Effects of seed traits variation on seedling performance of the invasive weed, Ambrosia artemisiifolia L.

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1. Introduction

The juvenile stage represents the most vulnerable period in a plant’s life cycle (Simons and Johnston, 2000; Vange et al., 2004). During this time, seedling performance is crucial and can have an impact on later life stages, and therefore affect the overall fitness of the parents (Gross, 1984; Renata D Wulff, 1986). The successful development of a seedling increases the chances for effective establishment of opportunist species or plant invaders in new areas (Fenesi et al., 2014; Skalova et al., 2012). It is therefore very important to understand what the factors are that can influence seedling performance. Variation in such performance may result from genetic variation (Biere, 1991), have a plastic origin in response to environmental conditions (Hotchkiss et al., 2008), or be caused by seed trait variation (Dolan, 1984; Harper et al., 1970; Monty et al., 2013; Roach and Wulff, 1987; Stanton, 1984).

Seed traits, e.g., seed mass, seed size, seed colour, are known to vary considerably within various species, even among populations or individuals (Harper et al., 1970; Lopez et al., 2008; Roach and Wulff, 1987; Simons and Johnston, 2000; Stanton, 1984; Susko and Lovett-Doust, 2000). This phenotypic variation often comes about from environmental constraints. For example, evidence has been found of clinal variations in seed traits as a consequence of local climatic conditions (Moles et al., 2007; Monty and Mahy, 2009). When the resources become limiting, a variation may appear because of trade-offs in resource allocation between seed size and seed number (Smith and Fretwell, 1974; Venable, 1992). This differential resource allocation is known as the “bet-hedging” strategy.

A large intraspecific variation in seed traits can also be an adaptive response of the plant to environmental conditions, e.g. to habitat characteristics (Tautenhahn et al., 2008), to the competition intensity (Kleunen et al., 2001), or to predation (Moegenburg, 1996). Selection can also directly promote seed variation in order to enhance the ability of a plant to survive in a wider range of environmental conditions (Fenner and Thompson, 2005).
production of a ‘range’ of seed traits is an effective evolutionary strategy that can minimize risk and increase the probabilities of reproducing in an unpredictable environment (Venable and Brown, 1988). This is particularly true for annual ruderal plant species that colonize disturbed habitats (Harper, 1977).

The consequences of seed trait variation on seedling performance may depend on environmental conditions experienced by the progeny, with performance differences between large and small seeds being greatest under adverse conditions and lower in more favourable environments (Dolan, 1984; Gross and Smith, 1991; Gross, 1984; Monty et al., 2013; R D Wulff, 1986a, 1986b). These differences would be explained by a stronger advantage of seed resources in aiding seedling development in adverse conditions versus favourable conditions where the seedlings are less stressed.

Seed traits may have different influence on seedling development. For example, seed mass may be considered a proxy of the reserves that the mother had provided to the embryo, and it is often used to account for seed variation (Simons and Johnston, 2000; Vange et al., 2004). Seed mass variation can be directly connected to germination kinetics (Harper et al., 1970; Schutte et al., 2008), but may also have an impact on seedling performance (Baskin and Baskin, 2001; Dolan, 1984; Harper et al., 1970; Monty et al., 2013; Roach and Wulff, 1987; Stanton, 1984). Another example is seed colour that has been documented to be linked to seed dormancy, with darker or more coloured seeds having a thicker seed coat and a greater dormancy than lighter seeds (Durán and Retamal, 1989; Khan et al., 1997; Powell, 1989; Wyatt, 1977). The seed coat exerts its germination-restrictive action most of the time by being impermeable to water and/or oxygen, by its mechanical resistance to radicle protrusion, or by the presence of phenolic compounds with antioxidant properties that play a protective role against degradation processes (Debeaujon et al., 2000).

The study of seed traits variation is especially important in the case of plant invasion. The seed is often the dispersal vector of invasive plant (Cain et al., 2000), and is then to the invasion process. Seed trait variation could be a major feature explaining invasive plant success, as it not only can enhance colonization at both local and regional scales, but also facilitate the exploitation of spatial and temporal heterogeneous environments (Mandák and Pysk, 2001; Monty et al., 2013; Willis and Hulme, 2004). A better understanding of how plant invaders colonize and establish in new areas is therefore critical to prevent further invasion. However, to date, just a few studies have examined the influence of seed variation and its consequences for plant invaders (Söber and Ramula, 2013; Susko and Lovett-Doust, 2000).

