The Effects of Varying Light Exposure Times on the Colonization and Sporulation of **Gigaspora margarita** in the California Pepper

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A Thesis

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The Faculty of the College of Arts and Sciences
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In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

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by

Mary E. Kirker

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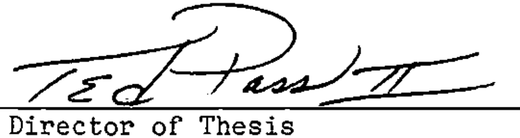
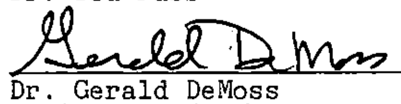
Accepted by the Faculty of the College of Arts & Sciences, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.


Director of Thesis

Master's Committee: , Chairman
Dr. Ted Pass

Dr. Gerald DeMoss

Dr. Howard Setser

July 24, 1989
Date

Abstract

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in the California Pepper.

Mary E. Kirker, M.S.
Morehead State University, 1989

Three hundred and sixty California Pepper plants (a variety of **Capsicum frutescens**) were inoculated with **Gigaspora margarita,** a vesicular-arbuscular mycorrhizal (VAM) fungus, to compare root colonization, sporulation and endophyte morphology at three photoperiods (8, 12 and 16 hours). In addition, plant response was evaluated after a 3 month observation period. The average root length, shoot length and total green weight of 35 inoculated plants were compared at each light exposure time (and within individual photoperiods to 10 uninoculated quality control plants) to assess the impact of this host/endophyte relationship on plant growth in low phosphorus (34 ppm) poor clay soil.

Root samples were collected, carefully cleaned of soil and held in Formalin-Aceto-Alcohol (FAA) killing and fixing solution to await clearing and staining by an acid fuchsin - lactic acid staining procedure. After staining the root systems were evaluated microscopically.

The 16 hour photoperiod showed very poor root colonization results (7.5% colonized) and sporulation was not observed. The average root length (10.2 cm), shoot length (23.0 cm) and green weight of roots and shoots (4.8 g) did not differ significantly from the uninoculated quality control plants in that photoperiod.

A high percentage of colonization occurred at the 12 hour photoperiod (63%). Sporulation was 67% in the final 35 root samples that were infected. The average root length (10.2 cm) did not differ significantly from the quality control samples (9.9 cm). However, the average shoot length (31.2 cm) was 14.2 cm longer than the quality control shoots and the average green weight (5.6 g) was 2.4 g heavier than the quality control samples.

The 8 hour photoperiod showed no colonization or sporulation and poor plant growth. The average root length (5.0 cm), shoot length (16 cm) and green weight (2.7 g) did not vary significantly from the uninoculated quality control plants.

Inadequate light at 8 hours and excessive drying between waterings at 16 hours are possible causes of poor colonization in these sections. Morphologically, no soil-borne vesicles and only a moderate amount of arbuscules were observed in infected samples.

Light and moisture conditions between waterings were adequate enough at the 12 hour photoperiod to produce a high percentage of infection at a moderate rate and significant improvement in plant growth. Based on the results at this photoperiod, **G. margarita**

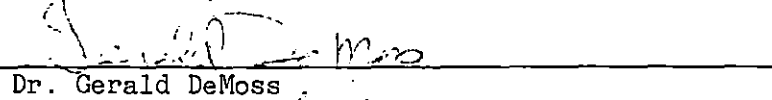
and the California Pepper proved to have a reasonably successful host/endophyte relationship and this endophyte could be considered as a possible source of commercial inoculum to enhance the growth of pepper crops in low phosphorus clay soils.

The Effects of Varying Light Exposure Times on the Colonization and Sporulation of **Gigaspora margarita** in the California Pepper.

Director of Thesis: Dr. Ted Pass

Accepted by:

Dr. Ted Pass, Chairman



Dr. Gerald DeMoss

Dr. Howard Setser

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TABLE OF CONTENTS

Page

I. Introduction 1

Significance 1
Need for Study 2
Objectives of Study 3

II. Literature Review 4

Historical Information and Future Goals.. 4
Taxonomy and Morphological Features of VAM Fungi 5
Requirements for Successful Colonization.. 6

III. Materials and Methods 11

General Information.. 11
Physical Set-Up 11
Light 11
Temperature 13
Water 13
Growth Medium 13
Inoculation 15
Root Collection and Preservation 17
Clearing and Staining Procedure 20
Assessment of Colonization and Endophyte Morphology.. 21
Assessment of Plant Response 22
Quality Control 23

IV. Results and Discussion.. 24

V. Conclusion 38

VI. Summary 41

VII. Literature Cited 43

LIST OF FIGURES

FIGURE		PAGE
1.	Structures Associated with **Gigaspora margarita** in a Root X-Section	7
2.	Summary of Procedure	12
3.	Soil Test Results	16
4.	Shoot Length Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** To Uninoculated Samples at Three Photoperiods	28
5.	Root Length Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** (after sporulation) To Uninoculated Samples at Three Photoperiods	29
6.	Green Weight Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** (after sporulation) To Uninovulated Samples at Three Photoperiods	30
7.	Overall Summary of Colonization and Sporulation Results at Photoperiod A (16 hrs.), B (12 hrs.) and C (8 hrs.) Including Quality Control Results on Uninoculated Samples	33
8.	Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod A (16 hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil	35
9.	Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod B (12 hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil	36
10.	Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod C (8 hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil	37

LIST OF TABLES

TABLE PAGE

1. Average Temperature and Water Schedule for Section A, B and C.................................... 14

2. Results of Seeding Presamples for Observation of Initial Colonization.................................... 19

3. Colonization and Sporulation and Results of Section A (16 hr Photoperiod)........................... 26

4. Colonization and Sporulation and Results of Section B (12 hr Photoperiod)........................... 31

5. Colonization and Sporulation and Results of Section C (8 hr Photoperiod)............................ 32

Introduction

Significance

Overwhelming evidence has been published on the beneficial properties of vesicular-arbuscular mycorrhizal (VAM) fungi (Schenck, 1984). According to Schenck, one of the most important aspects is the growth promoting ability of these endophytes. They have been shown to increase the absorption of relatively immobile elements in the soil (such as phosphorus, copper and zinc) and generally increase the absorptive surface area of host roots (Campbell, 1983). In addition, Campbell states that VAM fungi give host plants a greater tolerance to various adverse conditions such as toxic heavy metals contamination, high salinity, adverse pH, drought and transplant shock.

For these reasons, researchers are examining VAM fungi for use in reclaiming spoiled or inhospitable land such as coal and copper mine wastes and badly eroded areas. In the tropics these endophytes are being extensively studied for use in reclaiming spoiled rain forest areas. Many tropical plant species can not establish or maintain growth without VAM infections (Janos, 1987, by personal communication).

The agricultural benefits could be numerous especially in lesser developed areas of the world where expensive fertilizers are difficult to obtain.

Need for Study

Though there has been a considerable increase in the interest of vesicular-arbuscular mycorrhizal (VAM) fungi over the last 15 years, there is still a great deal to be learned. The complex processes by which plant growth is affected by these symbiotic fungi is still not understood (Miller, 1988, by personal communication). In addition, taxonomic confusion exists because colonization patterns and endophyte morphology may vary depending upon the host/endophyte combination used and the conditions to which they are subjected.. Attempts to monoculture VAM fungi on artificial media have been unsuccessful (Miller, 1988).

No work has been published on the host/endophyte combination used in this study. The experiment was conducted to determine whether **Gigaspora margarita** might be considered a suitable inoculum to enhance growth of pepper crops in low phosphorus clay soils and to contribute to needed information on colonization and sporulation patterns of **G. margarita**.

Objectives of the Study

The objectives of this study revolved around the host/endophyte relationship and the effects of varying photoperiods on that relationship under the given test conditions. They were as follows:

1. to compare the percentage of colonization and sporulation at three photoperiods (8, 12 and 16 hours);
2. to evaluate the effects of varying light exposure times on colonization rates and sporulation;
3. to compare host plant growth response (root length, shoot length and total green weight) in colonized and uncolonized samples;
4. to observe any morphological variations in the endophyte under the given test conditions;
5. to determine whether or not the test host and endophyte are able to establish a successful relationship in low phosphorus clay soil at the varying light levels;
6. to contribute much needed data on this host/endophyte interaction.

