

# Emergence of a new salt-tolerant alien grass along roadsides? Occurrence of *Diplachne fusca* subsp. *fascicularis* (Poaceae) in Hungary

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**Abstract** – This paper reports the occurrence of a North American salt-tolerant taxon, *Diplachne fusca* subsp. *fascicularis* (Lam.) P.M.Peterson et N.Snow in Hungary (Central-Europe). Two earlier Hungarian observations of *D. fusca* were known from 1915, near Győr (West Transdanubia), later the taxon was collected by Péntzes in 1958, in downtown Budatétény (central Hungary). Both observations seem to be occasional. Recently, the taxon has started spreading in Europe, mainly on rice paddy fields, with a serious invasion potential. In North America its appearance on ruderal habitats, as well as along roads and other linear infrastructures is a well known phenomenon. The Hungarian population was found near Cegléd (central Hungary) on the roadside of the E40 primary main road in September 2018. In July 2019 more than one thousand (mostly vegetative) individuals were detected. The salt content of the habitat shows remarkable temporal and spatial variability. At one meter distance from the edge of the paved road soil salt content was higher in spring (after the winter de-icing regime), than in autumn. Salt concentration was highest in the vicinity of the road, and decreased with increasing distance from it. Germination tests revealed a significant negative effect of NaCl concentration on germination rates, but germination occurred even on extremely saline substrates with 1.5% NaCl concentration. Considering its biology and reproduction strategy, the further spread of *Diplachne fusca* is highly presumable.

**Keywords:** Central Europe, de-icing salt, plant-invasion, roadside verges, salt-tolerance, seed dispersal

## Introduction

Biological invasion is one of the recent global challenges (Drake et al. 1989, Perrings et al. 2002). Increase of global cargo and passenger transport, as well as the growth of global road networks, now 64 million kilometres long in total (van der Ree et al. 2015) is remarkable even from a biological point of view, especially because roads play an important role in the spread of invasive species worldwide (Forman 2000, Gelbard and Belnap 2003, Kalwij et al. 2008). Long-distance dispersal of native and alien plant species by vehicles is frequent, but appears to be more common among alien species (von der Lippe and Kowarik 2007).

The construction and maintenance of roads are usually associated with anthropogenic disturbances, including chemical de-icing, use of herbicides, mowing verges, trampling, introduction of a range of pollutants (e.g. petroleum

products) and modified soils used for construction, the last of which may contain propagules of alien species (Šerá 2008, van der Ree et al. 2015). These factors act in synergy, favouring the spread of alien plant species, since native species are usually less able to adapt to the altered, anthropogenic conditions at roadsides (Greenberg et al. 1997). In the case of plant species producing lightweight seeds, dispersal may be facilitated by the air turbulence caused by cars (von der Lippe et al. 2013), but seeds may also travel long distances in mud attached to cars (Clifford 1959, Ross 1986, Schmidt 1989, Zwaenepoel et al. 2006, Ansong and Pickering 2013). Moreover, machines used for mowing verges have also been shown to transport seeds, potentially aiding dispersal in some taxa at least (Strykstra et al. 1997, Vitalos and Karrer 2009).

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Winter de-icing (using mostly NaCl, with a small proportion of CaCl<sub>2</sub>, rarely MgCl<sub>2</sub>; Houska 2007) became widespread in Europe during the second half of the 20th century. De-icing salts have complex effects on the roadside environment (Amrhein et al. 1992). The resulting increased soil salt content in the vicinity of roads can facilitate the spread of halotolerant, or even of halophytic plant species (Davison 1971). For instance, the east- and northward spread of a Mediterranean coastal halophyte species, *Plantago coronopus* L., was detected in Hungary between 2013 and 2016 (Schmidt et al. 2016). Similarly, the spread of the Atlantic coastal halophyte, *Cochlearia danica* L. was also documented in continental Europe (Fekete et al. 2018).

