A standard protocol for testing viability with the Triphenyl Tetrazolium Chloride (TTC) Test

Uwe Starfinger[1], Gerhard Karrer[2]

[1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,
Institute for National and International Plant Health, Messeweg 11/12, 38104 Braunschweig, Germany,
e-mail: uwe.starfinger@julius-kuehn.de;
[2]Institute of Botany, Department of Integrative Biology and Biodiversity Research (BOKU), University of Natural Resources and Life Sciences, Gregor Mendel Str. 33, 1180 Wien, Austria; e-mail: gerhard.karrer@boku.ac.at

DOI 10.5073/jka.2016.455.18

A standard protocol for the TTC Test was developed in order to conduct ringtests with 3 different common ragweed (*Ambrosia artemisiifolia*) populations among project partners (JKI, KU, BOKU, KIS, AU, for abbreviations, see introduction of this volume) and interested institutions (Ministry of Economic Affairs, Agriculture and Innovation; Iğdır Üniversitesi Turkey and UMR Biologie et Gestion des Adventices France). After the first ringtest the viability results showed more variability between laboratories than between the populations (Starfinger *et al.,* 2012). Therefore the protocol was adapted concerning the categories of dyed seed tissue and a second ringtest was carried out.

Triphenyltetrazolium chloride, TTC, is a redox indicator used to indicate cellular respiration. Its solution in water is colorless but in living tissues the TTC is reduced to a red substance thus dyeing living tissues in red.

The test is commonly used for testing seed quality with various instructions produced by, e.g., the International Seed Testing Association. Certain adaptations for specific seeds are commonly made. This protocol was developed after pre-trials.

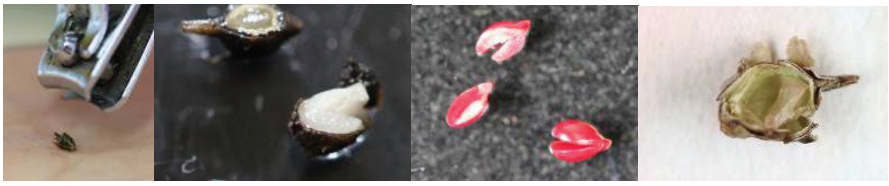
Three populations of Ambrosia seeds were tested (2 from BOKU and 1 from KU). These samples were sent to each partner by BOKU and KU. 100 achenes per population are required (4 samples, each with 25 achene halves).

Materials:

- 100 achenes of each population. Choose randomly, i.e., do not exclude small or light ones that you might suppose to be less viable.
- Tap water
- An instrument to cut achenes in halves. A nail clipper was very reliable or a surgical scalpel or similar instrument
- Distilled water
- 12 glasses of 5-10ml volume which can be covered
- Incubator or drying chamber
- Refrigerator
- 1% TTC-solution (ca. 100ml)
- dissecting microscope/binocular
- Implementation:
- Ambrosia achenes are imbibed in tap water at room temperature over night (i.e., for ca. 12 -15 hours).
- The achenes are cut open with a surgical scalpel or similar instrument in a vertical line (top to base).
- The bigger part of the seed is used for testing, the other part is discarded.

- 25 achene halves are put into one glass and filled up with TTC solution (4 times per population).
- Glasses are tightly closed and put to react at 30°C for 6 hours in darkness. Because TTC is light sensible, avoid unnecessary light input.
- If it is not possible to keep on with the protocol after these 6h, the closed glasses can be stored in a refrigerator (~6-8°C) over night.
- TTC solution is poured off and halves are rinsed under distilled water.

Under a dissecting microscope the seedhalves are removed from the integument (outer shell). Seeds are counted in 3 classes: a) stained (=alive), b) not stained resp. no fully developed embryo present (=dead), c) intermediate cases that are only lightly or partly stained. For the decision on intermediate see below.



Figures 1. – 4.: Illustrating the procedure

1. potential tool for cutting open the achenes (nail clipper); 2. achenes after opening; 3. well-stained embryos; 4. no embryo developed

How to assign "alive" (1); "dead" (0) and "intermediate" (0.5) to staining results

The staining of different tissues in the seed may have different implications for the interpretation of the test. A dead (= not stained) radicula in a otherwise stained seed will mean that the seed is dead. For the sake of simplicity and ease of judgment in the ring test, all seeds that are completely stained shall be deemed alive (1), seeds without any trace of staining will be deemed dead (0) and the rest intermediate (0.5). Figure 5. shows examples and how they should be rated.

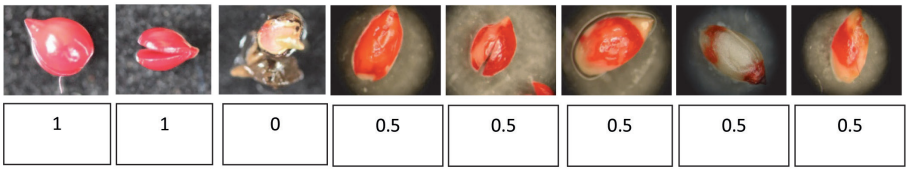


Fig. 5.: Examples of different staining results and how they should be rated; 1 – alive, 0 – dead, 0.5 – intermediate.