Field survey of the population dynamics of common ragweed (Ambrosia artemisiifolia) Version 2

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Abstract

Background: Common ragweed, *Ambrosia artemisiifolia* L., is an economically important worldwide plant invader, due to its huge production of seeds and allergenic pollen, while its range and impact are expected to increase in future. Knowledge of the population dynamics of this annual species is essential to understand what factors drive spatio-temporal variation in population growth, and can help assessing efficacy of management interventions at the population level. Detailed demographic surveys over multiple years are labour-intensive, but when multiple research groups join forces and work according to standardized protocols, it may be feasible to collect such in-depth data in multiple habitat types across a large geographic range and over larger time spans.

Aim: We developed this field survey protocol to monitor the population dynamics of common ragweed (*Ambrosia artemisiifolia* L., Asteraceae) across Europe. It is designed to estimate all vital rates of a population (establishment of plants, plant survival, plant growth, reproduction, and seed survival), and study how they relate to plant density and individual plant size. It targets unmanaged field populations (to create a baseline), but could also be used for assessing managed populations with some adaptations.

Methods: Plots of 0.25m^2 are established in a field population of common ragweed. The population, plots and (randomly selected) marked common ragweed plants within these are monitored early in the growing season and are re-assessed at seed set, when in addition soil samples and reproductive plants are collected (adjacent to but outside the plots) for further analysis in the laboratory. Repeating the procedure on the same plots in multiple years is essential to derive estimates of seed survival in the soil seed bank from year to year. Replicated monitoring in time (multiple years) and/or space (multiple populations), and measuring (additional) environmental factors, is needed to relate the dynamics to environmental variables.

Protocols provided: i) list of materials, ii) the selection of a site, iii) setting up the survey, iv) monitoring, v) sampling of plants, soil and seeds, vii) lab analyses of samples (plant biomass, soil seed bank, soil texture and content, cleaning of aerial seed samples)

Record forms provided: i) for registration of meta-data of the site, ii) for all measures taken in the field and in the lab; all in a pdf-version for printout to use in the field and in an excel-version

for digitising data

Other material provided: i) field sheets (checklist for field work, legend to the record forms), ii) help tools (for mapping plants, for assigning unique numbers to plants)

Optimisation: The protocol has been used to monitor over 50 populations across the European continent in 2014-2016 by members of the SMARTER Task Force Population Dynamics, within the framework of the EU-funded COST Action 'SMARTER' (FA1203, 'Sustainable management of *Ambrosia artemisiifolia* in Europe', 2013-2016). By yearly Task Force meetings, the protocol has been evaluated and improved to the current version.

Citation: Suzanne T. E. Lommen,Caspar A. Hallmann,Bruno Chauvel,Melinda Leitsch-Vitalos,Gerhard Karrer,Peter Tóth,Heinz Müller-Schärer,Eelke Jongejans Field survey of the population dynamics of common ragweed (Ambrosia artemisiifolia). **protocols.io**

dx.doi.org/10.17504/protocols.io.mmyc47w

Published: 15 Jan 2018

Guidelines

The pdf 'Protocols...' contains an introduction and overview of the study design and includes detailed protocols for all steps.

In short:

- The study has been developed for populations of common ragweed, *Ambrosia artemisiifolia*
- The protocols are based on the phenology of the species in Europe
- The study was designed to estimate all vital rates of a single population (establishment of plants, plant survival, plant growth, reproduction, and seed survival), and study how they relate to plant density and individual plant size in that population.
- As this is an annual plant species with a seed bank, estimates of seed survival can only be made when the monitoring is performed during 2 consecutive years. Note that this estimate is age-independent.
- The protocol targets unmanaged field populations (to create a baseline), but could also be used for assessing managed populations with some adaptations.
- It does include, however, measures of plant damage and presence of natural enemies, in particular of the ragweed leaf beetle *Ophraella communa,* a candidate biocontrol agent for ragweed in Europe which is already used for biocontrol on other continents.
- The protocol assumes no migration of seeds, and therefore does not provide methods to assess movement, immigration or emigration of seeds. The selection of sites is based on this requirement.
- The protocol monitors plants in (permanent) plots of 0.25 m2 representing the range of densities of common ragweed at the site (in order to study the effect of plant density). Hence, the plots are not entirely randomly selected and are therefore not the optimal representation of the site.

