# What drives grassland-forest boundaries? Assessing fire and frost effects on tree seedling survival and architecture

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## Abstract

1. Fire and frost represent two major hurdles for the persistence of trees in open grassy biomes and have both been proposed as drivers of grassland-forest boundaries in Africa. 2. We assess the response of young tree seedlings, which represent a vulnerable stage in tree recruitment, to traumatic fire and frost disturbances. 3. In a greenhouse experiment, we investigated how seedling traits predicted survival and resprouting ability in response to fire vs frost; we characterised survival strategies of seedlings in response to the two disturbances, and we documented how the architecture of surviving seedlings is affected by fire vs frost injury. 4. Survival rates were similar under both treatments. However, different species displayed different levels of sensitivity to fire and frost. Seedling survival was higher for older seedlings and seedlings with more basal leaves. Survivors of a fire event lost more biomass than the survivors of a frost event. However, the architecture of recovered fire and frost treated seedlings were mostly similar. Seedlings that recovered from fire and frost treatments were often shorter than those that had not been exposed to any disturbance, with multiple thin branches, which may increase vulnerability to the next frost or fire event. 5. Synthesis. Fire caused more severe aboveground damage compared to frost, suggesting that trees in these open grassland systems may be subjected to a seedling release bottleneck maintained by fire. However, the woody species composition will almost certainly be influenced by phenomena that affect the timing and frequency of seedling exposure to damage, as mortality was found to be dependent on seedling age. Therefore, changes in fire regime and climate (esp. changes that bring about less frost and reduced fire intensity and frequency) are likely to result in changes in the composition and the structure of the woody components of these systems.

## Introduction

Open grasslands have long been underappreciated for their biodiversity and ecosystem services (Carbutt et al., 2017) and many are under threat due to pressures such as agriculture, urbanisation and bush encroachment (the increase of indigenous woody cover in open grassy systems) (Hoekstra et al., 2005, Neke and Du Plessis, 2004, O'Connor and Kuyler, 2009, Skowno et al., 2017). Often mistaken for degraded woodlands and forests, grasslands have been underrepresented in conservation networks, and, more recently, have been targeted as potential sites for forest "rehabilitation" by forestation (Veldman et al., 2015), even though they are often ancient systems (predating anthropogenic activities) and harbour a rich diversity of species (Bond, 2016, Mucina et al., 2006).

Despite extensive research (e.g. Mills et al., 2006, Scholes and Archer, 1997, Higgins et al., 2000), there is still no real consensus about what determines the boundaries between grassland and wooded (savanna or forest) vegetation due to the complexity and interactive effects of the various factors affecting these systems. Demographic bottlenecks may be created by several factors that ultimately limit the presence of trees in grasslands (Midgley and Bond, 2001)(Figure 1).

Winter frost has been proposed as a major limiting factor for tree establishment in grasslands. Frost may

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be especially relevant in African grasslands, since African trees are of sub-tropical origin and are generally frost sensitive (Bredenkamp et al., 2002, Bannister and Lord, 2006). Paleontological evidence suggests that changes from warm interglacial phases to cooler glacial maxima were mirrored by shifts from woodland savanna to open grassland in Africa (Scott, 1982, Partridge et al., 1993). Elsewhere, frost events prevent tree recruitment by killing saplings or maintaining them in a suppressed state in 'frost traps' by repeated annual top-kill (Whitecross et al., 2012, Finckh et al., 2016, Holdo, 2007, Wakeling et al., 2012).

Fire may also play an essential role in excluding trees from grasslands since it is a common disturbance in open grasslands (Bond et al., 2003, Wakeling et al., 2012). Experimental work illustrates noticeable increases in woody biomass and cover with the exclusion of fire (Swaine et al., 1992, De Villiers and O'Connor, 2011, Bond and Keeley, 2005, Pinheiro et al., 2010). Similar to frost, fire may kill juvenile trees, or keep them suppressed in fire traps to the advantage of grasses, which are better adapted to survive top-kill (Higgins et al., 2000, Gignoux et al., 2009). Where fire keeps woody cover low enough to prevent grass exclusion through light competition, grasses can be effective competitors with trees for below-ground resources, especially at the juvenile stages (Morrison et al., 2019).

Whether fire or frost represent the major limiting factor for tree recruitment is widely debated (e.g. Maurin et al., 2014, Finckh et al., 2016), and it is often difficult to distinguish between the effects of fire and frost, since both often occur together in open grassy systems. Both mostly cause top-kill (i.e. damage to aboveground buds and shoots) in small seedlings, and little damage to underground structures below 10 cm (Finckh et al., 2016). Fire may damage plants by canopy scorching, by causing cambium necrosis or by phloem death (ringbarking) (Midgley et al., 2010). However, recent evidence suggests that hydraulic system failure (xylem damage) caused by extreme heat spikes may be a more common cause of plant death (Midgley et al., 2011, West et al., 2016). Hydraulic failure is also typical of frost damage (Midgley et al., 2010), resulting from ice crystal formation and cavitation of the xylem sap due to repeated freeze and thaw events (Dickison, 2000). Although the outcomes of fire and frost damage for plants are similar (Midgley et al., 2010), the physiological stresses caused by extreme heat and cold may in fact be very different (Dickison, 2000), and it may be expected that different adaptive mechanisms apply to these thermal stressors.

Woody species may adopt different strategies to survive fire and frost. One way is to avoid damage to aboveground structures through specialized protective measures. These 'disturbance resistor' species may avoid damage by rapid growth to reach escape height (Higgins et al., 2000, Balfour and Midgley, 2006, Bond, 2008, Burrows et al., 2008), by producing a thicker stem to prevent hydraulic xylem failure (Midgley et al., 2011, Michaletz et al., 2012) or by producing thicker bark that protects the internal cambium and bud meristems (Gignoux et al., 1997, Hoffmann et al., 2009, Midgley et al., 2010, Waters et al., 2010). Alternatively, 'disturbance tolerators' do not invest in specialized defence mechanisms to avoid damage, but have the ability to resprout from remaining structures after topkill (Clarke et al., 2013, Gignoux et al., 1997, Archibald et al., 2019). Resprouting ability is dependent on the development, maintenance and protection of a viable bud bank (Clarke et al., 2013) and belowground reserves (Gignoux et al., 2009, Tomlinson et al., 2012).

Vast areas of African savannas are regularly disturbed by fire and frost, affecting vegetation composition and structure (Bond et al., 2003, Bond and Keeley, 2005). Investigating trait responses of woody seedlings to fire and frost events in this context is essential, as it gives an indication of how different trait complexes may provide advantages to surviving these two different disturbances. This is also important, considering recent vegetation shifts towards increased woody biomass in previously grass-dominated areas, which have partially been put down to decreased fire frequency and intensity (Trollope et al., 2002, Bond et al., 2003) and fewer frost events (Wakeling et al., 2012, O'Connor et al., 2014), and pose a major threat to grassland biodiversity and rangeland productivity (Eldridge et al., 2011, Devine et al., 2017, O'Connor and Crow, 1999, O'Connor et al., 2014). Mitigating unwanted changes in tree:grass ratios due to anthropogenic global change requires a good understanding of the factors constraining woody cover at all demographic stages (Ward, 2005).

