Persistence of Ecologically Similar Fungi in a Restricted Floral Niche

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Abstract

Fungi in the genera *Knoxdaviesia* and *Sporothrix* dominate fungal communities within *Protea* flowerheads and seed cones (infructescences). Despite similar ecologies, they show strong host recurrence and often occupy the same individual infructescence. Differences in host chemistry explain their host consistency, but the factors that allow co-occupancy of multiple species within individual infructescences are unknown. *Sporothrix splendens* and *K. proteae* often grow on different senescent tissue types within infructescences of their *P. repens* host, indicating that substrate-related differences aid their co-occupancy. *Sporothrix phasma* and *K. capensis* grow on the same tissues of *P. neriifolia* suggesting neutral competitive abilities. Here we test the hypothesis that differences in host-tissues dictate competitive abilities of these fungi and explain their co-occupancy of this spatially restricted niche. Media were prepared from infructescence bases, bracts, seeds, or pollen presenters of *P. neriifolia* and *P. repens*. As expected, *K. capensis* was unable to grow on seeds whilst *S. phasma* could. As hypothesised, *K. capensis* and *S. phasma* had equal competitive abilities on pollen presenters, explaining their co-occupancy of this resource. Growth of *K. proteae* was significantly enhanced on pollen presenters while that of *S. splendens* was the same as the control. *Knoxdavesia proteae* grew significantly faster than *S. splendens* on all tissue types. Despite this, *S. splendens* was a superior competitor on all tissue types. For *K. proteae* to co-occupy infructescences with *S. splendens* for extended periods, it likely needs to colonize pollen presenters before the arrival of *S. splendens* and may consequently depend on different spore vectors.

Introduction

A high diversity of saprobic fungi colonizes senescent plant materials such as leaf litter and wood (Kodsueb *et al.* 2008) and form integral parts of ecosystem processes such as decomposition and nutrient cycling (Kumar *et al.* 2012). Many factors contribute to the maintenance of high saprobe diversity on senescent plant parts, including differences in chemical composition and physical structure of different hosts (Lodge *et al.* 1997; Mille-Lindblom *et al.* 2006; Paulus *et al.* 2006; Hyde *et al.* 2007; Osono 2011; Wolfe and Pringle 2012; Tedersso *et al.* 2013). However, numerous saprobic fungal species also colonise substrates that originate from a single host and thrive in very close proximity. High numbers of fungal species on senescent parts of the same host may be maintained by differences in nutrient source usage, differences in colonising times related to differences in spore dispersal and differential competitive abilities, all of which may drive succession (Hyde *et al.* 2007; Bleiker and Six 2009; Zhao *et al.* 2013; Kubicek *et al.* 2014). In addition, plant structures usually contain many different tissue types that may each be exploited by different fungi (Hyde *et al.* 2007; Paulus *et al.* 2003a, b).

After pollination, the outer involucral bracts of the colourful inflorescences of *Protea* L. (Proteaceae) enclose the old flowers in compact cone-like infructescences (Fig. 1). Infructescences persist for several years as above-ground seed storage organs (Rebelo 1995) with living tissues comprising only the disc-like bases and fertile seeds (Fig. 1). The rest of these structures consist of dead material in the form of hundreds of infertile seeds, bracts, and senescent flower parts (including tepals and pollen presenters).

Infructescences provide a moist, protected environment (Roets *et al.* 2012) in which numerous micro-fungi (Marais & Wingfield 1994, 2001; Lee *et al.* 2003, 2005) and arthropods (Coetzee and Giliomee 1987a, b; Roets *et al.* 2006b) thrive. They represent a unique aerial niche for saprobic fungi that house communities that are strongly divergent from those on senescent *Protea* twigs and leaves (Lee *et al.* 2003, 2004; Marincowitz *et al*. 2008).