- Approximate time (but this is highly dependent on the population and the local conditions) needed for 2 people per population per year (excluding time needed to select a suitable population and to travel):
    - 1 day to set up the site and collect meta-data (in the first year, after that 1-4 hours, depending on the need for additional plots)
    - 1 day field monitoring after establishment, early during vegetative growth
    - 1 day field monitoring and sampling during seed set
    - (0.5-1 day for each optional additional field census)
    - 1-2 days lab analysis
    - 0.5 day digital data entry
- If interested in explaining the dynamics, replicates in time (multiple years) and/or space (multiple populations) are required, and we recommend measuring/obtaining additional environmental variables (e.g. weather, soil moisture) as well as more data on vegetation composition.
- This publication provides:
    - detailed **protocols** for conducting the study: pdf 'Protocols...'
    - **forms for collection of meta-data** of a population: pdf 'Form_Ambrosia_Population_Record' for printout and use in the field, and excel 'DigitalForm_Ambrosia_Population_Record' for digitisation
    - **record forms for data collection** in the field and the lab: pdf 'RecordForms...' for printout and use in the field, excel 'DigitalRecordForms...' to digitisation for standardized further processing of data
    - **field sheets** with a summary of the setup and the guidelines for the standardized measures: pdf 'FieldSheets...'
    - **help tools** that can assist tagging and refinding individual plants in the field: pdf 'Help-tools
- Note: The current version on protocols.io (Version 2) has a corrected author name, an improved abstract and clearer guidelines, but the protocol itself has not changed.

Before start

- Read the pdf document 'Protocols ....'.
- We have experienced that the selection of suitable populations, and obtaining permisison to work on-site, can be very time-consuming. We recommend reserving plenty of time for this.
- The protocol has been developed for a 3-year field survey of an international work group, the SMARTER Task Force Population Dynamics, within the framework of the European EU-funded COST Action 'SMARTER' (FA1203, 'Sustainable management of *Ambrosia artemisiifolia* in Europe', 2012-2016). Members of the Task Force have used it to collect demographic data on >50 populations across the European continent in 2014-2016, and have collected corresponding seed samples for the SMARTER Ambrosia Samples Bank at the University of Fribourg, Switzerland. During the project, the protocol has yearly been evaluated and improved by the Task Force. The currect protocol is the public version of the last version (V5, 2015). The protocol can surely be further improved and refined, and should carefully be adapted to the purpose of future users.
- The joint study of the SMARTER Task Force Population Dynamics has ended, so new users can neglect information about sending data or material to the SMARTER project. The email address SMARTER@unifr.ch is not used anymore, and the website www.ragweed.eu has been archived

within the website of the International Ragweed Society http://internationalragweedsociety.org/smarter/. The archive of the website of the Task Force Population Dynamics can be found at http://internationalragweedsociety.org/smarter/task-forces/task-force-population-dynamics/

- People that still want to contribute seed samples to the SMARTER Ambrosia Samples Bank can contact Prof. Heinz Müller-Schärer of the University of Fribourg (Heinz.Mueller@unifr.ch).
- For all questions about the SMARTER Task Force Population Dynamics or about the protocol, please contact Suzanne Lommen at suzannelommen@hotmail.com

Materials

✓ See the protocol for lists of material needed in the field and equipment needed for lab analyses of samples. Materials and equipment are pretty basic and are generally present in ecological laboratories. by Contributed by users

Protocol

Select a suitable population

Step 1.

Summary: Populations need to fulfill a set of requirements in order to be suitable to be monitored by this protocol (e.g. sufficient numbers of common ragweed plants, sufficient surface covered by the population)

Details: See Protocol Selection & registration population, 'Checklist for suitability of site' (Protocols are found in the pdf 'Protocols...')

Registration of the population (record meta-data of the site, only once per population)

Step 2.

Summary: record meta-data of the site

Details: See Protocol Selection & registration population, 'Registration of populations'

Record form: Form_Ambrosia_Population_Record (printable field version as pdf)

Digital form: DigitalForm_Ambrosia_Population_Record (excel file for digitisation of data)

Setup the site

Step 3.

Summary: in the first year permanent plots within the site are selected for monitoring, and their position recorded. Plots for soil sampling are chosen, and a photo reference point is defined. In consecutive years the same plots are re-assessed, and if necessary, additional plots are set up.

