Acta Scientific Agriculture (ISSN: 2581-365X)

Volume 2 Issue 7 July 2018

Research Article

First Report of Arbuscular Mycorrhizal Fungi Associated with Different Varieties of *Cucurbita maxima* grown in the United States

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Received: May 04; 2018; **Published**: June 08, 2018

Abstract

Nearly 80% of herbaceous plants form relationships with Arbuscular Mycorrhizal Fungi (AMF), which are often beneficial to the host, by acquiring limited resources, such as phosphorus and water. The cucurbit, *Cucurbita maxima* is an incredibly diverse species, and suggested to have more cultivated forms than any other crop species. *C. maxima* has many medicinal uses, including anti-diabetic, anti-oxidant, anticancer, and anti-inflammatory properties, and is also a major food source due to its fiber content, carbohydrates, β-carotene, vitamins, alkaloids, minerals, fatty acids, flavonoid, and diverse polysaccharide content. Currently, the scientific literature is missing definitive documentation of an AMF association with *Cucurbita maxima* in the United States of America. This study utilizes light microscopy as supportive evidence to show an arbuscular mycorrhizal relationship with different varieties of *Cucurbita maxima.*

Keywords: Mycorrhizae; Fungi; Cucurbita maxima; Cucurbitaceae; Glomeromycota; Glomeromycetes; AMF; Arbuscule; Vessicle

Introduction

The genus Cucurbita includes 13 species, with *Cucurbita maxima* producing the most morphologically distinct fruit of any species in this economically important clad [1]. The Latin binomial name suggests vigorous growth of fruit, which is the biggest fruit on Earth [2]. *Cucurbita maxima* is an incredibly diverse species and it is suggested to have more cultivated forms than any other crop species [3]. The polymorphic nature of *C. maxima* can result in a misidentification of the species, and lead to confusion when using this cucurbit for botanical research. *C. maxima* originated in South America and are thought to have been domesticated 4,000 years ago [3]. Various forms of *C. maxima* were disseminated to Europe in the 16th century, and subsequently were taken by European explorers to the Indian sub-content, and Southeast-Asia [3].

Cucurbita maxima has been the subject of research for medicinal uses, including anti-diabetic, anti-oxidant, anticancer, and anti-inflammatory properties [4]. It is a major food source throughout the world, and cultures across the globe use *Cucurbita maxima* for its fiber content, carbohydrates, β-carotene, vitamins, alkaloids, minerals, fatty acids, flavonoid, and polysaccharide content [4]. Studies have shown bioactivity of certain polysaccharides extracted from *C. maxima*, and much of this activity is derived from the molecular structure of these compounds, which directly affects the hypoglycemic and anti-oxidant properties of the fruit [4].

Arbuscular Mycorrhizal Fungi (AMF) are categorized into the phylum Glomeromycota and are known to inhabit the root systems of many herbaceous plant species [5-8]. An ecological mutualism forms where an endosymbiotic relationship creates structures that span between the fungus, and the host plant [5-8]. Akhtar., *et al.* reported that 80% of herbaceous plants form relationships with AMF [5]. The herbaceous plant receives nutrients, water, and protection from pathogenic invaders, whereas the fungus receives protection and nutrients in which to survive [5-8] AMF are identified by the presence of branched haustoria like structures, which are found in association with the plasma membrane of cortical cells in the host plant and are termed arbuscules [5]. These modified hyphal structures are the location where nutrient exchange occurs between the host plant and the AMF symbiont [5]. Once the arbuscule is formed, the AMF hyphae will grow from the root surface and penetrate the surrounding soil, expanding the zone in which water and nutrients can be acquired [5]. Dickson and Kolesik state that AMF are dichotomized into two distinct groups based upon the morphology of their arbuscules [9]. The first group of AMF is the 'Arum' type, which has highly branched arbuscules, and the second group is the 'Paris' type, which has regions of coiled intracellular hyphae and intercalary arbuscules [9]. Currently, there are discrepancies with this nomenclature, which will not be discussed in this report [10]. Additionally, some species of AMF produce vesicles, which are lipid storage structures, and are important for survival during adverse conditions [11].

