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Technical Bulletin

082 09/21/06

Surface Inoculation with VAM Fungi Spores Evaluation Based on Biology and Physics

VAM Fungi spores are living entities. As such, they can be killed by harsh conditions or improper handling. In addition, since they can only live and grow by colonizing a plant root, these spores need to effectively reach a root in order to survive. Frequently, clients want to inoculate their plants by surface application of VAM fungi. Plant Health Care, Inc. scientists have performed numerous tests with VAM fungi spores to answer the following practical question related to surface application:

Is surface application of VAM fungi spores an effective method of inoculation?

To answer this question, several experiments were conducted addressing both the delivery and viability of VAM fungi spores when applied to the soil surface.

Test 1: Will rain water or irrigation carry spores down through the soil?

Test Procedure:

Six pots were filled with standard dry sand-soil potting mix. Enough water was applied to moisten the soil from the top to the bottom of the pot. Two thousand (2000) VAM fungi spores (*Glomus clarum*) were placed on the soil surface of each pot.

Irrigation: The pots were irrigated by misting or by sprinkler to receive the equivalent of $\frac{1}{4}$ inch of rainfall for each irrigation, according to the following protocols:

- **Pot 1** was irrigated only once through a misting nozzle ($\frac{1}{4}$ inch of water).
- **Pot 2** was irrigated twice at a one-hour interval through a misting nozzle ($\frac{1}{2}$ inch of water).
- **Pot 3** was irrigated three times at one-hour intervals through a misting nozzle ($\frac{3}{4}$ inch of water).
- **Pots 4-6** were placed on a lawn with a rain gauge next to the pots. The sprinkler was turned on until the rain gauge registered $\frac{1}{4}$ inch (about 4 minutes). The water dropped from a height of about 8 feet.
 - **Pot 4** was irrigated by sprinkler only once ($\frac{1}{4}$ inch of water).
 - **Pot 5** was irrigated by sprinkler twice, at a one-hour interval ($\frac{1}{2}$ inch of water).
 - **Pot 6** was irrigated by sprinkler three times at one-hour intervals ($\frac{3}{4}$ inch of water).

Determination of how far the spores moved: The top 2 inches of soil was removed from each pot in separate layers, about $\frac{3}{8}$ inches thick. The spores were separated from each layer and counted.

Result: In all six pots, the spores remained in the top $\frac{3}{8}$ inch layer.

This is not surprising, considering that VAM fungi spores are rather large, making it difficult for them to fall through spaces between soil particles.

Conclusion: **VAM Fungi will not reach the root zone if applied to the soil surface, despite irrigation or rainfall.**

Test 2: Is the location of VAM in the soil important for the inoculum to be effective?

Test Procedure:

Three pots were filled with standard sand soil potting mix so that each pot received the same number and mix (cocktail mix of 4 species) of spores of VAM fungi. However, in each pot, the spores were placed at a different depth, according to the following protocol:

- **Pot A:** 30 cc (1.8 cubic inches) of spore inoculum (about 2200 spores) was placed on the soil surface.
- **Pot B:** 30 cc (1.8 cubic inches) of spore inoculum (about 2200 spores) was placed about $\frac{3}{4}$ inch below the soil surface.
- **Pot C:** 30 cc (1.8 cubic inches) of spore inoculum (about 2200 spores) was placed about $1\frac{1}{2}$ inches below the soil surface.

Planting the seed: Twenty-two millet seeds were placed on the surface of each pot, and covered with a wet mulch suspension made of macerated paper towels. The seeded pots were watered with mist until moist.

Growing the plants: The seeded pots were incubated at room temperature under wide spectrum fluorescent light (16 hour/day), and watered when necessary.

Assaying the roots for VAM: After 22 days, the roots of the resulting millet plants were harvested, cleared, stained and examined microscopically to determine the degree of VAM fungi colonization.

Results:

- Pot A: 0% colonization
- Pot B: 14% colonization
- Pot C: 32% colonization

Conclusion: The effectiveness of an inoculum is strongly influenced by its location in the soil. Inoculum must be optimally located in the root zone to be effective.