Literature Review

Historical Information and Future Goals

In a brief overview of early mycorrhizal studies Cooke (1977) describes that in the late 1800's, several individuals observed the presence of fungi in plant roots. They noted that some grew predominately intercellularly and developed extensively outside the root (ectomycorrhizae) while others developed predominately within the root cells (endomycorrhizae). Frank, in 1885, coined the term "mycorrhizae" for these fungus-root associations. In these early studies there was some disagreement on whether this association was parasitic or mutually beneficial.

Up to the 1950's most studies involved ectomycorrhizae. Between 1950 and 1960 the wide-spread occurrence, extensive host range (including most agronomic crops) and growth benefits of endomycorrhizae became apparent (Schenck, 1984). Schenck noted that by 1979 the number of publications on endomycorrhizae increased substantially with 96 papers in 1979 alone.

Evidence now shows that mycorrhizal associations are critical to plant nutrition (especially in infertile soils) and that using vesicular-arbuscular mycorrhizal (VAM) fungi in a compatible host-soil-fungus combination can lead to more economical exploitation of expensive fertilizers and better use of marginal land (Rhodes, 1980).

The benefits VAM fungi could contribute to land reclamation

and food production in the future are tremendous. There are, however, several drawbacks in mycorrhizal research. Aside from a shortage of information on the physiological, biochemical and ecological processes of host/endophyte interactions, VAM fungi have not, to date, been successfully monocultured on artificial media making it difficult to evaluate individual species requirements. Host/endophyte combination variability is also difficult to sort-out, due to the complex influence of the environment and rhizosphere. In addition, without the ability to artificially culture VAM fungi, agricultural experiments must remain on a small scale due to lack of inoculum (Miller, 1988, personal communication).

Siqueira and Hubbell (1982) met with limited success in the artificial culture of VAM. Using spores of **Glomus**, **Endogone** and **Gigaspora** species they induced the spores to germinate under artificial conditions by varying pH, phosphorus, nitrogen and various other chemical parameters. The spores did not, however, develop beyond the germ tube stage.

Taxonomy and Morphological Features of VAM Fungi

Endogonales (Zygomycotina) presently consists of a single family, the Endogonaceae. There are seven known genera separated at the first level by the manner of spore production and speciated by sporocarp morphology (Miller, 1988, by personal communication). **Gigaspora margarita** forms azygospores, asexual spores resembling

zygospores, borne singly in the soil from a bulbous, suspensor-like tip of a hypha. Spore dimensions range from 30 - 50 microns and have one to several separable membranes within the primary spore wall. There is very little ornamentation on the spore surface though it can appear reticulated at times. **G. margarita** often produces soil-borne vesicles (function unknown) and these are frequently used to confirm the identity of this species (Cooke, 1977).

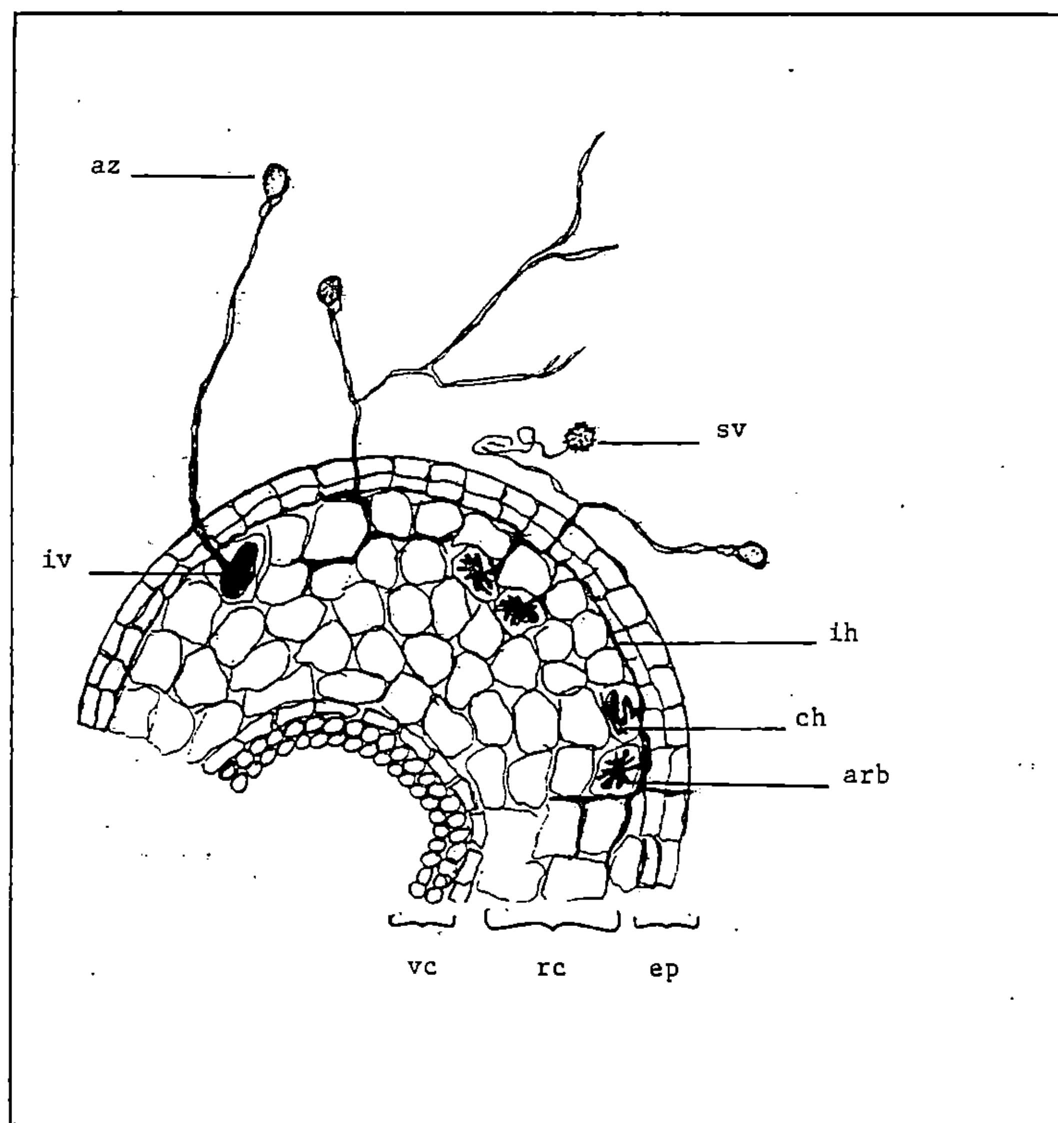
Other structures associated with **G. margarita**, illustrated in Figure 1, are inter - and intracellular, oil-rich vesicles (believed to function as endophytic storage organs), haustoria-like arbuscules (considered to be the primary structures involved in the bidirectional transfer of nutrients between host and endophyte) and nonseptate, dimorphic hyphae which can be inter- or intracellular or soil-borne (Gerdemann and Trappe, 1974).

The hyphae penetrate the root epidermis and enter the cortex (the vascular cylinder is not colonized) where they may differentiate into arbuscules, vesicles or become coiled intracellular hyphae. Eventually, according to Gerdemann and Trappe (1974), these structures disintegrate, probably being digested by the host, and their contents are released into the host cell.

Requirements for Successful Colonization

Furlan and Fortin (1977) studied the effects of light on the formation of VAM fungi in **Allium cepia** by **Gigaspora calospora.**

Figure 1. Structures Associated with **Gigaspora margarita** in a Root X-Section.



