Journal of Arid Environments 132 (2016) 60–62

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Think note

Inferring field performance from drought experiments can be misleading: The case of symbiosis between grasses and *Epichloë* fungal endophytes

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form
18 April 2016
Accepted 29 April 2016
Available online 6 May 2016

Keywords:
Plant-fungus interaction
Mutualism
Aridity
Productivity
Mean annual precipitation

Epichloë fungal endophytes (Clavicipitaceae, Hypocreales) form persistent symbioses with grasses of the subfamily Poöideae (Poaceae), including many important cultivated and wild forage species (Leuchtmann et al., 2014). The interaction has been labelled as a defensive mutualism, since fungal alkaloids protect host plants against herbivores (Clay, 1988; Schardl et al., 2007; Saikkonen et al., 2013). Along with herbivore resistance, tolerance to drought is commonly mentioned as an additional benefit for host plants. However, while the bioactivity of fungal alkaloids on herbivores is well-established (see e.g., Wilkinson et al., 2000), the physiological mechanisms underlying the endophyte effect on host plant tolerance to drought are not so clear (reviewed in Malinowski and Belesky, 2000). The idea of positive effects of endophytes on plant tolerance to drought has been used to explain ecological patterns in which high symbiosis incidence is associated with low levels of precipitation (Lewis et al., 1997; Malinowski and Belesky, 2006; Afkhami et al., 2014). However, a recent global survey of endophyte incidence including a larger number of grass and fungal endophyte species in a wider environmental gradient revealed a different pattern (Semmartin et al., 2015). By reviewing the literature and adding information to the drier extreme of the gradient, they found a positive relationship between endophyte incidence and mean aboveground net primary productivity (ANPP), a variable closely associated with mean precipitation (Sala et al., 1988). Moreover, increasing ANPP leads to an increase in herbivore pressure and biological complexity (McNaughton et al., 1989; Thrall et al., 2007, Fig. 1). In clear opposition with the notion of fungal endophytes conferring tolerance to drought, the low endophyte average incidence in the arid extreme of the gradient (which was associated with a large variance) seems to be more than an exceptional rarity among patterns of symbiosis prevalence and environmental variables (see Novas et al., 2007; Gundel et al., 2011a).

Here, we propose a reinterpretation that can help solving some of these apparent contradictions from the literature and takes into account three issues: (*i*) past biases in experimental systems and species choice; (*ii*) an influence of those experimental results in the interpretation of regional patterns; and (*iii*) the dynamics of the interaction between grasses and their endophytes in arid ecosystems.

Regarding the first issue, we point out that the evidence used so far to predict a positive endophyte effect on the ecological performance of host wild grasses in arid environments is mostly being taken from a few short-term drought experiments carried out with cultivated, mesophytic forage species. In a wider context, Saikkonen et al. (2006) had already warned about this species bias and its failure to represent natural variability in wild grass−endophyte symbiotic interaction. In order to uncover the bias, we conducted a search in Scopus (http://www.scopus.com, visited December, 2015) looking for articles containing: ["endophyte" AND ('*Acremonium*', OR '*Epichloë*' OR '*Neotyphodium*') AND "drought"] in their title, abstract or keywords. *Acremonium* was included to identify, and then filter out, some early articles that used this name for designating *Epichloë*/*Neotyphodium* endophytes. We found 190

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http://dx.doi.org/10.1016/j.jaridenv.2016.04.008

