

Review

# Invasive Alien Plant Species—Raising Awareness of a Threat to Biodiversity and Ecological Connectivity (EC) in the Adriatic-Ionian Region

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**Abstract:** Improving ecological connectivity (EC) within landscapes by establishing corridors and ecological networks has been proposed to counteract the negative effects of habitat fragmentation and climate change on biodiversity. To be functional, ecological networks should be kept free of opportunistic invasive species that can disrupt EC between protected areas and cause biodiversity loss. The present study focused on perennial herbaceous species whose occurrence in the Adriatic-Ionian region has increased in the last two decades, namely common milkweed (*Asclepias syriaca*), Jerusalem artichoke (*Helianthus tuberosus*), Japanese knotweed (*Reynoutria japonica*), Bohemian knotweed (*Reynoutria × bohemica*), giant hogweed (*Heracleum mantegazzianum*), giant goldenrod (*Solidago gigantea*), Canadian goldenrod (*Solidago canadensis*), and Bermuda buttercup (*Oxalis pes-caprae*). All species have a high potential to spread in grasslands, abandoned agricultural fields, forest edges, and riparian areas and pose a significant threat to native plant communities and biodiversity. Restoring heavily infested sites is a major challenge because these perennial invaders are very persistent and tend to alter the soil environment in invaded habitats and prevent the recolonization of native plant communities. Therefore, early action should be taken to prevent the spread of these environmental weeds in ecological networks and protected areas with high conservation value.

**Keywords:** environmental weeds; perennial weeds; dispersal; grasslands; riparian areas; abandoned agricultural fields; Balkan Peninsula; eradication



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## 1. Introduction

Improving ecological connectivity (EC) within landscapes through the establishment of corridors and ecological networks has been proposed to counteract the negative effects of habitat fragmentation and climate change on biodiversity [1]. At a time when biodiversity loss has become a serious issue in Europe, the conservation of EC has become a major challenge [2]. This is also true for a large part of the Balkan Peninsula, where political and economic circumstances have created transboundary barriers between associated countries, posing a major challenge for the conservation of EC and biodiversity [3]. In this context, the “Interreg Adrion DINALPCONNECT” project was launched in 2020 with the aim of improving EC by combating environmental vulnerability and habitat fragmentation and securing ecosystem services in the Adriatic-Ionian region. Seven countries in the Adriatic-Ionian region, namely Italy, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Albania, and Greece, are included in the broader project area. Four pilot regions have

been established as linkages between Natura 2000 sites and protected areas on the borders between Italy and Slovenia, Slovenia and Croatia, Croatia and Bosnia and Herzegovina, and Albania and Greece (Figure 1).



**Figure 1.** Countries of the Adriatic-Ionian region where ecological connectivity (EC) will be explored and consolidated. The four circles represent pilot regions selected as linkages between Natura 2000 sites and protected areas by the “Interreg Adrion DINALPCONNECT” project team. Abbreviated names of associated countries: IT; Italy, SI; Slovenia, HR; Croatia, BA; Bosnia and Herzegovina, ME; Montenegro, AL; Albania, GR; Greece.

By enabling the movement of organisms, corridors can reduce the likelihood of species extinction by increasing genetic exchange between populations and promoting recolonization of empty habitat patches in spatially structured populations [4,5]. However, to be sustainable and functional, ecological networks should be kept free of opportunistic invasive species that can disrupt EC between protected areas and lead to biodiversity loss [6]. To achieve this goal, the status of biological invasions in larger areas should not be overlooked but continuously monitored, and eradication programs should be initiated in a timely manner [7]. According to the Driver, Pressure, State, Impact, and Response (DPSIR) models developed in [8], extensive urbanization and improper management practices in agricultural areas, forests, grasslands, and riparian zones generate serious threats to biodiversity and EC in the Balkan Peninsula. Among others, the risk of further spread of exotic non-native plant species has been identified as one of the major threats to biodiversity in the Adriatic-Ionian region [8].

Biological invasions by alien plant species are among the main causes of biodiversity loss, as the presence of invaders leads to the displacement of native vegetation and can have negative impacts on the soil environment and associated fauna taxa [9–12]. In addition to alien invasive trees and herbaceous species with an annual life cycle, there is much evidence that the occurrence of perennial herbaceous species with high invasive potential has increased in the Adriatic-Ionian region in the last two decades [13–19]. Perennial herbaceous species have a strong invasive potential, as most of them can reproduce both by seeds and by underground vegetative organs. Vegetative propagation allows for the rapid establishment of dense, monospecific, clonal populations in invaded habitats, which makes eradication a very challenging goal because it requires sufficient control of the belowground vegetative organs that give rise to new clonal plants. If only the aboveground vegetative parts are removed, plants regrow from their belowground vegetative organs [20–24]. In addition, seed production and dispersal facilitate effective colonization of new areas for

species that can reproduce both by seed and vegetatively [15,21,25]. Therefore, measures to prevent the spread of these species and eradication programs should be implemented in the wider Adriatic-Ionian region before invasions become problematic in ecological networks and protected areas of high conservation value.

The present study focused on common milkweed (*Asclepias syriaca* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), Japanese knotweed (*Reynoutria japonica* Houtt.), Bohemian knotweed (*Reynoutria × bohemica* Chrtk et Chrtková), giant hogweed [*Heracleum mantegazzianum* Sommier & Levier], giant goldenrod (*Solidago gigantea* Aiton), Canadian goldenrod (*Solidago canadensis* L.), and Bermuda buttercup (*Oxalis pes-caprae* L.). Our first objective was to raise public awareness of the spread status of these perennial herbaceous alien plant species in the countries of the Adriatic-Ionian region, as described in the recent literature. Another objective was to focus on the main ecological features contributing to the success of these species as invaders and to present the impact of their invasions on biodiversity, based on data from case studies conducted in the wider European region. The importance of raising public awareness and taking preventive measures to prevent further spread of these species is emphasized. Available eradication methods that can be applied to infested sites are also presented.

## 2. Spread of the Selected Alien Plant Species in the Adriatic-Ionian Region

The alien plant species included in the current study are perennial herbaceous species that have been classified by the European and Mediterranean Plant Protection Organization (EPPO) as having a high potential to spread and are capable of altering the environment in invaded habitats, thus posing a significant threat to native plant communities, the environment, and biodiversity in the EPPO region. All species are already present in at least four countries in the Adriatic-Ionian region (Table 1).

**Table 1.** Summary of the presence of the selected alien plant species in the countries of the Adriatic-Ionian region.

Binomial Name	Botanical Family	Common Name	Origin	Infested Countries <sup>1</sup>
<i>Asclepias syriaca</i>	Apocynaceae	Common milkweed	North America	IT, SI, HR, BA, ME
<i>Helianthus tuberosus</i>	Asteraceae	Jerusalem artichoke	North America	IT, SI, HR, BA, ME, AL, GR
<i>Reynoutria japonica</i>	Polygonaceae	Japanese knotweed	East Asia	IT, SI, HR, BA
<i>Reynoutria × bohemica</i>	Polygonaceae	Japanese knotweed	East Asia	IT, SI, HR, BA
<i>Heracleum mantegazzianum</i>	Apiaceae	Giant hogweed	Eastern Europe	IT, SI, HR, BA
<i>Solidago gigantea</i>	Asteraceae	Giant goldenrod	North America	IT, SI, HR, BA, ME
<i>Solidago canadensis</i>	Asteraceae	Canadian goldenrod	North America	IT, SI, HR, BA, ME
<i>Oxalis pes-caprae</i> L.	Oxalidaceae	Bermuda buttercup	South Africa	IT, HR, AL, GR

<sup>1</sup> Country names are represented by three-code abbreviations—e.g., IT; Italy, SI; Slovenia, HR; Croatia, BA; Bosnia and Herzegovina, ME; Montenegro, AL; Albania, GR; Greece.

Common milkweed has been naturalized in Italy and occurs in seven administrative regions; it spreads in all northern regions of the country, except in Liguria and Aosta Valley [17]. In Slovenia, this species has been recorded in the central part of the country along roadsides, near Ljubljana, east and northeast of it; there are also reports from Brežice, Podravje, and Pomurje regions, and two other cases of occurrence in the northeastern part of the country, near watercourses along the Mura River on the border with Croatia [26]. In Croatia, Boršić et al. [16] found that common milkweed occurs mainly in the northwestern and eastern parts of the country along roadsides and railways, riverbanks, pastures, and open forest areas. The presence of local populations has been confirmed in wet meadows in

the Posavina region of Bosnia and Herzegovina [27]. According to Stešević and Petrović [19], common milkweed is one of the alien plants detected in Montenegro, where only a few populations have been reported infesting wet meadows outside the city of Podgorica.

