






Article

Is *Ambrosia trifida* L. Preparing for a Wider Invasion? Changes in the Plant Morpho-Functional Traits over a Decade

Aleksandra Savić ¹, Maor Matzrafi ², Sanja Đurović ³, Rodolfo Gentili ^{4,*} and Sandra Citterio ⁴

¹ Department of Weed Research, Institute for Plant Protection and Environment, 11040 Belgrade, Serbia; aleksandra.m.savic@gmail.com

² Department of Plant Pathology and Weed Research, Agricultural Research Organization, Newe Yaar Research Center, Ramat-Yishay IL-30095, Israel; maorm@volcani.agri.gov.il

³ Department of Phytopharmacy and Environmental Protection, Institute for Plant Protection and Environment, 11040 Belgrade, Serbia; stojakovicsm@yahoo.com

⁴ Department of Earth and Environmental Sciences, University of Milano-Bicocca, 20126 Milano, Italy; sandra.citterio@unimib.it

* Correspondence: rodolfo.gentili@unimib.it; Tel.: +39-0264482700

Abstract: Plastic traits are argued to favor the establishment of invasive alien plants and to evolve during the invasion process, so that invasions may be favored by both plasticity and evolutionary changes in plasticity. Despite this aspect being pivotal to understanding invasion processes, no information is available about the effect of residence time on the evolution of phenotypic plasticity of aliens to produce locally adapted phenotypes. Therefore, we aimed to evaluate changes in the morpho-functional traits of the invasive alien *Ambrosia trifida* L. over time. Specifically, we grew *A. trifida* plants under laboratory (at different temperatures: 10 °C, 20 °C and 25 °C) and field conditions by using seeds collected from 2011 to 2020 from an Eastern European population. Data about seedling emergence, vegetative (laboratory and field) and reproductive (field) traits were collected, e.g., maximum plant height (cm), total dry biomass (g), number of flowers (n) and number of new seeds (n). Analysis of variance (ANOVA), linear regressions and a plasticity index were applied to assess differences in plant performances when grown from seeds of different ages. An auto-regressive integrated moving average (ARIMA) model was applied to predict future trends of some key plant traits (maximum height, total dry biomass and number of new seeds). Overall, the time of seedling emergence was not statistically different among seeds of different age, and regression analyses exhibited a positive relationship between residence time (seed age) and vegetative and reproductive parameters of *A. trifida*. Only male flower production exhibited a negative trend over time. At all temperatures (especially at 25 °C), the vegetative parameters increased with the decrease in residence time. The plasticity index of the measured traits tended to decrease over time for most traits both in the field and in the laboratory trials. The ARIMA model predicted that plant traits will continue to increase in size over time by 2030, suggesting further plastic adaptation of *A. trifida*. The results highlighted that residence time influences the evolution of phenotypic plasticity of *A. trifida*, which has strengthened its adaptability to the new conditions over a decade, also demonstrating a great adaptive and invasion potential for the future.

Keywords: giant ragweed; plasticity; adaptations; invasion; plant traits



Citation: Savić, A.; Matzrafi, M.; Đurović, S.; Gentili, R.; Citterio, S. Is *Ambrosia trifida* L. Preparing for a Wider Invasion? Changes in the Plant Morpho-Functional Traits over a Decade. *Agronomy* **2024**, *14*, 1601. <https://doi.org/10.3390/agronomy14081601>

Academic Editors: Bruce Osborne and Panayiotis G. Dimitrakopoulos

Received: 26 June 2024

Revised: 19 July 2024

Accepted: 20 July 2024

Published: 23 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Biological invasions, representing one of the main components of global change, can have negative consequences on the structure, function and dynamics of ecosystems [1]. The success of biological invasions depends on a species' potential to colonize new habitats (e.g., high dispersal efficiency, the persistence of the seed bank, and population density) and on its competitive ability against native plant species (i.e., fast plant growth in terms of higher biomass production and high regeneration capacity; see [2]). In general, the

impact of biological invasions on the resident community depends both on the biological characteristics of the invader and on those of the invaded community [2].

In particular, as highlighted by genetic studies, many invasive species show a quite rapid adaptation to novel environments, occurring within less than 20 generations [3,4]. However, the different speed of adaptive evolution across invasive species make it difficult to predict the potential range expansions of species, especially considering that this aspect has been scarcely considered until recently [5].

Recent studies have attempted to clarify how evolutionary changes are influenced by phenotypic plasticity, as the success of an invasive species has often been attributed to an all-purpose genotype that produces a suitable phenotype in different environments, implying a high level of phenotypic plasticity [6,7]. Plastic traits have been reported to evolve during the invasion process, so that invasions may be favored by both plasticity and evolutionary changes in plasticity [7]. Nevertheless, only a few studies have documented the divergence of traits in natural populations, after observing a gradient of plasticity in the same populations [6,8–10]. Specifically, the “plasticity first” hypothesis predicts that phenotypic plasticity may promote adaptive evolutionary changes by producing populations with increased fitness under different environmental conditions across the species’ distribution ranges (stressful or novel) [11,12]. Over time, environmentally modulated changes in traits can be optimized through a genetic accommodation (i.e., selection) [13]. In this framework, during the period of climate change, many invasive alien species are predicted to be in the process of adaptation over a short time, which could lead to increased populations with better fitness [14–16]. In turn, trait plasticity could confer a competitive advantage to invasive aliens compared to native species, augmenting their impact on ecosystems [17]. Therefore, some plant traits could be modulated by new environmental conditions (for species colonizing new territories) via phenotypic plasticity and could be responsible for the invasion success of alien species [17]. Considering such important issues, more studies predicting how invasive species will behave and adapt to new environmental conditions are needed. Specifically, during biological invasions, species commonly spread over large and climatically diverse areas [18]. Therefore, predicting how plants will respond to new areas needs to be understood in terms of adaptation of local populations to the new conditions. Temperature in particular can exert effects on plants directly, and also indirectly through environmental factors that covary with temperature [19]. *Ambrosia trifida* L. is one of the most noxious alien plant species in Europe. It is native to North America, and today it occurs in almost all United States and Canadian provinces, with a lesser presence in the western part of the continent [20]. It has been widely introduced into Europe [21] and reported in many regions of Europe and Asia [22,23]. *A. trifida* is mostly in the naturalization phase, with a tendency to spread to new areas. In Central Europe, the species’ populations have been recorded in wide areas in a phase of spreading [22,24]. The species is known to be highly variable morphologically and genetically and highly competitive for resources and space [25,26]. Indeed, it is among the first plant species emerging in early spring [27]. By late May, plants can reach a height of up to 4 m [28]. *A. trifida* is highly efficient in utilizing water and nutrients from soil and thus affects the availability of natural resources needed for the growth and development of other plant species [29,30]. As an agricultural weed, *A. trifida* may lead to significant yield losses of certain crops [31]. For example, Baysinger and Sims [32] and Harrison et al. [33] reported losses in maize and soybean yields of 13% and 50%, respectively, with only one *A. trifida* plant/m². In addition, Harrison et al. [31] found a yield loss in corn of 60% with 14 plants of *A. trifida*/10 m². In addition, in non-agricultural habitats, it can negatively affect the biodiversity of local flora and vegetation by suppressing autochthonous, non-native, rare and endangered species [34]. Finally, it is also an allergenic species. Its pollen is an important source of aeroallergens, and it has become the most serious and persistent cause of allergy-associated respiratory diseases in invaded areas [20,23]. Many researches have also demonstrated that plant diffusion and the prevalence of allergy are increasing worldwide due to all the drivers of global change and in particular to temperature rise [20].

Despite its potential for invasiveness, studies about the adaptation potential and plasticity of *A. trifida* are scarce. Previous research has mainly investigated its negative effects on agricultural production [32,35], herbicide resistance [36,37] and its distribution in Europe [22]. No information is available about the effect of residence time on the evolution of phenotypic plasticity to produce locally adapted phenotypes.