Legend. vc = vascular cylinder
rc = root cortex
ep = epidermis
az = azygospore on extracellular hyphae
sv = soil-borne vesicle on coiled hyphae
iv = intracellular vesicle
arb = arbuseule
ch = coiled intracelluar hyphae
ih = intercellular hyphae

They found that light intensity as well as photoperiod can strongly influence the development of VAM infection. Onion plants exposed to greater radiant energy under various light regimes had greater mycorrhizal infection than plants grown under lower radiant energy levels. When the mycorrhizal onions were grown under constant temperatures and light intensities but varying photoperiods much less infection was noted at the 6 hour photoperiod than at the 12 and 18 hour photoperiods. They observed that longer photoperiods appeared to have a greater influence on the extent of mycorrhizal infection than the variation of light intensity which produced more qualitative than quantitative results.

Hayman (1974) found results similar to Furlan and Fortin using various host/endophyte combinations and varying light exposure times and temperature. He concluded that higher temperatures and longer photoperiods generally increased VAM infections.

Daniels, McCool and Menge (1982) discussed the attributes of using mixed as opposed to pure spore inoculum. Since attempts to culture VAM on artificial media have met with little success these endophytes must be collected from field soils, identified and cultured in greenhouses on living host plants (nurse crops). From these pot culture soils, two types of inocula can be obtained, (1) a mixed inoculum consisting of spores, vesicles, hyphae and infected root pieces or (2) a pure spore inoculum. Wet sieving and decanting or density gradient centrifugation are just two methods of obtaining a pure spore inoculum.

There are advantages with each form of inoculum. Mixed inocula have repeatedly given faster, more reliable infection. However, if the inoculum is not used within 2 weeks the inoculum potential drops. There is also a greater chance of contamination by other fungal or bacterial forms. Pure spore inoculum is more difficult to obtain and does not colonize as rapidly as fresh mixed inoculum. However, this type is easier to quantify and one can be sure that only the desired species is present (Daniels, McCool and Menge, 1982).

Schenck (1984) reported a great deal of information on soil conditions and their effects on mycorrhizal infection and plant growth response. With few exceptions VAM fungi have been found world-wide. Very few natural infections by mycorrhizae have been found in temperature podzols, very wet soils or highly disturbed soils. Grasslands, rain forests and arid regions have shown to support variable levels of VAM fungi.

Soil penetration resistance (soil strength) influences the rate at which roots can grow to reach nutrients and water. Because the mycelium of mycorrhizal fungi may translocate nutrients (and possibly a small amount of water) they may promote plant growth when soil imposes limitations on root function.

The available soil phosphorus has been shown to have dramatic effects on mycorrhizal colonization and in turn improved plant growth. In most instances a high soil phosphorus level (greater than 75 ppm) inhibits mycorrhizal infections. The phosphorus

effects, however, are strongly influenced by the pH, nitrogen levels and host/endophyte combination used (Schenck, 1984).

Soil pH alters nutrient solubility and plant nutrition. The effects of soil pH on plant response to mycorrhizae are very complex and not completely understood. VAM infections have been observed over a wide range of pH levels (2 - 10).

Schenck (1984) also noted many direct and indirect effects of soil water status on mycorrhizal infection and plant growth response. High soil water potentials reduced growth and infection by many mycorrhizal fungi (possibly by affecting the plant indirectly via anaerobiosis). Low soil water potentials also reduced growth and infection by some endophytes, though to a lesser degree.

Reid and Boen (1979) in another study on soil moisture and its effects on VAM infection found similar results. They reported that exposure of infected plants to repeated drought cycles decreased mycorrhizal infection, spore production and plant growth response in some host/endophyte combinations.

Materials and Methods

General Information

The experiment was conducted under artificial conditions where light, growth medium, water and temperature could be controlled (See Figure 2 for Summary of Procedure).

Physical Set-Up

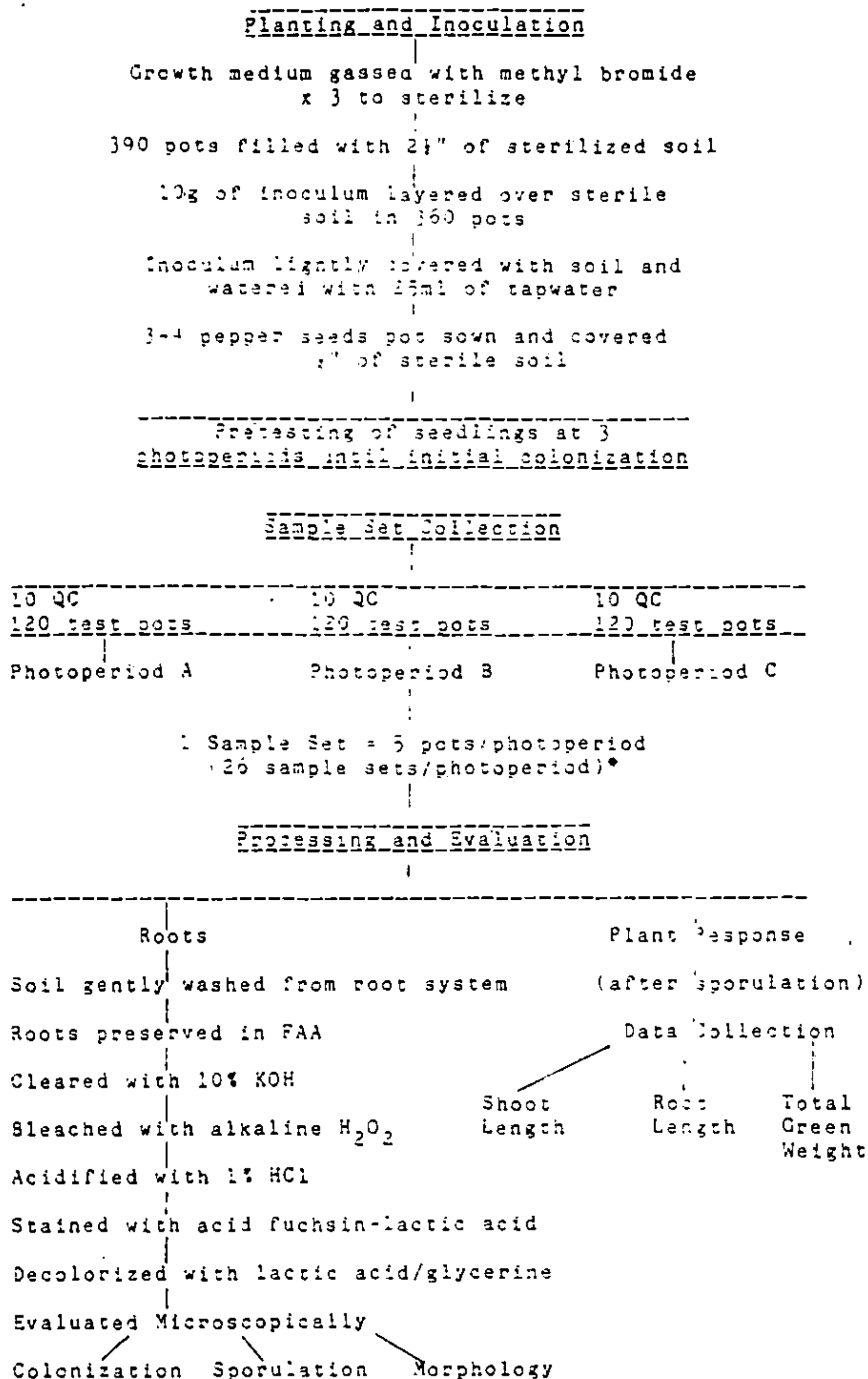
A plant table long enough to accommodate 390 pressed fiber pots (each 3½" square) was divided into three sections labeled A (representing a 16 hour photoperiod), B (a 12 hour photoperiod) and C (an 8 hour photoperiod). Each section contained 130 pots arranged in 26 rows with 5 pots per row. Each row represented a sample set per photoperiod. Rows 25 and 26 in all three sections were uninoculated quality control samples.

Light

Two fluorescent tubes running the length of each test area and three 150 watt incandescent lights (Gro-Lite) with reflectors were positioned 30 inches above the pots in each test area. To achieve the highest intensity possible, each section was lined with foil to further reflect the light.