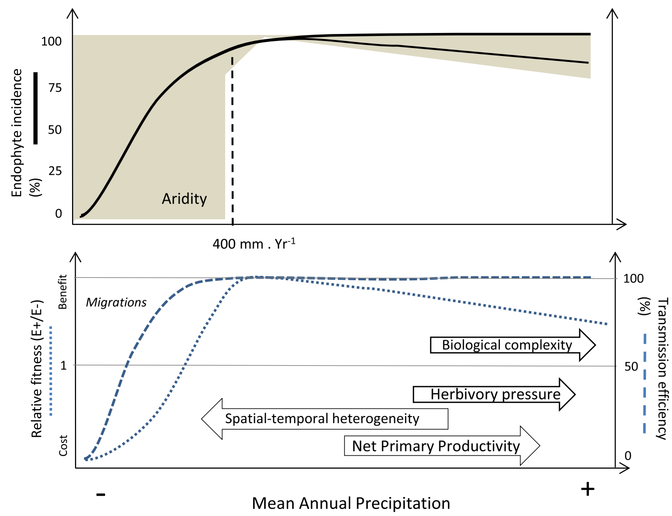

Fig. 1. Predicted curves showing the variation of the incidence of Epichloë fungal endophytes in host grass populations (upper panel) in relation to ecosystem mean annual precipitation (MAP), due to two main population processes: (1) the relative fitness of endophyte-symbiotic (E+) and non-symbiotic (E−) plants and (2) the transmission efficiency of endophytes and migrations (lower panel). Precipitation appears as the main control across wide type of ecosystems, while for each ecosystem (with a given MAP), other abiotic (e.g. drought and flooding intensity) and biotic (e.g. grazing pressure) control variables may appear as well. In the arid extreme of the precipitation gradient (arid ecosystems), the cost of the endophyte compromises the symbiosis which, additionally with the higher environmental and temporal variability, increases the probability of local extinction (*migrations* may play an important role in determining the re-colonization).

articles, including conference ones, and experimental and review articles. Nearly 66% (126 documents) did not describe any drought experiment or related information. Other 13% of the articles (25 documents) were about the topic but not experimental (e.g., review articles). Only 21% of them (39 documents) were experimental research articles with manipulative trials designed for evaluating the endophyte effect on plant tolerance to drought. We found a bias not only on the approach of these studies, mainly short-duration experiments, but also on the focus species: out of these 39 studies, 12 (31%) of them were carried out with tall fescue [*Schedonorus phoenix* (Schreb.), syn.: *Festuca arundincea* and *Lolium arundinaceum*] and 9 (23%) with perennial ryegrass (*Lolium perenne* Lam.). The other 18 (46%) were very diverse, and included other 14 species (*Achnatherum inebrians*, *Achnatherum sibiricum*, *Agrostis hyemalis*, *Elymus dahuricus*, *Elymus virginicus*, *Festuca arizonica*, *Festuca eskia*, *Festuca pratensis*, *Festuca rubra*, *Hordelymus europaeus*, *Poa alsodes*, *Poa autumnalis*). These species do not occur in arid ecosystems (even though some are from semiarid ecosystems); moreover most of them were included in just one or two of the 14 studies. Taken together, this strongly suggests that there is an unwarranted extrapolation which ascribes a general positive effect of fungal endophytes on drought tolerance to host plants in general and in the long term, and that has spread out in the literature to make predictions about the endophyte effect on wild species in natural arid ecosystems.

In relation to the second issue, our main point is that experiments showing larger fitness under drought for endophyte symbiotic than for non-symbiotic plants should not be directly translated to observed symbiosis incidence in natural populations. The mechanistic basis of this assertion will be expanded below, but first we need to draw attention to several influential regional surveys. In an article cited so far 73 times (source: Scopus, December 2015), Lewis et al. (1997) showed that populations of *Lolium* spp. closer to the Mediterranean Sea presented higher endophyte incidence than those located at higher latitudes in Western Europe (Lewis et al., 1997). Similar patterns (i.e., negative association between endophyte incidence and precipitation) had been reported in the same region for *S. phoenix* and *Festuca pratensis* (meadow fescue) (Malinowski and Belesky, 2006), and in semiarid South West of North America, for *Bromus laevipes* (Afkhami et al., 2014). Although some of these examples found support for an endophyte benefit on plant tolerance to drought in manipulative experiments (Gibert et al., 2012; Afkhami et al., 2014), none of them covered intense aridity. In fact, surveys that included the lower extreme of the gradient (<400 mm) unveiled an opposite pattern (Novas et al., 2007; Casas et al., 2015). Endophyte incidence has been exhaustively studied in two native wild species from arid Patagonia (South America), *Bromus setifolius* J Presl, and *Hordeum comosum* L (Novas et al., 2007; Iannone et al., 2012, 2015; Casas et al., 2015). Novas et al. (2007) found that the incidence of endophytes in populations of *Bromus setifolius* was positively associated with annual precipitation. Research on endophyte incidence in *Hordeum comosum* took two stages. Whereas the first survey showed a negative association between endophyte incidence and precipitation in a geographically narrow sampling of populations (see Iannone et al., 2015), the second one (which was based on a larger sampling design along four precipitation transects) unveiled the opposite through a positive relationship between both variables (Casas et al., 2015). Mean annual precipitation of the distributional range for *B. setifolius* and *H. comosum* is 259 and 471 mm, respectively, while for *L. perenne* and *S. phoenix* is 592 and 685 mm (data took from Semmartin et al., 2014, and Casas et al., 2015). Thus, it seems that the negative relationship between endophyte incidence and parameters of environmental quality (e.g., precipitation or ANPP) would be not valid in arid ecosystems (see Semmartin et al., 2015) or, at least, would be specific to species and/or ecosystems.

