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Daniel Gatch 12/1/11

The effects of Mycoleptodiscus terrestris on Cabomba caroliniana

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

The effects of Mycoleptodiscus terrestris on Cabomba caroliniana.
By Daniel Gatch

The purpose of this research project was to determine the effects of the fungus Mycoleptodiscus terrestris (Mt) as a biocontrol agent against the invasive aquatic plant Cabomba caroliniana (fanwort). Experiment one compared the final biomasses of treated vs. untreated fanwort after being exposed to Mt for 27 days. Experiment two and experiment three compared differences in damage in treated vs. untreated fanwort after being exposed to Mt for 27 and 19 days respectively. Damage in experiment one was determined quantitatively by comparing the final biomasses of treated vs. untreated fanwort. Experiments two and three used a qualitative damage rating to determine damage caused by Mt. Hydrilla verticillata, an invasive aquatic plant known to be affected by Mt, was used as a control in experiment one and three to determine if the prepared inocula were working properly. Experiment one and experiment three showed no significant difference between Mt treated vs. untreated fanwort, while in experiment two there was significantly higher damage recorded for Mt treated fanwort vs. untreated fanwort. Experiment three supports the idea that Mt does not have an effect on fanwort. Because of the contrasting results of the three experiments on the effects of Mt on fanwort, additional studies comparable to experiment one that use a quantitative approach need to be performed to confirm the absence of an effect of Mt on fanwort.

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Dr. Chris Beck has been my advisor and mentor at Emory University since I joined the Biology Department. He helped me choose my thesis topic, obtained the permit to transfer Mt (the fungus used in my experiment) across state borders, helped provide lab space to conduct my experiment, provided the funding for my project, advised me throughout my project, provided technical knowledge when I was lost, edited my thesis multiple times, and prepared me for my defense. More importantly, he has always been there to guide me in my scholastic endeavors whenever I have asked for help. Thank you Dr. Beck for taking me on as your honors thesis student.

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Introduction

Geographic barriers such as mountains, rivers, and oceans can create separate regions and prevent populations within regions from intermingling. Sometimes species cross barriers and reproduce in a different region. These species are known as non-native species (Lockwood et al. 2007). Charles Elton (1958) first coined the term "invader" to denote a non-native species that successfully moves into and takes over a region. Non-indigenous, exotic, and alien are other terms used to describe invaders (Lockwood et al. 2007). Short-distance colonizers are invaders that diffuse into an adjacent region and expand their range (Davis and Thompson 2000). In contrast, long-distance colonizers are invaders novel to a region that spread via saltation and diffusion (Davis and Thompson 2000). Saltation involves the abrupt movement of a species across a barrier, and diffusion is the gradual movement of this species from its focal point to other regions. Long-distance colonizers are generally influenced by human interactions and would be considered an invasive species if their introduction causes or is likely to cause economic, environmental, or human health harm (Clinton 1999*)*.

Humans have increased the ability of species to invade a region. Increasing international trade has led to increased invasions even with increased awareness and prevention efforts (Lockwood et al. 2007). Levine and D'Antonio (2003), found a direct correlation between a country's imports and the number of non-natives found in that country. Large transport ships, which unintentionally transport organisms via ship ballasts, are a well-known problem. Early cargo ships used soil in their ballasts to counteract the weight of ship cargo. Eighty-one non-native plants recorded in Pennsylvania came from discarded ballast soil in Philadelphia (Mack 2004 citing Rhoades and Klein 1993). Transport ships now use water in their ballasts, and it is

estimated that 7,000-15,000 species are transported within these ballasts daily (Carlton 1999). Increasing international trade, particularly of organisms in the horticulture trade, is becoming increasingly more difficult to control due to online trading (Kay and Hoyle 2001). In a study conducted by Maki and Galatowichka in 2004, participants were successful in obtaining federally-listed noxious weeds and Minnesota-prohibited exotic plants that are illegal to trade across state borders 92% of the time via online trading.

In addition to humans transporting potentially invasive species, human disturbances can increase the invasive species in certain regions (Lockwood et al. 2007). For example, when farms are abandoned, farmers often introduce novel crop species that are well adapted to the farm habitat left behind. In these cases, native species are driven out of the original habitat during the creation of the farm. Once the farm is abandoned, native species are unable to reestablish due to the new habitat and effective invasion of the non-native crop species (Lockwood et al. 2007).

According to the "tens rule" in general approximately 10% of introduced species become established and approximately 10% of established species become invasive species (Williamson 1996). Despite the low probability of a species invading a region, the few species that do invade can have vast consequences. Invasive species can alter genetic integrity among native species, out-compete native species, prey upon native species, and ultimately permanently damage the entire invaded ecosystem (Lockwood et al. 2007) This irreparable damage causes both intrinsic as well as monetary loses. Harmful non-indigenous species cost the US approximately $137 billion per year, and the invasion of the Mediterranean fruit fly in New Zealand cost over $15 million in monetary loss to exports and control (Pimentel 2000, Lockwood et al. 2007).

Although an invasive species has an overall negative effect, sometimes invasions have positive aspects. For instance, much of the food, clothes, and wood used by European cultures came from species grown out of their native range (Elton 1958). Also, changes in disturbance regimes can cause changes in community composition and structure (Vitousek 1990). Invasive species provide a unique opportunity for scientists to study these changes experimentally as they occur in the field (Vitousek 1990).