Jerusalem artichoke is one of the most noxious invaders in field crops in northern Italy, especially where it has been grown in the past as an energy or industrial crop, or when allowed to spread uncontrollably on abandoned agricultural land [28]. This species is also reported as invasive in Tuscany, where it occurs mainly on riverbanks [29]. This species is also introduced in Greece and Albania but is not yet invasive in these countries [13,14]. In Montenegro, Jerusalem artichoke is widely distributed on abandoned agricultural lands, ruderal areas, and riverbanks [19]. In addition, it is considered invasive in northwestern Bosnia and Herzegovina and also in the central part, including the Sarajevo Canton area, along roadsides, railways, riverbanks, and streams [15,27]. Jerusalem artichoke is a serious invader in riparian zones of streams in Slovenia [30]. Kuzmič and Šilc [18] reported its occurrence in six habitats of the European Nature Information System (EUNIS). Follak et al. [31] also mentioned that this weed has a high potential to spread across roadsides and railways in the country and colonize new areas. This is also supported by the recent studies in which dense populations were discovered along a roadside near Zbilje, a settlement in the Upper Carniola region of Slovenia [32]. In Croatia, escaped Jerusalem artichoke populations have spread aggressively along forest edges, drainage systems, and riverbanks in the continental part of the country, as well as in flooded woodlands on the riverbanks of Sava, Sutla, Krapina, and Drava [33]. Its occurrence on forest edges was also confirmed in Medvednica Nature Park by Vuković et al. [34].

According to Mincheva et al. [35], Japanese knotweed has been well distributed in grasslands in the Piedmont region of northwestern Italy during the last 30 years. Populations have also been observed in the western Italian Alps [36]. In Slovenia, this species is also widespread in urban areas, along watercourses, riparian corridors, roadsides, and railroad lines [31]. In particular, the southeastern part of the municipality of Ljubljana in central Slovenia is known as the most infested area in the country [37]. According to Vuković et al. [38], Japanese knotweed populations have infested at least 25 sites in Croatia. Most detections are from roadsides and railways, urban areas and areas along watercourses at several sites around the city of Zagreb in the north and sites in the Alpine biogeographical region on the Dinaric mountain of Velebit. There are also reports of grasslands infested with this species between the settlements of Krstinja and Brusovačanear near the border with Bosnia and Herzegovina. Dense populations of Japanese knotweed have also been found along roadsides and railways, watercourses, urban areas, and grasslands in several locations in Bosnia and Herzegovina, including the larger areas of Sarajevo, Zenica, and Žepce, as well as outside cities in the northeastern part of the country (Banja Luka, Derventa and Doboj) [15,27]. It should be noted at this point that Japanese knotweed may be confused with Bohemian knotweed, which is the hybrid of Japanese knotweed and giant knotweed (*Reynoutria sachalinensis* (F. Schmidt) Nakai) [39].

Jovanović et al. [40] recorded Bohemian knotweed populations in ruderal and urban areas in the cities of Celje and Ljubljana, in Slovenia. Galasso et al. [17] reported that Bohemian knotweed is invasive and widespread in Italy. Giuliani et al. [41] collected samples from three populations of Bohemian knotweed growing along artificial banks of the Ombrone River in Podere della Chiesa, along stream shores and artificial banks under woody riparian vegetation along the stream Vincio in Pontelungo, and also along the Arno River in Subbiano; in Subbiano, Bohemian knotweed was also widespread along cultivated areas and habitats disturbed by anthropogenic activities. According to Lazzaro et al. [42], the hybrid occurs in at least nine different regions and is classified as one of the most important invasive alien plants in the country. In Croatia, Vuković et al. [38] reported large populations growing along the wider area of the Kašina stream in the settlement of Kašina, eastwards of Medvednica and in a grassland located between the settlements of Dekanovec and Domašinec. The same authors detected Bohemian knotweed populations in urban areas, in the city of Zagreb, and also across roadsides in several localities throughout

the country. In Bosnia and Herzegovina, the hybrid is found in riparian habitats near the towns of Višegrad, Zvornik, Nova Kasaba, Dobrun, and Sarajevo; this weed is also found in ruderal sites in Dobrun, Rogatica, and in urban areas in Uvac [40]. In Montenegro, these researchers have detected Bohemian knotweed occurrences on riparian and ruderal sites near Pljevlja and in gardens in the small town of Žabljak.

In the Sava River basin, populations of giant hogweed have been found only in Slovenia near Ljubljana, probably as escapees from the Ljubljana Botanical Garden [43]. Although active eradication measures are implemented every year to control the spread of this invasive species, there is recent evidence that roadsides serve as corridors for further spread in Slovenia [31]. In Croatia, the occurrence of this species has been confirmed in disturbed habitats in Međimurje County and in Gornja Šemnica in Krapina-Zagorje County near roadsides [16]. As for its occurrence in Bosnia and Herzegovina, populations have been reported in the central part of the country and especially near Hadžići, southwest of Sarajevo, along the railway between Sarajevo and Mostar, and also near Lokve between Hadžići and Pazarić [44]. In Italy, populations of giant hogweed have been classified as naturalized in some areas and invasive in others [17]. According to maps provided by Jahodová et al. [45], this species also occurs in the northeastern part of the country, while Siniscalco and Barni [36] reported infestations in mountainous areas in the Aosta Valley and Alpine Botanical Gardens.

Giant goldenrod and Canadian goldenrod are among the most widespread invasive alien plant species in Slovenia. Zelnik [30] reported that extensive populations of both species infest the riparian zones along streams, disturbed habitats with standing waters, and the edges of floodplain forests. In the study by Marinšek and Kutnar [46], their occurrence was found in 37 out of 130 plots near the Mura River in the northeastern part of Slovenia, in the sub-Pannonian region. These species are also common in shrublands and afforestation areas. According to Kuzmič and Šilc [18], giant goldenrod has spread in 16 EUNIS habitats, while Canadian goldenrod is already present in 7 different EUNIS habitats. Šajna [47] found that giant goldenrod occurred in 65% and 91% of mowed and abandoned forest meadows, respectively, within the natural forests that developed along the riverbanks of the Drava river. Canadian goldenrod has a high potential to spread across roadsides and railways in Slovenia [31]. In Croatia, both species are invasive [48], but giant goldenrod seems to be more common. In the continental part of the country, it has been found on many different ruderal areas, usually disturbed due to human activities; the highest infestation rates are observed on abandoned agricultural land, riverbanks, and forest edges [49]. Novak and Novak [49] reported that at least 10 ha were infested by the giant goldenrod at such sites in Zagreb, Varaždin, Koprivnica-Križevci, Krapina-Zagorje, Međimurje, Bjelovar-Bilogora, and Karlovac counties. Maslo et al. [27] mentioned that both species were found along rivers and forest edges, on roadsides, and in gardens in Bosnia and Herzegovina; they also indicated that giant goldenrod populations have invaded the wider area of Trebević mountain. In Montenegro, two distinct occurrences of giant goldenrod were found near roadsides in the suburban areas of Nikšić and Mojkovac, near gardens where it is grown as an ornamental plant [50]. A population of Canadian goldenrod was found on the roadside in the village of Vir, near the town of Nikšić [51]. The giant goldenrod is the most common of the two species in northern Italy [52].

Bermuda buttercup occurs in Greece in all 18 habitat groups, namely marine habitats, coastal habitats, inland water bodies, fens and fogs, grasslands, shrublands, forests, rocky areas and scree, cultivated arable fields, cultivated areas in gardens and parks, perennial orchards and vineyards, man-made urban areas, transportation networks (roadsides and railways), artificial constructed boundaries, and abandoned places [13]. It is common in urban and suburban ruderal areas, olive groves, meadows, and forest edges, where it forms dense populations that prevent the growth of other species, especially during the growing season from autumn to early spring [13]. This species is considered invasive in similar habitats in Italy [17]. It was also introduced in Croatia in the last two decades [48]. In all mentioned countries, islands are highly susceptible to invasions of this species [13,17,48]. In

addition, Barina et al. [14] reported that Bermuda butter cup is well established in Albania, but not yet invasive.

### 3. Species Description and Key Ecological Characteristics

#### 3.1. Common Milkweed

Common milkweed is a perennial broadleaved weed species; it is diploid ( $2n = 22$ ) and belongs to the family Apocynaceae, subfamily Asclepiadoideae [53]. It is native to the northeastern, north central, and southeastern United States and adjacent areas of Canada [54]. The plants have a  $C_3$  photosynthetic pathway and grow up to 1.5 m tall. They grow in clusters of stout stems with short downy hairs; all plant parts contain a milky sap [55]. The conspicuously veined leaves are smooth on the upper surface, pubescent on the lower surface, 10–20 cm long and 5–11 cm wide, and appear in pairs on opposite sides of the stem [56]. Inflorescences are large spherical clusters (umbels) that arise in the upper leaf axils and at the tips of the stem [57]. The number of inflorescences per plant ranges from 2 to 5; the number of flowers per inflorescence ranges from 30 to 108 [58]. The flowers are fragrant, pink to white, and considered an excellent source of nectar for butterflies, bees, and other insects [59].

