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Biodegradable plastic degradation products alter germination and growth of Aspergillus

Taylor Cofer
Western Washington University, coferth@gmail.com

Biodegradable plastic degradation products alter germination and growth of *Aspergillus*

By

Taylor Cofer

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ADVISORY COMMITTEE

Dr. Marion Brodhagen, Chair

Dr. Mark Peyron

Dr. Matthew Zinkgraf

GRADUATE SCHOOL

David L. Patrick, Dean

Master's Thesis

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Taylor Cofer
November 5, 2020

Biodegradable plastic degradation products alter germination and growth of *Aspergillus*

A Thesis
Presented to
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In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Taylor Cofer
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Abstract

Although much work has been focused on micro and nano-plastics in soils, the bioactivity of common plastic additives (plasticizers) and monomers have been overlooked. One source of plastic pollution in agricultural soils is breakdown products from plastic soil covers called mulches. The plastics industry is attempting to make biodegradable plastic mulch (BPM) that have reduced environmental impacts and removal costs compared with those typically associated with conventional plastic mulches. Two common polymers added to BPMs are thermoplastic starch (TPS) and poly(butylene-adipate-co-terephthalate (PBAT). In this study, the effects of components that can leach from TPS and PBAT were investigated to determine their influence on growth and germination in four *Aspergillus* strains. Glycerol, a plasticizer that can leach from TPS, increased growth and germination for *A. flavus*. The PBAT breakdown product 1,4-butanediol did not alter germination in any of the four strains tested. The PBAT breakdown product adipic acid decreased germination in three strains, including *A. flavus*. The practical significance of these results is that within the microenvironment of BPMs, glycerol leaching from TPS could increase germination of a mycotoxin producing species of *Aspergillus* (*A. flavus*), increasing the potential for more toxins to enter the food system. Conversely, the release of organic acids from PBAT may cause acid stress to other microorganisms besides *Aspergillus*. Hydrolysis of ester linkages is a common mechanism for breakdown of most biodegradable polymers, so consideration of the effect of the resultant organic acids on microorganisms that colonize or break down these plastics is important.

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INTRODUCTION

Plastic mulches

With a growing world population there is the need to produce more food than ever and plasticulture has been one of our answers to this need. Plasticulture is the agricultural practice of using plastics to transport, store, cover, or assist in growing crops. A large market segment of plasticulture is plastic mulch (PM). Several attempts have been made over the past two decades to estimate PM acreage (e.g. 427,000 hectares in Europe (2011), 10 million hectares in China (1999), and 160,000 hectares in the U.S. (2006)) (Espí et al., 2006; Scarascia-Mugnozza et al., 2011). Since 2013, the coverage has been predicted to increase annually by 5.7% and is forecasted for continued growth (MarketsandMarkets, 2013; MarketsandMarkets, 2017). PMs help to protect growing plants, increase soil temperature, reduce weed growth, fertilizer run off, soil compaction, gas exchange, and reduce water usage by 25 % (Ingman et al., 2015; Lamont, 1993). Overall, PM increase yields (as much as 30 %) and produce higher quality foods in the short-term (Fan et al., 2017; Overbeck et al., 2013; van Ittersum et al., 2013). However, the short-term benefits of increased food production may not outweigh the long-term impacts of PMs.

After 60 years of researching PMs, their agronomical, environmental, and ecological impacts are still unclear (Steinmetz et al., 2016). PMs are used for one growing season and then must be removed, which is a time and cost intensive process ($440/hectare) (Galinato et al., 2012); creating a large amount of plastic waste: 117,700 tons in 2017 from the US alone (MarketandMarkets, 2017). Few landfills and recycling centers will accept PMs, due to their high vegetative matter, soil content (up to 50% w/w), and the potential that they carry pesticides (Hussian and Hamid, 2004). As an alternative, farmers sometimes burn PMs illegally to reduce removal costs, which releases pollutants, including dioxanes (Garthe and Kowal, 2012; Lemieux, 1997).

Even if PMs are removed and disposed of correctly, some of the material will remain in the field due to inefficient removal or the natural wear-and-tear on PMs by weather, animals, and machines. For polyethylene-based PMs, this residual plastic left in soil can be 5-10% of total PM used (Ghimire et al.,

2018). Remaining pieces of PMs then can persist in the environment for hundreds of years (e.g., there is a 0.175% decomposition per year for polyethylene mulches; Albertsson and Ranby, 1979) during which time they may become micro- and nanoplastics. The effects of these plastic fragments have been reviewed and were shown to disrupt soil structure as well as soil physical and chemical composition (Qi et al., 2020).

PM fragments and microplastics have been shown to decrease water incorporation into soils, disrupt soil aggregates, and decrease aeration (Diehl, 2013; Zhang and Liu, 2018). In addition to soil quality PMs also affect soil invertebrates, which are critical for maintaining healthy soil. For example, with increasing plastic concentration, earthworm mortality rates increased (Coa et al., 2017; George et al., 2017; Huerta Lwanga et al., 2016). The negative impacts of persistent PMs on soil ecosystems suggest that the long-term usage of PMs may not be sustainable for increasing food production.

Biodegradable plastic mulches composition

An alternative to PMs is biodegradable plastic mulch (BPM). While the BPMs previously and currently on the market typically degrade in compost, they do not degrade in soil completely or at all, often misleading farmers that these are benign products (Moore-Kucera et al., 2014). BPMs that are soil-degradable are being explored and improved upon with new regulations and standards (EN17033:2018). The ultimate end of life goal for soil-degradable BPMs would be to till them in after use, allowing them to enter the carbon cycle through biotic and abiotic degradation, and reducing waste and cost associated with PMs.

Two common materials used in BPMs are thermoplastic starch (TPS) and poly(butylene-adipate-co-terephthalate) (PBAT) (Hayes et al., 2012). TPS is commonly added to BPMs with the goal of increasing biodegradability of components that make up BPMs (Brodhagen et al., 2015). TPS is made from plant starch, the natural storage polymer for plants, meaning that microbes have evolved enzymes to break down and utilize starch for a carbon source. TPS also is hydrophilic, and therefore can attract water for abiotic hydrolysis, as well as soluble, microbially-secreted starch-degrading enzymes.

However, TPS alone is brittle making it unsuitable for BPM production unless modified Plasticizers are therefore added to TPS to create more flexible final product (Bocqué et al., 2015). Plasticizers are low molecular weight molecules that disrupt the hydrogen bonding and van der Waals forces between polymers chains within starch granules. This allows for increased molecular movement and creates a softer polymer suitable for BPM production (Mekonnen et al., 2013). Common plasticizers in TPS are water, glycerol, and sorbitol. These plasticizers vary in concentrations but can be commonly found from 8-40% (w/w); however, the exact polymer composition is proprietary information (de Vileger, 2000; Handbook of Plasticizers, 2012; Mekonnen et al., 2013). Because plasticizers added during processing are not chemically bound to the polymer, they migrate from the matrix to the surface of the plastic and out into the environment over time (Mohammadi Nafchi et al., 2013), and these organic molecules have the potential to be bioactive.

In addition to plasticizers, TPS is commonly added to other polymers for commercial use. This often reduces the ability for the TPS to be biodegradable (Ceuvas-Carballo et al., 2019; Shanks and Kong, 2012). Alternatively, native starch granules can be added to molten thermoplastics below the thermal degradation temperature of starch; in this case, starch granules are not plasticized, but serve as a filler for the polymer. Scanning electron microscopy of the commercial "starch-based" BPMs used in Moore-Kucera et al. (2014) revealed lumps in the films that disappeared after incubation with various fungi, suggesting that this was type of mixture used. In other cases, TPS is melt-mixed with other thermoplastic polymers. These more homogeneous polymer mixtures are called blends (Mohammadi Nafchi et al., 2013). A common blend for BPMs is TPS with PBAT (Hayes et al., 2012). PBAT is composed of adipic acid, 1,4-butanediol, and terephthalic acid as the base monomers (**Figure 1A**). Adipic acid and 1,4-butanediol are combined to create one co-polymer then 1,4-butaendiol and terephthalic acid are combined to make the second copolymer. PBAT is a statistically random copolymer, meaning that there is no order to how the two copolymers react with other another to create PBAT (see reaction scheme depicted in **Figure 1B**). Therefore, there are often domains with greater adipic acid or terephthalic acid content. When the polymer degrades, adipic acid, 1,4-butanediol, and terephthalic acid will be released

individually or in chains of the two copolymers (Künkel et al., 2016). Although much work has been done showing the impacts of microplastic on soil ecology, little is known of the bioactivity of these breakdown products of PBAT, or of the TPS plasticizers described above. In this study, we investigated the effects of plasticizers and monomers on a common soil fungus, *Aspergillus*.

***Aspergillus* grows on BPMs**

The connection between BPMs and *Aspergillus* is a previous study showed that BPMs buried in agricultural soils accumulated disproportionate numbers of fungi on their surfaces from the Trichocomaceae. This is a family of cosmopolitan, ubiquitous soil fungi that are saprobes with a broad range of enzymatic abilities, and that often are capable of withstanding extreme environmental conditions (Moore-Kucera et al., 2014). Within this group were members of the genus *Aspergillus*, which are able to grow oligotrophically; that is, on substrates that provide very little nutrition (e.g. BPMs) (Hirsch, 1986). Between their large catalogue of enzymes and the ability to grow oligotrophically, *Aspergillus* could outcompete other soil fungi on the surfaces of PMs and BPMs, and an unintended consequence of BPM use may be to increase *Aspergillus* in agricultural production systems. *Aspergillus* is undesireable not because it destroys them -it is only a weak plant pathogen. Rather, fungi in this genus produce potent toxins.

One species within the *Aspergillus* genus is *A.flavus*, which is able to produce aflatoxin (AF), a potent carcinogen, as a secondary metabolite. High levels of AF contamination in food resulted in hundreds of deaths in Kenya (2010), and annually causes losses of one and a half billion dollars to US farmers from crops exceeding the regulatory limits for AF (> 20 ppb in food, or > 0.5 ppb in milk) (Cast, 2003; Probst, 2009; National Grain and Feed Association, 2011; Robens and Cardwell, 2003). Consistent low dose exposure of AF has been correlated with hepatocarcinoma, and with stunted growth in children (Gong et al., 2004; Liu and Wu, 2010). Because *Aspergillus* thrives in warm climates, aflatoxin contamination of crops is predicted to increase with increased temperatures – whether due to climate change (Battilani et al., 2016) or the warmer soil beneath a BPM. If *A. flavus* populations increase on

BPM surfaces, conidia are likely to spread to the associated crops and set up infection, making them susceptible to greater aflatoxin contamination.

AF has been shown to adsorb to polyethylene and other plastics, (Scoppa and Marafante, 1971). Since AF adsorbs to conventional plastics, which are hydrophobic, then logically AF may adsorb to hydrophobic components of BPMs as well (i.e. PBAT). This could also allow AF to have a mode of transportation in soil. AF is not only toxic to humans but mammals, fish, birds, and insects as well (Yu et al., 2008). It has been shown that soil invertebrates eat common plastics used in BPMs. If AF has adsorbed to the material, then the results could be deadly for the invertebrates (Wood and Zimmer, 2014). Therefore, if BPM use in agroecosystems leads to an increase in *A. flavus* populations and thus an increase in aflatoxin, BPM fragments carrying AF could poison soil meso- and macrofauna. Over time, such agroecosystems might become less productive or suitable for agriculture.

Though the formulation was proprietary, the buried BPMs (Moore-Kucera et a. 2014) likely contained TPS and PBAT. Because *A. flavus* did not grow on pure PBAT or TPS in culture, it is not known what component of BPMs enhanced *Aspergillus* population growth. One possibility is growth without metabolic use of the BPMs: in this scenario, the fungus fills a unique niche on the surface of BPMs, using them for surface attachment but utilizing trace nutrients elsewhere in the environment to grow oligotrophically. A second possibility is that additives, or the co-polymer and monomers of PBAT, enhance *Aspergillus* germination and/or growth by serving as a carbon source for the fungus. Finally, the interaction of fungal conidia and hyphae with BPMs could be separate: conidia could germinate directly on BPM surfaces, but in search of better nutrient sources, colonize outward. Conversely, conidia might germinate in surrounding soil grow towards the films. Because germination is the first step in fungal development and necessary for colonization of BPMs, the effect of TPS plasticizers and monomers of PBAT on the germination of *Aspergillus* was investigated.

Germination

Aspergillus conidia that land on BPMs first have to germinate in order to colonize the films. Because plasticizers can leach out of plastics, and because polymers most commonly degrade on the

surface first, I hypothesize that additives and degradation products on BPM surfaces could be influencing germination rates of *Aspergillus* spp.

Germination of *Aspergillus* conidia is a complex, signal-induced process that could be potentially induced by plasticizers or BPM breakdown products and monomers. Conidia are used as an asexual reproductive mechanism by *A. flavus.* They lie dormant until they perceive signals for germinating (sugars, amino acids, and inorganic salts, and possibly oxidized fatty acids), through a G protein-coupled receptor and MAPK signaling pathways (Affeldt et al., 2012; Osherov and May, 2001; Tiwari et al., 2016). When germination occurs, it is broken up into three distinct phases 1) isotropic swelling, 2) establishment of cell polarity, and 3) formation of a germ tube and continual polar growth (Lamarre et al., 2008; Leeuwen et al., 2012).

During isotropic swelling, conidia gain volume and use stored carbohydrates, mainly mannitol and trehalose, for glycolysis (Thevelein et al., 1996; Witteven and Visser, 1995). The majority of differential gene expression between dormancy and actively germinating conidia occurs within the first 30 minutes of germination, during the swelling stage. The upregulation of genes for RNA binding proteins, protein complex assembly, translational regulatory proteins, and lipid biosynthesis occurs during isotropic swelling. The major changes during germination lead conidia to switch from a fermentative metabolism to aerobic respiration and start to uptake essential nutrients from the surrounding environment (Lamarre et al., 2008).

Cell polarity can be established through cell wall modification and a decrease in microviscosity of the cytoplasm (Dijksterhuis et al., 2007). Conidia have a complex extracellular structure consisting of various lipids, proteins, chitin, and sugars (Morozova et al., 2001). During swelling, glucanases and chitinases are upregulated, and they loosen the cell wall polymers (Tiwari et al., 2016). This allows for germ tube formation by the addition of new phospholipids by cellular machinery (Momany, 2002). Germ tubes will then continue to extend until hyphae are formed.

Study overview

The goal of this study was to investigate the bioactivity of small molecules that can leach from BPMs during the degradation process. One set of experiments focused on glycerol and other common plasticizers added to TPS. Another set of experiments focused on the water soluble breakdown products of PBAT, which include the monomers adipic acid and 1,4-butanediol. All of these components can leach from the plastic matrix to the surface and then into surrounding soil water, where they have potential to affect microorganisms. Relevant test microbes were chosen from previous studies: three *Aspergillus* strains previously isolated from a commercial BPM after soil burial (Kucera et al., 2014), and the model species *A. flavus* NRRL 3557. In the case of TPS, the approach was to test i) the effects of TPS plasticizers on growth of *A. flavus*, and ii) the effects of glycerol, the most common plasticizer, on germination of all four *Aspergillus* sp. For PBAT, experiments were designed to determine iii) how PBAT monomers (resembling degradation products) influence *Aspergillus* sp. germination, iv) how PBAT monomers influence isotropic swelling of *A. flavus* conidia, v) how adipic acid influences the pH of *A. flavus* during germination, vi) if *A. flavus* conidia can recover from adipic acid stress, and vii) how intact PBAT on glass microscope slides with additional adipic acid and/or 1,4-butendiol, resembling degradation of PBAT films, influences germination of *A. flavus* using visual techniques to determine timing and transitions between the various stages of germination and to determine potential long term impacts of using BPM on *Aspergillus* populations and overall soil ecology. The bioactivity of glycerol and adipic acid is demonstrated herein and the relevance of these observations to agroecology and food safety is discussed.

METHODS AND MATERIALS

1. Fungal strains and growth conditions

Four *Aspergillus* strains, *A. flavus* NRRL 3357 and three native soil fungal isolates from a previous study (M, K, and FF from **Table 1**, Moore-Kucera et al., 2014), were used for glycerol and PBAT monomer germination experiments. The native soil fungi are herein referred to as "BPM M", "BPM K", and "BPM FF", respectively, and were isolated from soil-buried biodegradable BioAgri and BioTelo mulch films - commercially available, starch-based biodegradable plastic films made from Mater-Bi® feedstock. It's likely, though not a certainty, that the Mater-Bi® used in this plastic product contained a mixture of PBAT and TPS (Aldas et al., 2020). The isolates were identified by 18S rRNA gene sequencing as *Aspergillus* spp. (Moore-Kucera et al. 2014). All fungal strains (**Table 1**) were isolated to single conidia colonies and subsequently propagated on Champes media (per liter: 5 g yeast extract, 20 g glucose, 1 mL Hunter's trace elements [per liter: 5.0 g $FeSO_4 \cdot 7H_2O$, 50.0 g EDTA, 22 g $ZnSO_4 \cdot 7H_2O$, 11 g H_3BO_3, 5.0 g $MnCl_2 \cdot 4H_2O$, 1.6 g $CoCl_2 \cdot 6H_2O$, 1.6 g $CuSO_4 \cdot 5H_2O$, 1.1 g $(NH_4)_6Mo_7)_{24} \cdot 4H_2O$]) and allowed to grow for three days at 28°C with continuous illumination (VitaLUME Plus Grow bulbs, Sunleaves) for conidia production. To avoid contamination of conidia with trace medium components that might alter germination, conidia were harvested dry. Petri plates containing fungal cultures were inverted over an empty, sterile Petri plate, and the two plates were taped together to prevent conidia escape. The culture plate was gently tapped to collect conidia, which were suspended in an aqueous 0.01% (v/v) Triton X-100 solution. The conidia suspensions were vortexed for one minute prior to dilution for enumeration using a hemocytometer. All experiments were performed twice and treatments with a minimum of three triplicates.