Knowing the invasion process can help scientists and managers determine the potential impact of a non-native. According to Elton (1958), the invasion process can be divided into three elements: historical, ecological, and consequential. The historical element involves the events that lead to the introduction of an invader. For example, in the case of the brown tree snake invasion of Guam, the historical element was the physical crossing of the Pacific Ocean by the brown tree snake during the 1940s from New Guinea to Guam via Navy airplanes (Lockwood et al. 2007). The ecological element involves the characteristics of an organism, its behavior, and how it interacts with its new introduced environment (Elton 1958). In the case of the brown tree snake, the ecological element was that the brown tree snake had no natural predators in Guam and small invertebrates became easy targets as its prey. The third element is the actual effects and outcome of the introduction (Elton 1958). After the brown tree snake invaded and established, native birds and bats were wiped out on the island by the 1970s, resulting in a dramatic change in the food web that favored other non-native species (Lockwood et al. 2007). According to Vitousek (1990), a non-native species will have a larger effect on a region if they 1) differ from natives in utilization and storage rates of resources, 2) alter an invaded areas trophic structure, or 3) change frequency and magnitude of disturbance. A non-native species will compete with a similar native species for the same niche and little change in

the ecosystem will be seen due to the addition of the invasive. On the other hand, a non-native that is dissimilar than other native species of an ecosystem will create a new niche and will have a greater effect on the surrounding ecosystem. One of the reasons the brown tree snake was such a good invader was because no other small vertebrate predators lived on the island of Guam. It created its own niche where it became the only top predator and swiftly established and invaded.

Propagule pressure is one measurement used to determine if a species will effectively invade (Lockwood et al. 2007). A set of introduced non-native individuals is known as a propagule. Propagule pressure describes the size of propagules, the number of propagules released, and the health of a propagule (Lockwood et al. 2007). Propagules are generally transported from donor regions to recipient regions. The distance between the donor and recipient regions often has an effect on propagule health. During transportation of propagules from a donor to recipient region, the living conditions are often poor. Therefore, greater distances between donor and recipient regions generally correlate to poor propagule pressure (Lockwood et al. 2007). In a given donor region, the propagule size generally increases at the core of a species range, which makes the location the propagule is taken from within the donor region important in determining propagule pressure (Lockwood et al. 2007).

In order to prevent an invader from taking over, early detection and quarantine are essential for an effective management plan (Lockwood et al. 2007). Each management strategy is specific for each invasion, but regardless it is best to eradicate the invading population while it is small. Mechanical means can be especially good for eradicating a small focal point population (Lockwood et al. 2007). If a population becomes established over a large area, chemical and mechanical controls can be used, but sometimes biocontrols are sought (Lockwood et al. 2007).

Biological control is the intentional release and use of a natural enemy to control an invasive species. Classical biocontrol, inundative biocontrol, and augmentation are the three main types of biocontrol. If an invasive species has been introduced in an area with no natural enemies and has a high population density, a classical approach should be taken (Lockwood et al. 2007). During a classical approach, one or more non-native enemies of an invasive species are found and established in the area of invasion. The idea is both enemy and invader will be permanently established at low non-costly levels (Williamson 1996). The inundative approach tests for a natural enemy of an invader, but the enemy does not establish permanently. Instead, the biocontrol agent is released at regular intervals as if it were a chemical pesticide (Williamson 1996). Augmentation involves the supplementation of an enemy native to the habitat the invasive species is invading (Cox 2004). During augmentation, a native enemy species is regularly released until its population levels are high enough to keep the invasive population at low levels.

The main advantage of classical biocontrols is that if it works, it works virtually forever. It also reduces the use of pesticides that are costly and dangerous to non-target species (Nentwig 2008). For example, the use of the alfalfa weevil reduced US pesticide use by 95% from 1968-1983 and saved farmers $100 million per year on insecticide usage (Nentwig 2008). The main disadvantage involved with the use of biocontrol agents is the potential risk of releasing an agent that is nonspecific for its desired host. A nonspecific biocontrol can potentially destroy an entire ecosystem. Early biocontrol programs had little or no specificity testing, which resulted in disastrous consequences. For instance, in 1977 the rosy wolf snail *Euglandina rosea* was released as a biocontrol agent against the invasive giant African snail *Achatina fulica* without proper specificity testing (Lockwood et al. 2007). Researchers later discovered that *E. rosea* ate

most native snails within the region, particularly the *Partula*. After its release, *E. rosea* ended up causing extinctions of the *Partula* land snail species on every island of the French Polynesia except Tahiti (Lockwood et al. 2007). The success of biocontrol agents has greatly improved with increased specificity testing. Vertebrates are rarely used anymore, while invertebrates tend to be the most common biocontrol agent, especially against noxious weeds and insects (Nentwig 2008). In general, three main questions are asked when choosing a biocontrol agent: 1) Should the target species be controlled with a biological control agent or are other better methods available? 2) How specific is a biological control agent? 3) Will a specific agent evolve to be less specific (Williamson 1996)?

If a biological control is being considered, the first thing to do is look for natural predators from the invasive species native home range (Mcfadyen 1998). Another approach is to find a natural predator of a species similar to the invasive species in a separate range (Mcfadyen 1998). This approach can be highly effective because the invader has never been introduced to this species before. The next step after locating a potential agent is to conduct specificity testing. Damage done by a chemical application can be harmful and sometimes permanent, but damage done by a biocontrol can be harmful and is almost always permanent. Therefore, great care must be taken into consideration when conducting specificity tests. If the agent is decided to be released, then a cost-benefit analysis must be run before and after release. Then, follow up studies should be conducted to determine the efficacy of the biocontrol agent and its effects on the environment (Mcfadyen 1998).

