Ph.D. Thesis

# Examination of the invasion of the common milkweed (*Asclepias syriaca* L.) and the possibility of control against it in open sand grasslands

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# INTRODUCTION

Invasive species are introduced from other geographical regions, by entering new habitats they can become predominant over natural flora and fauna and cause damages to the environment. Biological invasions are one of the most serious threats to biodiversity today, but they also cause economic and human health problems worldwide too. Despite the caused damages, the number of invasive species does not appear to be declining or even leveling off, moreover their numbers are constantly increasing. Thus, invasion biology is one of the most intensively researched subjects in ecology today. There are four main directions in plant invasions research: The first is the search for traits or combinations of traits that may be responsible for the successful invasion of these plant species. It is difficult to draw general conclusions from these, but one such common trait is the ability to grow vegetatively or clonally. It is based on the fact that clonally growing species are capable of long-term and large area dominance thus somewhat hindering the succession of vegetation. The second line of research is the study of the community invasibility, while the third is the study of invasion impacts on community. The consequence of the invasion is basically determined by the interaction of the invasive species and the attacked vegetation. A successful invasion requires two things: Susceptible community to invasion and invasive species with appropriate trait. In many cases, it has been demonstrated that the more disturbed a community is, the more invasible it is. The fourth direction of plant invasion research is protection against them, the importance of which is no longer limited to protected areas. Knowledge of the invasive species biology, their effects and their responses to managements plays an essential role in their effective control and thus in the conservation of biodiversity. Nevertheless, we have little longer-term knowledge to answer the behavior of an invasive species before, during, and after treatment and thus the effectiveness of treatment. In many cases, these research directions of invasive plants are treated independently of each other, however, their holistic approach requires, which is increasingly gaining a role in recent research.

Common milkweed (*Asclepias syriaca* L.) is one of the most aggressive invasive plant species on the open sand grasslands between the Danube and the Tisza, however this habitat is a very valuable from

a nature conservation point of view. Its establishment and spread is usually associated with anthropogenic disturbance, while it cannot be said to be successful in natural vegetations. It poses a nature conservation risk as it can inhibit the regeneration of the degraded association, it is able to maintain its disturbed state for a long time. The formation of dense, monodominant stands is promoted by its strong clonal propagation and its shading and allelopathic effects. One of the most effective methods of conservation management is chemical eradication, even in protected areas. In the present dissertation, on the one hand, I wanted to clarify the interactions between milkweed and open sand grassland, whereas the results of previous studies are rather contradictory. On the other hand, I want to contribute to a more effective control against the species with the experience and conclusions of a long-term study of one-time nature conservation (herbicide) treatment.

## AIMS

The aim of the present dissertation can be divided into two parts: on the one hand, we examined the interactions between the common milkweed and the vegetation, and on the other hand, the success of the control method against the species and the survival and regeneration ability of the species. It follows from the aims two methods were used to carry out examinations, which concerns both the documentation and data processing.

The first aim is to clarify the role of milkweed as an alien species in the organization of open sand grasslands.

- What interactions does it have with the invaded sand vegetation? Does this have an impact on the frequency of sand grassland species?
- Can the effect of species invasion be demonstrated in relation to life forms?
- Is there a significant difference in the beta diversity of the invaded vegetations compared to the original sand vegetation types (impoverishment, species pool change)?
- In which vegetations is the milkweed invasion more common?

The second aim is to know about the survival and regeneration ability of milkweed after a single herbicide treatment. To determine this, a milkweed stand was surveyed and analyzed over a seven-year period (2011-2017). The study of entire stand includes the before, during and after a single herbicide treatment period.

- How did the vegetative (shoot number, shoot cluster number) and reproductive characteristics (pod number, pod-bearing shoot number) of the stand change due to the single herbicide treatment?
- How did the treatment affect the structure of the stand (spatial extent, density)?
- Which strategies were used by the species for survival and regeneration, in the latter case, to regain its original area?
- How did the single herbicide treatment affect the further spread of the stand?

## MATERIALS AND METHODS

*Study site*

The study site was the core area of the Fülöpháza UNESCO biosphere reserve in the Kiskunság National Park for the examination of the *Asclepias syriaca* invasion by microcoenological methods, as well as for the study of the herbicide-treated stand.

