

First Report and Genetic Analysis of the Invasive Species *A. fruticosa* L. in Greece: A Combined Genetic and Regeneration Study

Evangelia V. Avramidou ^{1,*} , Evangelia Korakaki ^{2,*} , Ermioni Malliarou ³ , Alexandra D. Solomou ⁴ , George Mantakas ⁵ and George Karetos ⁴

¹ Laboratory of Forest Genetics and Biotechnology, Institute of Mediterranean Forest Ecosystems, ELGO-DIMITRA, Terma Alkmanos, Ilisia, 11528 Athens, Greece

² Laboratory of Tree Physiology, Institute of Mediterranean Forest Ecosystems, ELGO-DIMITRA, Terma Alkmanos, Ilisia, 11528 Athens, Greece

³ Institute of Plant Breeding and Genetic Resources, ELGO-DIMITRA, Themi, 60458 Thessaloniki, Greece

⁴ Laboratory of Forest Ecology, Institute of Mediterranean Forest Ecosystems, ELGO-DIMITRA, Terma Alkmanos, Ilisia, 11528 Athens, Greece

⁵ Laboratory of Landscape Architecture & Environmental Rehabilitation, Institute of Mediterranean Forest Ecosystems, ELGO-DIMITRA, Terma Alkmanos, Ilisia, 11528 Athens, Greece

* Correspondence: eavramidou@elgo.gr (E.V.A.); ekorakaki@elgo.gr (E.K.)

Abstract: A first assessment of genetic diversity and expansion of the invasive species *Amorpha fruticosa* L. was estimated in the 91E0* (Annex I Dir. 92/43/EEC) priority habitat of Nestos. *A. fruticosa* is a perennial deciduous shrub belonging to the family *Fabaceae* and is native to the south-western part of North America, which recently was introduced to Europe as an ornamental plant. Its expansion through the cross-border river of Nestos has significant negative effects on the 91E0* habitat (a habitat with *Alnus glutinosa*, *Populus alba*, and *Ulmus minor*). Herein, we studied the genetic diversity with amplified fragment length polymorphisms fragments of eastern and western populations in order to see if there is one genetic pool for the species. Furthermore, we estimated the regeneration index (IR) in order to see the potential expansion of this invasive species. Results showed that eastern and western populations have almost equal heterozygosity (H_e) and with a slightly higher value for the western population, which was in agreement with the results of the IR. This is an innovative study that discusses the implications of *A. fruticosa* of 91E0* habitat in Nestos and proposes management measures to eliminate its expansion and improve the resilience of the habitat.

Keywords: *Amorpha fruticosa* L.; genetic diversity; regeneration index; 91E0* habitat; Nestos Delta



Citation: Avramidou, E.V.; Korakaki, E.; Malliarou, E.; Solomou, A.D.; Mantakas, G.; Karetos, G. First Report and Genetic Analysis of the Invasive Species *A. fruticosa* L. in Greece: A Combined Genetic and Regeneration Study. *Ecologies* **2023**, *4*, 627–635. <https://doi.org/10.3390/ecologies4030041>

Academic Editor: José Ramón Arévalo Sierra

Received: 15 June 2023

Revised: 6 September 2023

Accepted: 8 September 2023

Published: 9 September 2023



Copyright: © 2023 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

Alien and invasive plants threaten the integrity of natural ecosystems because most of them are invasive, compete with native plants, and in many cases take their place in the ecosystem. Invasive species are characterized by rapid spread due to the production of large numbers of progeny, which can be dispersed over long distances [1]. Moreover, they have a high survival rate due to their plasticity to respond to different environments. In addition, some natural phenomena can further help them spread, such as floods, wind, surface erosion, etc.

The spread of invasive species has also been significantly affected by human activities [2]. Since ancient times, alien plants have been inadvertently or intentionally transported to new environments and cultivated as horticultural or ornamental plants [3]. Their spread is also facilitated by human activities, the role of which is twofold: transport propagating material and habitat alteration. The main advantage is that they do not have physical enemies that would control their populations so that they fully exploit their competitive potential. Some invasive plants also develop new mechanisms of interaction

with natural ecosystems [4]. One of these mechanisms is the production of allelopathic substances and their release in the ecosystem [5].