Cabomba caroliniana, also known as fanwort, is a freshwater perennial dicot native to the sub-tropic to temperate regions of North and South America (Orgaard 1991). Fanwort

reproduces mainly through vegetative processes, but has been known to produce seeds (Orgaard 1991). Fanwort grows best in depths under 3m, but can grow at depths up to 6m (Schooler and Julien 2006). Three varieties of *C. caroliniana* have been described*: C. caroliniana var. caroliniana, C. caroliniana var. pulicherrima*, and *C. carolininana var. flavida Orgaard (Orgaard). C. caroliniana var. caroliniana* has white flowers**.** It is the most widespread *Cabomba* species and is commonly found in southeastern North America, central Texas, Florida, Massachusetts, Kansas, southern Brazil, Paraguay, Uruguay, and northeastern Argentina (Orgaard 1991). *C. caroliniana var. pulcherrima* is distinguished by its purple flowers and is located in South Carolina, southwestern Georgia, and Florida. *C. caroliniana var. flavida Orgaard* is characterized by its relatively large yellow flowers and large ellipsoid seed. It is found in southern Brazil, Paraguay, and northeastern Argentina. These varieties were once considered three separate species, but DNA analysis supports they are one species (Orgaard 1991).

C. caroliniana is considered invasive to parts of the United States, as well as, Australia, Canada, Greece, Japan, and China (Schooler et al. 2006). *C. caroliniana* has the potential to outcompete native plants to produce monostands. *C. caroliniana* invasions have been shown to lower plant diversity in parts of China, including the biodiversity of rare plants such as *Ottelia alismoides* and *Najas* oguraensi (Ding 2007). In Australia, *C. caroliniana* has been known to outcompete native aquatic plant species such as pondweeds *Potamogeton* spp., stoneworts *Chara* spp., hornwort *Ceratophyllum demersum* and water nymph *Najas tenuifolia*, as well as, prevent native plant species from germinating (Schooler et al. 2006 citing Mackey and Swarbrick 1997). The alterations in macrophyte communities caused by *C. caroliniana* are hypothesized to have

reduced platypus *Ornithorhynchus anatinus* and water rat *Hydromys chrysogaster* populations in Northern Queensland (Schooler et al. 2006 citing Mackey and Swarbrick 1997).

Pesticides and mechanical management are not effective against *C. caroliniana* (Schooler et al. 2006 citing Anderson and Diatloff 1999). Herbivores, competition from other aquatic plants, and shade are known to reduce the abundance of *C. caroliniana* (Schooler et al 2006). However, herbivores and plant competition can be indiscriminate in what they control, and management via shade is only efficient for smaller areas. Fluridone was proposed as a control method for *C. caroliniana*. However, it was not found to be an effective treatment if other non-target plants such as *Megalodonta beckii* were present (Nelson et al. 2002). Due to recent cases of plant resistance to chemical treatments, biocontrol agents have been sought. Statistical studies have been undertaken to determine efficacy of the stem boring weevil *Hydrotimetes natans* and an aquatic moth *Paracles spp.*, but no field or lab studies have been conducted (Schooler et al. 2006).

Hydrilla verticillata is considered one of the most invasive plants in the world (Soerjani 1986), and is a pond plant similar to *C. caroliniana*. As a result, methods of biocontrol for *Hydrilla* might be effective with fanwort. Gastropods, fungus, and insects were among the first biocontrol agents tested against *Hydrilla*, but none were found effective (Langeland 1996). The Asian carp *Ctenopharyngodon idellus* can be an effective biocontrol agent (Van Dyke et al. 1984). However, Asian carp pose a problem in lakes with multiple aquatic plant species because they eat native plants as well as *H. verticillata* (Langeland 1996).

Mycoleptodiscus terrestris (*Mt*) is a fungus that naturally infects *H. verticillata* (Joye 1988, 1989). The exact pathways that make *Hydrilla* susceptible to *Mt* are still under study, but

it has been suggested that the phenylpropanoids released by *Hydrilla* for pathogenic defense do not inhibit the growth of *Mt* (Kees 1998). *Mt* can be stored as a dry preparation and can germinate hyphally and sporogenically after rehydration. This makes it suitable as a biocontrol agent against *H. verticillata* (Shearer and Jackson 2006). A study conducted by Shearer (2006) showed that the application of a liquid inoculum of *Mt* microsceleria had a 99% efficacy rate in killing *H. verticillata* stands over a four week period. Current studies are focusing on scaling-up inoculum production to be cost effective in its application (Shearer 2006).

In addition to *Hydrilla*, *Mt* has been found effective in controlling against Eurasian watermilfoil *Myriophyllum spicatum* (Netherland and Shearer 1996). It has recently been tried in combined applications with chemicals to reduce the use of chemical compounds in treatments against invasive plants (Shearer and Nelson 2009). During three separate pathogenicity specificity studies, parrotfeather *Myriophyllum aquaticum*, *Ceratophyllum demersu,* and *Hydrilla verticillata* were the only aquatic plant species found to be susceptible to *Mt* (Gunner et al. 1990, Joye and Cofrancesco 1991, Verma and Charduttan 1993). *C. caroliniana* has never been tested against *Mt,* viable biocontrol agents have never been found for *C. caroliniana*, and currently biocontrol agents for *C. caroliniana* are being sought. Therefore, to determine *Mt*'s viability as a biocontrol agent for *C. caroliniana*, this experiment examined the pathogenicity of *Mt* to *C. caroliniana*.

Materials and Methods

Study Specimens: *Cabomba caroliniana* was collected from the Lullwater Research Pond, Atlanta, GA. *Hydrilla verticillata* cultures were shipped from the University of Georgia. Before each experiment, all fanwort and *Hydrilla* fragments were rinsed with deionized water to remove

epiphytes. *Mycoleptodiscus terrestris* cultures on Potato Dextrose Augar (PDA) and Basal Salt Medium (Nelson and Shearer 2005) were shipped from Jackson, Mississippi under USDA Permit #P526P-10-03640.