Survival strategies of seedlings may be different to those of mature trees because of their small size and the lack of protective structures such as thick bark, which may only develop at a later stage of their life cycle.

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It is important to consider the adaptations of seedlings, given that seedling germination and establishment probably represent a significant bottleneck in tree persistence in many grassland systems (Jeltsch et al., 1998, Higgins et al., 2000, Sankaran et al., 2004, Van Wijk and Rodriguez-Iturbe, 2002).

The aim of this study was to compare the effects of fire and frost on the seedlings of a selection of woody species during their first growth year. Our specific objectives were to (1) compare survival rates between fire and frost treatments and determine which traits predict seedling survival, (2) explore which traits predict survival strategy (i.e. which traits aid resistance or tolerance), (3) determine the extent of stem regrowth (recovery) after fire and frost, and (4) assess how architectural traits of surviving seedlings compare between fire and frost treatments. We provide one of the first studies on trait related responses to traumatic disturbances at the young seedling stage of African woody species. This may ultimately enable predictions of species composition and distribution across open grassy systems under current conditions and in a changing world.

## Methods

*Experimental design*

Nineteen woody species representing a range of habitat preferences were selected (Appendix S1). These species are all native to one geographic area, namely Buffelskloof Private Nature Reserve (BKNR) (25°17'53.6"S 30°30'31.7"E) in Mpumalanga, South Africa. This reserve supports patches of grassland, savanna and forest, and is currently experiencing encroachment into the open grassy areas (Haddad, 2011). The area receives moderate frost, estimated to range between 9 and 21 mean frost days per annum (Mucina et al., 2006). Fire represents an essential part of the BKNR management strategy. However, because of the inherent risk of fires spreading to adjacent plantations, management block-burns are conducted only within the timeframe of fire belt burning in forestry areas, namely in autumn or early winter, before any significant frost. (J.E Burrows pers. comm. 2018).

Seeds for each species were ordered from a seed company (Silverhill Seeds), collected in BKNR, or collected from trees in garden cultivation. Seeds were sown in plastic seedling trays filled with standard nursery seedling soil mix early in the growing season (23 October 2017). Seedling trays were placed under 40% black shade-netting. The photosynthetically active radiation (PAR) measured on a clear day at midday (12:17 PM on 1 March 2018) with a ceptometer (Accu-PAR LP-80, Decagon Devices, Pullman, WA, USA) at seeding tray height averaged 770.67 μmol m$^2$ s$^{-1}$(SE = 27.21) compared to 1655.67 μmol m$^2$s$^{-1}$ (SE = 15.95) taken in full sun just outside the shade-net house. Germinations were recorded every second day over seven months. Within a week of germinating, newly germinated seedlings were transferred to 3.57 L soil bags filled with a growth medium composed of a 1:1:1 ratio of topsoil, compost and river sand.

At 74 days, seedlings were transferred to a plot covered by 40% white shade netting. The average PAR value taken at midday (12:20 PM on 1 March 2018) at soil bag height was 1287 μmol m$^2$s$^{-1}$ (SE = 13.91) compared to 1655.67 μmol m$^2$ s$^{-1}$ outside the shade-net house. For each species, up to 15 individuals were assigned to each treatment; assignment was done to ensure similar aged trees were assigned to each treatment.

On 3 May 2018 an experimental fire was applied to seedlings assigned to the fire treatment. The timing of the fire (May) was similar to management fires conducted at BKNR. The day was cool, clear and with low wind speeds. Seedlings in bags were transferred to a level, open field and packed in a rectangular 3 m x 6 m soil trench, approximately 30 cm deep. The seedlings were spaced ± 30 cm apart. The area between the bags in the trench was then filled up with soil so that the tops of the soil bags were level with the soil surface. A uniform fuel bed of dried grass (approximately 30 cm thick) was lightly strewn around the trees, taking care that the fuel bed had sufficient aeration to prevent smouldering. The fuel load based on the grass biomass in 0.5m$^2$ was estimated at 1708.08 kg ha$^{-1}$. The fuel bed was extended approximately 1 m upwind of the plot to enable the fire to gain momentum before reaching the first seedlings. A head fire (moving with the wind) was then set in a single line along the length of the plot and allowed to burn through the designated area. Directly after the burn, scorch height (height of highest scorched foliage) was measured for a random selection of seedlings in different positions throughout the plot to give an indication of flame height. The

seedling bags were dug out and transported back to the nursery the next day.

The frost treatment was applied to assigned seedlings from 16 to 17 July 2018. Seedlings were transported in a closed vehicle to Lanseria (25°56'13.5"S, 27°52'06.6"E), where they were packed in an open site next to a river, with spacing between individual trees of approximately 50 cm. To insulate the roots of the seedlings, each soil bag was placed in a larger (22 cm diameter) plastic pot and the space between the plastic bag and the pot stuffed with dried grass. Maxim temperature data loggers (iButtons) were placed at different positions throughout the demarcated plot (centre and periphery) to monitor soil and air temperatures. Air temperature loggers were placed on stands between the trees, with four at 90 cm and ten at 60 cm above the ground. Ten soil temperature loggers were placed inside the soil bags, between the soil and the edge of the bag, approximately 10 cm below the soil surface. Temperature was recorded at one minute intervals and 0.0625 °C resolution. Upon completion of the treatment, the seedlings were transported back to their original position in the nursery.

The control (no treatment) trees remained under 40% white shade netting for the duration of the experiment.

All seedlings were continually watered during the course of all the treatments by overhead sprinklers.

*Trait measurements*

Baseline trait measurements of all the seedlings were taken one day before each treatment was applied (Table 1). (Baseline measures for frost and fire treated individuals took place on different days because these two treatments happened on different days). Additionally, these, and some additional traits, were measured on the same seedlings two months after treatment, and again on 1 November 2018 at the end of the experiment (Table 1).

Survival status (dead or alive) was recorded at the end of the experiment to provide information on survival rates for different species under the different treatments. Live plant material present two weeks after the treatments was recorded as an indication of which seedlings retained aboveground live foliage after the disturbance (resprouting only started to appear approximately one month after treatment). 'Live foliage' was defined as any visibly live material such as leaves, stems and active buds. For seedlings that survived the disturbance, survival strategy was scored. Species that had live material two weeks after disturbance, indicating that they were capable of avoiding total aboveground tissue damage, were classified as 'disturbance resistors'. In contrast, seedlings that survived solely by resprouting after total aboveground biomass destruction were classified as 'disturbance tolerators' (see Archibald et al. (2019) for a discussion of these definitions).