Reproduction is by seed as well as by underground vegetative organs. The flowers are self-sterile and are pollinated by insects [60]. Only 2% of the flowers produce mature pods. On average, each plant produces 4–6 pods containing 150–425 seeds [55]. Consequently, the presence of 1–6 stems  $m^{-2}$  can lead to a production of up to 87 million seeds  $ha^{-1}$ . Csontos et al. [61] reported that at densities of 14 and 18.1 stems  $m^{-2}$ , seed production reached 7820 and 10597 seeds  $m^{-2}$ , respectively. In addition to seed production, seeds have inherent dormancy; they require at least a post-maturation period of 1 year to germinate [62]. Although seed survival is influenced by environmental conditions, the high seed production capacity and seed dormancy allow the formation of persistent seed banks in infested areas [63]. The seeds are pinnate, and their weight is very low (e.g., the weight of 1000 seeds is usually 7–8 g). Therefore, they can disperse over long distances by wind. For example, early studies by Platt and Weis [64] reported that the diameter of the plume was 5.59–5.73 cm and the seeds were dispersed by the wind at a distance of 13.3–14.3 m with a velocity (terminal velocity) of 0.0249  $m s^{-1}$  with a wind dispersion of 2.78–4.18  $m s^{-1}$ . In more recent studies, Moravcová et al. [65] reported that seeds were dispersed by wind at a velocity of 0.284–0.422  $m s^{-1}$ .

Seed dispersal is the main mechanism for milkweed to spread over long distances and colonize new areas, while establishment of dense stands over shorter distances occurs by vegetative propagation with rhizomes [21]. Seed germination occurs between April and May. Three weeks after germination, the formation of underground rhizomes occurs. Flowering does not occur during the first growing season [63]. The rhizomes contain buds that develop the next spring. New shoots develop from these buds under favorable conditions. The shoots are viable for one year but are formed in the same place each year [55]. Most rhizomes normally grow horizontally 10 to 40 cm below the soil surface at a growth rate of 0.01 to 0.25 m per year [63]. Some rhizomes penetrate deeper soil profiles (1–1.5 m depth) and reach groundwater, which provides water and nutrients to the shoots; new shoots appear from mid-spring to early summer, and plants flower from June to August [63]. Plants grow as single shoots or as groups of 2–5 shoots connected by rhizomes for many years after establishment [21]. Cultivation for ornamental purposes, cultivation for fiber production, cultivation as a nectar source by beekeepers, improper cleaning of agricultural machinery used near infested sites, transport of soil contaminated with rhizomes to new areas, abandonment of agricultural land, and other anthropogenic activities also contribute significantly to its spread [21,66–68]. In addition, this species spreads along roadsides and railways [31].

### 3.2. Jerusalem Artichoke

Jerusalem artichoke is a hexaploid ( $2n = 102$ ), erect, rhizomatous, perennial, broadleaved weed species originally from North America [69]. The plants have a  $C_3$  photosynthetic pathway, but also possess enzymes typical of the  $C_4$  type of photosynthesis [70]. Stems are light green or green-purple in color, hairy, sparsely to moderately branched in the upper half, woody in the years after establishment, and can reach a height of up to 3–4 m with a stem diameter of 1.6–3 cm [15,71]. Leaves are 10–25 cm long and 4–12 cm wide, broad near the base, and glabrous on the upper surface. In the lower part of the stem, they are opposite or arranged in whorls of three leaflets, while in the upper part of the stem, the leaves are alternate, simple, winged above, the blade ovate to ovate-lanceolate, with three prominent veins and a petiole 2–8 cm long [72]. Inflorescences are sunflower-like heads with bright yellow disk flowers; they are formed as single heads or in corymbs at the ends of the main stems and axillary branches and are 4–11 cm in diameter, while disk diameters range from 1.5 to 2.3 cm [71,72]. The bracts are lanceolate, green, finely pubescent dorsally, and hairy at the margin; the petals are oblong-spatulate, acute, and hairy in the upper part [73]. The flowers are insect-pollinated and are an excellent nectar source for honeybees, wasps, flies, and butterflies [74]. It should be noted that this species can be confused with wild and weedy forms of cultivated sunflower (*Helianthus annuus* L.) [75]. Fewer than five seeds are produced per flower head, forming glabrous and hairy achenes. The seeds are wedge-shaped and smooth achenes, usually gray or brown in color, 6–8 mm long, and 2 mm wide [15].

The root system is adventitious and fibrous and develops cord-like rhizomes that are 1 m long with a swollen apical portion and axillary buds. Tubers are formed by the thickening of the rhizomes [76]. Reproduction is vegetative, most commonly by tubers or tuber pieces, but also by rhizomes or rhizome pieces. The majority of seeds are infertile, but their production is an important means of maintaining the genetic diversity of the species. Seed dormancy is also a feature that contributes to persistence [72]. Growth begins from tubers or seeds in April or May and is followed by a period of rapid vegetative growth. Rhizome sprouting occurs at the beginning of the flowering period, from early July to late September. At this time, tubers are initiated [77].

It should be highlighted here that, although Jerusalem artichoke is a  $C_3$  species, the high aboveground biomass acts as a reservoir of carbohydrates that are stored in the tubers and rhizomes to maintain the clone throughout the winter and provide a ready energy source for rapid spring regrowth [78,79]. The main type of carbohydrates includes homologous series of polyfructofuranose units, comprised of linear chains of *D*-fructose molecules terminated by a *D*-glucose molecule; the dominant term used to describe all such polysaccharides consisting largely of fructose units is often referred to as inulin [77]. It should be also noted that plants are photoperiod-sensitive, requiring alternate cycles of long light followed by periods of shorter light to stimulate tuber formation and further development [80]. In any case, Jerusalem artichoke exhibits rapid vegetative growth and tuber development in a single growing season [78]. A single plant is alive for up to five generations, but stands are persistent after establishment since new ramets emerge from tubers continuously over years [72].

The plant thrives on riverbanks; water is an important means of dispersal over long distances. There is evidence that rivers, streams, and creeks can transport rhizomes and tubers to new locations [81]. Seed dispersal may also contribute to further colonization of new areas at a given time [15]. Dispersal by animals is an important aid in dispersal. Rodents feeding on tubers, rhizomes, and seeds of Jerusalem artichoke in late summer carry the invader's reproductive organs through their digestive system and subsequently release them in new locations—a phenomenon known as endozoochory [29,82]. An example is the study by Mori et al. [29], who reported that the initial presence of a pair of adult Crested porcupine (*Hystrix cristata* L., 1758) increased Jerusalem artichoke density by 41% in five years, which was observed in a total area of 212 hectares. In any environment, Jerusalem artichoke can spread rapidly through vegetative propagation and form dense, monospecific

stands once established. Thus, in the study by Žgančíková et al. [81], its density increased by 30–80% from 2010 to 2011 on ruderal areas, railroad lines, and riverbanks. The same authors recorded a population density of up to 265 plants  $m^{-2}$ . However, it should be noted that this species is self-limiting at high densities, and fewer tubers are produced per plant under crowded conditions [72]. In any case, human activities are also one of the most important reasons for the invasiveness of this species in non-native areas. In particular, Jerusalem artichoke is widely cultivated in Europe as a multipurpose crop for feed production and industrial, energy, and medicinal purposes [83,84]. Therefore, the plants can escape from cultivation and grow in the wild [85].

### 3.3. Japanese Knotweed and Bohemian Knotweed

Japanese knotweed is a rhizomatous, perennial, broadleaved weed species with a  $C_3$  photosynthetic pathway, native to Japan, Taiwan, and Korea [15]. In Europe, populations with a chromosome number of  $2n = 88$  are predominant [86]. Rhizomes are dark brown, nodular with annular structures spaced about 2 to 4 cm apart, thick and woody when old, up to 8 cm in diameter, and often bright orange inside [87]. Stems grow more than 3 m tall, branch, become woody with age, and often have reddish spots [88]. Leaves are 5–12 cm  $\times$  5–8 cm, broadly ovate, pointed at the apex and truncate at the base, coarse in texture, and glabrous with petioles 1–3 cm long [88]. Inflorescences are membranous ochreate that are initially erect and fall off at maturity. Flowers are cream-colored and appear in clusters of three to six on terminal and axillary inflorescences [15]. In male sterile plants, flowers have five tepals, the outer three of which are keeled, and eight stamens in the perianth [88]. The fruits are trigonous achenes, 2–4 mm long and 2 mm wide, dark brown in color and shiny [89]. Although most European populations reproduce vegetatively, propagation by seed can also be observed [90]. Seedlings emerge from late March to May. To survive, plants from seed should form at least five true leaves in the year of establishment (BBCH: 15) [91]. The potential of the seed bank is negligible because seeds do not exhibit significant dormancy levels [92].