Amorpha L. (*Fabaceae* Juss.: *Amorpheae* Boriss.) is a genus of 16 species native to North America [6]. These plants can be shrubs, subshrubs, or herbaceous perennials and occur in a variety of habitats, including riparian corridors, savannas, and grasslands. Many species of *Amorpha* have very restricted distributions, and several are considered threatened or endangered [7].

Amorpha fruticosa L. (indigo bush) is a perennial deciduous shrub belonging to the family *Fabaceae* and is native to the south-western part of North America [8]. The indigo bush was introduced in Europe as an ornamental plant [5]. It is used as a medicine for the treatment of burns and eczema [9]. In recent years, special attention has been paid to it due to its bioactivities. Studies have shown that it is a plant rich in polyphenols and contains bioactive ingredients. It also has anticancer, anti-inflammatory, and antimicrobial activity [10]. Indigo bush is a plant with great decorative value and is used in the construction of urban landscapes and in the protection of slopes. It can survive in dry soils, but prefers riverbanks and the edges of flooded forests, where it is easily spread by water as its seed pods are buoyant [11]. Its great tolerance to various climatic conditions as well as its great ability to multiply, gives it the advantage to grow outside its natural distribution with great ease [12]. Today, it is recognized as one of the most invasive species in Europe, with severe impacts on local ecosystems [11]. It is a photophilic plant that competes for light, forming dense thickets that overshadow native flora, and this leads to a decrease in biodiversity [11–13]. Due to the damage it causes to natural ecosystems and water management, measures should be taken immediately to reduce/control its populations.

In Greece, the cross-border rivers such as the Nestos, the Axios, etc., are transport corridors for alien plant species, which expand their range using water [14]. However, apart from the specific mode of transport, in recent years, many foreign plant species have been introduced into Greece, both intentionally (e.g., ornamental, and medicinal plants) and unintentionally, using various ways of introduction [2]. Although the number of alien species is large, not all are invasive. Out of the 343 alien species, only 50 show invasive behavior, and most of them are of American origin [15].

The Nestos is a cross-border river of Bulgaria and Northern Greece. It originates from Mount Rila in southern Bulgaria and passes between the mountain ranges of Aimos and Rhodope. Its length is 234 km, of which 130 km is located in Greece [16]. Before it empties into the sea, it stretches across the Chrysoupoli plain and expands as a deltaic system with freshwater lakes forming the Delta of Nestos. In that area, priority habitat 91E0 (Annex I Dir. 92/43/EEC) exists which shows two subtypes: the subtype of alder stands (*Alnus glutinosa*) and the subtype of white poplar (*Populus alba*) and elm (*Ulmus minor*) stands. The first subtype is composed of pure alder stands, which grow in light to moderately light soils with very good physical properties, which can be characterized as Calcaric fluvisols. Moreover, the subsoil water level is relatively high (90–95 cm). This subtype is found between the dykes in places next to the riverbanks or in places a few dozen meters away from them. The second subtype constitutes a significant part of the riparian forest and is located outside of the dykes. It is composed of white poplar and elm stands, which grow in deep light to moderately light soils with good physical properties, which belong to the Eutric Fluvisol soil type, without alkalinity and salinity problems. The subsoil water level is low but shows a large variation [17].

The area along the river has changed significantly since the Second World War [18]. In 1945, forests and wetlands dominated the area. In the 1960s, agricultural land increased, resulting in a decrease in forested areas. There was also a large reduction in wetlands due to their conversion to grassland. However, despite the great decline of the natural ecosystem, the area still maintains the most extensive riverine forest in Greece. Furthermore, the Nestos River Plain is of significant ecological importance due to its many important habitats. It has been classified as a wetland of international importance and has been included in the

Ramsar Convention. It is also protected by the Berne Convention, European Commission Directives, and Greek legislation. In addition, it belongs to the Natura 2000 Network [19].

In Greece, *Amorpha fruticosa* L. is considered as an invasive species because it is a plant that has the ability to spread over a wide area. In the present study, the genetic diversity of *Amorpha fruticosa* L. populations growing along the Nestos River was investigated. Our objectives were (1) to determine the level of genetic differentiation, if it exists, between the two sides of the river: west and east; (2) to estimate the degree of inbreeding in each population as well as the effective population size; and (3) to discuss the implications of the expansion of the specific species in the natural ecosystem and more specifically in the populations of native species in the area.