Inoculum Preparation: Following Jackson (personal communication) and Nelson and Shearer (2005), inocula were prepared as follows: 50 mL Basal Salt Medium (Nelson and Shearer 2005), 30 mL glucose (20% w/v), 6 mL solulysis, and 14mL deionized water were added to two 250mL shake flasks. Shake flasks were autoclaved, and 1/4 PDA plate of *Mt* was added to each flask. Shake flasks were shaken at 300 rpm at 20ºC for 5 days at which time microsclerotia had formed. The inoculum in both shake flasks was poured into a 1000mL beaker and hand shaken till mixed. After preparation, colony forming unit counts were determined by plating 1mL of inoculum onto a PDA plate and counting the number of initial colonies formed.

Experiment One: Experiment one was conducted to quantitatively determine the effects of *Mt* on fanwort growth. Twenty days into the experiment, tanks were moved to another lab. Water temperatures were dependent upon building temperatures and ranged between17ºC-22ºC throughout the experiment. We filled 32, 38 liter tanks with Smart and Barko solution (Smart and Barko, 1984). Plastic cups (473mL) were filled with 500g of garden loam. In each cup, a 10cm (±3 cm) fanwort or *Hydrilla* fragment was planted 3 cm deep. Cups then were covered with a top layer of Aquatic Planting Media. Four fanwort containing cups were placed in each tank assigned to the fanwort treatment (N=28) and six cups were placed in each tank assigned to the *Hydrilla* treatment (N=4). Fragments were allowed to grow out for 22 days under 14 hour light/ 10 hour dark conditions. After 22 days, twenty-eight tanks containing fanwort fragments

were randomly treated with prepared inoculum at application rates of 0mL, 5mL, 7 mL, and 11 mL, and two tanks containing *Hydrilla* were randomly treated with prepared inoculum at 0mL and 7mL. Four fanwort tanks originally designated as 11mL concentrations were left as 0mL due to a miscalculation in inoculum preparation, and one of the 0mL fanwort tanks broke during the experiment and was not counted in the final results. 27 fanwort tanks and four *Hydrilla* tanks were randomly treated at the same application rates seven days later. After 27 days stems were cut at the soil and wet weights of the stems were measured. The stems were air dried for several days and dry weights of the stems were measured. A One-way ANOVA was used to examine the effect of amount of *Mt* inoculum on final wet and dry biomasses.

Experiment Two: Experiment two was conducted to determine if any qualitative effects of *Mt* treatment on fanwort could be observed. For each replicate, an 8 cm (±3 cm) fanwort fragment was placed in a 473mL cup with 350mL of water. Eleven randomly selected replicates were inoculated with 1mL of inoculum from the prepared inoculum to which 100 mL of water were added. Plants were inspected weekly for signs of damage and fungus spread and a damage rating was assigned after 27 days as seen in Shearer (1998). Damage ratings were as follows: 1- no damage, 2- little damage, 3-medium damage (eg. fragmentation and wilting), 4- complete disintegration of fragment. Because of the low number of fragments in each category, fragments with a damage rating of 1 and 2 were grouped together and fragments with a damage rating of 3 and 4 were grouped together. The effect of *Mt* on the frequency of high versus low damage was analyzed using a Fisher's exact test.

Experiment Three: Experiment three was a repeat of experiment one with a larger sample size that included a *Hydrilla* (known to be affected by *Mt*) control group. For each replicate, an 8 cm (±3 cm) fanwort or *Hydrilla* fragment was placed in a glass jar with 350mL of water. Twenty-three randomly selected fanwort replicates and eleven randomly selected *Hydrilla* replicates were inoculated with 1 mL of inoculum. Twenty-four fanwort fragments and eight *Hydrilla* fragments were used as controls. Plants were weekly inspected for signs of damage, fungus spread and chlorosis-the loss of green chlorophyll pigment in plants. After nineteen days, damage ratings were assigned, grouped, and compared for fanwort and *Hydrilla* as described above.

Results

In experiment one, we found that the amount of *Mt* inoculum had no significant effect on the final wet mass of fanwort ($F_{3,23} = 2.102$, $p = 0.128$, Fig. 1).