In this paper, considering *A. trifida* ecological characteristics, we hypothesized that the residence time of its populations in the invaded area may have promoted the adaptation of the species to the new environment by changing the morpho-functional traits associated with invasiveness to maximize the plant fitness. Specifically, starting from the scientific evidence of the high level of phenotypic plasticity of *A. trifida*, we assumed that the initial invasive environment had induced a phenotype characterized by traits that allowed the species to satisfy its needs, which, over time and with the persistence of the same environmental conditions, became heritable.

To test the hypothesis, we examined the morpho-functional traits of *A. trifida* plants from 10 seed populations collected in the same agriculture area during 10 consecutive years (from 2011 to 2020). Trait changes detected under different temperatures in controlled conditions and in field were statistically elaborated to determine the effect of the residence time on the evolution of phenotypic plasticity of *A. trifida* and to estimate its local adaptive potential.

2. Materials and Methods

2.1. Sampling

Ambrosia trifida L. seeds were collected from an agriculture area in Central Bačka (Vojvodina Province), Republic of Serbia (45°30' N, 19°31' E), each year from 2011 to 2020. The seeds were collected at maturity (in autumn) from at least 100 different mother plants (every year), then brought to the lab and stored at room temperature (20 to 23 °C) until use. To determine the changes in morpho-functional traits of plants grown from the 10 seed populations, a laboratory experiment was performed at the Institute for Plant Protection and Environment (Belgrade), and a field experiment was carried out at a farm near Dobrić, Republic of Serbia (44°41' N, 19°34' E) in 2021. Over the period of seed collection (2011–2020), the climatic conditions during *A. trifida* vegetative season (mean temperature and total rainfalls) at the site of seed collection and at the experimental site were similar (about 18 °C and 500 mm).

2.2. Laboratory Experiment

All the 10 stocks of *A. trifida* seeds were tested for emergence and growth under controlled conditions. To break the dormancy of seeds, they were exposed to stratification treatments in three steps: low temperature (4 °C/7 days), high temperature (40 °C/2 days) and low temperature again with the addition of KNO₃ (4 °C/7 days).

Emergence tests were conducted in the soil substrate (Cultivo, Zeleni Hit, Belgrade, Serbia) (10 seeds/pot) at three different temperatures for 28 days and repeated three times: 10 °C, 20 °C and 25 °C. Such temperatures were chosen based on literature indicating that *A. trifida* germination occurs within a temperature range of 5–41 °C, with a maximum germination rate at 20 °C and an optimum between 10 and 25 °C [38]. Thus, we chose the three temperatures with the temperature of 20 °C corresponding to the peak of maximum germination and the 10 and 25 °C as the lower and upper optimum. With regards to 10 °C, we specifically chose this temperature also because *A. trifida* has been observed to be one of the first summer annual weeds to emerge under this soil temperature early in spring ([22] and according to the Republic Hydrometeorological Institute of Serbia). In addition, testing different temperatures can highlight the ability of the species to adapt to different climatic conditions, as already observed for *A. artemisiifolia* [17]. The sprouted seeds were then used to test plant growth and development at the three different temperatures (10 °C, 20 °C, 25 °C) in three growth chambers with identical photoperiods and light intensity (16/8 h light/dark). We used 150 pots (140 mm × 110 mm)

(3 temperatures \times 5 replications per year \times 10 years). Pots were arranged in a completely randomized design and were rotated weekly to minimize spatial variation. The seeds were planted at 0.5 cm depth in each pot and regularly watered to maintain soil moisture. After sprouting, one seedling was grown per pot for about 2 months, for each treatment. The experiment was set up on 12 April, and the following plant traits were recorded on three subsequent dates (3 May, 24 May and 14 June): maximum plant height (cm), plant number of leaves (n), maximum plant width (cm), fresh and dry biomass of roots (g) and total plant dry biomass (g).

2.3. Field Experiment

The experiment was performed between April and October 2021 (Dobrić, Serbia, 44°41' N, 19°34' E) in a fallow field where no crop had been sown for more than seven years. The results of the chemical analysis of powdered clay soil regarding the basic agrochemical properties, the experiment timeline and the mean daily air temperature and precipitation during the trial period are reported in the Supplementary Materials: Tables S1–S3; Figure S1.

The experiment was set up on the principle of a completely randomized block design with three replications (Supplementary Figure S2). In particular, 10 treatments, corresponding to seeds collected for 10 successive years, from 2011 to 2020, were considered. Each year, seeds were sown in three parcels of 1 m² \times 1 m² and grown at a density of five plants per square meter. The given plant density was maintained by thinning spontaneous vegetation growing in the parcels every 7 to 10 days over the season. The distribution of *A. trifida* within the parcels was kept uniform to minimize differences due to competition among individuals [34]. During plant growth, the following data about vegetative and reproductive traits were collected: maximum plant height (cm), maximum plant width (cm), number of leaves (n) (20 May, 20 June, 24 July, 24 August, 15 September), total dry biomass (g) at the end of the experiment (15 October), number of inflorescences (n) (24 August), number of male flowers (n) (24 August), number of seeds (n) (15 October) and seed mass (g) (15 October). Plant maximum height and width were measured with a wooden pullout meter, while dry biomass was measured with a precision scale in the laboratory. The seed mass was measured on an analytical balance, and the seed counter determined the exact number of seeds per plant (The Contador: Pfeuffer GmbH, Kitzingen, Germany, an optical seed counter with an integrated vibration channel).

2.4. Statistical Analysis

ANOVA and Bonferroni's test were applied to determine statistical differences in seed mass and time of emergences among populations (2011–2020) during the laboratory test. The relationships between the residence time and the recorded *A. trifida* traits in control and field conditions were analyzed by linear regression. Standard error of parameters and adjusted R² were used to show model fitness. Based on the data obtained, the behavior of the future generations of *A. trifida* were predicted by applying the ARIMA model [39]. Phenotypic plasticity of individual traits was calculated for the species for each observation year with the relative distance plasticity index (RDPI), as described by [40]. The RDPI is calculated as the Euclidian distances between the expressed trait values of individuals exposed to different temperatures in both control and field conditions. In particular, the RDPI considered interactions with temperature in lab and field conditions for all traits year by year. All analyses were performed in R version 4.2.2. [41].

3. Results

Overall, although the 10 seed populations of *A. trifida* of different ages showed the same average mass ($F = 0.789$, $p > 0.05$, Figure 1) and the same mean time of seedling emergence at 10 °C ($F = 1.202$, $p > 0.05$, Figure 2), 20 °C ($F = 2.189$, $p > 0.05$, Figure 2) and 25 °C ($F = 1.024$, $p > 0.05$, Figure 2), their derived plants varied in vegetative and generative traits under both laboratory and field conditions.

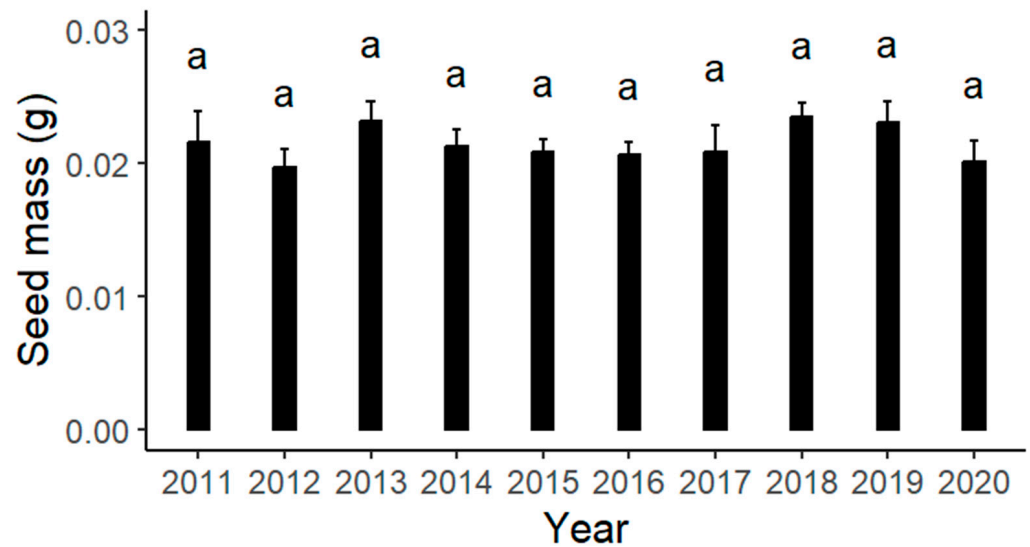


Figure 1. Mean mass of *A. trifida* seeds collected in successive years from 2011 to 2020. Identical letters indicate non-significant differences between years ($p > 0.05$).

