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Multi-Year Monitoring of *Asclepias syriaca* L. Spread in the Deliblato Sands Protected Reserve in Serbia

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Abstract: The invasion of non-native plant species has a detrimental effect on native biodiversity. In a seven-year research project, we investigated the occurrence of the invasive species *Asclepias syriaca* L.in the Deliblato Sands protected area, located at a south-eastern part of the Pannonian Plain, and identified the factors that contribute significantly to its colonisation. The distribution of this invasive species was monitored on more than 300 km of the accessory, bordering and selected internal roads. *A. syriaca* occurs within the protected area but is much more widespread on accessory and bordering roads. The number of locations of *A. syriaca* increased every year of the study, even within the protected area, indicating a further spread of this species. *A. syriaca* is much more abundant on the north-eastern edge than in the south-west. The reason for this is most likely the much larger area of abandoned agricultural land in the north-east. Roads used for public transport are the main entry points for the further spread of *A. syriaca*. In contrast, recreational trail use does not enhance the spread in the Deliblato Sands natural reserve. This study can be used to further analyse the ecological dynamics of *A. syriaca* and to develop timely strategies by which to prevent or slow down its spread.

Keywords: *Aclepias syriaca*; biological invasion; Deliblato Sands; invasive species; nature reserve

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1. Introduction

The biological invasion of non-native species is considered one of the greatest threats to native biodiversity [1–3]. The consequences of the ongoing introduction and spread of invasive non-native species are becoming increasingly clear in terms of their impact on autochthonous species, ecosystems and human wellbeing [3–7]. When species arrive, establish and spread outside their historical geographic range, they introduce new traits, behaviours and genes and alter ecosystems by introducing new biological interactions, species extinctions and habitat modification [8–10]. Hejda et al. [11] found that, in areas invaded by *Fallopia sachalinensis* (F.Schmidt) Ronse Decr., the number of species detected in the communities was reduced by almost 90%. Invasive non-native species could modify pollinator biodiversity and abundance through nutritional deficits for pollinators [12,13], shortening the period of availability of floral resources [14] or simply outcompeting native plants for pollinators [15]. In addition to the direct effects on human health and agricultural production, the ecological consequences of the biological invasion of plant species include changes in fire regimes, forest loss, transmission of diseases to native species, reduction of water flow and habitat modification [16].

Due to rapid global change and environmental degradation, protected areas are of crucial importance for the conservation of biodiversity and ecosystem function [17,18]. In recent decades, protected areas have been shown to be vulnerable to invasions. They suffer from impacts at the species and community level, through habitat modification and through various undesirable effects on the abundance, diversity and richness of native species [19,20]. Although the impacts of biological invasions are thought to be similar both inside and outside protected areas [21], the relative impacts may be more negative in protected areas than elsewhere, as these areas conserve key elements of global biodiversity [19]. It has been reported that protected area managers in Europe consider invasive species to be the second greatest threat after habitat loss [22]. This makes the monitoring and understanding of plant invasion in protected areas very important.

The number of recorded non-native invasive species in the region of the Pannonian Plain in Serbia represents less than 10% of the total flora of this area. However, their abundance, distribution and population density have a significant negative impact on the natural habitats and on the wildlife living there [23]. Over the last two decades, a significant spread of invasive species has been observed in the Deliblato Sands protected area, one of the most important centres of biodiversity in Serbia and Europe [24]. The Deliblato Sands is one of the largest continental European sandy areas and is located in the south-eastern part of the Pannonian Plain. It is the last and largest oasis of the sand, steppe, forest and swamp vegetation that once dominated the Pannonian Plain. The flora of this area consists of over 1000 species of higher plants, many of which are relicts and rarities, such as *Artemisia pancicii* (Janka) Ronniger (Pančić wormwood), *Paeonia officinalis* ssp. *banatica* (Rochel) Soó (Banat peony), *Rindera umbellata* (Waldst. & Kit.) Gürke, *Prunus tenella* Batsch, *Helichrysum arenarium* (L.) Moench, *Fritillaria meleagris* L. and *Juniperus communis* L. var. *communis*–the only autochthonous conifer of the Pannonia Plain. Some of these are listed in the Red Data Book of the Flora of Serbia [25].

One of the most widespread invasive species in the Deliblato Sands Nature Reserve, and in most places located in its vicinity, is *Asclepias syriaca* L. *A. syriaca*, the common milkweed, has been of interest to agriculturists for many years because of its potential economic value as a new crop [26–28], but also because of its negative impact as an invasive non-native species and weed, as it leads to a reduction in yields and biodiversity [26,29–31]. This species has already been naturalised in 27 European countries and it is predicted that expected climate changes will contribute to its spreading beyond its current range in the future [32]. The species is most widespread in the warmer areas of southern Europe, where it is categorised as invasive. Due to its mass occurrence, the common milkweed threatens native biodiversity and invades natural and semi-natural habitats. The risk assessment of *A. syriaca* for Europe [33] confirms that the invasion of this species poses a threat to the stability of several natural ecosystems in the continental, Mediterranean and Pannonian biogeographical regions of Europe. *A. syriaca* is widespread in Serbia and is classified as a quarantine weed species in the A2 list, the list of harmful organisms detected on the territory of the Republic of Serbia [34]. Therefore, the phenomenon of the spread of *A. syriaca* in the Deliblato Sands protected area is particularly worrying.

In the Deliblato Sands protected area, the densest stands of common milkweed have been observed on and near roads. Because invasive non-native plant species often occur along roads, it is assumed that dispersal along roads is an important mode of invasion [35–38]. Roadsides are usually well-drained habitats that receive more light and are often disturbed by maintenance activities (vegetation mowing, drainage, removal of surface layer), which creates environmental conditions in which invasive non-native plants thrive [39]. Due to the large extent of road networks and their proximity to natural communities, roads could be an important element of regional invasion [40,41].

Plant invasions have rarely been documented in real time at the population level [42]. Most evidence is indirect, derived from static distributions in conjunction with mathematical models describing potential factors and directions of dispersal [43–46]. The most accurate way to document non-native invasions should be direct observation of the spread, establishment and expansion of populations in real time, quantified by the distribution of individual plants [47]. This type of precise monitoring requires years of activity, as plant invasions usually occur over a long period of time [45,48]. In addition, an invasion may involve the dispersal of plant parts and seeds over large areas, so effective monitoring must also account for infrequent colonisation over long distances [49]. For these reasons, we monitored the occurrence of common milkweed in the Deliblato Sands area every second year for a period of seven years, from 2015 to 2021. It has been observed that the plants of the common milkweed are most numerous in the contact zone between the Deliblato Sands protected area and the neighbouring agricultural areas and that the type and dynamics of agricultural use differ on the two sides (the north-eastern and south-western) of the protected area. Therefore, we analysed the occurrence of *A. syriaca* on the accessory and bordering roads and on selected inner roads in the Deliblato Sands (more than 300 km). To determine how important seed dispersal is for the colonisation of the Deliblato Sands over large areas, the distances between new stands of common milkweed and the nearest existing stands were measured.