A number of traits were measured prior to disturbance to assess their contribution to seedling survival. These were plant height (height of the highest green material), diameter of the main stem at 1 cm above the soil surface), main stem length, and the number of leaves below 1 cm (Table 1). Taller plants may resist damage by having their foliage and buds above the zone of severe temperatures (Hoffmann et al., 2019). Larger basal diameters could increase survival to disturbance by providing insulation to stem tissues, making the stems more resistant to large temperature fluctuations (Midgley et al., 2010). Plants with longer stems may have higher numbers of epicormic buds, from which new shoots can resprout (Meier et al., 2012). Stem length is related to plant height, but compensates for skew-growing plants. The number of leaves below 1 cm indicates the extent of photosynthetically active resources and buds available to the plant in the basal area, and potentially its ability to survive via resprouting if top-killed by a major disturbance event (Peìrez-Harguindeguy et al., 2013, Clarke et al., 2013).

Plant height, stem length, lateral branching, and stem tip counts were measured post-disturbance to assess changes in architecture due to fire and frost exposure. Main stem length was used to determine the degree of recovery achieved at the end of the experiment. Plant height: stem diameter ratio was taken as an indicator of whether stem growth is long and slender or short and thick. This may indicate whether the seedlings invested more resources into height gain *vs.* lateral growth in response to the treatment; i.e. if they are following an "escape" strategy (Rozendaal et al., 2015, Dantas and Pausas, 2013). Also, plant height:stem

tip ratio was calculated to determine whether seedlings invested more resources in shorter cagey, or taller growth with fewer branches (Archibald and Bond, 2003).

*Analyses*

To test for collinearity between predictor variables, Pearson's correlation analysis was conducted between all traits using the control treatment data measured at all time periods. Only control data was used as treatment effects may influence relationships between variables. Seedling age was excluded here as all control seedlings were the same age when measured.

Pearson's Chi-square test was used to compare seedling survival between treatments (control, fire or frost) at the end of the experiment. A chi-squared goodness-of-fit test with "fdr" p-value adjustment was performed for pairwise comparisons between treatments.

A generalized linear mixed-effects model (GLMM) was used to test which traits were significant predictors of seedling survival, with species ID as random effect (Appendix S2). The predictors used at the outset of this analysis were baseline age (the age of the seedlings on the day they were treated), baseline plant height, baseline number of leaves below 1 cm baseline stem diameter and treatment (fire or frost – control plants not used). To determine the best subset of predictor variables to use in the GLMM model, a best subsets model selection was used. The combination of factors resulting in the lowest BIC score were then selected for the final GLMM model (Appendix S2).

For the seedlings that survived treatment, we tested which traits predicted survival strategy (resisting *vs.* tolerating). A GLMM model was run with treatment (fire or frost, without control plants), baseline age, baseline stem length, baseline stem diameter, baseline number of leaves below 1 cm, as well as the interaction terms between treatment and all traits, included as predictors. Species ID was included as random effect (Appendix S2). Best subset modelling, using the lowest BIC, was again used to select the best subset of predictors.

To determine the extent of stem regrowth after treatment, the average final stem length was calculated per species for each of the treatments. From this, average percentage stem regrowth was calculated for fire and frost treatment, using the equation $\frac{T}{C} \times 100$, where $C$ is the average final stem length for control plants and $T$ is the average final stem length of treatment plants. A linear mixed-effects model (LMM) with was fitted to test the effect of treatment on stem length regrowth. Transformed proportion values were used as model input (Appendix S2).

To test how architectural traits (plant height, stem diameter, number of lateral branches, plant height: stem tip ratio and final diameter:stem tip ratio) of surviving seedlings were affected by fire and frost, we ran LMM or GLMM models (Appendix S2). Traits were included as response variables, treatment (fire, frost or control) and seedling age (at the time of treatment) as predictor variables, and species ID as random effect. Pairwise post-hoc comparisons between treatments were done using general linear hypotheses with Bonferroni p-value adjustments to control for falsely rejected hypotheses (the false discovery rate) that could occur with multiple comparisons (Benjamini and Hochberg, 1995).

In addition, to test how architectural traits of resprouted seedlings compared between treatments, LMM or GLMM models were run on a dataset of only the tolerators (i.e. the seedlings that experience top-kill but resprouted after disturbance).

Variance inflation factor (VIF) analyses were conducted for each of the above models to test for multicollinearity of predictor variables. VIF measures how much the variance of a regression coefficient is inflated due to multicollinearity in the model (Quinn and Keough, 2002). Variables with a VIF value larger than 5 were removed due to collinearity (James et al., 2013).

All analyses were conducted in R version 3.5.2 (R Core Team, 2018) using packages 'lme4' version 1.1-20 (Bates et al., 2014), 'leaps' version 3.0 (Lumley, 2017), 'RVAideMemoire' version 0.9-73 (Hervé, 2019),

'multcomp' version 1.4-8 (Hothorn et al., 2008) and 'car' version 3.0-2 (Fox et al., 2012). Family, link functions and response variable transformations for all LMM and GLMM models are displayed in Appendix S2.

## Results

During the fire experiment (Table 2), the calculated fire spread rate was 0.4 m s$^{-1}$ and fireline intensity estimated at 1230.44 kJ s$^{-1}$ m$^{-1}$ based on the equation of Trollope et al. (2002). The lowest night-time temperature recorded during the frost experiment (Table 2) was -4.71 @C at 90 cm above soil level, and -7.85 at tree level (±60 cm above soil surface). Temperatures 10 cm below the soil surface in the soil bags averaged 6.65 @C (Table 2).

Pearson's correlation coefficient analysis of the control tree data indicated that there were few significant correlations between the measured traits at -0.7 [?] r [?] 0.7; (Appendix S3). All such correlations were between variables representing plant size, namely plant height, stem length and stem diameter.

Seedling survival was significantly higher in control (100%) than treated (frost: 75%; fire: 66.7%) seedlings ($X^2$= 70.35, df = 2, p < 0.001). Although the fire-treated seedlings had lower survival rates than the frost seedlings, this difference was not significant.

Seedling age at the time of treatment, number of leaves below 1 cm and the main stem diameter before treatment were retained in the best subset model predicting survival. Treatment was removed due to VIF scores above 5 as it was correlated with age (Table 3). This is because the two treatments happened at different times, corresponding to the times at which these disturbances may have happened in the field. The number of leaves below 1 cm and seedling age increased seedling survival (Table 3, Appendix S2). Diameter was not a significant predictor.