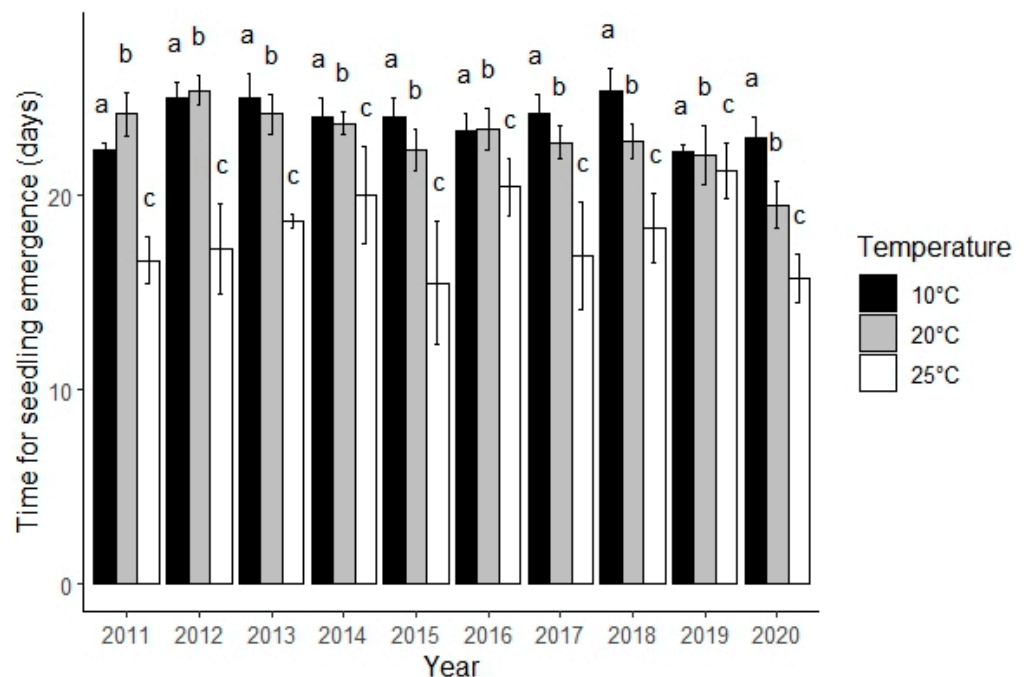


Figure 2. Time required for seedling emergence from the 10 seed populations of *A. trifida* at different temperatures (10 °C, 20 °C and 25 °C) under laboratory conditions. Identical letters indicate non-significant differences ($p > 0.05$) between the years.

3.1. Plant Traits Measurements in Controlled Conditions

Regression analyses exhibited a positive relationship between residence time (seed age) and vegetative parameters of *A. trifida* (Figure 3). At all temperatures, the vegetative parameters increased with the decrease of residence time; the increase was most pronounced at 25 °C (Figure 3). Specifically, a relatively marked positive relationship was recorded between the residence time of *A. trifida* and the trait of maximum height ($F = 6.835$, $p < 0.01$), which was most pronounced at temperatures of 10 °C ($R^2 = 0.231$, $p < 0.01$) and 25 °C ($R^2 = 0.355$, $p < 0.01$) (Figure 3A). A positive trend was also recorded for the parameters of maximum plant width ($F = 17.285$, $p < 0.01$) (Figure 3B), number of leaves ($F = 21.632$, $p < 0.01$) (Figure 3C), root dry biomass ($F = 14.34$, $p < 0.01$) (Figure 3E) and the total plant dry

biomass ($F = 72.065$, $p < 0.01$) (Figure 3F), which was most pronounced at 25 °C ($R^2 = 0.662$, $p < 0.01$) (Figure 3F). Significant interactions between temperature and year were recorded for number of leaves ($F = 3.91$, $p < 0.05$), fresh root mass ($F = 5.477$, $p < 0.05$), dry root mass ($F = 8.833$, $p < 0.01$) and dry mass ($F = 14.04$, $p < 0.01$).

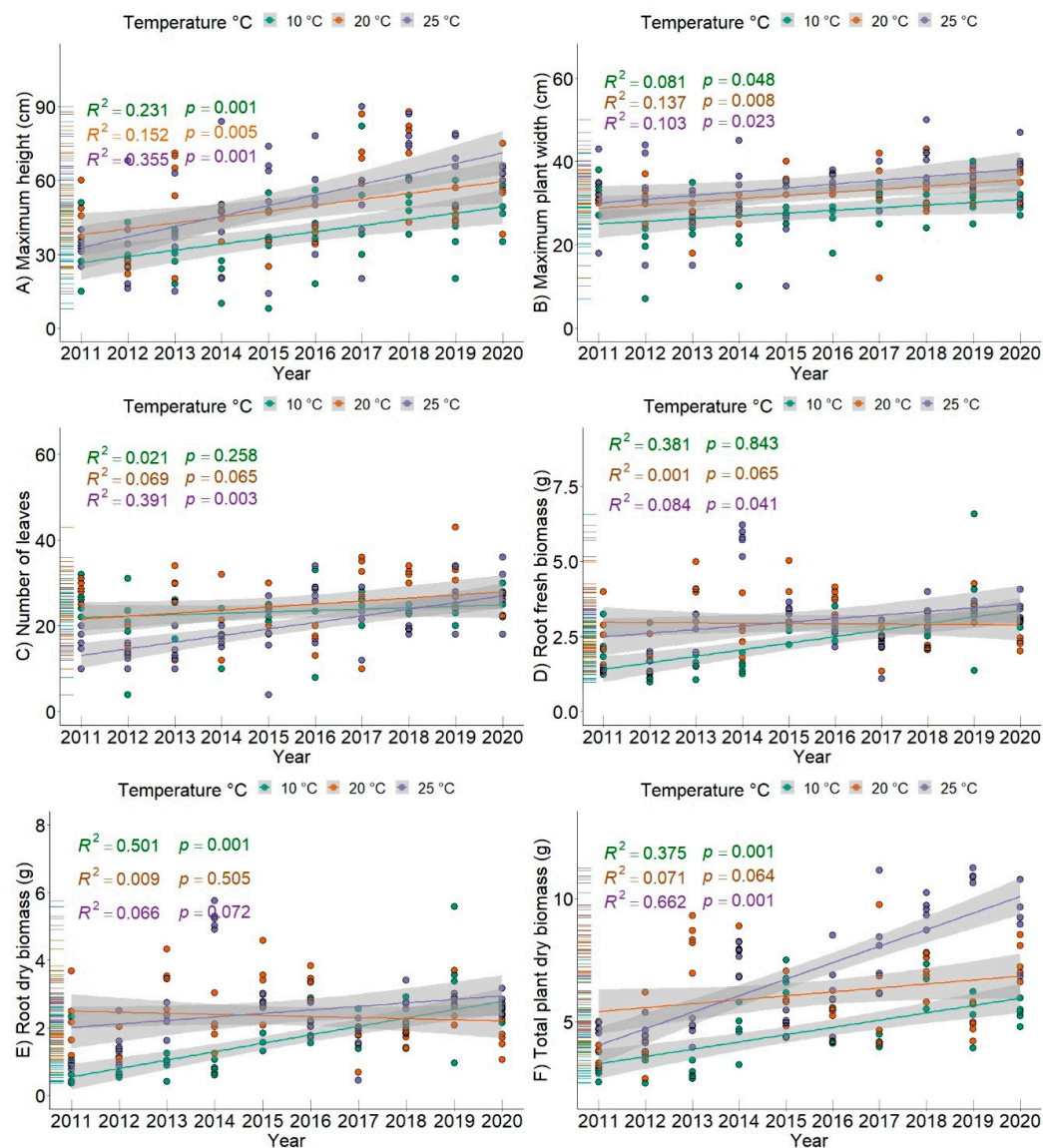


Figure 3. Influence of residence time on vegetative traits (A—maximum height; B—maximum plant width; C—number of leaves; D—root fresh biomass; E—root dry biomass; F—total plant dry biomass) of *A. trifida* plants grown in controlled conditions at different temperatures.