Based on previous research on the nature of invasion and the factors contributing to the spread of invasive non-native species in protected areas, we tested the following hypotheses. (1) The spread of invasive species into the protected area is caused by anthropogenic impacts from the surrounding unprotected areas. We therefore hypothesise that *A. syriaca* is much more abundant in the unprotected surroundings of Deliblato Sands, i.e., that there is more milkweed on the accessory roads and bordering roads than in the inner parts of the protected area. (2) The rapid colonisation of new areas, accompanied by an expansion of territory and an increase in numbers, is one of the main characteristics of invasive non-native species in areas where there are no active protection measures. We expect the number of newly established localities with *A. syriaca* to increase during the seven-year period. (3) The type and dynamics of agricultural utilisation in the vicinity of a protected area has a significant impact on biological invasions. Accordingly, we expect different dispersal pressures for common milkweed from different sides (north-east vs. south-west) in the neighbourhood of this protected area as well as in the protected area itself. (4) Different types of roads leading near or through protected areas may have different effects on the spread of invasive plant species. We will investigate whether there are differences in the spread of *A. syriaca* on the three types of roads passing through the Deliblato Sands protected area. (5) The strong invasive potential of *A. syriaca* results from its ability to reproduce both by seed and by underground vegetative organs. We will determine how widespread the long-distance colonisation by seeds of *A. syriaca* is in the Deliblato Sands and whether there are differences in dispersal over large areas between the north-eastern and south-western parts of this protected area.

2. Materials and Methods

2.1. Research Object and Study Area

A. syriaca is a perennial herbaceous plant that reproduces by seeds and rhizomes. The rhizomes can reach over 2 m in depth and have a high capacity for vegetative propagation. The plants produce many seeds, which have hairy appendages and are very easily blown away by the wind. Common milkweed is found in habitats such as grasslands, dunes, river valleys and edges of water bodies, forest edges and even wetlands, but more commonly in human-modified habitats such as roadsides, railway areas, wasteland, abandoned orchards, vineyards, abandoned farmland, with a wide range of soil conditions, especially in sunny, sandy places [50,51]. The Deliblato Sands are one of the windiest areas in Serbia, which favours the spread of common milkweed. The wind easily catches the fluffy part and carries the seed over long distances. Even the wheels of vehicles travelling in this area can spread the seeds. If we also take into account the rapid dispersal by underground rhizomes, we can assume a high dispersal potential of *A. syriaca* in the studied area.

The studies on *A. syriaca* were carried out for the purposes of this work in the Deliblato Sands nature reserve in northern Serbia, in the south-eastern part of the Pannonian Plain in central Europe (Figure 1).

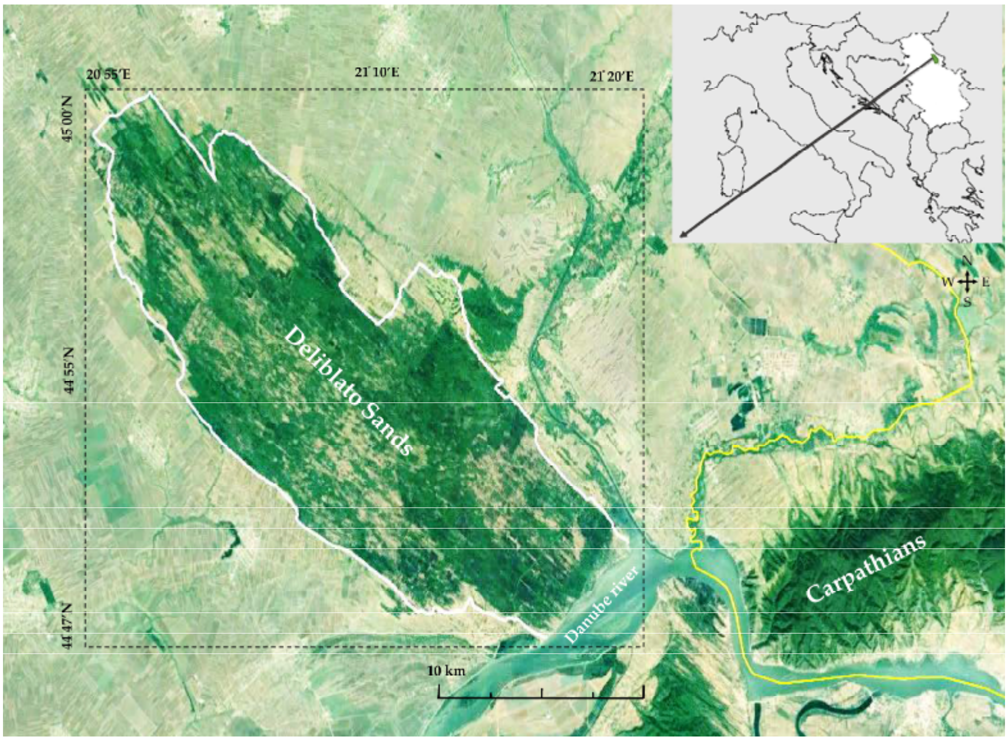

Figure 1. Location of the study area Deliblato Sands protected area in northern Serbia.

Deliblato Sands covers an area of more than 300 km^2 [52] and lies between the Danube and the south-western slopes of the Carpathians. In this area, the influences of the temperate–continental climate of the Pannonian Plain and the climate of the southern foothills of the Carpathians intertwine, while the influence of the sub-Mediterranean climate, which reaches the Deliblato Sands through the valley of the Južna and Velika Morava rivers, can also be felt [53]. The Deliblato Sands, with their thick aeolian sand deposits and pronounced dune relief, as well as the distinctive mosaic of animal communities and typical representatives of flora and fauna, are a unique natural phenomenon in Europe. The main landscape of the Deliblato Sands are elliptically shaped hills with a mosaic of steppe, shrub and forest habitats that can be defined as forest–steppe [54,55]. The steppe communities in the Deliblato Sands are *Festucetum vaginatae deliblaticum*, with some typical species that include *Stipa borysthenica* Klokov ex Prokudin, *Festuca vaginata* Waldst. & Kit. ex Willd., *Astragalus onobrychis* L, *Koeleria glauca* (Spreng.) DC., *Artemisia campestris* L, *Euphorbia seguieriana* Neck, and *Dianthus gigantiformis* (Borbás) Heinr.Braun [52]. The shrub vegetation is a succession of steppe vegetation and includes the occurrence of the species *Crataegus monogyna* Jacq. and *Juniperus communis* L. in stands with *Chrysopogon gryllus* (L.) Trin. Some characteristic species for this plant community are *Crataegus monogyna*, *Paeonia tenuifolia* L., *Chrysopogon gryllus*, *Orlaya grandiflora* (L.) Hoffm., *Medicago falcata* L. and *Adonis vernalis* L. The appearance of the forests is related to the more humid and less insolated exposures (north, north-east and north-west). There are some characteristic species in the oak–lime forest community, including *Tilia tomentosa* Moench, *Quercus pubescens* Brot., *Quercus robur* L., *Fraxinus ornus* L., *Cotinus coggygria* Scop., *Ligustrum vulgare* L., *Fritillaria montana* Hoppe ex W.D.J.Koch and *Convallaria majalis* L. Light, open woodlands with *Juniperus communis* and *Populus alba* L. contain a significant proportion of species of the sand community and some of the grass and shrub species are *Carex liparocarpos* Gaudin, *Polygonatum odoratum* (Mill.) Druce, *Fragaria vesca* L., *Hieracium umbellatum* L., *Euphorbia cyparissias* L., *Rhamnus cathartica* L., *Prunus spinosa* L., *Crataegus monogyna* and *Rubus caesius* Thunb. ex Maxim. The coniferous vegetation and black locust stands in this area are the result of plantations [56]. *Robinia pseudoacacia* L. stands cover a significant part of the Deliblato Sands. Pine plantations on the Deliblato Sands also occupy significant areas.