Images of arbuscular mycorrhizal fungi in *C. maxima* has not been documented in the scientific literature. In 1993, Ragupathy and Mahadevan published a manuscript that reported on the dis-

tribution of AMF in Tamil Nadu, India [12]. In this publication, *C. maxima* was sampled, but no image evidence of mycorrhizal association was presented or described [12]. In 2008, Akond and colleagues published a paper that surveyed southern India for the presence or absence of AMF in common vegetables and included a table of vegetable/mycorrhizal associations (Akond., *et al.* 2008). This table included *C. maxima* as having endosymbionts but did not include micrographs to supporting the inclusion. In 2012, Srivatava and colleagues published a manuscript that identified *Acaulospora mellea, Gigaspora margarita, Glomus citricola, G. macrocarpum* and *G. minuta* from root samples of *C. maxima* from southern India, but they did not include any images of AMF within the host tissues [13]. In 2010, Muthukumar and Tamilselvi published a manuscript that surveyed 45 species of crops, and visually documented AMF infection in *C. maxima*, although the variety was not stated [14]. Documentation of AMF in *C. maxima* from the United States of America is missing from the literature and is a crucial step in verifying the presence or absence of a fungal endosymbiosis in North America.

To date, the United States Department of Agriculture (USDA), Agricultural Research Service, does not acknowledge an AMF relationship with *C. maxima.* (http://nt.ars-grin.gov/fungaldatabases/). The purpose of this study is to provide a detailed first report of an AMF with *C. maxima* in the United States of America. Understanding root colonization by AMF in different varieties of *C. maxima* will allow researchers to make recommendations to agriculturalists that are interested in organic and sustainable production of gourds. Varieties that have more colonization by AMF could potentially have less disease prevalence and might be more adapted to specific geographical areas.

Methods

Preliminary Sampling

The first phase was a survey of *Cucurbita maxima* roots collected from farms in the eastern United States, and the use of traditional staining techniques to discern the presence or AMF in the host. Light Microscopy techniques are commonly utilized to image these fungi, and the use of differential stains is a critical step in the imaging process. The first phase was conducted in order to establish a justification for further exploration into the colonization mechanisms of AMF in *C. maxima.*

Root samples were collected from 3 farms in southeastern Ohio, 1 farm in West Virginia, and 2 locations in Maryland. Farms were selected based upon their different agricultural practices, ranging from certified USDA Organic, uncertified organic, unsprayed, and conventional treatment. Root samples were collected randomly from multiple plants of each cultivar, and then roots were chosen at random for staining and microscopic analysis. According to the growers at each farm, the cultivars included Blue Hubbard, Burgess Buttercup, Dills Atlantic Giant, Rouge Vif d'Etampes, Red Kuri, Sweet Meat, and Turk's Turban were sampled.

Staining Preliminary Samples

Root samples were processed and stained similarly to the Bundrett [15] methodology, with a few exceptions. Root samples were cut into 3 - 7 mm sections using a razor blade. Samples were heated in 10% (w/v) aqueous potassium hydroxide (KOH) solution for 20 minutes at 95°C. KOH was decanted off of the samples, and 5% hydrochloric acid (HCL) (v/v) was added to neutralize the pH. The samples were kept in 5% HCL for 3 minutes at 20°C. The aqueous HCL was decanted off of the samples. Then, acidified glycerol (200 µl of 5% HCL and 800 µl of 50% glycerol (v/v)) was pipetted to cover the samples, and 2 drops of 1% Trypan blue (w/v) was added to the storage tubes. The samples were incubated at 20°C for 3 minutes, and immediately washed with 20°C distilled water. The samples were de-stained twice with 50% glycerol (v/v) for 20 minutes each at 20°C. Samples were stored in 50% glycerol (v/v) at 4°C.

Experimental Design

Seedlings were planted on June 2nd, 2015 at Miami University's Ecology Research Center. Roots from *C. maxima* were sampled monthly, on July 2nd, August 2nd, September 2nd and 3rd. During each sampling event, 10 plants of each variety were randomly destructively-sampled, and five roots from each plant were sub-sampled from the total roots collected. The individual roots were randomly cut into 1 cm segments and selected for staining and quantification. Only one segment was used for each root, and the rest was saved for future analysis.

