

Survival and regeneration ability of clonal common milkweed (*Asclepias syriaca* L.) after a single herbicide treatment in natural open sand grasslands

László Bakacsy (✉ bakacsy@bio.u-szeged.hu)

Szegedi Tudományegyetem Természettudományi és Informatikai Kar <https://orcid.org/0000-0003-2593-1795>

István Bagi

Szegedi Tudományegyetem Természettudományi és Informatikai Kar

Research

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Abstract

Background: Invasive species are a major threat to biodiversity, human health, and economies worldwide. The cost of the damages and the fight against them exceeds 9.6–12.7 billion euros annually for European Union. The Pannonic open sand grasslands represent important endemic habitats in European Union and are threatened by the spread of several invasive plant species. Among them, the common milkweed *Asclepias syriaca* L. has already transformed large areas of natural vegetation and endangered the others. The need for management of alien plants is urgent in both agricultural and protected areas. Herbicide treatment may be a cost-effective method for controlling the extended stand of milkweed even in protected areas. Therefore, this study monitored the herbicide treatment effects on *A. syriaca* before, during, and after treatment in a strictly protected UNESCO biosphere reserve near Fülöpháza from 2011 to 2017. The entire stand was treated with glyphosate in May 2014. We used simple data processing methods to follow the fate of individual shoots.

Results: The 7-year data showed that treatment was successful for a short term (the year of treatment and the following year). The number of *A. syriaca* shoots in the stand decreased following herbicide treatment, with 73% of the shoots dying. In the first year after treatment, the number of shoots decreased continuously as herbicides were translocated by rhizomatic roots, thereby damaging dormant bud banks.

Conclusions: The surviving buds adjusted to the number of emerging shoots in the years after treatment, and growth of the milkweed stand appeared to show a slow regeneration for a longer-term period. We concluded that the successful control of *A. syriaca* after herbicide treatment depends on continuous management (e.g., further point spraying) of treated areas to suppress possible regrowth during subsequent seasons. Therefore, periodic control is highly recommended because one-time treatment is insufficient.

Background

Currently, invasive species are a major threat to biodiversity, human health, and economies [1–4]. It has been estimated that the fight against invasive species and the damage caused by them in European Union accounts for a minimum of 9.6–12.7 billion euros annually, and this amount is expected to rise to 20 billion euros annually [1, 5–7]. The most important elements of protection against invasive species are prevention of introduction and early detection. In the case of established invasive species, the most successful options are eradication or isolation [8–13]. Herbicide treatment is one of the most effective ways to control or eradicate invasive plants in large areas [10, 14–19]. Nevertheless, the prevention and control measures of invasive species with chemicals are rather arguable whereas their use endangers other species and the ecology and abiotic elements (above and below ground waters, soil and air) in protected areas [18, 20, 21]. Consequently, herbicides have to be carefully chosen (dosage, types and combination) based on the native species community [16, 19, 20]. Therefore, the herbicide application must be well planned and localized, the applied chemicals should be safe and effective. However, the use of these products in non-agricultural areas are very rarely accessible [cf. 10, 22, 23]. This knowledge gap also requires not only extensive research but also effective exchange of information and experience [cf. 10, 18, 19].

Clonality is common among invasive plants [24, 25, 26]. The common reed *Phragmites australis* [27], alligator weed *Alternanthera philoxeroides* [28, 29], Japanese knotweed *Fallopia japonica* [30–33], Solidago species [14, 34], and Canada thistle *Cirsium arvense* [35, 36] are examples of problematic invasive clonal species. Their success is partly due to translocation of water, nutrients, and photoassimilates among physically interconnected shoots [37–42]. However, pathogens can also be transported through the same clonal network [36, 43–45] as can heavy metals [46, 47] and herbicides [17, 48–51]. Bud banks on a clonal network play an important role in competition, vegetative multiplication, and resprouting [52, 53]. An extensive dormant bud bank can be activated, resprouted, and made able to colonize an empty niche or re-establish monospecific stands after disturbance. Subsequently, the succession of natural vegetation is impeded or completely obstructed. The mortality risk of clonal plants is low because death only occurs when both shoots

and bud banks are simultaneously destroyed [38, 54–60]. This possibly explains why management of clonal spreading species is difficult even with herbicide treatment [61], and knowledge of invasive plant biology is essential for effective management [8]. While most studies involved a single year of monitoring, examination of herbicide treatment for several years before, during, and after treatment can provide useful information that will help guide management programs [18, 62–65].