Intensity was measured with a General Electric light meter at several points in each section to ensure uniformity. The level measured 200 ± 5 foot candles in all areas of each section.

Figure 2. Summary of Procedure



* Sample sets 25 & 26 were uninoculated quality control plants processed at final sample set collection.

Automatic timers were used to control the lights; providing 16, 12 and 8 hours of light to sections A, B and C respectively.

Temperature

Thermometers (Scientific Products, Obetz, Ohio) ranging from 0° - 100°F were positioned at the front and back of each growth area. In addition, a thermometer measuring daily minimum and maximum temperatures was placed at the back of each section.

Temperature data are shown in Table 1, variations between test sections were not significantly different.

Water

A Scientific Products Repipet Dispenser (S/P, Obetz, Ohi) was used to ensure that all plants received equal amounts of water. Water was dispensed on an "as needed" basis to keep the soil moderately moist to dry (Table 1).

Growth Medium

Before choosing a growth medium for this experiment, several physical and chemical aspects of the soil, such as soil texture, pH, nitrogen, phosphorus, had to be considered. A soil was chosen that would allow the endophyte to alleviate some physical-chemical constraint on root acquistion of nutrients and thus enhance growth in infected plants. The constraints primarily considered in

Table 1. Average Temperature and Water Schedule for Section A, B, and C.

Date	Average Temps.* in degrees C			Water in ml
	A	B	C	A/B/C
6-02	25	25	25	25
5-04	23	24	23	40
5-06	23	23	23	25
5-08	27	27	26	40
5-10	27	27	27	25
5-12	30	29	29	25
5-13	30	30	30	25
5-15	27	27	27	25
5-17	28	28	28	10
5-19	28	28	28	25
5-21	28	28	28	10
5-23	28	28	28	0
5-24	28	28	28	40
5-25	30	30	29	0
5-26	29	29	29	40
5-27	29	29	29	0
5-28	28	29	28	50
5-30	28	28	28	50
5-31	32	32	32	0
6-01	30	30	30	50
6-03	30	30	30	50
6-05	30	29	29	50
6-06	30	30	30	0
6-07	28	28	28	50
6-09	32	32	32	50
6-10	30	30	29	0
6-11	30	30	30	50
6-13	30	30	30	50
6-15	30	29	30	50
6-17	34	34	34	50

Date	Average Temps.* in degrees C			Water in ml
	A	B	C	A/B/C
6-19	35	34	34	25
6-21	35	35	35	50
6-23	32	32	32	50
6-25	36	36	36	50
6-27	35	35	35	50
6-28	35	34	35	50
6-30	36	36	36	60
7-02	36	36	36	50
7-04	35	35	35	50
7-06	35	35	35	50
7-08	38	37	37	50
7-10	36	36	36	50
7-12	34	35	34	50
7-14	34	34	34	50
7-16	34	34	34	50
7-18	35	35	35	50
7-20	35	35	36	50
7-22	36	36	36	50
7-25	35	35	35	50
7-26	36	36	36	50
7-28	37	37	37	50
7-30	35	35	35	50
8-01	36	36	36	50
8-03	36	36	36	50
8-05	35	35	35	50
8-07	34	34	34	50
8-09	35	34	35	50
8-10	35	35	35	0
8-11	35	35	35	0

* Average Temperatures taken with the lights on. Low temperatures (taken with lights off) remained constant for all sections and averaged 21°C.

this experiment were poor soil texture and a low phosphorus level (less than 45 ppm).

Five soil samples were collected from poor, predominantly clay areas in Adams County, Ohio and were sent to the Ohio State University Research Analytical Laboratory (Columbus, Ohio) for analysis. From these samples, a soil was selected for this research on the basis of its moderately low phosphorus level, 68 lb/acre or 34 ppm (Figure 3).

An adequate amount of soil was collected from the appropriate area, and was sterilized by surrounding each sample in black plastic and gassed three times with methyl bromide (Southern States Cooperative, Morehead, KY) at two day intervals (Maronek, Hendrix and Kiernan, 1981). After sterilization, the soil was stored in clean, covered, plastic trash cans until ready for use.

Before test use, the soil was mixed with sterilized sand (five parts soil to one part sand) to slightly improve drainage. In addition, soil was cultivated three times during the experiment.

Inoculation

A mixed inoculum of **G. margarita** was used to introduce the endophyte. This type of inoculum was composed of bits of infected roots, spores and any soil borne structures produced by the species. A pure spore inoculum was not used in this research.

To enumerate the spores in the mixed inoculum, a plate method was used. One gram of soil (inoculum) was mixed with water in

Figure 3. Soil Test Results

Sample Information		Standard Test Results									
Plow Depth Inches	Lime Applied In Last 2 yrs. T/a	pH	Lime Test Index	Phos-phorus P lb/A	Potos-sium K lb/A	Calcium Ca lb/A	Magnes-ium Mg lb/A	meg/ 100g	Base Saturation % Ca	% Mg	% K
6	.0	8.1	70	68	682	8180	773	15	83	13	3.6

a petri dish and examined directly using a dissecting microscope. Counts of 20 spores per gram are considered to be high spore populations (Smith and Skipper, 1979). The spore count in the test inoculum averaged 16 spores per gram (based on a total of 5 grams examined).

The inoculation procedure was as follows:

1. 130 pots in each section were filled with equal amount of sterilized soil/sand mixture leaving one inch at the top of the pot for inoculum, seeds and cover soil;
2. 10 grams of inoculum were layered over the soil in 120 pots at each photoperiod so that the roots would have to penetrate the inoculum;
3. 10 pots per section were set aside as uninoculated quality control smaples;
4. A thin layer of soil was then placed over the inoculum and each pot was watered with 25 ml of tapwater;
5. Each pot was then sown with three to four California Pepper seeds and covered with a final ½" layer of soil.

The purpose of planting several seeds per pot was to allow for nonviable seeds and to have an adequate number of seedlings available for pretesting to observe initial colonization. After initial colonization, all pots were culled to contain no more than two plants per pot.

Root Collection and Preservation

Data collection began when initial colonization was observed

in at least one photoperiod. After germination, five seedlings were carefully removed from different pots at each photoperiod (whole pots not taken) to determine if roots had become colonized (Table 2). These presamples were processed in the same manner as the test samples discussed below. Test root sample sets were collected at the same intervals in all three sections. Sample sets consisted of 15 root samples (five per photoperiod). The remainder of the procedure was as follows:

1. Sample sets were recovered by removing the plant and roots from each pot, carefully removing as much soil as possible from the root system, then gently washing the roots in a large beaker with tapwater until all visible soil was removed. Careful root collection was important because the fine terminal feeder roots are primary sites of mycorrhizal development (Schenck, 1984).
2. Washed roots were cut from each plant at the soil level and placed in appropriately labeled Tissue-Tek plastic capsules (Fisher Scientific, Co., Pittsburgh, Pa.). The capsules served to minimize root handling and prevented mixing of samples. Root systems were not excessviely large so entire samples were used for evaluation.
3. Capsules containing the roots were then immersed in a standard Formalin-Aceto-Alcohol (FAA), in a 90:5:5 ration, killing and fixing solution to be batched for cleaning and staining. According to Miller (1988), root samples can be stored

Table 2. Results of Seeding Presamples for Observation of Initial Colonization (Whole Pots Not Taken)*

DATE	SAMPLE SET 15 SEEDLINGS/SET	RESULTS PER PHOTOPERIOD		
		SECTION A (16 HRS.)	SECTION B (12 HRS.)	SECTION C (06 HRS.)
5-17	SET # 1	NEGATIVE	NEGATIVE	NEGATIVE
5-21	SET # 2	NEGATIVE	NEGATIVE	NEGATIVE
5-25	SET # 3	NEGATIVE	NEGATIVE	NEGATIVE
5-28	SET # 4	NEGATIVE	NEGATIVE	NEGATIVE
5-31	SET # 5	NEGATIVE	NEGATIVE	NEGATIVE
6-01	SET # 6	NEGATIVE	NEGATIVE	NEGATIVE
6-03	SET # 7	NEGATIVE	NEGATIVE	NEGATIVE
6-07	SET # 8	NEGATIVE	NEGATIVE	NEGATIVE
6-10	SET # 9	NEGATIVE	POSITIVE	NEGATIVE

* Pre-Samples were used only to detect initial evidence of colonization and thus, began colonization rate comparisons at each photoperiod. The above results were not included in the final data report.

in this solution for up to 2½ years before assay with no adverse effects.