2. How do TPS plasticizers influence *Aspergillus* growth and germination?

2a. Growth of *A. flavus* NRRL 3357 on TPS additives

A. flavus NRRL 3357 (10^6 conidia/mL) were inoculated onto sterile one-inch square pieces of Whatman #1 filter paper, laid onto 25 mL solid medium containing 0.3% (w/v) carbon sources that are common TPS plasticizers (glycerol, maltose, glucose, and sorbitol), pure cornstarch (the starting material

for TPS, Argo, Summit, IL), or TPS itself. The base medium was glucose minimal medium (GMM) (50 mL 20X nitrate salts per liter [120 g $NaNO_3$, 10.4g KCl, 10.4 g $MgSO_4 \cdot 7H_2O$, 30.4g KH_2PO_4], 1 mL Hunter's trace elements [described above], pH 6.5). Glycerol (ThermoFisher, Carlsbad, CA), maltose (ThermoFisher, Japan), glucose (Acros, France), and sorbitol (ThermoFisher, Carlsbad, CA) were added after autoclaving. Pure cornstarch was added prior to autoclaving to ensure gelatinization. Thermoplastic starch (TPS) film was generated in-house (10 mL deionized water, 1.5 g corn starch (Argo, Summit, IL), 1.5g glycerol, 1 mL 5% acetic acid) according to published methods (Royal Society of Chemistry, 2015). TPS was not soluble, so a paper punch was used to obtain small round disks whose total combined weight was 0.075 g per sample (equivalent to 0.3% w/v) in 25 mL medium). Disks were surface sterilized by soaking in 0.6% hypochlorite (aqueous) for five minutes, followed by three rinses in sterile water, and then soaking in 70% ethanol, followed by five rinses in sterile water. Disks were placed atop GMM and beneath the one-inch square pieces of Whatman #1 filter paper prior to inoculation with fungal conidia. For all other treatments, conidia were added directly to the filter paper, which was laid on top of 25 mL solid GMM containing the appropriate carbon source. All treatments were incubated at 28 ˚C in darkness for 72 hours. Filters were harvested into 14 mL round bottomed plastic tubes filled with three mL 0.01% (v/v) Triton X-100, and conidia were dislodged by vortexing for one minute. Conidia were counted using a hemocytometer.

2b. Glycerol and *Aspergillus* germination

Because our TPS sample contained the common plasticizer glycerol, we focused on this plasticizer to determine whether its effects on growth occurred as early as germination. A concentration of 1% is used for carbon sources in this medium because it supports luxuriant growth, and is lower than the concentrations of plasticizers typically found in TPS, but high enough to observe physiological effects on the fungus. In the field, as plasticizers leach into the soil, their concentration will decrease with distance from the source from as high as 40% inside the plastic fragments, down to zero. Separate 1 mL conidia aliquots were used for each treatment and replicate. Conidia were vortexed for thirty seconds and diluted to 10^6 conidia/mL in semisolid (0.8% w/v agar) GMM containing 1% (w/v) glucose as a positive control,

1% (w/v) glycerol, or no carbon source as a negative control. Inoculated medium (200 μL) was pipetted onto sterile microscope slides as depicted in **Figure 2**. After agar solidified, slides were elevated on 24 mm racks in covered Pyrex containers containing 50 mL sterile Nanopure water to maintain 100% humidity. The samples were spread among Pyrex containers such that, as much as possible, all treatments were present in a single container, and each container represented one replicate for each treatment (at each timepoint). Samples were placed randomly in a Percival incubator held at 28 °C. At ten, twelve, fourteen, eighteen, and twenty-two hours, samples were observed. At each time point, all relevant replicate samples were removed from incubator and simultaneously cooled to 4 °C. Slides were removed from 4 °C and observed in the same order they were prepared. For each sample, at least one hundred conidia were counted, using an Olympus CH30 compound light microscope at 400x total magnification. Conidia were considered germinated if a germ tube was half the diameter of the conidia. Three replicate samples were prepared for each treatment and timepoint.

3. How does PBAT influence *Aspergillus* germination?

3a. PBAT monomers and *Aspergillus* germination

The method described in **2b** was followed with the modification of the treatments used. GMM was made with either 1% (w/v) glucose, 1% (w/v) adipic acid (Acros, Germany), 1% (w/v) 1,4-butanediol (Alfa Aesar, Ward Hill, MA), or no carbon. Terephthalic acid was excluded from the study for two reasons. First, its solubility properties rendered it difficult to mix in a uniform suspension into agar medium. Second, preferential uptake of adipic acid over 1,4-butanediol and especially over terephthalic acid was suggested by the results of Zumstein et al. (2018), and we focused on the components most likely utilized and therefore, bioactive.

3b. PBAT monomers on *A. flavus* NRRL 3357 conidial swelling

To determine the developmental stage at which PBAT monomers influence germination, isotropic swelling was also investigated. Conidia were examined for swelling in semisolid medium, where entrapment of conidia prevented them from clumping. *A. flavus* NRRL 3357 (10^6 conidia/mL) were

inoculated on semisolid GMM with 1% (w/v) glucose as a positive control, 1% (w/v) 1,4-butanediol, 1% (w/v) adipic acid, or no carbon as a negative control. Cultures were incubated at for twenty-two hours at 28 °C in a Percival incubator. Images were taken using a Lecia DMI6000 B microscope at ten, twelve, fourteen, and twenty-two hours. Conidial diameter was measured using the line tool in ImageJ version 2.00-rc-69/1.53p (Schneider et al. 2012). At least fifteen conidia were counted for each timepoint.

A. flavus NRRL 3357 conidia were also examined in medium identical to the semisolid medium above, but without agar. Cultures were incubated for six hours at 28 °C with shaking at 180 rpm (Excella E24 Incubator Shaker, New Brunswick Scientific). In liquid, conidia aggregation precluded visual measurements past six hours. Images were taken every hour for six hours using a Lecia DMI6000 B microscope. Conidia diameter was measured using the line tool in ImageJ version 2.00-rc-69/1.53p (Schneider et al. 2012). At least forty-one conidia were counted for each timepoint.

3c. Germination and pH with PBAT monomers on *A. flavus* NRRL 3357 conidia

GMM is normally adjusted to pH 6.5, but the addition of 1% (w/v) adipic acid lowered its pH to approximately 3.0. To distinguish effects of pH from other inhibitory effects of adipic acid on *A. flavus* NRRL 3357 germination, GMM containing adipic acid was buffered to a pH of 6.5 using 2-[4-morpholino]-ethane sulfonic acid (MES), which is poorly metabolized by fungi. Conidia (10^6 conidia/mL) were added to: i) GMM with no carbon at pH 6.5, or acidified with HCl to pH 3.0, ii) GMM containing 1% (w/v) adipic acid at pH 3.0, or buffered to pH 6.5 using 0.01 mmol MES (Sigma, St. Louis, MO), iii) GMM with 1% (w/v) glucose at pH 6.5, or acidified with HCl to pH 3.0, and iv) GMM containing 1% (w/v) glucose and 1% w/v adipic acid at pH 3.0, or buffered with MES to pH 6.5. Conidia suspensions (200 μL) were pipetted on to sterile glass slides as described in **2b.** above. Samples were allowed to incubate at 28 °C for twenty-two hours. At least one hundred conidia were counted for each treatment.

3d. *A. flavus* NRRL 3357 conidia recovery from adipic acid

To test the reversibility of the inhibition of adipic acid on ungerminated conidia, *A. flavus* NRRL 3357 conidia were inoculated at a density of 10^6 conidia/mL in 25 mL Champes broth containing 1%

(w/v) adipic acid or no glucose. After one, two, or six hours of incubation, conidia were washed two times with 0.01% (w/v) Triton x-100. A volume estimated to contain four hundred conidia was transferred to solid Champes agar. Plates were incubated at 28 °C for thirty-six hours and colonies were counted.

3e. Intact PBAT and *A. flavus* NRRL 3357 germination

To simulate conidia germinating on intact or degrading BPMs, slides were coated with a film of PBAT with or without free adipic acid and 1,4-butanediol, and conidia were overlaid in agar. To compare the ability of adipic acid and/or 1,4 butanediol to affect germination when in the film (distal, requiring diffusion) vs. while dissolved in medium in which conidia were germinating (proximal, requiring minimal diffusion), these components were added either to the film overlaid with conidia in carbon-free medium, or the same components were added to an agar overlay spread on bare slides. Glucose was a positive control for germination and GMM lacking carbon was a negative control. To make slides, 1% w/v PBAT (Daminer Scientific, Bainbridge, GA), or 1% w/v PBAT with adipic acid (1% w/v, 93 mM) and/or 1,4-butanediol at the molar equivalent of 1% w/v adipic acid were dissolved in a solution of acetone:chloroform (1:1 v/v). Solutions (50 μL) were pipetted on sterile, heated (100 °C) glass microscope slides, to evaporate the solvents and leave only PBAT treatments remaining on slides. For full evaporation, slides were subsequently placed in a vacuum oven (60 °C) for one hour.

A. flavus NRRL 3357 conidia aliquots were vortexed for thirty seconds immediately prior to adding to GMM containing no carbon, 1% w/v glucose, 1% w/v adipic acid, or 1% w/v butanediol for a final concentration of 10^6 conidia/mL. GMM/conidia suspensions (200 μL) were pipetted on top of polymer samples. Slides were then placed into Pyrex containers and the procedure described in **2b.** was followed. See **Table 2** for treatment combinations.

4. Statistical analysis

All analysis was performed using RStudio version 1.1.456 (RStudio Team, 2020). Time course experiments were analyzed with a generalized least square (GLS) regression model using the R package *nlme* version 3.1-137 (Pinheiro et al. 2020). Model selection was determined using significant terms,

determined through ANOVA, and Akaike information criterion. Significant differences between treatments were determined using least square means generated from the GLS model by *lsmeans* package version 2.30-0 (Lenth, 2016) with time as a covariate. To determine significant differences for TPS additives and pH experiments, a one-way ANOVA was performed. All analyses were checked for normality and homoscedasticity using histograms, residual plots, or Leven's test. A square root transformation was performed for (**3c.**) conidia germination and pH data. A log transformation was performed for (**2a**.) TPS germination and (**3d.**) conidia recovery data. Transformations were to correct for skewness or improving model fit. Tukey's honestly significant difference test was used to determine significance for one-way ANOVA results, using a confidence interval of 95%. An alpha level was set at 0.05 for all analyses. Percent germination was defined as germinated conidia per total conidia counted, times one hundred, and conidial recovery was defined as the log of the number of colonies counted per 400 conidia plated. For justification of statistical methods see **Supplemental Figures 1 - 8**.

RESULTS

1. How do TPS plasticizers influence *Aspergillus* growth and germination?

1a. Growth of *A. flavus* NRRL 3357 on TPS additives

Growth was measured by proxy via conidia numbers (**Figure 3**; **Supplemental Figure 1**). Conidial production was significantly greater when grown on TPS and cornstarch than in medium containing no carbon, by 10.5% and 29.9%, respectively. However, conidial production on media amended with common TPS additives was even higher ($0.05 < p < 0.001$) than on pure TPS: 24.2%, 25.5%, 26.8%, and 24.1% higher for glycerol, maltose, glucose, and sorbitol, respectively. Conidial numbers from medium amended with these common TPS additives were also significantly greater ($0.05 < p < 0.001$) than conidial numbers from medium supplemented with cornstarch as a carbon source: 5.7%, 6.6%, 7.9%, and 5.6% for glycerol, maltose, glucose, and sorbitol, respectively. Conidial production in the presence of TPS additives (glycerol, sorbitol, glucose, and maltose) were not significantly different from one another ($p > 0.70$; **Table 3)**.

1b. Glycerol and *Aspergillus* germination

The highest germination rates for all four strains were observed in GMM containing 1% (w/v) glucose, followed by 1% (w/v) glycerol, and lastly by GMM containing no carbon. In 1% (w/v) glucose, by twenty-two hours 99.7% of conidia were germinated for *A. flavus* NRRL 3357, 97.7% for BPM FF, and 50.1% for BPM K (**Figure 4**; **Table 4**; **Supplemental Figure 2**). Supplying glycerol as a carbon source resulted in significantly lower germination rates at twenty-two hours: 80.1% for *A. flavus* NRRL 3357, 82.3% for BPM M, 8.8% for BPM K, and 19.1% for BPM FF ($p < 0.01$). Germination rates in GMM containing glycerol were not statistically different from rates in GMM lacking a carbon source for BPM K, M, and FF ($p > 0.10$; **Table 4**). *A. flavus* NRRL 3357 had significantly more germination ($p < 0.0001$) when glycerol was present compared to all other strains used **(Table 5**, **Supplemental Figure 3**).

2. How does PBAT influence *Aspergillus* germination?

2a. PBAT monomers and *Aspergillus* germination

As above, conidia incubated in GMM containing 1% (w/v) glucose achieved the most germination by twenty-two hours: 99.7% for *A. flavus*, 98.4% for BPM M, 43.4 % for BPM K, and 99.7% for BPM FF. For *A. flavus* NRRL 3357, BPM M, and BPM K, the effect of substituting adipic acid for glucose was to inhibit germination nearly completely. By twenty-two hours, germination only was achieved 15.5%, 11.1%, and 5.94% of conidia, respectively. This was well below the rate of germination in GMM containing no carbon source, which supported 40.9%, 71.0%, and 15.2% germination by twenty-two hours for *A. flavus* NRRL 3357, BPM M, and BPM K respectively ($p < 0.01$). By contrast, for BPM FF, adipic acid supported a slightly higher germination rate than the no-carbon control (25.7% vs. 14.3 % at twenty-two hours, respectively). The effect of 1,4-butanediol on germination rates was statistically indistinguishable from that of the no-carbon control for all four strains over the entire time course ($p > 0.50$; **Figure 5**; **Table 6**; **Supplemental Figure 4**).

2b. PBAT monomers on *A. flavus* NRRL 3357 conidial swelling

Conidia grown in GMM with 1% (w/v) adipic acid appeared to never complete isotropic swelling, suggesting that the inhibition began in the earliest stages of germination. To confirm this, conidial diameters were measured for each of the treatments described above, but the scope of the experiment was reduced to the model fungus *A. flavus* NRRL 3357. Conidia incubated on solid GMM agar containing 1% (w/v) glucose exhibited the most swelling, increasing in diameter by 115% over twenty-two hours. *A. flavus* NRRL 3357 conidia treated with adipic acid were significantly less swollen compared to all other treatments ($0.05 < p < 0.01$), only increasing in diameter by 34.5% over twenty-two hours and smaller even than conidia incubated in GMM without carbon. By contrast, conidia incubated in GMM containing 1% (w/v) 1,4-butanediol swelled more than those in GMM without carbon ($p < 0.05$), increasing in diameter by 85% over twenty-two hours (**Figure 6-7**; **Table 7**; **Supplemental Figure 5**), although the difference in swelling was not correlated a difference in germination between these two treatments (**Figure 5**).

Conidial swelling was also tested in a liquid, complex medium (Champes). In that experiment, shrinkage of conidia during the first hour was observed for conidia incubated in Champes containing 1% (w/v) adipic acid, although swelling subsequently occurred. This phenomenon was not observed for conidia incubated in Champes containing 1% (w/v) glucose, or no additional carbon (**Figure 8**; **Table 8**; **Supplemental Figure 6**).

2c. Germination and pH with PBAT monomers on *A. flavus* NRRL 3357 conidia

Conidial germination on solid GMM containing 1% (w/v) adipic acid was statistically indistinguishable from germination on GMM containing no carbon and acidified with HCl to a pH of 3.0 ($p > 0.90$). Conversely, conidial germination on GMM containing 1% (w/v) adipic acid but buffered to a pH of 6.5 with MES permitted 172% more germination than conidia incubated on GMM containing unbuffered adipic acid. When buffered to decrease the negative effects of acidity, the adipic acid treatment actually permitted 22.5% more germination than the no carbon control ($p < 0.0001$). When GMM containing 1% (w/v) glucose was acidified to a pH of 3.0 by either HCl or adipic acid, germination rates not statistically distinguishable ($p > 0.90$) but both treatments lowered germination compared to conidia grown on GMM containing 1% (w/v) glucose at pH 6.5 ($p < 0.05$). When GMM containing 1% glucose was acidified with 1% (w/v) adipic acid but then buffered to pH 6.5 with MES, conidial germination rates were identical to those on GMM containing 1% (w/v) glucose (pH 6.5) ($p > 0.90$) (**Figure 9**, **Table 9, Supplemental Figure 7**).