Fungi in the genera *Knoxdaviesia* M.J. Wingf., Van Wyk & Marasas (Microascales) and *Sporothrix* M.J. Wingf., Van Wyk & Marasas (Ophiostomatales) dominate dead floral parts in *Protea* infructescences (Marais and Wingfield 1994, 2001; Lee *et al.* 2005; Roets *et al.* 2005). Three species of *Knoxdaviesia* (Wingfield *et al*. 1988; Wingfield and Van Wyk 1993; Crous *et al.* 2012) and 11 species of *Sporothrix* (Marais and Wingfield 2001; Roets *et al.* 2006a, 2008, 2010; Marais and Wingfield 1994, 1997; Ngubane *et al.* 2017) have been described from this niche. These show various degrees of host recurrence. For example, *K. proteae* M.J. Wingf., P.S. van Wyk & Marasas is exclusive to *P. repens* L. (Roets *et al.* 2009). In contrast, the closely related *K. capensis* M.J Wingf. & P.S. van Wyk is common on other host species such as *P. neriifolia* R. Br. and *P. lauriifolia* Thunb. and is only very rarely found on *P. repens* (Roets *et al.* 2009; Aylward *et al*. 2015b). *Sporothrix splendens* G.J. Marais & M.J. Wingf. is nearly omnipresent within infructescences of *P. repens*, but has occasionally also been found on other hosts such as *P. neriifolia* (Theron-De Bruin *et al.* 2018). In contrast, *S. phasma* (Roets, Z.W. de Beer & M.J. Wingf.) Z.W. de Beer, T.A. Duong & M.J. Wingf. is found on species such as *P. neriifolia* and *P. lauriifolia*, but never on *P. repens* (Roets *et al*. 2009). These strong host recurrence patterns are maintained even when the hosts grow sympatrically. It may be ascribed to differences in temperature and humidity within infructescences, differences in chemical composition of different *Protea* species, host-induced differences in competitive abilities and the actions of their spore vectors (Roets *et al.* 2012, Mukwevho *et al.* 2020, 2021).

Knoxdavesia and *Sporothrix* produce sticky spores adapted to arthropod vectored dispersal (Malloch and Blackwell 1993). Various mites are the primary vectors of all *Protea*-associated species (Roets *et al.* 2007, 2009) and some may even have mutualistic associations with their fungal partners (Roets *et al.* 2007; Theron-De Bruin *et al.* 2018). For long-distance dispersal, the mites are phoretic on *Protea*-pollinating beetles (Roets *et al.* 2008) and birds (Theron-De Bruin *et al.* 2018). This vectored mode of dispersal not only ensures that these fungi can colonize new flowers over vast distances (Aylward *et al.* 2014, 2015a, 2016a; Ngubane *et al*. 2018), but can do so very early on, as soon as the very first flowers open within individual inflorescences (Theron-De Bruin *et al.* 2018). This gives these competitively weak fungal species an advantage over other saprobic fungi for resources in the restricted infructescence environment (Mukwevho *et al.* 2021).

Multiple *Knoxdavesia* and *Sporothrix* species often grow within the same individual infructescence and even sporulate concurrently (Roets *et al.* 2005, 2013). Within *P. neriifolia* infructescences, *S. phasma* grows on seeds near the base, but also towards the tips of old pollen presenters (Roets *et al.* 2006a; Theron-De Bruin *et al.* 2018; Fig. 1). *Knoxdaviesia capensis* is confined to pollen presenters (Aylward & Roets *pers. observ*.). No *Knoxdavesia* and *Sporothrix* have been found on the hard *P. neriifolia* infructescence bases, except if these are damaged by boring insects (Roets *et al.* 2006). *Knoxdavesia* and

Sporothrix in *P. repens* infructescences seem to be more segregated in space, as *K. proteae* is only found on pollen presenters, while *S. splendens* is usually confined to the seeds (Roets *et al.* 2013). However, *S. splendens* has also been recovered from the involucral bracts that enclose the other floral parts (Fig. 1) and, occasionally, pollen presenters of *P. repens* (Human, Ngubane & Roets, *pers. observ.*). This co-occurrence of ecologically similar fungi (in terms of saprobic lifestyle, host recurrence and spore dispersal agents) within the restricted area provided within a single *Protea* infructescence is intriguing. Possible explanations for this could include differences in position where the different fungi are initially inoculated onto different tissues within infructescences, or that the different fungi may have different competitive abilities on the different tissue types. In terms of the former hypothesis, it is possible that some fungal species outcompete other species only on specific tissue types within infructescences. In terms of the latter hypothesis, inoculation on different tissue types within infructescences is only likely to happen when different fungal species have different main spore vectors.

Protea-associated *Sporothrix* species are mainly dispersed by mites in the genera *Tarsonemus* Canestrini and Fonzago, *Glycyphagus* Hering and *Proctolaelaps* Ryke between open flowers and by *Trichaouropoda* Berlese mites between infructescences and open flowers (Roets *et al.* 2007, 2009, Theron-De Bruin *et al.* 2018). Main spore vectors for the *Knoxdavesia* species in this system are not well-studied, but current evidence suggest that they are mainly dispersed by the *Trichaouropoda* mites between infructescences and open flowers (Roets *et al.* 2011). Secondary vectors of the *Tarsonemus*, *Glycyphagus* and *Proctolaelaps* mites include various *Protea*-pollinating insects and birds, while *Trichaouropoda* mites have only been collected from a single *Protea*-pollinating beetle species (Roets *et al.* 2011; Theron-De Bruin *et al.* 2018).