Ambrosia artemisiifolia L. (common ragweed, Asteraceae) is an annual plant that was introduced from North America to Europe more than a century ago (Heckel, 1906). The achenes of A. artemisiifolia have a central terminal beak surrounded by a ring of tiny spines (Fig. 1), probably having a dispersal role through soils and human transport (Bassett and Crompton, 1975). The species substantially spread in numerous European countries (Chauvel et al., 2006; Kazinczi et al., 2008; Smith et al., 2013; Solomon et al., 2007). It is both a weed colonizing spring crops and a ruderal plant invading open disturbed habitats, such as wastelands, roadsides or riverbanks (Bassett and Crompton, 1975). For convenience, the entire dispersal unit of A. artemisiifolia will be referred to as a seed.

A. artemisiifolia invasion in Europe is an especially interesting case study, as invasion success is known to be linked to seed characteristics (Funesi et al., 2014; Fumanal et al., 2006; Guillemin and Chauvel, 2011). On top of being highly variable (Fumanal et al., 2007a; Cebben, 1965), the seeds are able to survive for many years in the soil (Bassett and Crompton, 1975), allowing the creation of a long-lasting soil seed bank. This soil seed bank ensures the establishment of the species for years beyond once a single successful seed production has occurred in the area (Fumanal et al., 2007). Furthermore, the species produces allergenic pollen known to be causing a health crisis in Europe, making the understanding of the mechanisms involved in invasion success highly valuable for management (Kazinczi et al., 2008; Laaidi et al., 2003; Smith et al., 2013).

Both studying how seed variation is structured as well as gaining insight into its consequences on seedling performance are essential to better comprehend the critical factors influencing seedling development in the early stage of its life, and the subsequent successful establishment of the species. Specifically, the work presented here addressed the following questions: 1) How is the seed variation structured among mothers and populations and are there geographic patterns? 2) How do seed traits influence seedling performance? 3) Is the influence of seed traits on seedling performance dependent on environmental conditions (i.e. the temperature)?

2. Materials and methods

2.1. Seed collection

Seeds of A. artemisiifolia were collected from nine populations in the Netherlands, Belgium and France (Table 1). Populations were sampled in ruderal habitats (along roadsides, riverbanks or wastelands) during the autumn of 2013. In each population, all seeds of ten randomly chosen mature individuals (i.e. mother plant) having at least 30 seeds were collected and stored in separate paper bags. Pending the start of the experiment, the seeds were stored for 6 months at 4 °C.

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<td>I</td>
</tr>
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</table>
For each harvested mother plant, the total number of seeds was counted using a Contador® (Pfeuffer®, Kitzingen, Germany). Ten seeds from a mother plant were randomly selected. Seeds without an embryo were excluded by testing their resistance to light hand pressure on the seed surface (Fumanal et al., 2007b; Guillemin and Chauvel, 2011). Each excluded seed was replaced by another randomly chosen seed. In total, 900 seeds were selected.

2.2. Seed measurements

In order to best characterize seed variation structure, three seed traits were chosen that are known to have different origins and varying physiological implications in the development of the future seedling. First, the mass of each seed was measured to the nearest $10^{-4}$ g (XA105 DualRange, Mettler Toledo®, Viroflay, France). In addition to the seed mass, we assessed the seed functional area. This measurement corresponds to the area of the biggest ellipse inscribed in the seed picture. The aim is to have a measurement of the space available for the embryo without outgrowths that commonly encompassed in seed mass measurements. Seed functional area was determined with ImageJ (National institute of Health, Bethesda, Maryland, USA) from pictures of each seed taken using an SLR camera (Lens EF 50 mm f/1.8, Canon®, Tokyo, Japan) mounted on a tripod with a white background (Fig. 1). Each picture had a resolution of $3966 \times 1288$ pixels (2.49 megapixels). Finally, the seed coat lightness was measured in the same ellipse drawn for the seed functional area. It was recorded in shades of grey, on a scale from 0 (darkest black) to 255 (purest white). It is calculated as the mean sum of red, blue, and green values of each pixel included in the ellipse.

2.3. Growth chamber experiment

Each seed was sown in an individual pot on a substrate saturated with water. The substrate was housed in 20% universal potting soil (Terofofl, La plaine Chassart, Wagnéœé, Belgium) and 80% river sand (Dololux, Echt, Netherlands) with a total of 25 ml of substrate. The pots with the seeds were stratified over the course of 3 weeks at 4 °C and in darkness.

