

Letters

In defence of the selective transport and role of silicon in plants

A response to Exley & Guerriero (2019) 'A reappraisal of biological silicification in plants?'

With interest, we read the Letter by Exley & Guerriero (2019; in this issue of *New Phytologist*, pp. 511–513) 'A reappraisal of biological silicification in plants?' in response to our recent Tansley review on the biological roles of silicon (Si) (Coskun *et al.*, 2019).

As stated in our article's Conclusion, 'our aim is to stimulate critical thinking and positive advances towards a better understanding of Si properties'. Naturally, we are therefore open to constructive arguments and to divergent opinions and hypotheses, aimed at the improved understanding of the roles of Si in plants. In this context, we expected that the Letter by Exley and Guerriero would share in a spirit of collegial scholarly discourse. However, we found the Letter to assume an unnecessarily critical tone while propagating several misconceptions and producing only a small number of substantive arguments.

Exley and Guerriero contend that the view that Si does not act as an 'active cellular agent' had 'previously not been explicitly expressed' by our research groups. For those readers unfamiliar with our work, notwithstanding the many conferences where we expressed this view, our review charts out the progression of this school of thought, from as early as 1992 to the present (Chérif *et al.*, 1992; Fawe *et al.*, 1998; Fauteux *et al.*, 2006; Chain *et al.*, 2009; Vivancos *et al.*, 2015; Rasoolizadeh *et al.*, 2018). Vivancos *et al.* (2015), in particular, tested the hypothesis that silicic acid acts as an intracellular signaling agent and definitively ruled this out. In addition, as shown in Fig. 4 of our review, we have led the field in using the latest molecular techniques to demonstrate the lack of an intracellular biochemical role of Si in a variety of plant species (i.e. Arabidopsis, wheat, and soybean).

Exley and Guerriero also contend that our 'apoplastic obstruction hypothesis' lacks novelty and importance. It would seem this is largely based on the authors' confounding of the distinctions between the 'mechanical barrier hypothesis' and the 'apoplastic obstruction hypothesis'. As we exposit in our Tansley review (see Section IV), and in more detail later, the 'mechanical barrier hypothesis', which proposes that silica deposits in the apoplast impede the physical penetration of fungal pathogens from entering cells, is a largely deposited model in this system (Yoshi, 1941; Liang *et al.*, 2005; Van Bockhaven *et al.*, 2015). The 'apoplastic

obstruction hypothesis', by contrast, is a novel working model that attempts to unify diverse strands of evidence from the scientific literature by proposing Si's prophylactic role stems from its apoplastic presence obstructing, for example, signaling between fungal/insect effectors and host receptors and apoplastic fluxes of mineral toxicants. This is important and timely, since, as we note in our Tansley review, many contemporary researchers in the Si community are actively engaged in searching for a biochemical role of Si.

Exley and Guerriero further claim that 'much important past and recent research [was] either inexplicably by-passed or not appropriately cited', suggesting that we may have overtly overlooked studies because they were perceived as a 'challenge' to our model. Our review has 177 references spanning close to eight decades, already exceeding the typical 150 for a Tansley review, in addition to over 100 references in the Supporting Information. As any scientific author will attest, it is inevitable that some studies will not be cited, especially considering that well over 1000 papers have been published on Si and plant biology, as detailed in our Introduction. In the context of our review, it was our opinion that the issue of biosilicification was subsidiary to the four main areas of contention we outlined surrounding Si's biological role in plants. Simply put, we are of the view that the presence of highly selective Si transporters underlies the differences in Si accumulation observed across species, whereas Exley and Guerriero are of the opinion that biosilicification is of primary importance. The fact that we have a difference of opinion does not mean, however, that the topic of biosilicification was overlooked or related references were 'by-passed'. On the contrary, many noteworthy articles on this subject were cited and discussed, including works by Hodson, Flowers, Elbaum, Lux, Zhang, and indeed, Exley and Guerriero (Coskun *et al.*, 2019; references cited therein).

Exley and Guerriero claim that we, the authors of the Tansley review, 'for the first time in print to [their] knowledge', agree that (ortho)silicic acid ($\text{Si}(\text{OH})_4$) is the only bioavailable Si species, coupled to a surprising comment about our 'understanding of the chemistry of silicon' and their own discovery of this 'sacred and scientific truth'. Again, a closer reading of our collective works easily dispels their notion. For instance, Ma *et al.*'s seminal work in the discovery and characterization of Lsi1 and Lsi2 as $\text{Si}(\text{OH})_4$ transporters (Ma *et al.*, 2006, 2007) demonstrates this understanding, as do the numerous studies by our groups on Si transport and accumulation. By contrast, Exley and Guerriero attempt to take credit for this critical understanding by citing their own work, overlooking decades of foundational work by others, many of which we highlight in our Tansley review.