During systematic surveys of the roadside vegetation along Hungarian paved roads, on 25 September, 2018, we found *Diplachne fusca* subsp. *fascicularis* (Lam.) P.M. Peterson et N. Snow (syn: *D. fascicularis* (Lam.) P. Beauv., *Festuca fascicularis* Lam., *Leptochloa fusca* subsp. *fascicularis* (Lam.) N. Snow) on the roadside of the E40 primary main road near Cegléd (central Hungary). The taxon was identified according to Snow et al. 2018. This species has a native distribution range restricted to the Americas, spanning the area from southern Canada to Argentina. It has been previously reported from disturbed habitats of several countries in Europe: Belgium (Lambinon 1957, Verloove and Vandenberghe 1999), the Netherlands (van der Meijden 1975), Ukraine (Dubyna et al. 2003), Portugal (Valdés and Scholz 2009) and the Czech Republic (Pyšek et al. 2012). It is considered an invasive weed of rice paddy fields in Italy (Romani and Tabacchi 2000), Spain (Osca 2013), Turkey (Altop et al. 2015) and Bulgaria (Vladimirov and Delcheva 2016). Here we document the first European appearance of the species on roadside verges, which raises the possibility of further spread or even invasion of the taxon along European roads. Occupancy of this habitat could help the species to conquer new habitats (possibly even arable fields) and possibly reach new geographic regions.

The central aims of this paper were: (i) to document the circumstances of occurrence of *Diplachne fascicularis* along Hungarian roadsides; (ii) to examine soil salt content of this newly found habitat and to test the effect of salt content on seed germination of the taxon in an *in vitro* germination experiment.

## Materials and methods

The geographic coordinates and the elevation of the locality were determined using a Garmin eTrex Legend handheld GPS device and recorded in WGS84 format. The number of *Diplachne fusca* subsp. *fascicularis* individuals was estimated on 14<sup>th</sup> December 2018 and 22<sup>nd</sup> July 2019. The nomenclature of vascular plant taxa follows Király (2009).

Soil samples were collected from root depth (1.5–6.5 cm) before and after the de-icing season (on 18<sup>th</sup> September 2018 and 11<sup>st</sup> March 2019, respectively). They were collected at six different distances from the paved road. These distances

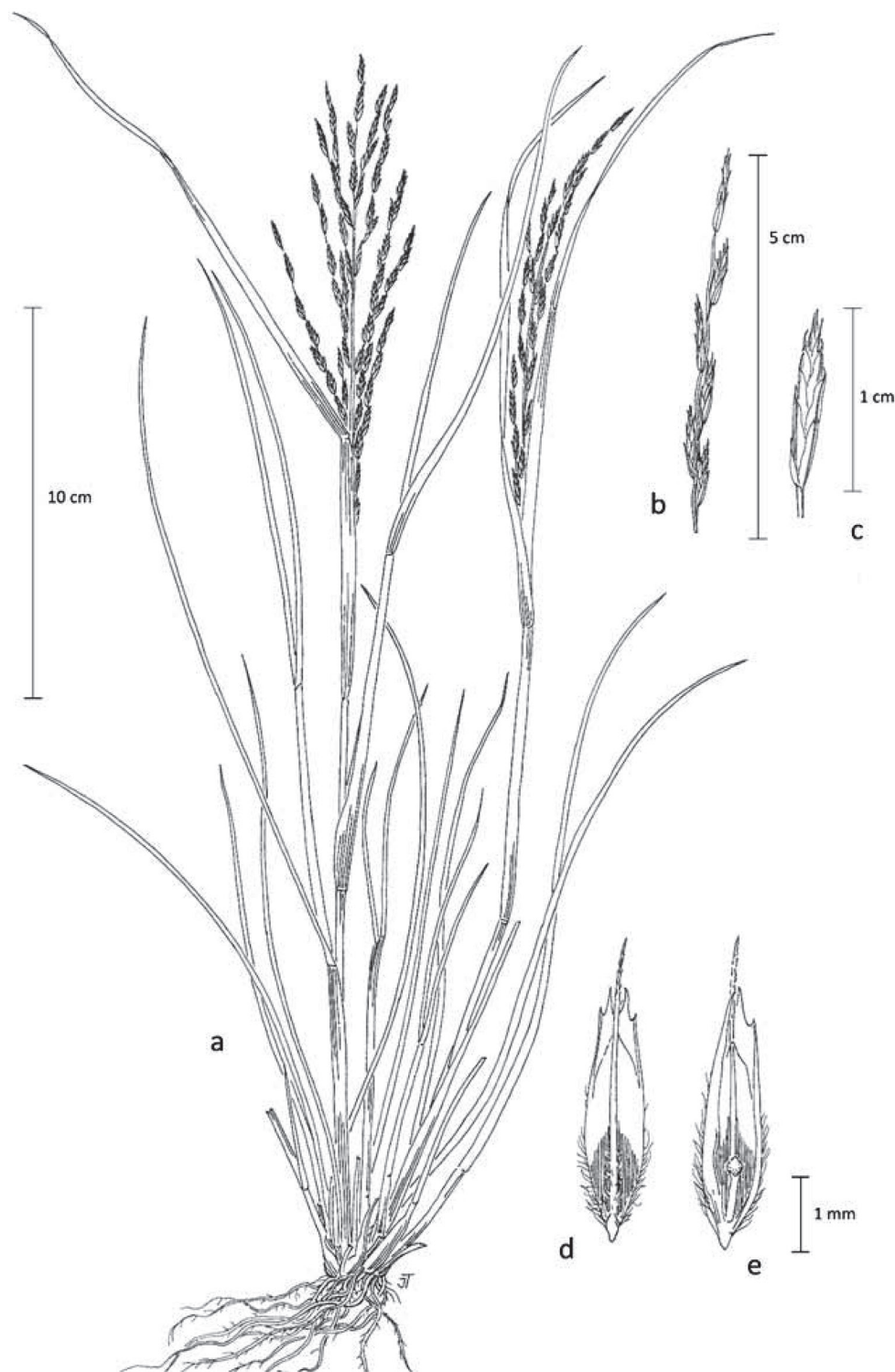
were 1, 2, 3 m in September 2018 and 0.1, 0.5 and 1 m in March 2019. Soil total soluble salt content was quantified by measuring electric conductivity of a saturated paste of soil and water using a conductivity meter (Tetra Con 325) (Hungarian technical standard MSZ-08-0213:1978 2.2). Soil analyses were carried out by the accredited laboratory of the Research Institute of Karcag of the Centre for Agricultural and Applied Economic Sciences of the University of Debrecen.