Documentation methods

*JNP information satistic documentation method*

For the comparative microcoenological study, I examined five invasion-free (as a controls) and five invaded stands in the May-June 2015. Each invaded stand was selected to be an invasion-free stand in the vicinity, allowing five standpairs to be formed. Thus, in the case of each standpair, a similar abiotic state and vegetation history can be assumed, and each stand can be examined and interpreted against its own pair. The selection of individual stands was based on the following considerations: They should be of a suitable size, so that place a 52 m long, self-closing line (circular transect) can be laid on them. Their vegetation should be homogeneous and free of any larger

visible disturbances. Every 52 m long circular transect consists of 1040 units of 5 cm x 5 cm contiguous microquadrats. I recorded the presence of plant species in these microquadrats. The relative total frequency and relative frequency of three main life-forms (perennials, annuals and cryptogams) also have been determined in the case of each transect.

*Herbicide treatment documentation method*

Herbicide treatment of milkweed in the protected area was carried out by the Kiskunság National Park Directorate within the framework of a KEOP tender. Manual lubrication was used for the study stand, because it is located in natural vegetation and thus minimizing damage of vegetation. The used herbicide was Medallon (the active substance was glyphosate) within a 50% aqueous solution (2 l $ha^{-1}$). The stand was treated with herbicide only once during the study period (7 years), in May 2014, i.e. the treatment was not repeated at all. The examination extended to the entire stand, so it was covered with several contiguous 2 m x 2 m quadrats. The exact position of the shoots, the number of solitary shoots, shoot clusters (using a maximum distance of 15 cm for this), and the number of pod-bearing shoots were recorded in the quadrats. The pattern of the stand was depicted based on the documentation of the shoot locations (5 cm accuracy). Its heat map (Kernel density) can be used to determine the distribution, spatial and temporal changes of the stand shoot density. This sampling procedure was repeated during the study duration (from 2011 to 2017) in every July.

Data processing methods

*JNP information statistic data processing method*

I used two JNP information statistical functions to analyze the data: the compositional (species combination) diversity (CD) and the number of realized species combinations (NRC). Both the maximum values and tha characteristic area (CA) of the two functions were used for further analyzes. The maximum values and characteristic areas of the two information statistics functions provide information of us on beta diversity. I used INFOTHEM 3.01 software to performing spatial

series steps, making random references and calculating JNP-functions. For these, I applied the following settings: species below 2% were excluded from data processing. I used complete randomization as random references (neutral models). Monte-Carlo method, 5000 randomizations and $\alpha = 0.01$ significance level were applied in each transect. During the modeling of the invaded stands, I performed these calculations with and without milkweed. I examined whether the invasive species have any interaction with the three main Raunkiaer life forms (perennials, annuals, cryptogams) and their combinations (annuals-perennials, annuals-cryptogams and perennials-cryptogams). To determine this, I also used JNP-functions in separate tests with the same settings as described previously in the basic analysis. I used two-way ANOVA to compare the JNP-functions values of the stands and their characteristic areas. GraphPad Prism software version 8.0.1.244 (GraphPad Software, La Jolla, California, USA) was used to create the significance tests and diagrams.

*Herbicide treatment data processing method*

Simple data processing and descriptive statistics were used to evaluate the effectiveness of herbicide treatment and the shoot characteristics of the stand. Deeper statistical analysis (e.g. one-way ANOVA) should not be used for pre- and post-treatment comparisons, due to a statistical error, the so-called pseudoreplication. GraphPad Prism software version 8.0.1.244 (GraphPad Software, La Jolla, California, USA) was used for descriptive statistics and charts. I used QGIS software version 2.18.24 to draw the shoot location schemes, and to analyze the Kernel density.

## SUMMARY

One of the most dangerous herbaceous invasive plant species of the Pannonian open sand grasslands is common milkweed. Numerous studies have already addressed the impact of the milkweed invasion on natural vegetation, but from these often contradictory conclusions can be drawn. Furthermore, we have relatively little information on effective control methods against the species.

Therefore, one of the aims of my research was to clarify the interactions between common milkweed and the plant community of the open sand grasslands. The other aim was to study what survival and regeneration capacity the invasive plant has against a single herbicide treatment at stand level.

The present dissertation provides the following new results in related to the study of the effect of milkweed on open sand grasslands:

- There was not show any significant effect on the frequency of sandy grassland species between the invaded and non-invaded communities.