2. Materials and Methods

2.1. Plant Material

Leaf sample collection was carried out on 20 *Amorpha fruticosa* individuals from 2 populations of the Nestos River area (indicative coordinates for west population A: X: 565671, Y: 4527927 and east population: X: 566918, Y: 4525481). DNA extraction from the fresh needles was performed with the Macherey Nagel Plant kit (Düren in North Rhine-Westphalia, Germany) according to the manufacturer's protocol.

2.2. AFLP Procedure

A total of 200 ng of genomic DNA was fragmented by digestion with 4 U of EcoRI and MseI enzymes at a temperature of 37 °C for 3 h. The digested DNA fragments were then combined with EcoRI and MseI adapters, and the resulting mixture was ligated using T4 DNA ligase from New England Biolabs (Ipswich, MA, USA). This ligation step was carried out at a temperature of 26 °C for 3 h. The resulting DNA from this process was used as the primary template for AFLP analysis.

For the pre-amplification step, a pair of primers based on the sequences of the EcoRI and MseI adapters, with an additional selective nucleotide at the 3' end (EcoRI+A and MseI+C), were used. The pre-amplification PCR was conducted in a total volume of 20 µL, which contained 1X Kapa Taq Buffer, 0.2 mM of each dNTP, 2.5 mM MgCl₂, 30 ng of each primer (EcoRI+A and MseI+C), 1 unit of Taq DNA polymerase from Kapa Biosystems (located in Wilmington, MA, USA), and 5 µL of the diluted DNA fragments obtained from the digestion and ligation reaction. The PCR cycling conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. To confirm the amplification, a 5 µL sample of the reaction was subjected to agarose gel electrophoresis, while the remaining 15 µL was diluted 5-fold with a TE buffer.

Selective amplifications were performed in 10 µL reaction volumes, consisting of 3 µL of the diluted preselective template, and using the same reaction conditions as the preselective amplification step, with the exception of using 30 ng of a MseI primer and 5 ng of an EcoRI primer per reaction. The selective amplification cycling program was carried out using a BioRad thermocycler (Hercules, CA, USA), and followed this pattern: an initial cycle of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 1 min. This was followed by twelve cycles of denaturation at 95 °C for 30 s, annealing starting at 65 °C for 30 s and decreasing by 0.75 °C each cycle, and extension at 72 °C for 1 min. Finally, twenty-three cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min were performed, with a final hold at 72 °C for 30 min.

The f-AFLP product mixtures were subjected to denaturation by treating them with formamide at a temperature of 94 °C for a duration of 2 min. Subsequently, these denatured mixtures were separated through electrophoresis using an ABI Prism 3730xl Genetic Analyzer, which is manufactured by Applied Biosystems (Waltham, MA, USA). Ten AFLP selective primer combinations were employed. Each individual hybrid was then examined to determine the presence or absence of specific fragments. The size of the detected fragments was determined by utilizing the GeneMapper 4.0 program, along with an internal

standard known as GS 500 LIZ, which was also provided by Applied Biosystems (Waltham, MA, USA). In order to minimize the impact of potential size homoplasy, only fragments within the size range of 150 to 500 bases were considered and subjected to further analysis and counting [20].

2.3. Index of Regeneration

A. fruticosa presence and density were assessed in priority habitat 91E0* within the Nestos area, north-eastern Greece (Figure 1). Fifteen (15) plots, each covering an area of 706.86 m², were selected at random and placed evenly throughout habitat 91E0*. Subsequently, an analysis was conducted to examine the composition and structure of tree species in these plots. *A. fruticosa* was present in four out of 15 plots, of which three were located in the west riverbank and one in the east. Tree height (m), density (individuals ha⁻¹), and the index of regeneration (IR) were measured for *A. fruticosa* individuals. IR was calculated by multiplying the *A. fruticosa* density (per m²) by the average *A. fruticosa* height (cm) and provides a measure of the regeneration extent [20].



Figure 1. *Amorpha fruticosa* extended by the 91E0* priority habitat in Nestos Delta, north-eastern Greece.