The common milkweed *Asclepias syriaca* originated in North America but is reportedly established in Continental, Mediterranean, and Pannonian Europe [66]. It is a perennial clonal plant [66–69], and despite the fact that its shoots die back every autumn, it can resprout in the same place for extended periods [68]. The clonal structure of *A. syriaca* comprises solitary or few (2–5) groups of shoots that develop vegetatively by buds of plagiotropic rhizomatic roots [68]. Milkweed is one of the most dangerous invasive transformer species currently widespread in Hungary and is spreading in Czech Republic, Romania, Poland, Serbia, and several other countries [66, 68, 70–72]. It primarily endangers psammophilous habitats where its structure differs from that of natural vegetation [73]. The problems arising from the invasion of milkweed were primarily attributed to the assumption that it can inhibit the regeneration of natural vegetation [20, 68, 73, 74]. Despite the harmful effects of *A. syriaca*, it was only recently added to the list of Invasive Alien Species of Union Concern [75]. To adequately control common milkweed, the bud banks of its roots and lateral roots must be eliminated. Control or eradication is an increasingly important action from both agricultural and conservation perspectives [20, 68, 76–78]. Complicating matters is the fact that extermination itself can create suitable conditions for colonization (e.g., soil disturbance), and large areas can become permanently milkweed-free only with coordinated efforts and at enormous costs [68]. Nevertheless, herbicide treatment may be a cost-effective method to control extended stands of milkweed in strictly protected areas [10, 20, 66, 68]. The most frequently used herbicides for *A. syriaca* management are glyphosate and triclopyr, whereas fluroxypyr or dicamba are rarely used. These are often used individually or in combination with each other or with some level of mechanical control [10, 20, 76, 77, 79]. Relatively little information is available on the long-term or semilong-term effects of postemergent herbicides on *A. syriaca*. Here we report one of the first and longest monitoring period of one-time herbicide treatment on a common milkweed stand and analyzed the before, after and during treatment effects. We hypothesized that clonality is an important factor for resistance to herbicide treatment and proposed the following three questions: a) How does a single herbicide treatment influence the further spread and pod production of the invasive plant? How much the single treatment reduces the shoot, pod and pod-bearing shoot number and how long-lasting these effects are? We examined the shoot, pod and pod-bearing shoot number changes in long term (and not only in the year of the treatment). b) Which strategies are used by the invasive species to recover? The recover strategies of the invasive plant mean: vegetative or generative propagation (or both of them) was used by the stand in the re-establishment. Based on the answers of the first two questions could be determined the third one: c) Was treatment a successful environmental management approach? The duration of the investigation also plays an important role, because what effect it has on the stand and for how long. This is a kind of cost effectiveness of the single herbicide treatment. d) Which can be potential herbicides for controlling common milkweed or other clonal plants?

Materials And Methods

Study Site

The study site is in the UNESCO biosphere reserve, Fülöpháza Sand Dunes in the Kiskunság National Park, Central Hungary (Fig. 1). According to the European Union Habitat Directive (92/43/CEE), Pannonic or open sand steppes (Natura 2000 code: 6260) represent prominent biomes [80]. Although these dry, nutrient-poor, calcareous sand habitats support only few communities, many rare, endangered, and endemic species can be found in this area. The site has the following abiotic characteristics: groundwater level is at a high depth [81, 82], mean annual precipitation is 530–565 mm [83–85], and mean annual temperature is 10.33 °C [85]. As a result, vegetation grows in a mosaic pattern. The 2000-ha study area has been protected from grazing since 1974. In the last quarter century, the site has been invaded by common milkweed

whose extended stands can be found throughout the protected area [20, 68, 72, 73]. It prefers mostly less heavy soils (well drained sandy or sandy-loess soils). The colonization of *A. syriaca* can be facilitated by some anthropogenic disturbance of the soils. In 2011, an isolated milkweed stand embedded in natural psammophilous vegetation units was mapped (GPS coordinates: N46°53.488' E019°24.771'). It had a manageable number of shoots, pods, and stand size (approximately 400 shoots, with a maximum extension of 1000 m²) and was separated from other clones (Fig. 1).

Herbicide Treatments

Herbicide treatment of common milkweed was conducted in the framework of a KEOP tender (KEOP-7.3.1.2-09-2010-0024). The Environment and Energy Operational Program (KEOP) was carried out by the Kiskunság National Park Directorate with the support of the European Union and co-financing from the European Regional Development Fund. The aim of this program was to suppress the invasive alien plants in the most valuable sand areas of the Danube-Tisza Interfluvium. Based on the existing Hungarian practical experiences two methods were applied for the treatment of the target vegetation in the study site: machine broadcast or a motor sprayer was applied in buffer areas (formerly arable areas where the target vegetation was very dense), while lubrication (manually) was applied in the more valuable areas (natural vegetations). In the latter case can be minimized to the active ingredient reaches the non-target vegetation. The optimal application time was when the target vegetation reached 20–40 cm tall, until it bloomed (from May to June 2014) [10, 86]. Moreover, the study site is rather big and there are some hard-accessible parts. Therefore, the study site was divided into parts when the treatments were done. In the machine broadcast or motor sprayer application two herbicides were used in a mix: Tomigan 250 EC (1 l ha⁻¹) and Banvel 480 (1.5 l ha⁻¹). Synthetic auxins were the active ingredients. Fluroxypyr is a pyridine carboxylic acid (Tomigan 250 EC), and dicamba is a benzoic acid (Banvel 480). In the lubrication case the used herbicide was Medallon, within a 50% aqueous solution (2 l ha⁻¹). Glyphosate was the active ingredient in Medallon (it is an EPSPG synthase inhibitor). Glyphosate belongs to category G of the Herbicide Resistance Action Committee and category 9 of the Weed Science Society of America.