Seed Germination

Seeds of *C. maxima* cv. Burgess Buttercup, Rouge Vif d'Etampes, Mariana de Chioggia, and Golden Hubbard were purchased from Seed Savers Exchange®. Ten seeds of each cultivar were placed in filter paper lined Petri-plates, moistened with de-ionized water. This was replicated 10 times, for a total of 100 seeds of each cultivar. Seeds were incubated at 22°C under 24 hours of florescent lights. Seedlings were transferred from filer paper to 3" peat pots, filled with moistened Farfard® 3B potting soil. Plants were grown on light carts or in light boxes under fluorescent lights at 22°C with a regime of 18 hours of light, and 6 hours of dark.

Field Cultivation

Research was conducted at Miami University's Ecology Research Center (ERC) on Somerville Road, north of Oxford, Butler County, Ohio. The field is approximately 1/2 hectare in size and is adjacent to the ERC access road. The field was left fallow for a year prior to this study. The field was disked twice and tilled before planting. No chemical treatments were applied to the field pre- or post-planting.

Four-week-old seedlings of *C. maxima* cv. Burgess Buttercup, Rouge Vif D'Etampes, Mariana de Chioggia, and Golden Hubbard were transplanted in a non-randomized pattern that contained rows of 26 individuals, with 2.4m spacing between the plants, and between the rows. Two rows of the same cultivar were planted adjacent to each other. Plants were irrigated by hand for the first 10 days post-planting.

Soil Sampling

Soil samples were collected once during the month of July from the field plot at the ERC, and these samples were processed for AMF Glomerospore extraction. A subsample of 50g of soil was processed from each original soil sample. A (20%/60%) sucrose solution was used for gradient separation of Glomerospores and was centrifuged at 600 x g for 3 minutes. Soil was sieved through a 500 µm and 43 µm stainless mesh sieve, and spores were washed three times with distilled water. Extracted spores were imaged with bright field light microscopy.

Root Sampling

During each sampling event, 10 plants of each cultivar were randomly destructively sampled, and five roots from each plant were sub-sampled from the total roots collected. Sampling took place during three evenly spaced times during the growing season. The sampling took place on July 2nd, August 2nd, and September 2nd - 3rd, 2015. Roots were stored in plastic bags at 4°C until processing within 48 hours' post-harvest. Only one, 1 cm segment was used from each root, and the rest of the sample was frozen at -80°C for future analysis

Modified Staining Techniques

Staining techniques were modified from the preliminary staining procedures in order to maximize Trypan Blue absorption into the roots, and this modification was used in the 2015 field study. Samples were heated in 10% (w/v) aqueous potassium hydroxide (KOH) solution for 50 minutes at 95°C. KOH was decanted off of the samples, and 5% hydrochloric acid (HCL) (v/v) was added to neutralize the pH. The samples were kept in 5% HCL for 5 minutes at 20°C. Then, 1% Trypan blue (w/v) was added to the storage tubes. The samples were incubated at 100°C for 5 minutes, and immediately washed with 20°C distilled water. Samples were cut into 1 cm sections and mounted on glass slides within 50% lactic acid - glycerol (v/v) and stored at 4°C.

Light Microscopy

Root segments were examined for distinct morphological features using bright field light microscopy and laser scanning confocal microscopy and imaged with an Olympus AX-70 light microscope. Definitive characteristics of AMF such as arbuscules and vesicles were used to distinguish Glomeromycota from other phyla of fungi.

Results

Preliminary Phase I sampling during the fall of 2014 of *C. maxima* roots shows a diversity of structures associated with Arbuscular Mycorrhizal Fungi and ever sample of *C. maxima* (Table 1). This initial survey provides light micrographs as evidence to show a microbe/host relationship between microbial organisms and *C. maxima.*

Cultivar	Features	Novel Associations	Horticultural Practices	State	Country	Farm
Burgess Buttercup	H, Ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Golden Hub bard	H, Ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Mariana di Chioggia	H, Ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Rouge Vif d'Etampes	H, Ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC

Table 1: Presence of Arbuscular Mycorrhizal Fungi morphological features associated with different cutivars of *Cucurbita maxima* sampled during Phase II in July, August, September 2015 in Butler Country Ohio.

