SHORT NOTE

Mycorrhizal colonization of *Palafoxia feayi* (Asteraceae) in a pyrogenic ecosystem

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Abstract Although it is known that a number of factors have predictable effects on mycorrhizal colonization, determining generalized patterns for some variables have remained elusive. In particular, fire has been identified as a major event that may influence plant–mycorrhiza interactions, yet efforts to date have yielded contradictory results. Here, we assess the impact of fire on mycorrhizal colonization in *Palafoxia feayi*, a plant commonly found in the fireswept, nutrient-poor scrub community of central Florida. We determined soil nutrient conditions and percent colonization patterns for plants growing in replicate plots that were burned 1 to 15 years previously. The results showed a negative relationship between mycorrhizal colonization and time since fire, but there was no effect of fire return interval (lapsed time between successive fires). Soil nutrient analyses corroborated previous studies and showed no change in soil nutrient following fire. In contrast to previous studies of mycorrhizal colonization in Florida scrub, we conclude that fire can affect arbuscular mycorrhizal fungi colonization and we speculate that this is mediated by light availability.

Keywords Mycorrhizal colonization · Fire ecology · Fire return interval · *Palafoxia feayi* · Soil analysis

Arbuscular mycorrhizal fungi (AMF) are crucial mutualists of many plant species (e.g., Hart and Klironomos 2002; Hartnett and Wilson 2002; Parniske 2008; Smith and Read 2008), especially in nutrient-limited ecosystems where plants may have enhanced mycorrhizal dependency (Harrison 2005; Smith and Read 2008; Johnson 2010). One such nutrient-limited environment is the scrub ecosystem of the Lake Wales Ridge in south-central Florida. Scrub is characterized by nutrient-poor, well-drained, sandy soils and is dominated by stands of xerophytic oaks, saw palmettos (*Serenoa repens*), and pines (*Pinus clausa* and *Pinus elliottii*). Not only are the soils nutrient-poor, but this ecosystem also experiences natural disturbance caused by relatively frequent fires. Fires are often stand-replacing, but do not generally affect species composition, as many species resprout from roots or regenerate from seeds (Abrahamson 1984; Schmalzer and Hinkle 1987).

Fire can influence a number of factors important to AMF colonization, such as the nutrient content of soils by rapid combustion and release of nutrients from herbaceous vegetation and accumulated organic matter. Ash deposition may create a nutrient pulse following fire (e.g., Kellman et al. 1985; Smithwick et al. 2005; Turner et al. 2007; Galang et al. 2010), or nutrients can be lost via volatilization and/or removal of ash by wind or water (Raison et al. 1985; Gray and Dighton 2006). Ultimately, soil nutrient content following fire will depend on fire intensity, duration of soil heating, and local conditions at a site (Raison et al. 1985; Certini 2005; Gray and Dighton 2006; Galang et al. 2010). In addition to changes in nutrient content, fire can also influence mycorrhizal inoculum potential or the ability of propagules to form mycorrhizal associations. Burning can reduce mycorrhizal inoculum potential (Vilariño and Arines 1991; Dhillon and Anderson 1993) and spore germination (Vilariño and Arines 1991) which, in turn, decreases AMF colonization. Alternatively, studies have also shown...
a range of effects from no change to increases in inoculum potential with burning (Allsopp and Stock 1994; Korb et al. 2003); thus, the effects of fire on AMF colonization involve a number of factors and may be ecosystem-specific.

This complexity makes it challenging to identify generalized AMF colonization patterns correlated with fire disturbance (Cairney and Bastias 2007), and not surprisingly, previous research on the role of fire in mycorrhizal colonization has yielded contradictory results. For instance, studies have identified increased colonization (Bentivenga and Hetrick 1991), decreased colonization (Klopatek et al. 1988; Vilarino and Ariñes 1991; Dhillon and Anderson 1993; Hartnett et al. 2004), and no change in AMF colonization following fire (Bellgard et al. 1994; Anderson and Menges 1997; Eom et al. 1999; Haskins and Gehring 2004; Treseder et al. 2004). Part of this variability in plant–AMF response to fire is likely a result of differences in conditions and methodology among studies, including the time frame of the study, study sites, and plant–AMF species involved (Cairney and Bastias 2007). The next step in resolving this issue is to tease apart the myriad factors that determine AMF colonization patterns following fire.

