

**Potential Organic Fungicides for the Control
of Powdery Mildew on *Chrysanthemum x morifolium***

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Abstract

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of Powdery Mildew on *Chrysanthemum x morifolium*

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Powdery mildews are some of the most common plant pathogenic fungi for greenhouse-grown ornamental plants. Millions of dollars are spent annually on fungicides to control these pathogens. There are multiple environmental and human health issues associated with some of the more common fungicides used. These problems dictate a demand for alternative, safe, and environmentally friendly fungicides available on the market. Two fungicide efficacy trials were conducted at the Douglas Research Conservatory at the University of Washington. Four fungicide treatments were evaluated: ammonium oleate, potassium laurate, malic acid, and sesame oil. A popular ornamental plant, *Chrysanthemum x morifolium*, was inoculated with a species of powdery mildew. The species was identified as *Euoidium chrysanthemi* at Washington State University Puyallup Research and Extension Center by sequencing its DNA and looking at key morphological features. This was the first case of *E. chrysanthemi* recorded on *C x morifolium* in the United States. Ammonium oleate and potassium laurate inhibited *E. chrysanthemi* from colonizing *C x morifolium* and suppressed *E. chrysanthemi* growth. However, potassium laurate cannot be recommended because it proved to be phytotoxic at the percent concentration tested. Sesame oil was found to suppress *E. chrysanthemi* growth.

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Introduction

A pesticide is “any substance or mixture of substances intended for defoliating or desiccating plants, preventing fruit drop, inhibiting sprouting, or for preventing, destroying, repelling, or mitigating any insects, rodents, fungi, bacteria, weeds, or other forms of plant or animal life or viruses” (Clemson University 2014). Some common pesticides used in the garden are insecticides, fungicides, herbicides, and nematicides. Pesticides can be beneficial to society in many ways, but they can also be harmful. Pesticides can be damaging to water, soil, wildlife, plants, and air within the environment (National Pesticide Information Center 2012).

Organic pesticides are becoming more and more prevalent. To be termed “organic,” the pesticide must have been produced by “approved methods that integrate cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity” (USDA 2014). Organic fungicides can provide effective control while limiting ecological impact. If they are used improperly, organic fungicides cause some of the same problems as synthetic ones. They may not provide adequate control of the fungus and they could harm non-target organisms. Organic pesticides often require more frequent applications than synthetic pesticides (Beckerman 2008).

A fungicide is a type of pesticide that affects fungi. Fungicides tend to be either preventative, creating a physical or chemical barrier to prevent an infection from taking place, or curative, inhibiting pathogenic fungi from spreading from the original infection site. Preventative fungicides must be applied before the onset of infection. Curative fungicides can be applied after the infection occurs (Calvert and Chalker-Scott 2014). Curative fungicides can act on a single protein, enzyme, specific location of metabolic pathway (single site mode of action) or a number

of different sites within the fungus (Iowa State University 2006). The frequent application of fungicide products with the same mode of action has been found to lead to resistance within pathogen populations (Brent and Holloman 2007). When applying fungicides, it is important to repeat applications every 7, 10, or 14 days to protect new growth that has developed (Beckerman 2008).

Powdery mildews are plant pathogenic fungi. They are obligate parasites, meaning they require living plant tissues to survive. Fungicides for the control of powdery mildew are the most commonly used fungicide in Western Europe. Millions of dollars are spent annually to control powdery mildew (Hewitt 1998). Powdery mildew is a widely distributed detrimental disease for vegetables, fruits, and greenhouse grown ornamental plants (Mueller et al. 2003). Signs and symptoms will appear within 3-7 days of initial infection in late summer or early fall. The fungus is spread through asexual spores known as conidia. Favorable conditions for the growth of the disease include dense plant growth, low light, and temperatures between 68-80 F°. High humidity can be favorable for infection and conidial survival; however, dryness is favorable for colonization, sporulation, and dispersal (Cornell University 2011).