Morphological Features designated by Ar = Arbuscule, H = Hyphae, S = Spore, V = Vesicle, Conventional/Unsprayed means that the fields were previously treated in the past decade but were not treated during these experiments. OH = Ohio, ERC = Ecology Research Center at Miami University in Butler Co. Ohio.

Micrographs of these groups of organisms provide evidence of a relationship with *C. maxima* and will help fill in the gaps that might be missing in the literature. Images of these organisms will serve as justification to further study the rolls of these organisms in and around *C. maxima.*

Every sampling location provided plant tissue bearing some mycorrhizal structures, although vesicles and arbuscules were less frequent than normal hyphae and inter-coiled hyphae. 'Blue Hubbard' grown under USDA Certified Organic conditions in Ohio, as well as the uncertified organic cultivation from West Virginia showed inter-coiled hyphae, but vesicles were only observed in uncertified organic pumpkins grown in West Virginia.

Samples from the cultivar 'Turk's Turbans' were collected from an unsprayed conventional farm in Ohio, and from an uncertified organic farm in West Virginia. The Ohio samples contained hyphae and arbuscules, whereas the West Virginia samples contained hyphae, vesicle, and arbuscules. Samples from 'Rouge vif d'etampes' were collected from an uncertified organic farm in West Virginia, as well as from a conventional farm in Maryland. The samples collected from West Virginia had both hyphae, and arbuscules present. The samples from Maryland contained hyphae, and vesicles. The cultivar 'Burgess Buttercup' grown under conventional farming techniques in Maryland, USDA Certified Organic conditions in Ohio, and from un-certified organic cultivation from West Virginia showed normal hyphae and inter-coiled hyphae, but vesicle were

only observed in un-certified organic pumpkins grown in West Virginia. Arbuscules, vesicles, and hyphae were observed from the conventionally grown 'Burgess Buttercup' from Maryland. The cultivar 'Dill's Atlantic Giant' was sampled from a conventional farm in Maryland and showed vesicles, arbuscules, hyphae, and intercoiled hyphae. 'Red Curi' samples were collected from a USDA Certified Organic farm in Ohio. The root samples only contained hyphae, and no other morphological structures were observed. The cultivars 'Sweet Meat' was sampled from a USDA Certified Organic Farm in Ohio, and only hyphae were observed. Arbuscular mycorrhizal fungi were found in every cultivar of *C. maxima* sampled.

