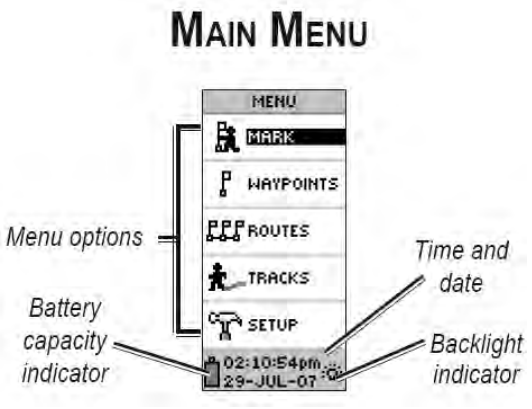
- Do not block the antenna (located just above the word “Etrex” on the front of Garmin GPS) with your fingers or head
- Always carry an extra set of two AA Alkaline batteries

6. Setting up your GPS for use

This only needs to be done *ONCE* – upon receipt of GPS units, prior to use in field survey. After settings are complete, NO further changes should be made. Properly setting up your GPS helps to ensure that standard GPS settings are used in every country. Every effort is made to standardize GPS units before distribution by the BGRI.

Standard Settings Procedure
This can be done indoors, no satellite signal is needed

1. Switch on GPS by pressing **Power** key
2. Press **Page** key several (4) times until the **Main Menu** screen appears



3. Press the **Down / Up** keys to select **Setup**
4. Press the **Enter** key to open the **Setup Menu**
5. Press the **Down / Up** keys to select **Units**. The **Units** option contains the key settings that you should check / change.
6. Use the **Enter** key to select a category, then the **Up / Down** Keys to select the correct option within a category. Finally, press **Enter** key to save change.
7. Repeat step 6 for each Units category

The required Units settings are:

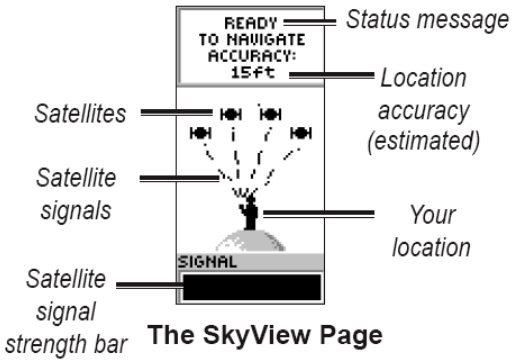
Position Format:	**Hddd.ddddd°** [this means that latitude / longitude coordinates are given in decimal degrees]
Map Datum:	**WGS 84**
Distance/Speed:	**Metric**
Elevation:	**Meters**
Vertical Spd:	**m/min**
Depth:	**Metric**

7. Using a GPS for field survey

1. Turning on the GPS and getting a satellite signal

1. At the field location. Switch on GPS unit by pressing the **Power** button
2. Wait for 2-3 minutes for the GPS to get a location fix using the satellites overhead.
3. Wait until the unit is **Ready to Navigate** – this means that it is receiving signals from at least 4 satellites. [*Note 1:*

   - *The first time you use a GPS in a completely new region it can take up to 5 minutes to receive satellite signals. After initial use, signal reception will be much faster – about a minute or less.*
   - *The more satellites you receive signals from the more accurate will be the location. So it is worth waiting a short time before recording a location. However, the maximum accuracy possible with these units is +/- 4 or 5 meters. Anything less than 10 meters is good enough*.



The SkyView Page

2. Recording and storing a location – "Marking a Waypoint"

There are two ways to do this:

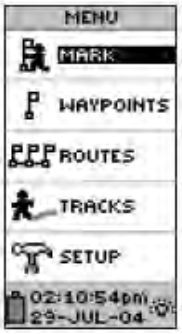
Option A: Via the Enter Key

1. Press and hold the **Enter key or Thumbstick.** This will make the **Mark Waypoint** page appear – showing a 3 digit waypoint number, latitude, longitude, elevation
2. Record the **Latitude** (N or S), **Longitude** (E or W) and **elevation** data on the survey form
3. Select **OK**, and press **Enter** key to record and save the waypoint

Mark Waypoint Page

Option B: Via the Main Menu

1. Press the **Page** key several times until the **Main Menu** appears.
2. Use **Up / Down** keys to select **Mark**
3. Press **Enter** key to open **Mark Waypoint** page
4. Record the **Latitude** (N or S), **Longitude** (E or W) and **elevation** data on the survey form
5. Select **OK**, and press **Enter** key to record and save the waypoint



Menu

3. Turning off the GPS

1. After recording the location ("Marking the Waypoint"), switch off GPS – press and hold **Power** key
2. Complete the *Global Cereal Rust Survey Form* and sampling
3. Move to next survey location

8. Protocols on rust sample collection and handling

Robert Park, Kumarse Nazari, Tom Fetch, Yue Jin and Zac Pretorius

As valuable as trap nurseries are in providing an indication of the presence of a disease in a region, plus initial information on the specific individual virulences present, they provide little or no information on virulence combinations and do not provide any indication of what pathotype (race) or pathotypes are present. The only way of doing this at present is to undertake bioassays under controlled conditions, using sets of differential testers ("race" analysis). Race analyses of cereal rust pathogens have been undertaken in many countries for many years using more-or-less standardized methodologies of inoculation, incubation and then "reading" the sets (data recording). The need for specialized facilities plus trained personnel can hinder efforts to undertake ongoing detailed race analyses.

What we must do

It is essential that we get as much information on (1) where stem rust is present, (2) the severity of stem rust infection when it is present, (3) an idea of the current distribution of "Ug99" and its derivatives, and (4) what variation exists in the stem rust pathogen. It is vital that information on these four points is obtained so that it can be incorporated into an operational surveillance and race analysis system. This can be done even in the absence of sophisticated facilities.