The present study sets out to test the hypothesis that co-occupancy of individual *Protea* infructescences by ecologically similar fungi is due to differential competitive abilities on different tissue types within these structures. We test the competitive abilities of *S. splendens* and *K. proteae* on media prepared from bases, pollen presenters, unfertilised seeds and bracts of their usual *P. repens* host. Similarly, the competitive abilities of *S. phasma* and *K. capensis* on media prepared from the bases, pollen presenters and unfertilized seeds from their usual *P. neriifolia* host was tested. Based on field observations, it was expected that no fungi will be able to grow on media prepared from the bases of their hosts. On *P. neriifolia*, it was expected that *S. phasma* can grow on seeds and pollen presenters. *Knoxdavesia capensis* was expected to only grow on pollen presenters and perhaps on seeds, but if it could grow on seed, it will be outcompeted by *S. phasma* on this tissue type. As both species often co-occur on pollen presenters, it was expected that they will have similar competitive abilities on these structures. From observations on *P. repens*, it was expected that *S. splendens* will grow on all structures (except infructescence bases) and that *K. proteae* will only be able to grow on media prepared from pollen presenters. If *K. proteae* can grow on media prepared from seeds and bracts, it was expected that *S. splendens* would be a superior competitor. On pollen presenters, *S. splendens* was expected to be a superior competitor (Mukwevho *et al.* 2021). Any deviations from these expectations may point towards a possible role of different vectors in the dispersal of the various fungal species.

Methods And Materials

Collection of fungi and preparation of growth media

Fungi used in this study were the same species and isolates used in previously published fungal competition studies (Mukwevho *et al.* 2020, 2021). *Knoxdavesia proteae* (Stellenbosch Mountain (-33.9466; 18.8805)) and *S. splendens* (Betty's Bay (-34.3315 18.9925)) were collected from *P. repens* and *K. capensis* (Betty's Bay (-34.35495; 18.90135)) and *S. phasma* (Jonkershoek Nature Reserve (33˚59'24.5"S, 18˚57'25.2"E)) were collected from *P. neriifolia*. For growth media (following Roets *et al.* 2012 and Mukwevho *et al.* 2020), two-year-old infructescences of *P. repens* and *P. neriifolia* were collected from the Jonkershoek Nature Reserve and air-dried in the laboratory until they opened *ca*. 3 weeks later. Hereafter infructescences were separated into the infructescence base (receptacle for bracts and florets), the bracts (for *P. repens* only, as the exposed and recurved bracts of *P. neriifolia* are not suitable for colonization by *Knoxdavesia* and *Sporothrix*), pollen presenters (including any remnants of tepals) and seeds. For media prepared from seeds, all fertile seeds, identified by their larger size (Theron de-Bruin *et al.* 2018), were removed as not to include antimicrobial compounds that they may contain into media. These separated dead floral parts were dried at 40˚C for 48 hours and ground into a fine powder using a milling machine (Monitoring and Control Laboratories (Pty) Ltd). One litre of water-based growth medium contained 300 ml prepared *Protea* tissue (powder) and 1.5 % MEA. Media was autoclaved at 115˚C for 20 min and poured into 90 mm petri dishes that acted as competition arenas (Mukwevho *et al*. 2021).

Fungal growth rates on different tissues

The growth of *Knoxdavesia* and *Sporothrix* was tested on media prepared from the different tissues following methods described in Roets *et al.* (2012). In short, plates were centrally inoculated with 5 mm diameter agar discs containing actively growing, 2-week-old hyphae of one of five different isolates of each of the four fungal species tested (n = 5 per tested species on the different media). As we were interested in the growth of the fungi on their usual hosts, *S. splendens* and *K. protea* were grown on tissues that originated from *P. repens* and *S. phasma* and *K. capensis* were grown on media prepared from *P. neriifolia*, respectively. In addition, all isolates were also grown on plates containing only MEA as a control. All inoculated plates were inverted and incubated at 25˚C in the dark. The diameter of each fungal colony on the various media was determined after 10 d of growth by calculating the average of two perpendicular diameter measurements. Growth for each fungal species on each of the test media was determined by calculating the mean radial growth (±standard error) of the five representative isolates of each of the four fungi. The radial growth for each fungus pair per host species on media from each tissue type were compared using linear models in R (R Development Core Team, 2013) after log transformation of the data. The model followed the formula: lm (Colony diameter ~ Tissue type + Fungal species + Tissue Type * Fungal species). Hereafter, a conservative Tukey post-hoc test in the R package *multcomp* was used to determine pairwise differences (Horthawn *et al*. 2020). Significant differences are reported when $P \leq 0.05$.