Clearing and Staining Procedure

1. Preserved root samples were gently washed in tapwater to remove the FAA, placed in glass beaker and covered with a 10% KOH solution. They were then autoclaved at 15 psi for 10 minutes. This part of the procedure cleared the host cytoplasm and nuclei and prepared the roots for stain penetration.
2. The KOH solution was poured off and the capsules were gently rinsed three times in tapwater (or until no brown color appeared in the rinse water).
3. The capsules were then transferred to a clean beaker and covered with alkaline H_2O_2 for 15 minutes at room temperature to bleach the roots. This solution lost its effectiveness overnight and had to made fresh for each batch of roots stained. It was made by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml of tapwater.
4. The capsules were gently rinsed three more times to remove the H_2O_2 and then immersed in a 1% HCl for four minutes to acidify the roots for proper staining. The HCl was poured off but the roots were not rinsed after this step.

5. The acidified roots were covered with 0.01% acid fuchsin-lactic acid staining solution and autoclaved for 10 minutes at 15 psi. The staining solution was made with 875 ml of laboratory grade lactic acid, 63 ml of glycerine, 63 ml of tapwater and 0.1 g of acid fuchsin mixed in that order. The stain was filtered and reused for several sample batches until the color lightened, at which time fresh stain was made.
6. After staining, the capsules were placed in petri dishes containing a destaining solution (made just as the staining solution minus the acid fuchsin). This step served to remove excess stain from the roots. The samples were transferred to glycerine after two weeks to prevent excessive destaining.

Assessment of Colonization and Endophyte Morphology

An American Optical dissecting microscope (10x) was used to visually determine the presence of infection, its extent and sporulation. A Swift Instruments compound microscope (10x and 40x) was used to more closely view morphological characteristics.

The following data were recorded:

1. the presence or absence of infection (for the purpose of evaluating overall percentages of plants colonized);
2. the extent of infection as it changed over time for the purpose of comparing the rate of colonization at photo-

periods A, B and C. (The following classification system, used by the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia, was used to evaluate the extent of mycorrhizal infection as listed below:

Class 1 = 0 - 5% Colonization

Class 2 = 6 - 25% Colonization

Class 3 = 26 - 50% Colonization

Class 4 = 51 - 75% Colonization

Class 5 = 76 - 100% Colonization);

3. any changes in morphological characteristics for the purpose of discerning possible growth pattern differences in **G. margarita** in this host/endophyte relationship under these particular test conditions;
4. initial sporulation marking the end point of the experiment;
5. the percentage of colonized roots that produced spores after the initial event of sporulation was observed.

Assessment of Plant Response

Three physical parameters were measured: root length, shoot length, and green weight of roots and shoots.

The total number of plants/section to be included in the plant response evaluation was to be determined by one of two events. One, the end of the three month observation period (in which case the last 25 plants per section along with the quality control plants would have been measured or two, initial sporulation (after

which all remaining plants were evaluated).

Quality Control

Quality control consisted of ten uninoculated plants per photoperiod. Quality control pots and samples were subjected to the sample conditions and processing as the test pots and root samples. These samples were used as a check on soil sterility and for comparison of plant response to test conditions in infected vs uninfected roots.

Results and Discussion

Three hundred and sixty California Pepper plants were equally divided among 3 photoperiods labeled section A (16 hours), B (12 hours) and C (8 hours) and were inoculated with 10 g of a mixed inoculum of **Gigaspora margarita** to compare colonization, sporulation and plant growth response at different light exposure times. In addition, 10 uninoculated quality control plants were included at each photoperiod to compare the results of colonized and uncolonized samples for the purpose of assessing this host/ endophyte relationship on plant growth in low phosphorus (34 ppm) clay soil. Endophyte morphology was also observed to determine if any variations in structures typically produced by **G. margarita** under these experimental conditions.

The experiment was conducted under artificial conditions where growth medium, temperature and the amount of water received per plant could be controlled, leaving the photoperiod as the only variable. However, the evaporation rate of water was faster under the warm incandescent lights causing the longer exposure time (16 hours) to dry considerably more than expected between waterings and leaving the soil moisture level obviously greater at the 8 hour photoperiod (growth chambers were not available for this experiment). The differences in soil moisture between waterings had to be considered in the results and evaluation of this host/endophyte relationship and its impact on plant growth.

The observation period of this experiment was designed to last three months or until initial sporulation was observed (sporulation occurred first). Under the test conditions discussed, germination was completed in 13 days and initial colonization was observed in the 12 hour photoperiod 26 days thereafter. Peak colonization occurred 54 days after initial colonization and sporulation occurred 60 days after initial colonization.

G. margarita typically produces inter- and/or intracellular hyphae, abundant arbuscles, soil-borne vesicles, azygospores and occasionally intracellular vesicles (Roncadori, 1988). In this experiment, no soil borne vesicles or intracellular vesicles were observed and only a moderate amount of arbuscules were formed.

The 16 hour photoperiod (section A) had very poor colonization results and no sporulation was observed (Table 3). Only nine of the 120 inoculated roots were colonized (7.5%). Of these nine root systems, only three reached a class three colonization level (26 - 50% colonization). This occurred 18 days after class three infection was first observed at the 12 hour photoperiod. quality control plants (at all photoperiods) were negative for VAM infection.

In the final 35 plants evaluated for growth response in section A, the average root length was 10.2 cm, average shoot length was 23.0 cm and the average total green weight of the roots and shoots was 4.8 g. These results did not differ significantly from the ten uninoculated quality control plants in this

Table 3. Colonization and Sporulation and Results
Section A (16 hr. Photoperiod)

Collection Dates

6-11	6-15	6-19	6-23	6-27	6-30	7-04	7-08	7-12	7-16	7-20	7-25	7-28	8-01	8-03	8-05	8-07	8-09	8-10	8-11	8-11	8-11	8-11	8-11	8-11	8-11
-	-	-	-	-	-	-	-	-	cl 1	-	-	-	-	cl 3	-	-	-	-	-	-	cl 2	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	cl 3	-	-	-	-	-	-	-	-	cl 2	-	-
-	-	-	cl 1	-	-	-	-	-	-	-	cl 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	cl 2	-	-	-	-
-	-	-	-	-	-	cl 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

Sample Sets (Vertical Blocks Represent 5 Pots Per Set)

Negative (-)		=	No Colonization
Class (cl.)	1	=	0 - 5% Colonization
Class (cl.)	2	=	6 - 25% Colonization
Class (cl.)	3	=	26 - 50% Colonization
Class (cl.)	4	=	51 - 75% Colonization
Class (cl.)	5	=	76 - 100% Colonization
	S*	=	+ Sporulation

* Classification system taken from the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia.

photoperiod (Figures 4, 5 and 6).

A high percentage of colonization occurred at the 12 hour photoperiod (section B) with 83 of the 120 roots colonized (63%) and 67% sporulation in the final 35 root samples that were infected (Table 4). At peak colonization (54 days after initial colonization) the highest level of infection per root system achieved was a class four (51 - 75% colonization).

With the exception of the average final root length (10.2 cm), plant growth response in colonized samples differed significantly from uninoculated quality control samples in section B (Figure 4, 5 and 6). The average shoot length (31.2 cm) was 14.2 cm longer than the quality control shoots and the average total green weight (5.6 g) was 2.4 g heavier than the quality control samples.