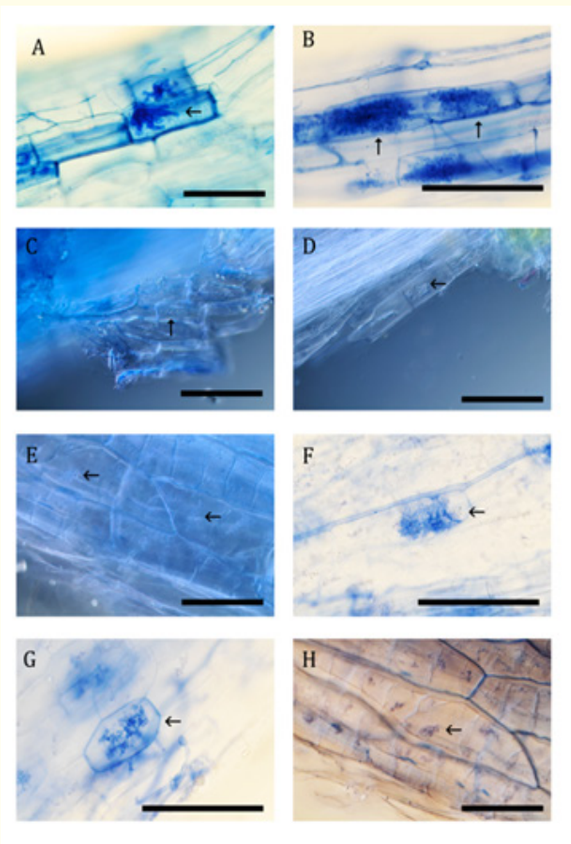

Figure 1: 'Arum' type arbuscules produced by AMF observed from Phase I and II sampling observed in the following cultivars. A. Developing arbuscule in *C. maxima* cv. Burgess Buttercup collected at Orr Farms. B. Pair of arbuscules imaged in *C. maxima* cv. Golden Hubbard collected at the ERC. C. Imaged with Differential Interference Contrast Microscopy in *C. maxima* cv. Dills Atlantic Giant collected at Mountain Valley Orchard farm. D. *C. maxima* cv. Rouge Vif d'Etampes collected at Kensho Farms. E. *C. maxima* cv. Burgess Buttercup collected at Five Oaks Farms. F. Mariana de Chioggia collected the ERC. G. *C. maxima* cv. Rouge Vif d'Etampes collected at the ERC. *C. maxima* cv. Burgess Buttercup collected at Five Oaks Farms.

'Arum' type arbuscules were observed with bright field light microcopy in *C. maxima* cv. Burgess Buttercup collected at Orr Farms (Figure 1A), *C. maxima* cv. Mariana de Chioggia collected at Miami University's ERC (Figure 1F), *C. maxima* cv. Rouge Vif d'Etampes collected at Miami University's ERC (Figure 1G), *C. maxima* cv. Golden Hubbard collected at Miami University ERC (Figure 1B). An arbuscule, shown with an arrow, was imaged with Differential Interference Contrast Microscopy in *C. maxima* cv. Dills Atlantic Giant collected at Mountain Valley Orchard farm (Figure 1C), *C. maxima* cv. Rouge Vif d'Etampes collected at Kensho Farms (Figure 1D), *C. maxima* cv. Burgess Buttercup collected at Five Oaks Farms (Figure 1E,1H).

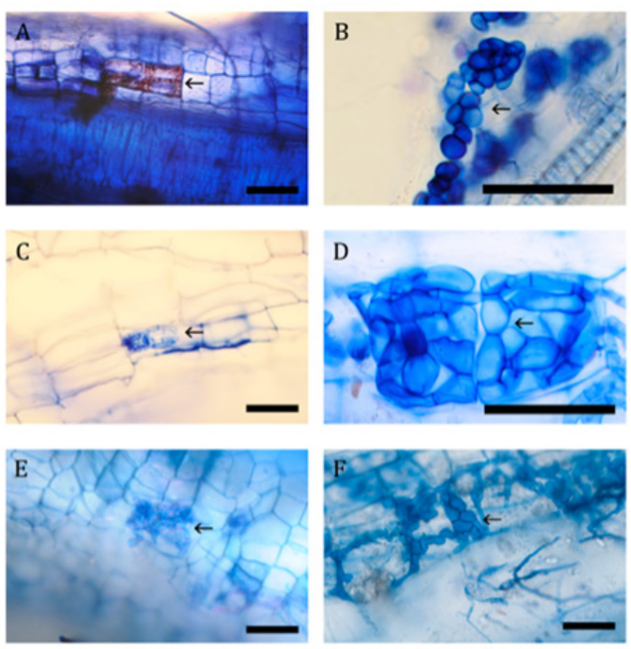

Figure 2: 'Hyphal coils' or 'Paris' type arbuscules were observed in *C. maxima* during Phase I and II sampling in the following cultivars. A. *C. maxima* cv. Rouge Vif d'Etampes at Kensho Farms. B. C. maxima cv. Mariana de Chioggia collected at Miami University's ERC. C. C. maxima cv. Turks Turban collected from Orr Farms. D. *C. maxima* cv. Golden Hubbard collected at Miami University' ERC. E. C. maxima cv. Turks Turban collected from Orr Farms, imaged with Differential Interference Contrast Microscopy. F. *C. maxima* cv. Golden Hubbard collected at Miami University ERC. Mag. bar is 50 µm.

