AUBURN UNIVERSITY
FOREST HEALTH
COOPERATIVE

RESEARCH REPORT 17-04

COMPETITIVENESS OF *AMYLOSTEREUM* SPP. FUNGI AGAINST *LEPTOGRAPHIUM* SPP. FUNGI

by
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ABSTRACT

Amylostereum spp. are basidiomycetes, which cause white rot fungi of pine trees. Spores of these fungi are vectored by *Sirex* spp. woodwasps, who infect host trees by ovipositing eggs into the xylem of affected trees. The invasive complex associated with *Sirex noctilio* and *Amylostereum areolatum* (native to Europe and Northern Africa) has been devastating to planted non-native pine forests in the Southern Hemisphere, but has not been problematic in North America where *S. noctilio* also has been introduced. While there is currently no evidence that *S. noctilio* is in the southeastern United States, studies are being carried out to determine how its symbiont, *A. areolatum*, might interact with other fungi that are already present within the southern pine ecosystem. For this study two species of *Leptographium* were chosen, since they are commonly found in industrial pine stands in Alabama. Both are ascomycetous root pathogens that behave differently than *Amylostereum* spp., and also would affect the overall tree vigor if inoculated into the same substrate. Isolates of *Amylostereum* spp. from around the world were plated on petri dishes with isolates of *Leptographium terebrantis* and *Leptographium procerum*. Growth rates were determined by measuring the leading edge of mycelia with a planimeter every other day for two weeks after inoculation. In most cases, *Leptographium* isolates outcompeted *Amylostereum* isolates, and in some cases completely overgrew them. This study suggests that *Amylostereum* spp. likely would not outcompete *Leptographium* spp. in a forest setting, although further studies need to be undertaken to see how the two fungi would compete in situ.

INTRODUCTION

Amylostereum is a genus of white rot fungi that affects *Pinus* spp. This genera is associated with *Sirex* spp. woodwasps, the obligate symbiont that vectors *Amylostereum* spores from tree to tree. This relationship between wasp and fungi is crucial to the development of the siricid larvae. *Amylostereum* hyphae decreases the moisture content of the tree, rotting the wood from within, until it is hospitable for larval development. Historically, *A. chailletii* (Pers.) Boidin has been linked with *S. nigricornis*, the species of woodwasp native to the southeastern United States. Both insect and pathogen are associated with declining stands, normally attracted to dead or dying wood. More recently, studies have shown that *S. nigricornis* F. might now be associated with *A. areolatum* (Chaillet ex. Fr) Boidin, a pathogen associated with the invasive *S. noctilio* F. The pairing of *S. noctilio* and *A. areolatum* are different than other woodwasp-fungal associations. The two species colonize fairly healthy trees, eventually causing mortality if the infestation is heavy.

Since commercial pine plantations are a sizable industry in the southeastern United States, many independent collection efforts have been waged to try to better understand woodwasp populations in this region. Recent studies (Wahl, Chapter 2; Barnes et al., 2014; Johnson et al., 2013) have surveyed southeastern United States forests, finding mostly native siricids. No known captures of *S. noctilio* have been reported in the area, but this species has been identified in the northeast United States since 2004 (Hoebeke et al., 2005). Other non-native siricids have been identified in the United States, specifically *Eriotremex formanosanus* Matsumura in the Southeast (Smith et al., 1996).

One objective of this study is to determine how isolates of native and non-native *Amylostereum* might compete with common root pathogens found in the Southeast (*Leptographium terebrantis* (Kendrick) Wingfield and *L. procerum* S.J. Barras & T.J. Perry). These and similar root pathogens are factors in a phenomenon known as loblolly pine decline (Eckhardt et al., 2007). The fungi that are part of this syndrome are vectored by root feeding beetles, such as *Hylastes* spp., *Hylobious pales* (Herbst), and *Pachylobious picivorous* Germar in the southeast (Matusick and Eckhardt, 2010), as well as phoretic mites (Hofstetter and Moser, 2014).