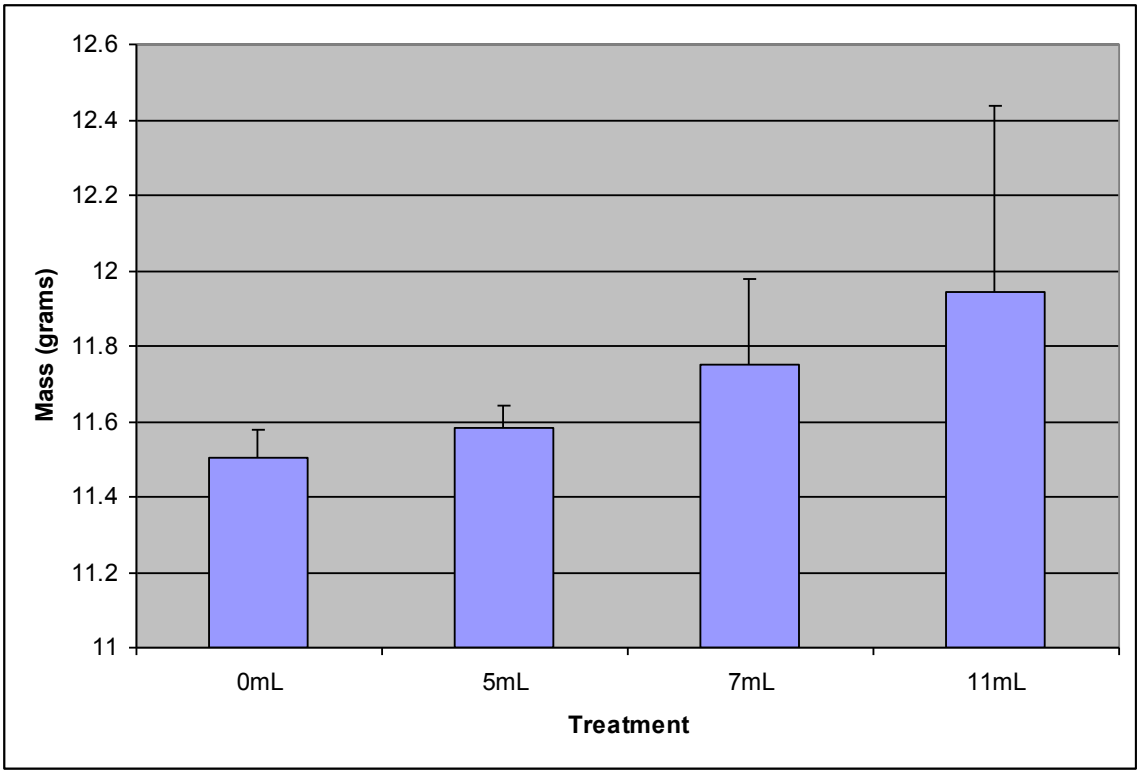

Fig. 1. Final wet mass of fanwort fragments treated with different volumes of *Mt* inoculum.

In addition, amount of *Mt* had no significant effect on the final dry mass ($F_{3,23} = 0.873$ $p = 0.470$, Fig. 2).

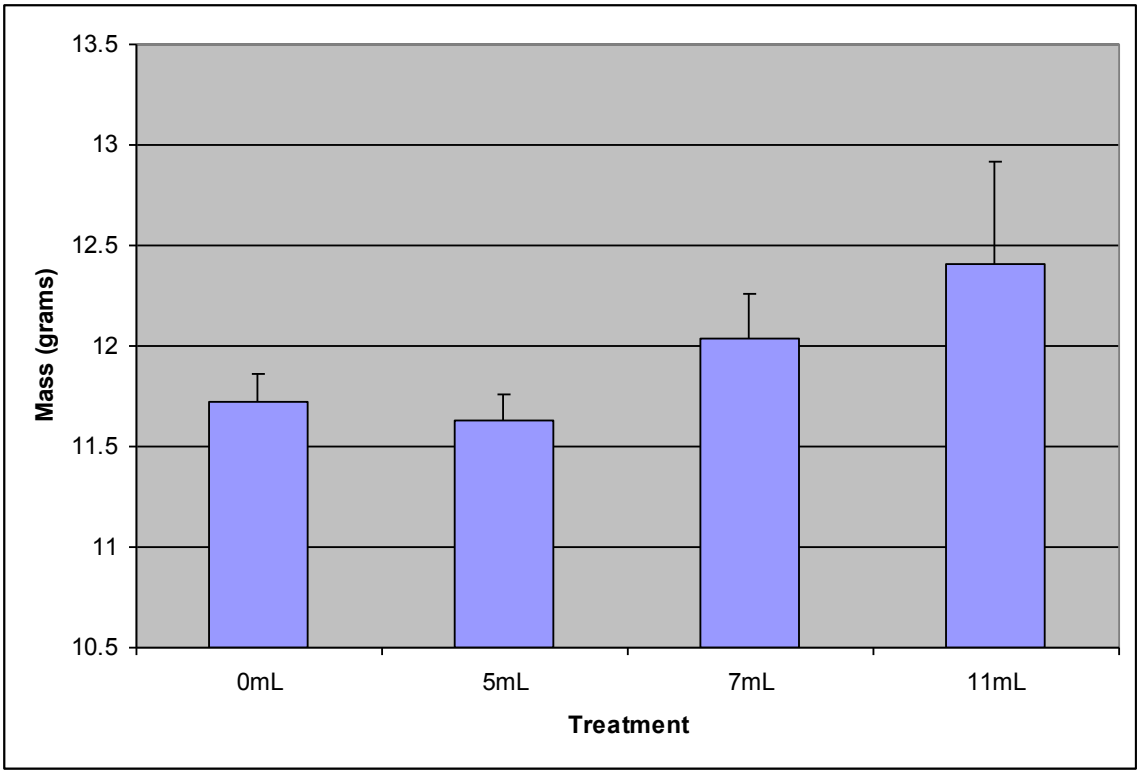

Fig. 2. Final dry mass of fanwort fragments treated with different volumes of *Mt* inoculum.

Interestingly, *Mt* did not seem to reduce final wet or dry mass of *Hydrilla* (Fig. 3, 4).