2.4. Data Collection and Statistical Analysis for AFLP Data

To transform allele size information obtained from GeneMapper 4.0 (Applied Biosystems, USA) into a binary format, an AFLP Excel Macro was employed. This macro assigned a value of “1” to fragments that were present and “0” to those that were absent. To minimize the impact of potential size homoplasy, only consistent fragments falling within the 150 to 500 base pair range were tallied and subjected to additional scrutiny [21]. In addition, GenAlex v6.0 [22] was used in order to calculate parameters such as percentage of polymorphic bands (P), Shannon’s information index (I), and genetic diversity (He). GenAlex v6.0 was also used to conduct an analysis of molecular variance (AMOVA) and to calculate genetic distances and principal coordinate analyses (PCoA).

In detail, principal coordinate analysis (PCoA) is a multivariate technique that allows one to find and plot the major patterns within a multivariate data set (e.g., multiple loci and multiple samples). The mathematics are complex, but in essence, PCoA is a process by which the major axes of variation are located within a multidimensional data set. For multidimensional data sets, each successive axis explains proportionately less of the total variation, such that when there are distinct groups, the first two or three axes will typically reveal most of the separation among them. Four different options are provided, two based on the conversion of the distance matrix to a covariance matrix, and two working directly from the input distance matrix. The distance matrix is used as the pool for calculating the distribution on axes [22].

2.5. Habitat Information of 91E0*

Habitat 91E0* in the Nestos Delta expands in low altitudes (<100 m above sea level), with surfaces of a mild to flat relief (gradient 0–10%) (Korakaki E. and Karetso G. personal data sampling). It constitutes from dominant tree species such as *Fraxinus excelsior* L. and *Alnus glutinosa* (L.) Gaertn., but also *Fraxinus angustifolia* Vahl, *Salix alba* L., *Populus alba* L., *Pinus nigra* J.F. Arnold, *Juglans regia* L., *Cornus sanguinea* L., *Ulmus minor* Mill., and *Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky. There is a profusion of climbing plants (e.g., *Periploca graeca* L., etc.), and the herbaceous layer invariably includes many species (e.g., *Aegopodium podagraria* L., *Hedera helix* L., *Lysimachia punctata* L., *Circaea lutetiana* L., *Arum maculatum* L., etc.) (Solomou A. and Karetso G. personal data sampling). The Nestos Delta has been created by alluvial deposits of Nestos River. Fluvial sediments consist of alluvial deposits of clays, sands, and gravels, whereas floodplain deposits consist mainly of clays and sands [23].

3. Results

3.1. Genetic Results

Seven AFLP primer combinations yielded 313 loci for the two populations. According to AFLP results, the eastern population had 83.39% polymorphism, while the western population had 83.07%. The mean expected heterozygosity (He) was 0.127, and the western population presented a mean heterozygosity slightly higher (0.136) than the eastern (0.119). Parameters for genetic diversity are being presented in Table 1 along with the epigenetic results.

Table 1. Mean and standard error (SE) for Na (number of alleles), Ne (number of effective alleles), I (Shannon information index), and He (expected heterozygosity).

Mean and SE over Loci for Each Population		Na	Ne	I	He
Western population	Mean	1.671	1.184	0.239	0.136
	SE	0.042	0.011	0.010	0.007
Eastern population	Mean	1.661	1.155	0.216	0.119
	SE	0.042	0.010	0.009	0.006

The AMOVA (analysis of molecular variance) portioned 97% of the genetic variation within populations and 3% only among populations (Figure 2). Principal coordinate analysis explained 16.89% of the variance (Figure 3).