These bark and ambrosia beetles are commonly found in the same forest ecosystem that siricids inhabit, and have the potential to be attracted to the same sort of pine material as female *Sirex* spp. (Ryan et al., 2012a). Both bark beetles and woodwasps tend to be attracted to volatilized chemicals given off by stressed trees (Franceschi et al., 2005; Böröczky et al., 2012). These chemicals not only give the insects an idea of which trees might be suitable substrate, but also have been shown to affect the growth of the associated fungi the insects inoculate the tree with (Eckhardt et al., 2009; Wahl, Chapter 4). Gaining a better understanding of how these complexes overlap in ecosystems is crucial to better manage pine forest systems.

An additional objective of this study is to determine the relationships between isolates of *Amylostereum* used in the competition assay. A method of determining relationships between isolates of fungi in the same species is testing for Vegetative Compatibility Groupings (VCGs). This is a test where multiple inoculums of the same species are grown on the same plate to determine how hyphae interact with each other. This is a useful method for determining relationships of isolates, as clones form the VCGs. Recognizing that multiple isolates are clones can allow pathways of fungal introduction to be traced back to its origins (Vasiliauskas et al., 1998).

MATERIALS AND METHODS

Competition Study Inoculation

Amylostereum areolatum and *A. chailletii* isolates from around the world were obtained from the culture collection at the Forestry and Agricultural Biotechnology Institute at the University of Pretoria, South Africa. Two isolates of *A. chailletii* were isolated from live female *S. nigricornis* wasps in Auburn, Alabama, United States. *Leptographium* isolates were from the culture collection of the Forest Health Dynamics Lab, Auburn University, isolated in previous chapters of this thesis.

Isolates of *Amylostereum* were inoculated onto plates of Potato Dextrose Agar (PDA) four days prior to adding the *Leptographium* inoculums to allow the *Amylostereum* isolates time to begin to grow in accordance with the methods of Ryan et al. (2011). *Leptographium* isolates were plated directly across from the growing *Amylostereum* isolate at the edge of the plate, so that they would have to grow towards each other over the duration of the study (Fig. 5.1).

Competition Study Measurement

Plates were kept in dark cabinets in a temperature controlled laboratory, to mimic the growth environment within the bole of a pine tree. The hyphal growth was traced onto transparencies, starting two days after the inoculation of the *Amylostereum* isolates. The plates were traced every other day for two weeks, or until the plate was completely grown over by one of the isolates. The surface area (cm^2) was computed by measuring the traced hyphal growth with a digital planimeter (Lasico 1281-12; Lasico, Los Angeles, CA, USA) as in Eckhardt et al., (2009).

Statistical Analyses

Statistical analyses were performed on the competition plates using SAS version 9.3 (2010; SAS Institute, Inc., Cary, NC). A repeated measures one-way ANOVA was performed on *Amylostereum* isolates plated against each of the *Leptographium* spp. An ANOVA between isolates of *Amylostereum* spp. also was performed. A post hoc Tukey's Standardized range Test, and a Paired T-tests were performed.

Vegetative Compatibility Grouping Study

The same *Amylostereum* spp. isolates used in the competition assay were plated in a similar fashion as the previously described plate study, this time placing different isolates of the same species on the same plate. Only isolates of the same species were plated together, in the cases where the species were determined before the assay. VCGs were scored 0 if a barrier formed between the hyphae of the two isolates, or 1 if the hyphae grew into each other. If a score of 1 was given, it was assumed that the two isolates were clonal. Isolates N1, S1, and 15B from Alabama were tested against each of the other isolates, regardless of species.

RESULTS

Competition Study

Amylostereum isolate growth rates differed significantly from each other. Certain isolates of *Amylostereum* such as 15102 (*A. chailletii*, Sweden) had no significant growth over the duration of the study (P=0.2790), whereas 15B (*A. chailletii* from Alabama) grew significantly at each of the measurements (P<0.0001) (Fig. 5.2). As expected, *Leptographium* growth was more uniform, and did not differ significantly from plate to plate, but did differ significantly over time (Fig 5.3).