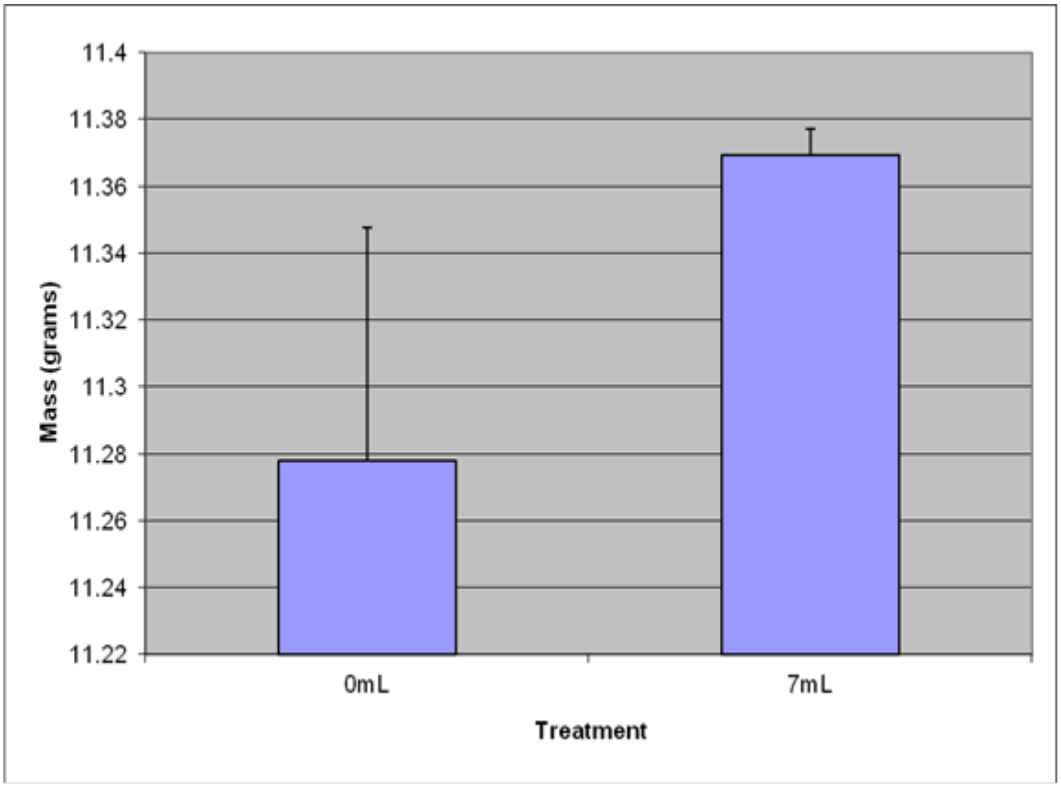

Fig. 3. Differences in final wet mass of *Hydrilla* fragments treated at 0 ml and 7ml.

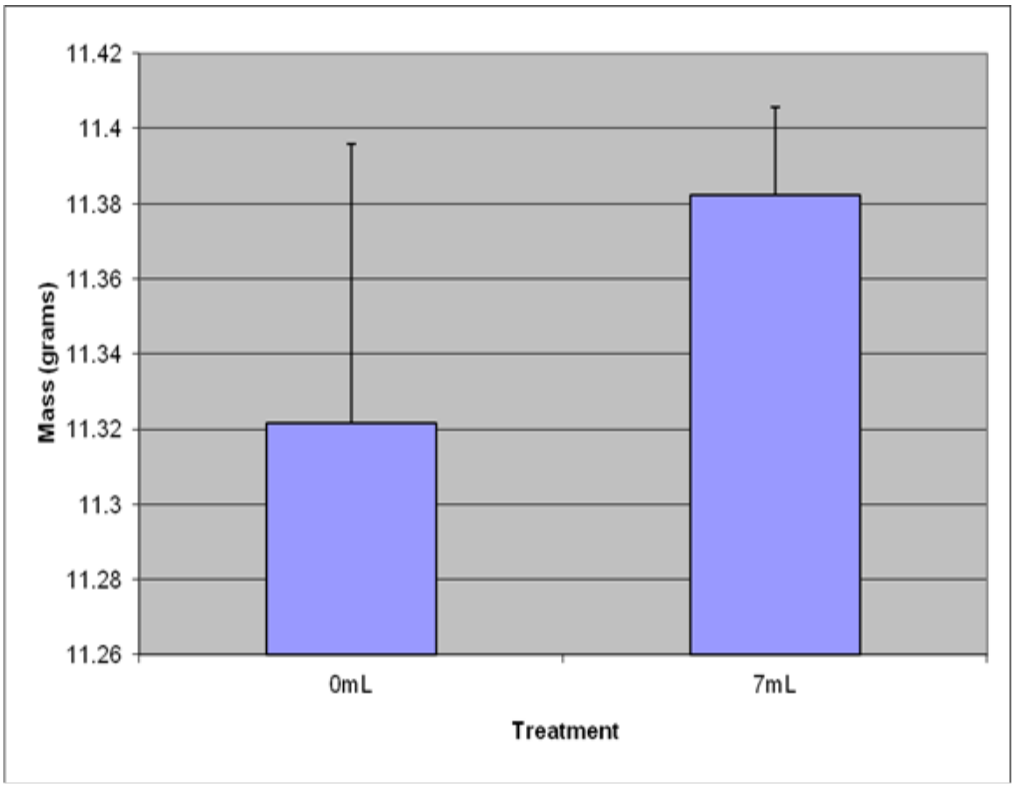

Fig.4. Differences in final dry mass of *Hydrilla* fragments treated at 0 mL and 7mL.

In experiment two, fragments of fanwort treated with *Mt* had significantly more damage than fragments not treated with *Mt* (Fisher's Exact Test, p=0.018). Five of the treated fragments showed high levels of damage, while none of the untreated fragments showed signs of damage (Fig. 5).