Whereas the examined stand embedded in natural vegetation the lubrication was the applied technology, therefore glyphosate was used. The examined stand was treated by herbicide only once (in May 2014) over the 7-year study period (the treatment was not repeated at all). The time of the treatment (phenology) and the used chemical was suitable for a recent study of a basic model for the control of invasive clonal plants [33].

Monitoring of Herbicide Effectiveness for Milkweed Stand

The investigation extended to the whole stand, and the entire occupied area of the stand was covered with 2 m × 2 m quadrats, in which the localization of the shoots, the number of solitary shoots and clusters (maximum distance between shoots of 15 cm), and pod production of shoots were recorded. The positions of the shoots (to an accuracy of 5 cm) were necessary to depict the pattern of the stand; this allowed monitoring of individual shoots. Pod production served as a measure of vitality. The sampling was repeated for 7 years (from 2011 to 2017).

Data Processing

We used simple data processing methods and basic descriptive statistics to follow the fate of shoots in the stand and demonstrate the efficiency of herbicide treatment. In this study deeper statistical analysis (e.g. One-Way ANOVA) was not applicable because it would lead to misleading results due to pseudoreplication [cf. 87–92]. GraphPad Prism version 8.0.1.244 for Windows (GraphPad Software, La Jolla, California, USA) was used for calculating descriptive statistics and

plotting of diagrams. QGIS version 2.18.24 [93] was used for drawing the study site map, shoot location schemes and Kernel density analysis.

Results

The investigation period was divided as follows: before (the first 3 years), during (year of treatment, 2014), and after (the last 3 years) treatment.

Before treatment: There were about 500 shoots in this period: 507 shoots were in 2011, 485 shoots were in 2012 and 536 shoots were in 2013. Most pod numbers showed annual fluctuations: 82 pods were in 2011, 305 pods were in 2012 and 87 pods were in 2013. The annual difference of the pod-bearing shoot number was small during that three years: 41 pod-bearing shoots were in 2011, 52 in 2012 and 56 in 2013). Most pods appeared in 2012 (Fig. 2). The number of solitary shoots increased, whereas the most common shoot clusters (2 and 3 shoots/cluster) decreased in the stand (Figs. 2 and 3).

During treatment: Of the original 388 shoots, 102 survived till 2014 and pod production completely ceased that year (both the viable shoots and dry shoots were included in the 388 shoot number as well). Due to the effects of the herbicide, 74% of the shoots died, although intact shoots were still observed in almost all areas of the stand (Figs. 2 and 3). Solitary shoots were the predominant pattern in the stand (Fig. 3).

After treatment: While the number of shoots and pods temporarily decreased in the first year after treatment, an increase was observed over the longer-term. A further decrease was observed in the number of shoots in 2015, with almost half of the shoots that has survived from the previous year dying (from 102 survived shoots to 65; Figs. 2 and 3). An increase in the number of new shoots and pods was observed from the second year after treatment, although it did not reach its original densities (Figs. 3 and 4). The number of solitary shoots increased moderately, whereas the number of those with 2 or 3 shoots/cluster decreased after treatment (Fig. 2). The proportion of solitary shoots never decreased below 60%. The solitary shoots represented a higher proportion than in the first period: in 2015, the proportion of the solitary shoots was 90.76%. In 2016, it was 82.53% and in 2017, it was 93.22%. The number of pod-bearing shoots were low: in 2015, there were not pod-bearing shoots, while there were three in 2016 and 2017.