Vegetative Compatibility Groupings

Isolates of *A. areolatum* formed three compatibility groups. All isolates from Alabama, *A. chailletii* (S1 and 15B), and the unknown N1 were not in the same vegetative compatibility groups as other isolates used.

DISCUSSION

In most cases, *Leptographium* isolates significantly outcompeted *Amylostereum* isolates, and in some cases completely overgrew them. *Leptographium* spp. performed in a more uniform manner, which is to be expected, as only one isolate of *L. terebrantis* and *L. procerum* were used throughout the duration of the study. Growth rates of *Leptographium* isolates were significantly higher overall than *Amylostereum* competitors during the first two growth measurements (p=0.0003, 0.0009 respectively). In some cases, *Leptographium* isolates overgrew their *Amylostereum* competitors, causing the *Amylostereum* surface area to decrease near the end of the study.

The isolate of *L. terebrantis* had significantly higher growth rates than most isolates of *Amylostereum* (all except 15B), while the results from the *L. procerum* trial varied much more (Fig. 5.4). Certain isolates of *A. areolatum* (3310, 8898, 8902, 13127, 37116, 37414, 37416) did not differ significantly overall in growth from the *L. procerum* isolate (Fig. 5.4). Interestingly, these are from three different VCG groupings. Isolate 13127 and 3309 are from the same VCG, yet did not behave in the same means when paired with *L. procerum*. This is unsurprising, as *L. procerum* does not tend to be as severe a pathogen (Eckhardt et al., 2004). In previous studies, *L. procerum* was found to be a less virulent competitor than *L. terebrantis* (Wingfield, 1986).

One isolate of *Amylostereum chailletii*, 15B isolated from a female *S. nigricornis* in Auburn, Alabama outcompeted both the *L. terebrantis* (p<0.0001) and *L. procerum* (p<0.0001) in all replications of the study. The performance of this isolate was an outlier from the normal behavior of *Amylostereum* isolates used in this study, as well as the findings of Ryan et al. (2011). This growth pattern differed even from the other *A. chailletii* isolates from the same area. This difference observed could be attributed to the fact that these two isolates were found in the same area, in arguably similar environmental conditions.

In the VCG study several distinct compatibility groupings were determined (Fig. 5.5). Unfortunately, this test was inconclusive to try to trace isolates of *A. chailletii* from Alabama back to a point of origin. This uniqueness would suggest that these isolates are endemic to the area where they were naturally located. Interestingly, the two isolates from Alabama that are molecularly confirmed as *A. chailletii*, 15A and S1, (Wahl, Chapter 3) did not form a VCG. However, isolate S1 did form a VCG with N1, the unknown isolate, which suggests that this is the same clone of *A. chailletii*. This theory supports the different reactions of isolate 15B versus S1 and N1 in the *Leptographium* trials.

CONCLUSION

This study shows that non-native isolates of *Amylostereum* spp. fungi tend to be poor competitors to *Leptographium* spp. that are found in the southern pine ecosystem. One isolate of *A. chailletii* from Alabama significantly outcompeted both of the *Leptographium* spp. isolates. This was especially surprising, as *Amylostereum* spp. are generally thought of as slow growing decomposers in a system. The more economically damaging *A. areolatum* when coupled with the invasive *S. noctilio* has been shown to cause eventual mortality of trees, but that is typically not the role of *A. chailleti* in the southern pine ecosystem.

Isolates of *A. chailleti* from Alabama did not all form a vegetative compatibility group, suggesting that the three isolates are not clonal. This is further supported by the difference in growth rates when in competition with a *Leptographium* spp. inoculum.