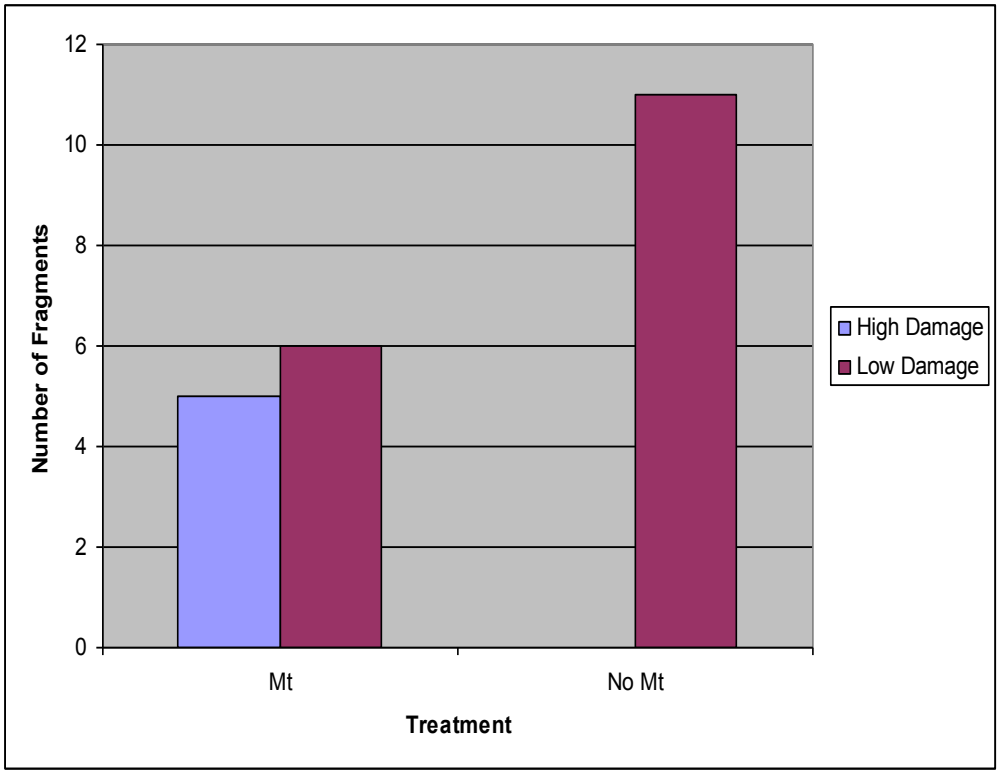

Fig.5. Difference in damage severity between *Mt* treated and untreated fragments.

In experiment three, *Mt* treated fanwort fragments did not have significantly more damage than fanwort fragments not treated with *Mt* (Fisher's Exact Test, p=0.138). Eight of the treated fanwort fragments showed high levels of damage, while four of the untreated fanwort fragments showed high levels of damage (Fig. 6).

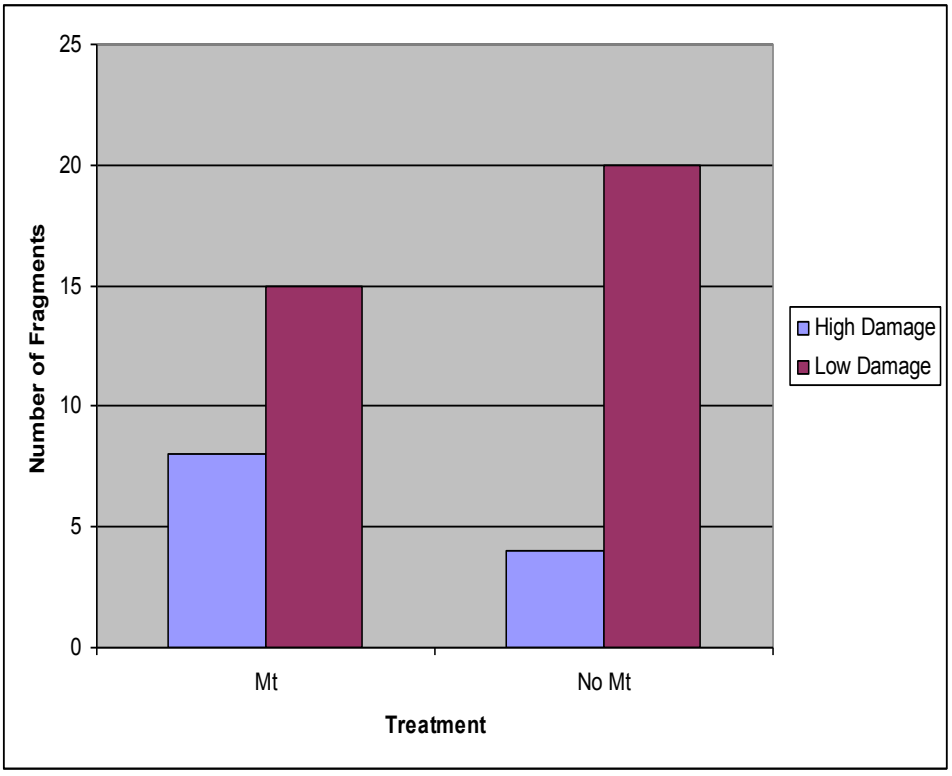

Fig.6. Difference in damage severity between treated vs. untreated fragments during the third experiment.

In experiment three, *Hydrilla* fragments treated with *Mt* had significantly higher levels of damage compared to those not treated with *Mt* (Fisher's Exact test, p=0.006). Ten of the treated *Hydrilla* fragments had high levels of damage, while two of the untreated *Hydrilla* fragments had high levels of damage (Fig. 7).