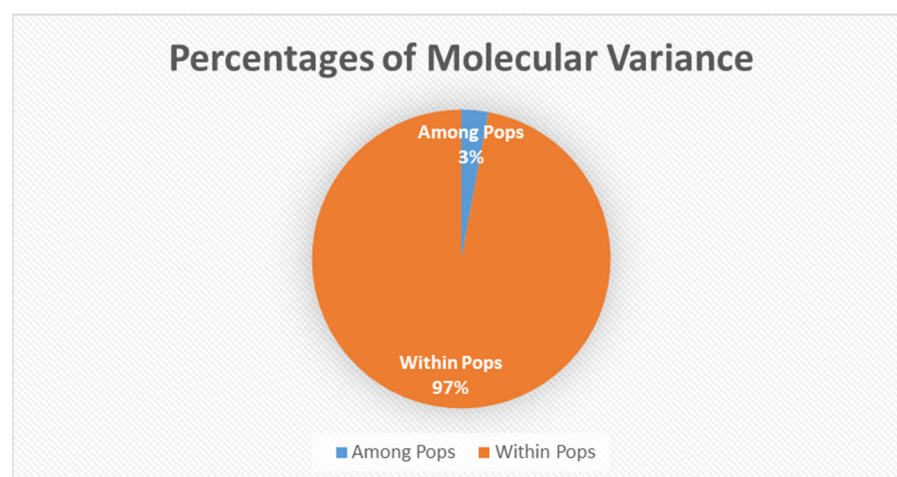


Figure 2. Analysis of molecular variance among and within populations, among population variance was 3% while within was 97%. Genetic variation from AFLP fragments used for the analysis of AMOVA.

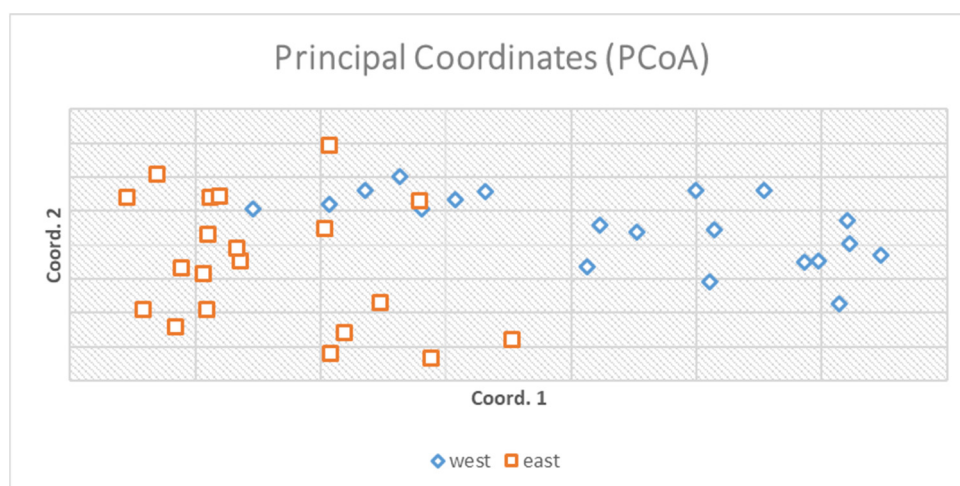


Figure 3. Principal coordinate analysis explained 16.89% of the total variation and two small groups according to the origin of populations were formulated. The figure indicates a small grouping for the two populations derived from the matrix of genetic distances of the genotypes.

3.2. Density and Index of Regeneration

Across the four plots, a total of 233 *A. fruticosa* individuals were documented, resulting in an average density of 824.07 plants per hectare. Notably, the density of *A. fruticosa* and the index of regeneration (IR) were found to be higher in the population residing on the west river side compared to the east river side. However, it is worth mentioning that the mean tree height was greater on the east river side, as indicated in Table 2. The regeneration of alien species *A. fruticosa* and *A. negundo* is most widespread in the alluvial forest (Korakaki personal communication).

Table 2. Mean height, mean density, and mean index of regeneration (IR).

River Side	Mean Height (m)	Mean Density (Trees ha ⁻¹)	Mean IR (cm m ⁻²)
West	1.01	1070.46	10.17
East	1.45	84.88	1.23

4. Discussion

False Indigo (*Amorpha fruticosa* L.) has been reported as non-native [15], and recent observations have documented its presence in significant areas along the Ardas, Evros, and Nestos rivers in north-east Greece, indicating its introduction from neighboring Bulgaria, possibly through river transport of genetic material. In the riparian forests of the Nestos River area in NE Greece, *A. fruticosa* exhibits significant regeneration dynamics and appears to suppress vegetation in important priority habitats.