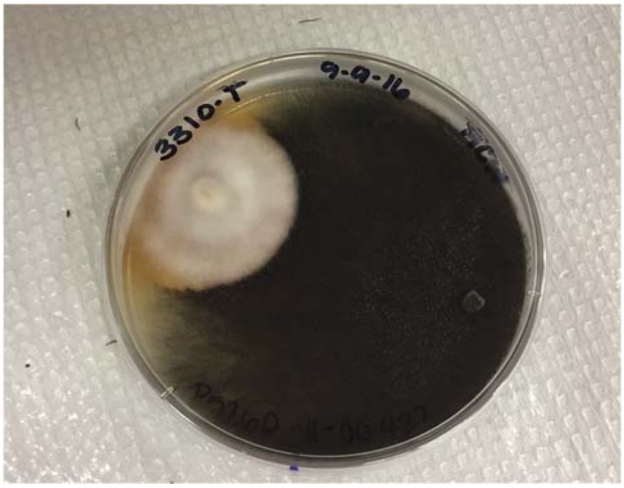

Figure 5.1 *Amylostereum areolatum* isolate from France (white hyphae) plated with *Leptographium terebrantis* isolated in Alabama (green hyphae).

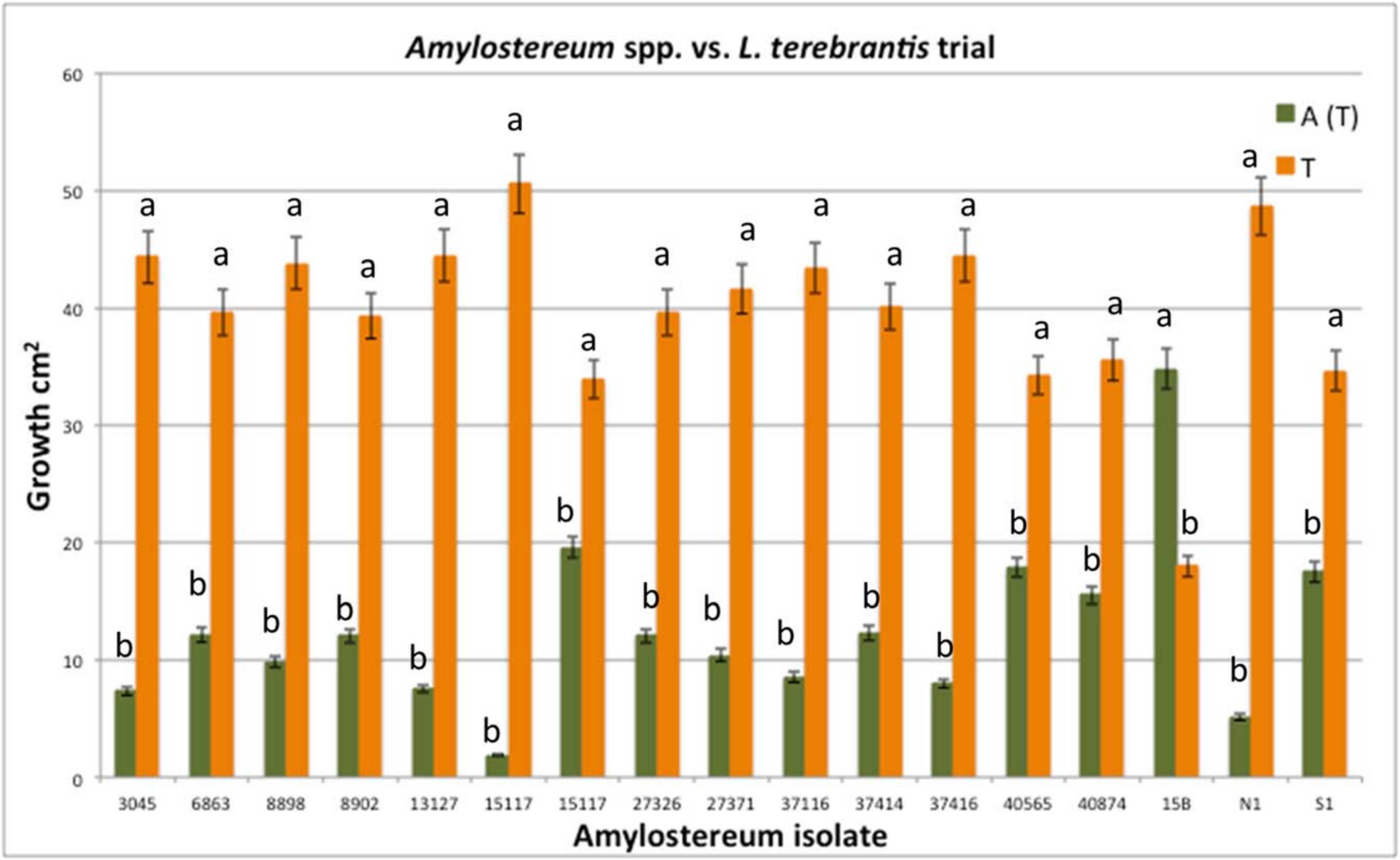

Figure 5.2. *Amylostereum* spp. growth in cm² plated against *Leptographium terebrantis*. Isolate 15B (*A. chailletii*) has a significantly higher growth rate through the duration of the study.

