



FEB 08, 2024

OPEN  ACCESS**DOI:**

[dx.doi.org/10.17504/protocols.io.  
ewov1q67kgr2/v1](https://dx.doi.org/10.17504/protocols.io.ewov1q67kgr2/v1)

**Protocol Citation:** Erin H Hill,  
Benjamin Schwessinger 2024.  
Wheat disease symptoms  
observation, capture, description,  
and evaluation. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.ewov1q67kgr2/v1>

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**Protocol status:** Working  
We use this protocol and it's  
working

**Created:** Feb 07, 2024

 Wheat disease symptoms observation, capture, description, and evaluation

 Forked from [Wheat disease symptoms observation, capture, description, and evaluation](#)

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BIOL3106/6106



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Last Modified: Feb 08, 2024

PROTOCOL integer ID: 94793

## ABSTRACT

This protocol is part of the ANU Biosecurity mini-research project #1 "Plant Pathogen Diagnostics: Visuals, subcultures, and genomics".

You will be provided four pots of 3-4 week old wheat plants that have been infected with different wheat pathogens. Each pot has been infected with one major pathogen. You will not know which pot has been infected with which pathogen. However, you will be provided a compendium of 10-15 wheat pathogens that will guide you to identify the infective agent for each treatment group. The fifth treatment group will be uninfected wheat plants which will be clearly identified. You can use treatment group #5 as negative control for your experiments.

In total, each group will obtain five pots each:

	A	B
	Treatment group 1	Unknown infective agent
	Treatment group 2	Unknown infective agent
	Treatment group 3	Unknown infective agent
	Treatment group 4	Unknown infective agent
	Treatment group 5	Uninfected control

This specific protocol is a step by step guide to capture and describe the disease symptoms the provided wheat plants display in week 2 and 3. You can then compare the observed disease symptoms with the provided compendium and other online sources to try to identify the causal infective agent.

The final goal is to achieve the following:

- obtain high quality images of your diseased leaves for each treatment group that are representative. These pictures can be taken with your mobile phone for the complete attached leaves lined up on black cardboard paper or via the stereo microscope as demonstrated by the support staff.

- provide a detailed description of the symptoms observed on the leaves. This includes the following: size, shape, form, color, microscopic structure, spore shape and color, and timing of disease symptom development post infection.
- Compare your observed disease symptoms with the uninfected negative control, the provided compendium, and online sources.

This protocol is applicable for week 2 and 3.

Protocols progress overview:

- Week 2 observe, capture, and describe wheat leaf disease symptoms.
- Week 3 observe, capture, describe, and evaluate wheat disease symptoms.

## GUIDELINES

You must have read, understood, and follow the health and safety instructions provided in the "Overview Mini-Research Project #1 BIOL3106/6106" provided on Wattle (ANU learning portal).

You must have signed and returned one copy of the "Student Safety Declaration Form For Practical Class Work" before starting the any laboratory work.

You must have read and understood the Hazard Sheets (Risk assessment) of all chemicals listed bellow in the "Safety Warnings" section. These Hazard Sheets are provided on Wattle as part of the "Overview Mini-Research Project #1 BIOL3106/6106" document.

## MATERIALS

As always you need to bring a lab notebook, a printed version of this protocol, and a pen to record your adventures in the lab.

Consumables and culture material needed:

- Black cardboard paper.
- Double sided transparent tape e.g. Sellotape Double-sided.

Equipment needed:

- Scissors; week 2 and 3.
- Ruler; week 2 and 3.
- Forceps; week 2 and 3.
- Pencil or light marker pen; week 2 and 3.
- Camera (only to be handled without gloves).

## SAFETY WARNINGS

 This protocol does not require any hazardous substances.

Greatest care needs to be given when using scissors.

You need to wear safety equipment at all times including lab coats, gloves, and safety goggles when handling chemicals and biological agents. While the major biological agents used for the wheat infection are pathogens commonly found in Australia, you must treat them as they were infective agents of general concern. Treat them with care. Do not remove them from the laboratory. Do not spread them via clothing. Use a dedicated notebook and pen to make notes during the mini-research project. Do not put anything into your mouth while in the laboratory. Wash your hands each time you leave the laboratory.

## BEFORE START INSTRUCTIONS

You must study the protocol carefully before you start. If anything is unclear post questions directly here on protocols.io.

## Disease symptom capture

- 1** You will receive five pots with wheat plants as detailed in the "Description" section. Please label each pot with your research group name, the date, and the treatment group.
- 2** Carefully, study the plants in each pot. Select four to five leaves for each treatment group that show representative symptoms [TG#1-4] or not in the case of the negative control [TG#5].
- 3** Perform the following steps [3.1 to 3.11 and 4] for each treatment group [#1-5].
  - 3.1** Layout your cardboard paper vertically [aka short side facing you] in front of you.
  - 3.2** Obtain one black cardboard paper. Label it with your research group name, the date, and the treatment group.
  - 3.3** Mark the bottom side closest to you of your black cardboard paper with a horizontal line approximately 1 cm from the edge. Make this line parallel to the edge. This will be called the baseline.
  - 3.4** For your each treatment group, one after another, cut the selected leaves at the base.
  - 3.5** Cut your selected leaves for the treatment group you are currently working on.

- 3.6** Line up your cut leaves with the leaf apex [tip of the leaf] pointing away from the baseline and the leaf base aligned to the baseline.
  - 3.7** Mark the position of the tip of the leaf with a pen. This mark is called the tip-mark.
  - 3.8** Attach one strip of double-sided tape at the bottom baseline.
  - 3.9** Attach one strip of double-sided tape parallel to the bottom baseline at a distance about 1 cm below the tip-mark.
  - 3.10** Attach your cut leaves to the cardboard with the leaf base affixed to the lower baseline double-sided tape and the tip affixed to the double-sided tape close to the tip-mark.
  - 3.11** Place a ruler next to your leaves and take a clear picture with a camera. Having clear labels and a ruler will allow you to easily identify the specific sample later on and add a scale bar to the picture digitally if required. You will know how long 1 cm is based on the ruler. Ideally, you take the picture holding your camera completely parallel to your leaves mounted to the cardboard.
- 4** In addition, you can also take pictures of the disease symptoms with the stereo microscope. Please see a demonstrater for hands on instructions. The microscopes will be connected directly to a laptop. Alternatively you can connect via the Leica Airlab app ([Google Play](#) or [App Store](#)).

Make sure to label your pictures as follows so we can share them online after class and you can clearly identify your pictures.

YYYYMMDD\_ResearchGroupName\_TreatmentGroupNumber\_PictureNumber

For example:

20230206\_BSPS\_TG1\_2.jpg

Pictures that do not follow this naming convention will not be shared with the class.

- 5 Repeat all steps for each treatment group in week 2 and week 3.