These stands are formed by *Pinus sylvestris* L. or *Pinus nigra* J.F.Arnold or a combination of both species. In young pine stands, the steppe elements of the habitat in which the plantations were created have been preserved. In the older white pine stands, a dense complex of shrubby and herbaceous plants forms, so that these phytocenoses resemble true secondary pine forests [56].

In the immediate vicinity of Deliblato Sands there are rural settlements whose inhabitants practise agriculture. The use of agricultural land differs on the two sides of the protected area. On the north-eastern side, much of the agricultural land is not cultivated every year and there are more abandoned agricultural areas, while the agricultural land on the south-western edge of the protected area is continuously cultivated every growing season. Paved and unpaved roads lead from some of these settlements into the protected area and we have marked these roads as accessory roads. The length of these roads was 29 km and the occurrence of *A. syriaca* was recorded along these roads (Figure 2).

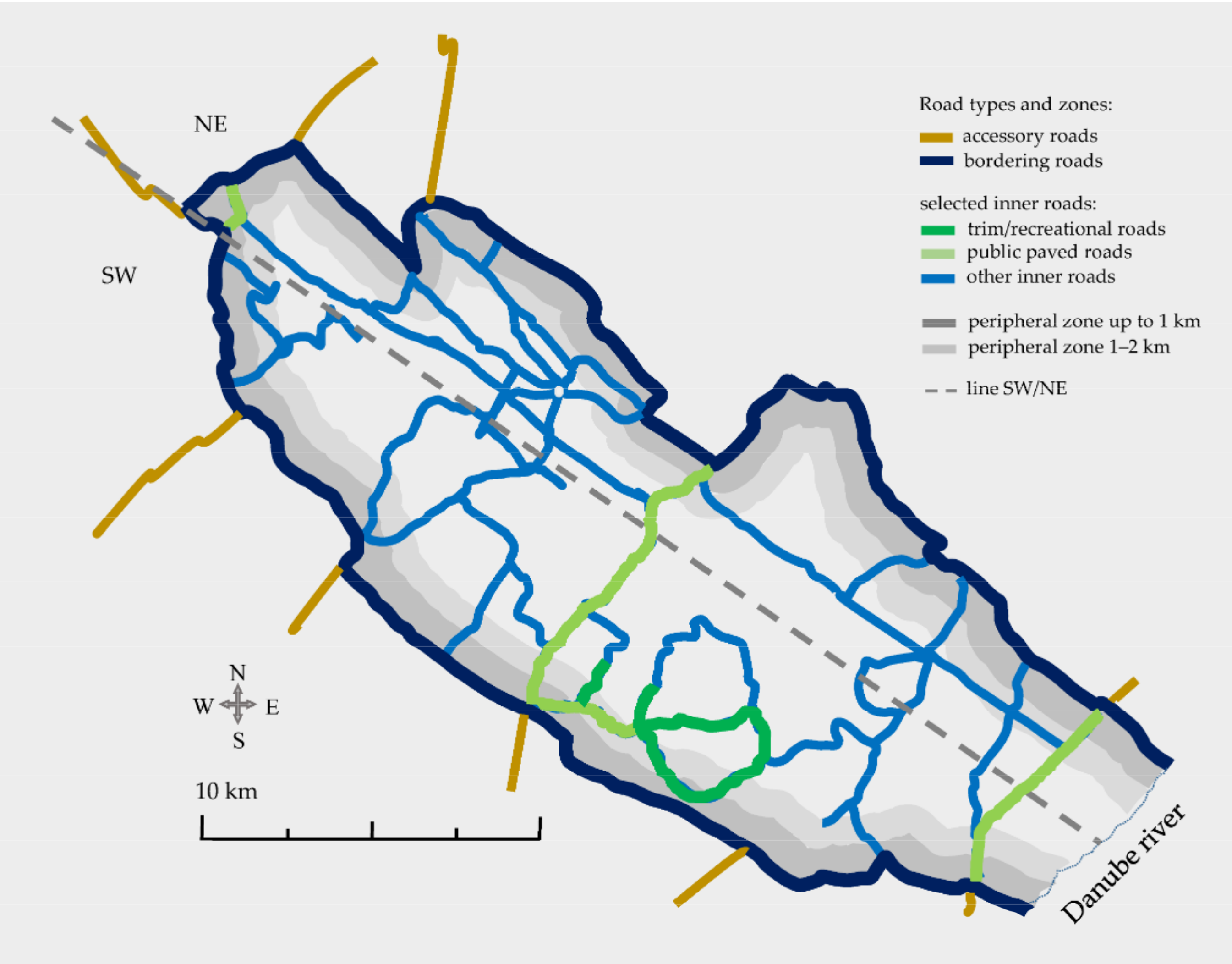

Figure 2. Deliblato Sands map with marked roads and zones used in the research.

There are also unpaved roads totalling 91 km along the boundaries of the Deliblato Sands protected area. We have marked these roads as bordering roads and recorded the occurrence of *A. syriaca* along these roads as well. Only on the banks of the Danube are there no such roads (Figure 2).

In the area of Deliblato Sands there are parts that are under the influence of various human activities. Of selected inner roads, only three roads passing through this area are paved and open to public traffic, but no activities are allowed outside the carriageways themselves (public roads). A significant portion of Deliblato Sands is intended for recreational purposes only (trim trails). Visitors coming for recreational purposes are allowed to walk on the designated trim trails, but without motor vehicles. The remaining majority of the selected inner roads (other inner roads) and the trim trails are unpaved roads. Unpaved roads in the protected area may only be used by foresters. With a special permit, some parts of these roads are used by tourists/recreationists, beekeepers and loggers (other inner roads). In the forests, next to the unpaved roads, narrow openings in the canopy (a 1–2-m-wide strip) are maintained by infrequent mowing and dominated by grasses and weeds. These parts of the roads in the forests receive more light than undisturbed forest sites. The total length of the selected inner roads where we observed the presence of this invasive species was 187 km (Figure 2).

2.2. Field Methods

In order to map the distribution of *A. syriaca*, data were collected directly in the field. Observations of plants outside the roads were possible at different distances, depending on the vegetation in the area, ranging from a few tens of metres to more than a hundred metres. The study was conducted over a period of seven years between 2015 and 2021. The surveys were conducted in autumn (October) every second year (measurement year), in 2015, 2017, 2019 and 2021. According to field experience, autumn was the best time to detect *A. syriaca* among other plants. Due to the large number of seeds in the fruits, which have characteristic, white-coloured appendages that are used for dispersal by the wind, the plants are easy to spot at this time, even from a great distance. To minimise the possibility of some plants being overlooked, the same field was observed by two researchers during each inspection. In each survey year, the site was inspected from different directions, which also minimises the possibility of some stands of this species not being noticed due to the surrounding vegetation. Distribution maps of all plants were created using the geographical coordinates recorded during the road survey. The locations were marked using GPS devices with an accuracy of 2 m (Garmin eTrex Touch 35, Garmin Ltd. 1200 E. 151st Street, Olathe, KS, USA). In the areas where there was a significant spread of this invasive species, we measured the size of the stands. Any expansion of the invasive area by more than 20 metres when measured year to year was recorded.