'Hyphal coils' or 'Paris' type arbuscules are were observed in samples from *C. maxima* cv. Rouge Vif d'Etampes at Kensho Farms (Figure 2A), *C. maxima* cv. Mariana de Chioggia collected at Miami University's ERC (Figure 2B), *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 2C), *C. maxima* cv. Golden Hubbard collected at Miami University' ERC (Figure 2D) and imaged with Bright Field Light Microscopy. Hyphal coils' or 'Paris' type arbuscules were observed in *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 2E) and was imaged with Differential Interference Contrast Microscopy. Hyphal coils' or 'Paris' type arbuscules, were observed with Bright Field Light Microscopy in *C. maxima*cv. Golden Hubbard collected at Miami University ERC (Figure 2F).

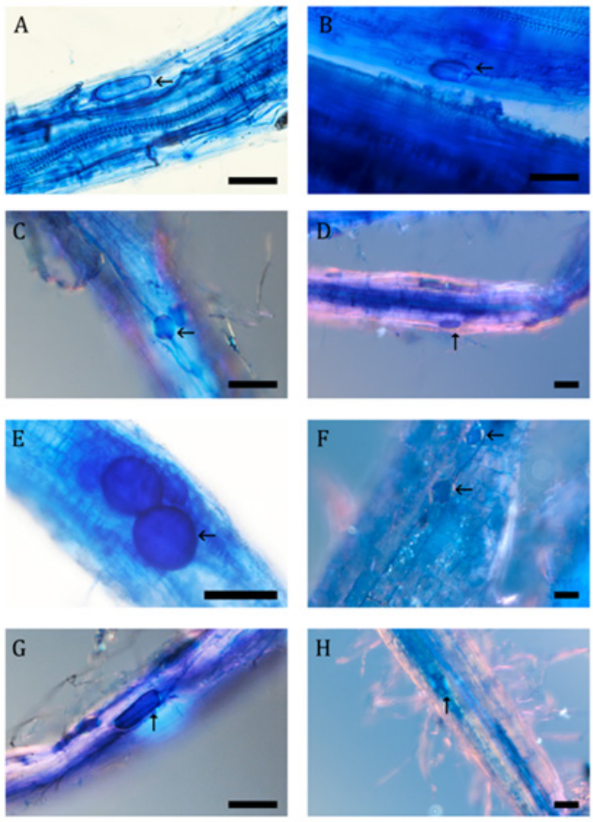

Figure 3: Vesicles observed with Differential Interference Contrast Microscopy during Phase I and II sampling. A. Micrograph of *C. maxima* cv. Burgess Buttercup from Orr Farms. B. Micrograph of *C. maxima* cv. Turks Turban collected from Orr Farms C. Differential Interference Contrast Micrograph of *C. maxima* cv. Turks Turban collected from Orr Farms, D – E. Micrographs of *C. maxima* cv. Blue Hubbard collected from Orr Farms. F. Micrograph of C. maxima cv. Turks Turban collected from Orr Farms. G. Micrograph of *C. maxima* cv. Turks Turban collected from Orr Farms. H. Micrograph of *C. maxima* cv. Turks Turban collected from Orr Farms. Mag. bar is 50 µm.

