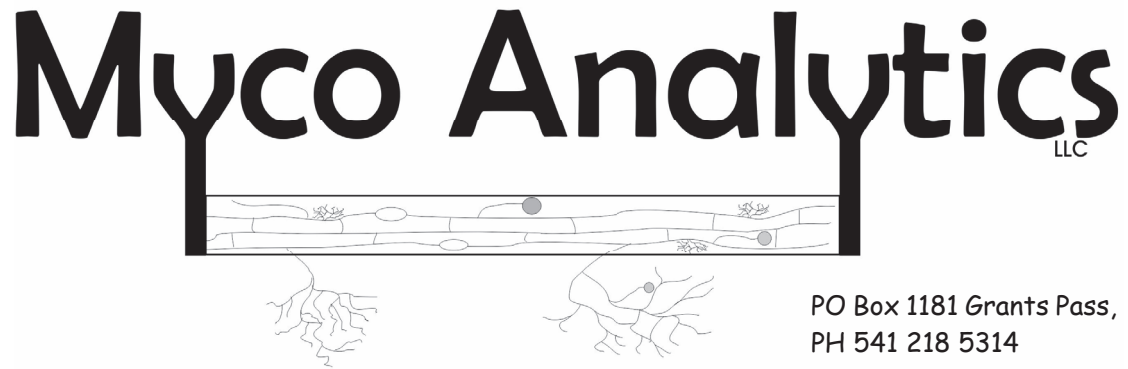




Certificate of Analysis

Date: 8/3/25

Product: Boomers Blend Root Hugger

Lot # : Sample for testing

Arbuscular Mycorrhizal propagules, 30/ml

Ectomycorrhizal Mycorrhizal propagules, 400,000/ml

Glomus intraradices, Glomus mosseae, Glomus aggregatum, Glomus etunicatum, Glomus deserticola, Glomus clarum, Glomus monosporum, Paraglomus brasilianum, Gigaspora margarita, Rhizopogon, Pisolithus, Scleroderma, Suillus, Laccaria sp..

Approved by: Mike Amaranthus PhD Date: 8.3.25

Methods for determining mycorrhizal propagule counts

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For purposes of determining the free spore count for Boomers Blend Root Hugger only free spores were analyzed by decanting the product and microscopy of several samples from the bottom, middle and upper third of the sample bag. However, propagules of arbuscular mycorrhizal (AM) fungi include spores outside of roots, spores inside roots, colonized root fragments and other colonized bulk material in soil. All of these propagule types can be important in the colonization of plant roots. Hyphal networks should not be considered as propagules in assays of dried or liquid commercial inoculum. We do not consider hyphal networks as propagules in dried or liquid commercial inoculum because they are more sensitive than other propagules to disturbance in inoculum preparations and have a short storage life. Spores outside of roots, spores inside roots, and colonized root fragments containing arbuscules and vesicles need to be empirically determined in deriving propagule counts. Diverse mycorrhizal mixes are achieved by careful blending individual mycorrhizal species by volume and density. Mycorrhizal inoculum is best assessed first before incorporation into formulated, soil or compost materials and in these cases counts are based on detection of the proportion of the concentrated inoculum used in the final product.

Dried or liquid inoculum is carefully examined for spores and root fragments. Subsampling of root fragments and determination of spore and colonization content is

essential. This is accomplished by weighing out .05 grams of inoculum and placing in a gridded petri dish. The number of root fragments observed are tallied and multiplied by 20 to get root fragments/gram.

After the tally of root fragments is completed, a sample of 20 root fragments are picked from the .05-gram sample for clearing and staining. This method includes examination for the development of spores, vesicles, arbuscules and fungal hyphae along the 20 sample roots.

Sampled roots are placed in capsules in a 10% KOH solution in a water bath at low heat for 36 hours (or until cleared). The KOH is poured off and capsules rinsed in three complete changes of tap water. Capsules are placed in a 1% HCL mixture for 30 minutes then immediately transferred into a water bath of Indian ink (ready to use from Alfa Aesar www.alfa.com is best) for 2 hours and lightly rinsed. Root segments from each capsule are examined and tallied for the presence of arbuscular spores, vesicles and arbuscules of mycorrhizal fungi using a dissecting microscope and sub sample with the compound microscope if necessary. The propagules represented by roots are then included in the total propagule count. For example, if 200 root fragments are present per gram of inoculum and 50% (10 of 20) of the root fragments are colonized by mycorrhizal structures then the roots contain 100 propagules/gram. For example, if during the examination of the 20 root fragments 40 spores were counted then the average spores per root fragment would be 2. Two hundred root fragments tallied x 2 spores/per root fragment would equate to 400 propagules per gram. Since the propagule count for spores in root fragments exceeds the propagule count for colonized root fragments this value is used in the total propagule count.

We use centrifugation to extract free AM spores in inoculum and include this tally in the total propagule number. For centrifugation we take 25 gm subsamples and using agitation wash material over a 1000 micron sieve. All materials are greater than 1000

microns are discarded. The remaining material is washed over a 40-micron sieve and all material retained decanted into a 50 ml plastic vial. Spores are separated from the decanted material using double centrifugation at 2500 rpm. The first centrifugation is for four minutes and all floating organic debris is removed. A sugar suspension is added to the material for the second centrifugation. After 15 seconds at 2500 rpm the sugar suspension with retained spores is gently washed again over a 40-micron sieve and decanted into a petri dish. Spore counts are made on a gridded petri dish. Taxonomic classification to individual species or genus is performed by microscopy and are compared to published descriptions of individual species. Propagules within root fragments and free spore counts are added together to get the total AM propagule count and the species determined morphologically and tallied proportionally from observations.

For ectomycorrhizal fungi 1 gram of inoculum is macerated with a blender for 90 seconds and one milliliter of solution is diluted in 400 ml of water. One drop of this final dilute suspension was assessed using a compound microscope and a gridded haemocytometer on an 80 square scale. Ectomycorrhizal inoculum is assessed first before incorporation into formulated products. Formulated products are examined for detection of taxa. Examination for taxonomic species is performed on a compound microscope.