As for vegetative propagation, a clonal patch of Japanese knotweed can arise from rhizome fragments as small as 1 cm with a fresh weight of 0.7 g [93]. Rhizome sprouting occurs from April and continues until the canopy closes between June and July [87]. The optimal soil depth for rhizome emergence is 2 cm, but emergence can occur from 1 m depth; rhizomes become 15–20 m long and penetrate the soil at 2–3 m depth [20]. Clonal populations develop very dense stands that accumulate large amounts of biomass [94]. A mature stand can produce up to 1467 g  $m^{-2}$  dry weight [95]. Flowering occurs between August and mid-September, when fruit set begins; storage of fruit in stems during winter is another common feature of populations in invaded areas [88]. Leaves are photosynthetically active until the onset of senescence and drop in winter [96]. When shoots die in October, a few dormant buds develop on the rhizome [97]. In May and June, 80–90% of the freshly synthesized carbohydrates remain in the shoots, while in August and September, proportions of 35% and 70%, respectively, are diverted to the rhizomes [98]. The stand can persist for more than 50 years as old and young rhizome parts remain physiologically connected [96].

Seed production per stem can reach 127,000 [89]. Seeds are reported to have high germination rates ( $\geq 90\%$ ) in central Europe [99]. They can be easily dispersed by wind in all directions at a distance of 16 m [90]. Seeds can also float in streams, which facilitates the colonization of riparian corridors [20]. Typical dispersal routes for seeds include roadsides and railways [90]. Regarding the population dynamics of clones, stems with at least two rhizome nodes can lead to the production of 2.3 new shoots  $m^{-2}$ , and the stem density per unit area can range from 8.9 to 42 stems  $m^{-2}$ , leading to the formation of 86–407 rhizome nodes  $m^{-2}$  [95]. Stems with rhizome fragments can spread via fresh and salt water, while anthropogenic activities such as illegal use as an ornamental plant or transport of soil profiles contaminated with rhizome fragments facilitate introduction into new areas [100]. Shallow tillage also leads to excessive spread at infested sites [88].

As for Bohemian knotweed, hexaploid ( $2n = 66$ ) populations of the hybrid are most common in Europe [86,90]. Correct identification of this weed is difficult given its similar-

ities to Japanese knotweed [101]. Bailey et al. [25] summarized the main morphological differences between the hybrid and Japanese knotweed. First, the leaves of Bohemian knotweed are larger (23 × 19 cm) than those of Japanese knotweed. Second, the leaves do not have the truncate bases typical of Japanese knotweed but have a weakly to moderately cordate base and are of medium shape. Furthermore, unlike Japanese knotweed, the leaves are characterized by a medium texture and appearance. Another difference is found on the lower abaxial leaf surface; there are very distinct, stout, celled pointed hairs in Bohemian knotweed, while there are single-celled “bumps” on the leaf veins of Japanese knotweed. In addition, the outlines of epidermal cells are clearly visible in Japanese knotweed, while they are clearly intermediate in Bohemian knotweed. The stems of Bohemian knotweed also vary more in their height (2.5–4.0 m) than those of Japanese knotweed. Both sexes occur in hybrid populations, and hermaphrodites, which are self-incompatible, appear to be outnumber male-sterile plants [87]. Flowering begins in mid-August to late September and continues for several weeks or until stems are cut down due to frost; reproduction occurs both by rhizomes and seed. Seed production is an important means of dispersal [102]. Seeds can be dispersed over long distances by wind [103]. The water of rivers and streams is also very important for seed dispersal as the seeds can float in water for more than two days and remain highly germinable [104]. As for its ability to propagate vegetatively by rhizomes, Bímová et al. [93] found that Bohemian knotweed has higher rhizome regeneration rates compared to Japanese knotweed and other closely related species of the genus *Reynoutria* spp. The spread of the hybrid is also favored by anthropogenic activities [105].

### 3.4. Giant Hogweed

Giant hogweed is a perennial broadleaved weed classified as a hemicryptophyte; it is diploid ( $2n = 22$ ) and belongs to the Apiaceae family [106]. Unlike other species included in this review, it reproduces only by seed. Because it is a monocarpic species, the life cycle is complete after flowering and seed formation. This forb originates from the southern part of the western Great Caucasus in Russia and northeastern Georgia [45]. The most striking feature of giant hogweed is its conspicuous appearance; plants are usually 3–4 m tall at flowering time but can reach a height of up to 5.5 m [107]. In fact, giant hogweed is among the tallest and largest herbs in Europe. Plants grow from branched taproots that develop at soil depths up to 45–60 cm; the crown develops 10 cm below the soil surface [106]. The crown surface is woody for years, and the crown diameter reaches 15 cm at flowering time [107]. The well-developed lower leaves of adult plants become huge, e.g., 3 m long and 1.7 m wide. Vegetative growth begins in winter and flowering is in early June, 3–5 years after the establishment year, with whitish flowers. The stems grow rapidly in the year of flowering. The diameter of the stem is up to 10 cm [106]. The terminal inflorescences are large, compound umbels that are 80 cm wide and contain 50–150 hairy umbel rays that are 15–40 cm long and terminate in smaller umbellets [107]. There is a compound terminal umbel surrounded by satellite umbels. The satellite umbels overtop the terminal umbel. Numerous umbels form below it, which branch. Tertiary and quaternary umbels are also formed in each branch [108].

A total of 81,519 flowers can be produced per plant [106]. They are insect-pollinated, hermaphroditic, and protandrous [108]. However, flower production is not a reliable indicator of fertility and seed production. The flowers of the main umbel are hermaphrodite, but those of the lower umbels mature earlier in the season, and many of them are male. According to Perglová et al. [109], a single mature plant had 1 terminal umbel, 4.3 satellite umbels, 3.5 branch umbels, 17.3 tertiary umbels, and 2.8 quaternary umbels. Seed production per umbel was 9216, 1288, 1157, and 32 seeds for terminal, satellite, branch, and tertiary umbels, respectively, giving an average production of 20,671 seeds per plant. Other studies determined an average production of 15,729 seeds per plant [110]. Under real field conditions, seed banks are only persistent in the short term, as most seeds ( $\geq 90\%$ ) germinate after the first winter [111]. The majority of seeds ( $\geq 95\%$ ) are concentrated in the top 5 cm of the soil layer. As for seed dispersal potential, although seeds are flat with

winged edges, wind is not the most effective means of long-distance dispersal. Most seeds (75%) fall near the parent plants within a 1.2 m radius [112]. There is evidence that seed release from 2 m height at wind speeds of 10 and 14 m s<sup>-1</sup> resulted in dispersal over 2 and 10 m only [113]. According to Moravcová et al. [111], seeds can float in water for at least 8 h, resulting in long-distance dispersal when seeds are dispersed by fast-flowing streams. Wadsworth et al. [114] suggest that dispersal by water can lead to seed dispersal over a distance of 10 km. Anthropogenic activities are also an important means of colonizing new areas. Seeds can adhere to car tires along roads, while the slip streams of trains also contribute to seed dispersal along railways [111]. In the European Union (EU), there is a ban on the sale, planting, and keeping of the plant, as any potential use as an ornamental plant is very likely to lead to further spread of giant hogweed in new areas [115].

### 3.5. Giant Goldenrod and Canadian Goldenrod

Giant goldenrod and Canadian goldenrod are perennial, herbaceous, rhizomatous species belonging to the botanical family Asteraceae. Both species are native to North America, are classified as hemicryptophytes, and have a C<sub>3</sub> photosynthetic pathway. Their base chromosome number is  $n = 9$ ; there are diploid, tetraploid, and hexaploid forms in both species [116,117]. Tetraploid giant goldenrod populations are most common in Europe, while diploids occur with lower frequency [116,118]. As with Canadian goldenrod, the dominant populations in Europe are diploid [119,120]. Stems are erect and unbranched, except in inflorescence. In giant goldenrod, the stems are up to 2.80 m tall, glabrous from base to inflorescence, and purple in color [23]. The stems of Canadian goldenrod are up to 2.5 m tall, glabrous at the base, often reddish, and weakly to densely hairy at least in the upper half [121]. Leaves are simple, alternate, and oblong to lanceolate, trifoliate, with the largest leaves in the middle of the stem and decreasing in size toward the top. They are 80–180 mm long and 10–30 mm wide in giant goldenrod and 30–150 mm long and 5–22 mm wide in Canadian goldenrod [23,24].