The Florida scrub ecosystem offers an opportunity to examine the role of fire in AMF colonization. Although fire can clearly have an impact on soil nutrient dynamics, in scrub, such changes may be less important in determining the extent of AMF colonization. Short-term increases in soil nitrogen and phosphorus have been observed; however, these changes in nutrient content are ephemeral, lasting only a few months before returning to pre-burn levels (Abrahamson 1984; Anderson and Menges 1997; Schafer and Mack 2010). Furthermore, mycorrhizal inoculum potential of Florida scrub also appears to be unaffected by fire (Anderson and Menges 1997). Consequently, the relatively constant nutrient status of soils and mycorrhizal inoculum potential of scrub allows us to examine alternative factors that influence AMF colonization in pyrogenic ecosystems.

Previous work in Florida scrub has shown no effect of fire on AMF colonization and three of four species examined were either not colonized or were only weakly colonized (Anderson and Menges 1997). These results were somewhat surprising given the expectation that this nutrient-limited ecosystem would facilitate strong dependency on AMF and that fire would impact colonization. The root structure of these species suggested that only Balduina angustifolia would be strongly dependent on AMF; however, comparisons of colonization before and after burning were limited by the short-term nature of the study and that B. angustifolia must regenerate from seed following fire (Anderson and Menges 1997). Furthermore, only one burned and unburned site were used in the study. Consequently, we currently lack statistical power to assess the effects of fire on AMF colonization in the Florida scrub.

Here, we take advantage of a series of controlled, replicate burns at the Archbold Biological Station (ABS) to determine the relationship between time since fire and AMF colonization. We assessed total colonization of Paloxia feayi (Asteraceae), a small, perennial shrub endemic to the scrub, scrubby flatwood, and pineland ecosystems of south-central Florida (Carrington 1999). This plant has adapted to fire by establishing seedlings between fires and resprouting postfire from the root system (Ostertag and Menges 1994; Menges and Kohfeldt 1995; Carrington 1999), thus making it an excellent candidate to assess the impact of fire on AMF colonization. We address three questions: (1) Is there a relationship between time since fire (time since the most recent burn) and AMF colonization patterns? (2) Is there a relationship between fire return interval (elapsed time between successive burns) and AMF colonization patterns? (3) Is there a relationship between AMF colonization and soil nutrient content?

Materials and methods

Site information and sample collection

P. feayi was collected from 24 sites at the ABS, Highlands County, Florida (27°10′50″ N, 81°21′00″ W). ABS maintains a 2,101-ha preserve located on the Lake Wales Ridge in south-central Florida that is dominated by fire-adapted scrub habitat with sandy, nutrient-poor soils. The station has been subdivided into a series of discrete plots of varying sizes; these plots are independently burned according to the ABS fire management plan (Main and Menges 1997). The fire return interval for each plot or the time lag between successive fires is determined following Abrahamson (1984) and the intervals range from 2 to 5, 6 to 9, 10 to 14, 15 to 19, 20 to 59, and 60 to 100 years between fires. Thus, sites with a 6- to 9-year fire return interval are burned every 6–9 years. The sites selected for this study spanned the breadth of fire return intervals and ranged from 1 to 15 years postfire (Table 1). As a consequence of the fire management plan at ABS, fire return interval and time since fire (the number of years passing since the most recent fire) vary independently. For example, a plot that was burned 1 year ago could have a fire return interval of 2–5 or 60–100 years depending on the prescribed burn schedule for that particular plot. This independence between the two measures of fire history was accounted for in our statistical analysis.