In order to test the ability of *Diplachne fusca* subsp. *fascicularis* seeds to germinate at different NaCl concentrations we conducted an *in vitro* germination test. Seeds were collected on 18<sup>th</sup> September 2018 and were stored in paper bags at room temperature until germination tests were initiated. We tested germination on a 1% agar substrate in Petri dishes with the following 13 NaCl concentrations (m/m% = mass percent): 0 (control), 0.15%, 0.30%, 0.45%, 0.60%, 0.75%, 0.90%, 1.05%, 1.20%, 1.35%, 1.50%, 2% and 2.50%. These concentrations were used to determine whether the species requires salt for germination and to assess the maximum concentration of NaCl at which it is able to germinate. In one Petri dish 25 seeds were placed on the agar-agar medium. Each concentration of medium was repeated three times. Thus, at one given concentration, 75 seeds were germinated in three Petri dishes with 25–25 seeds per Petri dish. Thus, a total of 975 seeds were tested at 13 different concentrations. Petri dishes were stored at room temperature under natural light conditions (i.e. near a window in a laboratory) and germination was followed from 16<sup>th</sup> October 2018 to 6<sup>th</sup> November 2018. Seedlings were counted on the 21<sup>st</sup> day of the experiment.

Data analyses were carried out in the R statistical environment (R Core Team 2018). To test the effect of substrate salt content on germinability, we performed a binomial generalized linear mixed model (GLMM), where germination status (0/1) was used as dependent variable and NaCl concentration was included as the sole explanatory variable in form of a second-degree orthogonal polynomial. The i. d. of the Petri dish was included as a random factor in the model. Prediction intervals were calculated using 'predictInterval' function from MerMod objects (Knowles and Frederick 2016). Results were visualized using a sunflower plot.

