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 apparently 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 tissue types 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, appearing to explain 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. Knoxdaviesia 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.

Keywords Fungal diversity · Interspecific competition · Knoxdaviesia · Spore vector · Sporothrix

Introduction

A high diversity of saprobic fungi colonizes senescent plant materials such as leaf litter and wood (Kodsbueb 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; Pauls 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

Fig. 1 (Top left)—Inflorescence of Protea repens, (Top right)—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. (Bottom left)—inflorescence of Protea neriifolia. (Bottom right)—cross-section of P. neriifolia infructescence 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.
of hundreds of infertile seeds, bracts, and senescent flower parts (including tepals and pollen presenters). Infuctescences provide a moist, protected environment (Roets et al. 2012) in which numerous microfungi (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 (Microscales) and Sporothrix M.J. Wingf., Van Wyk & Marasas (Ophiostomatales) dominate dead floral parts in Protea infuchescences (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, 1994, 1997); Roets et al. 2006a, 2008, 2010; Ngubane et al. 2018) 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. 2009a, b). 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. (Roets et al. 2006a, b, c). Sporothrix splendens G.J. Marais & M.J. Wingf. is nearly omnipresent within infuctescences 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. 2009a, b). These strong host recurrence patterns are maintained even when the hosts grow sympatrically. Host recurrence patterns may be ascribed to differences in temperature and humidity within infuctescences, 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; Mukwewho et al. 2020, 2021).

Knoxdaviesia and Sporothrix produce sticky spores adapted to dispersal by arthropod vectors (Malloch and Blackwell 1993). Various mites are seemingly the primary vectors of all Protea-associated species (Roets et al. 2007, 2009a, b) and some mites 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 vector mode of dispersal not only ensures that these fungi can colonize new flowers over vast distances (Aylward et al. 2014a, b, 2015a, 2016; 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 infuctescence environment (Mukwewho et al. 2021).

Multiple Knoxdaviesia and Sporothrix species often grow within the same individual infuctescence and even sporulate concurrently (Roets et al. 2005, 2013). Within P. neriifolia infuctescences, S. phasma grows on unfertilized 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 Knoxdaviesia and Sporothrix have ever been found on the hard P. neriifolia infuctescence bases, except if these are damaged by boring insects and then also only by S. proea-sedis and S. gemellus (Roets et al. 2006a, b, c). Knoxdaviesia and Sporothrix in P. repens infuctescences seem to be more segregated in space, as K. proteae is only found on pollen presenters, while S. splendens is usually confined to the unfertilized 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 infuctescence is intriguing. Possible explanations for this could include differences in position where the different fungi are initially inoculated onto different tissues within infuctescences, or that the different fungi may have different competitive abilities on the different tissue types and under different environmental conditions. In terms of the former hypothesis, it is possible that some fungal species outcompete other species only
on specific tissue types within infructescences under certain conditions. In terms of the latter hypothesis, inoculation on different tissue types within infructescences is only likely to happen when different fungal species have different spore vectors, possible including arthropod taxa not identified as primary or secondary spore vectors yet.

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, 2009a, b; 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 none of the tested fungi will be able to grow on media prepared from the bases of their hosts as these fungi have never been observed to grow on these, even if these were suffering from insect damage. On P. neriifolia, it was expected that S. phasma can grow on unfertilized seeds and pollen presenters. Knoxdavesia capensis was expected to only grow on pollen presenters and perhaps on unfertilized 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 unfertilized 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 the abiotic environment or additional spore vectors than those currently known in the dispersal of the different 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° 56' 47.76"S, 18° 52' 49.89"E)) and S. splendens (Betty’s Bay (34° 19' 53.4"S, 18° 59' 33"E)) were collected from P. repens and K. capensis (Betty’s Bay (34° 21' 17.82"S, 18° 54' 4.86"E)) and S. phasma (Jonkershoek Nature Reserve (33°59′24.5″S, 18°57′25.2″E)) were collected from P. neriifolia. For growth media, ca. six month-to-one year-old infructescences of P. repens and P. neriifolia were collected from the Jonkershoek Nature Reserve. Protea inflorescences take one year to mature from the bud stage to the open flower stage which lasts for another month or two before the involucral bracts start to close again. Only hereafter do the pollen presenters start to become senescent and the Protea-associated Knoxdavesia and Sporothrix proliferate within infructescences. Collected infructescences were 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 do not support the growth of Knoxdavesia and Sporothrix in vivo. These separated dead floral parts were dried at 40 °C for 48 h and ground into a fine powder using a milling machine (Monitoring and Control Laboratories (Pty) Ltd). Following Roets et al. (2012) and Mukwevho et al. (2020), one litre of water-based growth medium contained 300 ml prepared Protea tissue (powder) and 1.5% malt extract agar MEA (Biolab, Biolab, Midrand, South Africa). Control plates contained only 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).