2d. *A. flavus* NRRL 3357 conidial recovery from adipic acid

The log rate of revival (colony outgrowth) did not differ between conidia incubated in Champes containing 1% (w/v) adipic acid (2.15 ± 0.03 standard error at one hour and 2.40 ± 0.02 standard error at two hours) and conidia incubated in Champes containing no glucose (2.21 ± 0.08 standard error at one hour and 2.30 ± 0.06 standard error at two hours) (F-value = 0.057 , *p*-value = 0.81) (**Table 10**).

To determine hyphal sensitivity to adipic acid, germlings grown on Champes agar from single conidia were transferred to Champes with 1% (w/v) adipic acid or unaltered Champes agar. On adipic

acid, colonies showed a decrease in diameter and increase in conidiophore heights compared to germlings plated on Champes agar (data not shown).

2e. Intact PBAT and *A. flavus* NRRL 3357 germination

On slides with or without PBAT, conidia treated with adipic acid, exhibited significantly lower rate of germination compared to all other treatments ($p < 0.001$). All treatments containing adipic acid were statistically indistinguishable ($p > 0.90$) **(Figure 10**; **Table 11**).

Reflecting the lack of difference already shown in **Figure 5a**, germination rates of conidia in GMM with no carbon source or with 1,4-butanediol were not statistically different ($p>0.90$; **Figure 10; Table 11**). Similarly, in GMM with no carbon source, overlaid onto slides spotted with PBAT or PBAT plus 1,4-butanediol, germination was statistically indistinguishable ($p > 0.60$). However, comparing treatments where conidia were in the same type of medium but overlaid onto glass slides vs. PBAT revealed that PBAT reduced germination, permitting slightly but significantly less germination than a PBAT-free overlay ($0.05 < p < 0.01$) of GMM with no carbon source, and also permitting slightly but significantly ($0.05 < p < 0.001$) less germination in overlays containing 1,4-butanediol. All treatments permitted more germination than adipic acid treatments, suggesting that adipic acid (with or without PBAT) is more inhibitory than PBAT alone, and more inhibitory than 1,4-butanediol.

Conidia incubated in GMM containing glucose overlaid onto untreated slides, or slides spotted with PBAT, were both fully germinated by twenty-two hours. However, germination rates were greater in the absence of PBAT earlier in the time course. Conidia grown without PBAT in glucose had 59.1%, 64.4%, 35.1% more germination at ten, twelve, and fourteen hours respectively (**Figure 10**; **Table 11**; **Supplemental Figure 9**), causing the average effect of GMM plus glucose overlays onto PBAT to be significantly different from the same overlays on untreated glass slides ($p < 0.001$). These data provide evidence that PBAT may impede early stages of *A. flavus* NRRL 3357 germination.

DISCUSSION

1. How do TPS plasticizers influence *Aspergillus* growth and germination?

A. flavus NRRL 3357 grew more avidly on glycerol than on medium containing TPS or corn starch, as carbon sources. In fact, *A. flavus* grew less on TPS than on cornstarch, suggesting that the ability to grow on BPMs containing TPS may not stem from the ability to degrade starch polymers. Commercial TPS is often modified, e.g., through the covalent addition of monomers, polymers, or fatty acids (Cuevas-Carballo et al., 2019; Shanks and Kong, 2012). It could be the case that *A. flavus* is unable to efficiently utilize covalently modified TPS as a carbon source due to steric hindrance and an increase hydrophobicity of the material (Schlemmer and Sales, 2009) (**Supplemental Figure 9**). Some starch degradation was expected by *A. flavus* because of its alpha amylase production (Mellon et al., 2007). However, the fungus grew better on glycerol and sorbitol than on cornstarch, suggesting that growth of *A. flavus* on our TPS sample was due largely to it utilizing the plasticizer, glycerol. Glycerol is the primary plasticizer used in TPS products, and can migrate to the surface of the material, where it is readily available for use by microorganisms. (Mekonnen et al., 2013; Schlemmer and Sales, 2009). Thus, while addition of native starch to plastic (a "mixture", in plastic terminology) could encourage *Aspergillus* to utilize starch as a substrate, addition of TPS (alone or in "blends") may more likely encourage *Aspergillus* to use the plasticizers as substrates.

Conidia germinated better in glycerol than on no carbon for *A. flavus* NRRL 3357 but not for the three BPM *Aspergillus* strains (**Figure 4**). It is known that different *Aspergillus* species germinate at different rates (Araujo and Rodrigues, 2004). Thus, a universal prediction about the effects of glycerol on wild *Aspergillus* populations in agricultural soils cannot be made.

Glycerol is the primary polyol produced by conidia during isotropic swelling and induces has been seen to initiate citric acid production in *A. niger*, resulting in the change from a fermentative to aerobic respiration in two ways. First, glycerol inhibited $NADP^+$ dependent isocitrate dehydrogenase, allowing increased citrate production (Legisa and Mattey, 1986; van Laere and Hulsmans, 1987). Secondly, glycerol can be phosphorylated into glycerol-3-phosphate, which is an early intermediate of

glycolysis, starting the pathways for both aerobic respiration and fermentation. *Aspergillus* species use different proteins for metabolizing glycerol, which may be why glycerol enhances germination in *A. flavus* but not in the three other strains of *Aspergillus* tested. Salazar et al. (2009) showed that there were eighty-eight genes conserved among three *Aspergillus* strains for glycerol catabolism, but there was a large degree of variation among significantly expressed but non-conserved glycerol catabolism genes among the three strains. They also found that *A. oryzae*, an almost identical strain to *A. flavus*, had significantly more transcripts of glycerol kinase enzymes, responsible for converting glycerol to glycerol-3-phosphate, compared to *A. niger* and *A. nidulans*. This could explain why they saw a quicker accumulation of biomass for *A. oryzae* when grown on glycerol as a sole carbon source. Because *A. flavus* and *A. oryzae* are nearly identical species, a more robust glycerol metabolism may explain why, in this study, *A. flavus* was able to germinate at a faster rate compared to the BPM strains, which are different species.

Continued use of BPMs containing TPS with glycerol could lead to an increase in *A. flavus* population. One of the most effective pre-harvest strategies at reducing *A. flavus* and aflatoxin production has been seeding fields with non-mycotoxin producing strains of *Aspergillus*, with the hope to outcompete naturally-occurring *A. flavus* (Cleveland et al., 2003; Ehrlich et al., 2014; Mauro et al., 2018; Pitt and Hocking, 2006). However, in a field where BPMs are deployed, if a particular toxin-producing *Aspergillus* strain has the ability to utilize glycerol for germination more efficiently than the biocontrol (non-mycotoxin producing) strains, then this method for reducing aflatoxin contamination may be less effective if glycerol-containing TPS is a component of BPMs.

2. How does PBAT influence *Aspergillus* germination?

The effects of 1,4-butanediol and adipic acid on *Aspergillus* germination were investigated to determine how degradation products of PBAT may influence soil ecology and colonization of BPMs. These monomers may also remain in the polymers as by-products of synthesis. Either way, they may leach to the surface and out into the environment. Germination rates on 1,4-butanediol or no carbon were statistically similar for *Aspergillus flavus*, BPM M, and BPM K, and BPM FF (**Figure 5**). The uniform

pattern suggests that 1,4-butanediol is not recognized by conidia as a carbon source or germination induction signal. However, multiple studies demonstrated that *A. oryzae* and *A. niger* grow on 1,4-butanediol as a sole carbon source, although to my knowledge the effects of 1,4-butanediol on germination rates for these species remain unknown (Darby and Kaplan, 1968; Gardio et al., 2011; Maeda et al., 2005). Conidial diameters were significantly larger for 1,4-butanediol treated conidia compared to no carbon treated conidia (**Figure 6**). Therefore, if conidia had been incubated longer than twenty-two hours in the study herein, it is possible that increase in germination may have occurred. Alternatively, it's possible that in 1,4-butanediol has neutral effects on germination, but after germination, induces growth.

Although 1,4-butanediol did not influence germination, adipic acid was shown to influence germination. Germination for *A. flavus* NRRL 3357, BPM K, and BPM M (**Figure 5**) was lower on 1% (w/v), or 93 mM, adipic acid even than on the no-carbon control. By contrast, for BPM FF, germination on adipic acid was higher than on the no carbon control, although still significantly lower than on glucose. Therefore, the effects of adipic acid are not uniform across species for *Aspergillus* germination. To my knowledge, no previous germination studies have been performed using adipic acid with *A. flavus*. However, Vicedo et al. (2006) showed that adipic acid monoethyl ester, a commonly used fungicide, was able to prevent germination at 10 mM for *Botrytis cinerea,* a common mold found on grape vines. Karlsson et al. (2017) demonstrated that *A. niger* conidia were able to form mycelium but had decreased growth and conidiation with decreased pH (pH 6 and 5) using concentrations of adipic acid from 0 to 684 mM, suggesting that the fungus was stressed at lower pH and increased adipic acid concentration. From this study, germlings from individual conidia were transferred from Champes agar to Champes containing 1% (w/v) adipic acid, and were able to form colonies, although these were smaller than germlings transferred to GMM containing 1% (w/v) glucose (data not shown). These studies, taken together, and the presented data, give evidence that conidia are more susceptible to adipic acid than growing mycelium.

In addition, **Figure 5** demonstrates that adipic acid can either promote or inhibit germination, depending on the *Aspergillus* species. Therefore, if adipic acid is being released into the environment during PBAT degradation, the impact on *Aspergillus* species and other fungi may vary depending on

species and developmental stage. This is relevant to the same concerns about non-mycotoxigenic *Aspergillus* biocontrol strains.

The effect seen from adipic acid at various pH levels can be explained through weak acid theory. Weak acids have the ability to passively diffuse cross cell membranes in their undissociated form and acidify intracellular pH, causing acid stress. Many weak acids inhibit growth and germination of organisms differently. The effect of sorbic acid, acetic acid, propionic acid, lactic acid, and nonanoic acid have all been extensively studied on fungal conidial germination and growth (Araujo and Rodrigues, 2004; Breeuwer, 1996; Dijksterhuis et al., 2019; Novodvorska et al., 2016; Pillai and Ramaswamy, 2012; Stratford et al., 2009). Adipic acid is a diprotic weak acid (pK_a = 4.41 and 5.41). When 1% (w/v) adipic acid was added to GMM, the pH was reduced from 6.5 to 3.0, resulting in 99.4% of the acid being in the undissociated form (**Figure 11**; **Table 12)** and thus able to traverse the membrane.

During germination, conidia are vulnerable to stress (d'Enfert, 1997). When conidia are dormant, the internal pH was been reported to be 5.7-6.5, but as conidia germinate and develop a germ tube the pH increases to 6.3-7.2 (Breeuwer, 1997; Chitarra et al., 2005). This change in pH allows for key metabolic pathways and enzymes to begin to function (e.g., respiration pathways, phosphofructokinases, and transporters) (Liewen and Marth, 1985; Krebs et al., 1983). With the majority of adipic acid being in the undissociated form at a pH of 3.0, I postulate that adipic acid is disrupting this pH transition that occurs during germination. There is also evidence for disruption of the germination process, from the conidial diameter data. Conidia treated with 1% (w/v) adipic acid at a pH of 3.0 were able to start to swell, indicating that germination was initiated, but conidia were unable to continue swelling and produce a germ tube. Further supporting evidence comes from fluorescence microscopy, where conidia treated with adipic acid are no longer fluorescent when in the presence of fluorescence diacetate (**Supplemental Figure 10**). This indicates that adipic acid treated conidia are not metabolically active. However, adipic acid-treated conidia are able to germinate when washed and placed on a complex media without adipic acid, indicating that conidia can resume development and that any damage from adipic acid may be reversible, showing that the long-term impacts of adipic acid may not reduce fungal germination.

When 1% (w/v) adipic acid was buffered to a pH of 6.5, only 0.06 % of adipic acid was in the undissociated form. Therefore, the majority of adipic acid was charged, preventing diffusion across a phospholipid bilayer. There was significantly more germination in buffered adipic acid compared to no carbon media at pH 6.5, suggesting that the dissociated form of adipic acid could be used as a carbon source. This result supports Karlsson et al. (2017), where *A. niger* was shown to grow with adipic acid as a sole carbon source, with increased conidiation at increased pH. Non-specific dicarboxylic acid receptors, commonly used for the citric acid cycle, could import adipic acid in its dissociated form. This could allow for intracellular pH to stay consistent, and for adipic acid to be used as a carbon source. Although specific receptors are not known in *A. flavus*, yeast have both been reported to contain these transporters with a large degree of flexibility for chain lengths (C_1-C_4) (Aliverdieva et al., 2006; Camarasa et al., 2001; Casal et al., 2008). Therefore, it is not unreasonable to hypothesize that adipic acid (C_6) has the ability to be transported and used as a carbon source.

The monomers of PBAT (adipic acid, 1,4-butanediol, and terephthalic acid) would be the constituents released into the environment once the film starts to degrade individually or in chains of the two copolymers; therefore, PBAT-coated slides spiked with adipic acid and/or 1,4-butanediol were used to simulated surface degradation. The results were similar to germination of conidia in GMM with added monomers for *A. flavus* NRRL 3357 (**Figure 5**). PBAT spiked with adipic acid resulted in the lowest germination, and germination on PBAT spiked with 1,4-butanediol was similar to that on unspiked PBAT. Therefore, the effects of PBAT monomers seen in GMM seem to hold true for when *A. flavus* NRRL 3357 was grown on a PBAT film. However, there was a decrease in total conidia germinated or a lag in germination when conidia were incubated in the presence of PBAT, whether in the presence of glucose, or 1,4-butanediol, or no carbon.

I propose two hypotheses for why there is significant decrease or lag in germination when *A. flavus* NRRL 3357 was germinated in the presence of PBAT polymer. First, the decrease in germination could be explained through hydrolysis of adipic acid from the polymer from abiotic and biotic factors, resulting in a greater concentration of inhibitory adipic acid. Abiotically, PBAT undergoes a hydrolysis

reaction in the presence of water, which would lead to natural release of the two copolymers and monomer units (Muthuraj et al., 2015). Biotically, *A. oryzae* and *A. nidulans* possess cutinases with the ability to breakdown polybutylene succinate and poly-(butylene succinate-co-adipate), two structurally similar polymers (Gardio et al., 2011; Maeda et al., 2005). The cutinases are upregulated when conidia adhere to a hydrophobic surface (Garrido et al., 2012; Maeda et al., 2005). It has also been seen that adipic acid is preferentially degraded among the monomers in PBAT, most likely due to increased chain mobility (Zumstein et al., 2017). Therefore, when conidia are adsorbed to the PBAT film, cutinase enzymes could be secreted during germination, allowing for an increase in adipic acid and copolymer release. When BPMs are used in agricultural fields, they are exposed to high ultra-violet and thermal stress, which could lead to increased abiotic degradation. Therefore, more adipic acid may be released on the surface of the material in the field than from the PBAT coating used in these experiments, underlining the biological relevance of my results.

Second, the lag in germination could be caused by hydrophobic interactions between the polymer surface and signaling compounds produced by *A. flavus* NRRL 3357 conidia during germination. Lipids, often in the form of oxygenated polyunsaturated fatty acids (oxylipins), have been shown to be key signaling molecules in fungi for changes in morphology (Affeldt et al., 2011; Brown et al., 2008, 2009; Singh and Poeta, 2011). The oxylipins involved are long hydrophobic carbon chains, primarily eight carbons long, that would have the ability to adhere to hydrophobic plastic, which are known to adsorb hydrophobic compounds (Teuten et al., 2007). *A. flavus* NRRL 3357 conidia have been shown to produce 1-octen-3-one, 3-octanone, 2-octen-1-ol, and primarily 1-octen-3-ol (Miyamoto et al., 2014). It is thought that oxylipins may act as a quorum sensing mechanism for synchronous germination; however, no direct evidence has supported this claim. If oxylipins are used for germination cues, and are sequestered on PBAT film via hydrophobic interactions, then a decrease germination rate would be observed. To combat this effect, more oxylipins would need to be produced until the signal is strong enough to overcome the hydrophobic interactions and produced germination cues, thus causing a delay in germination.

It has been shown that soil fungi have the ability to mineralize adipic acid, 1,4-butanediol, and terephthalic acid as free monomers from PBAT polymer (Zumstein et al., 2018). It would appear that adipic acid does not hinder growth of certain fungal species after germination has occurred but can be used as a preferred carbon source (Karlsson et al., 2017).

The long-term impact of using BPMs with PBAT are still questionable and highly pH dependent. When *A. flavus* conidia land on BPMs in a field, the presented data show that the rate of germination could be lowered if adipic acid lowers the pH of soil water. It has been seen that the pH of soils under PM is decreased due to high decomposition and release of organic acid (Tisdale and Beaton, 1990). Therefore, in a microenvironment, close to BPMs, there could be an inhibition of germination due to a high concentration of undissociated adipic acid, however, further works needs to be done for confirmation. PBAT constituents that make it into the bulk soil could be used as a carbon source for *Aspergillus*, due to non-specific dicarboxylic acid receptors mentioned above. The experiments performed with PBAT could be extrapolated to other polyesters commonly used in BPMs [poly(3-hydroxybutyrate, poly(3-hyroxyvalerate, poly(ε-caprolactone), and poly(butylene succinate)], which would also release organic acids upon degradation (Brodhagen et al., 2014). Therefore, around the microenvironment of most polymers used in BPMs, there could be acid stress of microorganisms and fungi.