Many species displayed different survival responses to fire than to frost treatments (Figure 2). Some species that survived frost well were adversely affected by fire and *vice versa* . Most species could be classified as fire tolerators (i.e. > 50% of survivors resprouted). Only one species (*Dombeya rotundifolia* ) could be considered a fire resistor ( > 50% of individuals retaining live foliage after fire). In contrast, four species could be classified as frost resistors (Figure 2). Interestingly, *D. rotundifolia* was not amongst the frost resistors. Seven species displayed a frost tolerator response. Treatment, stem length and the number of leaves below 1 cm were significant predictors of survival strategy (Table 3). Increased stem length was associated with greater resisting capability to both treatments. We also found a significant interaction effect between treatment and leaves below 1 cm. More leaves below 1 cm resulted in greater resistance against fire damage, but not against frost. No other significant interaction effects were found between treatment and any of the other predictor variables used in the model (Appendix S2).

There was variation in the degree to which species recovered lost biomass after disturbance, but regrowth rates for any particular species after fire *vs.* frost were similar (Table 3, Figure 3). As for architectural trait differences of surviving seedlings two months post treatment, control seedlings were taller, with larger stem diameters and more lateral branches than treated seedlings (Appendix S4, S5; Figure 4). After two months of recovery, seedlings that were burned were shorter than seedlings that received frost, though stem diameter did not differ between treatments. At the end of the experiment burned seedlings had significantly higher plant height:stem tip ratios than frost-treated seedlings, indicating that they had a lower degree of branching in relation to height, compared to seedlings exposed to frost. However, the models containing only resprout data with the same equations indicated that there were no significant differences for any of the traits between the fire and frost treatment when only considering the individuals that resprouted (Appendix S6).

## Discussion

*Seedling survival*

Survival rates in response to fire and frost were similar under both treatments. Therefore, we cannot conclude whether fire or frost is more important in preventing the establishment of tree seedlings in these systems.

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To our knowledge, this study represents one of the first that explicitly contrasts the effects of fire and frost on young tree seedlings across a range of species. While the role of fire and frost in limiting woody cover is often acknowledged, few studies specifically contrast their respective effects without them being confounded by other factors such as herbivory, rainfall and soil moisture (Murphy and Bowman, 2012, Hirota et al., 2011). For example, Holdo (2007) demonstrated in a simulation model that fire and herbivory by elephants were particularly important drivers of savanna change, and that frost alone was less effective in causing change in woody cover. On the other hand, in a review about the drivers of woody cover, fire (but also grass competition and herbivory) were posited to be particularly important in maintaining grasslands globally, the effects of frost were not specifically interrogated (Bond, 2008). However, there is evidence for frost as the main driver of woody cover in some systems (Joshi et al., 2019, Hoffmann et al., 2019, Brando and Durigan, 2005).

Overall, seedlings were remarkably resilient to fire and frost damage. Our results suggest that a wide variety of indigenous tree species are potentially capable of surviving fire and frost damage during their first growth year. More than half of the seedlings considered in this study survived the treatments. Overall, 67% and 75% of seedlings survived fire and frost respectively and only four species showed less than 50% survival after a single fire or frost event (Figure 2; Appendix S1). Other studies have found mixed results: one study found that young seedlings of four African savanna tree species could survive three consecutive cooling events to -5@C in a cold chamber (O'Keefe et al., 2016), while another found relatively low survival rates for tree seedlings subjected to their first fire event in field observations (Gignoux et al., 2009). Differences between our observations and other studies could be due to the experimental nature of our study: seedlings were well-watered, grown in nutrient-rich soils and grew without grass or other competitors; therefore, seedlings had obtained fairly large sizes at the time of experimental treatment. Additionally, fire intensity of our burn was low (Trollope et al., 2002), with average leaf scorch heights of less than one meter recorded directly after the burn. Also, frost was applied as a single event rather than repeated throughout the dry cold season. Therefore, natural fire regimes and frost events may result in different mortality rates.

*Plant traits that aid seedling survival*

The ability of woody seedlings to survive disturbance will be influenced by certain plant traits. Seedling survival increased with increased number of leaves in the basal region prior to treatment. Fire and frost are mostly top-kill events (especially for seedlings smaller than 4 m) with less damage to foliage close to the soil surface than to aerial foliage (Finckh et al., 2016, Whitecross et al., 2012); therefore, photosynthetic sources and extensive bud banks in the basal region increase tolerance to these disturbance (Clarke et al., 2013). Basal resprouting seems to be a prominent fire tolerance strategy, even for mature woody species, in African savannas compared to other fire-prone systems (Clarke et al., 2013). However, this may be a particularly important character in young seedlings, as they have not had the time to build up some of the defences (e.g. height or stem diameter) that adult trees possess.

Our findings that older seedlings had higher survival rates agree with other studies assessing fire (Gignoux et al., 2009, Hoffmann, 2000, Radford et al., 2001) and frost (Gusta and Wisniewski, 2013, Molina et al., 2016) survival. Older individuals would have had more time to accumulate above- and belowground resources, providing capacity to either resist damage to stems or regrow from stored reserves after topkill (Gignoux et al., 2009).

We found that neither seedling height nor stem diameter were significant predictors of survival. Other studies have shown that the critical escape height for fire and frost ranges between 2-4 m in African savannas (Wakeling et al., 2012, Trollope et al., 2002, Whitecross et al., 2012). The mean height of our seedlings at time of treatment was 22.24 cm (std. dev: 17.39). The stem diameter above which stem survival after exposure to fire increases varies between species (Hoffmann et al., 2003, Gignoux et al., 1997); and diameters between 20-40 mm have been identified as thresholds for 100% survival of some species (Gignoux et al., 1997, Hoffmann et al., 2003), and diameters between 4.5 and 10.32 cm for 50% survival (Hoffmann et al., 2003); though these values will also vary with fire intensity. The mean diameter of our seedlings at time of treatment was 3.95 mm (std. dev: 3.12). Therefore, even though our seedlings were probably considerably

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larger than they would have been at the end of their first season under natural conditions due to the conducive experimental conditions under which they were grown, they still did not achieve sufficient height or thick enough stems to be completely resistant to fire and frost after one growing season.

Our results support the notion that the traits promoting survival in seedlings are fundamentally different from those of older saplings or mature trees. It is known that mature trees that are taller (Higgins et al., 2000, Balfour and Midgley, 2006, Bond, 2008, Burrows et al., 2008) with thicker stems (Midgley et al., 2011, Michaletz et al., 2012) and other traits not considered here such as thick bark and bud protection structures (Gignoux et al., 1997, Hoffmann et al., 2009, Midgley et al., 2010, Waters et al., 2010) have increased survival under fire and frost damage.

*Seedling survival strategies*

The traits that promote survival in response to disturbance may be subdivided into those that are helpful for damage resistance and those that aid damage tolerance (resprouting). Our results support the idea that different plant functional traits are associated with resisting and tolerating fire and frost. More basal leaves and longer stems aided resistance (Table 3, Table S2). None of the traits considered here were specifically associated with seedling tolerance. Other traits that were not measured, such as those associated with belowground resource allocation and basal bud morphology, may be specifically important for tolerating topkill (Gignoux et al., 2009, Charles-Dominique et al., 2015). Resprouters may benefit from having large belowground bud banks, belowground storage and well protected buds, which may be traded off against vertical growth to persist in damage-prone environments until a way is found to grow past the critical vulnerable height (Gignoux et al., 2009).