Essential activities:

- collect stem rust samples! At least 20 from each country
- record basic information on each sample
- keep them alive (with adequate back-up collections established)!
- undertake basic analysis of important virulences or send to an international laboratoryfor analysis

Desirable activities:

- samples collected from crops as well as trap plots
- more than 20 samples per country with good data on stem rust incidence and distribution
- comprehensive information on where the samples came from
- surveys to include not only the incidence of stem rust but also leaf rust and stripe rust
- where barberry populations are known to exist, at least some inspection and, if aecia are found, establishment of uredinial isolates from aecia with initial virulence testing

8.1 Live Sample collection

Rust samples are usually collected from crops, trap plots and other experimental plots, self sown cereals growing along roadsides, and even grass species. Rust surveys do not need to be extensive to provide valuable information – considerable value can be obtained by simply requesting samples from colleagues in different regions, rather than travelling hundreds or even thousands of kilometres. If you do not have access to a vehicle, you can contact colleagues and ask them to send samples to you by normal post. The minimum information they should provide you with is:

- Location (preferably with GPS co-ordinates)
- Date of collection
- Name of person who collected it
- Did it come from a crop, experimental plot (incl. trap plot) or a self sown plant?
- What cultivar did it come from (if known)
- Other relevant comments (incidence, severity, etc.)

If possible, you should supplement these collections by undertaking your own surveys. These are most fruitful if you target an area where rust has been reported. Normally, inspection stops can be made every 20 km, however, if something of interest is seen, stops can be more frequent.

Where rust is found in a crop, a good sample of rust, as illustrated below, should be collected:

Ideally, stem samples are obtained from between the nodes, and the core tissue removed to leave only the leaf sheath so that the sample will dry down easily. Samples should **never** be stored in plastic bags. Samples stored in a paper envelope under ambient conditions of between 10-25°C should retain good viability for about a week. The samples can be stored in an airtight container above dried silica gel in a refrigerator for up to 4 weeks, although there could be a gradual loss of viability even under these conditions. A major problem with sending samples to North America in past years has been lack of viability by the time the samples were received for analysis. Hence, unless you have access to long term storage facilities (minus 80°C freezer or liquid nitrogen), it is vital that samples are either stored (see section 8.2.3), sub-cultured, inoculated on differential sets, or sent to an international laboratory(after drying) **within 2 weeks of collection**.

Where a crop or plant is found that is moderately heavily to heavily rusted, it will also be of great value if you collect some healthy leaf tissue (3 to 5 segments of about 50mm in length), specifically from rust infected plants. This tissue will be used to check if the cultivar carries resistance genes including *Sr2*, *Sr24*, or *Sr31* by using linked DNA markers if the samples are sent to Australia (see below and **Appendix C** for details).NB: Samples must be killed before sending to Australia, no live samples are permitted.

DNA samples of single pustules can also provide extremely valuable information. Molecular diagnostics using SNP markers can now provide a rapid assay for the Ug99 race group and postulate individual races in the group. Full details of the sampling procedure are given in **Appendix F**.

A step-by-step summary of collecting and handling samples is provided in **Appendix A**.

8.2 Sample Processing

Where facilities permit race analysis, samples should be either sub-cultured onto a susceptible host or inoculated directly onto differential sets. The susceptible host and differential lines need to be planted 7-8 days prior to inoculation. Thus, if you anticipate that you will obtain samples you should plant pots of the susceptible host or of the differential sets so that inoculation can commence as soon as possible. If you anticipate that you will receive many samples, sometimes it is worth planting pots of susceptible seedlings on a weekly basis so that you always have a supply of healthy plants to inoculate as samples are received.

8.2.1 Sub-culturing

This can be done as a way of keeping isolates alive if race analysis will not be done immediately, to increase the amount of rust in a sample if there is not enough present to inoculate a differential set, or to allow single pustule isolates to be established. Sub-culturing is best done by transferring rust from infected tissue to healthy leaves using a spatula. A solution of water and Tween-20 (1 drop Tween for 1 liter water) will help in spore transfer. Wet the spatula lightly with the solution and scrape the spores from the stem tissue (A). Wet the seedling plants lightly with the solution using a hand-sprayer, then apply the spores that are on the spatula to the leaves (B). The spores will germinate quickly because they are exposed to water, so put the inoculated seedlings into incubation conditions (chambers, bags, or pop (soft drink) bottle tops) as soon as possible. A sterile spatula must be used for each sample, and extreme care be taken in keeping uninoculated plants free from contaminant stem rust spores. It is good practice to include a control to monitor potential contamination by incubating an uninoculated pot of Morocco seedlings with your inoculated plants or diff sets.

A

B

The inoculated plants should be given a dew treatment for about 12-16 hours under dark conditions (night) and as close to 21°C as possible. If incubated at cooler temperatures, the length of time needs to be extended. After the dew treatment, plants infected with stem rust

will need to be put into an area with bright light to finish the infection. This is best achieved by putting in sunlight or underneath bright lights, and uncovering the plants slightly to facilitate slow drying (3 hr) and to avoid overheating of the plants. Plants infected with leaf rust or stripe rust do not require this procedure and can be uncovered and put on greenhouse benches directly after the dew treatment. The dew treatment can be provided by placing the pot in a tray of distilled water (e.g. a Petri dish lid) and covering with a clear plastic bag or soft drink bottle (C). Alternatively, a cabinet can be constructed with wood/plastic sheeting or plexiglass (D) to house many pots. The cabinet should be painted or covered to exclude light during the incubation process. Seedlings/enclosures should be misted with water before covering to get good dew (E).