The density of the shoots changed after treatment; the former dense milkweed stand almost disappeared by glyphosate treatment in 2014, whereas the size of the occupied area by the stand remained almost unchanged, but all the parts remained occupied after treatment (Figs. 2 and 3). The precise location of the shoots was used to calculate a heat map (or Kernel density) to determine the shoot density. This spatial analysis was performed to estimate the probability of (dis)continuity in gradients of local shoot density interpolated over the whole stand. A comparison of the before, during and after treatment of the stand clearly shows differences in the heterogeneity and (dis)continuity of georeferenced spatial intensity of the clustered shoots. Before and during treatment, Kernel densities of the shoots had more continuity, as indicated by numerous internal gradients of aggregation that diminished to the minimum (white) density within the full range present. The stand had one main focus point in this two periods (Fig. 4). After the treatment, shoots had lower Kernel point densities and congregated into three smaller isolated foci as this period characterized by discontinuity of Kernel densities. Moreover, it had higher gradient connectivity with less discontinuity of kernel densities (Fig. 4).

Discussion

Our semilong-term case study showed that a large milkweed stand in an undisturbed semi-natural habitat not only can survive a single herbicide treatment but it can regain its vitality slowly.

First of all, it is important to determine how does a single herbicide treatment influence the further spread and pod production of the invasive plant. A study has reported that one-time cultivation (as a mechanical control) has no effects

on milkweed abundance in arable lands [77]. Zalai et al. [78] reported that resprouting only becomes stronger in such cases. Badalamenti et al. [16] found a similar effect in a study on *Ailanthus altissima*. The most likely explanation is that dormant bud banks are activated in the absence of apical inhibition. Conversely, herbicide treatment (even one-time application) is undoubtedly more effective, as reported by Zalai et al. [78] wherein damage from glyphosate in shoot numbers in the second season (resprouting in their study was half that of that in the previous year). Similarly, the resprouted shoots of *C. arvense* were weaker and less dense in the year following herbicide treatment or mechanical control [35]. Moreover, Dođramacı et al. [94] showed that foliar glyphosate treatment reduced the vegetative growth of *Euphorbia esula* and caused changes in transcript abundance in crown buds. Saunders and Pezeshki [51] reported that changes in leaf and shoot production in exposed *Ludwigia peploides* shoots may be caused by a translocation-induced hormesis effect. Glyphosate translocation in the roots towards root buds was also observed in *C. arvense* [95–97], which has a similar root and root bud structure and development to *A. syriaca* [cf. 35, 98, 99]. When Savini et al. [50] applied glyphosate to shoots, they found that it was translocated from treated to untreated shoots in the same clone, causing death in both of them. This mechanism would explain the short-term (1–2 years) effects observed in our study. This explains why the number of milkweed shoots in our study decreased in 2014 and 2015: because of herbicide translocated by rhizomatic roots and damaged dormant bud banks in the year after treatment. The inability of milkweed to resprout from a bud bank of rhizomatic roots following herbicide treatment indicates that herbicides translocate through rhizomatic roots.

The effects of herbicide treatment on pod or fruit production have been rarely monitored. Due to lack of important differences between pod numbers in the before- and during treatment periods, we assumed the stand remained relatively vital. Herbicide treatment did not alter the proportions of shoot clusters and pods; any observed effects were temporary and limited to the year of treatment and the second year after treatment. Therefore, one-time herbicide application is not considered an effective method to reduce seed production. Guo et al. [14] came to a similar conclusion in a study on *Solidago canadensis*.

The second question is that how the stand survives the large shoot-losses and which strategies are used by the invasive plant to recover. The species' resilience can be attributed to vegetative propagation, which produces adventitious shoot buds on rhizomatic roots [67, 68] and creates a large dormant bud bank. According to Schmid [100], activation of the bud bank depends on clone conditions. This was observed in *A. syriaca*; several buds appeared on its rhizomatic roots but only one or few of them are activated, whereas the others remain dormant [101] in a given year. Large numbers of solitary milkweed shoots of a clonal architecture may prefer density-dependent regulation to reduce or avoid intraclonal competition. In addition, these shoots (former activated buds) will be vegetative or pod-bearing [102]. The reproductive output (pod and seed production) of three milkweed species (including *A. syriaca*) is resource limited [103]. This may explain the annual fluctuations in the production of *A. syriaca* shoots and pods observed in our study before treatment and can be used as a sensitive and important indicator of annual vitality of milkweed stands and conditions of the bud bank. Survival of *A. syriaca* benefited greatly from its clonal characteristics and growth after treatment. An extensive dormant bud bank may be activated by disturbance [53, 58]. Waldecker and Wyse [104] found that buds of *A. syriaca* in the proximal part of the rhizomatic root system accumulated less radioactively labeled glyphosate than distal root buds. Therefore, proximal rhizomatic root buds are more dormant than distal ones and thus accumulate less glyphosate. The surviving and newly propagated buds on rhizomatic roots adjusted the numbers of emerging shoots in the years after treatment, and reconstruction of dormant bud banks in 2015 explains the spatial position of the shoots during that period (2015).