In this study, we assessed the genetic diversity of two populations from the west and east sides of the river Nestos in order to estimate if they exhibit differences in genetic parameters or share similar indexes of genetic diversity. AFLP loci estimated that heterozygosity for the western population was slightly higher but not significantly different from the eastern population. Furthermore, as the AMOVA analysis pointed out, the majority of genetic variation was within populations (97%). Principal coordinate analysis explained only 16.89% of the total variation, and two small groups according to the origin of populations were formulated. The main conclusion from the genetic analysis was that the western and eastern populations share a common genetic pool, which comes through the river Nestos with the water.

Regarding similar research, the authors did not find any reference to genetic diversity using molecular markers for plants of *A. fruticosa*. There is however one study by [24] in which they measured 47 agronomical and morphological traits for biomass energy. Results showed that all traits were influenced by accessions, a key that may also demonstrate genetic content that probably influences variation. Furthermore, studies have been conducted on the expression of genes associated with drought [25], phenolic constituents [26], proteomic analysis of symbiotic proteins [27], and cancer research [28,29]. The aforementioned research signifies from one point of view the significance of *A. fruticosa*, although we cannot eliminate the negative effects that it has on ecosystems due to its rapid expansion. This invasive species has the potential to invade meadow communities and rapidly transform the habitat into a dense and uniform thicket. It outcompetes native plants and disrupts natural plant succession and biodiversity. This poses a serious threat by suppressing native tree species in the Nestos Delta's priority alluvial forest (91E0*). Additionally, it puts the survival of species *Emys orbicularis*, *Testudo hermanni*, and *Callimorpha quadripunctaria** at risk, as they rely on these habitats.

According to our findings, *A. fruticosa* is more vigorous at the west riverside of Nestos, which poses a significant risk to biodiversity and the suppression of native tree species within the alluvial forest of the Nestos region. Thus, any conservation measures and forest management actions to limit the expansion of *A. fruticosa* should be prioritized on this river bank. Conservation measures are essential to safeguard and maintain this natural habitat, which is of significant interest to the community. Actions should also aim to raise awareness about the threats faced by the alluvial forest and ensure its long-term protection and preservation. This IR result gives us the first indication that *A. fruticosa* will be more expansive on the west side, and the result of the small but higher heterozygosity is in agreement with the current result. Since 2021, the Institute of Mediterranean Forest Ecosystems (ELGO-DIMITRA) has been establishing different experimental treatments in order to explore possible solutions to limit *A. fruticosa* expansion.

Finally, in this study, we found a similar genetic pool from the eastern and western sides of the river Nestos that probably originates from Bulgaria. Future research should also cover a genetic analysis of those Bulgarian populations. It is considered necessary to continue the research and study of *A. fruticosa* in the coming years so that there is more comprehensive knowledge of the species and the effect it has on the ecosystem so that a system of management of the species can then be created and its advantages can be exploited without having negative effects on the ecosystem.

5. Conclusions

A first report about genetic diversity and expansion of the alien species *Amorpha fruticosa* L. was employed across the river of Nestos for the first time in Greece. We studied two populations, one from the western side and one from the eastern side, with AFLP markers, and we did not find differentiation among them. This indicates that probably a single genetic pool was transmitted through the river from Bulgaria, and future research should also implement Bulgarian populations. Furthermore, the first assessment of the regeneration status was accomplished also indicates the expansion of the species. It is a mandatory step to evaluate and propose management actions in order to preserve and limit the expansion of the species, which significantly has negative effects in 91E0* habitat.

Author Contributions: Conceptualization, E.V.A. and E.K.; methodology, E.V.A., E.K., E.M., A.D.S., G.M. and G.K.; software, E.V.A., E.M. and E.K.; validation, all; resources, E.V.A. and E.K.; data curation, E.V.A. and E.K.; writing—original draft preparation, E.V.A., E.K., E.M. and A.D.S.; writing—review and editing, all; supervision, E.V.A. and E.K.; project administration, E.K.; funding acquisition, E.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the LIFE PRIMED project, LIFE17 NAT/GR/000511, which is co-financed by the Greek Green Fund (GRFU).

Data Availability Statement: Data not available.