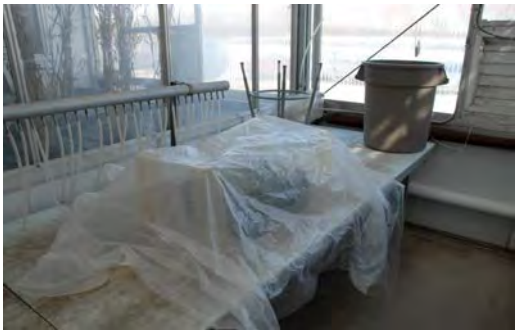
C

D

E

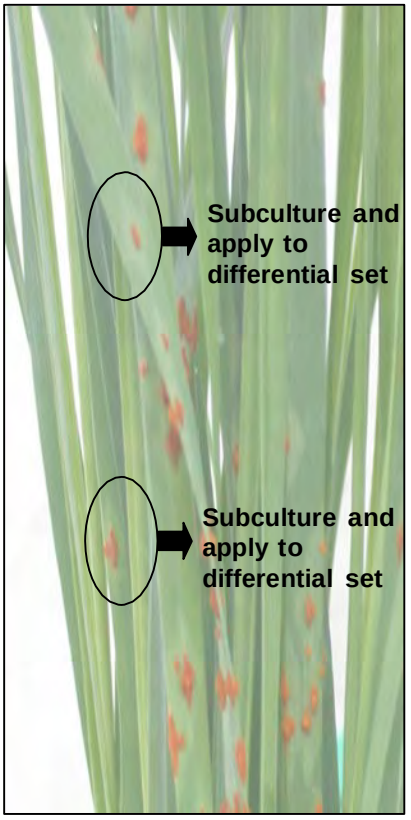
After incubation in the dark, plants must be put in bright light conditions for 3-4 hours to finish infection. If using bottles, unscrew the cap to avoid overheating and put under light. If using bags or chambers, remove bag or take pots out of cabinets and put on a bench with bright light. Tent the pots with plastic to avoid drying too fast (F, G). Mist the plastic tent before covering to keep plants wet. Remove the tent after 3-4 hours.

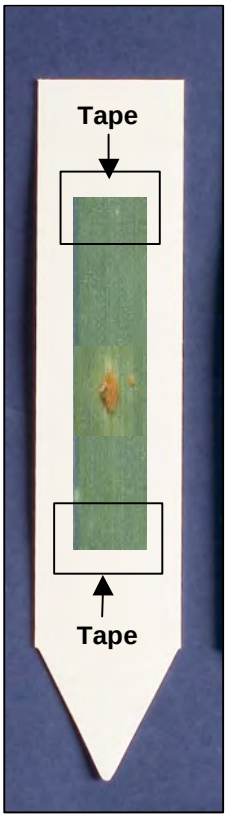
F



G

About 12-14 days after inoculation, large pustules should be present on leaves. Spores from the infected leaves can be collected and used to inoculate a differential set, or single pustule isolates can be established:





A convenient way of handling single pustules is to excise the section of leaf bearing the pustule and stick it onto a plastic pot label with adhesive cellotape. Freshly collected single pustules collected in this way can be stored in a tube and should remain viable for up to 3 weeks if kept over silica gel in an airtight container in a refrigerator. The adhesive tape will prevent the leaf segment from buckling as it dries, making the transfer to a susceptible plant very easy. The single pustules are sub-cultured on a susceptible genotype by direct wiping and /or using a spatula to do the transfer.

8.2.2 "Quicksets" – Preliminary testing of bulk samples for virulence for Sr24, Sr31 and Sr36

Whilst it is obviously very desirable to have state-of-the-art facilities that allow full race analysis of rust samples, it is still possible to do good rust work in basic facilities – after all, rust readily infects wheat in the field in nature. Because of the urgency of gaining some information on the distribution of Ug99, we are suggesting a low resolution baseline survey that will involve:

1. low (or if possible, high) resolution surveys in each country;
2. collection of representative stem rust samples from your country. This could be as few as 20. The samples can be forwarded to you by post from colleagues.
3. drying, packaging and storing individual samples after a bulk comprising a small sub-sample from all samples is prepared
4. inoculation of 'Quicksets' with the bulk
5. marker analysis using host tissue from all plants on which stem rust is found, using markers for genes including *Sr2*, *Sr24* and *Sr31* (in Australia)

6. if evidence of an unusual virulence is detected, a process of consultation will be undertaken with the country in question to request samples be sent to international laboratories for detailed race analysis
7. providing the basic survey information for testing and improving the global wheat rust monitoring system being developed by the BGRI with collaboration of CIMMYT and ICARDA

Ten key genotypes are included in the "Quickset":

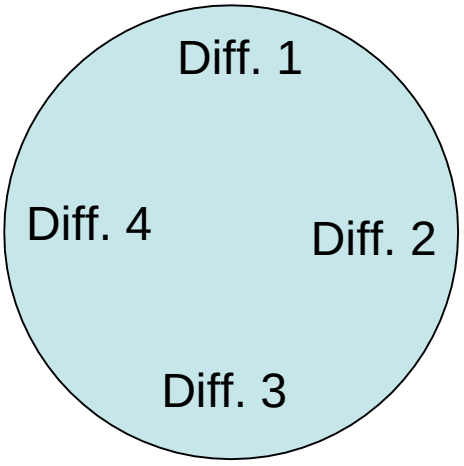
Number	Genotype	Resistance gene
1.	Morocco	Nil
2.	LMPG	Nil
3.	Seri 82	*Sr31*
4.	PBW343	*Sr31*
5.	Sr31/LMPG	*Sr31*
6.	LcSr24Ag	*Sr24*
7.	Sr24/LMPG	*Sr24*
8.	Cook	*Sr36*
9.	Sr36/LMPG	*Sr36*
10.	Eagle	*Sr26*