3. Conclusion

Based on these results, if BPMs containing TPS and PBAT are used in agricultural soils, *A. flavus* conidia that land on the material prior to tilling into the soil could experience opposing effects. Glycerol and/or other common plasticizers in TPS may increase germination. On the other hand, depending on its concentration and therefore its effect on the pH of the environment, adipic acid derived from PBAT may inhibit germination. Because most soils range between a pH of 5-7, if adipic acid concentrations in the microenvironment of the conidia were low enough to allow soil water to remain close to neutral, the main species of adipic acid would be in a dissociated form, which could then be used as a carbon source for germination. Other polyesters used in BPMs could potentially have the same effect on *Aspergillus* germination as PBAT degradation products, due to the release of organic acids during degradation.

Although this study reveals that *Aspergillus* germination and growth are affected by common constituents of BPMs, there is still more work needed to understand the overall ecological impact of BPMs on the genus *Aspergillus* and overall soil ecology.

Table 1: *Aspergillus* species and strains used in study

Strain	Description	Reference
Aspergillus flavus NRRL 3357	Model strain	Fungal Genetics Stock Center (University of Kansas, Kansas City, USA)
BPM M	Isolate from starch-based plastic tentatively identified as *Aspergillus* sp.	Moore-Kucera et al., 2014
BPM K	Isolate from starch-based plastic tentatively identified as *Aspergillus* sp.	Moore-Kucera et al., 2014
BPM FF	Isolate from starch-based plastic tentatively identified as *Aspergillus* sp.	Moore-Kucera et al., 2014

Table 2: Combinations of PBAT and its monomers in plastic film and in overlaid semisolid medium containing *A. flavus* conidia

Polymer film on slide	**Carbon Source (% w/v) in overlaid conidial suspension**
No polymer	No carbon, 1% glucose, 1% adipic, or 1% butanediol
PBAT	No carbon, or 1% glucose
PBAT + adipic acid	No carbon
PBAT + 1,4-butanediol	No carbon
PBAT + adipic acid + 1,4-butanediol	No carbon
Acetone:Chloroform (solvent control)	No carbon

Table 3: ANOVA results (conidia as a function of treatment) with test statistic and *p*-values for Tukey's HSD comparisons of conidia production by *A. flavus* NRRL 3357 grown on TPS additives

	df	**Sum Sq**	**Mean Sq**	**F value**	***p* value**
Treatment	6	22.679	3.780	107.9	< 2e-16
Residuals	33	1.156	0.035		
Pairwise Comparison					**T statistic, *p* value**
corn starch - glucose					-4.9, p <0.001
corn starch - glycerol					-3.3, p <0.05
corn starch - maltose					-4.1, p <0.005
corn starch - no carbon					14.1, p <0.001
corn starch - sorbitol					-3.5, p <0.05
corn starch - TPS					8.7, p <0.001
glucose - glycerol					1.3, p >0.80
glucose - maltose					0.8, p >0.90
glucose - no carbon					18.9, p <0.001
glucose - sorbitol					1.4, p >0.70
glucose - TPS					13.3, p <0.001
glycerol - maltose					-0.6, p >0.90
glycerol - no carbon					16.7, p <0.001
glycerol - sorbitol					0.03, p >0.90
glycerol - TPS					11.5, p <0.001
maltose - no carbon					18.1, p <0.001
maltose - sorbitol					0.6, p >0.90
maltose - TPS					12.6, p <0.001
no carbon - sorbitol					-17.5, p <0.001
no carbon - TPS					-4.7, p <0.001
sorbitol - TPS					12.0, p <0.002

Table 4: ANOVA results from generalized least squares model, least square means, and pairwise comparison with test statistic and *p*-values for germination in glycerol for *A. flavus* NRRL 3357, BPM M, BPM K, and BPM FF

		df	**F value**	***p* value**
A. flavus NRRL 3357	intercept	1	3203.6	< 0.0001
	tmt	2	264.0	< 0.0001
	time	1	543.0	< 0.0001
	$time^2$	1	117.5	< 0.0001
	tmt:time	2	42.2	< 0.0001
	residual	47		
BPM M	intercept	1	709.3	< 0.0001
	tmt	2	21.1	< 0.0001
	time	1	259.9	< 0.0001
	tmt:time	2	4.3	0.0185
	residual	48		
BPM K	intercept	1	137.8	< 0.0001
	tmt	2	36.6	< 0.0001
	time	1	110.2	< 0.0001
	$time^2$	1	36.0	< 0.0001
	tmt:time	2	25.3	< 0.0001
	residual	47		
BPM FF	intercept	1	1078.3	< 0.0001
	tmt	2	480.1	< 0.0001
	time	1	320.9	< 0.0001
	tmt:time	2	124.7	< 0.0001
	residual	48		

	Least square means with standard error			
Treatments	***A. flavus* NRRL 3357**	**BPM M**	**BPM K**	**BPM FF**
zero carbon	25.1 ± 1.65	36.6 ± 3.03	5.18 ± 1.21	7.4 ± 1.22
glucose	78.1 ± 1.65	62.5 ± 3.03	16.5 ± 1.21	51.4 ± 1.22
glycerol	58.8 ± 1.65	40.8 ± 3.03	2.9 ± 1.21	10.4 ± 1.22

	Test statistic, *p* values			
Pairwise Comparison	***A. flavus* NRRL 3357**	**BPM M**	**BPM K**	**BPM FF**
glucose - glycerol	8.27, p < 0.01	5.08, p < 0.01	7.99, p < 0.01	23.8, p < 0.01
glucose - zero carbon	22.7, p < 0.01	6.04, p < 0.01	6.63, p < 0.01	25.6, p < 0.01
glycerol - zero carbon	14.4, p < 0.01	0.96, p > 0.10	-1.36, p > 0.10	1.8, p > 0.10

Table 5: ANOVA results for generalized least squares model, least square means, and pairwise comparison with test statistics and *p* values for pairwise comparison between *A. flavus* NRRL 3357, BPM M, BPM K, and BPM FF for germination in glycerol

	df	**F value**	***p* value**
intercept	1	2314.1	< 0.0001
tmt	2	212.1	< 0.0001
time	1	658.5	< 0.0001
strain	3	238.0	< 0.0001
tmt:time	2	55.0	< 0.0001
tmt:strain	6	22.3	< 0.0001
time:strain	2	47.1	< 0.0001
tmt:time:strain	6	4.8	< 0.0001
residual	192		
Strain	**Glycerol least square means with standard error**		
A. flavus NRRL 3357			58.8 ± 2.37
BPM M			40.8 ± 2.37
BPM K			2.9 ± 2.37
BPM FF			10.4 ± 2.37
Glycerol Pairwise Comparison			**Test statistic, *p* value**
A. flavus NRRL 3357 vs BPM M			5.4, < 0.0001
A. flavus NRRL 3357 vs BPM K			16.7, < 0.0001
A. flavus NRRL 3357 vs BPM FF			14.4, < 0.0001

Table 6: ANOVA results for generalized least squares model, least square means, and pairwise comparison with test statistic and *p*-values for comparisons of germination in the presence of PBAT monomers for *A. flavus* NRRL 3357, BPM M, BPM K, and BPM FF

		df	**F value**	***p* value**
A. flavus NRRL 3357	intercept	1	1720.2	< 0.0001
	tmt	3	282.1	< 0.0001
	time	1	307.1	< 0.0001
	$time^2$	1	58.9	< 0.0001
	tmt:time	3	43.5	< 0.0001
	residual	63		
BPM M	intercept	1	1088.5	< 0.0001
	tmt	3	124.3	< 0.0001
	time	1	349.2	< 0.0001
	tmt:time	3	32.1	< 0.0001
	residual	64		
BPM K	intercept	1	416.5	< 0.0001
	tmt	3	67.1	< 0.0001
	time	1	286.5	< 0.0001
	$time^2$	1	70.4	< 0.0001
	tmt:time	3	46.2	< 0.0001
	residual	63		
BPM FF	intercept	1	3066.2	< 0.0001
	tmt	3	927.5	< 0.0001
	time	1	921.4	< 0.0001
	tmt:time	3	235.8	< 0.0001
	residual	64		

	Least square means with standard error			
Treatments	***A. flavus* NRRL 3357**	**BPM M**	**BPM K**	**BPM FF**
zero carbon	31.5 ± 1.71	39.4 ± 2.26	6.8 ± 0.8	7.9 ± 0.8
adipic acid	6.5 ± 1.71	5.4 ± 2.26	1.9 ± 0.8	15.0 ± 0.8
1,4-butanediol	28.9 ± 1.71	37.3 ± 2.26	7.3 ± 0.8	7.3 ± 0.8
glucose	75.4 ± 1.71	67.1 ± 2.26	17.5 ± 0.8	58.2 ± 0.8

	Test statistic, *p* values			
Pairwise Comparison	***A. flavus* NRRL 3357**	**BPM M**	**BPM K**	**BPM FF**
adipic acid – zero carbon	-10.3, p < 0.01	-10.6, p < 0.01	-3.9, p < 0.01	6.3, p < 0.01
adipic acid - glucose	-28.4, p < 0.01	-19.3, p < 0.01	-13.7, p < 0.01	-38.3, p < 0.01
1,4-butanediol – adipic acid	9.2, p < 0.01	9.9, p < 0.01	4.8, p < 0.01	-6.8, p < 0.01
1,4-buatendiol - glucose	-19.2, p < 0.01	-9.3, p < 0.01	-8.9, p < 0.01	-45.0, p < 0.01
1,4-buatendiol – zero carbon	-1.1, p > 0.50	-0.7, p > 0.50	0.9, p > 0.50	-0.5, p > 0.90
glucose – zero carbon	18.1, p < 0.01	8.6, p < 0.01	9.8, p < 0.01	44.6, p < 0.01

Table 7: ANOVA results for generalized least squares model, least square means, and pairwise comparison with test statistic and *p*-values for *A. flavus* NRRL 3357 conidia swelling in semi-solid GMM with 1% (w/v) PBAT monomers

	df	**F value**	***p* value**
Intercept	1	19905	<0.0001
Treatment	3	24.0	<0.0001
Time	1	1031.7	<0.0001
Treatment:Time	3	44.0	<0.0001
Residuals	808		
Treatments			**Least square means with standard error**
no carbon			3.3 ± 0.045
adipic acid			3.0 ± 0.047
1,4-butanediol			3.5 ± 0.049
glucose			3.7 ± 0.049
Pairwise Comparison			**Test statistic, *p* value**
1,4-butanediol – adipic acid			6.9, $p < 0.01$
1,4-butanediol - glucose			-2.7, $p < 0.05$
1,4-butanediol - no carbon			3.1, $p < 0.05$
adipic acid - glucose			-9.7, $p < 0.01$
adipic acid - no carbon			-4.2, $p < 0.01$
glucose - no carbon			5.8, $p < 0.01$

Table 8: ANOVA results from generalized least squares model, least square means, and pairwise comparison with test statistic and *p*-values for *A. flavus* NRRL 3357 conidia swelling in liquid Champes with 1% (w/v) PBAT monomers

	df	**F value**	***p* value**
Intercept	1	42972	<0.0001
Treatment	2	88.0	<0.0001
Time	1	1437.8	<0.0001
Treatment:Time	2	21.6	<0.0001
Residuals	1863		
Treatments			**Least square means with standard error**
zero carbon			3.3 ± 0.028
adipic acid			3.2 ± 0.030
glucose			3.6 ± 0.027
Pairwise Comparison			**Test statistic, *p* value**
adipic acid - glucose			-11.1, $p < 0.001$
adipic acid - zero carbon			-3.5, $p < 0.01$
glucose - zero carbon			7.8, $p < 0.001$

Table 9: One-way ANOVA (percent germination as a function of treatment) with test statistic and *p*-values for Tukey's HSD comparisons for *A. flavus* NRRL 3357 conidial germination in media of varying pH

	df	**Sum Sq**	**Mean Sq**	**F value**	**P value**
Treatment	7	200.4	28.6	419.5	<2e-16
Residuals	16	1.09	0.068		

Pairwise Comparison	**Test statistic, *p* value**
adipic acid - adipic acid + MES	-21.0, p < 0.001
adipic acid - glucose	-34.6, p < 0.001
adipic acid - glucose + acid	-30.4, p < 0.001
adipic acid - glucose + adipic acid	-30.6, p < 0.001
adipic acid - glucose + adipic acid + MES	-34.6, p < 0.001
adipic acid - no carbon	-14.9, p < 0.001
adipic acid - no carbon + acid	-0.02, p > 0.10
adipic acid + MES - glucose	-13.6, p < 0.001
adipic acid + MES - glucose + acid	-9.4, p < 0.001
adipic acid + MES - glucose + adipic acid	-9.6, p < 0.001
adipic acid + MES - glucose + adipic acid + MES	-13.6, p < 0.001
adipic acid + MES - no carbon	6.1, p < 0.001
adipic acid + MES - no carbon + acid	21.0, p < 0.001
glucose - glucose + acid	4.2, p < 0.05
glucose - glucose + adipic acid	4.0, p < 0.05
glucose - glucose + adipic acid + MES	0.00, p > 0.90
glucose - no carbon	19.7, p < 0.001
glucose - no carbon + acid	34.6, p < 0.001
glucose + acid - glucose + adipic acid	-0.10, p > 0.90
glucose + acid - glucose + adipic acid + MES	-4.2, p < 0.05
glucose + acid - no carbon	15.5, p < 0.001
glucose + acid - no carbon + acid	30.4, p < 0.001
glucose + adipic acid - glucose + adipic acid + MES	-4.0, p < 0.05
glucose + adipic acid - no carbon	15.7, p < 0.001
glucose + adipic acid - no carbon + acid	30.5, p < 0.001
glucose + adipic acid + MES - no carbon	19.7, p < 0.001
glucose + adipic acid + MES - no carbon + acid	34.6, p < 0.001
no carbon - no carbon + acid	14.9, p < 0.001

Table 10: *A. flavus* NRRL 3357 conidial recovery from adipic acid ANOVA from generalized least squares model

	df	**F value**	***p* value**
intercept	1	8075.2	< 0.0001
tmt	1	0.057	0.82
time	1	11.4	< 0.01
tmt:time	1	2.5	0.16
residual	8		

Table 11: ANOVA results for generalized least squares model and test statistic and *p*-values for Tukey's HSD Comparisons for *A. flavus* NRRL 3357 germination on PBAT-coated slides

	df	**F value**	***p* value**
intercept	1	3413.6	< 0.0001
tmt	9	296.6	< 0.0001
time	1	720.3	< 0.0001
$time^2$	1	52.8	< 0.0001
tmt:time	9	64.9	< 0.0001
residual	159		

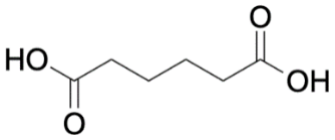
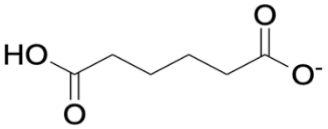
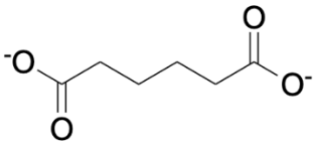
Treatment	**Least square means with standard error**
GMM w/ zero carbon	29.8 ± 1.41
GMM w/ 1,4-butanediol	29.8 ± 1.41
GMM w/ adipic acid	0.9 ± 1.41
GMM w/ glucose	72.8 ± 1.41
GMM w/ zero carbon + solvent	26.0 ± 1.41
PBAT + GMM w/ glucose	58.8 ± 1.41
PBAT + GMM w/ zero carbon	21.5 ± 1.41
PBAT w/ 1,4-butanediol + GMM w/ zero carbon	19.1 ± 1.41
PBAT w/ adipic acid + GMM w/ zero carbon	0.3 ± 1.41
PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	0.9 ± 1.41

Pairwise Comparison	**Test statistic, *p* value**
GMM w/ 1,4-butanediol - GMM w/ adipic acid	14.6, p < 0.001
GMM w/ 1,4-butanediol - GMM w/ glucose	-21.6, p < 0.001
GMM w/ 1,4-butanediol - GMM w/ zero carbon	0.05, p > 0.90
GMM w/ 1,4-butanediol - GMM w/ zero carbon + solvent	1.9, p > 0.60
GMM w/ 1,4-butanediol - PBAT + GMM w/ glucose	-14.6, p < 0.001
GMM w/ 1,4-butanediol - PBAT + GMM w/ zero carbon	4.2, p < 0.002
GMM w/ 1,4-butanediol - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	5.4, p < 0.001
GMM w/ 1,4-butanediol - PBAT w/ adipic acid + GMM w/ zero carbon	14.8, p < 0.001
GMM w/ 1,4-butanediol - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	14.6, p < 0.001
GMM w/ adipic acid - GMM w/ glucose	-36.1, p < 0.001
GMM w/ adipic acid - GMM w/ zero carbon	-14.5, p < 0.001
GMM w/ adipic acid - GMM w/ zero carbon + solvent	-12.6, p < 0.001
GMM w/ adipic acid - PBAT + GMM w/ glucose	-29.1, p < 0.001
GMM w/ adipic acid - PBAT + GMM w/ zero carbon	-10.3, p < 0.001
GMM w/ adipic acid - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	-9.2, p < 0.001
GMM w/ adipic acid - PBAT w/ adipic acid + GMM w/ zero carbon	0.3, p > 0.90
GMM w/ adipic acid - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	0.05, p > 0.90
GMM w/ glucose - GMM w/ zero carbon	21.6, p < 0.001
GMM w/ glucose - GMM w/ zero carbon + solvent	23.5, p < 0.001
GMM w/ glucose - PBAT + GMM w/ glucose	7.0, p < 0.001
GMM w/ glucose - PBAT + GMM w/ zero carbon	25.8, p < 0.001
GMM w/ glucose - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	27.0, p < 0.001
GMM w/ glucose - PBAT w/ adipic acid + GMM w/ zero carbon	36.4, p < 0.001
GMM w/ glucose - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	36.2, p < 0.001