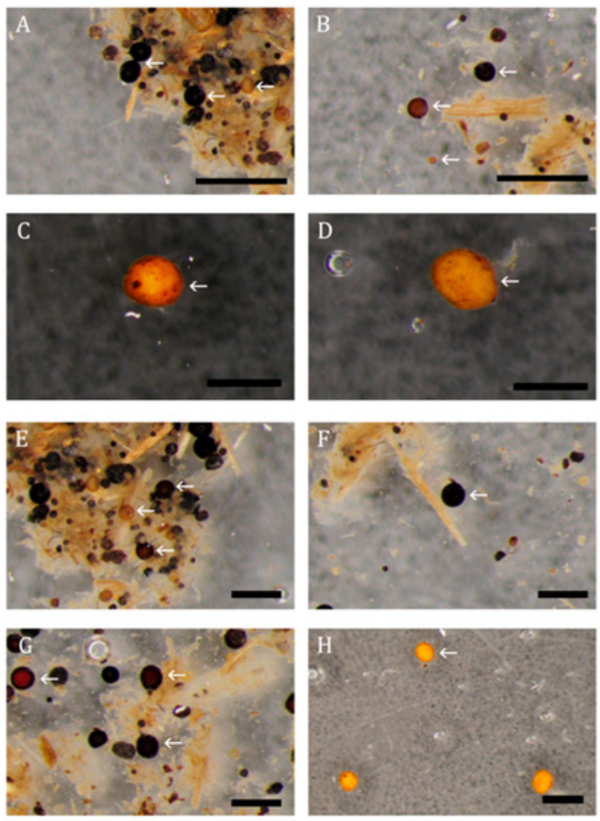

Figure 4: Asexual Glomerospores from Glomeromycota were observed during Phase II soil sampling with bright field light microscopy. A-B. Different glomerospore morphologies, shown with arrows, with 50 µm mag. bars. C-D. Parasitized Gigaspora glomerospores were observed amongst detritus with 100 µm mag. bars, E-G. Different glomerospore morphologies, shown with arrows, with 50 µm mag. bars. H. Three parasitized glomerospores shown with 100 µm mag. bars.

A vesicle was observed with Differential Interference Contrast Microscopy in *C. maxima* cv. Burgess Buttercup from Orr Farms (Figure 3A), *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 3C), *C. maxima* cv. Blue Hubbard collected from Orr Farms (Figure 3D), *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 3F), *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 3G), and in C. maxima cv. Turks Turban collected from Orr Farms (Figure 3H). A vesicle and lipid filled hyphae was observed with Differential Interference Contrast Microscopy in *C. maxima* cv. Turks Turban collected from Orr Farms (Figure 3B).

Microscopic evaluation of roots collected during the second phase of the experiments showed similar microbial associations with *C. maxima* (Table 2). A pair of vesicles was observed with bright field light microscopy in *C. maxima* cv. Golden Hubbard collected from Miami University's ERC (Figure 3E). A mixture of different glomerospores morphologies were observed from field samples collected at Miami University's ERC and shown with 50 µm magnification bars (Figure 4A-4G). Parasitized *Gigaspora* glomerospores were collected from Miami University's ERC, imaged with Bright Field Light Microscopy, and shown with 100 µm magnification bars (Figure 4C-4H). All of the images shown are provided with a 50 µm magnification bars. AMF with lipid filled hyphae and arbuscules are shown with arrows, imaged with Bright Field Light Microscopy in *C. maxim*a cv. Mariana di Chioggia (Fig-

ure 3B). AMF hyphae and 'Arum type' arbuscules are shown with arrows, imaged with Bright Field Light Microscopy in *C. maxima* cv. Mariana di Chioggia (Figure 1G). AMF hyphae with numerous darkly stained nuclei as well as 'Arum type' arbuscules shown with arrows, imaged with Bright Field Light Microscopy in *C. maxima* cv. Mariana di Chioggia (Figure 1H).

Cultivar	Features	Novel Associations	Horticultural Practices	State	Country	Farm
Burgess Buttercup	H, A ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Golden Hubbard	H, A ar, Par, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Mariana di Chioggia	H, A ar, Par, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC
Rouge Vif d'Etampes	H, A ar, S, V	Glomeromycota	Conventional/Unsprayed	OH	Butler	ERC

Table 2: Presence of Arbuscular Mycorrhizal Fungi morphological features associated with different cutivars of *Cucurbita maxima* sampled during Phase II in July, August, September 2015 in Butler Co. Ohio.

Morphological Features designated by A ar = Arum Arbuscule, Par = Paris Arbuscule, H = Hyphae, S = Spore, V = Vesicle, Conventional/Unsprayed means that the fields were previously treated in the past decade but were not treated during these experiments. OH = Ohio, ERC = Ecology Research Center at Miami University in Butler Co. Ohio.

