A standard protocol for sampling and handling of seed material

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Introduction

Some papers concerning ragweed biology include observations or experiments with seeds of *Ambrosia artemisiifolia*. Date (season) of seed collection and post-harvest treatment (sampling, drying, cleaning, storage) can have strong influence on the seed's viability and dormancy.

Following the fate of seeds we can distinguish papers on

- seed production (timing and quantity of seeds produced during the annual life cycle or up to a defined date of the season: i.e. Dickerson 1968, Basset and Crompton 1975, Kazinczi *et al*. 2008, Pixner 2012)
- seed dispersal by different vectors (Joly *et al,*. 2011; Vitalos and Karrer, 2008; 2009, EFSA 2010)
- seed persistence under different conditions: measured as germination rates of seeds, or test of seed viability (i.e. TTC-test) following specific treatments (including soil seed bank data, seed destruction by heating, burning, etc.) (Kazinczi *et al*. 2008; Karrer *et al*., 2011)

Influence of seed collection, storage and pre-treatment:

In Hungary seeds get ripened under natural conditions at the end of September (Kazinczi *et al*., 2008). The authors do not define what they meant by "ripened": it could be germinable seeds (tested for germination with or without stratification), or viable seeds (TTC-tested directly after collection, or tested after storage at defined conditions). Dickerson (1968) used for all his experiments seeds that were stored dry in an unheated building during wintertime. It can be assumed that low temperatures near or below 0°C were able to induce stratification.

Both examples show that any experiments on germination of ragweed must define the treatment (i.e., storage conditions) before the actual germination test is performed.

Post-harvest treatment up to the time of further tests or germination test must be documented exactly for conditions of air humidity, temperature and light.

In case of the different aims of studies on seed biology of common ragweed, we propose to apply the following seed treatments:

a) Number of viable seeds produced by the (living) individual plant:

a1: conditions of seed collection:

If the aim is to test for the number of viable seeds produced by individual plants the seeds must be taken in fully ripened condition. Following Kazinczi *et al*. (2008) natural ripening of the seeds only can be found from "end of September" onwards. Karrer *et al*. (2011) found germinable seeds already at the end of August. Spontaneous release of seeds may happen every time from the beginning of September to spring. Most seeds drop off latest after the first frost days. Few seeds stay fixed to the plant as long the stem is not pressed down to the ground by heavy rain, wind, or snow.

As female flowering starts from beginning of August and – mostly in case of cut plants – holds on

until October, the production of ripened seeds can be counted exactly in the field only with very high effort.

The two main options always have pros and contras:

(a) Picking seeds from the plant before frost may stop lately developed young embryos from ripening.

(b) Collecting seeds at the time of first frost - when all plants are killed - will also stop further development of ripening seeds.

Considering all experiences from various projects up to now we propose to sample the seeds in field experiments after the first frost killed the plants because the majority of seeds are still attached to the plants.

The number of "viable" seeds that drop off spontaneously could be counted exactly at that time, if the soil below the ragweed individuals is covered by any persistent material from early September onwards to keep all seeds for counting. This sampling net area should be twice the diameter of the individual plant. Thereby all seeds can be picked after frost has killed the plant.

a2: Conditions of storage until further experiments:

Further treatment of the collected seeds is different depending on the features to be measured.

Generally seeds should be cleaned from other vegetative parts of the plant. Such is done by different seed cleaning systems (sieves, gravity tables, upwind selection).

Commonly the seeds are dried before cleaning at room temperatures (20, 25, or 30°C) for about 1 to 8 weeks.

Some authors desiccate the seeds (i.e., at 30°C for about 2 days) before storing them at low temperatures. Such conditions might not simulate the real seed environment after seed set in the field correctly. Natural conditions in autumn include also low temperatures at night-time).

a3: Viability tests of seeds:

Viability is tested in two different ways:

By germinating the seeds, or by testing for viability by TTC-tests.

Germination tests commonly are undertaken with seeds that are stored after cleaning for at least 6 weeks under dry, cool and dark conditions (at around 0°C). Commonly used is 4°C. This treatment simulates the stratification period that is necessary to break the dormancy of ripened ragweed seeds (Payne and Kleinschmidt, 1961; Leiblein-Wild *et al*., 2014).

To study the induction of dormancy at early or later stages of development seeds must be tested directly after harvesting for germinability without any pre-germination treatment.

After collecting all seeds they may be counted for "number of viable seeds" directly after, using a standard TTC-test.

b) Number of germinable seeds produced by the (living) plant:

If the aim is to detect the number of germinable seeds for the next generation, the seeds must be stored immediately after collection under cool and dark conditions: 4°C in darkness is commonly used in several studies on seed persistence and soil seed bank analysis. In case of ragweed a storage period of 6 weeks is enough to stimulate germination afterwards (Karrer *et al*., 2011; Gebben, 1965). Other authors propose at least 8 weeks under such conditions (Kazinczi *et al*., 2008).

All temperatures lower than 4°C are allowed unless not deeper than minus 10°C. Very low temperatures below minus 10°C might have gradually increasing negative influence on the survival rate of ragweed seeds.

c) Number of viable seeds in the soil seed bank:

Soil seed bank can be analysed at different dates throughout the year. Generally the standard date for collecting soil samples is late winter/early spring (s. Fumanal *et al*., 2008), when dormancy of fresh or older seeds is interrupted. Kazinczi *et al.* (2008) report that winter dormancy commonly is broken already during January; such holds at least for Hungary.

New data (Schöberl and Lebernegg, 2013) show that the soil seed bank of ragweed shows some (not significant) losses during winter (4-5 months) at rates of 5 to 40 % (see trial B.2, deliverable DB2). The autumn soil sample was done in October (after first frost killed most of the plants). Soil was stored for 6 weeks at low temperature (stratification) and seeds were sieved in a wet sieving system for being counted and, directly after, tested for germinability. Spring samples from March needed no further stratification treatment and could be sieved, counted and tested directly after sampling.

The most relevant numbers for natural populations of summer annual crops are the germination rates of seeds in early spring. Thus we propose to study seed banks of ragweed always based on the early spring samples that do not need artificial stratification before germination tests. The number of seeds germinated at that time determines the success of ragweed in the raising season.

d) Long-time storage and seed exchange:

The longevity of seeds under the standard storage conditions in the lab (dry, darkness, ≤4°C) was tested also in trials B.2. Seeds from several sites and habitat types lost viability at an annual rate of about 5 to 20 % under standard storage conditions.

Experiments with buried ragweed seeds found that the seeds can survive up to 40 years (Toole and Brown, 1949). In order to standardize seed persistence measurements, seeds are tested in a joint long-term burial experiment of the HALT-Ambrosia team. In this experiment as well as in others all seeds are to be tested for germinability/viability before the start of the experiments (baseline germinability rates).

The first year data gave relatively inconsistent results with death rates (within 1 year) from 5 to 55 %. Further years will give clearer answers, hopefully.

Conclusions

Common ragweed is an interesting object for studying several physiological aspects of invasive plant seeds. Therefore, we call upon all scientists to define clearly the conditions of collection and storage of seeds used for answering different questions. For instance, analyses of the response of plants from seed lots of different geographical locations may be influenced very much by the seed treatment from field sampling up to the start of the experiment. Obviously, ragweed shows a rather complicated system of dormancy (Bazzaz, 1970). Thus the pre-experimental treatment of the seeds is expected to be very influencial.

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