Table 11: Continued

Pairwise Comparison	Test statistic, *p* value
GMM w/ zero carbon - GMM w/ zero carbon + solvent	1.9, p > 0.50
GMM w/ zero carbon - PBAT + GMM w/ glucose	-14.6, p < 0.001
GMM w/ zero carbon - PBAT + GMM w/ zero carbon	4.2, p < 0.01
GMM w/ zero carbon - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	5.3, p < 0.001
GMM w/ zero carbon - PBAT w/ adipic acid + GMM w/ zero carbon	14.8, p < 0.001
GMM w/ zero carbon - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	14.6, p < 0.001
GMM w/ zero carbon + solvent - PBAT + GMM w/ glucose	-16.5, p < 0.001
GMM w/ zero carbon + solvent - PBAT + GMM w/ zero carbon	2.3, p > 0.40
GMM w/ zero carbon + solvent - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	3.5, p < 0.05
GMM w/ zero carbon + solvent - PBAT w/ adipic acid + GMM w/ zero carbon	12.9, p < 0.001
GMM w/ zero carbon + solvent - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	12.7, p < 0.001
PBAT + GMM w/ glucose - PBAT + GMM w/ zero carbon	18.8, p < 0.001
PBAT + GMM w/ glucose - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	20.0, p < 0.001
PBAT + GMM w/ glucose - PBAT w/ adipic acid + GMM w/ zero carbon	29.4, p < 0.001
PBAT + GMM w/ glucose - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	29.2, p < 0.001
PBAT + GMM w/ zero carbon - PBAT w/ 1,4-butanediol + GMM w/ zero carbon	1.2, p > 0.90
PBAT + GMM w/ zero carbon - PBAT w/ adipic acid + GMM w/ zero carbon	10.6, p < 0.001
PBAT + GMM w/ zero carbon - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	10.4, p < 0.001
PBAT w/ 1,4-butanediol + GMM w/ zero carbon - PBAT w/ adipic acid + GMM w/ zero carbon	9.4, p < 0.001
PBAT w/ 1,4-butanediol + GMM w/ zero carbon - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	9.2, p < 0.001
PBAT w/ adipic acid + GMM w/ zero carbon - PBAT w/ adipic acid and 1,4-butanediol + GMM w/ zero carbon	-0.2, p > 0.90

Table 12: Fractional percentage of adipic acid at pH 3.0 and 6.5

Form	Structure	Amount (%)[a]	
		pH 3.0	pH 6.5
Undissociated		99.37	0.06
Semi-dissociated		0.63	7.35
Dissociated		0.00	92.59

[a] % fraction was calculated using adipic acid dissociation equilibrium. pKa values = 4.41 and 5.4

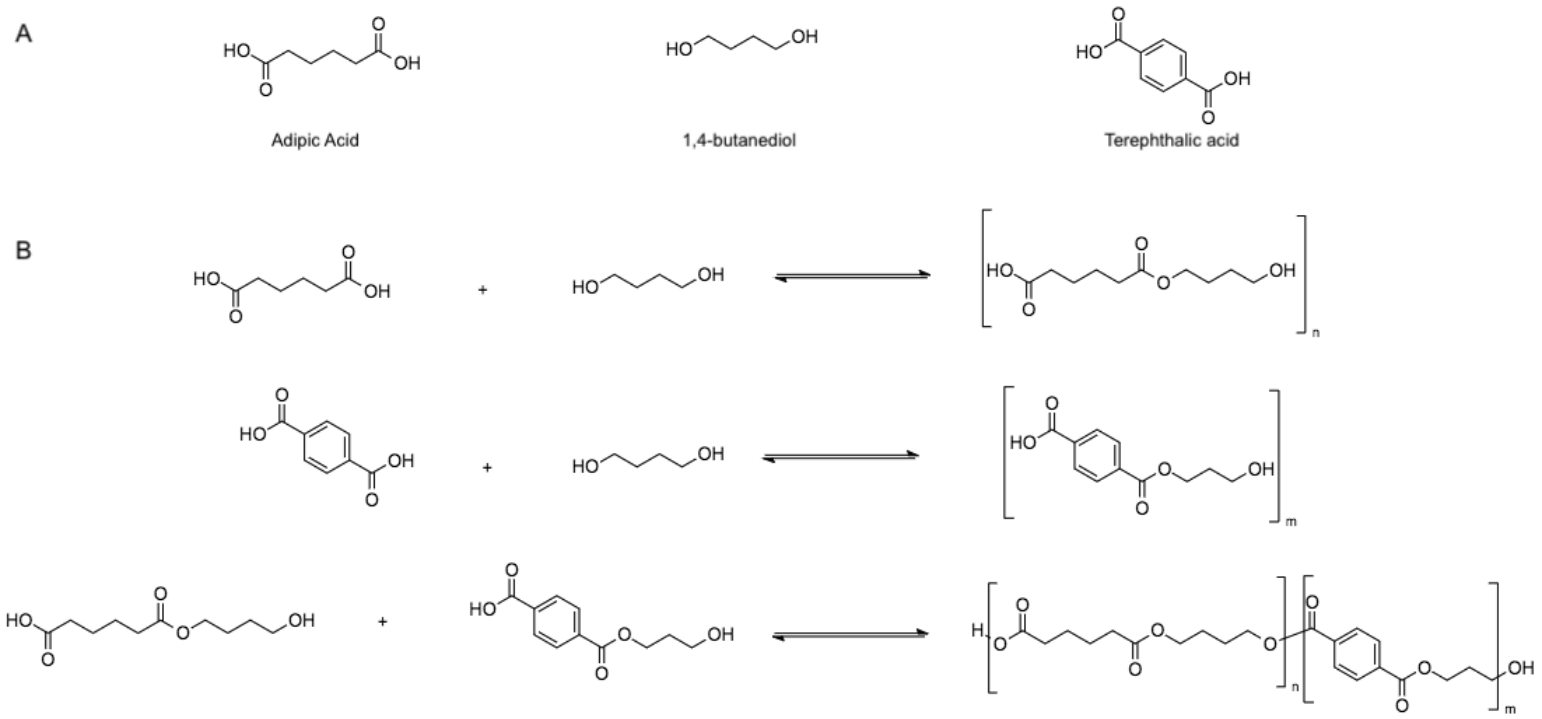


Figure 1: PBAT synthesis. (**A**) Monomers of PBAT. **(B**) Reaction scheme of the two copolymers and PBAT synthesis.

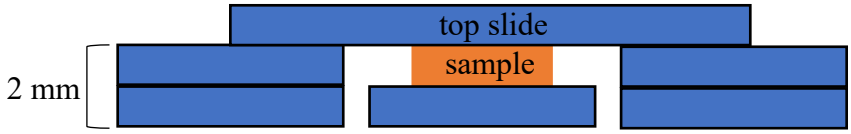


Figure 2: Side view of experimental setup for germination assay where blue rectangles represent microscope slides and the orange rectangle represents agar in which conidia were suspended. The purpose of this setup was to create flat GMM samples for improved optical quality.

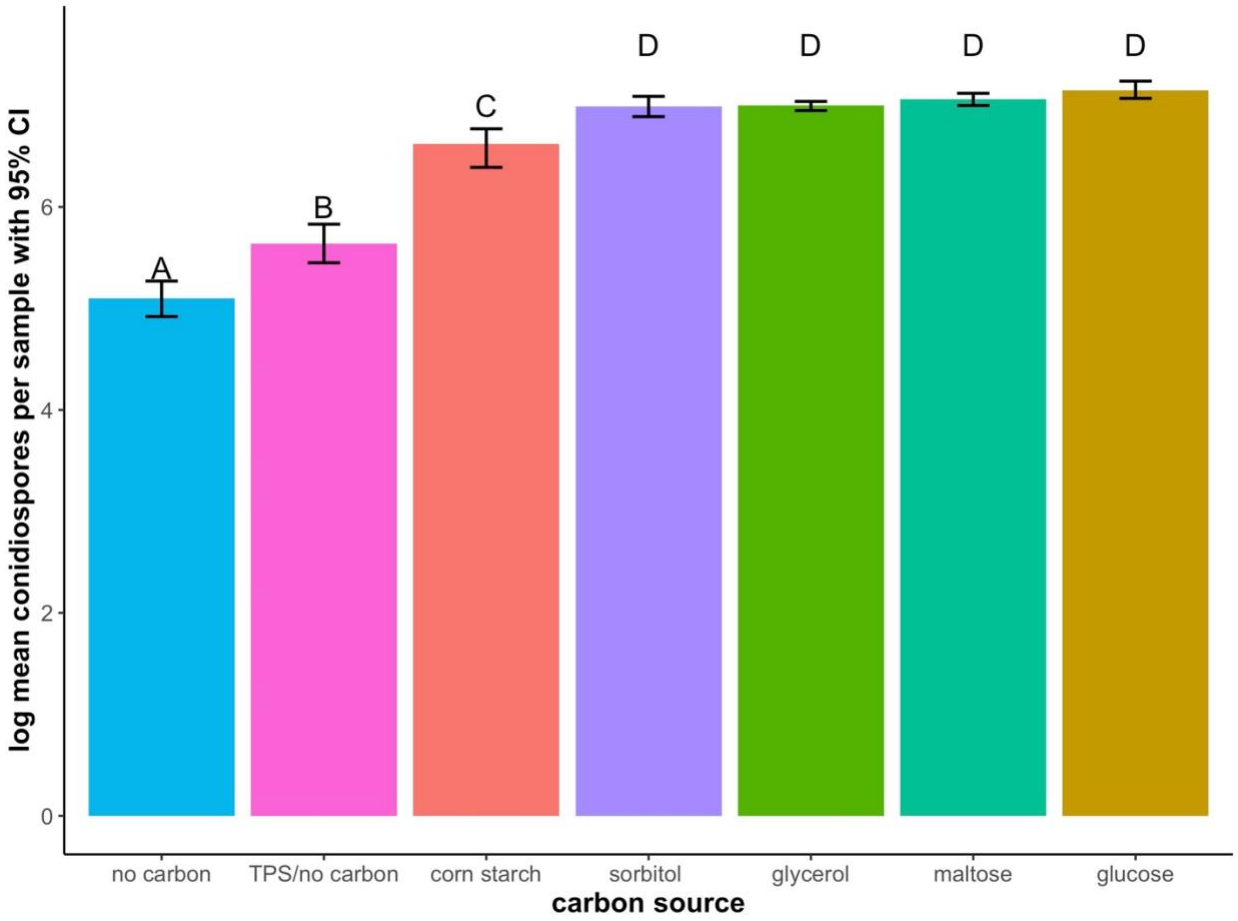


Figure 3: Conidial numbers (log transformed) of *Aspergillus flavus* NRRL 3357 grown on TPS and common TPS plasticizers at 0.3% (w/v). Letters represent significant differences from a one-way ANOVA using Tukey HSD for pairwise comparison with an alpha of 0.05. Five replicates per treatment were used. CI = confidence interval.

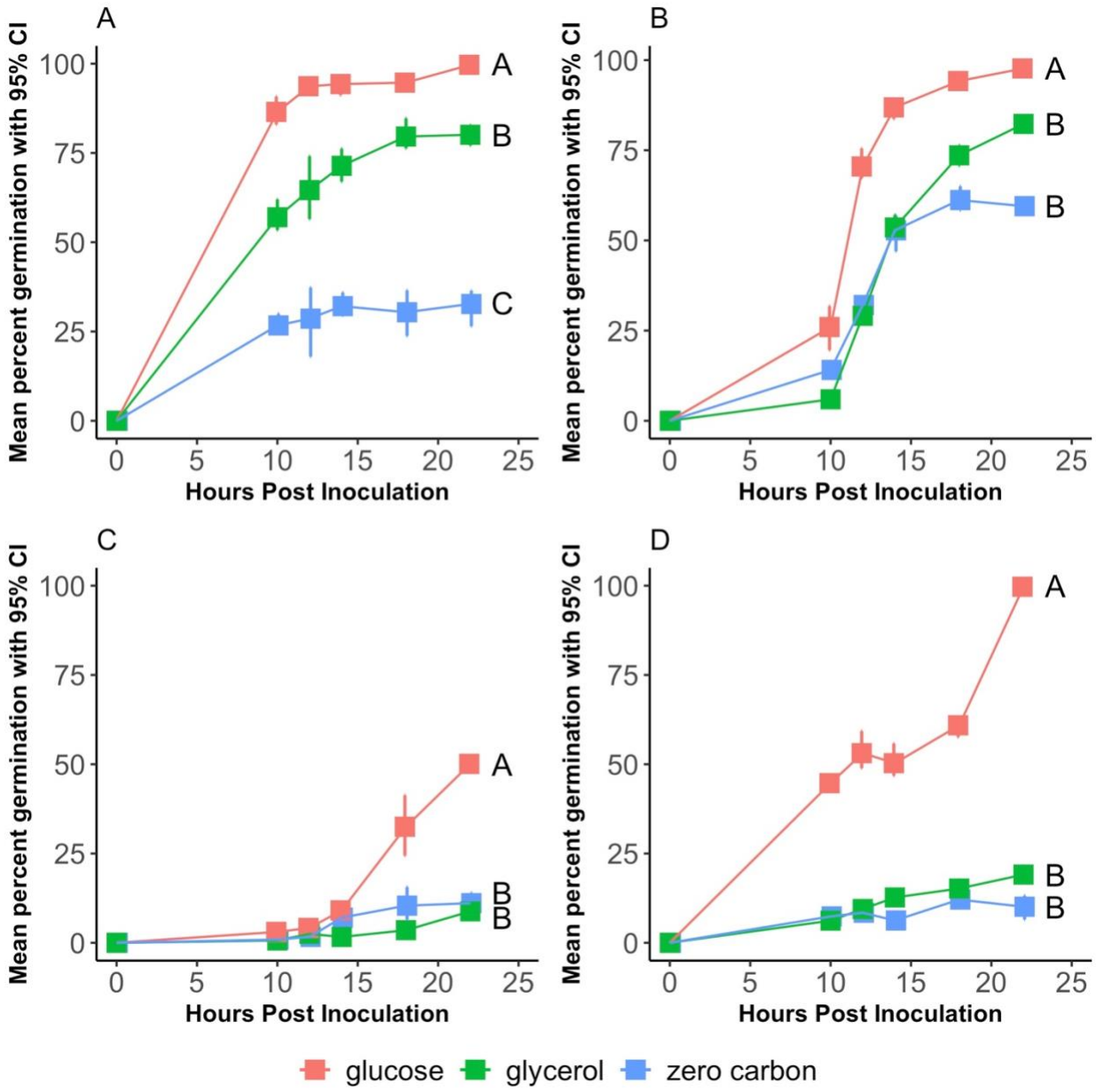


Figure 4: Germination time courses of *Aspergillus* strains grown for 22 hours on GMM with 1% (w/v) glucose, 1% (w/v) glycerol, or no carbon source (**A**) *A. flavus* NRRL 3357; (**B**) BPM M; (**C**) BPM K; and (**D**) BPM FF. Letters represent significant differences between least square means of treatments while controlling for time ($\alpha = 0.05$). Experiment was performed twice with three replicates per treatment per timepoint. CI = confidence interval.