Some traits that prevent or limit damage from extreme heat spikes as would be experienced during fires may simultaneously serve as protection against extreme cold (Gurskaya and Shiyatov, 2006, Molina et al., 2016, Treter and Block, 2004). Childes and Walker (1987) found that mature fire-resistant African tree species also tend to be frost-hardy. It is often difficult to establish direct causal relationships between the evolution of traits and their current function. For example, it has been suggested that traits that aid survival under frost may initially have evolved as an adaptation to fire (Lamont et al., 2017). On the other hand, it has often been suggested that fire-adaptations originally evolved in response to drought (Axelrod, 1980, López-Soria and Castell, 1992, Blödner et al., 2005).

However, our results illustrate that not all traits may be equally beneficial for resisting both disturbances. We found discrepancies in the species' abilities to resist fire and frost, and that none of the species could resist both treatments equally well. Seedlings with larger numbers of leaves in the basal region were better resistors to fire, while this trait was less helpful to resist frost. Tree seedlings are therefore able to resist fire by keeping their foliage low to the soil surface. In contrast, none of the traits we measured was specifically helpful for resisting frost. Other factors not examined here could affect resistance to frost. For example, caginess (i.e. a higher degree of branching) might provide a higher density of leaves, which could provide cold insulation to the main stem. Temperatures within canopies, at least for mature trees, can be significantly warmer inside than outside the canopies during frost (Whitecross et al., 2012), and frost-hardy species have been shown to possess shorter, more branched stems compared to frost-sensitive species (Palta and Li, 1979). In our study, frost survivors were overall more cagey compared to fire treated trees after the treatment (discussed in more detail below), although this caginess was not a reaction of resprouters to frost, but rather the result of less topkill. This change in architecture might however provide additional protection to a successive frost event.

Other adaptations not considered may be unique for either frost or fire resistance since there are differences in the way fire and frost cause physiological damage. While fire-adaptation traits reduce stem flammability and protect the cambium, vascular tissues and buds from heat damage (Gignoux et al., 1997, Hoffmann and Solbrig, 2003, Gignoux et al., 2009, Balfour and Midgley, 2006), adaptations to resist frost often involve preventing intracellular freezing and tolerating extracellular ice crystal formation in plant tissues (Gusta and Wisniewski, 2013). Apart from traits already mentioned such as thick bark (Schafer et al., 2015), well-protected buds (Charles-Dominique et al., 2015) and belowground storage organs (Maurin et al., 2014),

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trees with smaller leaf area possess lower time to ignition, burn duration and mean mass loss rate (Krix and Murray, 2018), which may decrease fire damage. Frost-hardy plants tend to have smaller, thicker leaves (Palta and Li, 1979) with decreased leaf wettability (through waxy cuticles or pubescence) (Aryal and Neuner, 2010). The ability of some frost-hardy woody species to retain liquid cellular water under sub-zero conditions (supercooling) by preventing ice nucleation has also been illustrated (Wisniewski et al., 2003). Hydraulic system failure resulting from cavitation after a freeze-thaw event occurs because air bubbles remain in the xylem sap after degassing of the frozen xylem, and this process therefore differs from cavitation caused by tension in the vessels during extreme drought or heat during a fire (Dickison, 2000). Small vessel diameter is particularly important in preventing cavitation due to freeze-thaw events since larger tracheary elements form larger air bubbles during sub-zero conditions (Dickison, 2000). Evidence that fire mortality also occurs through cavitation means that small vessel diameters (and particularly hydraulic segmentation) may also contribute to fire resistance (West et al., 2016).

*Changes in seedling architecture after fire and frost damage*

Tree architecture can take on a very different trajectory after damage compared to undamaged trees (Holdo, 2006, Klimes, 2007, Archibald and Bond, 2003). Although the recovery of stem length after fire and frost was similar under both treatments, which coincides with other studies (Brando and Durigan, 2005), interspecific differences existed, where some species recovered a larger percentage of their stem length after damage than others. Almost all of the species that could resist damage to their aboveground biomass under fire and frost still suffered more than 50% loss of their stem length after treatment. The plant height and stem diameter of seedlings after two months of recovery were also significantly reduced in seedlings damaged by both treatments. The build-up of height and stem thickness 'debt' may have implications for seedling survival during subsequent disturbance events: smaller seedlings with thinner stems are more vulnerable to large thermal fluctuations and may suffer increased mortality (Midgley et al., 2010, Gignoux et al., 2016, Lawes et al., 2011). Seedlings face trade-offs between investing in stem length and resisting damage, and building belowground storage or basal defence mechanisms (e.g. bark thickness and bud protection) to improve resprouting ability (Gignoux et al., 2009). Spending time investing in belowground resources for instance may limit the seedling's ability to gain height.

The architecture of frost-treated seedlings differed from those that received fire in that they had a higher degree of branching in relation to height. Since fire destroyed more biomass than frost, seedlings mostly created new stems from the base after fire, whereas they were more often able to retain their original branches after frost damage. These differences therefore appear to be due to the fact that more fire-affected trees resprouted, rather than intrinsic differences in response to each type of damage when the models were run with only the individuals that resprouted.

*Implications for grassland structure and management*

While seedling survival was high under fire and frost, the severe stunting effects of these treatments (especially fire) suggests that, once established, trees in these open grassland systems may be subjected to a seedling release bottleneck (i.e. seedlings can survive but not grow to adulthood). This is consistent with the findings of others who found that fire mainly suppresses seedling growth and has less effect on seedling mortality (Staver et al., 2009). However, the question remains whether seedlings are able to recruit in these grasslands (Russell et al., 2019). Competition for root space may be severe during the germination phase, especially considering the fact that most grass roots are concentrated within the first 10 cm of soil (Wakeling et al., 2015). It is therefore likely that the lack of root gaps may have contributed significantly to the relatively open structure of this system.

High-intensity fires are more sufficient in maintaining open grassy areas, as they kill more woody seedlings and cause more damage to aboveground biomass (Morrison and Renwick, 2000, Trollope et al., 2002). While fire rarely burns more than once a year due to fuel limitations, the cumulative effects of repeated frost events during a single season may further reduce survival probability (Joshi et al., 2019). Also, the combined effect of fire and frost may be more effective in killing seedlings than either disturbance alone (Holdo, 2007). In

areas where frosts have become less frequent, as has been observed in Africa and beyond (Midgley and Lotze, 2008, Rigby and Porporato, 2008), fire alone may not be able to prevent recruitment, and an increase in woody cover may be expected.