Differential competition between fungi on media prepared from different host tissues

A de Wit replacement series experimental design (Klepzig and Wilkens 1997; Klepzig 1998) was used to test the competition between *S. splendens* and *K. proteae* (on *P. repens* infructescence structures) and between *S. phasma* and *K. capensis* (on *P. neriifolia* infructescence structures) following a modified experimental procedure of Mukwevho *et al.* (2020, 2021). The two competing fungal species were introduced in a 90 mm diameter plate at different proportions of inoculum and left to compete for available space. Hereafter the total area occupied by each fungus was expressed as a log-linear function of its initial proportion inoculum. If both interacting species had similar competitive abilities, there would be no deviation from linearity. However, significant deviation from linearity for both species, one positive and the other negative, would indicate differential competition with one species dominating over the other. Inoculum covered disks (0.5 mm in diameter) of *Knoxdavesia* and *Sporothrix* were aseptically removed from the edges of actively growing fungal colonies and introduced face-down onto plates in a randomised block design (4 x 4 cm grid) following Mukwevho *et al.* (2020). Inoculation ratios used included: species A vs. species B: 0:1 (16 disks species B), 0.25:0.75 (4 disks sp. A and 12 disks sp. B), 0.5:0.5 (1:1) (8 disks sp. A and 8 disks sp. B), 0.75:0.25 (12 disks sp. A and 4 disks sp. B) and 1:0 (16 disks species A). The procedure was repeated for all five tests (5 different ratios) per pairwise species combination and replicated five times per tested medium type, each time using different isolates. Plates were incubated at 25°C in dark for ten days. Hereafter the areas occupied by each fungus were measured using image J software (LOCI, University of Wisconsin). Deviations from linearity were calculated by performing an analysis of variance (ANOVA) on log-transformed means of the area data (Wilson & Lindow 1994) in R (R Development Core Team 2013). Relative crowding coefficients (RCC) were also calculated for all pairwise combinations as [(mean area of species A at 1:1)/(mean area of species A at 1:0)] and [(mean area of species B at 1:1)/(mean area of species B at 1:0)]. The interacting species with a higher coefficient is considered as dominant. If the product of the coefficients was one, then fungal competition was neutral. If the product of the coefficients was less than one, then the fungi negatively affect each other and if it was greater than one the taxa benefit from growing together (Willey and Rao 1980).

Results

Fungal growth rates on different plant tissues

The model for fungal growth rate of *K. proteae* and *S. splendens* on the different media types prepared from *P. repens* hosts were significant (F-statistic: 930.9 on 9 and 50 df, p-value: < 0.001). Similarly, these factors had a significant influence on the growth rate of *G. capensis* and *S. phasma* on media prepared from *P. neriifolia* tissues (F-statistic: 237.6 on 7 and 40 df, p-value: < 0.001). Post hoc tests showed that the two fungal species per host plant always differed in their growth rates on the different tissues with the two *Knoxdavesia* species always outgrowing the *Sporothrix* species from their respective hosts (Fig. 2). *Knoxdaviesia proteae* did not grow on media prepared from *P. repens* bases (Fig. 2). It also had a significantly reduced growth rate on media prepared from bracts of this species. It grew well on media prepared from seeds and pollen presenters. As described in Roets *et al.* (2012), *K. proteae* produced denser hyphae when growing on media prepared from *P. repens* pollen presenters than on media prepared

from the seeds and bracts. *Sporothrix splendens* also failed to grow on media prepared from *P. repens* infructescence bases and its growth rate was supressed on most other infructescence structures. It grew at similar rates on all tissue types of *P. repens* (Fig. 2) and like *K. proteae*, it produced denser hyphae on pollen presenter media than on media prepared from the seeds and bracts. *Knoxdaviesia capensis* could only grow on media prepared from pollen presenters of *P. neriifolia* (Fig. 2). The growth of *S. phasma* was significantly inhibited on media prepared from *P. neriifolia* infructescence bases. It grew well on seed media, but optimally on pollen presenter media, where it also had the densest colony morphology.