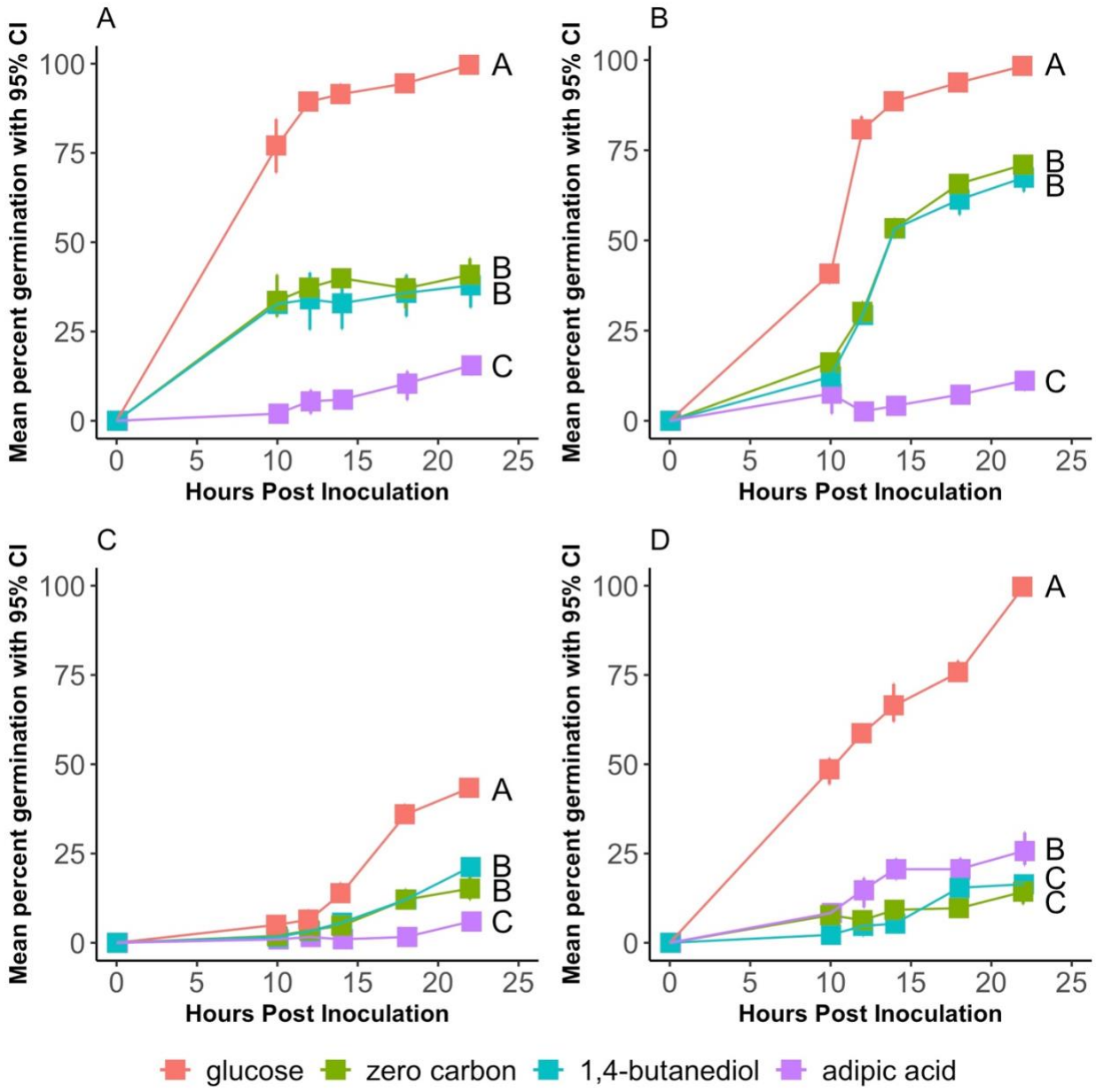


Figure 5: Germination time courses for *Aspergillus* strains grown for 22 hours on GMM with 1% (w/v) adipic acid, 1% (w/v) 1,4-butanediol, 1% (w/v) glucose, or no carbon source (A) *A. flavus* NRRL 3357; (B) BPM M; (C) BPM K; and (D) BPM FF. Letters represent significant differences between least square means of treatments while controlling for time ($\alpha = 0.05$). Experiment was performed twice with three replicates per treatment per timepoint. CI = confidence interval.

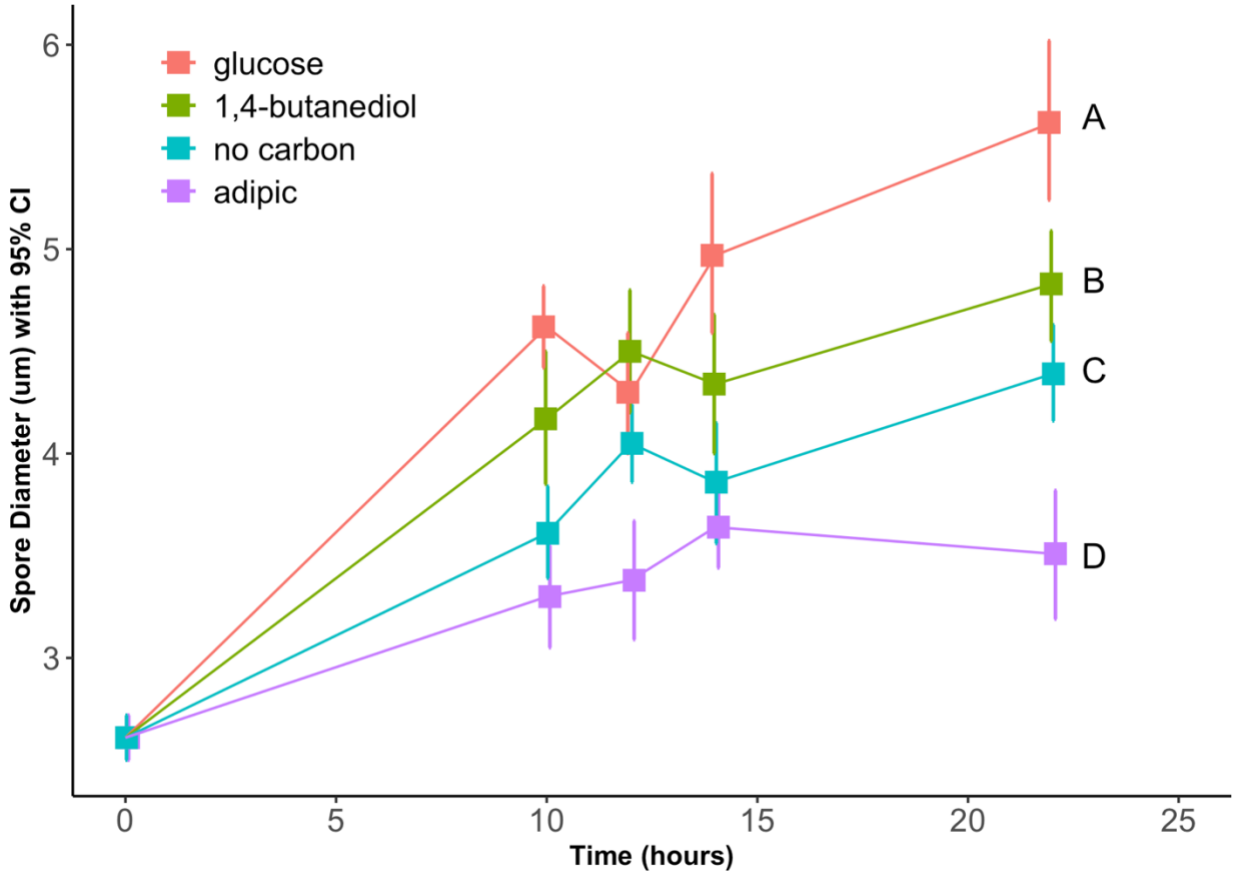


Figure 6*: A. flavus* NRRL 3357 conidial diameter on solid GMM with no carbon, 1% (w/v) glucose, 1% (w/v) adipic acid, or 1% (w/v) 1,4-butanediol. Letters represent significant differences between least square means of treatments while controlling for time ($\alpha = 0.05$). The experiment was performed twice with similar results. CI = confidence interval.

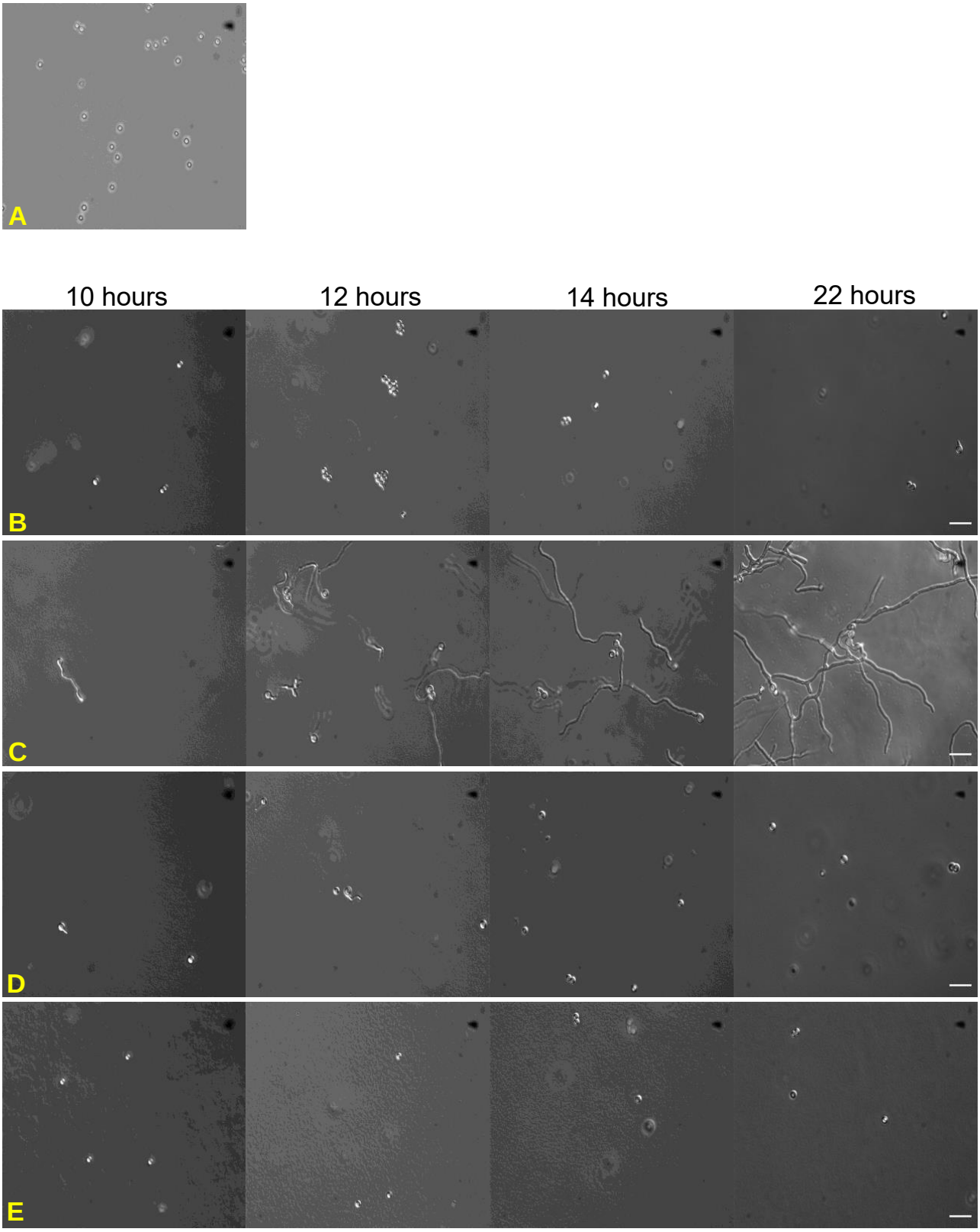


Figure 7: Micrograph of *Aspergillus flavus* NRRL 3357 germination in solid GMM with monomers of PBAT. Harvested conidia no treatment 0 hours (**A),** GMM with no carbon (**B**), GMM with 1% (w/v) glucose (**C**), GMM with 1% (w/v) 1,4-butanediol (**D**), GMM with 1% (w/v) adipic acid (**E**). Scale bar = 20 μm

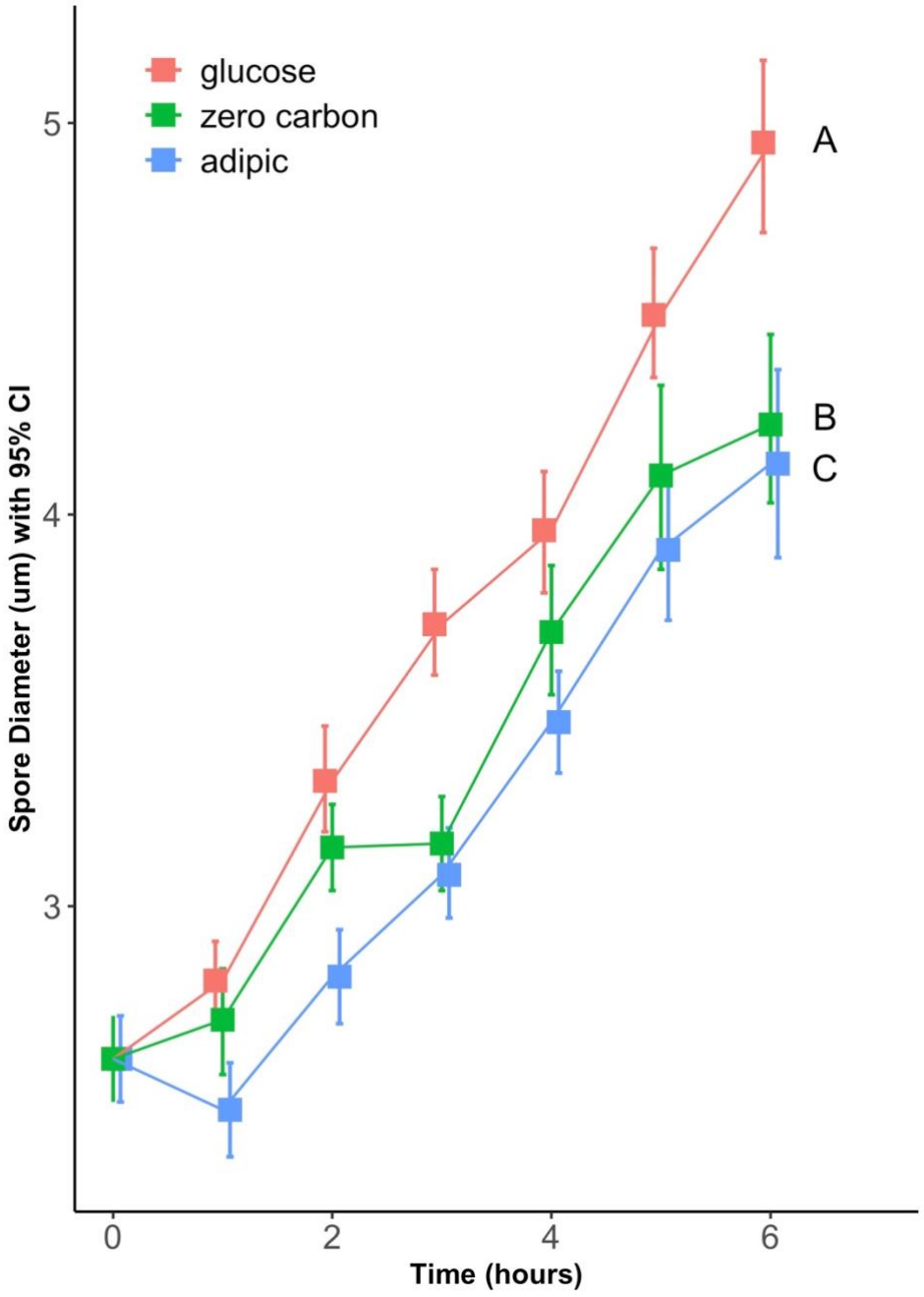


Figure 8*: A. flavus* NRRL 3357 conidial diameter liquid Champes media with 1% (w/v) glucose, glucose omitted, or replaced with 1% (w/v) adipic acid. Letters represent significant differences between least square means of treatments while controlling for time ($\alpha = 0.05$). CI = confidence interval.

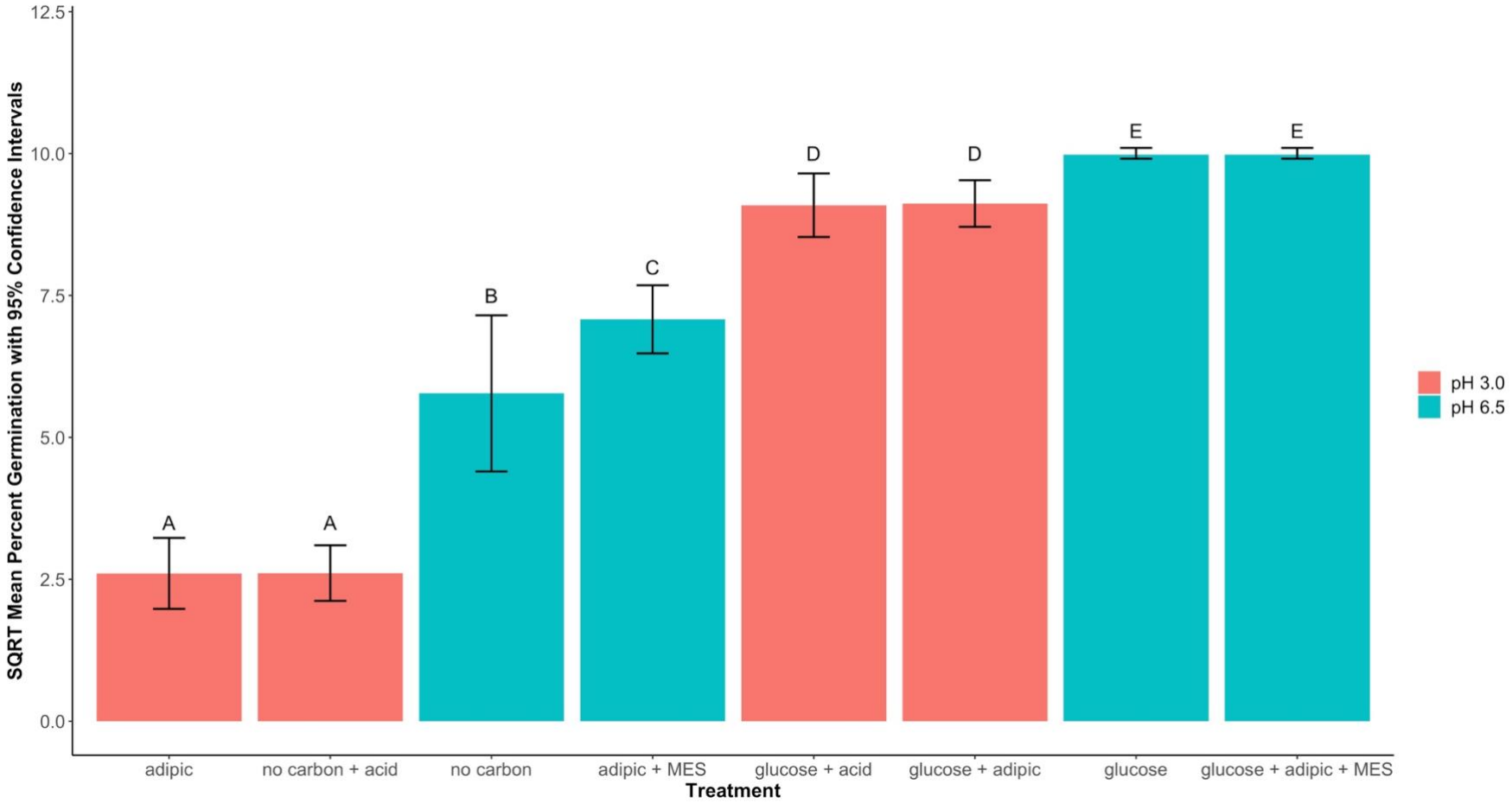


Figure 9: *Aspergillus flavus* NRRL 3357 conidial germination rate after 22h on solid GMM with no carbon source, 1% (w/v) adipic acid and/or glucose at pH 3.0 and 6.0. A square root transformation was taken for % germination to correct for skewness from several influential data points. Letters represent statistical difference 1-way ANOVA and Tukey HSD for pairwise comparison (alpha = 0.05). Experiment was performed twice with three replicates per treatment. SQRT = square root.