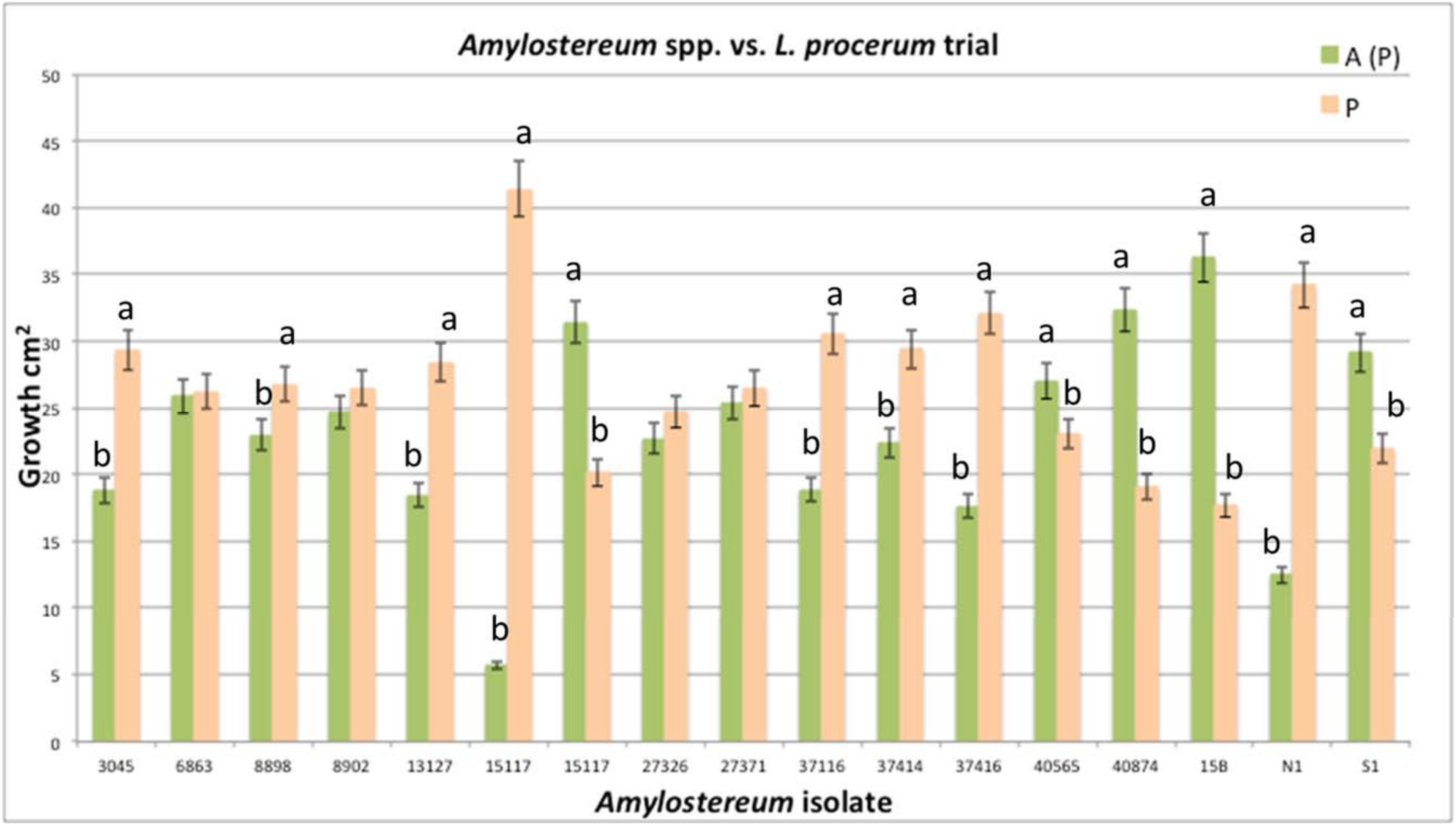

Figure 5.3. *Amylostereum* spp. growth in cm² plated against *Leptographium procerum.* Isolate growth rates differ significantly in response to their competitors.