A special note on Guerriero *et al.* (2018) is warranted, as (1) the authors give it much weight in their arguments, claiming that it 'supersedes and augments' our model, and (2) it was indeed, we readily admit, not cited in our review. The reasons for this are as

follows and pertain not to the chemistry of Si but to fundamentals of plant pathology. In short, the scientific approach used in Guerriero *et al.* (2018) violates numerous basic tenets of plant pathology, which renders the conclusions with respect to the mode of action of Si and biotic stress *deeply* problematic. In their work, the authors propose the ‘existence of a synergistic mechanical protection by callose and silica against pathogens in horsetail’ without showing any direct interaction between these. Indeed, the model rests on the serendipitous observation of a ‘fungal infection’ on dying or highly stressed plants, without any attempt to identify the fungus or to fulfill Koch’s postulates, the required procedure to establish the pathogenicity of an organism. The most common fungi reported on horsetail are *Ascochyta equiseti* (Polec & Ruskiewicz-Michalska, 2011) and *Stammaria personii* (Haelewaters *et al.*, 2018), both of which are observed on dead or dying material, which classifies their presence as saprophytic or opportunistic infections, as is the case in Guerriero *et al.* (2018), based on their own Fig. 1 and description. In addition, there is no inoculation treatment on either Si-supplied or -non-supplied plants, rendering their claims of ‘investigating . . . resistant plants’ scientifically inaccurate. The final deficiency in their model is presented in their Fig. 8, where, after describing (evidently unbeknownst to them) a case of saprophytic fungal infection, they propose a model whereby a haustorium, a fungal structure unique to biotrophic pathogens attacking living tissues exclusively, is involved. To date, no biotrophic pathogen has ever been reported to attack horsetail. We refer the reader to Table 2 of our Tansley review to appreciate the importance of this distinction. Based on these inadequacies, the proposed model by Guerriero *et al.* (2018) is not only incongruent with that proposed by Coskun *et al.* (2019), but is flawed in its fundamental understanding of fungal pathogen lifestyles, a point discussed at length in our review. Therefore, for Exley and Guerriero to claim any precedence over our model based on such work is unfounded.

Regarding the issue of describing Si-permeable aquaporins as ‘silicon transporters’, Exley and Guerriero engage in a simple matter of semantics, despite the fact that ‘aquaporins’ are widely accepted in the literature as transporters; indeed, any integral transmembrane protein involved in the transport of substrates across membranes is a transporter. Aquaporins, like any other class of membrane channels, are thermodynamically passive transporters, not to be confused with carriers or (primary/secondary) active transporters (White, 2012). We have previously described that Si uptake is cooperatively mediated by Lsi1, a passive channel-type transporter, and Lsi2, an active transporter. To make such claims as Exley and Guerriero have, exposes some of their own basic misconceptions in the field of transport physiology. Even the paper cited by the authors (Marron *et al.*, 2016), in support of their argument, describes Lsi1 and related mammalian aquaporins as ‘passive transporters’, discrediting the authors’ argument. Definitions aside, it is evident that the authors do not acknowledge the importance of the selectivity filter of channels, which bestows, in this case, aquaporins with a high level of discrimination against various substrates, including water and Si (Maurel *et al.*, 2008; Hove & Bhawe, 2011). The authors appear to stand alone in their belief that water fluxes and transpiration dictate Si absorption, and in their refusal to recognize the high degree of


selectivity of Lsi1, as well as Lsi2, in determining a plant’s ability to absorb Si, in spite of all the evidence related to Figs 2 and 3 of our Tansley review.

To Exley and Guerriero’s credit, we did incorrectly refer to aluminum as a ‘heavy metal’, a term that is indeed used too frequently with too much latitude in the biological literature. While we thank them for bringing this to our attention (and have made the necessary corrections), it is disappointing that they should seize on such a minor oversight as an opportunity to denigrate the credibility of the authors. In a further attempt to discredit, Exley and Guerriero claim that we ‘have little experience in silicon and abiotic stress’. Dr Ma’s record as a leading expert in Si and aluminum biology and abiotic stress is recognized worldwide (see for example, Ma, 2004; Xia *et al.*, 2010). Dr Kronzucker is also a world-renowned expert in abiotic stress, with recent articles on Si and abiotic stress standing out and being lauded as ‘paradigm-shifting’ by journal editors and colleagues (Coskun *et al.*, 2016; Flam-Shepherd *et al.*, 2018; Santa-Maria & Rubio, 2018). Typically, in our view, such *ad hominem* assaults are counterproductive to scientific discourse.

In summary, it appears that Exley and Guerriero’s major criticism of our Tansley review is based on a perceived lack of appreciation of the process of biosilicification and of their work. As we acknowledged here and in our Tansley review, biosilicification remains an interesting, although scantily understood, phenomenon, and we will continue to observe it closely. However, to afford it primacy over all other aspects of Si biology would be undermining the overwhelming evidence in support of the critical nature of the selectivity of Si transporters in the plant kingdom.

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