To standardize fire intensity, plots were selected that were heavily burned, with fire consuming surface litter, dead leaves, small twigs, and palmetto leaf blades (Main and Menges 1997). At this intensity, the aboveground portions of P. feayi are completely removed, although the root system is typically left intact. Plants quickly regrow following fire.
Mycorrhiza

Table 1 Site information for *P. feayi* roots

<table>
<thead>
<tr>
<th>Plot ID</th>
<th>No. plants sampled</th>
<th>Time since fire (years)</th>
<th>Fire return interval (years)</th>
<th>Plot size (m²)</th>
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and often reproduce within 1 year (Ostertag and Menges 1994). Due to a paucity of recently burned plots with fire return intervals of 10–14 and 15–19 years, we combined these two categories in our analyses. One plot in the 6- to 9-year fire return interval (plot 18D) was not burned on schedule due to poor burning conditions.

To test whether time since fire and fire return interval affect AMF colonization in *P. feayi*, roots were collected from approximately 5 plants per plot, sampling across 24 plots differing in fire history. Because the treatment (fire) was applied at the plot level, plots represent the experimental unit in this study; thus, replication was achieved by sampling from 24 plots (Table 1). Multiple plants were collected per plot to obtain a plot mean. To standardize plant collections, a line transect was established in each plot. On each transect, roots were harvested from plants separated by at least 10 m. Prior to root collection, the maximum height and number of lateral branches were determined on each individual to assess plant size. These measures were included in subsequent statistical analyses to account for potential effects of plant size on AMF colonization (see below). Following measurement, each plant was partially uprooted, approximately 30 cm of root length was sampled, and each plant was carefully buried again. This sampling scheme was used to minimize impact to the *P. feayi* population at ABS. Once the roots were harvested, they were placed into plastic bags and were immediately returned to the laboratory. Roots were thoroughly rinsed with tap water and stored in 70 % ethanol at 4 °C until analysis (Brundrett et al. 1996). All collections were made during late May to mid-June 2009.

Analysis of AMF colonization patterns

Roots were cut into approximately 1 cm pieces and cleared in 10 % potassium hydroxide by soaking them for 20 min at 121 °C in an autoclave. Once cleared, the roots were stained by autoclaving at 121 °C for 15 min in a 0.03 % w/v Chlorazol Black E solution (Brundrett et al. 1996). After clearing and staining, segments were stored in 50 % glycerol at 4 °C. Thirty-centimeter segments were haphazardly selected from each plant and mounted in 50 % glycerol, and each segment was viewed at ×40 magnification using a light microscope. Following McGonigle et al. (1990), we used the cross-section intersection method to assess percent root colonization. Each plant was viewed 150 times (30 segments viewed 5 times each), with the exception of 1 plant that had insufficient root material (viewed 105 times on 21 segments). For each view, the presence of AMF intraradical
hyphae, coils, vesicles, and arbuscules were scored separately. In total, 16,305 views were examined across 109 plants in 24 plots.

Soil analysis

To ensure that soil nutrient content (carbon, nitrogen, and phosphorus) did not differ significantly across plots, one soil sample was collected per plot. Samples were taken from the rhizosphere at approximately the root depth of P. feayi and were located near the center of the transect line used to collect root material. Samples were dried at 50 °C before transport to Syracuse, NY for analysis. Total carbon and nitrogen in each soil sample was determined by Dumas combustion in a CE Elantec Flash EA 1112 CN analyzer. Total carbon was measured to determine if there were any differences in organic matter and soil quality among plots differing in fire history. The methods of Bickelhaupt and White (1982) and Lajtha et al. (1999) were used to determine the concentration of inorganic (plant available) phosphorus in each sample.

Statistical analysis

Because the application of the treatment (fire) was conducted at the plot level, the experimental units were the plots (N=24 plots); thus, mean percent colonization per plot was used as the response variable for the analyses. Percent-age data were arcsine square root transformed prior to analysis. A general linear model (GLM) was used to assess the relationship between the total percent root length colonized by AMF and fire history. The model included time since fire, fire return interval, and measures of plant size (height and lateral branching) as factors. An additional analysis with time since fire, fire return interval, and plot size was also conducted to ensure that plot size had no influence on colonization. Similar GLMs were used to determine whether the percentage of root length with arbuscules, coils, vesicles, and intraradical hyphae were affected by fire history. These models also included time since fire, fire return interval, and plant size as factors. To determine whether the concentration of carbon, nitrogen, and phosphorus varied among plots differing in fire history, we used a GLM that included time since fire and fire return interval. Finally, linear regression was used to determine the relationship between soil nutrients and AMF colonization and between plant size and time since fire.