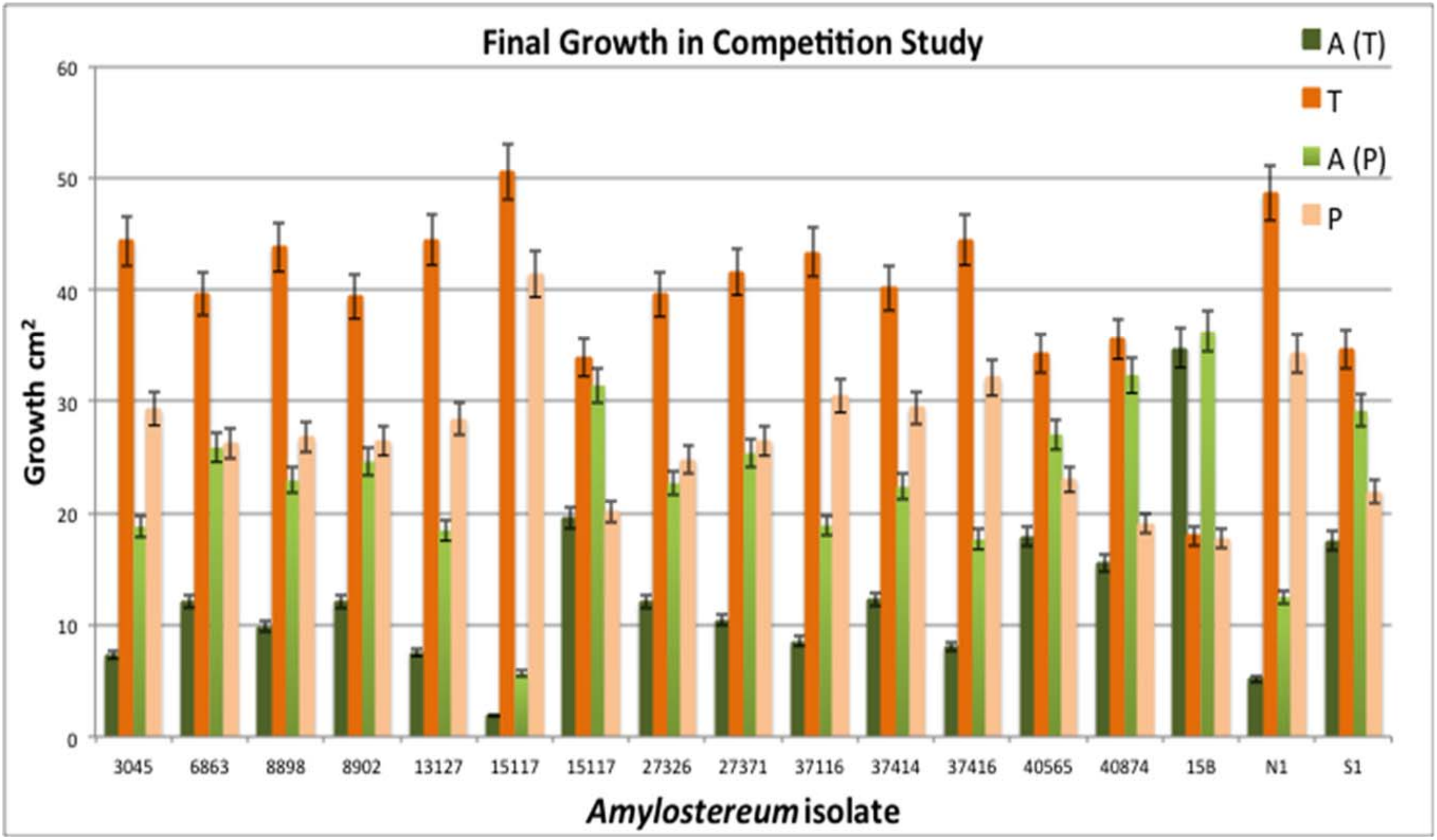

Figure 5.4. Overall results from competition study. Treatment effect from *Leptographium* spp. can be seen.

Table 5.1. Results from the VCG plate study. 1 represents that the two isolates paired together as a VCG, suggesting they are clones. 0 represents that the two isolates did not form a VCG, and therefore are not clonal.

	3309	3310	6863	8898	8902	13827	27371	37116	37414	37416	40565	40874	S1	N1	15B	15102	
3309	--																
3310	0	--															
6863	0	1	-														
8898	0	1	1	--													
8902	0	0	0	0	--												
13827	1	0	0	0		--											
27371	0	0	0	0	1	0	--										
37116	0	0	0	0	0	0	0	--									