3.2. Plant Traits Measurements in Field Conditions

In the field experiment, the effect of residence time on vegetative and reproductive traits was similar to that observed in control conditions (Figure 4). With decreasing seed age, vegetative and almost all generative parameters increased. Regression analysis confirmed the significant influence of residence time on the examined parameters. Throughout the growing season, height, width and number of leaves of the plants exhibited a positive trend (Figure 4A–C). Specifically, in the last assessment, a statistically significant positive relationship ($p < 0.01$) was found between residence time and plant maximum height ($F = 58.521$, $R^2 = 0.283$, $p < 0.01$) (Figure 4A), total dry biomass ($F = 141.03$, $R^2 = 0.488$, $p < 0.01$) (Figure 4D), number of inflorescences ($F = 83.618$, $R^2 = 0.487$, $p < 0.01$) (Figure 4E) and number of newly produced seeds ($F = 66.467$, $R^2 = 0.309$, $p < 0.01$) (Figure 4G).

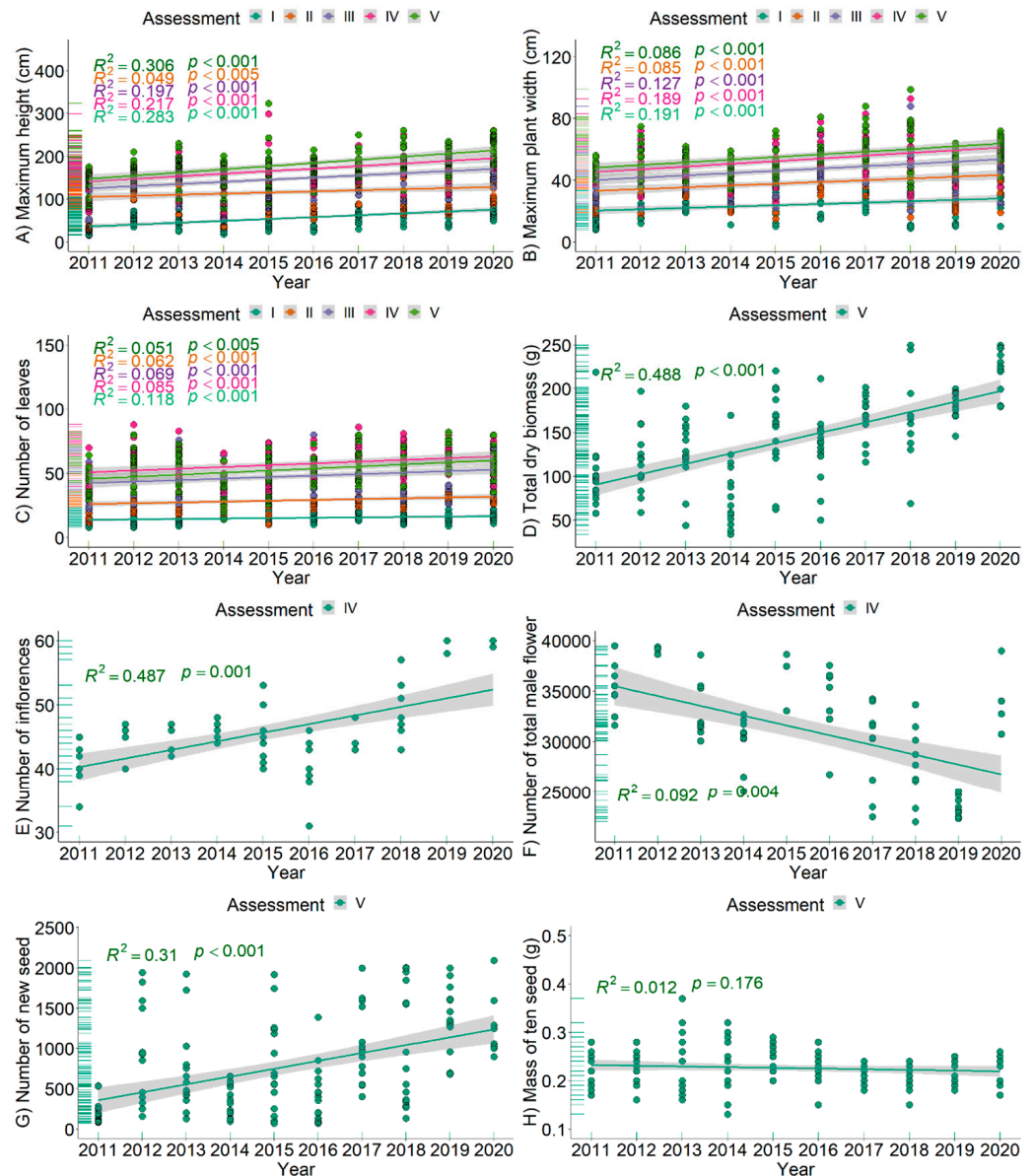


Figure 4. Influence of residence time on morpho-functional vegetative (A—maximum height; B—maximum plant width; C—number of leaves; D—total dry biomass) and reproductive traits (E—number of inflorescences; F—number of total male flower; G—number of new seed; H—mass of ten seed) of *A. trifida* plants grown in field.

A less marked relationship was found for the parameters of plant width ($F = 34.812$, $R^2 = 0.191$, $p < 0.01$) (Figure 4B) and number of leaves ($F = 19.746$, $R^2 = 0.118$, $p < 0.01$) (Figure 4C). Conversely, a negative correlation in the total production of *A. trifida* male flowers per plant was recorded ($F = 8.953$, $R^2 = 0.092$, $p < 0.05$) (Figure 4F), and the relationship between residence time and mass of 10 newly produced seeds was not significant ($F = 1.468$, $R^2 = 0.012$, $p = 0.176$) (Figure 4H). Moreover, significant positive Pearson's correlations between plant mass and seed production were recorded for all 10 consecutive years of seed populations ($F = 39.14$; $R^2 = 0.756$, $p < 0.01$ for 2011, $F = 130.53$; $R^2 = 0.911$, $p < 0.01$ for 2012, $F = 21.76$; $R^2 = 0.626$, $p < 0.01$ for 2013, $F = 128.1$; $R^2 = 0.91$, $p < 0.01$ for 2014, $F = 48.97$; $R^2 = 0.791$, $p < 0.01$ for 2015, $F = 45.26$; $R^2 = 0.77$, $p < 0.01$ for 2016, $F = 65.25$; $R^2 = 0.83$, $p < 0.01$ for 2017, $F = 154.6$; $R^2 = 0.92$, $p < 0.01$ for 2018, $F = 139.13$; $R^2 = 0.91$, $p < 0.01$ for 2019, $F = 58.88$; $R^2 = 0.82$, $p < 0.01$ for 2020) (Figure 5).