To determine whether there is pressure to expand the range of this species from areas of high human impact around the protected area, we defined three zones. The first zone in the protected area is the edge zone, which is 1 km wide and borders the agricultural (non-protected) area. The second zone is a part of the protected area that extends up to 2 km into the Deliblato Sands. The third zone is the central area, which is more than 2 km from the edge of the Deliblato Sands (Figure 2).

To assess how common the spread was over large areas, medium distances and neighbouring areas, we measured the distances between new stands of common milkweed and the nearest existing stands. This measurement was carried out for all newly established plants in each measurement year. Although distances were measured with an accuracy of one metre, four distance categories were introduced to facilitate the comparison of results where (a) the distance to the nearest site was up to 100 m, (b) between 100 and 500 m, (c) between 500 and 1000 m and (d) over 1000 m from the nearest location site.

During this multi-year inspection of the site, we found that some common milkweed plant stands had been lost, i.e., that there were no more plants in some localities. As there was a possibility that the plant had gone unnoticed during the site inspection, criteria for removing a locality from the map were established. If it was determined that no more plants were found at a site during two consecutive surveys, it was assumed that these data should be removed from the map (“confirmed loss”).

2.3. Data Analysis

The Google Earth Pro 7.3.6.9345 programme was used to create the maps [57]. The distance between the locations, the length of the roads and other necessary data were determined using additional options in the Google Earth Pro programme. The length of all roads on which plants were recorded was determined and the locations with plants on each of these roads were counted. The frequency of locations on three types of roads was compared: accessory roads, bordering roads and selected inner roads of the Deliblato Sands protected area. A chi-square test was used to test the significances of the differences between the frequencies of plant locations in the different parts of Deliblato Sands (SAS PROC Freq) [58]. The test compares the observed and expected number of localities within each group. Observed values are data collected in the field. The expected values are the numbers that would result from a uniform distribution of locations in the different parts, i.e., on the tested roads. The threshold value for p in this test was 0.05.

The same test was used to test the statistical significance of the differences in plant distribution between the north-eastern and south-western parts of Deliblato Sands. The differences between the three types of selected inner roads within the protected area (paved public roads, trim trails, and other inner roads) were also tested using the chi-square test. The same test was used to test the differences in distribution between three zones in the protected area: 1 km from the boundary, 2 km from the boundary, and central zone (more than 2 km from the boundary). This test was performed for both the north-eastern and south-western parts of Deliblato Sands. Testing the rate of occurrence of new localities of this species in three time intervals (2015–2017, 2017–2019 and 2019–2021) with the chi-square test provided information on whether the invasion process is significantly accelerating or slowing down.

3. Results

Near the north-eastern side of the Deliblato Sands protected area, the presence of *A. syriaca* was monitored on 67 km of accessory and bordering roads and on over 90 km of selected inner roads (Figure 2). Plants were found at 608 locations on the accessory and bordering roads, while on selected inner roads these plants were observed at 267 locations.

It is clear that, on the north-eastern side of Deliblato Sands, the frequency of common milkweed is significantly higher on accessory and bordering roads (nine locations per km) than on the selected inner roads, i.e., within the protected area (three locations per km) ($p < 0.05$, Table 1 (a)).

On the south-western side of the zone surrounding Deliblato Sands, we observed the occurrence of this species on over 53 km of accessory and bordering roads (Figure 2). On accessory and bordering roads, we recorded 33 sites where common milkweed grew. In the south-western part of the protected area, we monitored an area surrounding 96 km of selected inner roads, where 34 locations with these plants were observed. These data clearly show that the number of locations of *A. syriaca* is significantly lower on the south-western side than on the north-eastern side, both in the surrounding area and within the protected area itself. Additionally, given that the number of plant locations outside and inside the protected area is approximately the same even as the length of the internal roads is almost twice as long, there is a significant difference (0.6 locations per km versus 0.3 locations per km). The frequency of locations of this species was significantly higher in the unprotected, surrounding areas ($p < 0.05$, Table 1 (a)).

During this study, which spanned seven years, we monitored the occurrence of common milkweed in the Deliblato Sands protected area four times. On the first inspection, in 2015, we recorded 267 locations with one or more plants (Figure 3). On the next visit, in 2017, the number of sites observed increased by 148, an increase of 55%. The next observation of the site showed that the number of new locations continued to increase. In 2019, the number of locations increased by 161, an increase of 39%. The next year, 2021, another 151 new locations with *A. syriaca* were recorded. This time the increase compared with the previous monitoring was 26%. In each measuring year, we saw a significant increase in the number of locations (from 55 to 26%), so that, after seven years, the number of locations increased from 267 to 727 (Figure 3). Although the increase in the number of new locations was significant over the entire period, tests showed that the relative increase was not the same in each measuring year ($p < 0.05$, Table 1 (b)). This means that the rate of occurrence of new locations has decreased significantly over time.

Table 1. Chi-square statistics (χ^2) used to test the significance of differences between the frequencies of *Asclepias syriaca* L. locations in Deliblato Sands. (a) Differences between accessory and bordering roads compared with the selected inner roads in the north-eastern and south-western parts, (b) differences between the frequency of occurrence of new locations in the three time periods, (c) differences between the frequencies of locations in the north-eastern and south-western parts in the area adjacent to the protected area and in the peripheral zone (1 km) of the protected area.

	Observed	**Expected**	χ^2	*p*
(**a**)	Accessory and bordering roads vs. inner roads			
NE part				
Accessory and bordering roads	608	358	303.473	0.0001
Selected inner roads	235	485		
SW part				
Accessory and bordering roads	33	23.9	5.425	0.0199
Selected inner roads	34	43.1		
(**b**)	Rate of emergence of new locations			
2015–2017	55%	40	10.55	0.0051
2017–2019	39%	40		
2019–2021	26%	40		
(**c**)	Dispersal pressure north-east vs. south-west, accessory and bordering roads			
North-east	608	356.2	400.681	0.0001
South-west	33	284.8		
1 km peripheral zone				
North-east 1 km	212	136	97.480	0.0001
South-west 1 km	29	105		

On 67 km of accessory and bordering roads near the north-eastern side of the protected area, 608 locations with *A. syriaca* were recorded. On 53 km of accessory and bordering roads near the south-western edge of Deliblato Sands, only 33 localities were recorded. According to our data from the beginning of the survey, *A. syriaca* was drastically more abundant on the north-eastern edge than on the south-western edge of the protected area ($p < 0.05$, Table 1 (c)).