The woody species composition of an open grassland system will almost certainly be influenced by phenomena that affect the timing and frequency of seedling exposure to damage, as mortality was found to be dependent on seedling age. Therefore, changes in fire regime and climates (esp. changes that bring about less frost and reduced fire intensity and frequency) are likely to result in changes in the composition and the structure of the woody components of these systems (Enright et al., 2015).

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### Data availability statement

In prep. Data will be deposited in Dryad upon acceptance of manuscript

### Author contributions

M. Botha – conceptualisation, methodology, data curation, formal analysis, writing – original draft

S. Archibald – conceptualisation, methodology, supervision, writing – review and editing

M. Greve - conceptualisation, funding acquisition, methodology, supervision, writing – review and editing

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**Figure 1:** Simplified diagram summarizing some of the main demographic bottlenecks for seedling establishment in open grassy systems. Limiting factors for seedling establishment may act by either preventing seedling recruitment (through failure to disperse or germinate and by seed predation) or by preventing seedling release (seedlings are able to recruit but fail to reach escape height due to grass competition or top-kill events). Animals, fire and abiotic environmental conditions (temperature, rainfall and atmospheric $CO_2$ concentration) may ultimately have positive (+) or negative (-) effects on seedling recruitment or release. Adapted from Midgley and Bond (2001).
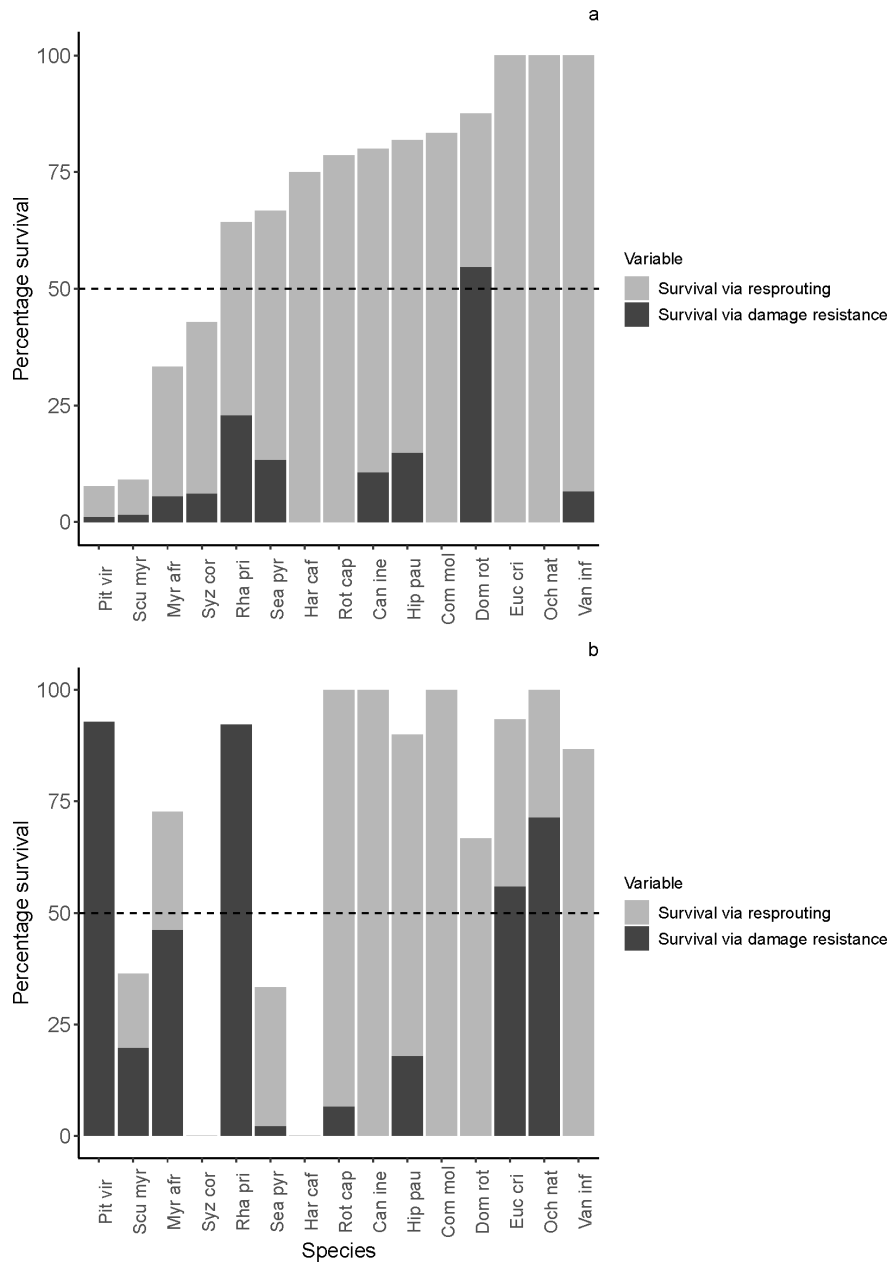


