ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTES IN THE SUGARCANE CROP CYCLE: RATES AND EXTENT OF DEVELOPMENT

By

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Abstract

SUGARCANE ROOT SYSTEMS host a variety of fungal symbionts including arbuscular mycorrhizal (AM) fungi and dark septate endophytes (DSE). The potential impacts of root-associated fungal symbiosis in sugarcane crops remains poorly understood. A field study was undertaken to assess the colonisation dynamics of AM/DSE in sugarcane sett and adventitious shoot roots from first and second ratoon crops over 28 weeks of development. Significant differences in the rate of sett root colonisation over time were observed between fungal types at both sites. Differences in AM fungal sett root colonisation between sites were not significant. DSE sett root colonisation was significantly lower in the second ratoon compared with the first ratoon crop. For both fungal types, sett root colonisation was significantly higher than for shoot roots at both sites. Maximum colonisation levels of AM/DSE occurred between 5–7 weeks, prior to shoot root establishment and rapid development phase of first and second ratoon crops. These data provide the basis for sampling strategies and further research to assess the impacts of AM/DSE in sugarcane cropping systems.

Aim and background

The multifunctional associations between plant roots and symbiotic fungi have the potential to influence the productive capacity of sugarcane cropping systems in a variety of ways (Magarey *et al.*, 2005b; Rutherford *et al.*, 2002). Among these associations, arbuscular mycorrhizal (AM) fungi have been widely scrutinised for their purported agronomic benefits (Smith and Read, 2008). The impacts of AM associations in sugarcane, however, are largely inconclusive (Kelly *et al.*, 2005; Magarey *et al.*, 2005a; Pankhurst *et al.*, 2001).

As such, the mechanisms influencing the rate and extent of AM symbiosis in sugarcane remain poorly understood (Claassens *et al.*, 2017). The dark septate endophytes (DSE) represent another group of fungal symbionts colonising sugarcane root systems (Pankhurst *et al.*, 2003; Ling *et al.*, 2016). Despite being less well characterised than AM associations, the potential of DSE to enhance plant biotic and abiotic stress tolerance has attracted considerable research attention (Rodriguez *et al.*, 2009).

As a critical first step to understanding AM/DSE fungal communities in sugarcane, a time sequence that follows fungal colonisation throughout the critical early developmental stages of the cropping cycle was proposed. The aim was to assess the rate and extent of AM/DSE colonisation of sugarcane sett roots and shoot roots from cropping sites with different histories over 28 weeks of crop development.

Our hypotheses were:

(1) Fungal colonisation in sett roots would differ over 28 weeks of crop development.

(2) Fungal colonisation in sett roots and shoot roots would differ between crops previously grown for one year and two years.

(3) Fungal colonisation would differ between sett roots and shoot roots.

Methodology

Two nearby sites with different cropping histories on the Richmond River floodplain in northern New South Wales were chosen for comparison. Both sites were initially planted with the Q208(ф) cultivar in October 2014, after a six-month soybean (*Glycine max*) break crop. At Site 1, a one-year plant cane crop was followed by a one-year ratoon. At Site 2, plant cane was continuously grown for two years. Burning and harvesting of both sites occurred on the same day in 2016. From October 2016 to May 2017, sugarcane root systems were sampled from a 15 m^2 area within each site at the following intervals: pre-harvest (0), 1, 2, 3, 5, 7, 11, 17 and 28 weeks.

At each interval, five randomly selected replicates consisting of whole root systems were excised and processed. At successive sampling intervals, adjacent plants were selected and the processing procedure was repeated. Sett root samples (about 2 g FW) from each root system were randomly selected from each sample for the duration of the experiment. Shoot roots samples (about 2 g FW) were collected at 7, 11 and 17 weeks, from the time of their contact with soil until they were indistinguishable from sett roots. All root samples were prepared for microscopic examination according to the procedures of Phillips and Hayman (1970). Prepared samples were quantified for fungal colonisation following the procedures of McGonigle *et al*. (1990). Statistical analyses to compare the interaction of time, site, and root type were undertaken with R software v 3.3.2 (R Core Team, 2016).

Results

Harvesting did not negatively impact the levels of AM sett root colonisation observed at both sites. Maximum AM and DSE colonisation was observed at week seven at Site 1 and week five at Site 2 (Figure 1). Lower levels of DSE colonisation were observed at Site 1 compared with Site 2 (Figure 1). The interaction for AM fungi for site and time was significant (Table 1). However, specific contrasts showed no significant differences between sites at each time point. Significant differences were observed for DSE fungi at each time point except week seven (Table 2).