Differential competition between fungi on media prepared from different host tissues

Differential competition was detected between *K. proteae* and *S. splendens* on *P. repens* pollen presenters, seeds and bracts (Table 1). *Sporothrix splendens* was always the strongest competitor, as was confirmed also by their relative crowding coefficients. Both fungal species were also always at a disadvantage when competing, as indicated by the product of their respective relative crowding coefficients. Neither *K. capensis*, nor *S. phasma*, was a superior competitor when growing on media prepared from *P. neriifolia* pollen presenters (Table 1). In addition, both species were at a disadvantage when competing on this medium.

Discussion

Here we provide evidence that factors related to differences in host infructescence tissues help maintain co-occupancy of multiple fungal species with similar ecologies within individual *Protea* infructescences. This builds on previous data by showing that different senescent structures in plants may each be exploited differentially by different fungal species, leading to enhanced overall biodiversity levels (Paulus *et al.* 2003a, b; Hyde *et al.* 2007). However, differences in infructescence tissues did not explain co-occupancy of all fungi tested, and the actions of spore-vectors may also have a significant influence on the persistence of comparatively weaker taxa within this restricted niche. The immense diversity of saprobes in general may therefore be explained by combinations of numerous factors that include host related differences (Hyde *et al.* 2007; Roets *et al.* 2012; Mukwevho *et al*. 2020), differences in substrate colonisation times and differential competitive abilities (Hyde *et al.* 2007; Bleiker and Six 2009; Zhao *et al.* 2013; Kubicek *et al.* 2014).

Results of experimental studies presented here mostly reflected colonization patterns observed in the field. For example, the lack of growth of most fungi on media prepared from infructescence bases was expected from observational studies (Roets *et al.* 2006; 2013). *Sporothrix splendens* could grow on all parts of *P. repens* infructescences (except infructescence bases) and *K. capensis* was only able to grow on media prepared from pollen presenters of *P. neriifolia*. *Sporothrix phasma*, the species with which *K. capensis* mostly shares space within individual *P. neriifolia* infructescences, was able to grow on both the non-fertile seeds and the pollen presenters, confirming field observations (Roets *et al.* 2006; Theron-De Bruin *et al.* 2018). *Sporothrix phasma* and *K. capensis* therefore only compete for resources on pollen presenters, where they have a neutral competitive interaction. A previous study also indicated that both

species are also able to capture uncolonized space at similar rates when inoculated at the same point (*i.e.,* when using the same spore vectors), but importantly, they can maintain this space, as they are not able to overgrow each other (Mukwevho *et al*. 2020). These data thus neatly explain their co-existence on this *P. neriifolia* resource.

In contrast to the other species evaluated here, *K. proteae* was able to grow on media prepared from infructescence structures of *P. repens* with which it is not known to be associated in field-collected infructescences (*i.e.,* non-viable seeds and bracts). As *S. splendens* can also grow on all structures, *K. proteae* will be in direct competition with *S. splendens* on this host. It is a significantly weaker competitor than *S. splendens* on all these structures, thereby excluding differential competitive abilities as explanation for their co-existence in individual *P. repens* infructescences. Even though a previous study indicated that *K. capensis* can capture at least some space on pollen presenter media when in competition with *S. splendens*, *S. splendens* would likely eventually overgrow *K. proteae* colonies (Mukwevho *et al.* 2020). For *K. proteae* to maintain area within *P. repens* infructescences for extended periods in the presence of *S. splendens*, it would need to exploit different available nutrient sources than *S. splendens*, or it would need to capture initial space rapidly before colonization by *S. splendens*. *Knoxdavesia proteae* is known to colonise *P. repens* infructescences at least as early as *S. splendens* and can use *Protea* nectar sugars as main source when no senescent floral parts are available yet (Aylward *et al.* 2017). Nutrient sources for *S. splendens* are unknown, but likely include these nectar sugars (Rodriguez-Del Valle *et al.* 1983), excluding differential nutrient resource usage as explanation for co-occupancy. *Knoxdavesia protea* is a much faster coloniser of pollen presenter media than *S. splendens* in the absence of the latter (Roets *et al.* 2012), but to colonise pollen presenters sooner than *S. splendens* it would likely also need to rely on a different main vector. *Sporothrix splendens* is mainly dispersed between inflorescences (flowers) by mites in the genera *Tarsonemus*, *Glycyphagus* and *Proctolaelaps* on *Protea* pollinating beetles and birds (Roets *et al*. 2007, 2009; Theron de-Bruin *et al.* 2018). They may be dispersed from infructescences to inflorescences on *Tarsonemus*, *Proctolaelaps* and a *Trichaouropoda* species vectored by a *Protea* pollinating beetle (*Genuchus hottentottus*) (Roets *et al*. 2007, 2009). Although significantly understudied compared to *Sporothrix* from this environment, the main vector for *K. proteae* is thought to be the same *Trichauropoda* mite, but it has also been detected on many other arthropod taxa in infructescences (Roets *et al.* 2011). Future studies may therefore need to re-examine the main vectors for *K. proteae* considering the evidence presented here.