After stratification, the pots were placed in two contrasted temperature treatments in controlled growth chambers (Fitotron®, SGC 120, Weiss Technik UK, Loughborough, United Kingdom). Five seeds of each mother plant were placed in a chamber. The experiment lasted from February 24th 2014 to April 22nd 2014. One chamber had colder conditions, 15 °C day/10 °C night cycle, and the other had warmer conditions, 25 °C day/20 °C night cycle. Both chambers were set up to have a photoperiod of 16 h day/8 h night with a luminosity of 450 μE during the day. A relative humidity of 70% was imposed to avoid a rapid desiccation of the watered pots. After four weeks of life, each seedling received 3.15 g/0.70% was imposed to avoid a rapid desiccation of the watered pots. At the end of the experiment, then weighed to the nearest $10^{-4}$ g.

2.4. Data analyses

The seed traits and seedling response variations were analyzed with descriptive statistics (mean, standard error of the mean, minimum, and maximum). The Pearson correlation between each seed trait was calculated. To analyze the influence of the population, the identity of the mother plant, and the intra-mother variability on seed traits, a two-ways nested analysis of variance (ANOVA) was performed for each trait using population as a random factor and the mother plant as a random factor nested in population. To test if there was a geographical pattern of variation of the seed traits, the Pearson correlation between each seed trait was calculated with latitude. It was also tested whether seed trait variation was correlated to the number of seeds that the mother plant produced by calculating the Pearson correlation between each seed trait on the number of seeds.

To analyze the influence of the population, the identity of the mother plant, and the temperature on seedling responses, a three-way ANOVA was performed for each of the three seedling responses, using population as a random factor, the identity of the mother plant produced by calculating the Pearson correlation between each seed trait on the number of seeds.

During the experiment, traits that translate different components of overall performance were measured. Firstly, time to germination was measured. The ability of seeds to quickly germinate when the appropriate environmental conditions were met may facilitate the establishment of invasive plants (Rice and Dyer, 2001). This was considered especially relevant for A. artemisiifolia as it has an opportunist behaviour (Bazzaz, 1974). The germinations were recorded daily and a seed was considered germinated when a radicle was visible. The time to germination was measured as the number of days from the start of the experiment in a growth chamber to germination of each seed.

Secondly, foliage cover was measured exactly fourteen days after seed germination. This was thought to be a proxy of early competitive ability that would correspond to a plant’s capacity to quickly impose competition to other plants. As it is independent of germination kinetics, these values would be related to the intrinsic development speed of the seedling. Photographs were taken from the top of the seedling in order to view the entirety of the foliage on a black background. Then, the foliage cover (cm²) was measured using the threshold function of ImageJ that separated the green colour from the background (Fig. 2).

Finally, the above ground dry biomass was measured as a proxy for overall seedling performance. It is a solid predictor of pollen and seed production (as demonstrated by Fumanal et al., 2007a). The plants were cut and dried for 48 h at 65 °C at the end of the experiment.

![Fig. 2. Illustration of the method of measurement for seedling foliage cover of Ambrosia artemisiifolia.](image-url) (A) The standard picture taken on a black background. (B) The corresponding output from ImageJ with the threshold function used to separate foliage cover from the black background. The resulting red area is the surface measured by ImageJ and corresponds to foliage cover measurement. Scale bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
mother plant as a random factor nested in population, and the temperature as a fixed factor. The interaction of the population and mother identity factors with the temperature were added to the model to evaluate whether the population and the mother had the same response to the temperature treatment. Four mothers were observed to cause a rank deficiency because of a lack of germination in one of the temperature conditions. To eliminate this problem, the four mothers were removed from this analysis. To investigate the influence of seed traits on seedling responses, a linear regression of each seed trait on each seedling response was performed. The regressions were conducted separately for each temperature treatment to allow comparison.

In order to test the influence of the temperature treatment on the relationship between seed traits and seedling responses, an analysis of covariance (ANCOVA) was performed for each seed trait influence on every seedling response with the considered seed trait as a covariate and the temperature treatment as a fixed factor.