Inflorescences form broad pyramidal panicles with recurved branches and a central axis; they are produced from June. One difference between these two species is that the inflorescence architecture is denser in giant goldenrod [23]. The main flowering period is between mid-August and late September, but flowering may continue into October. The flowers of giant goldenrod are golden yellow, and those of Canadian goldenrod are lemon yellow. Both species are self-sterile, insect-pollinated, and have fertile female flowers and fertile bisexual disk flowers; achenes are pubescent and have a pappus. Another morphological difference is the brownish-white color of the pappus in giant goldenrod and the whitish color in Canadian goldenrod [23]. New seeds, shoots, and rhizomes are produced each year, and all aboveground shoots die in the fall. Most plants of giant goldenrod reproduce in the first growing season, and a single branch produces up to 19,000 achenes. Seed germination is highest in spring but continues through summer. Flowering in the first year, on the other hand, is not common in Canadian goldenrod; up to 13,000 achenes can be produced per branch. In dry weather, wind is an important aid in colonizing new areas. Seedlings sprout in early summer. In both species, the seeds of European plants do not show dormancy [23,24]. It should be noted that both species can hybridize with European goldenrod (*Solidago vigaurea* L.), which is native to Europe [122]. In particular, hybridization between giant goldenrod and European goldenrod results in the hybrid *Solidago* × *snarskii* Gudžinskas & Žalneravičius, while outcrosses between Canadian goldenrod and European goldenrod produce the hybrid *Solidago* × *niederederi* Khek [122].

Shoot elongation begins in spring, and shoot height increases almost linearly until the end of July [123,124]. In giant goldenrod, rhizomes can be formed within four weeks of germination. In contrast, rhizomes of Canada goldenrod are usually formed after the first growing season [24]. They are much longer compared to those of Canadian goldenrod, and this is an important distinguishing feature between these two species [23]. The root system is branched, and secondary rhizomes are formed. Rhizome buds appear in spring, and new rhizomes (from the same crown) begin to sprout in summer, forming circular stem clusters.

Jakobs et al. [125] reported a production of 16.8 rhizomes per ramet in giant goldenrod. In Canadian goldenrod, the production of rhizomes per ramet ranges from 2 to 11 [126]. Rhizomes break dormancy in mid-April of the following year after production, and each rhizome produces a shoot from its tip; after a short period as a rosette with scale leaves, the shoot expands rapidly, and the total leaf area is largest in midsummer [127]. New rhizome buds are usually visible in August, but most rhizome growth occurs in the fall after fruit set is complete [23,24]. For both species, vegetative propagation is very important for spreading over shorter distances (up to 0.8 m per year for giant goldenrod) and establishing dense populations. In the study by Jakobs et al. [125], the mean density of giant goldenrod reached up to 78.5 stems  $m^{-2}$ , while Dudek et al. [128] found a mean density of 24.7 stems  $m^{-2}$  for Canadian goldenrod. Populations of giant goldenrod spread by rhizome propagation along riverbanks, while populations of Canadian goldenrod thrive best in loose and drier soils found near urban areas, roadsides, and railways [125,129]. Giant goldenrod spreads more rapidly due to increased rhizome length (up to 90 cm), increased secondary rhizome production, and rhizome development over a larger radius [130].

### 3.6. Bermuda Buttercup

Bermuda buttercup is a perennial  $C_3$  geophyte native to South Africa with an underground bulb system. Biotypes of this broadleaved weed species include diploids ( $2n = 14$ ), tetraploids ( $2n = 28$ ), and pentaploids ( $2n = 35$ ) [131]. Pentaploid biotypes dominate in invaded areas worldwide, while tetraploids are also found in the Mediterranean region [132]. Shoots arise from short vertical stems attached to pale brown underground bulbs [133]. Bulbs are borne at the tip of vertical rhizomes and form contractile roots annually that pull bulbs deeper each year, and bulbils are formed in axillary buds of vertical rhizomes [134,135]. Bulbs and bulbils can be buried in the soil at a depth of 2–50 cm or even deeper [22]. Plants grow up to 40 cm tall, and their aerial parts are bright green in color; leaves are trifoliolate, arise from an enlarged basal petiole apex, and are arranged in a loose basal rosette [136]. The petioles are up to 12 cm long, and the clover-like leaves bear 3–4 leaflets that are 3.5 cm long, which are often hairy on the underside [137]. Each plant bears 1–5 axillary inflorescences on a 15–30 cm long peduncle arising from the basal leaf rosette [138]. The umbel-like inflorescences bear 4–10 funnel-shaped flowers of 2.5–3.8 cm in diameter with 5 obovate petals that are 2.0–2.5 cm long and intensely yellow in color [133]. The fruits are pointed, pubescent capsules that are about 0.6 cm long [137].

This is a tristylous species with a heteromorphic self-incompatibility system [139]. The plants exhibit the phenomenon of heterostyly, with at least two flower types—some with short styles that do not exceed the length of the anthers, others with longer styles. This hinders self-fertility and makes cross-pollination necessary [140]. In the Mediterranean region, only the type with a short style occurs, so no seeds are produced, and reproduction is only by vegetative means [131]. Bermuda buttercup bulbs sprout around mid-October before the first autumn rains, when soil temperatures are low enough to overcome bulb dormancy [141]. This usually coincides with the formation of contractile roots [22]. Flowering begins in early February and lasts until April. At the end of flowering, in late spring, the aboveground part of the bulb disappears to reappear in autumn [132]. Paspatis [22] found that each plant produces 1–40 bulbs during a single growing season. Verdaguer et al. [142] recorded a total production of more than 35 bulbs per plant, of which more than 20 were hypogeous, larger bulbs, and the rest were epigeous, smaller bulbs. The same authors also noted that the larger the plant, the more bulbs are produced. More than 20 bulbils are formed from each bulb per year, usually between 80 and 114 days after the sprouting of the parent bulbs [133]. Damanakis and Markaki [137] reported that bulb density can exceed 841 bulbs  $m^{-2}$ .

The spread of the bulbs can be localized due to the action of the contractile roots, which pull the bulbs away from the mother plant at distance of 40 cm; in just a few years, the soil is completely covered with a dense carpet of Bermuda buttercup [22]. In three years, plant density will exceed 100 plants  $m^{-2}$  [141]. Once established, the stand is robust and

persistent for many years. Bulbs and bulbils, located in the uppermost soil layers, are light and can be dispersed over long distances by wind and birds; water is another important dispersal medium because the vegetative organs can float [133]. Anthropogenic activities greatly facilitate the spread of Bermuda buttercup. The transportation of contaminated soil or garden waste, spread by mechanical tillage, and use of contaminated tillage equipment are some examples of human-assisted spread [143].

#### 4. Impact on Biodiversity—Evidence from the Broader European Territory

All selected alien plant species pose a major threat to biodiversity and consequently EC in the Adriatic-Ionian region, as representative case studies from across Europe show (Table 2).

Common milkweed is a drought-tolerant, hardy species that thrives on dry, sandy soils and adapts well to loamy, fertile, and even moist soils [55]. It can also grow in poor soils with low nitrogen and phosphorus availability and form dense populations, varying the degree of mineralization of organic carbon and nitrogenous compounds [63]. The plants tolerate pH values between 4 and 7.6, while seedlings can survive in soils with pH values between 2 and 12 [144,145]. Its competitiveness in invaded habitats is attributed to its ability to form dense canopies that shade the soil and suppress the emergence and growth of native plants, as well as its effective use of resources and allelopathic potential [21]. This species is a noxious invader in Europe and is on the list of Invasive Alien Species of Union concern, provided by the EU Commission [115]. In a recent study conducted on abandoned agricultural land in Hungary, common milkweed significantly reduced cover of native grassland species [146]. In Slovakia, common milkweed caused a significant reduction in ground cover of white clover (*Trifolium repens* L.), common dandelion (*Taraxacum officinale* L.), meadow brome (*Bromus commutatus* Schrad.), and orchard grass (*Dactylis glomerata* L.) on permanent grasslands and abandoned vineyards [10]. This species also alters the soil environment by increasing humus, phosphorus, and nitrate levels and reducing pH and carbonated lime in Pannonic open sand grasslands [21]. Aside from its effects on native plant communities and soil health, the presence of common milkweed may also be harmful to some arthropods. An example of this is the study by Gallé et al. [147], who investigated the effects of common milkweed infestation on ground-dwelling arthropod fauna in a poplar forest in Hungary. These authors showed that the presence of common milkweed at a density of 59–86 stems<sup>-2</sup> negatively affected the abundance of two spider species.