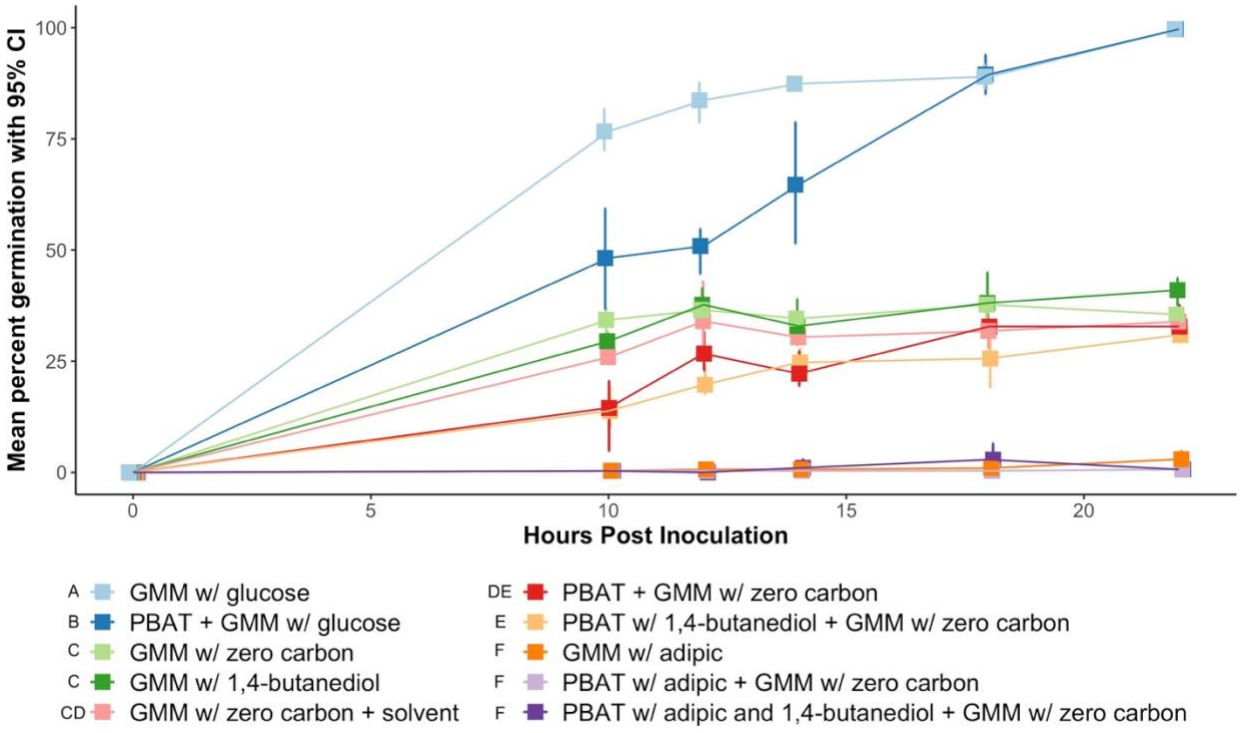


Figure 10: Germination timecourse of *Aspergillus flavus* NRRL 3357 grown for 22 hours on GMM with no carbon, with 1% (w/v) adipic acid, 1% (w/v) 1,4-butanediol, 1% (w/v) glucose and PBAT polymers spiked with monomers. Letters to the left of the legend represent significant differences between least square means of treatments while controlling for time ($\alpha = 0.05$). The experiment was performed twice with similar results. CI = confidence interval.

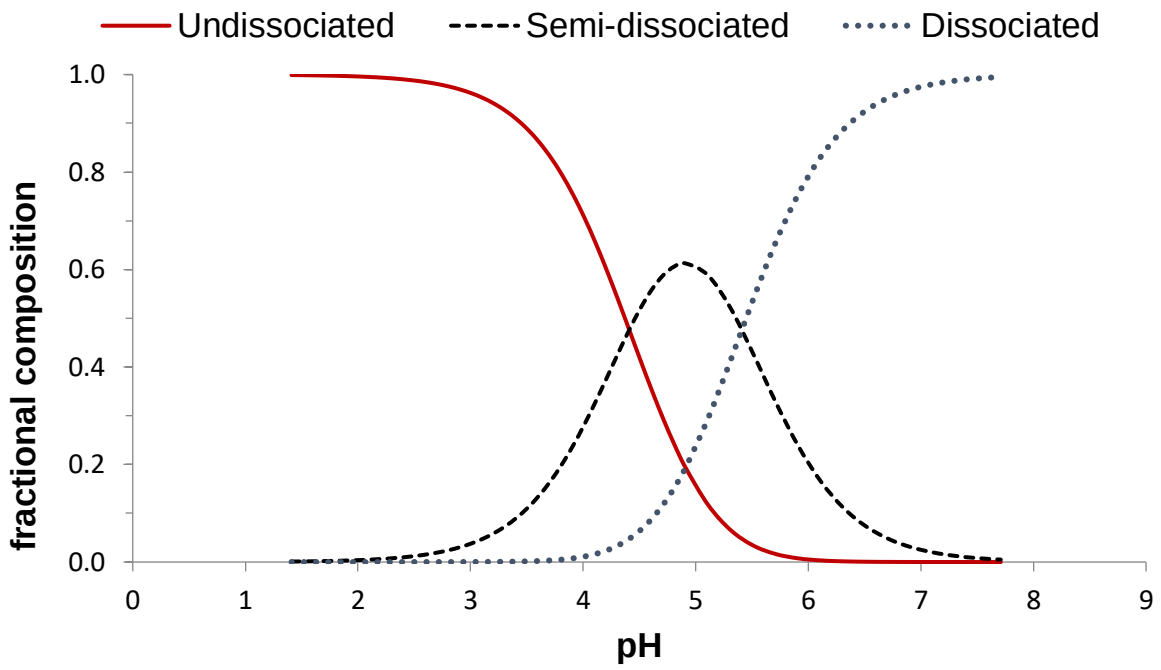


Figure 11: Adipic acid dissociation curve used to calculate values in **Table 12**. pka_1 =4.41 and pKa_2 = 5.41.

CITATIONS

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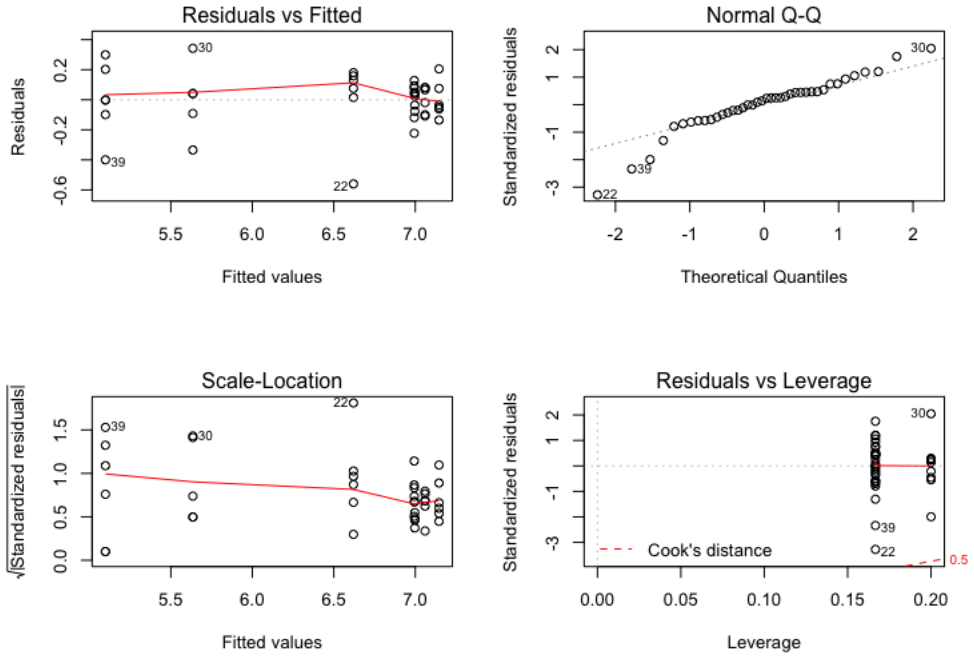
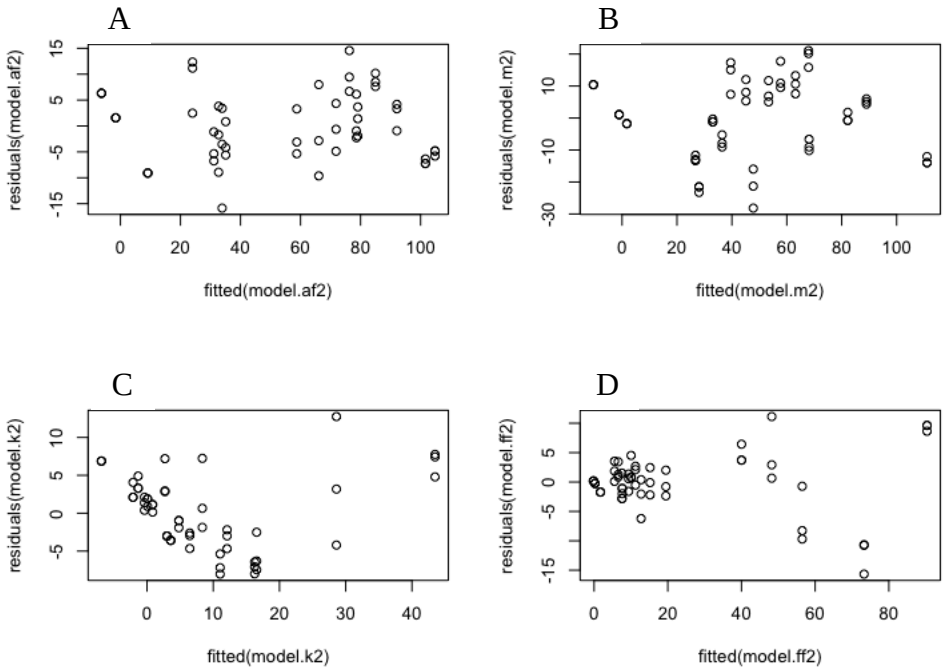
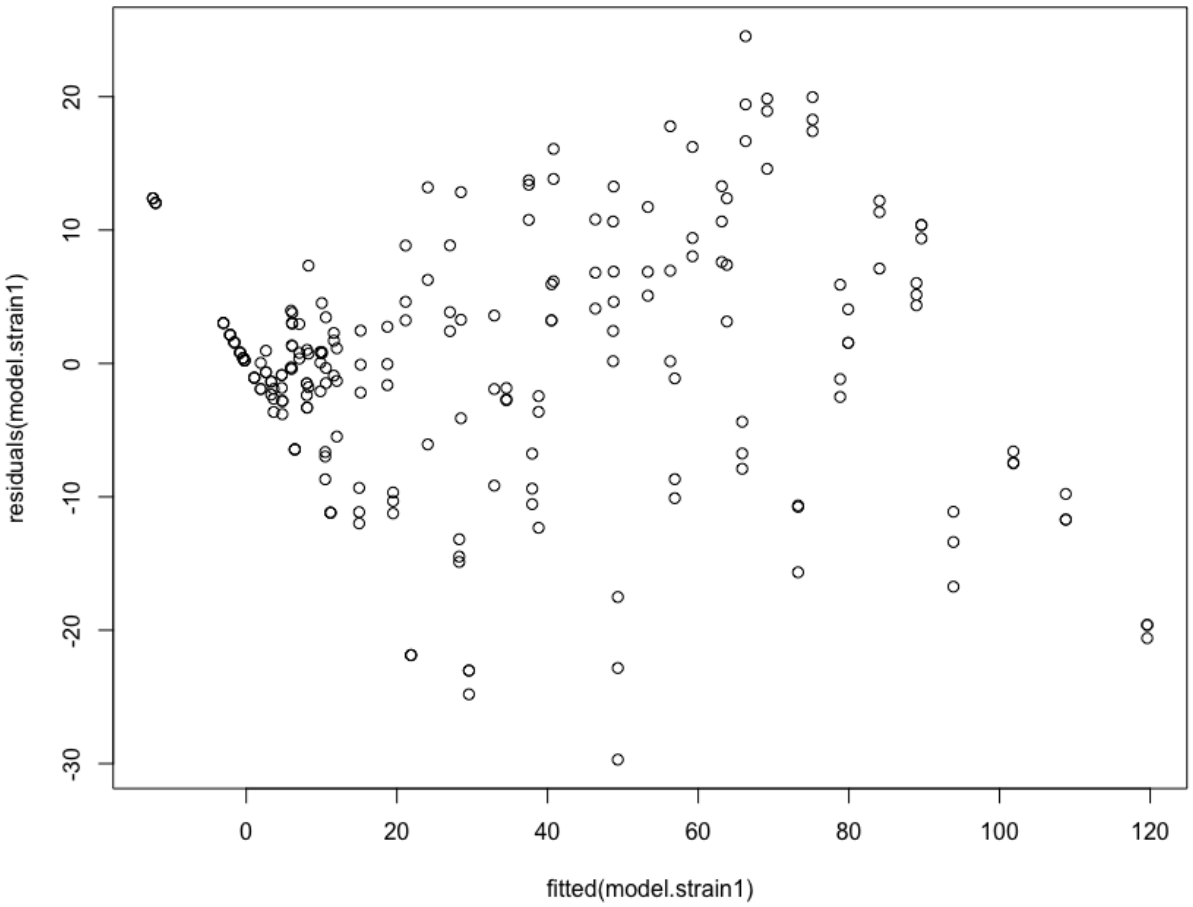
SUPPLEMENTAL FIGURES



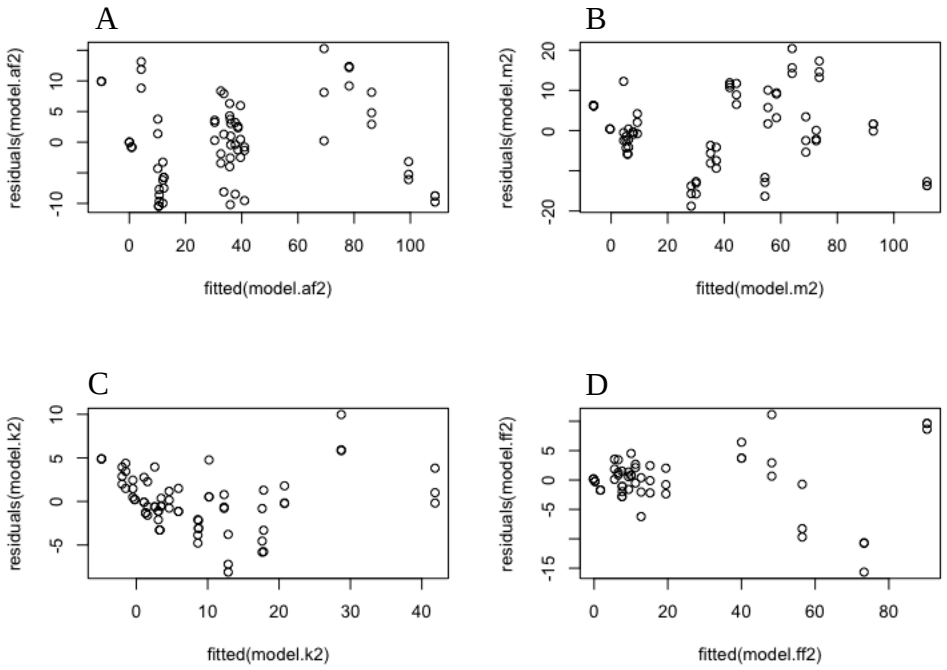
Supplemental Figure 1: Residuals of log transformed one-way ANOVA for TPS additive growth experiment for *A. flavus* NRRL 3357. ANOVA model used was log mean of conidia production as a function of treatment. From Levene's test: F-value = 0.66, p-value = 0.69. Residuals are randomly distributed (showing homoscedasticity), follow a normal distribution, and no data points fall are over leveraged shown by no data points fall in the Cook's distance, allow an ANOVA model to be run and pairwise comparison to be made.