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

References

- Richardson, D.M.; Pyšek, P.; Rejmanek, M.; Barbour, M.G.; Panetta, F.D.; West, C.J. Naturalization and invasion of alien plants: Concepts and definitions. *Divers. Distrib.* **2000**, *6*, 93–107. [\[CrossRef\]](#)
- Korakaki, E.; Legakis, A.; Katsanevakis, S.; Koulelis, P.; Avramidou, E.; Soulioti, N.; Petrakis, P. Invasive alien species of greece. *Invasive Alien Species Obs. Issues Around World* **2021**, *3*, 124–189.
- Miyawaki, S.; Washitani, I. Invasive alien plant species in riparian areas of japan: The contribution of agricultural weeds, revegetation species and aquacultural species. *Glob. Environ. Res.-Engl. Ed.* **2004**, *8*, 89–101.
- Callaway, R.M.; Aschehoug, E.T. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science* **2000**, *290*, 521–523. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ivanescu, B.; Lungu, C.; Vlase, L.; Gradinaru, A.C.; Tuchilus, C. Hplc analysis of phenolic compounds, antioxidant and antimicrobial activity of *Amorpha fruticosa* L. Extracts. *J. Plant Dev.* **2019**, *26*, 77. [\[CrossRef\]](#)
- Straub, S.C.; Sorrie, B.A.; Weakley, A.S. *Amorpha confusa*, a new name for an old *Amorpha* (Fabaceae: Amorpheae). *J. Bot. Res. Inst. Tex.* **2009**, 151–155.
- Straub, S.C.; Doyle, J.J. Molecular phylogenetics of *Amorpha* (Fabaceae): An evaluation of monophyly, species relationships, and polyploid origins. *Mol. Phylogenetics Evol.* **2014**, *76*, 49–66. [\[CrossRef\]](#)
- Liu, J.; Wang, E.T.; Chen, W.X. Diverse rhizobia associated with woody legumes *Wisteria sinensis*, *Cercis racemosa* and *Amorpha fruticosa* grown in the temperate zone of China. *Syst. Appl. Microbiol.* **2005**, *28*, 465–477. [\[CrossRef\]](#)
- Chinese, P. *A Dictionary of Chinese Materia Medica*; Chinese Science and Technology Press of Medicine: Beijing, China, 1997.
- Mitscher, L.A.; Park, Y.H.; Alshamma, A.; Hudson, P.B.; Haas, T. Amorfrutin a and b, bibenzyl antimicrobial agents from *Amorpha fruticosa*. *Phytochemistry* **1981**, *20*, 781–785. [\[CrossRef\]](#)
- Krpan, A.P.; Tomašić, Ž.; Bašić Palković, P. Biopotential of indigobusch (*Amorpha fruticosa* L.)—second year of investigation. *Šumarski List.* **2011**, *135*, 103–112.
- Kozuharova, E.; Matkowski, A.; Woźniak, D.; Simeonova, R.; Naychov, Z.; Malainer, C.; Mocan, A.; Nabavi, S.M.; Atanasov, A.G. *Amorpha fruticosa*—a noxious invasive alien plant in europe or a medicinal plant against metabolic disease? *Front. Pharmacol.* **2017**, *8*, 333. [\[CrossRef\]](#) [\[PubMed\]](#)
- Szigetvári, C. Initial steps in the regeneration of a floodplain meadow after a decade of dominance of an invasive transformer shrub, *Amorpha fruticosa* L. *Tiscia* **2002**, *33*, 67–77.
- Pedashenko, H.P.; Apostolova, I.I.; Vassilev, K.V. *Amorpha fruticosa* invasibility of different habitats in lower danube. *Phytol. Balc.* **2012**, *18*, 285–291.
- Arianoutsou, M.; Bazos, I.; Delipetrou, P.; Kokkoris, Y. The alien flora of Greece: Taxonomy, life traits and habitat preferences. *Biol. Invasions* **2010**, *12*, 3525–3549. [\[CrossRef\]](#)
- Samaras, A.G.; Koutitas, C.G. Modelling the impact on coastal morphology of the water management in transboundary river basins: The case of river Nestos. *Manag. Environ. Qual. Int. J.* **2008**, *19*, 455–466. [\[CrossRef\]](#)
- Décamps, H.; Décamps, O. *Mediterranean Riparian Woodlands*; Conservation of Mediterranean Wetlands; Tour du Valat: Arles, France, 2001.