Fungicides should be used for powdery mildew on a need basis when environmental conditions are most conducive to disease development (Mueller et al. 2003). Several chemical fungicides are recommended for use against powdery mildews. One of the most widely used organic fungicides is sulfur. There are three major disadvantages to the use of sulfur: (1) sulfur can be phytotoxic in the greenhouse when temperatures exceed 30 C°, (2) sulfur negatively affects beneficial organisms (Belanger and Labbe 2002), and (3) sulfur can accelerate the degradation of greenhouse plastic covers (Giotis 2006). In the United States, powdery mildew populations have shown resistance to the synthetic fungicides benomyl and triadimefon

(McGrath 2001). Research on alternative organic fungicides is needed. In this study the fungicidal potential of ammonium oleate, malic acid, potassium laurate, and sesame oil were evaluated. These compounds were chosen from the list formulated by Calvert and Chalker-Scott (2014). Each of these potential fungicides is used in a multitude of fungicide formulations (Washington State University 2015) even though there has been no scientific evidence showing that they have fungicidal abilities. It is currently unclear if any of them have an effect on the growth of powdery mildew. Sound scientific evidence is needed to deter the public from using potentially harmful broad-spectrum compounds with little to no fungicidal properties.

The powdery mildews have been reported to infect over 10,000 species of plants (Amano 1986). *Chrysanthemum* is a genus of plants in Asteraceae that has been found to be a host for powdery mildew. *Chrysanthemum x morfolium* is the most popular species for use as an ornamental. It is an autumn blooming perennial that can be grown from Zones 2-14. This hybrid species is used for borders, containers, and cuttings. *C. x morfolium* exists in many colors, sizes, and growth habits. The size of this hybrid species ranges from 1 to 6 feet (Brenzel 2012).

Literature Review

Salts of Fatty Acids

Ammonium salts of fatty acids (ammonium oleate) and potassium salts of fatty acids (potassium laurate) are two types of soap salts. Fatty acids are naturally occurring substances that are metabolized into simple compounds that supply energy used in all living cells. They also function as gene regulators, and are essential structural components of all membranes (Ruston and Drevon 2005). Potassium salts of fatty acids are commonly used as algacides, fungicides, herbicides, and insecticides (National Pesticide Information Center 2001). The EPA states that

there are some risks to applicators and consumers of soap salts. Some of these risks include minor skin irritations and eye damage. Reproductive issues have also been recorded in laboratory studies of animals. Soap salts have also been found to be toxic towards fish. Because of this each label must contain the following statement, “This product may be hazardous to aquatic invertebrates. Do not apply directly to water areas where surface water is present or to intertidal areas below mean high water mark. Do not contaminate water by cleaning of equipment or disposal of water.” (EPA 1992).

Potassium salts used as insecticide work by penetrating an insect’s cuticle and disrupting an insect’s cell membranes. This causes the insect to dehydrate due to its intercellular fluid flowing out. Soft tissue insects that are damaging to plants such as aphids, mealy bugs and white flies are more susceptible to this broad spectrum fungicide than some beneficial insects such as bees and ladybugs (National Pesticide Information Center 2001).

As a fungicide, potassium salts tested at 2.0% were found to increase yield of winter squash and muskmelon by suppressing powdery mildew growth. It was found to be most effective on the upper leaf surfaces (McGrath and Shishkoff 1999). M-Pede Insecticide/Fungicide, M-Pede Insecticide-Miticicide-Fungicide, Safer Brand 3-in-1 Concentration, and Safer Brand 3-in-1 Garden Spray are commercially available products advertised to control powdery mildew with the active ingredient potassium laurate. The concentration of potassium laurate in these products ranges from 0.75% up to 49.0%. The M-Pede products contain 49.0% potassium laurate. The Safer Brand products have a lower concentration of potassium laurate but list sulfur as an active ingredient. There are sixteen pesticides that list ammonium salts of fatty acids as an active ingredient. The active ingredient concentration of ammonium salts in these products ranges from 0.66% up to 22.0%. Hinder, is a

product that uses 0.66% ammonium oleate. It claims that their product is safe for use on vegetables, fruits, flowers, ornamentals, vine plants, and shrubs. The products that have a 3.68% or higher concentration of ammonium salts of fatty acids are advertised as herbicides (Washington State University 2015).

Organic Acids

Malic acid is an organic dicarboxylic acid found in all living organisms. Organic acids are involved in many biochemical pathways such as the production of energy, amino acid biosynthesis, and plant defense mechanisms. They are mainly produced in mitochondria through tricarboxylic acid or the Krebs cycle. Malic acid is found in a higher concentration in plants than in any other organism. This is most likely because of its role as photosynthetic intermediates in CAM and C4 plants. The U.S. Environmental Protection Agency considers malic acid a “minimum risk pesticide.” Minimum risk pesticides are “pesticides that are not subject to federal registration requirements because their ingredients, both active and inert, are demonstrably safe for the intended use” (EPA 2013).