Seed can be obtained from:

Dr. Kumarse Nazari, ICARDA
Email: k.nazari@cgiar.org

8.2.2.1 Growing Quicksets

About 10 seeds of each differential genotype should be sown in a clump in a good quality potting mix. We sow 4 clumps per 90mm diameter pot – this would mean each "Quickset" would be 3 pots, two with 4 genotypes and the third with two genotypes:



8.2.2.2 Inoculating Quicksets with bulk isolates

A single "Quickset" can be inoculated using a bulk of as many samples as you like. The bulk should be prepared using a sub-sample from each sample (retaining some of the original sample in storage will mean that if anything potentially important is found in the bulk, it can be traced back to an individual sample; see section 8.2.3 for details on storage protocols). A spore suspension is prepared from this bulk by shaking the combined samples in distilled water with Tween 20 added (1 drop Tween for 1 liter water). The suspension can then be sprayed over the "Quickset, using a pump action sprayer – these are available from Dr. Nazari at ICARDA:

Alternatively, if a sample has good amount of rust, simply scrape the rust from pustules into Tween 20 solution. Because spores can germinate rapidly in the water/Tween 20 suspension, inoculations should be done within 30min or less after preparation.

The inoculated "Quickset" can then be incubated under high dew conditions as described previously above.

8.2.2.3 Recording infection types; *typical low infection types associated with key resistance genes*

If the inoculation has worked, you should see pronounced flecking (small light green patches) on the leaves of the susceptible genotypes (Morocco, LMPG) within 7 days. The rust should be fully developed by 12 days, depending on the post-inoculation temperature. The lines 'Morocco' and 'LMPG' are susceptible, i.e. Infection Type (IT) "3+" or "4" (see **Appendix D** for a description of rust ITs). The remaining 8 genotypes carry the resistance genes *Sr24*, *Sr31*, *Sr36* and *Sr26*. To interpret results on these resistance genes, you must know the typical low IT that they produce. The three images below illustrate the typical low ITs associated with *Sr24*, *Sr31* and *Sr36* (*Sr26* generates a typical low IT of from "1" to "2"):

Figure 1. *Sr36* typically produces a "0;" to ";1" infection type. The first four leaves on the left are from lines carrying *Sr36*, and the 5th is a susceptible wheat lacking *Sr36*.

Figure 2. *Sr24* typically produces a "1" to "2" infection type. The first three lines on the left carry *Sr24*, and the 4th and 5th are susceptible lines that do not.

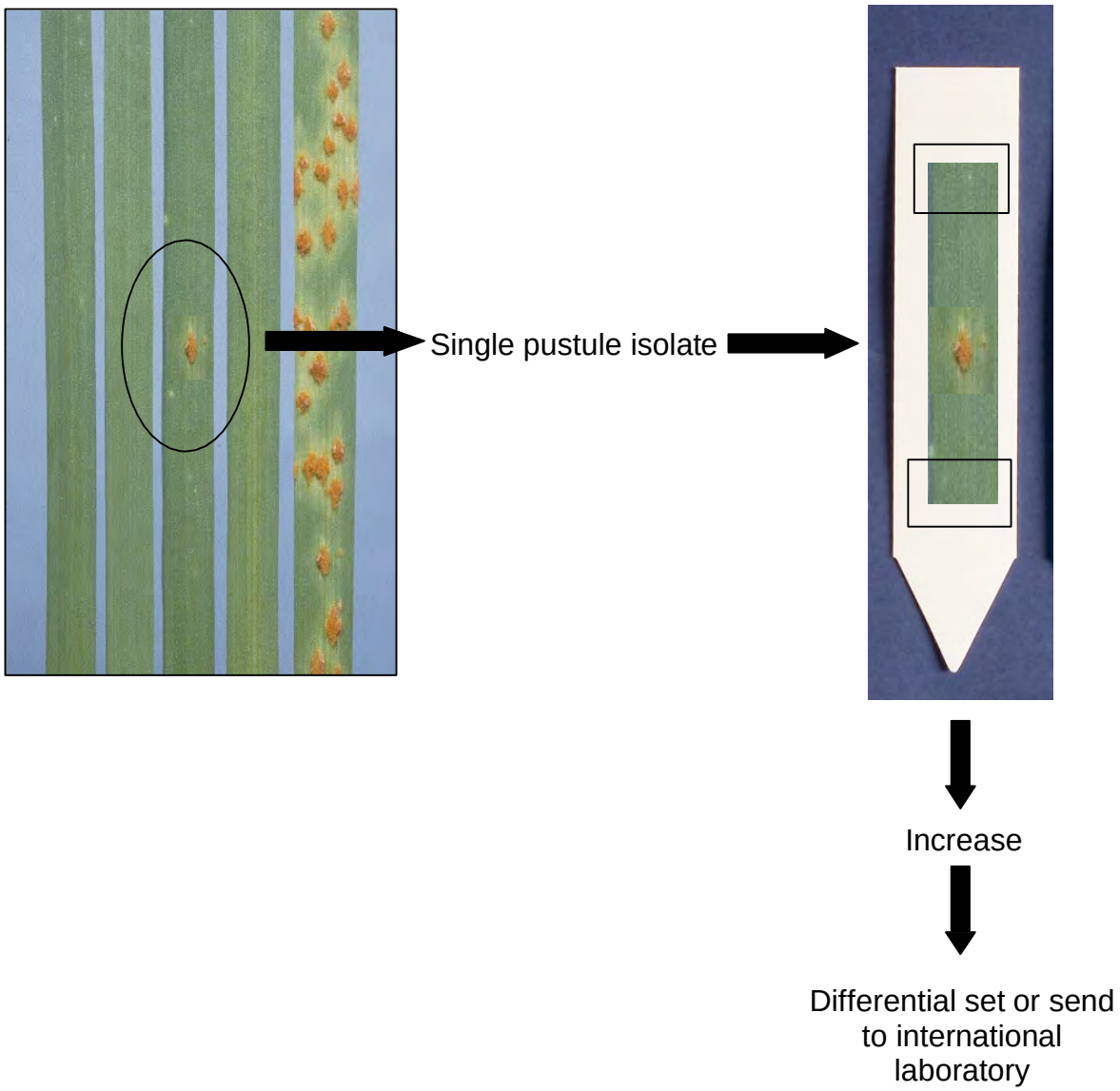
Figure 3. *Sr31* typically produces a ";1" to "2" infection type. The first four lines on the left carry *Sr31*, and the 5th and 6th are susceptible lines that do not.