Prior to conducting the ANOVAs and ANCOVAs, the seed mass, time to germination, foliage cover, and above ground biomass were square root transformed and the seed functional area was log-transformed. The results of the variance of each seed trait variation. Population also (Table 2). The identity of the mother plant explained more than 34% of the variance of each seed trait variation. Population also (Table 2). The identity of the mother plant explained more than 34% of the variance of each seed trait variation. Population also (Table 2). The identity of the mother plant explained more than 34% of the variance of each seed trait variation. Population also (Table 2). The identity of the mother plant explained more than 34% of the variance of each seed trait variation. Population also (Table 2).

The results of the fully-nested ANOVA showed significant variations among seed traits, populations, and temperature effects (Table 2). The Pearson correlation calculated to test if the seed traits varied with latitude did not elicit any significance (seed mass: $r = 0.005$, seed coat lightness: $r = 0.239$, seed mass: $r = 0.005$, seed coat lightness: $r = 0.224$). In contrast, the Pearson correlation calculated to test if the seed traits varied with the number of seeds that the mother produced exhibited a significantly negative impact of seed number on seed mass ($r = -0.258$, $P = 0.014$), and seed functional area ($r = -0.293$, $P = 0.005$), but not seed coat lightness ($r = -0.506$, $P = 0.597$).

3.2. Variation in seed performance

Mean, standard error of the mean, minimum, maximum, and the standard deviation of the time to germination, foliage cover and above ground biomass are listed in Table 3. Of the 900 seeds, 780 were germinated (86.7%), with 418 seeds germinating in warmer conditions (92.9%) and 362 seeds germinating in colder conditions (84.4%).

The results of the three-ways ANOVAs performed to investigate the influence of population and the identity of the mother and the interaction with temperature treatment on seedling responses are shown in Table 4. Significant differences were found among populations and mothers for the time to germination and foliage cover. The temperature treatment had an impact on all traits, and this impact varied among populations. While there was no significant above ground biomass differences among populations and mothers, a significant interaction with temperature was highlighted.

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### Table 3

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<td>19.8 0.519 3 54</td>
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<td>Above ground biomass (g)</td>
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### Table 2

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Note: Significant $P$-values are in bold.
seed functional area in colder conditions ($F_{1, 763} = 8.49$, $P = 0.004$, Fig. 3F), not being significant for the other relationships ($P$-values ranged from 0.157 to 0.885).

4. Discussion

This study sought to understand how seed variation is structured, as well as its consequences on seedling performance. Such knowledge is essential to better grasp the critical factors that influence seedling development in the early stage of life and, thus, the successful establishment of a species.
4.1. The structure of seed variation

A high variation range in the three measured traits was found: a six-fold variation for seed mass and a three-fold variation for both seed coat lightness and seed functional area. A large range of variation of seed traits had already been observed for other plant species (Michaels et al., 1988; Pitelka et al., 1983; Thompson, 1984), and is consistent with other studies on this particular species (Washitani and Nishiyama, 1992; Fumanal et al., 2007b). Sako et al. (2001) examined a large selection of seed traits of the congeneric species, *Ambrosia trifida* L., including seed area and colour, and also found high variability among seeds but did not test their influence on seedling performance. As is best presently known, no study has looked into the range of variation of either seed functional area or seed coat lightness in *A. artemisiifolia*. This variability of seed traits may have been favoured by selection in shifting environments (Fenner and Thompson, 2005; Pitelka et al., 1983; Thompson, 1984).

As for the European invasive species, *Impatiens glandulifera* (Willis and Hulme, 2004), the success of *A. artemisiifolia* in colonizing heterogeneous environments could be partially explained by such seed variability (Fumanal et al., 2007b). The diversification of phenotypes with the goal of increasing fitness in variable conditions is used by a bet-hedging strategy (Slatkin, 1974) and is commonly observed in the case of biological invasions (Hotchkiss et al., 2008; Tayeh et al., 2015).