## Results

*Diplachne fusca* subsp. *fascicularis* (Lam.) P.M. Peterson et N. Snow was found on 25<sup>th</sup> September 2018, near Cegléd, on the roadside of the E40 primary main road (coordinates 47.19151 N, 19.91483 E, elevation 89 m a.s.l.), in the immediate vicinity of road reconstruction and overpass construction works. In total, 117 individuals were found, distributed along a 250 meter-long section of road, in a narrow lane (at 100–380 cm distance from the paved road margin). On 22<sup>nd</sup> July 2019 the latter site was revisited and altogether 1082 (mainly vegetative) individuals were recorded, along a 200 m-long section (at 5–330 cm distance from the paved road). Illustration of the taxon (Fig. 1) was made based on herbarium specimens and photographs. Voucher specimens were



**Fig. 1.** *Diplachne fusca* subsp. *fascicularis* (Lam.) P.M. Peterson et N. Snow. a – habit, b – one branch of the panicle, c – spikelet, d, e – diaspore (caryopsis with lemmas) (drawn by Jana Táborská).

deposited in the herbaria of the University of Debrecen (DE), Hungarian Natural History Museum (BP) and Eszterházy Károly University (EGR).

Morphological description of the taxon, based on Vladimirov and Delcheva (2016) and on our own observations can be summarized as follows:

Annual plant. Generative stems 20–40(–100) cm tall. Leaf sheaths rolled up, 2–4(–5) mm wide, pointed, scabrid

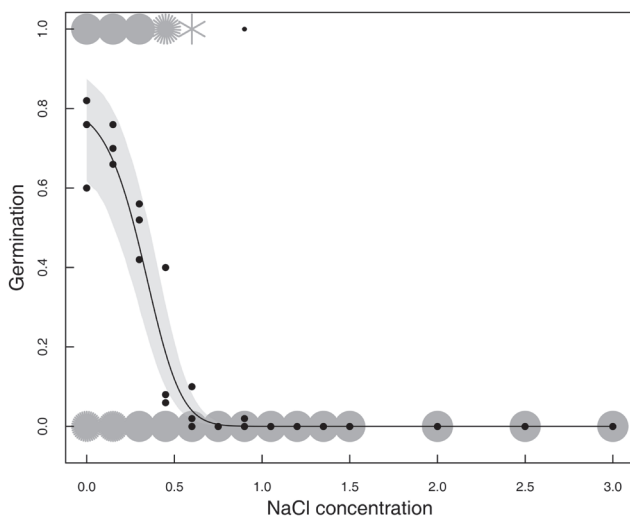
to subglabrous; ligules 2–8 mm, membranous, becoming lacerate at maturity; the uppermost leaf exceeds the panicle. The panicle is generally narrow, elongated. Panicle partly enclosed in the uppermost leaf sheath (even at maturity), 10–60 cm long, with 8–25 branches, with (1)2–7(8) spikelets per branch; branches (2)3–12 cm long, erecto-patent to suberect (Fig. 1). Spikelets sessile (peduncle 0.4–0.6 mm), (4)5–11 mm long, 5–11-flowered; lower glume 2–2.5 mm,

lanceolate, upper glume ca. 4 mm, elliptic; lemmas lanceolate, 3-veined, with silky hairs at base and along the margin in the lower half, bifid at apex, with 0.5–2.5 mm long apical awn arising from the notch, midrib keeled, usually scabrid.

*Diplachne fusca* subsp. *fascicularis* was found to co-occur with the following taxa (halophytes are marked with asterisks, other grass species also spreading characteristically along roads are marked with hashtags): *Achillea collina*, *Ambrosia artemisiifolia*, *Artemisia santonicum*\*, *Atriplex prostrata*\*, *A. tatarica*\*, *Bromus inermis*, *Chenopodium album*, *Cichorium intybus*, *Cirsium arvense*, *Cynodon dactylon*, *Daucus carota*, *Elymus elongatus*#, *E. repens*, *Festuca pratensis*, *F. pseudovina*\*, *Inula britannica*, *Limonium gmelinii*\*, *Phragmites australis*, *Plantago lanceolata*, *Podospermum canum*\*, *Polygonum aviculare*, *Populus × euramericana* (seedling), *Populus alba* (seedling), *Portulaca oleracea*, *Puccinellia distans*\*, *Setaria glauca*, *Sorghum halepense*#, *Taraxacum* sect. *Ruderalia*, *Tragus racemosus*#.