Results

Palafoxia feayi roots were consistently colonized by AMF, with more than 98 % of plants colonized. Total colonization within plants ranged from 0.7 to 29.3 %. Percent root length containing arbuscules was 0 to 8 %, coils was 0 to 23 %, and intraradical hyphae was 0 to 11 %. The GLM assessing the relationship between total colonization, fire history, and measures of plant size was significant ($F_{7,16}=2.77; P=0.04$); however, only the time since fire factor had a significant effect ($F_{1,16}=10.98; P=0.004$). Fire return interval, plant height, and lateral branching were nonsignificant ($P>0.6$ for all factors). There was a negative relationship between time since fire and total colonization (Fig. 1), and there was no relationship between time since fire and plant height ($F_{1,22}=2.22; P=0.16$). Similarly, the GLM assessing the relationship between total colonization, fire history, and plot size was significant ($F_{6,17}=3.16; P=0.03$) with time since fire having the only significant effect ($F_{1,17}=10.49; P=0.005$. Plot size had no effect on colonization levels ($F_{1,17}=0.10; P=0.75$).

Fire history also had significant effects on the presence of specific AMF structures. The GLMs examining the role of fire history on the percent root length containing intraradical hyphae ($F_{7,16}=5.00; P=0.02$) and coils ($F_{7,16}=2.68; P=0.05$) were significant. In both instances, time since fire and plant height were the only significant effects (all $P<0.05$). The presence of coils and intraradical hyphae declined with increasing time since fire (Fig. 1), and the roots of taller plants contained fewer of these structures. Neither of the GLMs examining the relationship between fire history and percent root length containing vesicles or arbuscules were significant (vesicles: $F_{7,16}=1.56; P=0.22$; arbuscules: $F_{7,16}=1.52; P=0.23$).

Soil nutrient levels were similar across sites. Nitrogen content ranged from 0 to 0.06 %, carbon content had a range of 0.17 to 1.23 %, and phosphorus content ranged from 3.37 to 9.31 μg/g. The GLMs testing for differences in soil chemistry associated with fire history were not significant (nitrogen: $F_{5,18}=0.81; P=0.56$; phosphorus: $F_{5,18}=0.82; P=0.55$; carbon: $F_{5,18}=6.65; P=0.66$). There was also no relationship between soil nutrient content and total AMF colonization (nitrogen: $R^2=0.001; P=0.88$; phosphorus: $R^2=0.003; P=0.80$; carbon: $R^2=0.001; P=0.86$).

Discussion

The results indicated a strong decrease in total AMF colonization with time since fire that cannot be explained by changes in nutrient availability across sites. Total colonization was greatest 1 year postfire, gradually declining as time since fire increased, and as expected, we observed no differences in soil nutrients across plots. The decrease in total percent colonization also cannot be explained by changes in plant size as we found no relationship between plant height and fire regime. P. feayi quickly resprouts following...
fire-preventing stratification of plant height across plots differing in burn history. The decline in total colonization was also mirrored by a decline in specific AMF structures. The results showed a negative relationship between fire and colonization by coils and intraradical hyphae. Interestingly, not all AMF structures changed with time since fire (e.g., arbuscules and vesicles), although the reason for this is unclear.

In contrast, there was no effect of fire return interval, the time lapse between subsequent fires. Given that scrub undergoes no successional change following fire, this result was expected. Scrub fires can completely eliminate aboveground vegetation, as was the case in our experimental plots, but most of the vegetation resprouts from protected root systems. As a consequence, the regrowth of scrub vegetation following a fire returns the system to the prefire community (Abrahamson 1984; Schmalzer and Hinkle 1987); thus, the length of time between successive fires is unlikely to affect colonization patterns.