Jerusalem artichoke is a hardy and drought-tolerant species that grows well in a variety of soils with pH values between 5.1 and 8.2 [83]. As an invader, it affects biodiversity in wet habitats, near riverbanks and watercourses, and in wet meadows and abandoned agricultural lands by suppressing the growth of native plants [15]. In Hungary, Romania, and Ukraine, Filep et al. [85] observed that Jerusalem artichoke reduced native species richness by nearly 40% at densities of 92–100 stems m<sup>-2</sup> along riverbanks. Aside from its ability to develop tall, dense canopies that stifle the growth of native plants, this species is known for its strong allelopathic potential. For example, the incorporation of Jerusalem artichoke residues from populations found in agricultural fields in northwestern Italy reduced the growth of barnyardgrass and large crabgrass seedlings by 30 and 70%, respectively [28]. Main allelochemicals of leaf tissues include 2-OH-cinnamic acid, salicylic acid, and 4-OH-benzaldehyde, while 2-OH-cinnamic acid is the main allelochemical released by plants into the soil as root exudate [148]. In the laboratory experiments of Filep et al. [148], leaf extracts from populations collected along a stream in southern Hungary significantly reduced the germination rate of bedstraw (*Galium mollugo* L.), while root extracts inhibited the plumule growth of couch grass (*Elymus repens* (L.) Gould). The same authors also found mortalities of up to 60% and 90%, respectively, in plants of hedge bedstraw and tansy (*Tanacetum vulgare* L.) grown in competition with Jerusalem artichoke. In addition to its competitive nature, this troublesome weed also removes nutrients from the soil, slows natural colonization by trees, and promotes erosion of riverbanks [71].

Japanese knotweed is a resilient species that tolerates pH values between 3.0 and 8.5 and survives in soils polluted with heavy metals and salt or in areas with low nitrogen availability [88]. Light is the only requirement for optimal growth, and therefore this weed cannot colonize forests, but grasslands, riparian zones, and watercourses are susceptible to invasion, as are urban environments [87]. This environmental weed causes major ecological changes in invaded communities by forming persistent and extensive monospecific stands [20]. Recent pot experiments with Japanese knotweed populations from northwestern Italy indicate that this species is extremely competitive with native plant communities in grasslands. Specifically, Mincheva et al. [35] found that competition from Japanese knotweed resulted in 13%, 20%, and 68% lower specific leaf area, growth height, and aboveground biomass in white clover, respectively, and 22% lower specific leaf area in perennial ryegrass (*Lolium perenne* L.). It should be noted that the weed density was only one plant per pot, whereas the native species had been established at a density of 10 plants per pot. The authors attributed the competitiveness of Japanese knotweed to its more efficient and aggressive use of resources compared to the native species. In Slovenia, Dorigo et al. [37] mapped 10.4 ha along the Ljubljana river where Japanese knotweed had displaced native plant species; the actual proportion of Japanese knotweed to the total species composition ranged from 50% to 100%, with an average of 85%. At five of the six sites studied by Dassonville et al. [149], the number of native plant species was 75% lower in infested plots compared to control plots. The same authors also noted that the invader can alter soil properties, particularly concentrations of the nutrients Cu, K, Mg, Mn, P, and Zn, in ways that promote its survival and self-renewal and inhibit the growth of native plant communities. Similar observations were made in a riparian zone in Normandy, France, where the presence of Japanese knotweed affects bacterivorous nematodes and microarthropods belonging to the Collembola class by releasing nutrients and root exudates (i.e., secondary metabolites) into the soil [9].

Bohemian knotweed, like Japanese knotweed, is highly adaptable to a wide range of soil and climatic conditions; the hybrid can be extremely competitive with native plant communities [150]. The study by Gerber et al. [151], conducted in riparian areas in Switzerland, Germany, and France, found that invasion by Japanese and Bohemian knotweed significantly reduced the species richness of native plant communities. It should be noted that both species have strong allelopathic potential in addition to their ability to compete for resources (light, water, and nutrients). Allelopathic compounds can be released into the soil as root exudates or through the decomposition of aboveground plant parts and can affect the germination and growth of native plants [152]. Gerber et al. [151] also found that total invertebrate biomass (including herbivorous insects, predators, and detritivores) was 45–60% lower in *Reynoutria* spp. infested plots than in non-infested plots. In the recent study by Neupert et al. [153], invasion of Bohemian knotweed resulted in 39% lower species richness for belowground invertebrates and 64% lower species richness in native plant communities in grasslands in northwestern France. Similar to its parent species, Japanese knotweed, Bohemian knotweed is also capable of altering the soil environment in favor of its growth in invaded sites. Dassonville et al. [154] observed that the hybrid reduced soil moisture and soil pH and increased soil carbon-to-nitrogen (C:N) ratio in a willow forest in Belgium and a wasteland in east-central France; soil moisture decreased and soil C:N ratio increased in a mesic-rough grassland located in the east-central part of the country. Furthermore, in all of the above sites, invasion by Bohemian knotweed resulted in lower activity of denitrifying bacteria in soil and thus nitrogen retention in the invaded ecosystems to its own advantage—a mechanism that can be seen as a kind of niche construction [154].

Giant hogweed is another species on the list of Invasive Alien Species of Union concern in the EU [115]. It is usually found on sandy or silty soils with a pH of 6.0 to 8.5. It is a nitrophilous species that grows best in nitrogen-rich soils. It is found along riverbanks, in grasslands, and in abandoned, disturbed habitats where it is very competitive with native species. In grasslands in the northwestern Czech Republic, giant hogweed reduced

species richness by 24% and native plant biomass by 40%, averaged over 24 different sites in the study by Dostál et al. [155]. In three riparian zones in Ireland, the presence of 1 plant  $m^{-2}$  resulted in 46–60% and 27–44% lower species richness in May and October, respectively [156]. In Germany, Thiele and Otte [157] showed that unmanaged grasslands are more prone to invasion by giant hogweed compared to tall-herb communities and forests, and that a 50% increase in giant hogweed ground cover reduces native species richness by 2.4. Although the main competitive characteristic of giant hogweed is to shade lower-growing species, Jandová et al. [158] also pointed out that plants of this species can also release allelochemicals into the soil through their root system. It can also be assumed that these compounds are toxic to nematodes and other soil organisms [12]. Renčo et al. [12] reported that the average number of nematode species at infested sites was up to 20% lower than the diversity of nematode species at uninfested sites. The same authors also found that giant hogweed invasion increased soil pH from 6.23 to 7.20 and decreased soil carbon and nitrogen content by 24% and 31%, respectively, on riverbanks in the eastern part of Slovakia. The adverse effects of this alien species on soil health are also evident in the study by Koutika et al. [159], in which the invasion in waste lands and valleys in the central part of Belgium resulted in a significant reduction in soil organic matter content by reducing carbon mineralization.

Giant goldenrod and Canadian goldenrod are species with a broad tolerance to biological stressors and high adaptability to different soil types and environmental conditions [23,24]. One difference between these closely related species is that giant goldenrod grows best on riverbanks and watercourses, while Canadian goldenrod prefers looser, drier soils and is most common along roadsides and railways, and near urban areas, forest edges, abandoned fields, etc. [129]. In any case, both species are strong competitors of native vegetation and pose a serious threat to biodiversity in general. The results of a field study conducted in riparian zones in Hungary showed that giant goldenrod can reduce native species diversity by 68% at densities of 340–350 stems  $m^{-2}$  [160]. In the Netherlands, invasive giant goldenrod populations resulted in species richness reductions of 46% and 50% on riverbanks and in semi-natural grasslands, respectively [161]. In the above study, the presence of the invader promoted the growth of certain soil fungi and nematode groups over others. According to Koutika et al. [159], giant goldenrod delivers large amounts of residues to the soil surface. The residues are characterized by lower lignin content compared to the residues of native species and decompose rapidly, increasing the carbon content in the soil and altering the carbon and nitrogen cycles. The negative impacts of Canadian goldenrod invasion on biodiversity are similar. In abandoned agricultural fields in southern Transylvania, Romania, Canada goldenrod invasion has reduced native plant species richness, bee abundance, and visitation by wild bees, honeybees, and hoverflies at the expense of native species [162]. Fenesi et al. [163] collected populations of Canadian goldenrod that had infested an abandoned grassland, a disturbed grassland and an abandoned agricultural field in Romania to be used in competition with native species, i.e., the couch grass and health false brome (*Brachypodium pinnatum* (L.) P.Beauv.). The results of the pot experiments showed that the invader suppressed the growth of native grasses by reducing their light availability and competing strongly for soil resources. In the study by De Groot et al. [164], conducted in ruderal areas near the city of Ljubljana, the species richness of plants decreased by more than 50% due to the invasion of Canadian goldenrod. Another finding of these researchers was that the presence of Canadian goldenrod in the field resulted in 60% and 70% lower species richness and abundance of butterfly species, respectively. In addition, a very recent study by Bielecka et al. [165] on grasslands in southern and eastern Poland found that Canada goldenrod invasion leads to an increase in soil carbon content and C:N ratio, resulting in soil degradation. In the same study, plant species diversity was 20–30% lower in the invaded plots, indicating high competitiveness of the invaders over native species. It should also be mentioned here that both species are considered allelopathic to native vegetation; secondary metabolites with

potential allelopathic effects have been isolated from root extracts of both species [166,167].