At the 8 hour photoperiod (section C) no colonization or sporulation was observed (Table 5). Plant growth response was poor under these conditions (Figure 4, 5 and 6). The average final root length was 5.0 cm, shoot length was 16 cm and total green weight was just 2.7 g. These results did not differ significantly from the uninoculated quality control plants within this section. Figure 7 shows a summary of colonization and sporulation results for all three photoperiods.

Colonization rates of each section could not be compared due to insufficient data at the 8 and 16 hour photoperiods. The colonization rate at the 12 hour photoperiod showed a pre-

Figure 4. Shoot Length Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** (after sporulation) To Uninoculated Samples at Three Photoperiods.

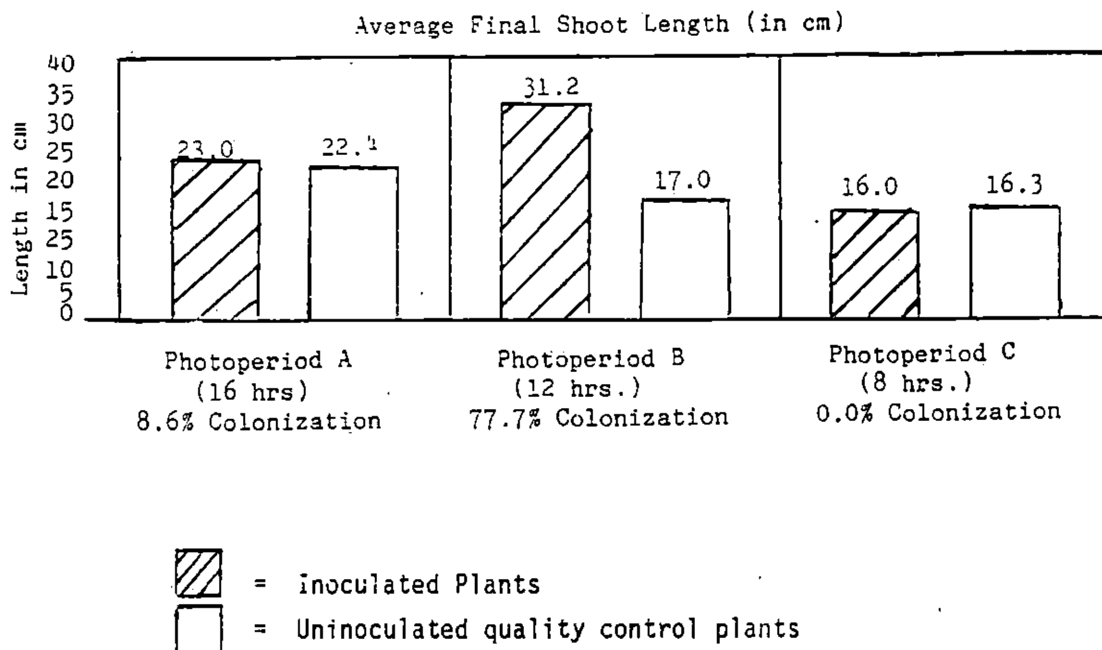


Figure 5. Root Length Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** (after sporulation) to Uninoculated Samples at Three Photoperiods.

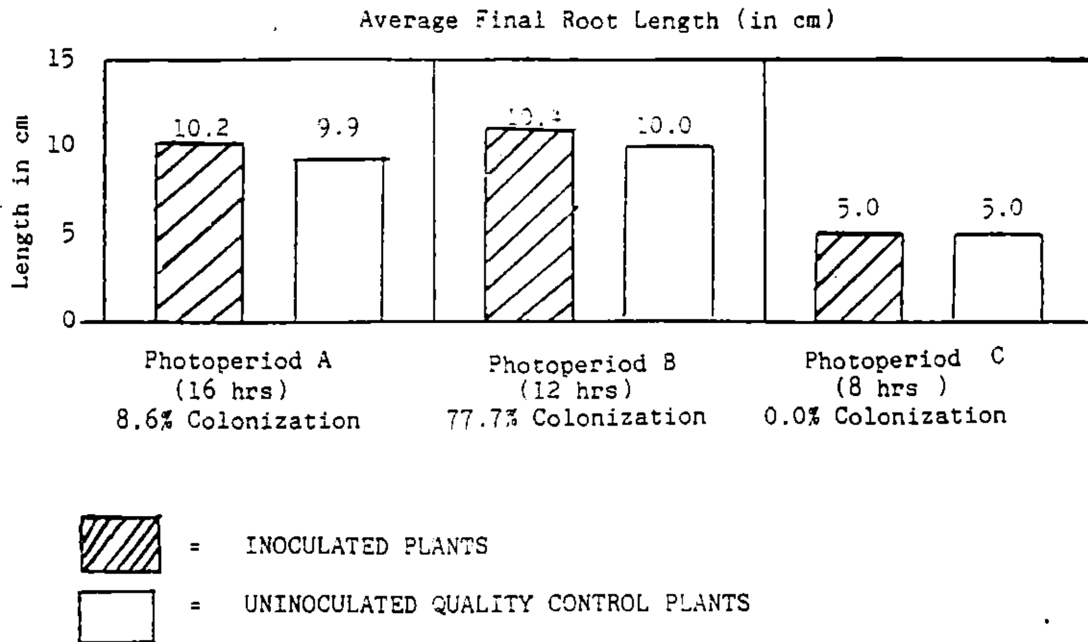


Figure 6. Green Weight Evaluation Comparing Percentage of Plants Colonized with **Gigaspora margarita** (after sporulation) to Uninoculated Samples at Three Photoperiods.

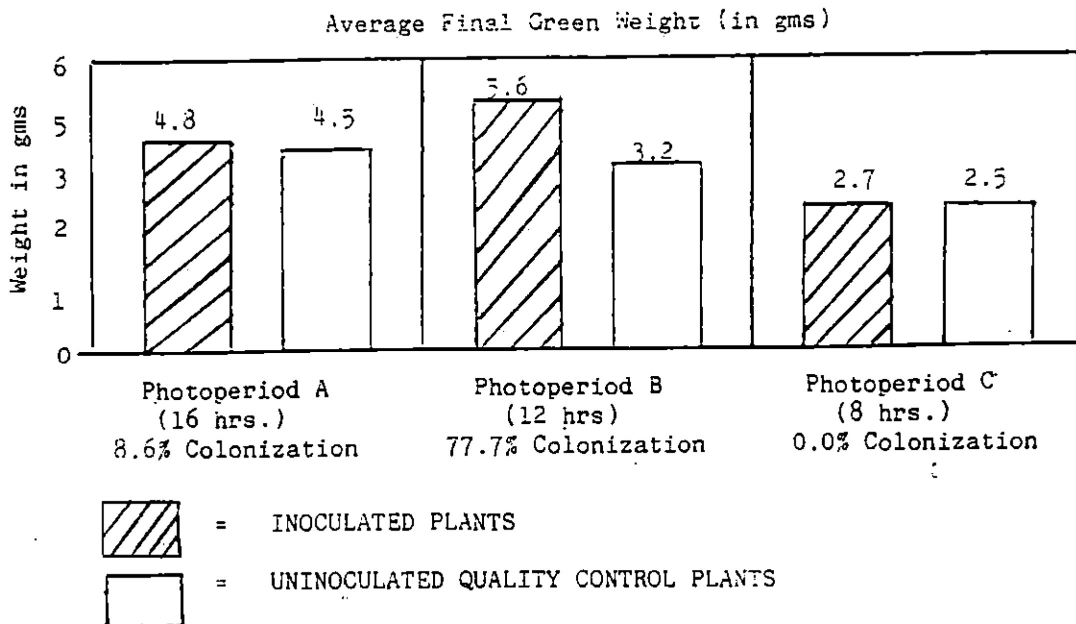


Table 4. Colonization and Sporulation and Results
Section B (12 hr. Photoperiod)