Elevated levels of AM shoot root colonisation were observed for both sites at week seven. Maximum colonisation levels were observed by week 11 and were lowest at week 17 at Site 1. In contrast, maximum levels of AM colonisation were observed at week seven and fell continuously until week 17 at Site 2. Low DSE colonisation of shoot roots was observed at both sites (Figure 2). Significant differences were observed for time and root type (Table 3). Actual root differences showed that sett root colonisation was significantly higher than shoot root colonisation for both fungal types (Table 4).

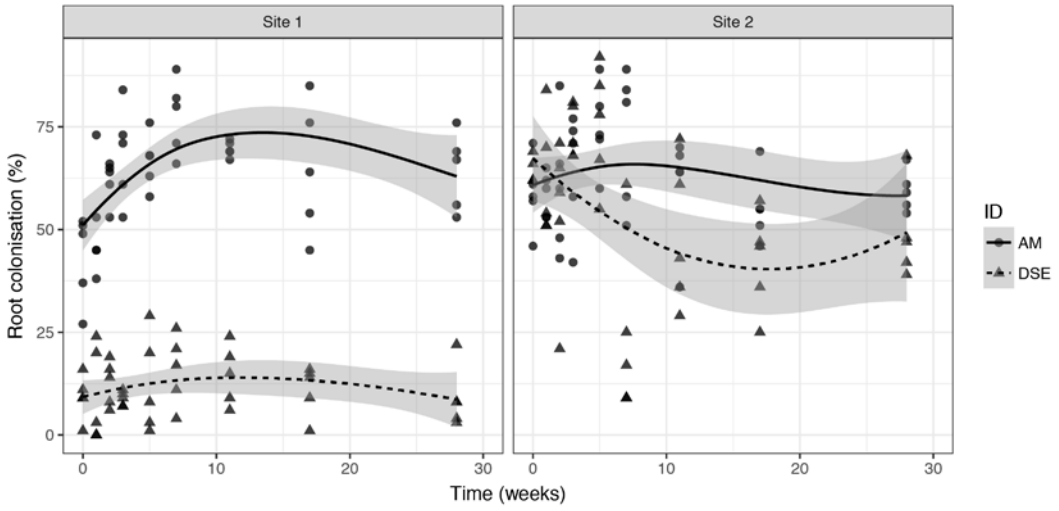

Fig. 1—Total colonisation (%) of (AM) fungi and dark septate endophytes (DSE) in sugarcane sett roots at sites 1 and 2 over 28 weeks of development.

Table 1—GLM table of significance (p-values) for AM/DSE fungi.

Fungi	Site	Time	Interaction
AM	0.047	<0.001	0.015
DSE	<0.001	NS	0.035

Table 2—Differences in sett and shoot root colonisation by AM/DSE fungi at Site 1 and Site 2 over time.

Week	AM root colonisation (%)		Pr(>|z|)	Significance
	Site 1	Site 2		
0	43.2 ± 4.9	58.6 ± 4.0	>0.05	NS
1	50.8 ± 6.0	58.6 ± 2.4	>0.05	NS
2	61.8 ± 2.4	60.4 ±7.4	>0.05	NS
3	68.4 ± 5.3	64.4 ± 6.5	>0.05	NS
5	66.6 ± 3.0	74.8 ± 4.8	>0.05	NS
7	77.6 ± 4.1	72.6 ± 7.6	>0.05	NS
11	69.6 ± 0.9	60.4 ± 6.2	>0.05	NS
17	64.8 ± 7.2	55.2 ± 3.8	>0.05	NS
28	64.2 ± 4.3	59.4 ± 2.2	>0.05	NS
Week	DSE root colonisation (%)		Pr(>|z|)	Significance
	Site 1	Site 2		
0	9.2 ± 2.4	64.2 ± 1.4	<0.01	***
1	9.4 ± 5.2	62 ± 6.5	<0.01	***
2	12.6 ± 2.4	53.6 ± 8.7	<0.01	***
3	8.8 ± 0.8	73.6 ± 2.9	<0.01	***
5	12.2 ± 5.3	75.4 ± 6.6	<0.01	***
7	15.8 ± 3.8	24.2 ± 9.7	>0.05	NS
11	14.6 ± 3.3	48.2 ± 8.0	0.013	*
17	11 ± 2.8	42.2 ± 5.4	0.023	*
28	9 ± 3.4	48.8 ± 5.1	<0.01	**