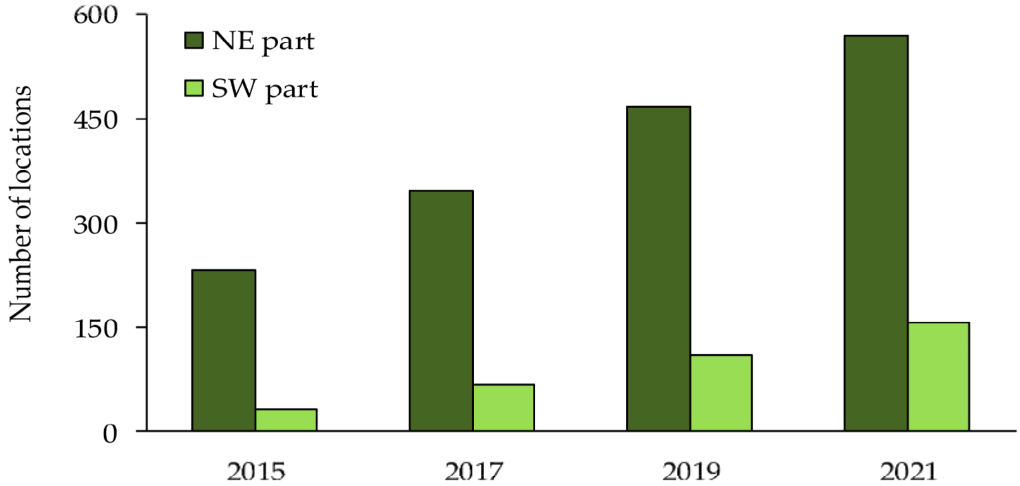

Figure 3. Number of localities of *Aslepias syriaca* L. in the north-eastern and south-western parts of the Deliblato Sands protected area during the seven-year period (2015–2021).

Due to the significantly greater number of *A. syriaca* on the north-eastern side of the unprotected area, we also checked whether there were differences in the density of common milkweed in the protected area bordering the surrounding unprotected zone. This peripheral zone of the protected area extended from the edge to 1 km inside the protected area (Figure 2). In the 1 km wide contact zone in the north-eastern part of the protected zone, 212 locations with this species were recorded along 18 km of roads. In the south-western part of the 1 km contact zone, 29 locations were recorded along 14 km of roads. As expected, the results are similar to the neighbouring unprotected area—a significantly higher number of locations were found in the north-eastern part of the 1 km contact zone of Deliblato Sands ($p < 0.0001$, Table 1 (c)).

In order to verify whether the inner parts of Deliblato Sands are equally occupied by *A. syriaca* as the contact zone between the protected and unprotected areas, two analyses were carried out. The first analysis, in which Deliblato Sands was divided into two zones (the peripheral zone, which extends up to 1 km inland, and the remaining internal zone), confirmed that the number of locations with this species in the peripheral zone of the protected area is significantly higher than expected (Figure 2). The peripheral zone accounts for about 17% of the area, but more than a third of all locations are found there. The number of locations in the internal zone was significantly lower than expected based on the area ($p < 0.0001$, Table 2 (a)).

Table 2. Chi-square statistics (χ^2) used to test the significance of differences between the frequencies of *Asclepias syriaca* L. locations in different parts of Deliblato Sands. (a) Differences between peripheral zone (up to 1 km) and the rest of the inner zone and between the wider peripheral zone (2 km) and the rest of the inner zone and (b) differences between the frequency of occurrence of new locations in the three types of selected inner roads within Deliblato Sands.

	Observed	**Expected**	χ^2	***p***
(**a**)	Peripheral (1 km) vs. remaining internal zone			
The entire protected zone				
Peripheral zone 1 km	241	125.6	128.202	0.0001
Remaining inner zone	486	601.4		

Table 2. *Cont.*

	Observed	Expected	χ^2	p
NE part				
Peripheral zone 1 km	212	114.9	102.775	0.0001
Remaining inner zone	358	455.1		
Peripheral zone 2 km	393	282.2	86.160	0.0001
Remaining inner zone	177	287.8		
SW part				
Peripheral zone 1 km	31	22.6	3.647	0.0562
Remaining inner zone	126	134.4		
Peripheral zone 2 km	67	55.4	3.753	0.0572
Remaining inner zone	90	101.6		
(**b**)	Frequency of occurrence of new locations			
Type of selected inner roads (three roads)				
Public (paved) roads	122	89.8	52.498	0.0001
Recreational roads	8	55.4		
Other inner roads	597	581.8		
Type of selected inner roads (two roads)				
Public (paved) roads	122	96	8.127	0.0044
Other inner roads	597	623		
Recreational roads	8	52.6	41.418	0.0001
Other inner roads	597	552.4		

The second, more detailed analysis was conducted separately for the north-eastern and south-western half of the Deliblato Sands (Figure 2, Table 2). Two comparisons were made for the north-eastern part and two comparisons for the south-western part of the protected area. First, we compared the obtained and expected number of locations between the 1 km wide peripheral zone and the rest of the internal area. Secondly, we tested the significance of the differences between the wider peripheral zone, up to 2 km, and the rest of the inner area (Table 2 (a)). The results obtained are significantly different for the north-eastern and south-western parts of Deliblato Sands. In the north-eastern part of Deliblato Sands, the number of locations was found to be higher than expected in the peripheral zone up to 1 km and also in the wider peripheral zone up to 2 km. In both cases, less than the expected number of locations with *A. syriaca* were found in the inner zone ($p < 0.0001$, Table 2). In the south-western part of Deliblato Sands, no significant differences were found either in the narrower peripheral zone (up to 1 km) or in the wider peripheral zone (up to 2 km) compared with the inner, central part ($p > 0.05$, Table 2 (a)).

In Deliblato Sands, there are three types of selected inner roads where different human activities take place (Figure 2). In this study, we tested whether there are differences in the number of locations of invasive *A. syriaca* between these road types. The chi-square test for differences between public paved, trim and other inner roads confirmed significant differences in the number of locations ($p < 0.0001$, Table 2 (b)). A significantly lower number of locations than expected was detected on trim roads (Figure 4). Only 1% of the plants were found on about 8% of the roads belonging to the recreational trails (Figure 4). When comparing public paved and other inner roads, which do not include the recreational roads, it was found that significantly more than the expected number of locations (17%) were recorded on public roads (Figure 4), which comprise about 13% of all selected inner roads ($p = 0.0044$, Table 2 (b)). When comparing trim trails with other inner roads (excluding public paved roads), a significantly lower than expected number of locations were confirmed on trim trails ($p < 0.0001$, Table 2 (b)).