Timing (very important!): The site should be set up when you expect that the majority of the germinated seeds have been established as seedlings/young plants, but are still small (several pairs of true leaves, plant height max. 30 cm).

Details: See Protocol Setup site

Record forms: Form_1_site-map, Form_2_plot-map

Other forms: Field_sheet_1_Checklist-field-work, Field_sheet_2_Legend-to-forms

Census Establishment (census e)

Step 4.

Summary: the site and the plots are monitored, and individual plants within plots are marked and measured.

Timing (very important!): immediately or shortly after having set up the site. The census should be executed when you expect that the majority of the germinated seeds have been established as seedlings/young plants, but are still small (several pairs of true leaves, plant height max. 30 cm).

Details: See Protocol Census Establishment

Record forms: Form_3_monitor-study-area, Form_4_monitor-plot (14 or more copies needed)

Other forms: Field_sheet_1_Checklist-field-work, Field_sheet_2_Legend-to-forms, help_tool_plant-map, your completed Form_2_Plot-map

Optional: Extra censi (census x1 and x2)

Step 5.

Summary: extra monitoring after the Census Establishment to follow the individually marked plants at smaller time intervals (ca. each month). It is equal to the protocol Census Seed set hereafter, but without the sampling.

Details: See Protocol Census Extra

Record forms: Form_3_monitor-study-area, Form_4_monitor-plot (14 or more copies needed)

Other forms: Field_sheet_1_Checklist-field-work, Field_sheet_2_Legend-to-forms,

your completed help_tool_plant-map, your completed Form_2_Plot-map

Census Seed set (census s)

Step 6.

Summary: the site, the plots, and the individually marked plants are re-assessed. Soil samples are taken around the plots for estimates of the soil seed bank, and 21 matured plants are sampled from outside the plots for estimates of reproduction.

Timing (very important!): The census should be executed around seed set: as soon as possible after all female reproductive structures have been formed, but before seeds ripen, and before plants

dry out

Details: See Protocol Census Census Seed set

Record forms: Form_3_monitor-study-area, Form_4_monitor-plot (14 or more copies needed), Form_5_plants-outside-plots

Other forms: Field_sheet_1_Checklist-field-work, Field_sheet_2_Legend-to-forms,

your completed help_tool_plant-map, your completed Form_2_Plot-map

Optional: Collection of aerial seeds

Step 7.

Summary: optional but recommended collection of seeds directly from the plants (to store for future research)

Timing: During census Seed set or any time later when seeds are ripe and can easily be collected from the plants

Details: See Protocol Aerial seed collection

Optional: extra census (census x4)

Step 8.

Summary: extra monitoring after the Census Seed set to monitor the individually marked plants once more (e.g. to assess their decay). It is equal to the protocol Census Seed set, but without the sampling.

Details: See Protocol Census Extra

Record forms: Form_3_monitor-study-area, Form_4_monitor-plot (14 or more copies needed)

Other forms: Field_sheet_1_Checklist-field-work, Field_sheet_2_Legend-to-forms,

your completed help_tool_plant-map, your completed Form_2_Plot-map

Lab analyses of samples

Step 9.

Summary: processing, measuring and storing of the field samples.

Four protocols are available:

I. Analyse plant samples - Assess biomass

II. Analyse plot soil samples - Assess the seed soil bank

III. Analyse extra soil sample - Assess soil content and soil texture

IV. Process and send the seed sample

Details: See Protocol Lab Analysis samples

Record forms: Form_6_soil-samples

Other forms: Field_sheet_2_Legend-to-forms

Digitise data from step 3-9

Step 10.

Summary: digitising data collected in step 3-9

Details: Data that have been collected in steps 3-9 and have been recorded manually on Record Forms 1-6 can all be digitized in one excel file 'DigitalRecordForms...', which groups data at different organisational levels. Detailed instructions for filling out the data can be found on the first work sheet. The format has been designed for further (standardized) processing of data in R. Use one file for each population and for each year.

Digital form: excel file 'DigitalRecordForms...'

Next year

Step 11.

Repeat step 3-10 each year of the study, have fun!

Warnings

- The wind-dispersed pollen of the study species are highly allergenic. Mind to take care of personal protection when monitoring during pollen release.
- The study species is invasive on many continents, and has a quarantine status in some countries. Please avoid dispersing seeds after monitoring and ensure complying with local/national/international laws when transporting plants and/or seeds.