Interactions with other microbes may help shape the co-occurrence of fungi in individual infructescences. Most other fungal species likely arrive within infructescences after colonization by *Knoxdavesia* and *Sporothrix*, and these may have contrasting impacts on the persistence of *Knoxdavesia* and *Sporothrix* at a later stage (Mukwevho *et al*. 2021). Interactions of *Knoxdavesia* and *Sporothrix* have been evaluated with very few other fungal taxa to date and only on pollen presenter media. It is possible that an entire network of differential interactions is needed to help maintain the co-existence of multiple fungi in this niche. In addition to fungi, bacteria also abound within these structures, and they can colonise infructescences at a very early stage (Human *et al.* 2016, 2018, 2021). Many *Protea*-associated species produce antifungal agents such as fungichromin and actiphenol that inhibit the growth of both

Knoxdavesia and *Sporothrix* and other saprobes (Human *et al.* 2016). It was shown that the *Protea*-associated *Knoxdavesia* and *Sporothrix* varied in their sensitivity towards these components (Human *et al.* 2016) and even though no benefit to *Knoxdavesia* could be deduced, it is possible that fungus-bacterial interactions help maintain co-occupancy of multiple *Knoxdavesia* and *Sporothrix* taxa in individual *Protea* infructescences.

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Data is available from FR.

Code availability

Not applicable.

Authors' contributions

V.O.M: Study design, data collection, laboratory work, statistical analyses, writing of first draft; F.R., L.L.D: Study concept, study design, acquired funding, statistical analyses, writing of manuscript

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Table

Table 1: ANOVA statistics for tests of deviation from linearity in relationships between the areas occupied by competing fungal species in a de Wit replacement series on media prepared from senescent tissues within infructescences of *P. repens* and *P. neriifolia*. The competitive influence of each separate species in an interacting pair, or relative crowding coefficient (RCC) and the product of the RCC values of the interacting pairs (in brackets) are also provided. *df* = Degrees of freedom, SS = Sum of squares, MS = Mean square

Comparison	Source	*df*	SS	MS	F value	P value	RCC
On *P. repens* pollen presenters							
S. splendens vs K. proteae							(0.257)
S. splendens area	Proportion	3	0.09	0.030	22.54	<0.001	0.675
	Residual	11	0.01	0.001			
K. proteae area	Proportion	3	0.16	0.055	8.33	0.005	0.381
	Residual	10	0.07	0.007			
On *P. repens* bracts							
K. proteae vs *S. splendens*							(0.212)
K. proteae area	proportion	3	0.50	0.167	4.80	0.034	0.296
	Residual	8	0.28	0.035			
S. splendens area	proportion	3	1.03	0.344	457.30	<0.001	0.717
	Residual	8	0.01	0.001			
On *P. repens* seeds							
K. proteae vs *S. splendens*							(0.175)
K. proteae area	Proportion	3	0.65	0.218	17.32	<0.001	0.335
	Residual	10	0.13	0.013			
S. splendens area	Proportion	3	1.59	0.529	177.6	<0.001	0.523
	Residual	10	0.03	0.003			
On *P. neriifolia* pollen presenters							
S. phasma vs *K. capensis*							(0.222)
S. phasma area	Proportion	1	0.01	0.002	0.35	0.567	0.041
	Residual	12	0.07	0.006			
K. capensis area	Proportion	1	0.00	0.000	0.03	0.864	0.547
	Residual	10	0.07	0.006			