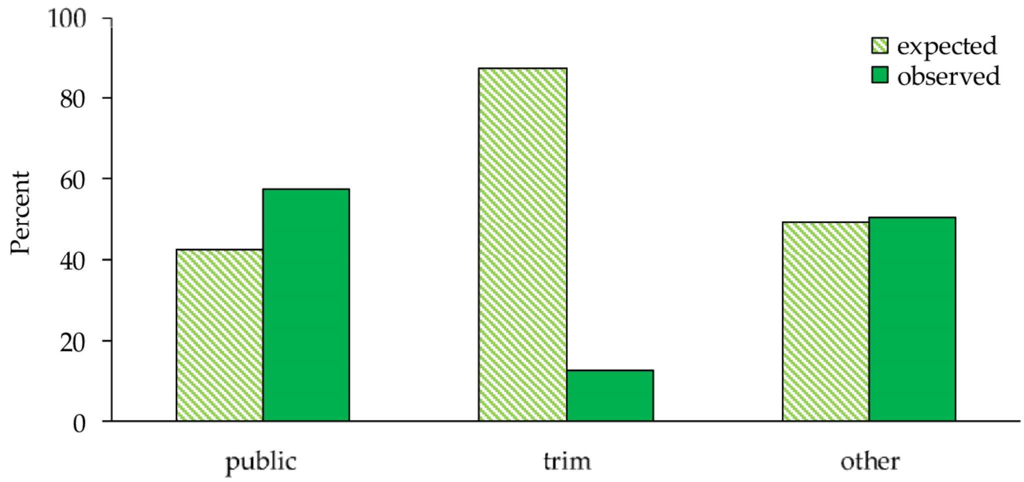

Figure 4. The observed and expected percentage of *Aslepias syriaca* L. localities on each type of road in Deliblato Sands (public roads, trim trails and other internal roads).

It has been observed that new localities do not occur uniformly in Deliblato Sands, i.e., that there are large differences in distance between new and established colonies. Distances between newly established plants and existing colonies ranged from a few metres to over 3 km. Distances of up to 100 m were the most common, and distances of over 1000 m accounted for the smallest proportion (Figure 5a). Over the course of seven years, this trend became more and more pronounced. With each subsequent measurement, the frequency of distances up to 100 m increased, while the frequency of distances of 100–500 m, 500–1000 m and distances of more than 1000 m decreased. Distances between new and existing locations were also analysed separately for the north-eastern and south-western halves of Deliblato Sands. In the north-eastern part, the data show a similar trend to the analysis carried out for the whole protected area, with the frequency of distances up to 100 m being highest throughout the period and increasing year to year over the measured period (Figure 5b). In the south-western part, the proportion of distances between locations of up to 100 m also increased (Figure 5c). The difference was that, in the south-western part between 2015 and 2017, the largest proportions had distances between 100–500 m (Figure 5c). In addition, the frequency of distances over 1000 m was significantly higher in the south-western part (almost 20%) than in the north-eastern part (less than 5%).

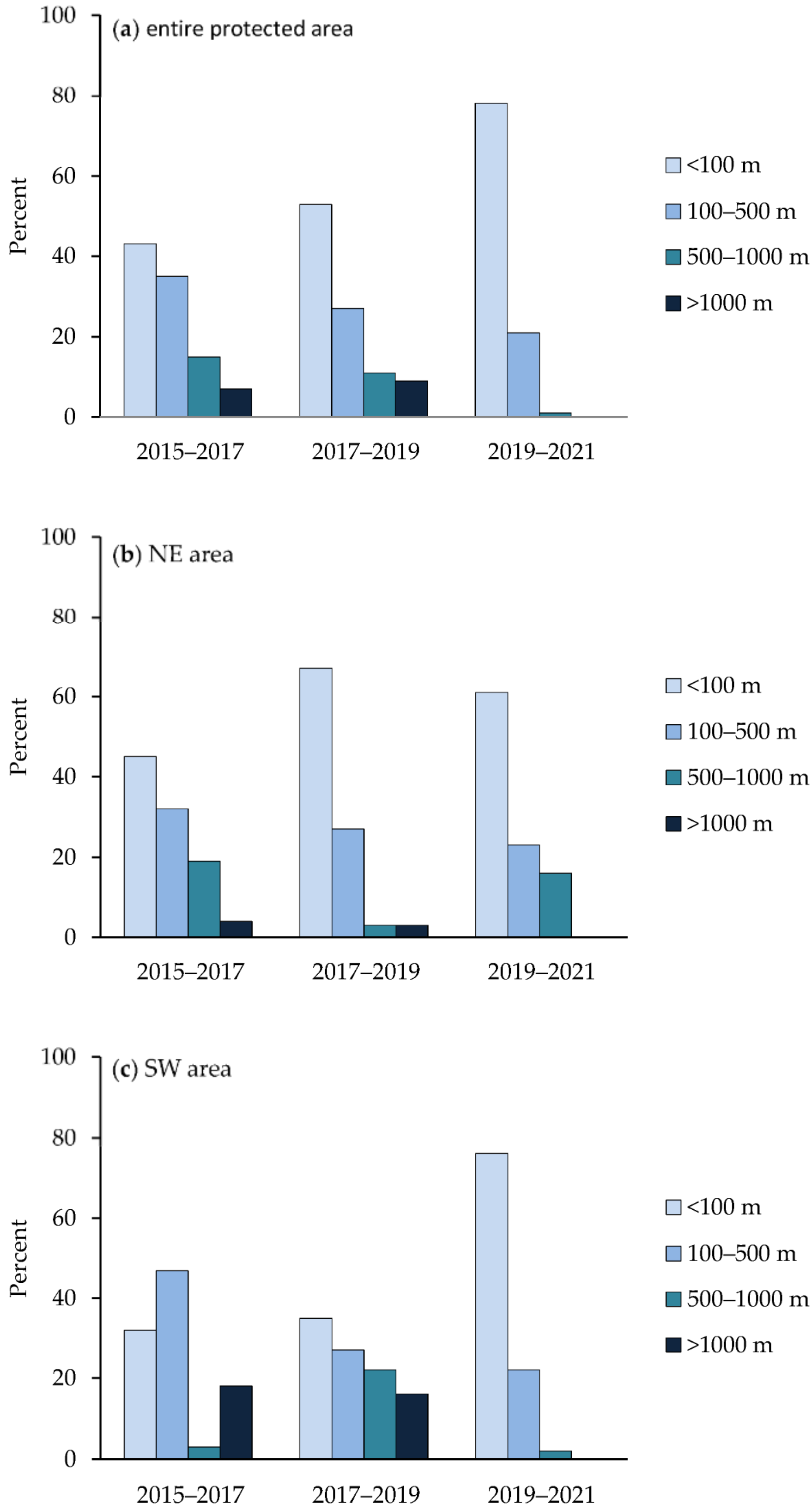

Figure 5. Distances between new locations of *Asclepias syriaca* L. from the nearest existing locations in Deliblato Sands from 2015 to 2021. The four categories of distance are indicated by the differently coloured columns.

4. Discussion

As Deliblato Sands contains a considerable number of sensitive ecosystems (sand, steppe, forest and marsh vegetation), it is highly endangered by the spread of invasive species. For some of these species, such as *A. syriaca* (with wide distribution, dense stands and large amounts of seeds), it is very difficult to implement an efficient and reliable control programme [59,60]. For these reasons, it makes more sense to control such invasive non-native species at the very beginning of the invasion process than to try to eradicate them later when they are abundant and widespread [61]. This early detection should be based on a system of regular surveys to detect newly established populations. Simberloff [62] has stated that there are reasons to believe that many invasive plant populations could be eradicated, especially if eradication is coupled with a monitoring system that detects invasions at an early stage. In addition to early detection, monitoring of existing populations of invasive non-native species is also part of an effective invasion control strategy [63–66]. This early data can help to understand how these invasive plants are affected by land cover, disturbance regimes and local climatic conditions [67], which could be crucial to ensure a timely response and create efficient prevention plans.