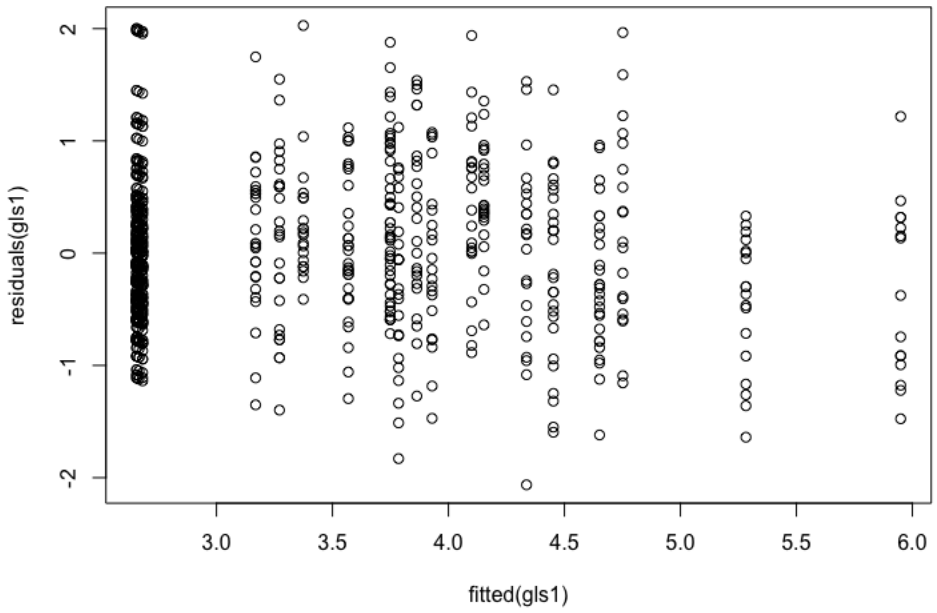
Supplemental Figure 2: Residuals for (**A**) *A. flavus* NRRL 3357, (**B**) BPM M, (**C**) BPM K, (**D**) BPM FF germination on glycerol. For model terms see **Table 4**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.



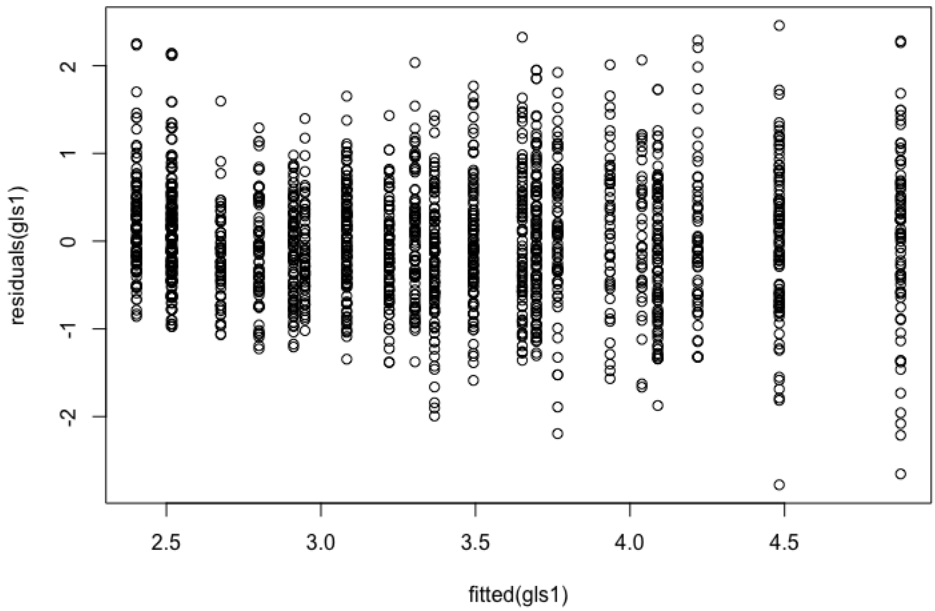
Supplemental Figure 3: Residuals for between *A. flavus* NRRL 3357, BPM M, BPM K, and BPM FF model for germination on glycerol. For model terms see **Table 5**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.



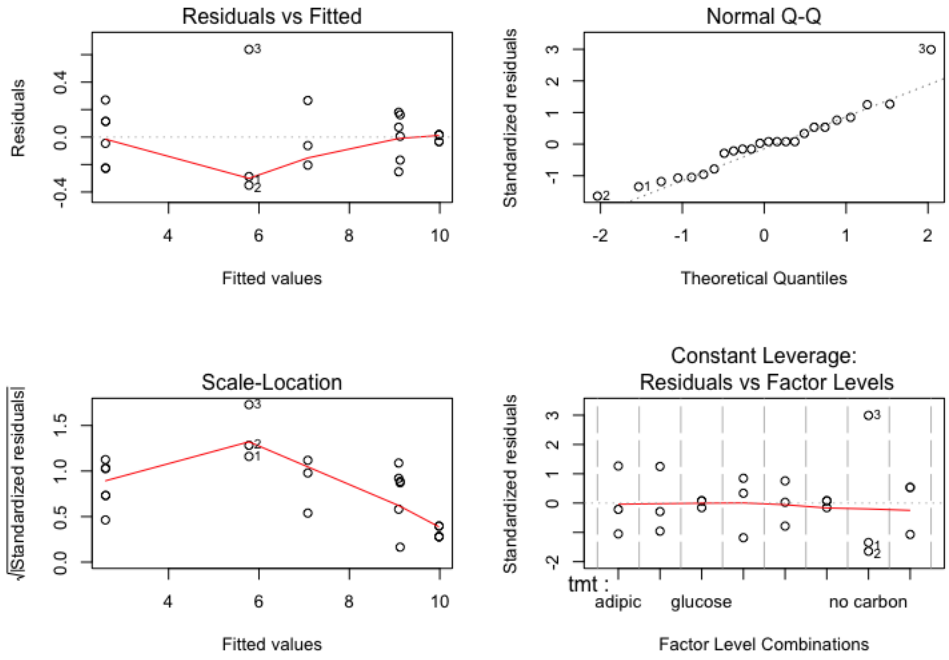
Supplemental Figure 4: Residuals for (**A**) *A. flavus* NRRL 3357, (**B**) BPM M, (**C**) BPM K, (**D**) BPM FF germination on PBAT monomers. For model terms see **Table 6**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.



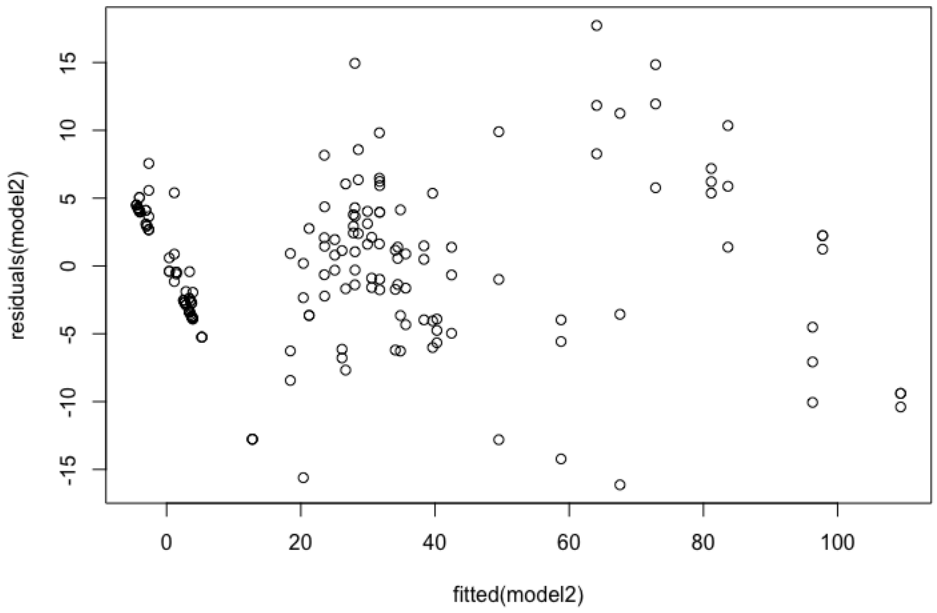
Supplemental Figure 5: Residuals for *A. flavus* NRRL 3357 conidial diameter in solid GMM. For model terms see **Table 7**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.



Supplemental Figure 6: Residuals for *A. flavus* NRRL 3357 conidial diameter in liquid Champes medium. For model terms see **Table 8**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.



Supplemental Figure 7: Residuals for *A. flavus* NRRL 3357 germination and pH one-way ANOVA (percent germination as a function of treatment) with square root transformation. From Levene's test: F-value = 0.59, p-value = 0.75. Residuals are randomly distributed (showing homoscedasticity), follow a normal distribution, and no data points fall are over leveraged shown by no data points fall in the Cook's distance, allow an ANOVA model to be run and pairwise comparison to be made.



Supplemental Figure 8: Residuals from *A. flavus* NRRL 3357 germination on PBAT coated slides. For model terms see **Table 11**. Residuals are randomly scattered, showing model homoskedasticity in from the generalized least squares model, validating the model and allowing pairwise comparison to be made.

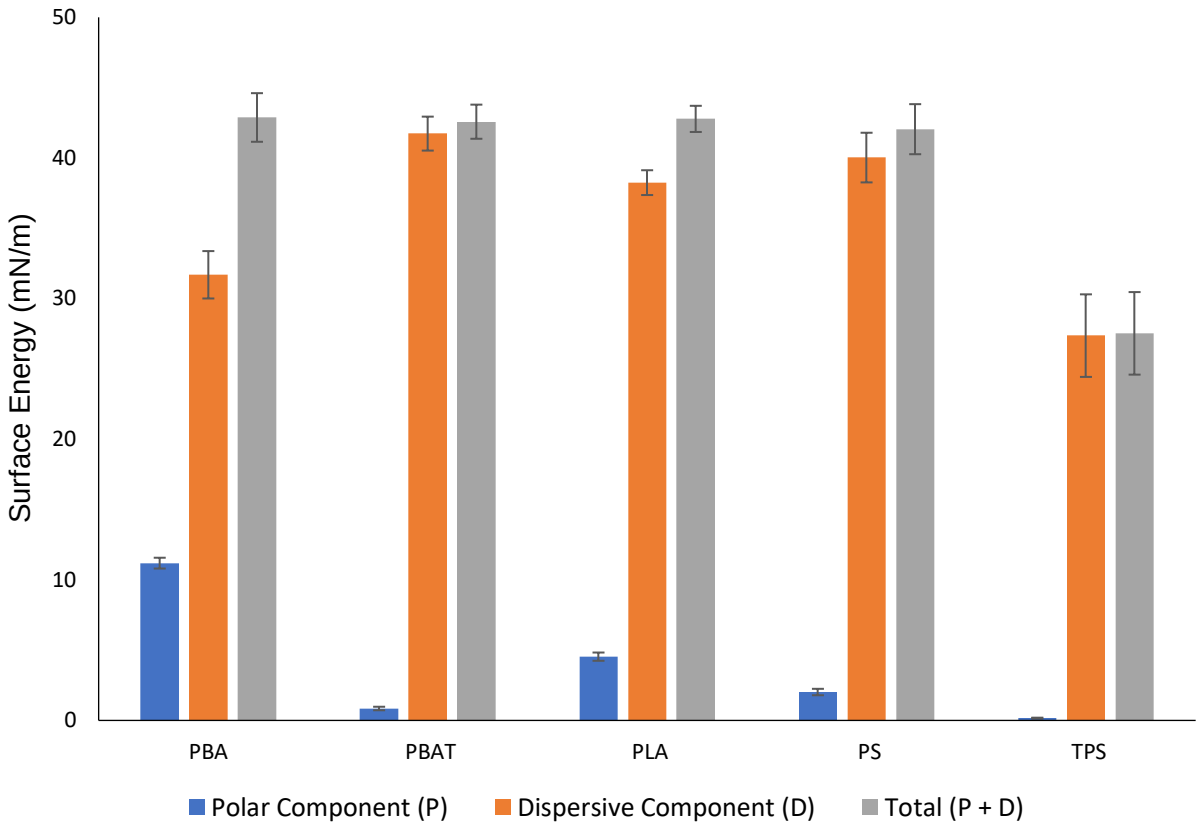
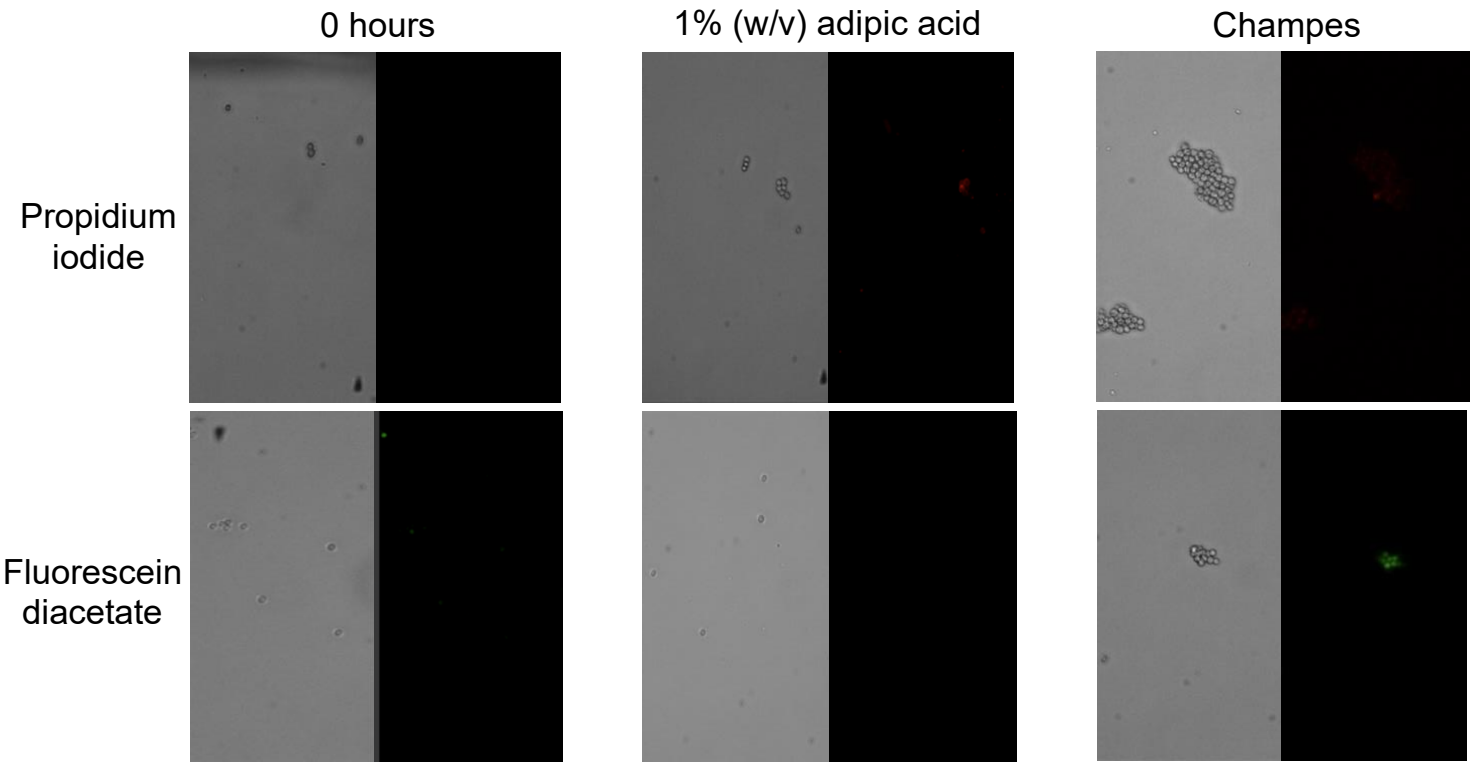


Figure 9. Summary of surface energies of various study materials using Kruss Drop Shape Analyzer (Model DSA 100) with a drop size/speed of 20 µL at 2.67 µL/s, table temperature of 20 °C, and a needle size of 0.51 mm in diameter. For each sample, twelve disks were used with six repeated measurements.



Supplemental Figure 10: Fluorescence microscopy of *A. flavus* NRRL 3357 conidia in no treatment (time = 0), two hours of incubation in liquid Champes medium with 1% (w/v) adipic acid, or unaltered liquid Champes medium. Conidia were washed three times with 0.01% (w/v) Triton X-100 at 2,000 rpm for 30 seconds. Dyes were added at a 20 μg/mL in a 1:1 solution with conidia suspension. Dyes were allowed to incubate in the dark at room temperature for five minutes and fifteen minutes for fluorescein diacetate (FDA) and propidium iodide (PI) respectively. FDA passively diffused across cell membranes fluoresces when metabolized, indicating that spores are metabolically active. PI crosses damaged cell membranes and has increased fluoresces when attached to nucleic acids, indicating that cells are dead or the out membrane has been compromised. Three frames were taken with similar results.