Michaels et al. (1988) observed that in 39 species, intra-mother variability was often the larger contributor to total variance. In fact, Fumanal et al. (2007a,b) found that intra-mother variability was the most important component of total seed mass variance in *A. artemisiifolia*. In comparison, the results here suggest that seed traits were strongly dependent on population and identity of the mother plant, which together explained more than 60% of total variance in traits. These influences lasted till the point of seedling development and also impacted time to germination and foliage cover. The strong effects of population and the mother could be made sense of by the very high genetic diversity within populations and mother plants, by genetic differentiation among populations, or by a plastic response to local environmental conditions. High genetic diversity of French populations has been described by Genton et al. (2005) as a result of multiple sources of introduction in France. In the present work, it was observed that there were significant interactions of the population with the temperature treatment for all seedling responses. This could also be a clue to population differentiation in response to environmental conditions in the original locations. However, significant effect of latitude was not seen on seed traits, supporting the idea that variation originated from factors other than climate. The differences among populations could then emanate from other environmental factors, such as edaphic differences, for example. In addition, evidence of active gene flow and population admixture has been documented (Chun et al., 2010), which tends to disprove the hypothesis of population differentiation at the geographic scale this study was based on. These observations are corroborated by previous studies on the same populations (Ortmans et al., submitted).

A significant negative correlation between seed number and seed size was observed. This trade-off suggests a strategy of energy allocation from the parent to the offspring (Smith and Fretwell, 1974) that depends on parent size and of the pool size of resources available for seed production (Venable and Burquez, 1990). The fact that seed size was dependent on the number of seeds produced by the parent is another explanation for the strong impact of the mother plant on seed traits. As well, this could be a clue that there was a resource limitation in certain original locations influencing parent size or seed production (Smith and Fretwell, 1974; Venable, 1992).

4.2. The influences of seed traits on seedling performance

The seed mass did not impact the time to germination, but did indeed affect foliage cover and above ground biomass. Heavier seeds also had faster foliage development and produced a larger final biomass. Seed mass variation is known to frequently influence germination kinetics (Harper et al., 1970; Roach and Wulff, 1987). Seed functional area had an effect on all seedling responses — seeds with a larger functional area germinated earlier in colder conditions, had quicker foliage development and produced a higher final biomass than seeds with a smaller functional area. These observations suggest that the place available for the embryo in the seed and the resources included have strong consequences for further development of the plant.

It is interesting that there was a very minimal effect of seed traits on time to germination as a relationship between seed size and time to germination has been found for the congeneric species, *A. trifida* (Schutte et al., 2008).

It was seen that seed functional area had approximately the same effect that seed mass, with a slightly supplementary impact, exerted on time to germination. This is probably a consequence of the strong correlation between these two traits that was highlighted with the Pearson correlation test ($r = 0.829$, p < 0.001).

In contrast to the other seed traits, seed coat lightness had a very negligible effect on the seedlings. This may reflect a previously reported germinative-restrictive influence of seed coat on seedling performance (Debeaujon et al., 2000).

4.3. The influence of the temperature treatment

Temperature treatment had a very strong impact on all seedling responses, denoting an important phenotypic plasticity of the species (Sultan, 2000), this plasticity already having been highlighted by several authors (Paquin and Aarsnes, 2004; Qin et al., 2012). The temperature selected for the colder condition had a negative effect on plant development, possibly a signal that establishment could slow down in countries with colder spring temperatures. However, these aspects cannot be studied without taking into account other climatic factors, such as the photoperiod and the first frost occurrence known to limit the species (Baskin and Baskin, 1980; Chapman et al., 2014; Deen et al., 1998), as well as field observations (Leiblein-Wild et al., 2014; Ortmans et al., submitted).

Surprisingly, an effect of the temperature on the relationship between seed traits and seedling response was barely detected. This could indicate that: 1) the seed reserve is not especially beneficial to the seedling in stressful conditions; 2) despite the strong impact of temperature treatment, the colder condition was not harsh enough to induce stress; 3) apart from temperature, the controlled conditions were too favourable versus those outdoors for truly marked physiological stress.

In this study, very high variability in seed traits and seedling performance was observed that could be one of the *A. artemisiifolia* L. species’ characteristics explaining invasion success, especially in variable environments (Willis and Hulme, 2004). A large part of the variation in seed traits and seedling performance was attributable to the population of origin and to the identity of the mother plant. These factors even had an impact on seedling responses to temperature treatment. In contrast, seed trait variation was not influenced by latitude of the original location, though this excludes the role of climate in seed trait expression. Seed mass and seed functional area appeared to be correlated and to have strong consequences for seedling performance.
Contributions
Conceived and designed the experiments: Monty, Ortmans, Mahy.
Performed the experiments: Ortmans, Monty.
Analyzed the data: Ortmans, Monty.
Contributed reagents/materials/analysis tools: Mahy, Monty.
Wrote the paper: Ortmans, Monty, Mahy.

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