The total soluble salt content of the habitat showed a remarkable temporal and spatial variability. At a one meter distance from the edge of the paved road soil salt content was remarkably higher (0.58%) in spring (after the winter de-icing), than in autumn (0.07%). In both cases the highest salt concentration (0.07% in autumn and 1% in spring) was detected in the sampling point closest to the road margin (1 m and 0.1 m).

Altogether, 486 seeds (51.4%) germinated from the 975 seeds tested. Binomial generalized linear mixed model (GLMM) showed a significant negative quadratic effect ( $P < 0.001$ ) of substrate NaCl concentration on germination (Fig. 2). The maximum concentration of NaCl where germination was detected was 1.5%. In the latter case, only 2.7% of the seeds germinated (Fig. 2). The highest germination rate (97%) was detected with the 0.3% NaCl concentration.



**Fig. 2.** Sunflower plot illustrating the relationship between germination rate and germination substrate supplemented with NaCl. Black dots show average germination rate in each Petri dish and each petal of sunflowers represents a single observation (germination of individual seeds).

## Discussion

The species *Diplachne fusca* has a secondary cosmopolitan range. It can be divided into four subspecies (Snow et al. 2018). The native range of subspecies *fascicularis* extends from Southern Canada to Argentina. In these areas it occurs in different marshy habitats, on wet, muddy surfaces and in ditches (Broyles 1987, Bartgis and Hutton 1988), but it also appears on ruderal sites, such as places where cars are parked, or on roadsides (Stevens 1917), being documented in both Southern Florida (Atlas of Florida plants) and Wisconsin (Virtual Flora of Wisconsin 2020).

The occurrence of *D. fusca* (without it being recognised as a subspecies) in Hungary was published by Sándor Polgár (Polgár 1918), in Győr (NW Hungary) from the area of a vegetable oil factory. This record was also mentioned by Jávorka (1924–1925). No recent herbarium voucher specimen was found in the following Hungarian natural history collections (on January 9, 2020): Natural History Museum, Budapest (BP); herbarium of the University of Eötvös (BPU, Nótári et al. 2017); University of Debrecen (DE, Takács et al. 2014) and Eszterházy University, Eger (EGR, E. Vojtkó et al. 2014). The only Hungarian specimen of *Diplachne fusca* available in Herbarium Carpato-Pannonicum (BP-380842) of the Hungarian Natural History Museum was collected in 1958 by Antal Péntzes in a private garden in Budatétény (Central Hungary), but our revision has shown that this specimen surely represents another subspecies and not the one the presence of which in Hungary is described above.

In Southern Europe, *Diplachne fusca* subsp. *fascicularis* is considered as a quickly spreading, potentially invasive alien plant species (Weber and Gut 2005). It is considered a problematic weed in California, as well as in Europe, interfering with rice production (Driver et al. 2019, Romani and Tabacchi 2000, Osca 2013). The newly found occurrence is located 60 km west from the closest rice paddy fields near Kisújszállás.

The number of individuals near Cegléd (central Hungary) increased almost 10 times from 2018 to 2019. This suggests that *D. fusca* subsp. *fascicularis* is able to reproduce under the habitat and climatic circumstances present at roadsides. However, we note that the species was able to colonize only otherwise open, gravel surfaces in the close vicinity of the road, but not the roadside verge, where dense, perennial grassland vegetation was dominant.

*D. fusca* shows some important biological and physiological characteristics, which raise the strong possibility that it will continue to spread. With the help of its symbiotic bacteria *D. fusca* subsp. *fascicularis* is able to fix  $N_2$  (Zafar et al. 1986, Reinhold-Hurek et al. 1993), furthermore it is a plant species of the C4 photosynthetic pathway (Snow et al. 2018). These characteristics can help the species to adapt even to extreme habitat conditions. Moreover, its ability to build a persistent seedbank (McIntyre et al. 1989), its cleistogamy and anemochory (Jurado et al. 1991) can enhance its reproductive success and dispersal ability, as shown by the case of two *Sporobolus* species, *S. neglectus* and *S. vaginiflorus* (Jogan 2017). Finally, its high salt tolerance (Myers and Morgan 1989, and this study) facilitates its spread along roads.

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