Differential competition between fungi on media prepared from different host tissues

A de Wit replacement series experimental design (Kleczwig and Wilkens 1997; Kleczwig 1998) was used to test the competition between S. splendens and K. proteae (on P. repens infructescence tissues) and between S. phasma and K. capensis (on P. neriifolia infructescence tissues) 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 × 4 cm grid, containing 16 1 cm3 grid cells, that was drawn on the back of the Petri dish) 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 experiment with all five tests (5 different ratios) was replicated five times per tested medium type and per pairwise species combination, each replicate using different isolates of the species pair. Therefore, the entire experiment was replicated 5 times per species combination per media type, with each species combination/pairing consisting of two different competing 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 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 two fungal species associated with different host plants 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). *Knoxdavesia 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 unfertilized seeds and pollen presenters. As described in Roets et al. (2012), *K. proteae* produced denser hyphae when growing times the interquartile range and dots represent outliers. Different letters above bars denote significant differences per medium type for the respective fungal species (lower case for *Knoxdavesia* and upper case for *Sporothrix*). For both comparisons, the *Knoxdavesia* species always had significantly larger colony diameters than the *Sporothrix* species on all tissue types (data not shown).

![Fig. 2](image-url)

**Fig. 2** Mean radial growth (mm diameter after 10 d at 25 °C) of *Knoxdavesia proteae*, *Knoxdavesia capensis* (in grey bars) and *Sporothrix phasma* and *Sporothrix splendens* (in white bars) on media prepared from senescent tissues from the infructescences of *Protea repens* (left) and *Protea neriifolia* (right) respectively. Controls consisted of malt extract agar only. Boxes indicate 95% data range, whiskers indicate 1.5
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 suppressed 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. *Knox-daviesia 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, unfertilized 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.

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**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 *Protea repens* and *Protea neriifolia*

| Comparison                      | Source       | df | SS   | MS   | F value | P value | RCC  | (0.257)  |
|---------------------------------|-------------|----|------|------|---------|---------|------|----------|
| **On *P. repens* pollen presenters** |             |    |      |      |         |         |      |          |
| *S. splendens* vs *K. proteae*  |             |    |      |      |         |         |      |          |
| *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*  |             |    |      |      |         |         |      |          |
| *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*  |             |    |      |      |         |         |      |          |
| *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*    |             |    |      |      |         |         |      |          |
| *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|         |         |      |          |
Discussion

Here we provide evidence that factors related to differences in host infructescence tissues may 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 or environmental conditions 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, difference in microclimatic conditions within infructescences (Roets et al. 2012) 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. 2006a, b, c; 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. 2006a, b, c; 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., primary resource capture when using the same spore vectors), but importantly, they can maintain this space, as they are not able to overgrow each other (i.e., secondary resource capture, Mukwevho et al. 2020). These data thus appear to 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. 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 (i.e., secondary resource capture, 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. Nutrient usage have to date only been studied in Knoxdavesia proteae (Aylward et al. 2017). 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 may 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, 2009a, b; Theron-De Bruin et al. 2018). They may be dispersed from infructescences to inflorescences on Tarsonemus, Proctolaelaps and a Trichauropoda species vectored by a Protea pollinating beetle (Genuchus hottentottus) (Roets et al. 2007, 2009a, b). 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. However, if the vectors prove to be the same for both fungal species, factors such as microclimatic conditions within infructescences may aid the sympatric
co-existence of these. Changes in temperatures or humidity can, for example, modify growth rates of the fungi relative to each other (Roets et al. 2012) and change the outcome of the competition studies on different tissue types reported on 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.

A possible confounding factor in our study is the use of nutrient enriched media in the competition studies. A previous study by Roets et al. (2012) investigated the growth rates of the various fungi using nutrient enriched and nutrient deficient media and concluded that the outcome of growth studies may differ depending on the nutrient levels in the media. However, when grown on a nutrient deficient media, all fungal taxa formed little aerial mycelial biomass and increased their radial growth. This precludes the use of area occupied by a specific fungus as basis of measure for competitive ability and a different measure would be needed. Also, it is possible that the autoclaving process may alter the chemical composition of plant tissues that may influence the growth of the fungi tested. In a previous study, Roets et al. (2012) investigated the role of temperature and humidity on the growth of these Protea-associated fungi. All fungal species grew optimally at 25 °C and the growth rate of al increased with an increase in relative humidity. There was little variation in the relative performance of the different fungal species between the different temperatures, but some variation occurred between growth rates of the different species when varying the relative humidity. All these experiments were however conducted on artificial media, without incorporating possible host-tissue effects. Future studies should therefore combine the effects of unaltered host tissue-type, temperature, and relative humidity on the outcome of the fungal competition studies reported here in vivo.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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