Collection Dates

Collection Date	6-11	6-15	6-19	6-23	6-27	6-30	7-04	7-08	7-12	7-16	7-20	7-25	7-28	8-01	8-03	8-05	8-07	8-09	8-10	8-11	8-11	8-11	8-11	8-11	8-11	8-11
Pot 1	cl 1	cl 1	cl 1	–	–	cl 2	cl 2	cl 2	cl 2	cl 3	–	cl 3	cl 3	cl 3	cl 3	cl 4	cl 3	cl S*	cl 4 S*	–	cl 3 S*	cl 3 S*	cl 4 S*	cl 4 S*	–	–
Pot 2	–	cl 1	cl 1	cl 1	cl 1	cl 2	–	cl 2	–	cl 3	cl 2	cl 3	–	cl 3	cl 3	cl 4	–	cl 3	–	cl 4 S*	cl 4	cl 3 S*	cl 3	cl 3 S*	–	–
Pot 3	–	–	–	cl 1	cl 2	cl 1	–	cl 2	cl 2	–	cl 2	cl 3	cl 3	–	cl 3	cl 3	cl 3	–	cl 4	cl 4 S*	cl 4 S*	cl 3 S*	cl 3 S*	–	–	–
Pot 4	cl 1	–	cl 1	cl 1	cl 1	–	cl 1	–	cl 2	cl 2	–	–	cl 4	–	cl 3	–	cl 4	cl 3 S*	–	cl 3 S*	cl 3	–	–	cl 3	–	–
Pot 5	–	–	–	–	cl 1	–	cl 2	cl 2	cl 2	–	–	–	cl 3	cl 3	cl 4	cl 3	cl 4	cl 3	cl 3 S*	cl 3 S*	cl 3 S*	–	cl 3 S*	cl 3 S*	–	–
Sample Set	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

Sample Sets (Vertical Blocks Represent 5 Pots/Per Set)

Negative	(–)	=	No Colonization
Class (cl.)	1	=	0 - 5% Colonization
Class (cl.)	2	=	6 - 25% Colonization
Class (cl.)	3	=	26 - 50% Colonization
Class (cl.)	4	=	51 - 75% Colonization
Class (cl.)	5	=	76 - 100% Colonization
	S*	=	+ Sporulation

* Classification system taken from the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia.

Table 5. Colonization and Sporulation and Results
Section C (8 hr. Photoperiod)

Collection Dates

Sample Set	Collection Date	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5
1	6-11	-	-	-	-	-
2	6-15	-	-	-	-	-
3	6-19	-	-	-	-	-
4	6-23	-	-	-	-	-
5	6-27	-	-	-	-	-
6	6-30	-	-	-	-	-
7	7-04	-	-	-	-	-
8	7-08	-	-	-	-	-
9	7-12	-	-	-	-	-
10	7-16	-	-	-	-	-
11	7-20	-	-	-	-	-
12	7-25	-	-	-	-	-
13	7-28	-	-	-	-	-
14	8-01	-	-	-	-	-
15	8-03	-	-	-	-	-
16	8-05	-	-	-	-	-
17	8-07	-	-	-	-	-
18	8-09	-	-	-	-	-
19	8-01	-	-	-	-	-
20	8-11	-	-	-	-	-
21	8-11	-	-	-	-	-
22	8-11	-	-	-	-	-
23	8-11	-	-	-	-	-
24	8-11	-	-	-	-	-
25	8-11	-	-	-	-	-
26	8-11	-	-	-	-	-

Sample Sets (Vertical Clocks Represent 5 Pots/Per Set)

Negative	(-)	=	No Colonization
Class (cl.)	1	=	0 - 5% colonization
Class (cl.)	2	=	6 - 25% Colonization
Class (cl.)	3	=	26 - 50% Colonization
Class (cl.)	4	=	51 - 75% Colonization
Class (cl.)	5	=	76 - 100% Colonization
	S*	=	+ Sporulation

* Classification system taken from the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia.

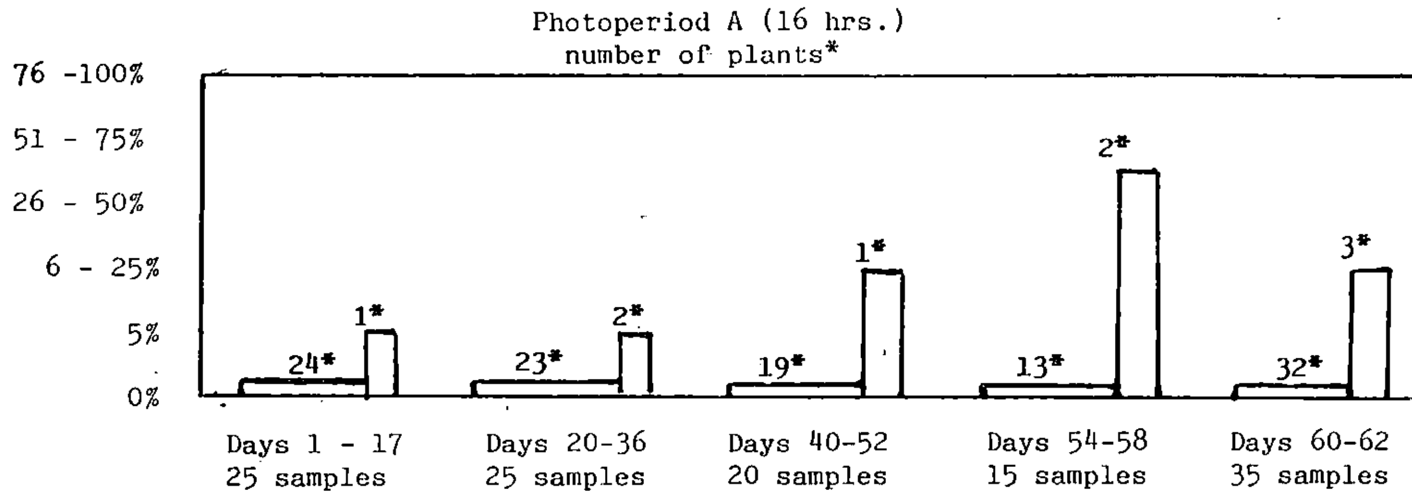
Figure 7. Overall Summary of Colonization and Sporulation Results at Photoperiod A (16 hrs.), B (12 hrs.) and c (8 hrs.) Including Quality control Results on Uninoculated Samples

	Number of Plants Inoculated	Total Number of Plants Colonized	% of Plants Colonized	% Sporulation in Final 35 Samples	Colonization in Uninoculated QC Samples
Section A (16 hrs.)	120	9	7.5%	0.0%	0.0%
Section B (12 hrs.)	120	83	69.5%	67.0%	0.0%
Section C (8 hrs.)	120	0	0.0%	0.0%	0.0%

dominance of 0 - 5% infection by day 17, 6 - 25% by day 36 and 26 - 50% by day 54 (Figure 8, 9 and 10).

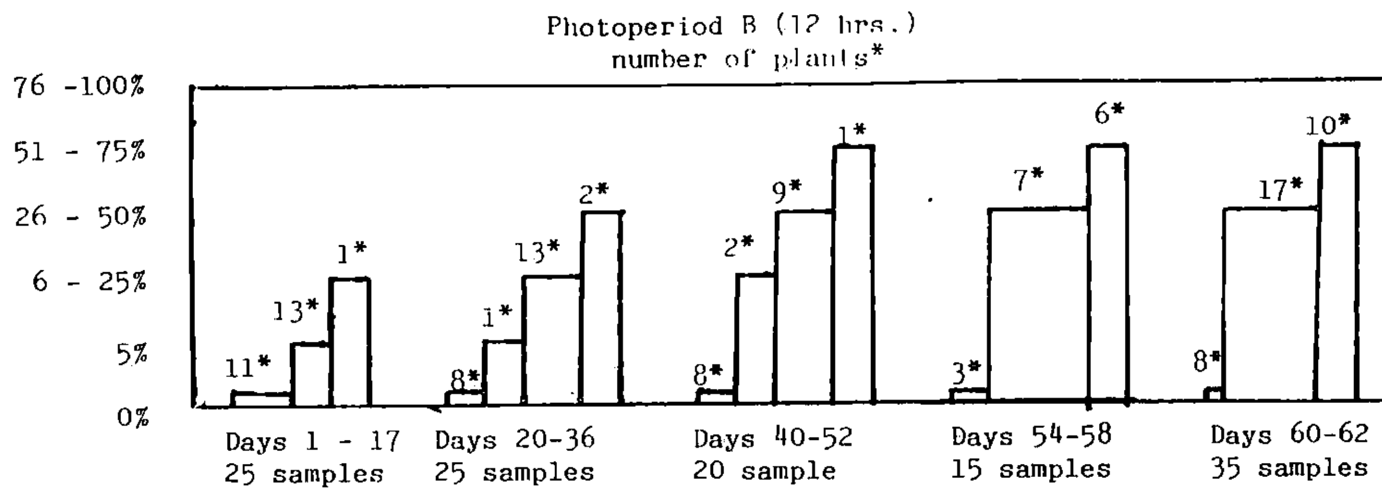
Compared to the 8 hour photoperiod, colonized plants at the 12 hour photoperiod exhibited nearly twice the root length, shoot length and total green weight. The root length in sections A and B differed only by 0.2 g but the shoot length was significantly greater in the colonized plants in section B compared to those in section A (8.2 cm longer). The average green weight was also significantly different; 5.6 g at the 12 hour photoperiod and 4.8 g at the 16 hour photoperiod. Figures 4, 5 and 6 compare the plant response evaluations at the three photoperiods in addition to the quality control results.