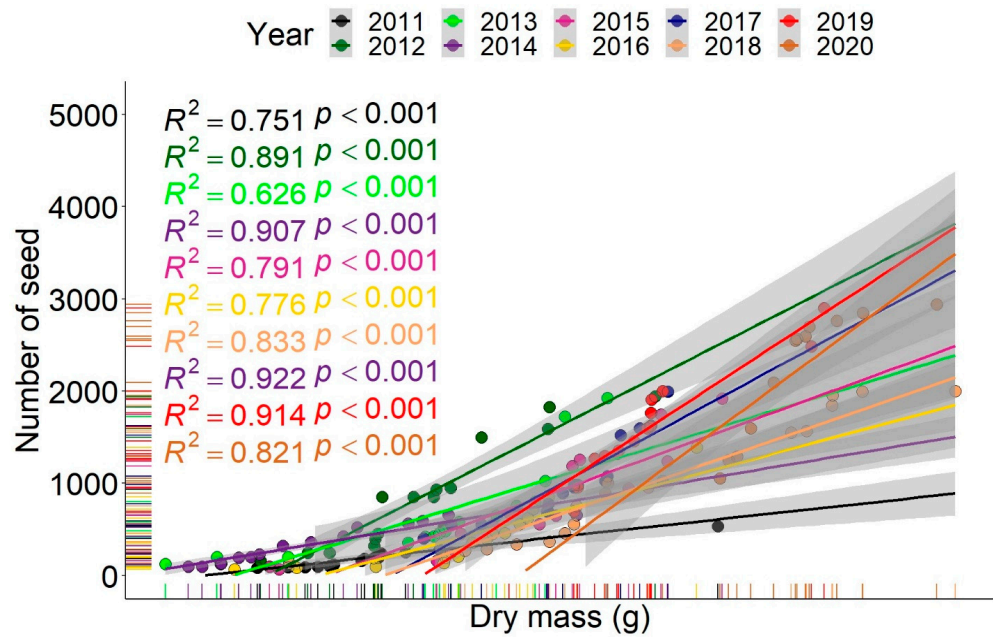


Figure 5. Correlations between vegetative and reproductive traits of *A. trifida*.

3.3. Plasticity of Traits

The phenotypic plasticity according to the RDPI, considering interactions with temperature in lab and field conditions, was calculated for all traits (Figures 6A and 6B, respectively). Overall, all traits exhibited significant differences (all with $p < 0.001$) over the investigated period, most notably for the highest and the lowest RDPI values across years (ANOVA and post hoc tests are shown in the Supplementary Materials). In the lab conditions, under the effects of different temperatures, the traits exhibited RDPI values ranging between 0.074 (plant width) and 0.539 (dry root mass). In the field conditions, the traits exhibited RDPI values ranging between 0.015 (number of inflorescences) and 0.471 (number of new seeds). Overall, both in the lab and the field, the plasticity of most of the traits tended to decrease through two main patterns over time: (a) a linear decreasing trend (e.g., root dry biomass in the lab; plant height in the field) and (b) a downward parabola trend, with a peak in the intermediate years of observation (e.g., plant height in the lab; total dry biomass and number of seeds in the field). A few traits showed a stable trend (total dry biomass in the lab; number of inflorescences in the field).

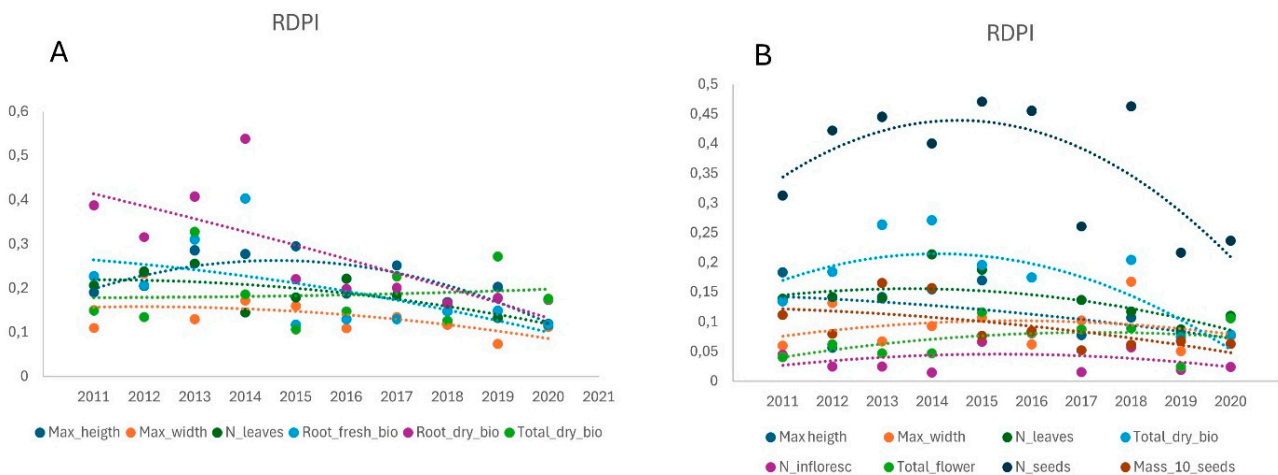


Figure 6. Relative distance plasticity index (RDPI) of *A. trifida* over time and considering the interaction with temperatures in (A) laboratory conditions and in (B) field conditions.

3.4. Prediction (ARIMA Model) of the Future Performance of *A. trifida*

Measurements in field of the maximum plant height, plant dry biomass and number of produced seeds were used for predicting the future performance of *A. trifida*. Predictions revealed that all three parameters were statistically significant with positive trends. In the case of the maximum height, the ARIMA model was best fitted, and the expected maximum plant height is estimated to be 287.86 cm at 2030. For the same year, the expected dry mass and number of seeds are estimated to be 359.12 g and 2944.37/plant, respectively (Figure 7, Supplementary Materials: Table S4).

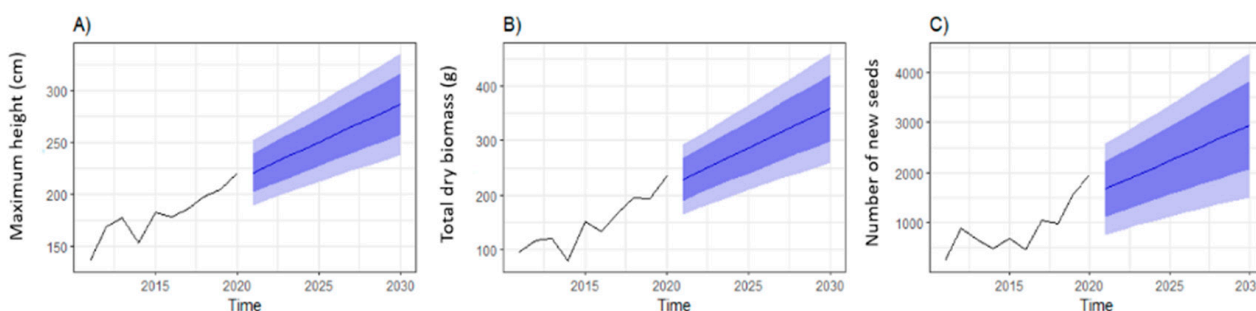


Figure 7. Time forecast (ARIMA model) for parameters (A) maximum height, (B) total dry biomass and (C) number of newly produced seeds. Purple area refers to the prediction of estimated parameters for the future.