Malic acid, tartaric acid, citric acid, lactic acid, and acetic acid have shown promising antimicrobial properties. It has been tested for its use against food borne pathogens (Dickson 1992 and Eswaranandam et al. 2004). Jafari and Hadavi (2012) conducted experiments with malic acid and citric acid. They found that applying 0.1% malic acid three times every 10 days on *Anethum graveolens* (Dill), a plant in Apiaceae, significantly increased the mean plant height from 82.6 cm to 92.1 cm. The authors stated that this increased growth could be due to malic acid enhancing the proton pumping capabilities of roots. Jafari and Hadavi (2012) found citric acid to significantly reduce the incidence of powdery mildew on *A. graveolens*. In this experiment they found citric acid to decrease the incidence of powdery mildew. The authors

assume that the increased disease resistance is due to citric acid altering the carbohydrate partitioning towards pathways that are more related to resistance to unfavorable conditions. When only malic acid was used, the disease resistance observed was minimal. Jafari and Hadavi (2012) conclude that “citric acid and malic acid could be considered as readily available tools for manipulation of natural plant resistance against unfavorable conditions.” Monterey All Natural Disease Control RTU, a product that claims to control powdery mildew, uses 0.02% malic acid along with rosemary oil, cloves/clove oil, and peppermint. Dr. Earth Final Stop Disease Control Fungicide lists malic acid as one of its active ingredients.

Sesame Oil

Sesame oil is extracted from the seed of *Sesamum indicum*. The seed has a high oil percentage constituting 50% of its weight. It is mainly used as a food source throughout the world and is predominantly made up of the fatty acids oleic and linoleic acid. Sesame oil has been reported as having a multitude of antioxidant properties (Wan et al. 2015).

Organocide, a fungicide that uses a 5.0% solution of sesame oil, showed no phytotoxic effects on coleus plants (Cloyd et al. 2009). Ex-icute/Rapid-O Rid-Bugs claims to control “fungal diseases like powdery mildew” and has a 5.0% concentration of sesame oil (Washington State University 2015). Organocide fungicidal ability has yielded mixed results (McGrath 2005, McGrath and Davey 2007, and Keinath and Dubose 2012). Keinath and Dubose (2012) found Organocide to have curative and preventative fungicidal properties. Organocide provided 85% control of powdery mildew on the upper surface of pumpkin cotyledons. In one of their experiments, Organocide also controlled the disease on the lower surface of the cotyledons. Organocide is 92% edible fish oil which makes it unclear whether the fish oil and/or the sesame oil have fungicidal properties.

Inoculation Methods

As mentioned earlier, powdery mildew is an obligate parasite and requires a living host to survive. A common way to preserve the fungus is to propagate it on susceptible hosts while occasionally transferring it to fresh material (Barden et al. 2007). There is no published optimal method for transferring conidia for germination and inoculation from one host to another. The methods used vary enormously (Nicot et al. 2002). Some methods mentioned used an artist's brush to dislodge conidia by tapping on an infected leaf and pressing spores from a leaf surface onto a substrate. Incubation is usually performed at room temperature in a dark chamber at 100% relative humidity (Cook and Braun 2009). Barden et al. (2007) successfully used a sterile paintbrush to spread spores of *Golovinomyces cichoracearum*, isolated from a melon, on the entire surface of fresh melon cotyledons. The maximum number of viable spores produced was observed between 12 and 19 days after inoculation. Cotyledons from the species *Lagenaria* cv. 'Minibottle' were used successfully as a culture medium. Spore viability was highest at 10 days of culture on the cotyledons. After 10 days, the germination rate decreased significantly (Barden et al. 2007). Twenty-one degrees Celsius is reported as the optimal temperature for the development of powdery mildews (Jarvis et al. 2002).

Grading Scale

The Horsfall-Barratt Scale is a commonly used tool to estimate the severity of a disease on a plant. The Horsfall-Barratt Scale is the most widely used and cited scale in plant pathology. It is an interval scale that divides percent disease severity into categories based on a logarithmic function. The scale was based on a logarithmic function because of the assumption that estimated disease was logarithmically related to true disease (Bock et al. 2009). The scale is meant to increase accuracy while subjectively analyzing the presence of disease. The symmetry of the scale was developed below and above 50% due to the assumption that when subjectively assessing disease severity, the eye reads infected tissue below 50% disease and healthy tissue above 50% disease. Each leaf is graded on the scale referenced in Table 1. The percent midpoint is calculated and averaged with the rest of the leaves on a given plant. This calculation yields the estimated mean percent disease severity for each plant (Horsfall and Barrett 1945).