Was treatment a successful environmental management approach? Our semilong-term study demonstrated that recovery of original shoot numbers in a milkweed stand takes longer in natural open sand grasslands. Whereas, the proximal rhizomatic root buds of *A. syriaca* are more dormant (accumulate less glyphosate and shows reduced respiratory rates) [104] they survive and can be activated in larger numbers from the second year after treatment. The surviving and later

activating of these more dormant rhizomatic root buds are showed by the increasing shoot and pod number from the second year after the treatment in our study. Moreover, the heat map shows that three foci developed in this period. Thus, in spite of that single herbicide treatment is really suitable for density control in short-term [cf. 78], but it seems the growth of common milkweed stand shows a slow regeneration for a longer-term period.

Which herbicides can be potential suitable for controlling common milkweed or other clonal plants? The complete destruction of a clone can only be caused by factors that affect the entire bud bank and shoots because the mortality risk is distributed among the interconnected bud bank and shoots in a clone [38, 54–60]. This indicates that rhizomatous roots and bud banks can create a successful and persistent colonization system, thus making periodic control more effective than one-time treatment. This phenomenon was also reported by a study on invasive clonal *Fallopia japonica* control, wherein the species was resistant to all forms of treatment [33]. Because treatment during periods of low shoot numbers may only result in herbicide-translocation at dilute concentrations to a dormant bud bank, beginning herbicide treatment in years with high shoot numbers may be more effective. Moreover, because bud banks can develop on rhizomatic roots [68, 98, 99], complete extirpation is difficult and can only be successful and efficient if rhizomatic roots and buds are destroyed. Herbicides that are easily absorbed through the leaves can be translocated to the roots by the phloem, but this process occurs mainly in the roots and not just in meristematic tissues. Dicamba accumulates strongly in meristematic tissues [48, 105] such as dormant buds, and similar effects have been reported with glyphosate treatment in several weeds [106] and on *A. syriaca* [49]. Application of a dicamba and tritosulfuron mix on invasive plants resulted in one-tenth of shoot numbers in the previous year [cf. 78]. Other example, hemp dogbane (*Apocinum cannabinum*), which has a similar biology and belongs to the *Asclepiadaceae* family of common milkweed, is sensitive to fluroxypyr [107] a herbicide that is usually favored during early reproductive stages because it can translocate toward roots [108, 109]. In addition, the phenological state of the invasive clonal plant must be considered when applying any herbicides [36, 102]. For example, herbicide treatment can be more efficient when assimilates translocate to the roots in the late summer, as reported in several studies on other invasive clonal plants [110, 111]. However, the substances and the treatment's half-life must be considered in protected areas, and a break of at least 1 year between treatments is advisable to allow regeneration of natural vegetation [19, 20].

The findings of the present study confirm that monitoring of invasive plant control should be continued for more than 1 year, especially in the case of clonal plants.

Conclusions

In our study the total shoots of a common milkweed stand were monitored over a 7-year period before, during and after a single herbicide application. The regeneration ability of the invasive clonal plant was calculated based on the precise location of the shoots, and the trait of shoot (clustering or solitary, reproductive or not). Overall, the results showed herbicide treatment was successful in the sense of short-term: number of shoots of *A. syriaca* reduced considerably (73% of shoots died). In the first year after treatment, the shoot number decreased due to herbicide translocated by rhizomatic roots and caused the damage of dormant bud bank. Number of surviving buds adjust number of emerging shoots at the years of after treatment. It seems the common milkweed stand regain its vitality slowly because rhizomatous-roots and bud bank create a very successful persistent (re)colonization system. This study is the first to demonstrate that one-fold herbicide treatment could weaken an invasive clonal plant but it is not enough for the complete extirpation. Periodic control methods are needed, where the first useful applied method could be a rhizome translocatable herbicide treatment. This information is applicable for each invasive clonal plant management.

Declarations

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Authors' contributions

IB and LB conceived and designed the study. LB conducted the analysis and drafted the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