4. Discussion

Our results show that in a 10-year experiment in both an agricultural area of Serbia, within its invasive range, and in lab conditions, *A. trifida* has modified most of its vegetative and reproductive traits, increasing its performance over time. This suggests that the species is adapting to local conditions through a genic and/or non-genic control of the main phenotypic traits. In our experiment, the residence time did not affect the germination time but modulated the plant development, generating ever larger individuals capable of producing more and more seeds. This trend was observed in both laboratory (at three different temperatures) and field experiments, suggesting an environmentally induced trait change refined through selection over time. Indeed, when undergoing change to adapt to new environments, populations that express a variety of phenotypes, as shown for *A. trifida* [42], are more likely to be able to adapt quickly, since beneficial phenotypes are more likely to be present and selected for [43]. We point out that the mechanisms enabling plant adaptation are still unclear [43,44] and the analysis of trait variation alone is not sufficient to determine the contribution of genetic and epigenetic factors. However, the reduction of the phenotypic plasticity that we observed in our experiments for most traits within a period of only 10 years suggests the heritability of those traits that allow the species to meet its needs in that specific environment and then its adaptation to local conditions. It is important to underline that we excluded the influence of seed collection and/or storage on our results. The ever larger plants that we obtained from the populations of seeds collected in the subsequent 10 years were not from larger seeds, which are reported to have higher germination and survival rates [45], as seeds were collected from over 100 different plants each year, and no statistical differences in their mean mass, viability and germination (percentage and time) was observed among the seed populations at the beginning of the experiment.

In any case, our results are different from those reported by [46]. They found that young natural populations of the invasive species *Mikania micrantha* were competitively inferior to the old ones, also considering an increasing distance from the source population, suggesting that invasive plants can achieve invasion success by modulating phenotypic plasticity and then changing their traits and genetic characters [46]. On the other hand, in our results in both field and lab conditions, a decrease in seed age corresponded to an increase of vegetative traits at all the tested temperatures (lab conditions), indicating a

certain equilibrium between plasticity and trait heritability (genetic or epigenetic mechanisms) over time. Accordingly, Hovick et al. [47] suggested that the increasingly successful spreading of this species in new environments and regions can be attributed, at least in part, to its adaptive potential. Interestingly, so far, the average height of *A. trifida* plants in Europe, its invasive range, is about half of that recorded in America [48], its native range (2 vs. 4 m), and is increasing and expected to reach 3 m in 10 years according to our data and the predictive ARIMA model. It seems that adaptation to the invasive range tends to make the species regain the size and, in general, the better performance it displays in its native range. We can speculate that the better native population performance, at a vegetative but also reproductive level, relates to genotypes that are probably similar to those that characterized the populations of *A. trifida* at the beginning of its introduction into Europe, which did not perform as well in this new environment, and which started a gradual adapting process.

Across the literature, several plants and, in particular, invasive species show evidence of plasticity and genetic adaptation to environmental gradients [17,49,50]. In fact, in variable environments, invasive species are reported to rapidly adjust those traits generally associated with invasive potential, such as generation time, growth rate, and dispersal ability, which result in phenotypic plasticity and new genetic architectures or adaptation abilities to new regions through genetic accommodation that can subsequently occur [4,51]. In our results, plasticity occurs over time with a multi-temporal shift of traits and growth adjustments. Of particular interest in our experiment was the different resource allocations for reproduction. We observed that the residence time of *A. trifida* was negatively correlated with male flower production, whereas it was positively correlated with plant biomass and seed production. In species belonging to the genus *Ambrosia*, the pattern of male and female flowers is peculiar, with a more significant number of male than female inflorescences [52]. This trend can indicate that different conditions may cause different resource allocations in the studied species, likely acting on phenotypic plasticity [42,53].

Although there is not a great amount of data in the literature about morphological differences within the same population at the multi-year age level of the species, some investigations have reported that many biotic and abiotic factors (of which some are implicated in the environmental changes) might result in the rapid selection and evolution of plant phenotypes. Some species can adapt to local climatic conditions after only one generation [54]. Franks et al. [55] found rapid evolution of flowering time in *Brassica rapa* under the influence of climate change, after just three generations. Additionally, Nowak et al. [56] demonstrated the adaptive evolution of *Noccaea caerulea* via trait variation after one generation of exposure to various levels of Zn contamination in the soil. In the same way, adaptive changes in life-cycle length in response to water stress have been observed over a few generations in *Arabidopsis thaliana* [57]. In the case of *A. trifida*, many different abiotic and biotic environmental factors can have contributed to the species' adaptation to the new environment by driving changes in the morpho-functional traits in order to maximize the plant's fitness. However, at the state of the art, it is difficult to determine what environmental factors have driven this change. Climate matching is a basic requirement for persistence in a new area. According to its global distribution, *A. trifida* comes from temperate areas. Although with several exceptions, this species mainly "moves" from/to warm temperate climate regions, generally avoiding equatorial, arid and snow climates, and apparently shows a limited shift of climatic niche between the native and adventive range [23]. In agreement with this, our laboratory test showed a better performance of the species at 20–25 °C than at 10 °C, regardless of the seeds' age. In addition, a limited shift of climatic niche is also supported by the analysis of mean daily temperatures and precipitations in the *A. trifida* native range and during its residence time in the invasive range, which did not change significantly. However, in addition to climate, many other abiotic and biotic factors can contribute to the difference between native and invasive niches [42]. Thus, further specific studies are needed to understand the factors that have determined the lower performance of *A. trifida* at the early stage of introduction and its

need for adaptation by changing the morpho-functional traits generally associated with invasive potential [58]. The concern, of course, is that the acquisition of adaptive traits associated with invasiveness may favor the spread of *A. trifida* in Europe in the next several years with negative impacts at the environmental, social and economic levels. As several studies have highlighted, plasticity across generations would influence the ecological and evolutionary dynamics of weed adaptation [59–61].

Considering that our results were produced in a short observation period, it is reasonable to assume a positive effect of changes in plant size rather than a negative one, as already observed for most invaders, since they are generally highly adaptable and successful in new environments. The cases reporting that invasive species can experience declines in fitness and adaptability are rare across the literature and generally occur over medium to long time periods (see, for instance, [62]).

In conclusion, our results showed that a 10-year residence time of *A. trifida* populations in the invasion area under steady-state conditions has likely promoted a continuous trend of trait adjustment, allowing the adaptation of the species to the new environment through more vigorous vegetative growth and more efficient reproduction. Indeed, while the mechanisms enabling plant adaptation are still unclear, the reduction of the phenotypic plasticity that we observed in our experiments for most traits over time suggests the heritability of the favorable traits that allow adaptation to local conditions. The results seem to confirm the hypothesis that the adaptability potential of *A. trifida* was drastically strengthened over a decade, and that in the future we can expect plants with a larger vegetative performance and fitness that may greatly influence the spread of this species. Nevertheless, further studies about the genetic mechanisms involved in the accommodation of invasive plant traits are needed.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14081601/s1>, Figure S1: Weather conditions during the 2021 field experiment (Dobrić); Figure S2: Field experimental design; Table S1: Chemical properties of soil in the experimental field Table S2: Timeline and additional information about the field trials during the season 2021; Table S3: Temperature and Rainfall data for the localities mentioned in the article; Table S4: Time forecast (ARIMA model) for parameters Maximum height, Total dry biomass and Number of new seeds of *A. trifida*.

Author Contributions: A.S.: conceptualization, designed the study and methodology, responsible for the investigation (field and control conditions), resources, funding acquisition, data curation, bioinformatics analysis, visualization, writing—original draft, validation. M.M.: writing—review and editing. S.Đ.: help with the field sampling. R.G.: conceptualization, designing the study and methodology, funding acquisition, resources, supervision, validation, writing—review and editing. S.C.: conceptualization, designing the study and methodology, funding acquisition, resources, supervision, validation, writing—review and editing. All authors revised the manuscript and approved it for submission. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of the Italian Ministry of University and Research, funded by the European Union—NextGenerationEU. Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022, adopted by the Italian Ministry of University and Research, CUP, H43C22000530001, Project title “National Biodiversity Future Center—NBFC”. The Ministry of Science, Technological Development and Innovation Serbia (451-03-66/2024-03/200010) supported a very small piece of this project.

Data Availability Statement: Information about the data presented in this study are available from Aleksandra Savić (aleksandra.m.savic@gmail.com). The data are not publicly available due to privacy.

