Comparing spore collection techniques of *Nothopassalora personata*

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ABSTRACT

Nothopassalora personata is a fungal pathogen that causes late leaf spot of peanut. Because this fungus does not sporulate on media, laboratory inoculation studies rely on spores collected from field-infected peanuts as the inoculum source. Recent studies have shown that inoculum solutions prepared using a vacuum spore collector do not germinate as well as inoculum solutions prepared using sporulating leaf spots that have been cut from peanut tissues and dried. An experiment was conducted to see if the poor germination rates were caused by the vacuum process or by a toxic effect of the dissolvable capsule that the vacuumed spores are stored in. Treatments included a negative control inoculum solution (vacuumed spores prepared in a dissolvable capsule), a positive control solution (spores from dried leaf spots prepared in a microfuge tube), a solution prepared in the dissolvable capsule using spores from dried leaf spots, and a solution prepared in a microfuge tube using vacuumed spores. The results demonstrated a higher percent germination of N. personata spores from both unvacuumed treatments versus the ones that were vacuumed. There was no effect from the dissolvable capsule. These results suggest that the vacuum process reduces spore health.

INTRODUCTION

Nothopassalora personata is a fungal pathogen that causes late leaf spot of peanut. Infected peanut leaves display small, dark brown circles that have fungal sporulation on the underside of the lesion (Figure 1). Controlled inoculation assays are frequently used to study this disease [1] [2]. There are two techniques to collect spores of *N. personata*. The first uses a spore vacuum (Figure 2A). Vacuumed spores are stored in pill capsules that dissolve when the inoculum solution is prepared (Figure 2B). The second technique consists of dried, sporulating leaf spots, cut from diseased leaves using a razor blade. Inoculum solutions prepared using these leaf spots are made in plastic tubes (Figure 2C).

In our laboratory, germination rates have been lower for inoculum solutions using the vacuum and dissolvable capsule stored spores than the spores from the dried leaf spots.

The purpose of this experiment was to test the hypothesis that poor germination from vacuumed collected spores is due to the dissolved pill capsule having a toxic effect on the spores.

Figure 1. Late Leaf Spot of Peanut

MATERIALS AND METHODS

An experiment was conducted to determine if spore germination using the vacuum collected spores is poor because of the vacuum process, perhaps the result of mechanical damage, or because the dissolvable capsule the inoculum solution is prepared in is toxic to the spores. Four inoculum solution treatments, prepared in 0.005% Tween water, were compared, and three replications were conducted.

TREATMENTS

1 - Vacuumed spores, solution prepared in a dissolvable capsule

2 - Spores from dried leaf spots, solution prepared in a microfuge tube

3 - Spores from dried leaf spots, solution prepared in a dissolvable capsule

4 - Vacuumed spores, solution prepared in a microfuge tube using a dry q-tip for transfer

Each solution was transferred to water agar, allowed to dry and incubated for 3 days. Agar sections were then moved to microscope slides, stained with Trypan Blue stain and covered with a cover slip. The number of fully germinated spores were counted to compute percent germination. Data were analyzed using Analysis of Variance in SPSS with Spore Source (vacuumed or dried leaf spots) and Tube Source (dissolvable capsule or microfuge tube) as fixed factors.

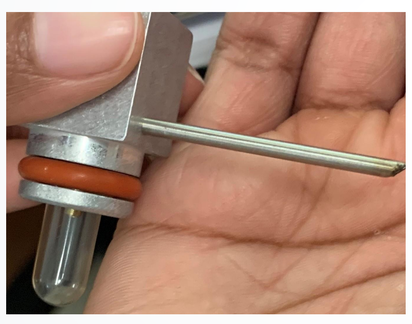


Figure 2A. Vacuum used to collect spores. Long, pointed end vacuums the spores off peanut leaves. The dissolvable pill capsule attaches at the bottom to store the spores.

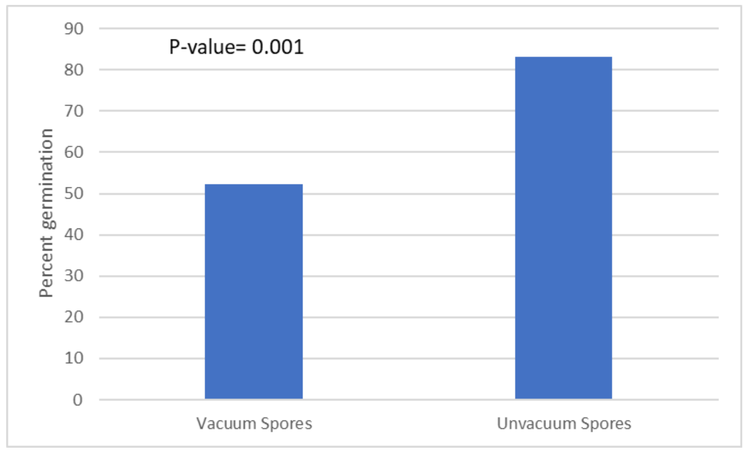
Figure 2B. Photos of both dissolving pill capsule with spores.

Dissolvable pill capsule after treatment with tween water.

Dissolvable pill capsule before treatment.

Figure 2C. Dried, sporulating leaf spots placed in a non-dissolvable microfuge tube when preparing solution.

RESULTS



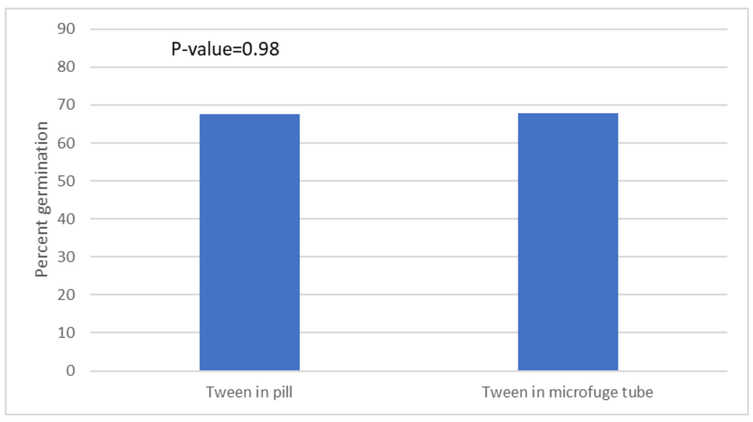


Figure 4. Spore germination between pill capsule and microfuge tube solutions.

The results demonstrated a higher percent germination of *N. personata* spores from both unvacuumed treatments versus the ones that were vacuumed (Figure 3). There was no effect from the dissolvable capsule (Figure 4).

CONCLUSIONS

The results did not support the hypothesis. There was no evidence that the dissolvable pill capsule was toxic to spores. Instead, the vacuuming process appears to the reason for poor germination. Our updated hypothesis is that vacuum is causing physical damage to the spores. Further studies will be conducted to see if reducing vacuum pressure improves germination.

ACKNOWLEDGEMENTS

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