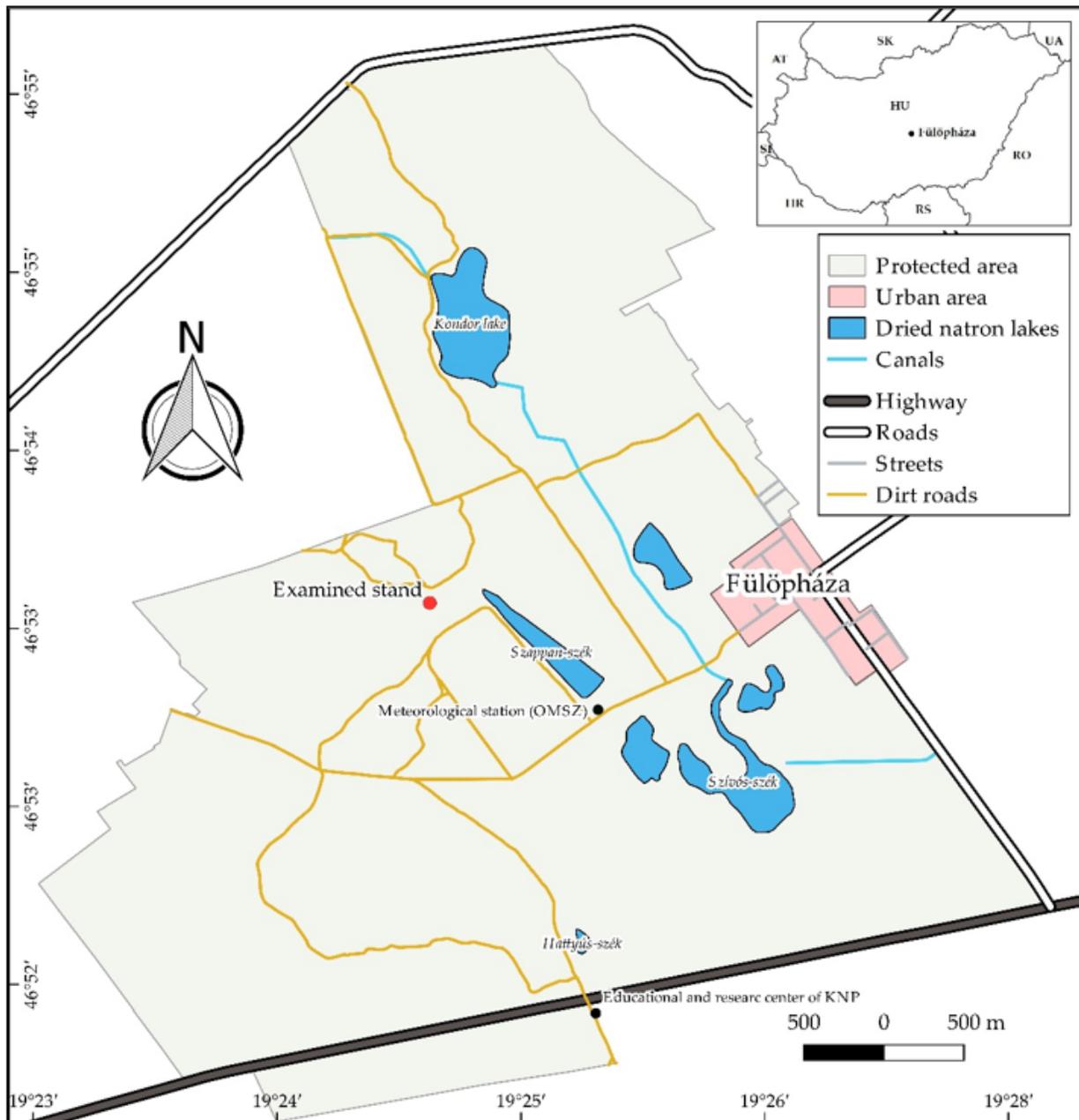


Figure 1

Location of the milkweed stand (red dot). The inserted image in the upper right corner shows the special protected Fülöpháza Sand Dunes, which are a part of Kiskunság National Park in Central Hungary.