Figures

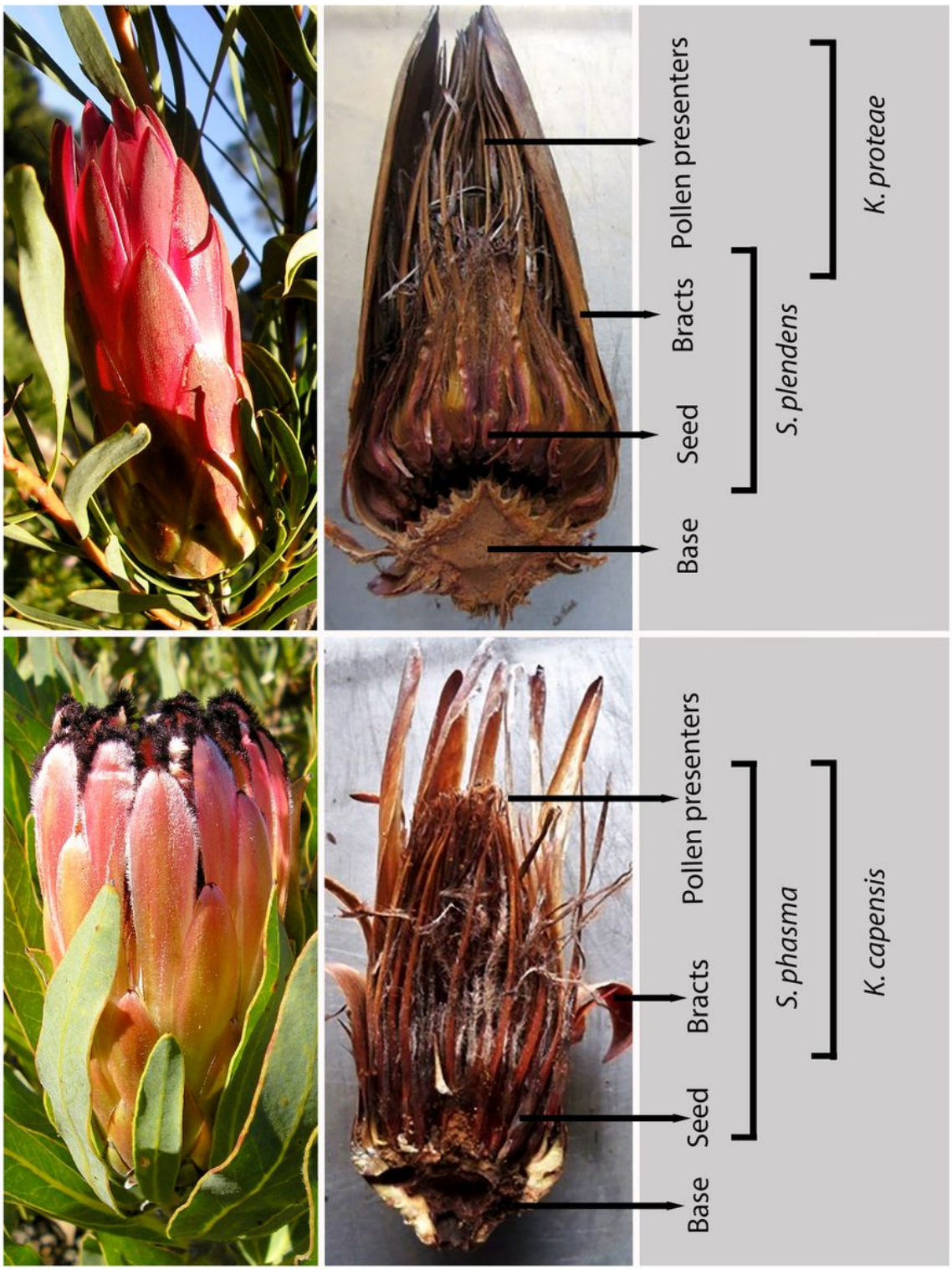

Figure 1

(Top left) - Inflorescence of *Protea repens*, (Top middle) - cross-section of *Protea repens* infructescence showing the hard receptacle (base) at the bottom with attached seeds, extended pollen presenters, and the surrounding bracts. Areas occupied by *S. splendens* (seeds, bracts and occasionally also pollen presenters) and *K. proteae* (pollen presenters) are indicated at the top right. (Bottom left) - inflorescence of *Protea neriifolia*, (Bottom middle) - cross-section of *P. neriifolia* infructuscence showing the hard base

at the bottom with attached seeds, extended pollen presenters, and the recurved bracts. Areas occupied by *S. phasma* (seeds and pollen presenters) and *K. capensis* (pollen presenters) are indicated at the top right.