Fig. 1

Fig. 2

Fig. 3

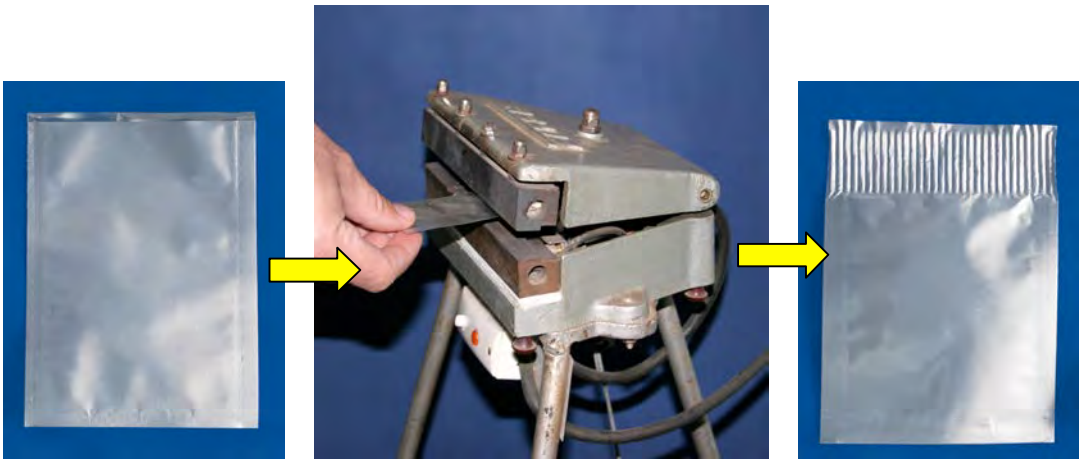
If you see large pustules that look like “3+” or “4” on any of these differentials, it likely indicates the isolate you are working with is virulent for the corresponding resistance gene. Sometimes a mixture of two races is found, one of which is virulent and the other avirulent for the gene in question. The image below gives an example of a mixed infection, of which the predominant race is avirulent for *Sr36*, but has a single “contaminant” pustule of a second race that is virulent for *Sr36*. The racial identity of this *Sr36*-virulent isolate can be determined by sub-culturing onto a susceptible host and then applying the new isolate to a full differential set:



8.2.3 Sample storage

Rust isolates can be preserved by either storing urediniospores **or** dried rust infected plant material. Long-term storage is ideally conducted in a minus 80°C freezer or liquid nitrogen, however, where these facilities are not available, storage for up to 4 weeks can be successful in a refrigerator or cool room (e.g. seed store), or longer in a freezer. If rust isolates are stored in a minus 80°C freezer, liquid nitrogen or a freezer, they must be heat shocked in a water bath before use. We normally do this by 4 minutes at 42°C. It is best practice to do this **immediately upon removal**, before the sample has had a chance to warm.

Before storing, it is **vital** that samples are first dried. This can be done over silica gel for about 3 to 5 days for urediniospores or about 7 days for infected plant material. Samples should then be sealed in an airtight container; this could be a cryovial, a glass tube that is heat sealed, or a plastic lined aluminium packet that can be sealed with a heat crimping device:



8.2.4 Sending samples to International Laboratories for race analysis

If rust samples are to be sent to international labsfor race analysis, it is very important that the correct protocols are followed to ensure:

1) sample viability is optimised
2) adherence to biosecurity procedures in sending samples
3) reinforcement of the expectation that information generated will be shared, but only following vetting and consultation and national approval. This procedure will include if necessary notification and input byBGRI members.

Samples (stem rust and yellow rust) can be sent to Dr Mogens Hovmøller at the Global Rust Reference Center, Aarhus University, Denmark at anytime, following the protocol given in **Appendix B**. Dr. Tom Fetch at AgriFood Canada can only receive stem rust samples from October-February using the protocol described in Appendix C and Dr. Jin, USDA-ARS Cereals Disease Lab, Minnesota, USA can only receive stem rust samples from October-December. Permits must be obtained in advance, for all of the international laboratories and the required protocols followed.

Dr. Fetch has suggested that samples bearing a good amount of rust be dried over silica gel for about 3 days, and once thoroughly dry, the tissue should be put into a plastic cryovial with a screw-type cap and an "o" ring (rubber) washer, along with some silica gel crystals if possible to keep dry. The vials can then be sent packed in a sturdy box. A larger cryovial (see image below) is preferable to allow a good amount of sample and to keep contained from the box. Samples should not be sent in paper envelopes for biosecurity reasons; the plastic vials with caps allow samples to be fully sealed. A step-by-step summary for sending samples to North America is provided in **Appendix C**.