**Figure 2:** Stacked plot of tree seedling survival strategies. Bars illustrate percentage seedling survival and percentage individuals with live foliage remaining two weeks after treatment for tree seedlings treated by (a) fire and (b) frost. Species represented by fewer than six individuals were not included in this figure.
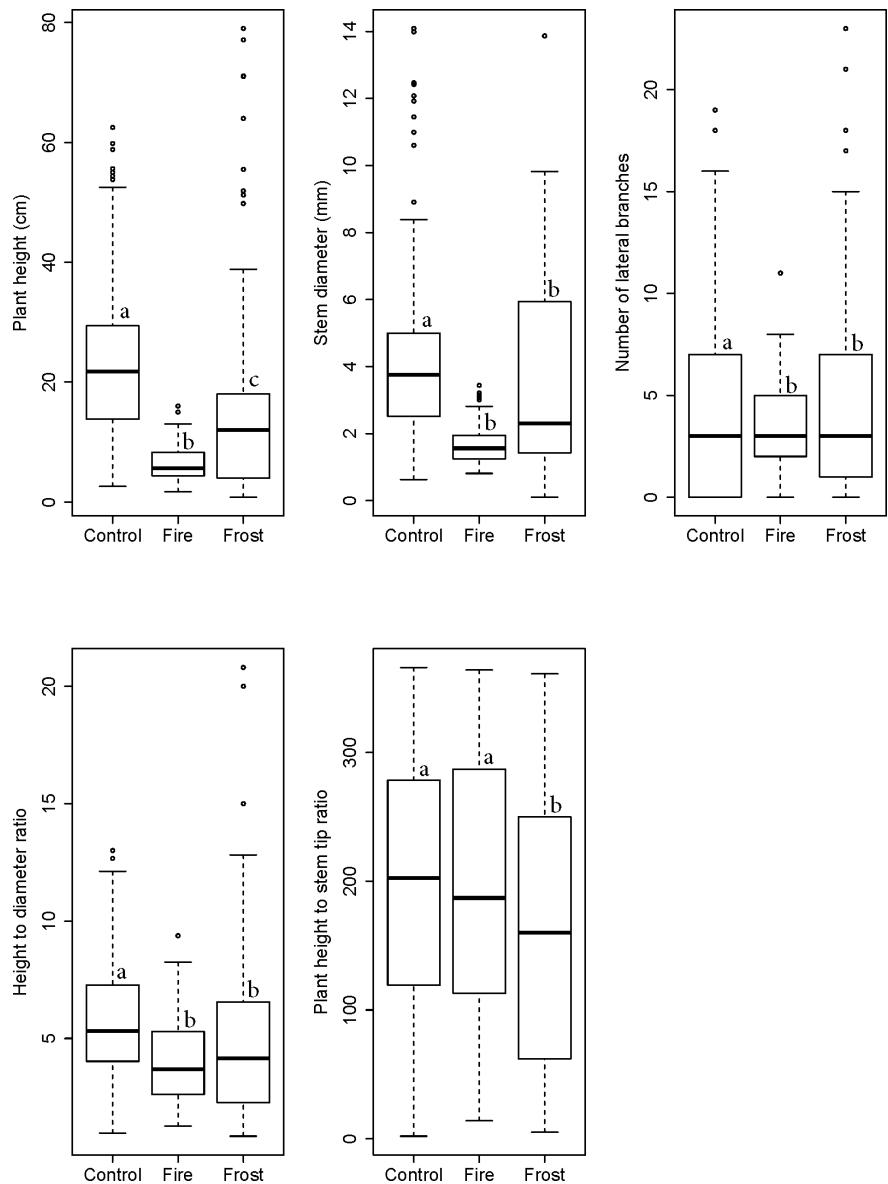
Species were considered 'resistors' if the percentage individuals with live foliage directly after treatment exceeded the 50% threshold (dashed line). Species were classified as 'tolerators' if the percentage resprouted plants exceeded the 50% threshold. Can ine = *Canthium inerme;* Com mol =*Combretum molle;* Euc cri = *Euclea crispa;* Dom rot =*Dombeya rotundifolia;* Har caf = *Harpephyllum caffrum;* Hip pau = *Hippobromus pauciflorus* ; Myr afr = *Myrsine africana* ; Och nat = *Ochna natalitia;* Pit vir = *Pittosporum viridiflorum;* Rha pri = *Rhamnus prinoides;* Rot cap =*Rothmannia capensis* ; Scu myr = *Scutia myrtina;* Sea pyr =*Searsia pyroides* ; Syz cor = *Syzygium cordatum;* Van inf =*Vangueria infausta* .
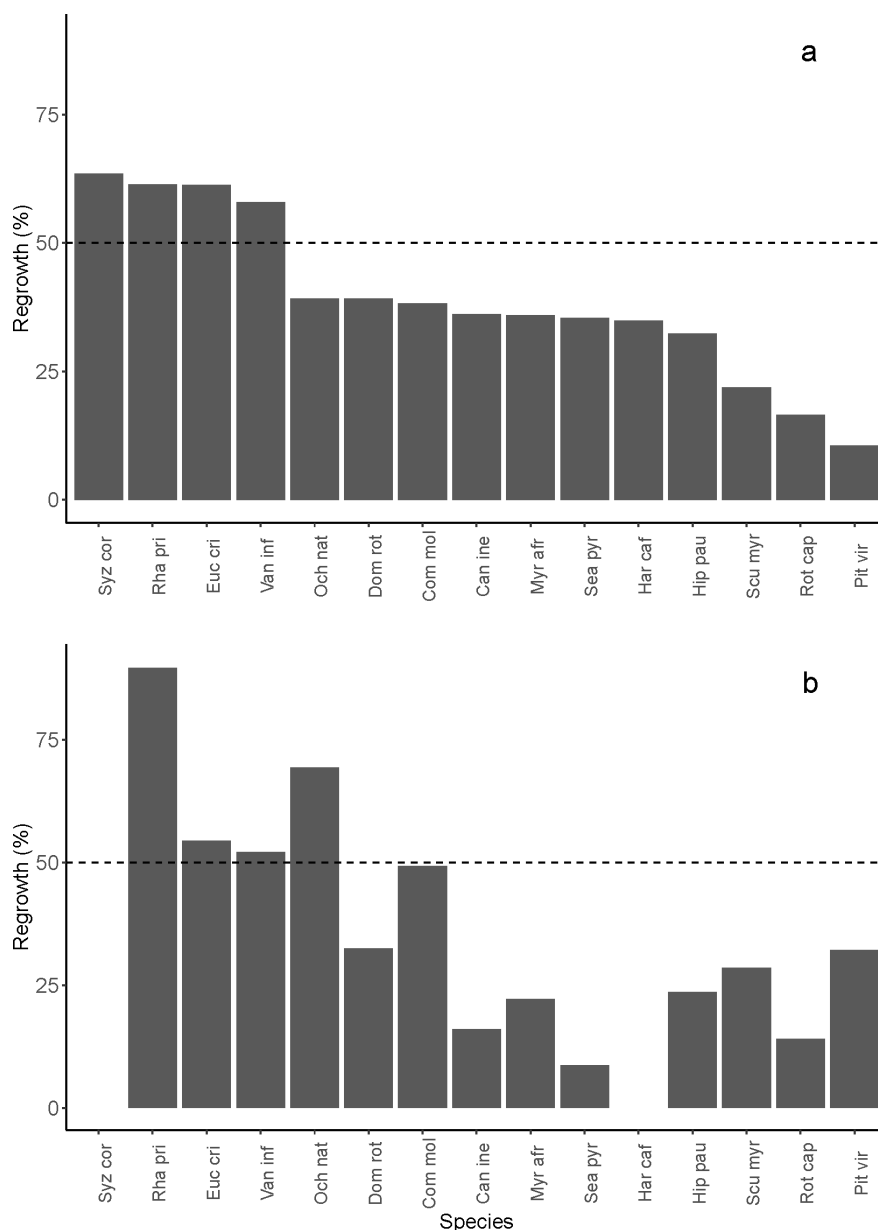
**Figure 3:** Percentage stem length regrowth for tree seedlings as a result of fire (a) and frost (b) damage. Species represented by fewer than six individuals were not included. Dashed line represents 50% cut-off for stem regrowth. Can ine = *Canthium inerme;* Com mol =*Combretum molle;* Euc cri = *Euclea crispa;* Dom rot =*Dombeya rotundifolia;* Har caf = *Harpephyllum caffrum;* Hip pau = *Hippobromus pauciflorus* ; Myr afr

= *Myrsine africana* ; Och nat = *Ochna natalitia;* Pit vir = *Pittosporum viridiflorum;* Rha pri = *Rhamnus prinoides;* Rot cap =*Rothmannia capensis* ; Scu myr = *Scutia myrtina;* Sea pyr =*Searsia pyroides* ; Syz cor = *Syzygium cordatum;* Van inf =*Vangueria infausta* .