Discussion

Cucurbits are cultivated worldwide for nutrition, medical, and cultural uses, and the species *Cucurbita maxima* was used as a model organism for this research project due to a void in information about microbial communities in the scientific literature. *Cucurbita maxima* is an economically important crop and its microbial communities should be studied in more detail because they can either be beneficial to the host plant or can harbor parasites and pathogens that may potentially kill or weaken the host organism and reduce agricultural yields.

Members of the Cucurbitaceae are often susceptible to fungal, viral, bacterial, and nematode plant pathogens, including: *Cladosporium cucumerinum Ellis* and Arth., *Colletotrichum, Monosporascus cannonballus Pollack* and Uecker, Rhizoctonia solani J.G. Kühn, members of the Ascomycota, *Choanephora cucurbitarum* (Berk. and Ravenel) Thaxt., a member of the Zygomygota, *Sclerotium rolfsii* (Curzi) C.C. Tu and Kimbr., a member of the basidomycota, Erwinia spp., a bacterial pathogen, *Pseudoperonospora cubensis* Berkeley and Curtis, *Pythium* spp., a member of the Oomycota, as well as Melodogyne and *Pratylenchus* spp., which are nematode pathogens (Seebold., *et al.* 2009).

Arbuscular mycorrhizal fungi have been proposed as biological control agents to combat soil borne pathogens of herbaceous plants and have been shown to reduce disease symptoms caused by pathogenic fungi and nematodes [16]. Saldajeno and Hyakumachi [16] use the term Plant Growth Promoting Fungi (PGPF) to describe beneficial fungi, which includes AMF, which stimulate plant growth, and decrease disease occurrence. [8] gave the term Mycorrhiza-Induced Resistance (MIR) for a type of pathogen resistance that arises from the colonization of plant roots by numerous types of mycorrhizal fungus. MIR increases the host's resistance to a diversity of pathogens and has been shown to decrease feeding by insect predators [8]. In the review by Akhtar's., *et al.* [5], AMF, Rhizobium, and fungal pathogen interactions are explored, and a direct correlation was shown between colonization of the host plant by AMF and decreased disease severity. Decreased disease severity in plants was attributed to various AMF species, including species of *Glomus Gigaspora* and *Sclerocystis* [5].

Selecting cultivars of *Cucurbita maxima* that have the highest levels of AMF colonization would be beneficial for agriculturalists, due to the protective and growth promoting potential by the Glomeromycota. This in turn can reduce synthetic chemical inputs in the field, generating more profit for landowners at less of an economic expense.

This study provides a first report of a relationship between Glomeromycota, in *Cucurbita maxima* in the United States. The images provided in this manuscript are the first photographic documentation of these organisms in *Cucurbita maxima* and provide a valuable piece of evidence that was missing from the literature. Distribution of fungi was observed over two seasons, and Glomeromycota was observed in sampling location and in every variety of *C. maxima* sampled [17-29].

Concussion

Cucurbita maxima grown in the United States showed associations with members Glomeromycota. This study provides a first report and images of a relationship between Glomeromycota in *Cucurbita maxima* in the United States. The images provided in this manuscript are the first photographic documentation of these organisms in *Cucurbita maxima* to date. Further research into the molecular identification of what species of AMF are living in the host is needed. Showing a presence of AMF indicates that once molecular characterization is completed, any species that was previously described to have beneficial properties could be selected for to grow exclusively with *Cucurbita maxima* and provide growth promoting properties.

Acknowledgments

I want to graciously thank Matt Duley for his training and technical expertise in the Miami University Center for Advanced Microscopy and Imaging. I want to thank Kristi Hutchinson at Five Oaks Organic Farm, John Krowka at Kensho Farms, Scott Downing at Downing Fruit Farm, Leroy Tracey at Mountain Valley Orchard, and Mike and Mark Orr Farm for allowing samples to be collected and processed for imaging. Thanks to Miami University for providing internal support for the Center for Advanced Microscopy and Imaging and the Willard Sherman Turrell Herbarium during my time at Miami University. I want to send many thanks to Jeremy Fruth for field assistance at the Ecology Research Center during the 2015 field season. I want to thank Jack Keegan for his horticultural expertise.

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