Acknowledgments: Special thanks go to farmer Vlastimir Savić for his support and assistance during the field experiment.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Richardson, D.M.; Pyšek, P. Plant invasions: Merging the concepts of species invasiveness and community invasibility. *Prog. Phys. Geogr.* **2006**, *30*, 409–431. [[CrossRef](#)]
2. Pyšek, P.; Richardson, D.M. Invasive species, environmental change and management, and health. *Annu. Rev. Environ. Resour.* **2010**, *35*, 25–55. [[CrossRef](#)]
3. Prentis, P.J.; Wilson, J.R.; Dormontt, E.E.; Richardson, D.M.; Lowe, A.J. Adaptive evolution in invasive species. *Trends Plant Sci.* **2008**, *13*, 288–294. [[CrossRef](#)] [[PubMed](#)]
4. Whitney, K.D.; Gabler, C.A. Rapid evolution in introduced species, invasive traits and recipient communities: Challenges for predicting invasive potential. *Divers. Distrib.* **2008**, *14*, 569–580. [[CrossRef](#)]
5. Neve, P.; Villa-Aiub, M.; Roux, R. Evolutionary-thinking in agricultural weed management. *New Phytol.* **2009**, *184*, 783–793. [[CrossRef](#)] [[PubMed](#)]
6. Ghalambor, C.K.; McKay, J.K.; Carroll, S.P.; Reznick, D.N. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **2007**, *21*, 394–407. [[CrossRef](#)]
7. Kelly, M. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos. Trans. R. Soc. B* **2019**, *374*, 20180176. [[CrossRef](#)]
8. Ghalambor, C.K.; Hoke, K.L.; Ruell, E.; Fischer, E.K.; Reznick, D.N.; Hughes, K.A. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **2015**, *525*, 372–375. [[CrossRef](#)] [[PubMed](#)]
9. Hendry, A.P. Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J. Hered.* **2016**, *107*, 25–41. [[CrossRef](#)]
10. Walter, G.M.; Clark, J.; Cristaudo, A.; Terranova, D.; Nevado, B.; Catara, S.; Paunov, M.; Velikova, V.; Filatov, D.; Cozzolino, S.; et al. Adaptive divergence generates distinct plastic responses in two closely related *Senecio* species. *Evolution* **2022**, *76*, 1229–1245. [[CrossRef](#)]
11. West-Eberhard, M.J. *Developmental Plasticity and Evolution*; Oxford University Press: Oxford, UK, 2003.
12. Levis, N.A.; Pfennig, D.W. Evaluating ‘plasticity-first’ evolution in nature: Key criteria and empirical approaches. *Trends Ecol. Evol.* **2016**, *31*, 563–574. [[CrossRef](#)] [[PubMed](#)]
13. Lalejini, A.; Ferguson, A.J.; Grant, N.A.; Ofria, C. Adaptive phenotypic plasticity stabilizes evolution in fluctuating environments. *Front. Ecol. Evol.* **2021**, *9*, 715381. [[CrossRef](#)]
14. Clements, D.; Ditommaso, A. Climate change and weed adaptation: Can evolution of invasive plants lead to greater range expansion than forecasted? *Weed Res.* **2011**, *51*, 227–240. [[CrossRef](#)]
15. Sun, Y.; Brönnimann, O.; Roderick, G.K.; Poltavsky, A.; Lommen, S.T.; Müller-Schärer, H. Climatic suitability ranking of biological control candidates: A biogeographic approach for ragweed management in Europe. *Ecosphere* **2017**, *8*, e01731. [[CrossRef](#)]
16. Sun, Y.; Bossdorf, O.; Grados, R.D.; Liao, Z.; Müller-Schärer, H. Rapid genomic and phenotypic change in response to climate warming in a widespread plant invader. *Glob. Chang. Biol.* **2020**, *26*, 6511–6522. [[CrossRef](#)] [[PubMed](#)]
17. Gentili, R.; Ambrosini, R.; Augustinus, B.A.; Caronni, S.; Cardarelli, E.; Montagnani, C.; Müller-Schärer, H.; Schaffner, U.; Sandra, C. High phenotypic plasticity in a prominent plant invader along altitudinal and temperature gradients. *Plants* **2021**, *10*, 2144. [[CrossRef](#)] [[PubMed](#)]
18. van Boheemen, L.A.; Atwater, D.Z.; Hodgins, K.A. Rapid and repeated local adaptation to climate in an invasive plant. *New Phytol.* **2019**, *222*, 614–627. [[CrossRef](#)] [[PubMed](#)]
19. Kardol, P.; De Long, J.R.; Wardle, D.A. Local plant adaptation across a subarctic elevational gradient. *R. Soc. Open Sci.* **2014**, *1*, 140141. [[CrossRef](#)] [[PubMed](#)]
20. Montagnani, C.; Gentili, R.; Citterio, S. Ragweed is in the air: *Ambrosia* L. (Asteraceae) and pollen allergens in a changing world. *Curr. Protein Pept. Sci.* **2022**, *24*, 98–111. [[CrossRef](#)]
21. Lawalree, A. Les *Ambrosia* adventices en Europe occidentale. *Bull. Jard. Bot. L'état À Brux.* **1947**, *18*, 305–315.
22. Follak, S.; Dullinger, S.; Kleinbauer, I.; Moser, D.; Essl, F. Invasion dynamics of three allergenic invasive Asteraceae (*Ambrosia trifida*, *Artemisia annua*, *Iva xanthiifolia*) in central and eastern Europe. *Preslia* **2013**, *85*, 41–61.
23. Montagnani, C.; Gentili, R.; Smith, M.; Guarino, M.F.; Citterio, S. The Worldwide Spread, Success, and Impact of Ragweed (*Ambrosia* spp.). *Crit. Rev. Plant Sci.* **2017**, *36*, 139–178. [[CrossRef](#)]
24. Obradov, D.P.; Radak, B.Đ.; Bokić, B.S.; Anačkov, G.T. Floristic diversity of the central part of the South Bačka loess terrace (Vojvodina, Serbia). *Biol. Serbica* **2021**, *42*, 5–25. [[CrossRef](#)]
25. Patzoldt, W.L.; Tranel, P.J. Molecular analysis of cloransulam resistance in a population of giant ragweed. *Weed Sci.* **2002**, *50*, 299–305. [[CrossRef](#)]
26. Sako, Y.; Regnier, E.E.; Daoust, T.; Fujimura, K.; Harrison, S.K.; McDonald, M.B. Computer image analysis and classification of giant ragweed seeds. *Weed Sci.* **2001**, *49*, 738–745. [[CrossRef](#)]
27. Chauvel, B.; Fried, G.; Follak, S.; Chapman, D.; Kulakova, Y.; Le Bourgeois, T.; Marisavljevic, D.; Monty, A.; Rossi, J.P.; Starfinger, U.; et al. Monographs on invasive plants in Europe N° 5: *Ambrosia trifida* L. *Bot. Lett.* **2021**, *168*, 167–190. [[CrossRef](#)]
28. Malidža, G.; Vrbničanin, S. Novo nalazište alohtone korovske vrste *Ambrosia trifida* L. na području Vojvodine. In Proceedings of the VIII Savetovanje o Zaštiti Bilja, Zbornik Rezimeja, Vojvodina, Serbia, 2006; pp. 44–45.
29. Abul-Fatih, H.A.; Bazzaz, F.A. The biology of *Ambrosia trifida* L. II. Germination, emergence, growth and survival. *New Phytol.* **1979**, *83*, 817–827. [[CrossRef](#)]