One possible explanation for the observed relationship between time since fire and patterns of colonization is that fire temporarily removes dominant vegetation and increases light availability for *P. feayi*. The sites selected for this study were heavily burned with aboveground vegetation completely removed; thus, light availability will be greatest immediately postfire and will decline as the vegetation resprouts. Indeed, at our field sites, canopy cover is positively correlated with time since fire, and plots with increased canopy cover have lower light availability (Abrahamson 1999). Shading has been shown to decrease AMF colonization as plants allocate more resources to aboveground parts and have fewer resources to support AMF (e.g., Whitbeck 2001; Gehring 2003). Canopy cover strongly influenced flowering in the scrub plants *S. repens* and *Sabal etonia*, suggesting that light availability is an important factor limiting growth and reproduction in this ecosystem (Abrahamson 1999). Furthermore, there is also evidence that growth patterns of *P. feayi* change in response to fire; *P. feayi* exhibits peak reproduction during the first year following fire and sharply declines 1 year postfire (Ostertag and Menges 1994). This growth response may be caused by the ephemeral nutrient pulse associated with scrub fires (e.g., Schafer and Mack 2010) and/or by changes in light availability. The combination of maximal light availability and a nutrient pulse may explain the increased AMF colonization patterns immediately following fire, and thus, we might expect to observe a gradual decline in colonization over time if plant carbon production is reduced by shading.

Even so, the hypothetical scenario described assumes that root colonization level reflects the mutualistic functioning of the interaction. Unfortunately, the relationship between colonization and mycorrhizal function is complex (e.g., Johnson 2010), thus our conclusions are somewhat limited. The colonization levels observed in *P. feayi* are relatively low, suggesting that the modest changes in colonization following fire may have little functional consequence for the plant. The present study is also limited in the sense that we have examined only one measure of the interaction: root colonization. Assessing extraradical hyphal abundance or AMF species composition across the fire timeline may help to explain the observed patterns in colonization. The finding of decreased colonization associated with time since fire was surprising given the results from a previous study conducted at the same sites. Anderson and Menges (1997) showed no change in colonization associated with fire in three scrub herbs (a fourth species was examined, but plants did not survive the fire). Contradictory results among studies of AMF colonization have been attributed to a number of factors including differences in plant community composition, plant adaptation to fire, and soil nutrient conditions (Cairney and Bastias 2007). However, since both studies were conducted at the same field sites on plants that respout following fire, none of these factors can explain the strikingly different pattern observed in *P. feayi*. Two obvious differences between these studies are plant growth form and AMF colonization levels. *P. feayi* is a small

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**Fig. 1** Relationship between mycorrhizal colonization of *P. feayi* roots and time since fire (years). Each circle represents the mean value for a plot (±SD). a Total colonization ($R^2_{adj}=0.50$; $P<0.0001$), b colonization by coils ($R^2_{adj}=0.19$; $P=0.03$), c colonization by intraradical hyphae ($R^2_{adj}=0.29$; $P<0.01$).
woody shrub in contrast to the herbs examined by Anderson and Menges (1997). *P. feayi* also had higher levels of colonization as more than 98 % of plants examined were colonized, and total colonization ranged upwards of 29 %. Very low (2–2 %) colonization was observed in both *Liatris tenuifolia* and *Pityopsis graminifolia*, and *Aristida stricta* lacked AMF completely (Anderson and Menges 1997). Consequently, differences between the studies may be attributed to differences in growth form and colonization level.

In addition to the dissimilarities among the study plants themselves, there also exist several differences in experimental design. Anderson and Menges (1997) compared colonization of plants in two plots: one site that was burned about 8 months prior to the final sampling date and one “unburned” site (burned previously but not as recently as the burned site). In contrast, the present study used 24 plots ranging from 1 to 15 years postburn. Since the timing of the last fire for the unburned site is unknown, comparing these two studies is challenging. If Anderson and Menges’ (1997) burned and unburned sites were more similar in burn age (e.g., the unburned site was burned about 5 years previously), detecting differences in colonization may have been difficult. By comparing multiple plots differing in fire history, the present study has greater statistical power.

Overall, the present study shows a negative relationship between percent colonization and time since fire in this pyrogenic ecosystem. We hypothesize that this pattern may be a result of increased shading as vegetation resprouts after a burn. Controlled experiments to assess this possibility will be useful in making progress towards determining the factors most important in shaping colonization patterns.

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