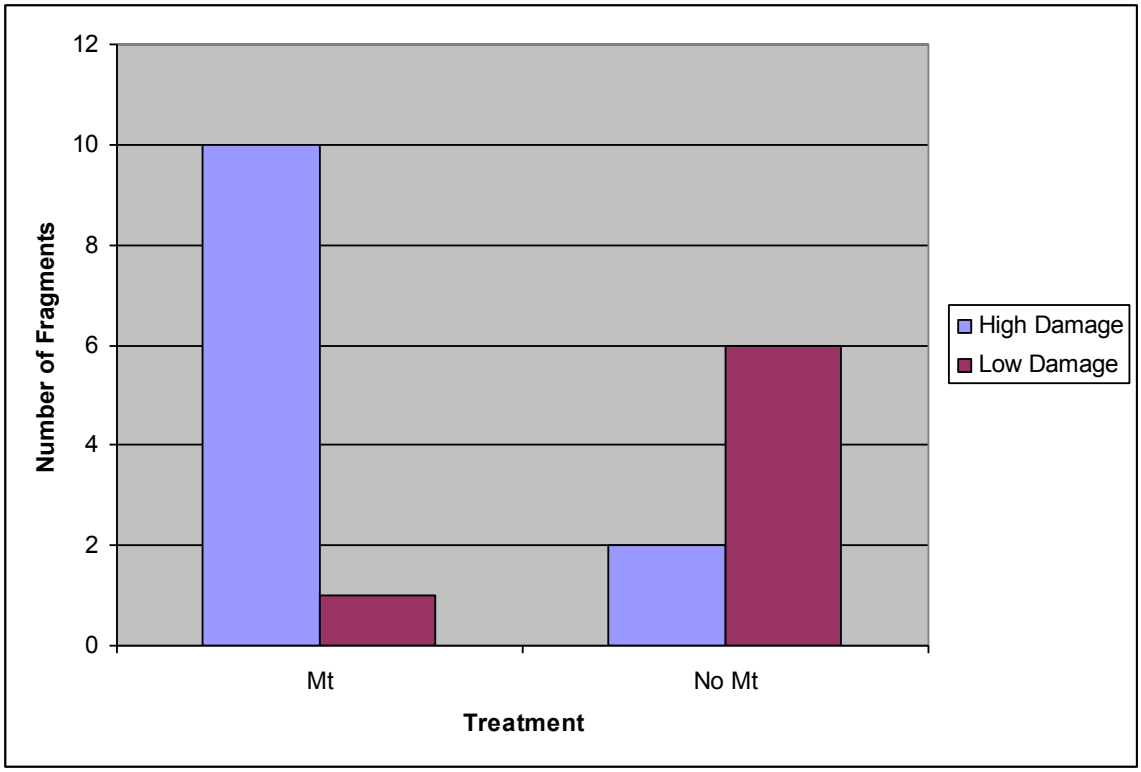

Fig.7. Difference in damage severity between treated vs. untreated *Hydrilla* fragments during experiment three.

Discussion:

Fanwort is becoming a serious invasive weed worldwide (Schooler et al. 2006). Despite its invasive threat, no effective management strategy has been found to control fanwort, and biocontrol agents have been sought (Schooler et al. 2006). *Mt* has been found effective in controlling *Hydrilla* and *Myriophyllum spicatum (*Eurasian watermilfoil). However, the pathogenicity of *Mt* to fanwort has never been tested. Therefore, this research project examined the potential of *Mt* as a biocontrol agent against fanwort.

In experiment two, fanwort treated with *Mt* had significantly higher damage than untreated fanwort. None of the untreated fanwort had signs of damage, whereas five of the treated fanwort had visible signs of damage, as well as fungus spread. This suggests that *Mt* has

an effect on the viability of fanwort fragments. However, during experiment three, fanwort treated with *Mt* did not have significantly higher levels of damage as compared to untreated fragments. Due to the fact that experiment three had twice the sample size of experiment two, the findings of experiment three are much better supported than experiment two. As expected, the *Hydrilla* in experiment three showed greater damage when treated with *Mt*, which suggests the inoculum was prepared correctly and administered at a lethal level. Therefore, assuming the results of experiment three are more accurate than experiment two, the findings of experiment three support the idea that *Mt* has no significant effect on the viability of fanwort fragments. However, due to the limited number of samples within the experiments and the subjective nature of the observations, these results should be viewed with caution. In order to diminish potential bias in the results, we also used a more quantitative approach.

Experiment one attempted to quantitatively assess the effect of *Mt* on fanwort growth. We found no significant difference in final dry or wet masses between the treated and untreated groups of fanwort. However, the *Hydrilla* control group also failed to show a significant difference in growth between treated and untreated groups. At the levels of inoculum used during treatment, we should have found a significant difference in growth between the treated and untreated control groups of *Hydrilla* (Nelson and Shearer 2006). Therefore, it is likely the inoculum was not prepared correctly, or the inoculum was not administered at a lethal dose. The fungal batches used had been sitting out for over 3 months, and it is possible they could have lost their potency. In similar experiments where *Hydrilla* was treated with *Mt*, the *Mt* was administered in a dry inoculum form (Nelson and Shearer 2008). Due to experimental constraints, we did not prepare a dry inoculum and instead a liquid slurry was used. Shearer (1998) used liquid slurries to test for *Mt* effects on *Hydrilla*. However, in Shearer's experiment a

lethal dose of slurry was found to be at 1x 10^5 cfu/ml. Despite strict preparation protocol, the colony forming unit counts in this experiment were only ~50 cfu/ml. Therefore, it is likely the *Mt* concentration was too low to affect the growth of *Hydrilla* or fanwort.

Even if the effect of *Mt* on fanwort shown in experiment two are indeed accurate, this still does not show how *Mt* affects fragments that have taken root and begun to grow. Also, the subjective nature of damage measurements and limited sample size in experiment two support the need for a larger experiment testing for quantitative effects of *Mt* on fanwort. Therefore, in order to more accurately determine the effects of *Mt* on fanwort growth, experiment one needs to be repeated using a larger sample size (for both experimental and control groups) and using a dry inoculum. Dry inoculum is easier to administer in a more standard quantifiable form as compared to liquid slurry, which will make it easier to determine the levels at which *Mt* is able to inhibit fanwort growth, if at all.

The results of this research project suggest *Mt* does not inhibit the viability of fanwort fragments. However, these observations need to be reassessed. I propose conducting an experiment similar to that of experiment one using 50 tanks (50 experimental and 50 control) and dry inoculum. Repeating several trials of this experiment should provide enough quantitative data to support or refute the results of this research project and provide enough information to determine the effect of *Mt* on the growth of fanwort. This will help in determining the potential of *Mt* as a biocontrol agent for fanwort.

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