30. Mun, S.; Lee, E.J. Litter decomposition rate and nutrient dynamics of giant ragweed (*Ambrosia trifida* L.) in the non-native habitat of South Korea. *Plant Soil* **2020**, *449*, 373–388. [CrossRef]
31. Webster, T.M.; Loux, M.M.; Regnier, E.E.; Harrison, S.K. Giant ragweed (*Ambrosia trifida*) canopy architecture and interference studies in soybean (*Glycine max*). *Weed Technol.* **1994**, *8*, 559–564. [CrossRef]
32. Baysinger, J.A.; Sims, B.D. Giant ragweed (*Ambrosia trifida* L.) interference in soybeans (*Glycine max*). *Weed Sci.* **1991**, *39*, 358–362. [CrossRef]
33. Harrison, S.K.; Regnier, E.E.; Schmoll, J.T.; Webb, J.E. Competition and fecundity of giant ragweed in corn. *Weed Sci.* **2001**, *49*, 224–229. [CrossRef]
34. Savić, A.; Oveisi, M.; Božić, D.; Pavlović, D.; Saulić, M.; Schärer, H.M.; Vrbničanin, S. Competition between *Ambrosia artemisiifolia* and *Ambrosia trifida*: Is there a threat of a stronger competitor? *Weed Res.* **2021**, *61*, 298–306. [CrossRef]
35. Harrison, S.K.; Regnier, E.E.; Schmoll, J.T. Postdispersal predation of giant ragweed (*Ambrosia trifida*) seed in no-tillage corn. *Weed Sci.* **2003**, *51*, 955–964. [CrossRef]
36. Vink, J.P.; Soltani, N.; Robinson, D.E.; Tardif, F.J.; Lawton, M.B.; Sikkema, P.H. Occurrence and distribution of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in southwestern Ontario. *Can. J. Plant Sci.* **2012**, *92*, 533–539. [CrossRef]
37. Kaur, S.; Aulakh, J.; Jhala, A.J. Growth and seed production of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in response to water stress. *Can. J. Plant Sci.* **2016**, *96*, 828–836. [CrossRef]
38. Ruziev, F.; Park, K.I.; Umurzokov, M.; Khaitov, B.; Bo, A.B.; Jia, W.Q.; Hien, L.T.; Choi, J.S.; Park, K.W. Seed Germination Ecology of Giant Ragweed (*Ambrosia trifida*) in Korea. *Weed Turfgrass Sci.* **2020**, *9*, 21–28. [CrossRef]
39. Box, G.E.P.; Jenkins, G.M. *Time Series Analysis: Forecasting and Control*; Holden-Day: Oakland, CA, USA, 1976.
40. Valladares, F.; Sanchez-Gomez, D.; Zavala, M.A. Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications. *J. Ecol.* **2006**, *94*, 1103–1116. [CrossRef]
41. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022. Available online: <https://www.R-project.org/> (accessed on 20 June 2024).
42. Wang, R.; Sun, B.; Li, J.D.; Wang, G.J.; Sun, J.N.; Wang, X.R.; Zhong, R.T. Effects of light intensity on the phenotypic plasticity of invasive species *Ambrosia trifida*. *J. Appl. Ecol.* **2012**, *23*, 1797–1802.
43. Lee, Y.; Chen, A.S.; Tajwar, F.; Kumar, A.; Yao, H.; Liang, P.; Finn, C. Surgical fine-tuning improves adaptation to distribution shifts. *arXiv* **2022**, arXiv:2210.11466.
44. Ng, E.T.H.; Kinjo, A.R. Plasticity-led evolution as an intrinsic property of developmental gene regulatory networks. *Sci. Rep.* **2023**, *13*, 19830. [CrossRef] [PubMed]
45. Meyer, S.E.; Carlson, S.L. Achene mass variation in *Ericameria nauseosus* (Asteraceae) in relation to dispersal ability and seedling fitness. *Funct. Ecol.* **2008**, *15*, 274–281. [CrossRef]
46. Huang, F.; Peng, S.; Chen, B.; Liao, H.; Huang, Q.; Lin, Z.; Liu, G. Rapid evolution of dispersal related traits during range expansion of an invasive vine *Mikania micrantha*. *Oikos* **2015**, *124*, 1023–1030. [CrossRef]
47. Hovick, S.M.; McArdle, A.; Harrison, S.K.; Regnier, E. A mosaic of phenotypic variation in giant ragweed (*Ambrosia trifida*): Local- and continental-scale patterns in a range-expanding agricultural weed. *Evol. Appl.* **2018**, *11*, 995–1009. [CrossRef] [PubMed]
48. Bassett, I.J.; Crompton, C.W. The biology of Canadian weeds. 55. *Ambrosia trifida* L. *Can. J. Plant Sci.* **1982**, *62*, 1003–1010. [CrossRef]
49. Turner, K.G.; Fréville, H.; Rieseberg, L.H. Adaptive plasticity and niche expansion in an invasive thistle. *Ecol. Evol.* **2015**, *5*, 3183–3197. [CrossRef] [PubMed]
50. Gentili, R.; Ferré, C.; Cardarelli, E.; Caronni, S.; Montagnani, C.; Abu El Khair, D.; Citterio, S.; Comolli, R. Performing as a transformer species? The invasive alien *Reynoutria bohemica* changes ecosystem properties in a riparian woodland. *Weed Res.* **2022**, *62*, 446–456. [CrossRef]
51. Fierst, J.L. A history of phenotypic plasticity accelerates adaptation to a new environment. *J. Evol. Biol.* **2011**, *24*, 1992–2001. [CrossRef]
52. Wayne, P.; Foster, S.; Connolly, J.; Bazzaz, F.; Epstein, P. Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia* L.) is increased in CO₂-enriched atmospheres. *Ann. Allergy Asthma Immunol.* **2002**, *88*, 279–282. [CrossRef]
53. Zhao, W.; Liu, T.; Liu, Y.; Wang, H.; Wang, R.; Ma, Q.; Bi, X. The significance of biomass allocation to population growth of the invasive species *Ambrosia artemisiifolia* and *Ambrosia trifida* with different densities. *BMC Ecol. Evol.* **2021**, *21*, 175. [CrossRef]
54. Skråppa, T.; Kohmann, K. Adaptation to local conditions after one generation in Norway spruce. *Tree Genet. Genomes* **1997**, *4*, 171–177. [CrossRef]
55. Franks, S.J.; Sim, S.; Weis, A.E. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1278–1282. [CrossRef] [PubMed]
56. Nowak, J.; Frérot, H.; Faure, N.; Glorieux, C.; Liné, C.; Pourrut, B.; Pauwels, M. Can zinc pollution promote adaptive evolution in plants? Insights from a one-generation selection experiment. *J. Exp. Bot.* **2018**, *69*, 5561–5572. [CrossRef] [PubMed]
57. Brachi, B.; Aimé, C.; Glorieux, C.; Cuguen, J.; Roux, F. Adaptive value of phenological traits in stressful environments: Predictions based on seed production and laboratory natural selection. *PLoS ONE* **2012**, *7*, e32069. [CrossRef] [PubMed]
58. Schutte, B.J.; Regnier, E.E.; Harrison, S.K. The association between seed size and seed longevity among maternal families in *Ambrosia trifida* L. populations. *Seed Sci. Res.* **2008**, *18*, 201–211. [CrossRef]

59. Herman, J.J.; Sultan, S.E. Adaptive transgenerational plasticity in plants: Case studies, mechanisms, and implications for natural populations. *Front. Plant Sci.* **2011**, *2*, 102. [[CrossRef](#)] [[PubMed](#)]
60. Matzrafi, M.; Osipitan, O.A.; Ohadi, S.; Mesgaran, M.B. Under pressure: Maternal effects promote drought tolerance in progeny seed of Palmer amaranth (*Amaranthus palmeri*). *Weed Sci.* **2020**, *69*, 31–38. [[CrossRef](#)]
61. Osipitan, O.A.; Matzrafi, M.; Ohadi, S.; Mesgaran, M.B. Maternal water stress reduces sensitivity of offspring to herbicides in *Amaranthus palmeri* (Palmer amaranth). *Weed Res.* **2021**, *61*, 486–495. [[CrossRef](#)]
62. Sádlo, J.; Vítková, M.; Pergl, J.; Pyšek, P. Towards site-specific management of invasive alien trees based on the assessment of their impacts: The case of *Robinia pseudoacacia*. *NeoBiota* **2017**, *35*, 1–34. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.