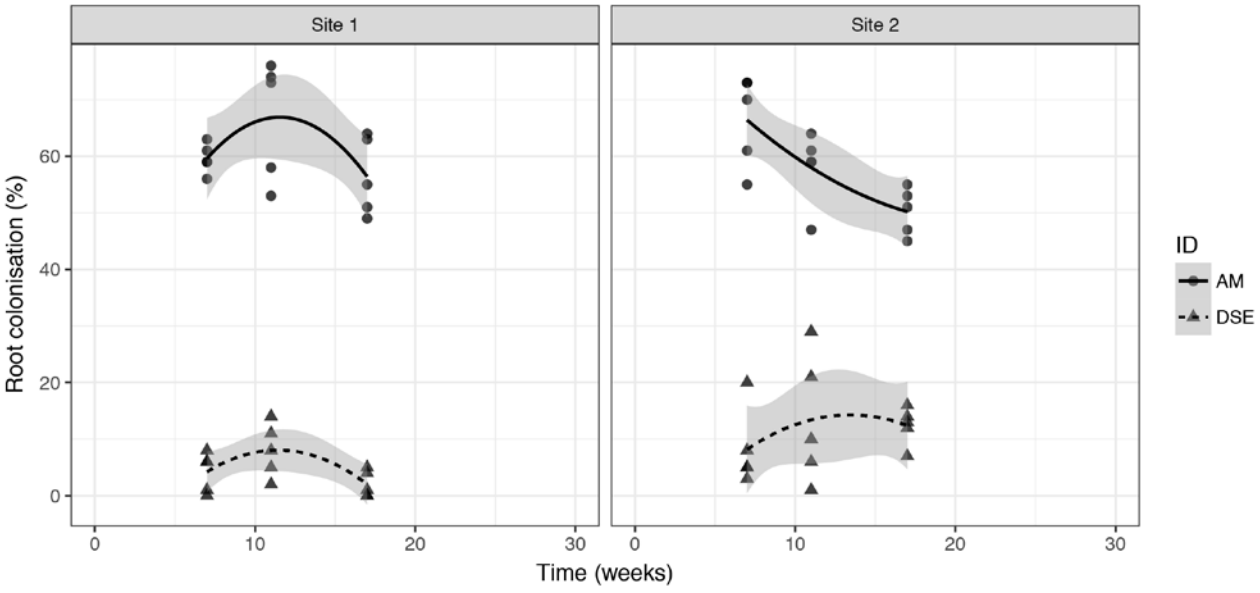

Fig. 2—Total colonisation (%) of (AM) fungi and dark septate endophytes (DSE) in sugarcane shoot roots at sites 1 and 2 over 28 weeks of development.

Table 3—GLM table of significance (p-values) for AM/DSE fungi testing for differences in root colonisation between sampling dates, sites and root types and the interactions between them.

Interaction	Fungi	
	AM	DSE
Time	<0.001	<0.001
Site	NS	NS
Root	0.006	0.037
Time × root	NS	NS
Time × site	NS	NS
Root × site	NS	NS
Time × root × site	NS	NS

Table 4—Colonisation (%) of different root types by AM and DSE, averaged over time and site (± are the SE of the mean).

Root type	AM	DSE
Sett	67±2	24±2
Shoot	60±2	7±1
P-value	0.004	0.03

Conclusions

Our results confirmed our first hypothesis with significant changes in root fungal colonisation observed over time. Our second hypothesis was partially supported, with significantly lower levels of DSE recorded colonising roots at Site 1 compared with Site 2. No significant differences were observed in AM colonisation of sett roots between sites and AM/DSE colonisation of shoot roots between sites were also not significant. Our third hypothesis was supported as significant differences in AM/DSE colonisation were observed between root types.

The sampling protocol developed herein provides an effective approach to assessing the colonisation dynamics of fungal root symbiosis is sugarcane systems. Given the diversity of AM and DSE taxa occupying sugarcane roots (Mehnaz, 2013; Pankhurst *et al*., 2003), utlising such a protocol will allow for comparison of root-fungal associations between sites and across years. Our results indicate that substantial levels of root colonisation are carried over between ratoons

These observations have been confirmed elsewhere (Magarey, 1996) and suggest that management strategies that support effective levels of beneficial fungal root symbionts may be critical, particularly in systems where these levels are suboptimal.

The differential colonisation of shoot roots and sett roots has been observed in glasshouse conditions (Pankhurst *et al.*, 2001). The present results demonstrate that these differences may be exacerbated under field conditions. As such, accounting for the contrasts in sett root and shoot root characteristics should be emphasised in future assessments of root fungal symbiosis in sugarcane.

Here, we have provided a more detailed understanding of root symbiotic functioning in sugarcane as plants approach physiological maturity. Further research is required to more fully elucidate these relationships from a range of different management contexts.

Varying climatic and edaphic factors, variety selection and the diversity and functional roles of specific fungal taxa may all influence observed outcomes. In light of these knowledge gaps, these data set the foundation for the targeted, hypothesis-driven experiments required to address below-ground production restraints in sugarcane systems.

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