**Title**: Plant symbiotic fungal isolate *Alternaria alternata* grows better with an inorganic nitrogen source than an organic nitrogen source

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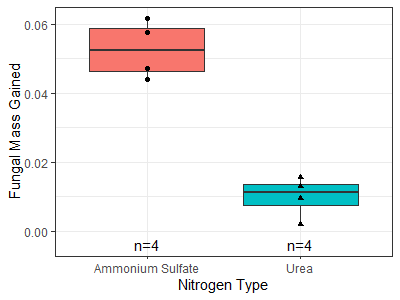
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**Abstract**

<100 words

*Alternaria alternata* is a fungus which causes spot diseases on agriculturally important crops, but it is also part of a fungal group (Dark Septate Endophytes) which can also improve the growth of host plants under certain conditions. To allow for future experimental testing of this relationship, it is necessary to know if this fungus can use both organic and inorganic nitrogen. We found that this *A. alternata* isolate not only grows when provided with an inorganic source of nitrogen, but it also grows better in an inorganic nitrogen media than in the organic nitrogen media. These data provide grounds for future studies testing more DSE taxa and more forms of organic and inorganic nitrogen to assess how different fungal groups utilize different sources of nitrogen.

**Figure**



**Figure caption**: Fungal biomass growth was significantly higher in media containing an inorganic nitrogen source compared to an organic nitrogen source (Welch Two Sample t-test; MAmmonium Sulfate = 0.0525975; MUrea = 0.0099475; t(5.3753) = 8.3487, p = 0.0003). Points are shaped according to nitrogen type and indicate each sample in the experiment.

**Description**

*Alternaria alternata* is a fungus well-known for causing a spot disease on plants, of economic interest because of the massive declines in crop productivity that it can cause (Troncoso-Rojas & Tiznado-Hernández, 2014). *A. alternata* is considered a Dark Septate Endophyte (DSE), which is a polyphyletic group of ascomycete fungi defined by regular cross-walls among cells (septa) and dark-pigmented hyphae (DeMers, 2022). While many DSE members have been categorized as plant pathogens, there is increasing evidence that under certain conditions, these fungi can form mutualisms with plants and improve plant growth instead of causing disease (Berthelot et al., 2019; Netherway et al., 2024; Schulz & Boyle, 2005). Given that *A. alternata* is a globally-occurring fungus (DeMers, 2022), it is of interest to understand which conditions are conducive to its being a mutualist instead of a pathogen so that management of the fungus’s effects on plants can be explored.

In this experiment, we assess the ability for an *A. alternata* isolate (GenBank Accession Number SUB14593255 germinator\_fung\_10x\_B\_2\_F12 PQ284877) to utilize inorganic and organic forms of nitrogen. It is vital to know if this fungus can use both forms of nitrogen because this will impact which forms of nitrogen are used and monitored in experiments. For example, stable isotopes of nitrogen are useful in tracing nitrogen movement in plant-fungal symbioses. However, it is unclear which form of nitrogen is best to use for these experiments despite this work being done on other groups of fungi (Finlay et al., 1992; Hawkins et al., 2000; He et al., 2003; Newsham, 2011; Upson et al., 2009).

In this laboratory experiment, we grew the *A. alternata* isolate in nitrogen-free media and amended it with either organic or inorganic nitrogen. We hypothesized that the fungus would be better able to use organic forms of nitrogen because of the fungus being a plant-symbiont which garners nutrition from the plant. In contrast to our expectation, the fungal isolate’s growth was significantly higher in the inorganic nitrogen media than in the organic nitrogen media (Welch Two Sample t-test; MAmmonium Sulfate = 0.0525975; MUrea = 0.0099475; t(5.3753) = 8.3487, p = 0.0003). While other sources of inorganic and organic nitrogen should be tested to determine fungal preference between the two types of nitrogen, it is clear that the fungus not only utilized an inorganic nitrogen source, but it also grew better with the inorganic nitrogen source than with the organic nitrogen source in this experiment.

This finding is relevant to a multitude of experiments, including those which aim to use stable isotopes of nitrogen as a way to trace the movement of nitrogen, as the fungus’s ability to use the nitrogen source is vital in such a method. Additionally, while more DSE isolates should be tested for their abilities to use inorganic and organic nitrogen, this experiment provides foundational evidence for DSE being able to use inorganic nitrogen sources.

**Methods**

We prepared two nitrogen-free media solutions by dissolving 0.0274 grams of Nitrogen free minimal media (MyBioSource Edinburgh) per 1 mL of autoclaved RO water. These solutions were amended with either an organic or an inorganic nitrogen source. For the inorganic N treatment, we added 0.0019 grams of Ammonium Sulfate (Mallinckrodt Chemical Works) per 1 mL of solution. For the inorganic N treatment, we added 0.0009 grams of Urea (Fisher Scientific U-15) per 1 mL of solution. Ratios were calculated based on the number of N atoms by weight so as to ensure the same amount of nitrogen was in each treatment. Both solutions were autoclaved and brought to room temperature over 24 hours.

50 mL Erlenmeyer cell culture flasks with metal caps were autoclaved, and 15 mL of either media type was added to each flask with sterile serological pipettes in a sterile biosafety cabinet using aseptic technique. A XXX mm diameter cork borer was used to punch same-sized inoculum plugs from the growing edge of the fungal culture (GenBank Accession Number SUB14593255 germinator\_fung\_10x\_B\_2\_F12 PQ284877), plated two weeks prior on Potato Dextrose Agar. The dry weight of these plugs is approximately XXX. One fungal plug was added to each flask. Flasks were incubated at 19C shaking at 80 RPM for 7 days. Filter papers were labeled and their individual weights were recorded. After the 7 days of incubation, the contents of each flask were filtered through the filter papers. Flasks were rinsed with autoclaved RO water, and the contents were filtered through the same filter papers until the visible contents of all flasks were cleared. Once filtering was complete, we placed folded filter papers upright in a rack in a 59.2 C drying oven for 48 hours. After drying, we weighed each sample. Fungal mass was calculated by subtracting each filter paper weight and the average dry fungal plug weight from the final mass of each filtrate.

**Reagents**

Alternaria alternata fungus (GenBank Accession Number SUB14593255 germinator\_fung\_10x\_B\_2\_F12 PQ284877). Isolated from a *Sorghum bicolor* seed by Dr. Ron Deckert in Dr. Catherine Gehring’s laboratory at Northern Arizona University.

MyBioSource Edinburgh minimal media, Nitrogen free Catalog #MBS652833

Mallinckrodt Chemical Works Ammonium Sulfate

Fisher Scientific Urea U-15

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**Statistical analyses**

Fungal mass measurements between nitrogen treatments were compared with a Welch Two Sample t-test; MAmmonium Sulfate = 0.0525975; MUrea = 0.0099475; t(5.3753) = 8.3487, p = 0.0003). We conducted all data analyses in R version 4.3.0 with RStudio (R Core Team, 2023).

**Data availability**

All code and data are available freely at <https://github.com/beabock/Org_vs_Inorg_N>.