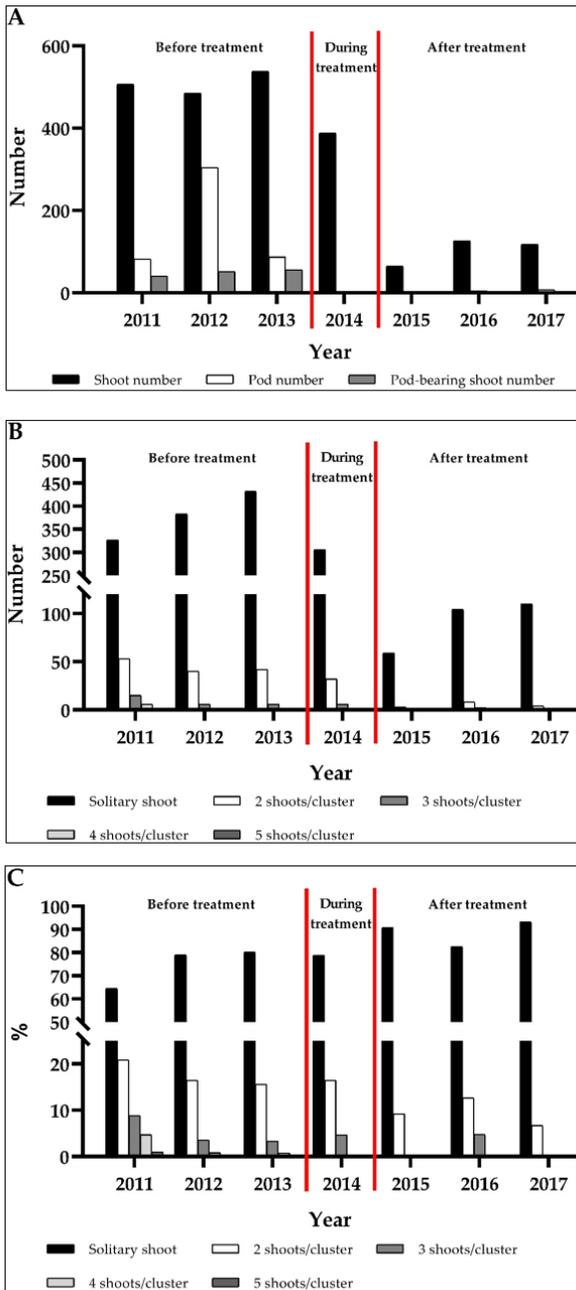


Figure 2

a Changes in the numbers of shoots, pod-bearing shoots, and pods b numbers of solitary and clustering milkweed shoots, c percentage of solitary and clustering shoots.

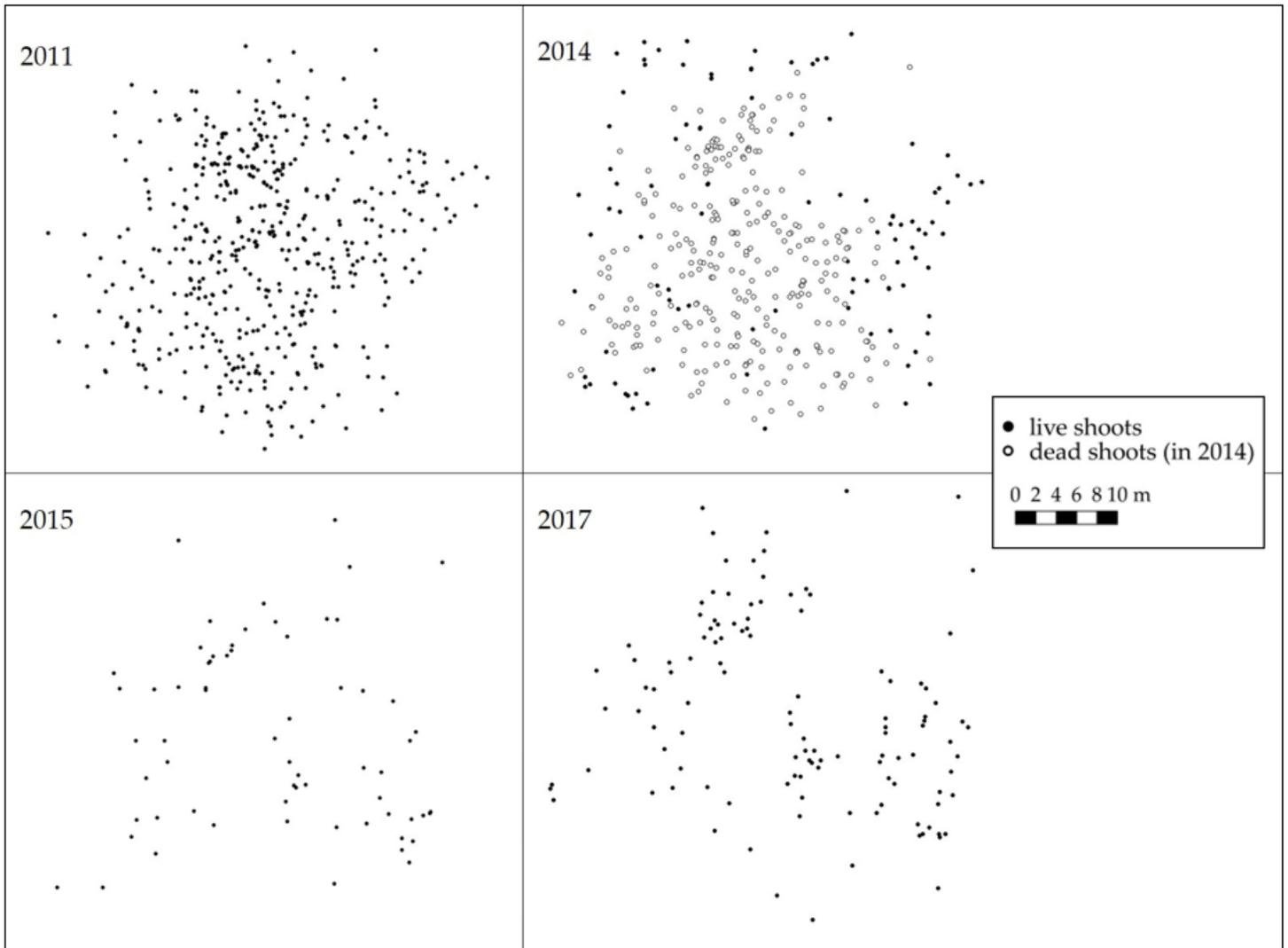


Figure 3

Shoots location of the stand illustrate changes in the arrangement of shoots on the test surface in time: before herbicide treatment (in 2011), during herbicide treatment (2014), and during two years after herbicide treatment (in 2015 and 2017).