**Table 2.** Representative case studies on the impact of invasion of selected alien plant species on biodiversity in different habitats in European countries.

Species	Invaded Habitats	Countries	Affected Organisms/Parameters	Case Studies
<i>Common milkweed</i>	Grasslands, abandoned agricultural fields	Slovakia	Native plant communities	[10]
	Poplar forest	Hungary	Soil arthropods	[147]
<i>Jerusalem artichoke</i>	Riparian areas	Hungary, Romania, Ukraine	Native plant communities	[85]
<i>Japanese knotweed</i>	Forest edges, abandoned agricultural fields, field edges, grasslands	Belgium	Native Plant communities, soil properties	[149]
	Riparian areas	France	Soil properties, arthropods	[9]
	Riparian areas	Switzerland, Germany, France	Native plant communities, invertebrates	[151]
<i>Bohemian knotweed</i>	Grasslands	France	Native plant communities, invertebrates	[153]
	Willow forest, grasslands, wastelands	Belgium, France	Soil properties	[154]
	Riparian areas	Switzerland, Germany, France	Native plant communities, invertebrates	[151]
	Grasslands	Czech Republic	Native plant communities	[155]
<i>Giant hogweed</i>	Riparian areas	Slovakia	Soil properties, soil nematodes	[12]
	Abandoned agricultural fields, grasslands	Belgium	Soil properties	[159]
<i>Giant goldenrod</i>	Riparian areas	Hungary	Native plant communities	[160]
	Grasslands	Netherlands	Native plant communities, Soil properties, Soil fungi, Soil nematodes	[161]
<i>Canadian goldenrod</i>	Ruderal areas	Slovenia	Native plant communities, insect-pollinators	[164]
	Grasslands	Poland	Native plant communities, soil properties	[165]
<i>Bermuda buttercup</i>	Orchards	Greece	Native plant communities, soil properties	[143]
	Abandoned agricultural fields	Italy	Native plant communities	[11]

Bermuda buttercup is best adapted to Mediterranean habitats, where it is often found in grasslands, abandoned fields, unmanaged orchards, and other types of disturbed habitats [11,168]. It tolerates a variety of soil types but performs best in heavy, well-drained, fertile soils that are slightly acidic, neutral, or slightly alkaline [133]. There is much evidence that the plant competes with native plant species in invaded areas. For example, in the study by Lazzaro et al. [11], native species richness in abandoned orchards was severely affected by Bermuda buttercup cover. These authors observed an initial decline in native species richness when Bermuda buttercup infestations reached up to 25% and an even more significant decline in native species richness when Bermuda buttercup infestations reached 75–100%. In Greece, heavy infestations of olive groves with Bermuda buttercup reduced plant species diversity by 75% in winter and completely displaced native plant

communities in April and May [143]. Apart from its ability to compete for resources, the plant may also have allelopathic effects on native flora in Mediterranean habitats [22]. In addition, the flowers of Bermuda buttercup have been reported to attract insects and reduce their visits to the flowers of native white rocket (*Diplotaxis eruroides* (L.) DC.) by about 33% in abandoned agricultural fields in Spain [168]. Even if it does not displace native plants, it has detrimental effects on soil health by enriching the soil and altering ecosystem nutrient cycling [22]. For instance, Petsikos et al. [143] noted that Bermuda buttercup invasion can reduce the decrease of carbon stored in above-ground vegetation in olive groves by 68%, suggesting that soil organic content will decrease in infested areas.

Apart from their direct negative impact on biodiversity, these perennial invaders also pose a serious threat to EC in the Adriatic-Ionian region and the wider European area. Given their high dispersal potential, it is highly likely that once these species occur in habitats adjacent to corridors, they will also invade the corridors and develop dense, monospecific populations that displace native flora, alter ecosystem functions and properties, and disrupt EC between protected areas [7,169]. As Vicente et al. [170] have shown, invasive populations of perennial alien plants pose a major threat to the establishment and maintenance of functional ecological linkages between areas of high conservation value. Species with such high dispersal potential and a competitive nature may exploit the establishment of corridors and use them as pathways for further dispersal into areas of high conservation value [52,171,172]. In such cases, the design of ecological corridors should be redesigned [172]. It should be noted here that the relationship between invasive plants and ecological linkages is a research objective that should be further explored in species-specific case studies in the near future [173]. Further work should also determine the extent to which the perennial invasive plants examined in this study reduce habitat connectivity for species that rely on displaced native plant species [7]. In any case, a general fact highlighted by Glen et al. [169] is that the establishment of ecological corridors between areas of high conservation value is not sufficient to halt biodiversity decline unless the corridors and their surrounding larger areas are protected from biological invasions. Vilà and Ibáñez [174] also stated that seed sources of alien plants should be kept at a safe distance from areas where ecological corridors have been established. In a more recent study, Gregory et al. [7] emphasized that invasive species management is one of the best management practices to create and maintain functioning ecological corridors. Therefore, it can be assumed that invasive species management should be considered as one of the priorities to improve EC throughout the Balkan Peninsula. As for the perennial herbaceous alien plants examined in the current study, their management should include both the adoption of measures that can prevent the spread of invasive populations in the Adriatic-Ionian region and the implementation of available eradication methods, as presented in the following section.

## 5. Available Management Practices

### 5.1. Preventative Measures

Recent literature shows that the occurrence of the species considered in this study has significantly increased in recent years in different habitats in several countries of the Balkan Peninsula. Considering their ecological characteristics, the occurrence of small patches in new locations can lead to massive infestations in subsequent years. Moreover, the restoration of heavily infested sites is a major challenge because these perennial invaders are very persistent and tend to alter the soil environment in invaded habitats and prevent recolonization of native plant communities [15,23,24,63,88,107,133]. Therefore, early management actions should be taken to prevent the spread of these environmental weeds in ecological networks and protected areas with high conservation value. The early identification and eradication of new invasive populations in the wider Adriatic-Ionian region is the first proactive step to protect EC. Experts in ecology, forestry, agriculture, and weed science should organize transboundary networks to continuously monitor the invasion of these alien plants in all associated countries. As shown by Dorigo et al. [37] in Slovenia, the use of remote sensing methods and extensive field surveys is essential to assess the extent of

current invasions and to effectively map new infestations. The development of potential Decision Support Systems (DSS) capable of conducting risk assessments and predicting the further spread of these species in the Balkan Peninsula would also help decision-makers and land managers to mitigate this threat to biodiversity and EC [175].

Researchers, land managers, and decision makers also have a special responsibility to raise public awareness of the harmful effects of invasions of these species on biodiversity and EC. Various tools such as developing websites, setting up forums, organizing workshops, creating informational films, and publishing scientific articles can help inform the public and ensure that plant invasions are not further overlooked by society [176]. Public participation plays an important role in mitigating plant invasions because they are partly due to human activities. For example, the horticultural industry was one of the driving forces in the introduction of common milkweed, giant hogweed, and Japanese knotweed in the Adriatic-Ionian region [21,37,43]. Therefore, it is important to inform the public about the environmental impact of these invasive species to prevent their further spread through illegal ornamental cultivation. Another example is the growing interest in the use of Jerusalem artichoke as an energy crop [83]. Society and industry should be aware of the risks associated with cultivating a species that has great potential to escape cultivation and become a troublesome invader and encourage the cultivation of energy crops that have lower invasive potential and are suitable for cultivation on marginal lands [177]. In addition, Bermuda buttercup should not be perceived by the public as a beneficial nectar source for honeybees in the Mediterranean region but as an invasive species that affects biodiversity and should not be allowed to spread uncontrollably in orchards and grasslands [22]. Apart from raising public awareness of the consequences of their actions, it is likely that local residents and volunteer groups, properly trained by experts, can actively contribute to invasion monitoring and implementation of eradication programs. This hypothesis was recently confirmed in the study by Cordeiro et al. [178], conducted in Portugal.

It should also be noted that abandoned agricultural fields, unrestored grasslands, riparian areas, and forests are typical examples of disturbed habitats that are vulnerable to invasion by these opportunistic species. Therefore, policy measures should be taken to prevent the further increase of such disturbed habitats. Providing farmers with better access to land, new sources of investment, and incentives to diversify implement crop diversification practices (i.e., crop rotation, intercropping, and cover cropping) are critical to prevent agricultural land degradation and abandonment [179]. Reseeding of native species to maintain the diversity of native plant communities or strategic grazing are also recommended to restore grasslands and increase their resistance to plant invasions [180,181]. Maintaining the diversity of native vegetation is also important to protect riparian areas from alien invaders [182]. Forests should also be protected from wildfires during the dry summer months because fire is a form of habitat disturbance that promotes the establishment of invasive alien plants [183].