8.2.5 Sending leaf tissue to Australia for marker analysis

If a crop is found that is moderately to heavily infected with stem rust, considerable value will be gained in testing the cultivar for the presence of resistance genes such as *Sr2*, *Sr24* and *Sr31*. If for example a crop is heavily infected with stem rust and the cultivar is found to carry *Sr31*, this is a good (but not necessarily conclusive) indication that the rust race or races causing the rusting are virulent for this gene. Leaf tissue (3 to 5 segments of about 50 mm in length) should be collected specifically from rust infected plants. The leaf tissue can be air dried and stored under ambient conditions for up to several months. The tissue must be stored in 70% ethanol for at least a week before being sent to Australia.

A step-by-step summary for sending leaf tissue to Australia is provided in **Appendix D**.

Appendix A. Sample collection and handling

1. Sample collected from field [3-5 infected stems, cut into 3-4cm lengths, with core stem tissue removed] , stored in paper or glycine envelope (**no** plastic) under ambient conditions.
2. Record details and assign an accession number according to the following format INIYYCCCNN, where INI is the collector initials (2 or 3 letters), YY is the year , CCC is the ISO country code, , NN is the sample number. Example: DPH12IRN01 (Collector DPH, 2012 Iran sample 1)
3. Air dry samples for 12-24 hours at ambient temperature
4. Within 5-7 days of collection: either send directly to an international lab (**only if all permits have been obtained**) or process in-country as follows:
    a. Store above dried silica gel in a sealed container in a refrigerator or cool room
    b. Subculture to a susceptible genotype (see Section 8.2.1), or
    c. Inoculate a full differential set, or
    d. Bulk with other accessions & inoculate "Quickset" (see Section 8.2.2), or
    e. After at least 5 days storage (and no more than 7 days), seal in airtight vials (cryovials) and:
        i. Store in liquid nitrogen, minus 80°C freezer, or normal freezer, or
        ii. If approved, send to international labs(see **Appendix B, C**)

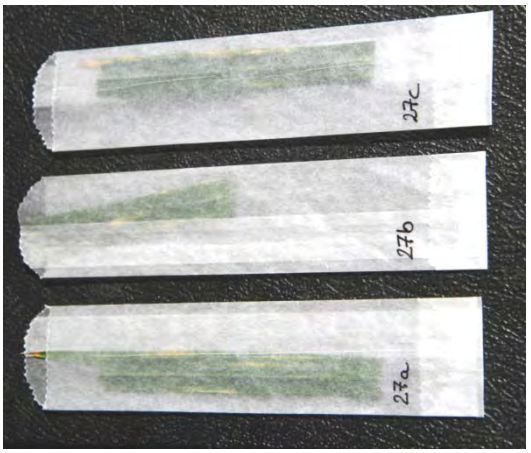
Appendix B. Sending samples to Global Rust Reference Center, Denmark for race analysis

Submission of stem rust samples

1. For submission of stem rust samples you should follow the guidelines developed in the phase I of DRRW project using sealed tubes containing silica gel to keep moisture low.
2. Alternatively, follow the guideline for submission of yellow rust samples which are given below:

Submission of yellow rust samples

Take 3 - 5 leaves/stems from each plot where you wish a sample, younger (upper) leaves are usually better than older leaves (more viable and green). Whenever possible take leaves/stems with clearly separated lesions/pustules. Fold leaves separately and put them individually in glycine bags or similar to promote rapid drying and to avoid curling, - pustules should be inside the folded leaf (photo below).



Press leaves while they dry 12-24 hours at room temperature. Put together samples from a single plot/site into a SINGLE paper envelope and label with unique ID number. After drying, each envelope must be sealed with tape. To increase diversity, we recommend taking samples from different locations and varieties (e.g., some heavily infected and some light infected), up to 20-25 sites/varieties (i.e., up to 25 envelopes with 3-5 individual leaves).Before sending samples to GRRC, you should put additional two layers of sealed envelopes (increasing in size). Each new envelope layer (absolute clean from rust spores) must be added in physical separate rooms, and handled in a lab bench/clean environment using separate lab coats and clean gloves. The final package must be wiped using 70% ethanol to avoid spread of spores during transportation – avoid to store in a fridge at any point due to high humidity – **NEVER** use plastic bags.

We can only guarantee processing incoming samples according to available (limited) resources. State clearly outside the envelope: ***"scientific material without commercial value"*** to avoid unnecessary delays in the customs.

We must receive a request by email prior to submission of samples. If we have sufficient capacity, we will send an import permit stating that GRRC can receive your samples, the permit to be enclosed along with sample submission. Information about details of collector (person), host variety, sampling date, location, disease severity in each plot from where samples are taken must be given (sheet is enclosed). This information should be sent electronically in Excel format or similar, **using all of the following email addresses**:

- Ellen.frederiksen@agrsci.dk
- AnnemarieFejer.justesen@agrsci.dk
- mogens.hovmoller@agrsci.dk

In addition, you should include our note of acceptance of wheat rust samples “GRRC Letter of import statement”, by which we accept the samples of wheat rust from outside Denmark.

Samples must be sent by courier to: Ellen Frederiksen, Dept. of Agroecology, Science and Technology, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark

Appendix C. Sending samples to North America for race analysis

Samples can be sent to Dr. Tom Fetch at AgriFood Canada only during the period October-February. Dr. Jin (CDL, Minnesota) can only receive samples from October-December.