18. Mallinis, G.; Emmanoloudis, D.; Giannakopoulos, V.; Maris, F.; Koutsias, N. Mapping and interpreting historical land cover/land use changes in a natura 2000 site using earth observational data: The case of Nestos delta, Greece. *Appl. Geogr.* **2011**, *31*, 312–320. [[CrossRef](#)]
19. Dafis, S.; Papastergiadou, E.; Georghiou, K.; Babalonas, D.; Georgiadis, T.; Papageorgiou, M.; Lazaridou, T.; Tsiaoussi, V. The Greek 'habitat' project Natura 2000: An overview. In *Life Contract B4-32000/94/756*; Commission of the European Communities, The Goulandris Natural History Museum-Greek Biotope/Wetland Centre; Springer: Berlin/Heidelberg, Germany, 1996.
20. Bianchi, L.; Paci, M. Dinamica evolutiva e gestione delle abetine toscane: Sintesi di quarant'anni di ricerche. *For.-J. Silv. For. Ecol.* **2008**, *5*, 122. [[CrossRef](#)]
21. Vekemans, X.; Beauwens, T.; Lemaire, M.; Roldán-Ruiz, I. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Mol. Ecol.* **2002**, *11*, 139–151. [[CrossRef](#)]
22. Peakall, R.O.D.; Smouse, P.E. Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **2006**, *6*, 288–295. [[CrossRef](#)]
23. Taftoglou, M.; Valkaniotis, S.; Papathanassiou, G.; Klimis, N.; Dokas, I. A detailed liquefaction susceptibility map of nestos river delta, thrace, Greece based on surficial geology and geomorphology. *Geosciences* **2022**, *12*, 361. [[CrossRef](#)]
24. DeHaan, L.; Ehlke, N.; Sheaffer, C.; Wyse, D.; DeHaan, R. Evaluation of diversity among north american accessions of false indigo (*Amorpha fruticosa* L.) for forage and biomass. *Genet. Resour. Crop Evol.* **2006**, *53*, 1463–1476. [[CrossRef](#)]
25. Li, M.; Liu, Z.; Liu, C.; Zhu, F.; Wang, K.; Wang, Z.; Li, X.; Lan, X.; Guan, Q. Drought resistance of tobacco overexpressing the afnac1 gene of *Amorpha fruticosa* Linn. *Front. Plant Sci.* **2022**, *13*, 980171. [[CrossRef](#)] [[PubMed](#)]
26. Dat, N.T.; Lee, J.-H.; Lee, K.; Hong, Y.-S.; Kim, Y.H.; Lee, J.J. Phenolic constituents of *Amorpha fruticosa* that inhibit nf-kb activation and related gene expression. *J. Nat. Prod.* **2008**, *71*, 1696–1700. [[CrossRef](#)] [[PubMed](#)]
27. Song, F.; Qi, D.; Liu, X.; Kong, X.; Gao, Y.; Zhou, Z.; Wu, Q. Proteomic analysis of symbiotic proteins of *Glomus mosseae* and *Amorpha fruticosa*. *Sci. Rep.* **2015**, *5*, 18031. [[CrossRef](#)] [[PubMed](#)]
28. Todorova, T.; Boyadzhiev, K.; Shkondrov, A.; Parvanova, P.; Dimitrova, M.; Ionkova, I.; Krasteva, I.; Kozuharova, E.; Chankova, S. Screening of *Amorpha fruticosa* and *Ailanthus altissima* extracts for genotoxicity/antigenotoxicity, mutagenicity/antimutagenicity and carcinogenicity/anticarcinogenicity. *BioRisk* **2022**, *17*, 201–212. [[CrossRef](#)]
29. Cvetković, D.M.; Jovankić, J.V.; Milutinović, M.G.; Nikodijević, D.D.; Grbović, F.J.; Ćirić, A.R.; Topuzović, M.D.; Marković, S.D. The anti-invasive activity of *Robinia pseudoacacia* L. and *Amorpha fruticosa* L. On breast cancer mda-mb-231 cell line. *Biologia* **2019**, *74*, 915–928. [[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.