The results of this study clearly show that the invasive species *A. syriaca* is strongly represented on the arable land around the Deliblato Sands nature reserve. More than 640 locations on accessory and bordering roads indicate that there is a high dispersal pressure for the spread of this species into the interior of the protected area. The dispersal pressure for this species was significantly higher in the north-east than in the south-west of the unprotected surroundings. The reason for these large differences is most likely the fact that there are large agricultural areas in the north-east that are not regularly farmed. As there is no natural vegetation on these areas, the spread of the common milkweed is strongly favoured. Similar results have been published by Paukova et al. [68]. They found that *A. syriaca* spreads into natural habitats from abandoned vineyards as well as from uncultivated areas where monocultures such as sunflowers were grown. Vila and Ibanez [67] also cite several confirmatory studies in their meta-analysis, showing that landscape dynamics (changes in land use over time) significantly influence biological invasions, especially after the abandonment of agricultural land. Of particular concern is that *A. syriaca* can also spread to natural, anthropogenically undisturbed habitats. Bakacsy [31] has shown that the common milkweed can also spread in natural vegetation where it causes degradation of the recipient community, and its spread is mainly favoured by disturbance of natural vegetation.

A. syriaca has long been present in the region of Pannonian Serbia. According to Obradović [69], its presence was first recognised by Kovács [70] in the vegetable crops of Novi Bečej. At the beginning of the 2000s, several authors recognised its invasive potential [23,71–75]. Considering the size and number of stands of this species, especially in the north-east, we can assume that *A. syriaca* has been present in the vicinity of the Deliblato Sands protected area for a long time and is well adapted. From 2015 to 2021, the occurrence of this species has increased significantly. Despite the large number of locations at the beginning of this period, the number has increased more than two and a half times after just seven years. This means that the invasion of *A. syriaca* is still ongoing. At the same time, the increase in the number of new locations has slowed down, so we can assume that the spread is continuing, but at a slower pace. The reason for this could be that, at least in the north-eastern part, this species has already taken over most of the habitats favourable to its growth. This is not the case in the SW part of Deliblato Sands, where there are significantly fewer *A. syriaca* locations (seven times fewer in the peripheral zone than in the NE part). This suggests that effective control and eradication is possible, at least in this part of the protected area.

If we compare the peripheral zones with the central part of the protected area, it becomes clear that there are considerable differences between the north-eastern and south-western parts of Deliblato Sands. In the north-eastern part, the number *A. syriaca* is significantly lower in the areas further away from the periphery, i.e., closer to the central part. In the SW part of Deliblato Sands, there is no significant difference between the areas closer or further away from the centre of the protected area. This difference between the NE and SW parts suggests that the appearance of new sites in the SW part is related to the migration of seeds from the NE part and not to an increase in pressure from the unprotected area near Deliblato Sands.

Roads differ from other types of disturbance, they provide a linear structure, serve as corridors [45,76] or facilitate access for non-native invasive species. Different types of roads vary in their degree of environmental disturbance. Paved roads have a greater impact on neighbouring ecosystems. In Deliblato Sands, there are three types of roads that are intended for different purposes. The highest density of *A. syriaca* locations was observed near public paved roads. These are roads that are used for motorised traffic. Similar results were obtained by Joly et al. [36] for *Ambrosia artemisiifolia* L., which occurs more frequently on asphalted roads than on unpaved roads. There may be several reasons for the large number of *A. syriaca* along public roads in Deliblato Sands, but the most important could be the large number of passing vehicles, which are able to transport large numbers of seeds to potentially suitable habitats. Similar results, namely a significant influence of traffic volume, have been obtained by Lemke et al. with regard to *A. artemisiifolia* [38]. The edges of paved roads are subject to frequent disturbance from vehicles and track maintenance, as well as from roadside pruning. This could also favour the spread of *A. syriaca*, as it may be less sensitive to pruning than native species [26,77]. Our results also show a significantly lower number of locations on unpaved roads. Unpaved roads may cause less disturbance in neighbouring ecosystems [78,79]. In contrast, the results of Follac et al. [80] confirm that the probability of detecting *A. syriaca* in some parts of Austria was significantly higher along local unpaved roads than along the various paved roads. In contrast with our results, Taylor et al. [81] have found that unpaved roads provide greater opportunities for the spread of the seeds of invasive plants because they require more frequent maintenance, which requires shifting of pavement and substrate. In their study of *A. syriaca* in Hungary, Szilasi et al. [82] concluded that roads and railway lines are not factors that contribute significantly to the spread of this invasive species. Differences in the results regarding the colonisation of unpaved and paved roads may be due to different ecophysiologies and evolutionary strategies of the different invasive species, but also to differences in the way roads are managed, in the adjacent vegetation cover, in the edaphic features of the terrain, etc. Roads used for recreational purposes had the lowest number of *A. syriaca*. Although these recreational roads are frequently used from spring to autumn, this does not seem to have a positive influence on the spread of this species. Anderson et al. came to different conclusions in their meta-analysis [83]. They show that the abundance and richness of non-native species could be significantly higher in places with intensive tourist activities. The difference between the results is probably due to the fact that tourism activities in this meta-analysis include numerous activities that do not occur in the Deliblato Sands protected area, such as the use of various vehicles or the construction of related infrastructure (resorts, hotels, car parks, etc.). Tourism activities in this analysis include tourism in terrestrial, freshwater and marine environments and not all studies differentiated between invasive and non-invasive non-native species. Furthermore, the meta-analysis considered different species with different evolutionary strategies in terms of dispersal ability, germination, allelopathy, phenotype plasticity, etc. Based on our research results, we can state that paved roads used for public transport are the main gateways for the further spread of *A. syriaca*.

It is clear that the frequency of various human activities is one of the most important factors determining the rate and direction of spread of this invasive non-native species in Deliblato Sands. It appears that short-distance dispersal has become the dominant mechanism for the range expansion of *A. syriaca*. The results show that the largest number of new common milkweed plants was observed in close proximity (often only a few tens of metres) to already established stands. This habitat expansion can be caused both by the formation of new plants by germinating seeds and by the spread of rhizomes of existing plants. A small but potentially important proportion of new locations appear to have been created by the transport of seeds by wind or vehicles over long distances (in some cases more than 3 km). Similar to our results, Rauschert et al. [47] found that dispersal over intermediate and local distances is much more common in *Microstegium vimineum* (Trin.) A.Camus, but they suggest that even infrequent long-distance dispersal can have a significant impact on dispersal rates. The proportion of new locations of *A. syriaca* that emerged more than 1 km from the nearest existing location was higher in the south-eastern part of Deliblato Sands during the first two measurement periods. Because, in this part, there is no significant pressure of *A. syriaca* from the area around Deliblato Sands into the protected area itself, we can assume that part of these remote locations in the south-western part could originate from plant seeds that themselves originate from the north-eastern part of Deliblato Sands. As this long-distance dispersal can be influenced by other factors and can accelerate dispersal rates [47], it must be taken into account and included in the models that describe the spread of invasive and potentially invasive species. The application of advanced methods in research could provide additional information on the mode of spread of *A. syriaca* [84]. For example, remote sensing using drones with a multispectral camera could facilitate the observation of parts of the Deliblato Sands protected area that are very difficult to access due to the density of autochthonous vegetation.