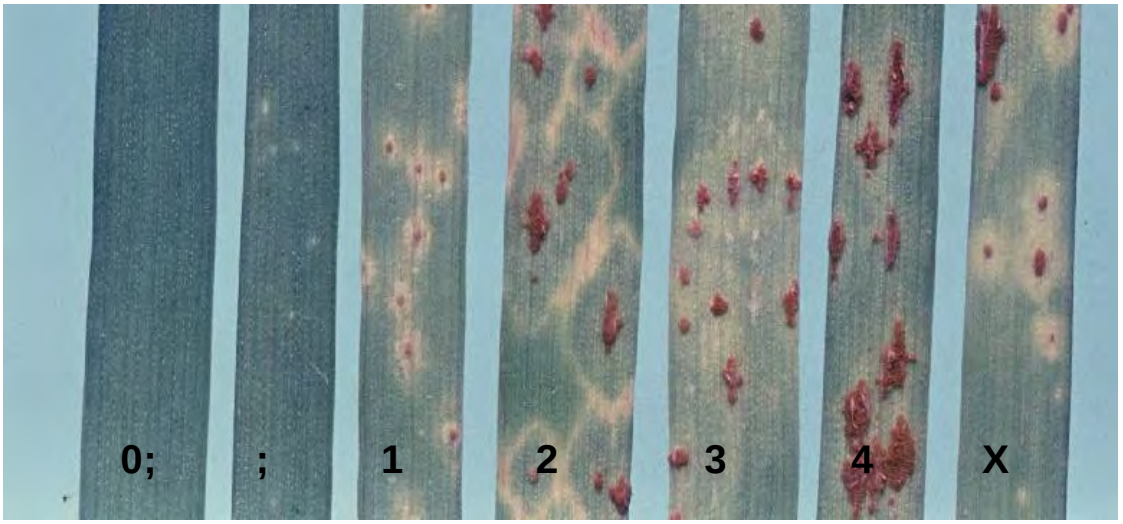
1. Contact Drs Fetch (tfetch@AGR.GC.CA), Park (r.park@usyd.edu.au) and Nazari (K.Nazari@cgiar.org) well in advance if you intend to send samples, and notify that you either have or will have samples to be dispatched
2. You will then be notified if shipment to North America is permissible. Dr Fetch will apply for an import permit to allow him to receive the samples. This normally takes 3-4 weeks for approval and it is vital that you do not send any samples until this permit has been received.
3. Once Dr. Fetch gets the permit, he will FAX or email a PDF file to the person shipping the culture. The permit is good for one year.
4. Collect rust samples as described above (store in paper)
5. Record details as described in **Appendix A.**
6. Within 1-2 days from collection, samples should be dried above silica gel in a refrigerator or cool room for about 5-7 days.
7. The culture(s) is prepared for shipment. Put several silica gel crystals into a cryovial, and then enclose spores or the infected stem tissue of the isolate. Tighten the cap securely, and if possible seal with Parafilm or masking tape. Put all cryovials into a sturdy box and enclose a copy of the import permit. Tape the box securely so to reduce the chance of damage. Affix another copy of the permit on the outside of the box and the address labels for shipment.
8. Ship the box to Dr. Fetch at the address on the permit (AAFC, Winnipeg) using a courier such as Federal Express or DHL. Do not use common post service. Canada Customs will inspect at the airport and send along to Dr. Fetch in Winnipeg. Contact Drs Fetch, Park, or Nazari if you have questions on shipping.

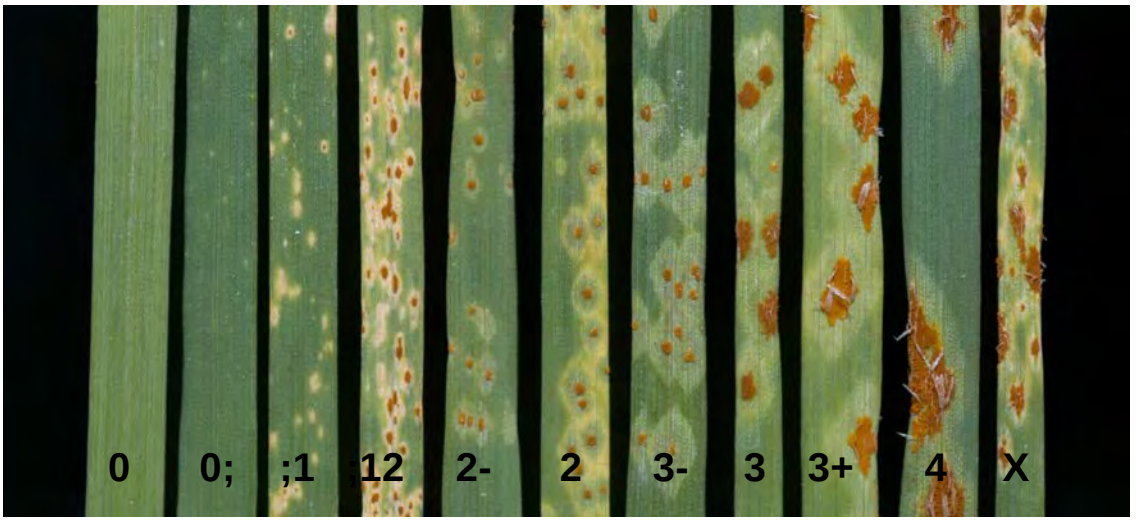
Appendix D. Sending leaf tissue to Australia for DNA marker analysis

1. Collect from 3-5 50mm long sections of leaf tissue (they do not need to be infected with rust) from rust infected plants
2. Record details on where the samples were collected (see sample collection form and envelope provided)
3. Store in paper envelope
4. Immerse leaf sections in 70% ethanol in an airtight container and leave for at least 7 days (container should be filled to at least 80% capacity)
5. Contact Prof. Robert Park (r.park@usyd.edu.au) and Dr. Kumarse Nazari (K.Nazari@cgiar.org) and notify them you have leaf samples to send. Details on how to dispatch material will then be provided by Prof. Park.