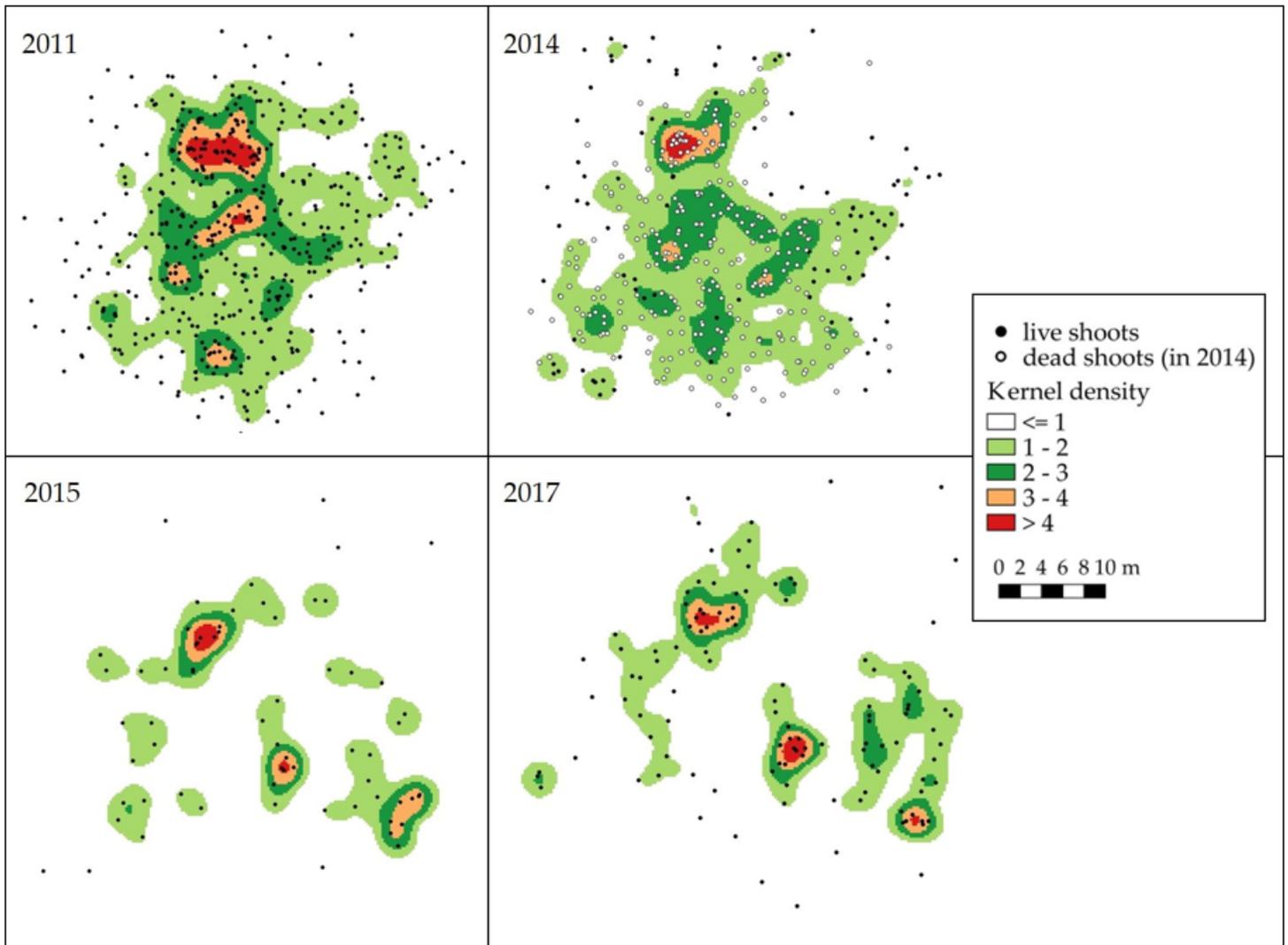


Figure 4

Kernel density of the stand illustrate the arrangement and extension of shoot density changes on the test surface in time: before herbicide treatment (in 2011), during herbicide treatment (2014), and during two years after herbicide treatment (in 2015 and 2017).