As environmental heterogeneity is of great importance for biodiversity [85], future research should consider the interaction between the biological traits of *A. syriaca* and the spatial and temporal variation in environmental complexity, in order to determine the potential for the spread of common milkweed in the Deliblato Sands protected area [86]. In some studies, higher landscape heterogeneity is positively correlated with resistance to invasion [87], while other studies have shown that environments with greater spatial and temporal variation are more susceptible to plant invasions [88].

Deliblato Sands is a protected area that, like most protected areas in Europe, is largely surrounded by agricultural land. This study shows that, given the nature of the land use, the greatest pressure for the spread of *A. syriaca* in the protected area comes from abandoned agricultural land. The first step to prevent the spread of this invasive species should be to reduce its presence on land adjacent to the protected area. For this purpose, the use of herbicides could be an effective method. Because a single treatment with herbicides is not sufficient to eradicate *A. syriaca* [89,90], the chemical treatment should be repeated if the land is not used for agricultural crops the following year. In protected areas, it is quite complicated to apply different chemical protection measures, so this measure is not recommended. In this case, an important contribution to slowing the spread of *A. syriaca* could be the reduction of damage to natural habitats, which would reduce the number of favourable sites for the establishment of new populations. The full recovery of native plant communities, despite some alelopathic effects of *A. syriaca* [91,92], could limit the spread and potentially reduce the area occupied by this invasive species. As the removal or reduction of common milkweed may not be sufficient to restore native communities and ecosystems, active restoration should also be undertaken [93].

Furthermore, given that there is evidence of high invasion pressure of *A. syriaca* from the surrounding unprotected area in Deliblato Sands, especially from the north-east side, it seems that the buffer zone of undisturbed vegetation surrounding the entire park could slow down the spread of non-native plant species. The same measure to protect the Kruger National Park is proposed by Foxcroft et al. [94]. In this buffer zone, it is possible to significantly reduce the anthropogenic factors contributing to the invasion and at the same time use appropriate chemical measures that cannot be applied in the protected area.

This study on dispersal and range expansion can serve as a basis for the further analysis of the ecological dynamics of *A. syriaca* populations and localities in the Deliblato Sands natural reserve. The significant spread of *A. syriaca* in the unprotected, but especially in the protected area, of Deliblato Sands shows how strong the negative effects that invasive species can have in this part of the Pannonian Plain is. These results could also be important because Deliblato Sands is located in the transition region between central and southern Europe, where many species of southern origin are currently in the process of adapting and spreading to northern areas. Therefore, these data can help to develop timely strategies by which to prevent or slow down the spread of these invasive species in the currently less threatened parts of the European continent.

5. Conclusions

Natural ecosystems are overwhelmed by non-native invasive species introduced by human activities outside their natural range. The introduction of non-native invasive species into protected areas, whose main objective should be the conservation of native biodiversity, is particularly problematic. The Deliblato Sands Special Nature Reserve is one of the most important centres of biodiversity in Serbia and Europe. It is one of the largest continental sand areas in Europe and one of the few large oases of the sand, steppe, forest and marsh vegetation that once dominated the Pannonian Plain. As Deliblato Sands is under great anthropogenic influence, it deserves great attention for biodiversity conservation studies.

Over the last ten years, the invasive non-native species *Asclepias syriaca* L., the common milkweed, has been observed spreading in the Balkan region, including Deliblato Sands and most of the surrounding areas. Numerous studies have already analysed the impact of common milkweed invasion on the natural vegetation, but they often lead to contradictory conclusions. Considering all of the negative aspects of the uncontrolled spread of *A. syriaca* in ruderal, agricultural and protected areas, the aim of the work was to observe the spread over a longer period of time and to investigate the factors contributing to its spread.

The results of this study show that *A. syriaca* is much more abundant in the unprotected surroundings of Deliblato Sands. There is more common milkweed on the accessory roads and bordering roads than in the inner parts of the protected area. We concluded that the spread of *A. syriaca* in the Deliblato Sands protected area is largely due to anthropogenic factors from the surrounding unprotected areas. Although we have demonstrated the importance of anthropogenic factors in this study, it is important to determine whether and to what extent certain abiotic factors, primarily changes in local climatic factors, contribute to the spread of common milkweed.

The number of newly established localities with *A. syriaca* has increased more than two and a half times in just seven years. This rapid expansion of common milkweed indicates that the colonisation is not yet complete. If appropriate protective measures are not taken, *A. syriaca* is likely to have a much greater impact on the natural functioning of this ecosystem. The further rate of spread of this species could largely depend on some indirect biotic and abiotic influences on the natural vegetation, as it has been confirmed that this species spreads more intensively when the natural vegetation is disturbed. Considering that biodiversity is significantly influenced by environmental heterogeneity, it is important to determine whether landscape heterogeneity in Deliblato Sands has a positive or negative effect on the dispersal of *A. syriaca*.

The strong invasive potential of *A. syriaca* also results from its ability to expand its habitat both through the formation of new plants by germinating seeds and through the dispersal of rhizomes of existing plants. Our results show that dispersal over medium and short distances is much more common for *A. syriaca* in Deliblato Sands. The spread, i.e., territory extension, over longer distances can be regulated in other ways. To assess the future spread of common milkweed, this small but potentially important proportion of new locations created by wind or vehicle transport over long distances should also be considered.

The study confirmed that there were significant differences in the distribution of *A. siriaca* on the three types of roads passing through Deliblato Sands. *A. syriaca* was found most frequently on paved roads and least frequently on recreational trails. These differences are most probably due to the more frequent vehicle use on paved roads as well as the greater environmental awareness of people using Deliblato Sands for tourist activities. The results of other authors are controversial when it comes to the impact of different road types on the spread of invasive species. To confirm our assumptions and determine the reasons for the differences, it would be interesting to investigate in more detail how the spread of *A. syriaca* and other invasive species is affected by some other factors. For example, the way the roads are maintained, the degree of destruction of the native plant communities surrounding the roads, and the changes in local climatic conditions caused by the roads.

One of the important anthropogenic factors influencing the range and rate of the spread of *A. syriaca* is the type and dynamics of agricultural utilisation in the vicinity of the protected area. Agricultural areas that are not regularly cultivated and that are mostly located on the north-east side of Deliblato Sands represent a greater pressure for the spread of invasive species. As there is no natural vegetation on these uncultivated areas, the spread of common milkweed is strongly favoured. Given the importance of the area in the immediate vicinity to the spread of *A. syriaca* in the Deliblato Sands, an important contribution to its protection could be the establishment of a buffer zone around the protected area. Some of the conservation measures could be applied in this buffer zone and negative anthropogenic factors reduced. In this way, the occurrence of invasive species at the borders of the protected area would be much less frequent.

There is evidence that the invasion of *A. syriaca* could jeopardise protected areas in the Pannonian Plain, especially protected areas in whose immediate vicinity are territories already affected by anthropogenic impacts or natural disturbances. As there is not enough information on effective control methods against *A. syriaca*, probably one of the most dangerous invasive herbaceous species, it is important to obtain more results in the future. Combining tested and new technologies can help to develop timely strategies by which to prevent or slow down the spread of this species.

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