Appendix E. Stem rust infection types

- Infection Types (IT) “3+” and “4” are regarded as compatible (i.e. susceptible host, virulent pathogen)
- All other ITs are incompatible (i.e. resistant host, avirulent pathogen)
- *Sr24* typically gives IT “2” to avirulent isolates
- *Sr26* is typically between IT “1” and “2” to avirulent isolates
- *Sr31* is typically between IT “1” and “2” to avirulent isolates
- *Sr36* is typically between IT “0;” to “1” to avirulent isolates





Appendix F: Schema for Stem Rust DNA sample, single pustule protocol

Collect samples from as widely dispersed and representative locations as possible (cover as many environmental zones as possible. 20-25 samples are usually a good representative sample). Include as many farmer field samples as possible. If possible, collect single pustule sample from the same stem (plant) as a live sample sent for race analysis. [NB: Label the live sample envelope with the same code as used for the dead DNA sample!] Include the same sample code on the field survey form and indicate on the survey form that live and/or dead DNA samples were collected.

1. Collect single pustule isolate

a. Identify a distinct single pustule (largest unique pustule possible)
b. Using sharp scissors, cut stem immediately above and below pustule. Cut sheath tissue at sides of pustule, remove core stem tissue. [NB: Single pustules excised from leaves are also fine]
c. Put sheath tissue with pustule into tube containing 80% ethanol {NB: tube filled to 75-80% volume}
d. Label tube, with permanent marker, using following naming convention (NB: use pre-labelled tubes if possible): 2 number year, 3 letter country code, 3 number code e.g., 11YEM001 – year =2011, country = Yemen, sample = 001
e. Clean scissors thoroughly after each sample –use alcohol wipes, wipe scissors thoroughly and discard wipes
f. Repeat above process for each sample

NB: ONLY ADD 1 UNIQUE SINGLE PUSTULE TO EACH INDIVIDUAL SAMPLE TUBE

2. Store samples in ethanol for at least 7 days

3. Pour off ethanol, air-dry tubes for 1-2 days, seal tubes

4. Sending samples

Before sending samples email Dr Szabo (lszabo@umn.edu) and Dr Hodson (d.hodson@cgiar.org) to inform them of intent to send.

• Place sample tubes inside 2 sealed plastic ziplock bags
• Send samples via DHL (or equivalent) to:

Dr. Les Szabo
Cereal Disease Laboratory
U.S. Department of Agriculture
Agricultural Research Service
1551 Lindig St, University of Minnesota
St. Paul, MN 55108-6052
(612) 625-3780 FAX (651) 649-5054
Email: lszabo@umn.edu

• Describe contents on package as: "Herbarium samples (dead, sterilized) for DNA analysis".

NOTE: ONLY DEAD, STERILIZED SAMPLES CAN BE SENT TO THE USDA-ARS CEREALS DISEASE LABORATORY, MINNESOTA

Appendix F: Schema for Stem Rust DNA sample, single pustule protocol

DNA Sampling Protocol

[NB: Clean scissors + hands with Ethanol after each sample]

1. Select a single, large, isolated pustule. Cut the stem either side of the pustule. [NB: **Only 1 pustule per tube**]

2. Remove the core stem tissue

3. Place pustule (and sheath tissue) into a cryovial. Add 70% Ethanol (or absolute alcohol) to ¾ fill the cryovial (ensure pustule is completely covered). Label tube + record unique code on survey form

+

4. Store in ethanol for 7 days. Decant the ethanol, air dry (with tube cap off) for 12-24 hours. When dry, seal the tube, place in zip lock bag and send via courier to Cereals Disease Lab, Minnesota

Please return completed forms to the National Focal Point in your country

Global Cereal Rust Monitoring Form 2013

Surveyor name: _____

Country/Institution: _____

Date of survey (dd/mm/yy): ____/____/____

Location name: _____

Latitude (decimal degrees): **N S** | | | . | | | | | | |

Longitude (decimal degrees): **E W** | | | | . | | | | | | |

Elevation: _____ meters

Survey site: Farmer field ☐ Weed ☐ Trial Site ☐

Crop: **Bread wheat** **Durum wheat** **Barley** **Triticale** **Oats** **Other**

Growth stage: **Tillering** **Boot** **Heading** **Flowering** **Milk** **Dough** **Maturity**

Field area size: _____ ha Variety: _____

Disease	**Stem Rust**	**Leaf Rust**	**Yellow Rust**	**None**
Incidence at field level	**L M H**	**L M H**	**L M H**	
Severity (on infected plants)	**L M H**	**L M H**	**L M H**	
Reaction (R,MR,MS,MSS,S)				

L (low) = 5-20% *M (moderate) = 20-40%* *H (high)= more than 40%*

Samples:

Stem Rust live sample collected: **Y N** SR Sample ID: _____

Stem Rust DNA sample collected: **Y N** SR DNA Sample ID: _____

Yellow Rust live sample collected: **Y N** YR Sample ID: _____

Other Diseases:

Other Disease	**Incidence (at field level)**	**Severity (on infected plant)**
	L M H	**L M H**
	L M H	**L M H**
	L M H	**L M H**

Comments / Observations:

Main stage	Description	Sub-stage
0	Germination	0.0-0.9
1	Main stem leaf production	1.0-1.9
2	Tiller production	2.0-2.9
3	Main stem node production (stem elongation)	3.0-3.9
4	Booting	4.0-4.9
5	Heading	5.0-5.9
6	Anthesis	6.0-6.9
7	Grain milk stage	7.0-7.9
8	Grain dough stage	8.0-8.9
9	Ripening	9.0-9.9

Growth stages follow Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974)