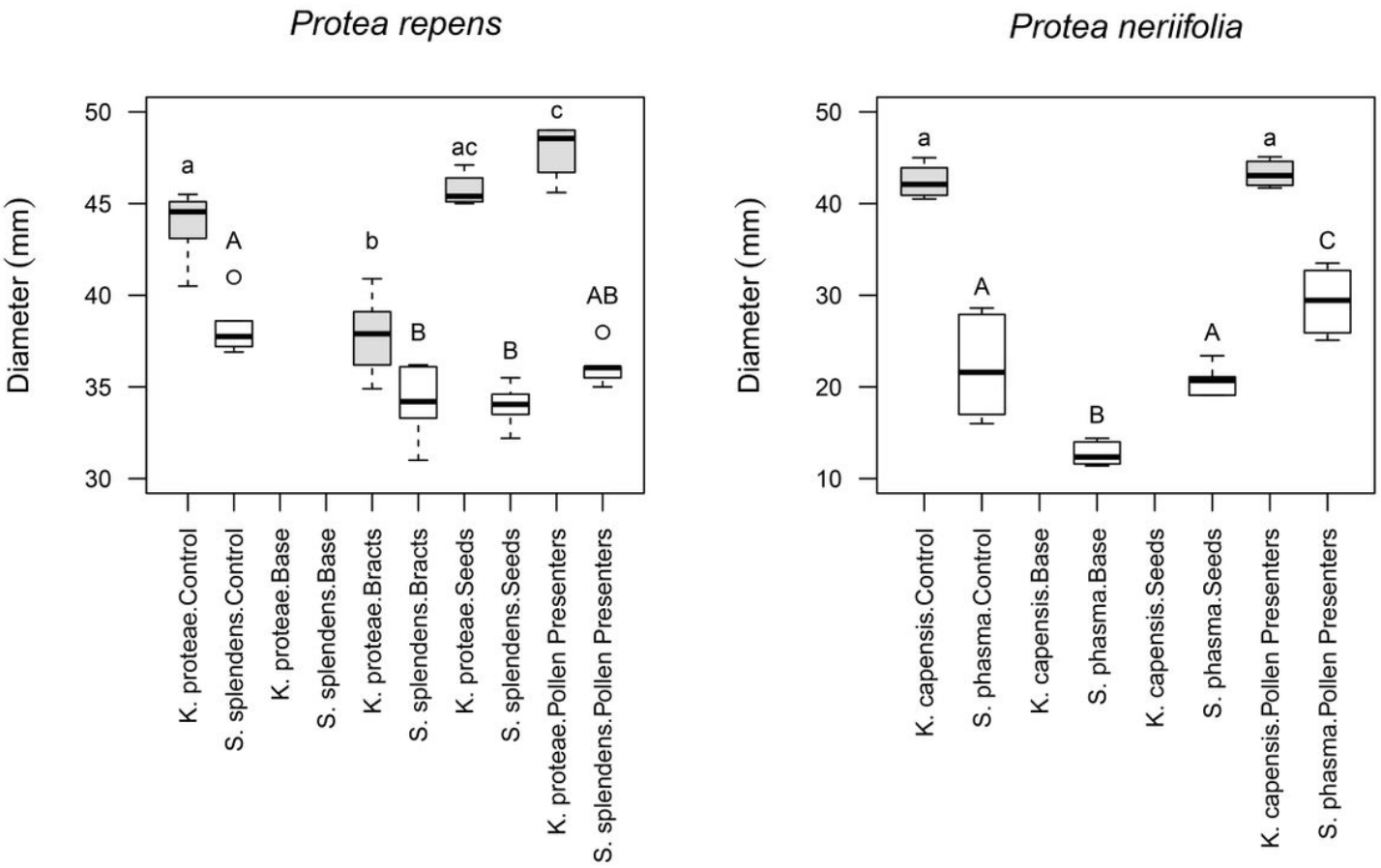

Figure 2

Mean radial growth (mm diameter after 10 d at 25˚C) of *K. proteae*, *K. capensis* (in grey bars) and *S. phasma* and *S. splendens* (in white bars) on media prepared from senescent tissues from the infructescences of *P. repens* (left) *P. neriifolia* (right) respectively. Controls consisted of malt extract agar only. Boxes indicate 25–75% data range, whiskers indicate 1.5 times the interquartile range and dots represent outliers. Different letters above bars denote significant differences per media type for the respective fungal species (lower case for the two *Knoxdavesia* species and upper case for the two *Sporothrix* species). For both comparisons, the *Knoxdavesia* species always had significantly larger colony diameters than the *Sporothrix* species on all tissue types (not indicated on the graphs).