## 5.2. Eradication Methods

Although the use of herbicides is not the most environmentally friendly method of weed control in natural environments, chemical weed control may be necessary to eradicate perennial invasive species from sites where they have become dominant and negatively impact biodiversity [184]. Glyphosate is the most common systemic herbicide used to eradicate perennial invasive species [11,21,96,185–189]. This herbicide is a substituted glycine that controls weeds by inhibiting the activity of enolpyruvyl shikimate phosphate synthase (EPSPS), which is involved in the synthesis of phenolic amino acids. It is taken up relatively rapidly by treated plant surfaces and translocated through the phloem into young roots and leaves, storage organs, and any other actively growing tissue or organ [190]. However, the possible phase-out of this active ingredient in Europe suggests that research should focus on alternative weed control methods to eradicate perennial invasive alien plants in the countries of the Adriatic-Ionian region [191–193].

Synthetic auxins are compounds that mimic the action of natural plant auxins. They are another group of systemic herbicides that are expected to play an important role as glyphosate-alternative weed control options to eradicate invasive populations of broadleaved herbaceous weeds. For example, the application of a pre-package mixture of clopyralid, fluroxypyr, and MCPA proved to be highly effective in controlling Jerusalem artichoke in riparian areas in the Czech Republic [194]. Svehlakova et al. [195] suggested that triclopyr is a very good option to control this weed at flowering. Triclopyr, 2,4-D, aminopyralid, and clopyralid can achieve a good level of control of Japanese knotweed [96]. The efficacy of triclopyr against giant hogweed has also been reported [189]. Herbicides that inhibit acetolactate synthase (ALS) such as metsulfuron-methyl or protoporphyrinogen oxidase-inhibiting herbicides such as oxyfluorfen provide good control of Bermuda buttercup [22,133]. Regardless of the active ingredient used, the timing of treatment is critical to achieve optimal control. Treatments should be applied near the phenological growth stage of senescence, when herbicides can be better translocated into plants by the flow of photosynthetic assimilates from leaves to the underground vegetative parts of perennial weeds [22,96]. In addition, multiple herbicide treatments repeated over time are necessary to eradicate a well-developed stand [20,21].

It should be noted at this point that foliar applications of herbicides have some strong disadvantages. Foliar applications, especially of synthetic auxins, are not suitable for all weather conditions, and off-target movement of herbicide spray-drift may cause injury to nontarget plants [196]. In addition, broadcast application of herbicides can pollute soil, groundwater, and surface water and have negative side-effects on the environment [197]. Therefore, herbicide application methods other than broadcast foliar applications should be investigated. Apart from conducting applications as site-specific spot treatments, stems with a diameter of  $\geq 2.0$  cm can be pierced with a hollow needle, allowing the highly concentrated herbicide to enter the interior of the stem [198]. This technique, known as stem-injection, provided excellent control of Japanese knotweed in the study by Delbart et al. [186]. Another method is to cut the stems and immediately apply phloem-mobile herbicides to the freshly exposed cambial tissue [199]. The potential of both methods to control herbaceous perennial species that become woody with age should be further investigated as they pose lower environmental risks compared to conventional methods because herbicides are applied to specific parts of the target plants.

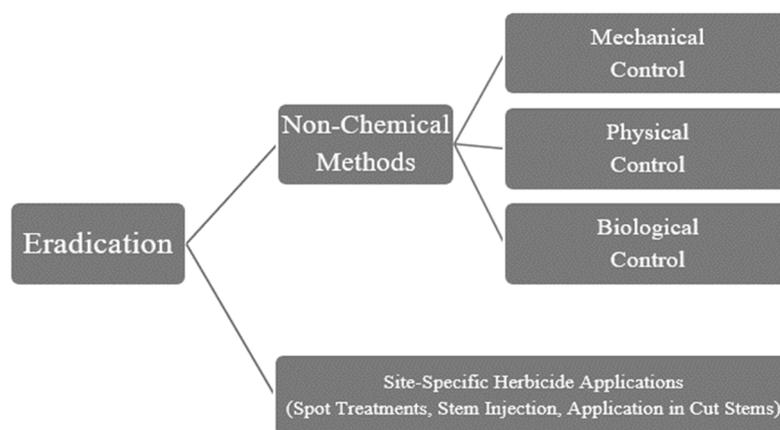
As for the mechanical methods evaluated to control the studied species, Nagy et al. [200] reported that mowing reduced the stem density of giant goldenrod by 72–81%. Conducting mowing for five consecutive years is recommended to deplete stands of Canadian goldenrod [201]. Regular mowing twice a year reduces both aboveground and belowground biomass of Jerusalem artichoke [202]. Monthly mowing treatments have been reported to reduce the biomass of stems of Japanese knotweed [186]. One explanation could be that repeated mowing depletes the resources of the underground vegetative organs of perennial weeds. Mowing plants just before flowering may also reduce the seed production of common milkweed [21]. Tillage is a non-chemical way to mechanically control giant hogweed. Cutting the taproot of plants at 15 cm soil depth in mid-spring prevents regrowth and results in good control of this species [189]. Tillage in early spring, when vegetative growth is complete, interrupts the formation of new bulbs and bulbils in Bermuda buttercup plants [22]. According to Švehláková et al. [202], digging up the tubers was among the effective non-chemical practices for the control of Jerusalem artichoke. However, as was also emphasized for this and other perennial species in this study, uprooted vegetative parts should be removed from water on riverbanks, as they can float in water and spread over new sites [15,81].

There is also evidence that mowing can be combined with physical weed control methods such as grazing and flooding. A recent example is the study by Nagy et al. [200], in which flooding with a water level of 10–15 cm above the soil surface for one month, together with a single mowing operation, reduced the stem density of giant goldenrod by 83%. The same authors observed that cattle grazing from mid-spring to early winter combined with

a single mowing reduced giant goldenrod stem density by 87%. In previous studies, long-term sheep grazing reduced giant goldenrod populations and allowed native plant species to become established in meadows [203]. Ducs et al. [204] also recommended that rabbit grazing could be an effective method to facilitate the control of common milkweed. Zhang et al. [205] used a bioherbicide isolated from the fungal pathogen *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. and reduced Canadian goldenrod density by 89% in abandoned agricultural fields and artificial forests. Hagner et al. [206] used a mulch material consisting of peat fibers, tap water, and birch (*Betula* spp.) slow pyrolysis liquids. The results showed that the above mulch material completely eliminated giant hogweed seedlings. Therefore, further research should be conducted on the development of bioherbicides as an environmentally friendly, physical method to suppress noxious invasive weed species.

Biological control, i.e., the release of biological agents associated with the species considered invasive at a particular site, is another non-chemical option for eradicating perennial invaders in invaded habitats. For example, the psyllid *Aphalara itadori* Shinjii serves as a biological agent against Japanese knotweed, while the stem-boring weevil *Lixus iridis* Olivier, 1807 is considered a natural enemy of giant hogweed [207,208]. Tóth et al. [209] pointed out that *Fusarium sporotrichioides* Sherb. is a fungal plant pathogen that facilitates biological control of giant hogweed. Podroužková et al. [210] showed that two snail species, namely *Succinea putris* Linnaeus, 1758 and *Urticicola umbrosus* (C. Pfeiffer, 1828), climb up the hairy stems of Jerusalem artichoke and feed on the aerial parts of the plants. In the Mediterranean region, boomrapes (*Orobanche* spp.) emerge at the time of flowering of Bermuda buttercup. These phanerogamous parasites attach to the root system of Bermuda buttercup plants and utilize the nutrients necessary for bulb formation. As a result, Bermuda buttercup plants form small bulbs and show poor vegetative growth the following fall [22].

To summarize, both herbicides and non-chemical weed control options should be considered as components of integrated eradication programs (Figure 2).



**Figure 2.** Weed control practices that should be combined to eradicate populations of the selected invasive perennial herbaceous alien plant species included in the current study.

## 6. Conclusions

The occurrence of the perennial herbaceous species studied in this review has increased in the last two decades in the Adriatic-Ionian region. All species have a high dispersal potential on grasslands, abandoned agricultural lands, forest edges, and riparian areas and pose a significant threat to native plant communities, the environment, and biodiversity. Therefore, early action should be taken to prevent the spread of these weeds in ecological networks and protected areas of high conservation value. Researchers have a special responsibility to raise public awareness of the harmful effects of invasions of these species on biodiversity and EC. Other objectives should be the early identification and mapping of invasive populations and the development of successful eradication programs

integrating site-specific herbicide application practices, mechanical, physical, and biological control methods.

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