- Based on results of the studies, which were performed on fine spatial scales, using microcoenological methods, it can be cloncluded that the invasive species is negatively related to the invaded sandy vegetations. There is a reduction in the beta diversity of invaded vegetations. Among the Juhász-Nagy Pál's information theory functions (hereafter JNP functions), the species compositional diversity (CD) shows that the organization of the invaded vegetations by common milkweed lag behind from the invaded-free vegetations ones. Under the invasive species, the characteristic species of sandy grasslands and their combinations are the same as invasion-free vegetations, but they are located in a significantly larger space. While the number of species combinations (NRC) the other JNP functions there was not any effect between invaded-free and invaded stands.

- There are biotic interactions between species in stands, whether they are invaded or not, which indicate that the randomization tests of JNP functions are significantly different from the field value of JNP functions.

- Examining the effect of invasion with the JNP-function on the three main Raunkiaer life forms (annuals, perennials and cryptogams) and their combinations (annuals-perennials, annuals-cryptogams and perennials-cryptogams), only the annual-cryptogams combination was (negatively) affected by the invasion in characteristic area of the CD function.

- It has been proven that the invasion of milkweed endangers the Pannonic open sand grasslands, especially those communities are exposed to its invasion which have already been degraded by an anthropogenic or natural disturbances (e.g. drought).

The following new results were gained by the examination of the single herbicide (glyphosate) treatment of milkweed at stand-level:

- As a result of the single herbicide treatment, the vegetative and reproductive characteristics of the studied stand have undergone significant changes. Shoot death in the year of treatment and the reduced number of shoots after the treatment year also occurred as a result of the single chemical treatment. In the latter case, glyphosate may have been translocated through the rhizomatic roots of the milkweed to the dormans root buds, thus causing their destruction. The pods completely disappeared in the year of treatment and the following year, however, the increasing shoot number and the re-appearance of the pods show that a single herbicide treatment is only a temporary and non-permanent solution to the management of invasive clonal plants.

- As a result of the single treatment, the examined stand disintegrated and became fragmented, these fragments initially showed a rarer so-called guerrilla growth, and later as they become stronger, they used the denser phalanx type. The species is thus able to use two growth forms: a sparse shoot structure (guerrilla) to maintain previously occupied areas, while a denser shoot structure (phalanx) is used to colonize new areas.

- The complete disappearance of the pods in the treatment year and the next year after treatment suggests that the species reallocates resources to vegetative growth in the case of significant damages. The survival and regeneration ability of milkweed is basically ensured by the dormant bud bank developing on rhizomatic roots, which play a role in vegetative growth. Regarding the growth forms of milkweed, it can be stated that it mainly uses the rarer, guerrilla growth

form for survival, while in regeneration processes, the species can use both growth forms to regain original areas.

- As a result of a single herbicide treatment, the remaining fragments of the previous stand may function as starting points for the re-establishment of a new stand. Therefore, a single herbicide treatment of larger populations of the species is not sufficient.

- Overall, it can be concluded that a single herbicide treatment is not suitable for eradicating clonal plant species, it is advisable to repeat the threatment within two years in order to significantly weaken them, or at least limit their seed production. In addition, the remaining fragments can be weakened, thus preventing their further vegetative spread by the repeated herbicide treatment.


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LIST OF PUBLICATIONS

László Bakacsy MTMT ID: 10055585
https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10055585

**The 2 mandatory publications for the doctoral procedure:**

Bakacsy, L. (2019). Invasion impact is conditioned by initial vegetation states. *Community Ecology*, 20(1), 11-19. https://doi.org/10.1556/168.2019.20.1.2
IF: 0,851

Bakacsy, L., & Bagi, I. (2020). Survival and regeneration ability of clonal common milkweed (*Asclepias syriaca* L.) after a single herbicide treatment in natural open sand grasslands. *Scientific reports*, 10(1), 14222. https://doi.org/10.1038/s41598-020-71202-8
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Bús, C., Kúsz, N., Kincses, A., Szemerédi, N., Spengler, G., Bakacsy, L., ... & Vasas, A. (2020). Antiproliferative Phenanthrenes from *Juncus tenuis*: Isolation and Diversity-Oriented Semisynthetic Modification. *Molecules*, 25(24), 5983.
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