Concerning the third issue, we put forward a mechanistic hypothesis that would account for the observed regional patterns of incidence showing an overall growing trend with ANPP (Semmartin et al., 2015), but also for the apparent larger variance of incidence towards low-precipitation locations (see Novas et al., 2007; Iannone et al., 2015; Casas et al., 2015). Local population endophyte incidence depends mainly on three processes: (1) the relative fitness of symbiotic vs. non-symbiotic plants, (2) same for net migration rates, and (3) the efficiency with which the endophyte is vertically transmitted (Saikkonen et al., 2002; Gundel et al., 2008, 2011a). The majority of studies have taken relative fitness as the main mechanism explaining the variation in population endophyte incidence, establishing an implicit relationship between the magnitude of the symbiosis benefits and endophyte incidence, disregarding the other two processes, migration events, and transmission efficiency (Gundel et al., 2008). Taking into account that the endophyte is only transmitted through seeds and that grass seeds have low dispersion (Williams and Bartholomew, 2005), we propose that the symbiosis is highly susceptible to be affected by stochastic processes. As strictly vertically transmitted symbionts, founder effects would then be critical in the dynamics of grass-endophyte interaction; a population that is established as endophyte free would only become symbiotic by the arrival of an endophyte-symbiotic propagule. On the other hand, it has been shown that the likelihood for an endophyte-symbiotic host to produce endophyte-free individual seeds is higher the lower its biomass (the spike or entire plant) (Gundel et al., 2011a, b). This pattern of endophyte vertical transmission with plant size resembles the relationship between population endophyte incidence and precipitation or ANPP (Novas et al., 2007; Casas et al., 2015; Semmartin et al., 2015), suggesting that control at both scales (population and ecosystem) could be associated with resource availability (Fig. 1). Therefore, we hypothesize that, in arid and semiarid ecosystems, chronic water shortage and low resource levels may affect the grass-endophyte symbiosis by impairing fungal transmission efficiency. Under these conditions, not only the fitness of endophyte-symbiotic plants relative to that of endophyte-free plants can be very low, but also the endophyte transmission efficiency can be low as well (Fig. 1) (Gundel et al., 2011a,b).

We conclude that the effect of *Epichloë* endophytes on plant tolerance to drought has been mostly studied in two worldwide distributed cultivated species. Therefore, to extrapolate results from these manipulative experiments to infer symbiosis incidence in arid ecosystems can be misleading. It well could be that, even when in more stressful environments there are potential (fitness) benefits of infection for the host, these often cannot be realized for failures in the transmission process (Gundel et al., 2008, 2011a,b). Thus, future studies should address the ecological performance of symbiotic (including endophyte performance, e.g., transmission) and non-symbiotic plants towards the arid extreme of the precipitation gradient. We have just suggested a sufficiently detailed hypothesis that could be taken into account in the design of such experiments and observations.

Acknowledgements

We thank Maria Semmartin and Roberto Fernández for their fruitful comments on earlier versions of this paper.

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