Figure 8. Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod A (16 Hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil.



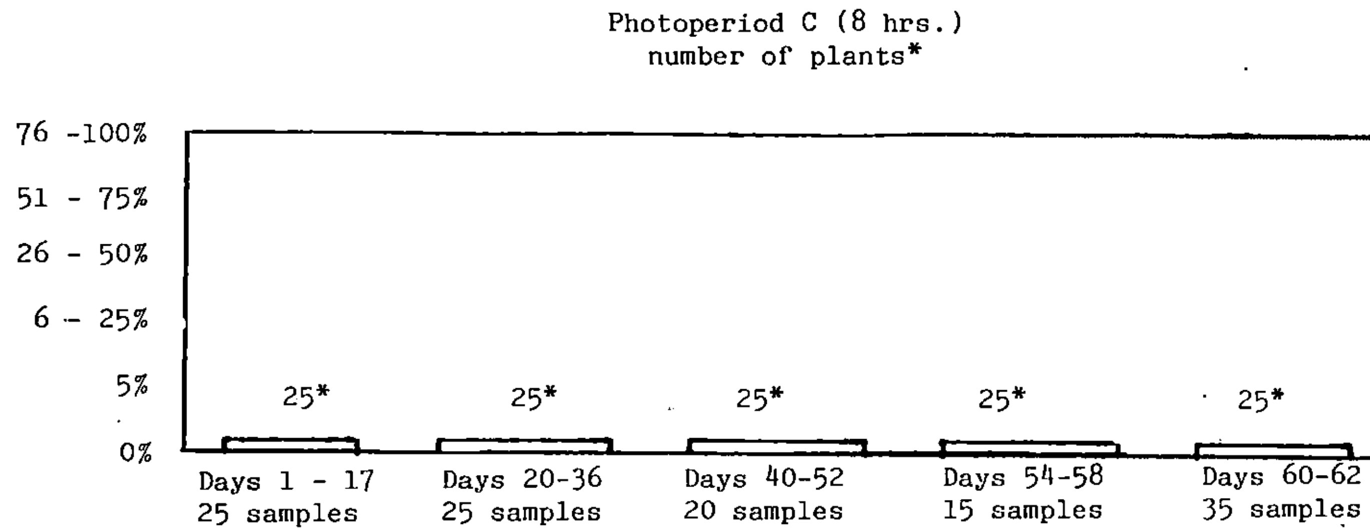
* Initial Colonization on Day 1, Peak Colonization on Day 54 and Sporulation on Day 60.

Figure 9. Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod B (12 hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil.



* Initial Colonization on Day 1, Peak Colonization on Day 54 and Sporulation on Day 60.

Figure 10. Root Colonization Rates of **Gigaspora margarita** in the California Pepper Compared at Photoperiod C (8 hrs.) from Initial Colonization to Peak Colonization and Sporulation in Low Phosphorus Clay Soil.



* Initial Colonization on Day 1, Peak Colonization on Day 54 and Sporulation on Day 60.

Conclusion

Overall generalizations can not be made about the influence of test conditions on endophyte success and host plant response. Host/endophyte combinations may interact differently depending on the condition of the rhizosphere, soil texture, pH, phosphorus, light and moisture conditions and numerous other factors.

It can simply be said that under the given conditions of this experiment, **G. margarita** exhibited a more successful host/endophyte relationship with the California Pepper at the 12 hour photoperiod than at the 8 and 16 hour photoperiods. In addition, plant growth response (root length, shoot length and green weight) was significantly improved in the colonized samples at the 12 hour photoperiod compared to the uninoculated quality control samples in that section and to inoculated samples in sections A and C.

A possible reason for the poor colonization in the 16 hour photoperiod (where highest rate was expected) could have been the longer exposure time to the warm lights causing the soil to dry out noticeably more between waterings than at 12 hours. This exposed the plants to repeated light drought cycles which may have been detrimental to endophyte colonization. Reid and Boen (1979) reported that using different host/endophyte combinations and exposing plants to several cycles of moderate drought decreased spore production and plant growth stimulation. It has also been demonstrated that extreme fluctuations of soil moisture can drastically

affect development of external hyphae and sporulation (Schenck, 1984).

According to Schenck, though there are many exceptions, moderately moist to slightly dry, well aerated soil is most conducive to successful endophyte colonization. The soil moisture level of the 8 hour photoperiod was considerably higher between waterings than in the 12 hour photoperiod. In addition the 8 hour photoperiod was probably too short. Longer photoperiods stimulate greater mycorrhizae infection and growth response than do shorter photoperiods (Hayman, 1974).

Lag time between germination and initial colonization, as well as peak colonization and sporulation was approximately two weeks longer than expected using **G. margarita** mixed inoculum (Roncadori, 1988).

There were also morphological variations observed in this host/endophyte combination. No soil-borne or intracellular vesicles were produced and arbuscule formation (typically abundant in this endophyte species) was moderately reduced. Morphological variations and colonization rate patterns, however are not unusual and do not constitute an unsuccessful host/endophyte relationship (Bevege and Bowen, 1975).

Based on the results at the 12 hour photoperiod, light and moisture levels between waterings were both adequate enough to produce a high percentage of VAM infection at a moderate rate. The plant growth response in this section showed significant improvement over the uninoculated quality control samples.

G. margarita and the California Pepper, therefore proved to be a reasonably successful host/endophyte relationship and **G. margarita** could be considered as a possible source of inoculum to enhance the growth of pepper crops in low phosphorus clay soils.

Summary

The results of this evaluation are summarized as follows:

1. The 16 hour photoperiod showed poor colonization results (7.5%) and no sporulation. Plant growth response did not differ significantly from uninoculated samples. The most likely cause for this was excessive drying of soil between waterings.
2. The 12 hour photoperiod showed a high percentage of colonization (63%) and sporulation (67%). Plant shoot length and total green weight was significantly greater in colonized samples as compared to uninoculated samples.
3. The 8 hour photoperiod showed no colonization and no sporulation. Plant growth response did not differ significantly from uninoculated samples. Possible causes were too short of a photoperiod and high soil moisture levels.
4. The 12 hour photoperiod exhibited a moderately fast rate of colonization with a predominance of 0-5% infection by day 17, 6-25% by day 36 and 26-50% by day 54.
5. Colonization rates could not be compared due to insufficient colonization in the 8 and 16 hour photoperiod.
6. Morphological variations under test conditions showed a moderate decrease in arbuscule production and no vesicles were produced.

7. Based on the results of the 12 hour photoperiod, light as well as the soil moisture level between waterings proved adequate enough to display a successful host/ endophyte relationship.

8. **Gigaspora migarita** could be considered as a possible inoculum to enhance growth of pepper crops in low phosphorus clay soils.

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