37414	0	0	0	0	0	0	0	1	--								
37416	0	0	0	0	0	0	0	1	1	--							
40565	0	0	0	0	0	0	0	0	1	1	--						
40874	0	0	0	0	0	0	0	0	1	1	1	--					
S1	0	0	0	0	0	0	0	0	0	0	0	0	--				
N1	0	0	0	0	0	0	0	0	0	0	0	0	1	--			
15B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--		
15102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	--	
27326	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	--

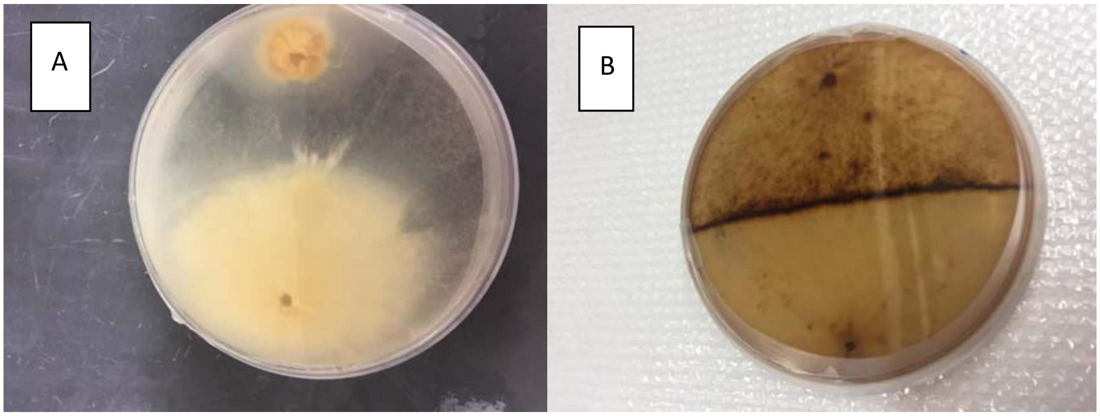

Figure 5.5. VCG trial results. (A) Isolates 37414 and 37416 form a VCG, suggesting they are clonal. (B) Isolates 6863 and 8902 have boundary separating hyphae, and do not form a VCG, suggesting they are not clonal.