**Figure 4:** Architectural trait differences of surviving tree seedlings between three treatment groups (C= control, FI = fire and FR = frost). All response variables were measured two months after treatment, except diameter:stem tip ratio which was measured at the end of the experiment. Different letters above plots indicate significant differences in means between treatments at p [?] 0.05.

**Table 1:** Summary and methodology of the traits measured, and calculated, for tree seedlings. The format of the trait measurement, the method used to measure the trait, and how the trait was hypothesised to affect seedling survival are indicated. Times at which measurements were taken, i.e. immediately prior to the experimental treatment ("Baseline") with fire or frost, two weeks after treatment, two months after treatment and/or at the end of the experiment ("Final") approximately one year after seeds were sown,

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is also provided. Additionally, whether the trait measure was used as predictor or a response variable in analyses is indicated. Baseline measures were often used as co-variates in models, as it was expected that initial measures of traits would affect post-treatment traits. For more details on analyses see the text.

| Trait | Data format | Method |
|---|---|---|
| Survival | Categorical (alive/dead) | Recorded as dead if no visible live tissue could be observed |
| Surviving green material | Categorical (present/absent) | Presence or absence of green leaves and active buds. |
| Survival strategy | Categorical (tolerator/resistor) | Classification of live individuals based on presence or absenc |
| Plant height | Continuous (cm) | Vertical measurement from the soil surface to the highest gr |
| Stem diameter | Continuous (mm) | The diameter of the main stem at 1 cm above the soil surfac |
| Main stem length | Continuous (cm) | The length of the main (tallest) stem from the soil surface t |
| Leaves below 1 cm | Continuous (count) | Count of the number of true leaves with petioles emerging fr |
| Number of lateral branches | Continuous (count) | Count of the number of lateral branches emanating from the |
| Number of stem tips | Continuous (count) | Count of the total number of stem tips of the plant |
| Plant height:diameter ratio | Continuous | The plant height divided by the main stem diameter |
| Plant height:stem tip ratio | Continuous | The plant height divided by the total number of stem tips |
| Stem regrowth | Continuous (%) | Calculated by the equation ($T/C)100$, where $C$ is the averag |

**Table 2:** Summary of conditions during fire and frost treatments. Rate of spread and fire intensity were calculated from formulas by Trollope et al. (2002).

| Fire conditions | Fire conditions | |
|---|---|---|
| Date of treatment | Date of treatment | 3 May |
| Time | Time | 12:00-13:13 |
| Average relative humidity (%) | Average relative humidity (%) | 46.00 |
| Average air temperature (@C) | Average air temperature (@C) | 25.00 |
| Average wind speed (km h$^{-1}$) | Average wind speed (km h$^{-1}$) | 4.00 |
| Fuel load (kg ha$^{-1}$) | Fuel load (kg ha$^{-1}$) | 1708.08 |
| Average fuel moisture (%) | Average fuel moisture (%) | 7.77 |
| Total burn time (s) | Total burn time (s) | 73.00 |
| Rate of spread (m s$^{-1}$) | Rate of spread (m s$^{-1}$) | 0.40 |
| Fire intensity (kJ s$^{-1}$ m$^{-1}$) | Fire intensity (kJ s$^{-1}$ m$^{-1}$) | 1230.44 |
| Average scorch height (cm) | Average scorch height (cm) | 46.57 |
| **Frost conditions** | **Frost conditions** | |
| Date of treatment | Date of treatment | 16-17 July |
| Time | Time | 17:00 -5:00 |
| Average relative humidity (%) | Average relative humidity (%) | 11.958 |
| Average wind speed (km h$^{-1}$) | Average wind speed (km h$^{-1}$) | 0.00 |
| Air temperature- 90 cm (@C) | **Mean:** | 5.379 |
| | **SE:** | 0.086 |
| | **Min:** | -4.705 |
| | **Max:** | 24.102 |
| Air temperature- 60 cm (@C) | **Mean:** | 5.471 |
| | **SE:** | 0.070 |
| | **Min:** | -7.847 |
| | **Max:** | 36.703 |
| Soil temperature- 10 cm below soil in soil bags (@C) | **Mean:** | 6.650 |
| | **SE:** | 0.051 |
| | **Min:** | -1.097 |

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| Fire conditions | Fire conditions | |
| --- | --- | --- |
| | **Max:** | 26.057 |

**Table 3:** Results for linear mixed-effects models (LMM) or generalized linear mixed effects models (GLMM) (after selecting for the best subset of predictors) testing for the best predictors of seedling survival, survival strategy (resistance vs. tolerance) and stem regrowth. Variance inflation factors (VIF) indicates multi-collinearity of predictor variables. $R^2$ and model p-values are given in Appendix S3. Treatment = Fire or Frost; Age = seedling age at which treatment was applied; Number of leaves = the number of leaves below 1 cm before treatment, Diameter = main stem diameter at 1 cm before treatment. Stem length =main stem length before treatment. NS: not significant; **: $p < 0.01$.

| Survival (GLMM) | **Fixed effects:** | **Estimate** | **Std. error** | **z-value** | **p-value** | **VIF** |
| --- | --- | --- | --- | --- | --- | --- |
| | Intercept | -1.592 | 0.916 | -1.739 | NS | - |
| | Age | 0.019 | 0.007 | 2.690 | ** | 1.455 |
| | Leaves below 1 cm | 0.308 | 0.116 | 2.649 | ** | 1.049 |
| | Diameter | -0.082 | 0.074 | -1.104 | NS | 1.419 |
| | **Random effects:** | **Variance** | **Std. dev.** | | | |
| | Species ID | 1.486 | 1.219 | | | |
| Survival strategy (GLMM) | **Fixed effects:** | **Estimate** | **Std. error** | **z-value** | **p-value** | **VIF** |
| | Intercept | -3.484 | 0.808 | -4.314 | *** | - |
| | Treatment | 1.918 | 0.497 | 3.859 | *** | 1.331 |
| | Stem length | 0.038 | 0.019 | 2.030 | * | 1.055 |
| | Leaves below 1 cm | 0.765 | 0.180 | 4.261 | *** | 2.044 |
| | Treatment × Leaves below 1 cm | -0.779 | 0.233 | -3.347 | *** | 1.935 |
| | **Random effects:** | **Variance** | **Std. dev.** | | | |
| | Species ID | 3.635 | 1.907 | | | |
| Stem regrowth (LMM) | **Fixed effects:** | **Estimate** | **Std. error** | **t-value** | **p-value** | **VIF** |
| | Intercept | -0.51228 | 0.25610 | -2.000 | NS | - |
| | Treatment | -0.02277 | 0.26877 | -0.085 | NS | - |
| | **Random effects:** | **Variance** | **Std. dev.** | | | |
| | Species ID | 0.498 | 0.706 | | | |
| | Residual | 0.486 | 0.697 | | | |