Viability Considerations

In addition to physical placement of the spores, are there other conditions associated with surface application that affect the viability of VAM fungi spores? What about lighting? Surface application necessarily involves prolonged exposure to sunlight. Since VAM fungi spores are not pigmented and spend their entire life cycle below ground, these spores may not have developed a resistance to the damaging effects of sunlight. Plant Health Care, Inc. performed the following tests to determine the degree of sensitivity, if any, of VAM fungi spores to natural sunlight.

Test 3: Does Sunlight Effect VAM Fungi Spore Infectivity?

Test Procedure:

Approximately 18,000 spores of our 4-species VAM cocktail were suspended in 4-ml of water. One ml (4500 spores) was transferred to each of 4 "boats" made of filter paper. Each "boat" was placed inside a covered petri dish.

All four dishes were exposed to direct sunlight for 2 hours, but each dish was covered with light-blocking foil at different times into the treatment:

- **Dish A:** Light-blocking foil applied at the start, so no sunlight exposure occurred.
- **Dish B:** Light-blocking foil applied after only 15 minutes sunlight exposure.
- **Dish C:** Light-blocking foil applied after 1 hour of sunlight exposure.
- **Dish D:** No light-blocking foil applied during 2 hours of sunlight exposure.

The spores from all four treatments were washed into individual beakers with about 20-ml of tap water. Each beaker was used to inoculate the roots of separate 3-day-old, pregerminated sweet corn seedlings (3-ml per seedling, 6 seedlings per treatment). One week later, the seedlings were transferred to 1-liter cups containing a sand-based medium. Four weeks later, the root samples were cleared, stained, and assessed for the degree of VAM colonization.

Results:

Treatment	Exposure Time	Percent Colonization of Six Replicates						Avg.
		1	2	3	4	5	6	
A	0	70	78	75	68	54	72	70
B	15 min	54	50	45	37	52	68	51
C	1 Hour	33	27	18	39	23	20	27
D	2 Hours	20	<10	<10	<10	14	25	15

Conclusion: Sunlight was detrimental to VAM fungi spores, having a cumulatively negative effect on spore viability with increased exposure times.

This further complicates attempts to inoculate via surface application. The sensitivity of VAM fungi spores to sunlight is not surprising, considering the fact that these spores develop entirely below ground, have no protective pigments, and never see the light of day under natural conditions.

What About Artificial Light?

The above test results indicate that natural sunlight is detrimental to VAM fungi spore viability. However, does artificial fluorescent light have a similar effect? Plant Health Care, Inc. performed tests to determine the degree of sensitivity of VAM fungi spores to fluorescent light. The results appear on the following pages.

Test 4: Does Fluorescent Light have an Effect on VAM Fungi Spore Infectivity?

Plant Health Care, Inc. performed the following test to determine the degree of sensitivity of VAM fungi spores to artificial fluorescent light.

Test Procedure:

Three grams of VAM fungi spores (3412 spores/g) were placed in each of 8 dry glass test tubes. Four of these tubes were wrapped with aluminum foil to block out the light. All 8 tubes were held for 2 days in the lab under ambient lighting. An amount containing 300 spores (0.088 g) was removed from each test tube and inoculated onto roots of pregerminated sweet corn seedlings. After 38 days, the roots were harvested, cleared and stained for degree of mycorrhizal colonization.

Results: Results appear in tabular form on the following page.

Percent Colonization of Corn Roots by VAM Fungi Spores With and Without 2-Day Exposure to Fluorescent Light

Treatment	Percent Colonization of Four Replicates				
	1	2	3	4	Avg.
Dark	38	34	33	62	42
Fluorescent Light	46	53	65	38	51

Conclusion: Ambient room fluorescent lighting had no detrimental effect on VAM fungi spore infectivity after 2-days exposure.

Summary Conclusion:

Based on these four tests, Plant Health Care, Inc. has confidently concluded that:

Surface application of VAM fungi spores is not an effective method of inoculation, because:

1. VAM Fungi spores will not reach the root zone if applied to the soil surface, despite rainfall or irrigation.
2. VAM fungi spores must be optimally located in the root zone to be effective.
3. Prolonged sunlight exposure is detrimental to VAM fungi spores, having a cumulatively negative effect on spore viability with increased exposure times.