AUDPC Curves

Area under the disease progress curves (AUDPC) are commonly used in plant pathology to assess the severity of a disease. It is calculated by the formula below which looks at the disease severity over time (Agrios 2005). A lower AUDPC score correlates to a plant being less susceptible to the disease.

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

ti=time interval

yi=disease severity

(American Phytopathological Society 2015)

Fungal Identification

The first objective of this study was to identify the powdery mildew species used. There are approximately 20 genera consisting of 100 species of powdery mildew. Species tend to be host specific and can vary anatomically (Braun and Cook 2012). It is important to know where different species are found across the globe, the host range of each species, the growth rate of each species, and successful ways to control outbreaks of each species.

Materials and Methods

Powdery mildew was collected off of *C. x morifolium* plants growing outside at the Center for Urban Horticulture at the University of Washington in the Fall of 2014. The fungus was brought to Washington State Puyallup Research and Extension Center. At the center Kathryn Coats took samples of the fungus, and had its DNA sequenced, using the PCR technique (White et al. 1990). Photographs were taken of different morphological features using a Leica Digital Camera hooked up to a compound and dissecting microscope.

DNA sequencing was done using a polymerase chain reaction (PCR) technique. This technique is a quick and inexpensive way to amplify small segments of DNA (National Human Genome Research Institute 2015). MEGA 6 software was used to align the ITS region sequence with closely related species that have ITS data in GenBank (2015).

The powdery mildew was later identified with a compound microscope using the descriptions and diagrams from Braun and Cook (2012). The spores were spread onto the upper surface of the leaves with the tip of a sterile paintbrush (Bardin et al. 2007). After each inoculation, the plants were placed in plastic bags in the dark for 24 hours (Whipps and Heyler 1994). When applying the fungicide treatments, the plants were taken outside of the greenhouse

into the head house. This ensured that no cross contamination occurred between the treatments.

Results

Identification of the fungi was first done through DNA sequencing using the PCR technique (White et al. 1990). DNA segments are isolated from the genome and amplified. Short DNA molecules are chemically synthesized. This is done in a reaction catalyzed by a purified DNA polymerase that is attained from a bacterium that is stable at extremely high temperatures. Multiple cycles of DNA synthesis are completed in this technique, creating billions of copies of the specified DNA molecules. The products of each cycle are used as the template for DNA synthesis for the next cycle. A brief heat treatment is applied at each cycle to separate the two strands of the DNA (Albets et al. 2002). The Internal Transcribed Spacers (ITS) 4 and 6 primers of nuclear DNA were used to identify the fungi.

Four samples of the fungi were collected off of four separate plants. The DNA sequence from the first two samples seemed to be of poor quality because there was plant and fungal DNA present. The other two only consisted of a fungus. They were amplified and had an identical sequence. The sequence is listed below:

```
CAGAGCGTGAGACTCTGCCCGGGCTCGTCCCGCGCGCCGAGTTGACCCTCCACCCGT
GTTGACTTATTTTCATGTTGCTTTGGCGGGCCAGGCGCTGAGCGTCTACCGGCTTCGG
GCTGGCTCGTGTCCGCCAAAGACCCAACCTAACTCGTGTTATCGTGTAGTCTGAGGA
AATAACTATTGAATTGTTAAAACCTTTCAACAACGGATCTCTTGGCTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTTAGTGAATCATC
GAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTCGAGC
GTCGTCACACCCCTCAAGCCGTGCCCTGTGTATGGCTTGGTGTGGGGCTCGCCAGT
```

CTGGCGGCCCTTAAAGACAGTGGCGGTGCCGGTGTGGTCTCTACGCGTAGTACAAGT
CTCGCGACAGAGCTCCAGCGGCTGCTTGCCAATCAATCTTTCAATTCAAGG.

The fungi were also examined under a compound and dissecting microscope to try to find a match through its morphology.

Figure 1: Pictures of conidia taken with a Leica Digital Camera hooked onto a compound microscope:

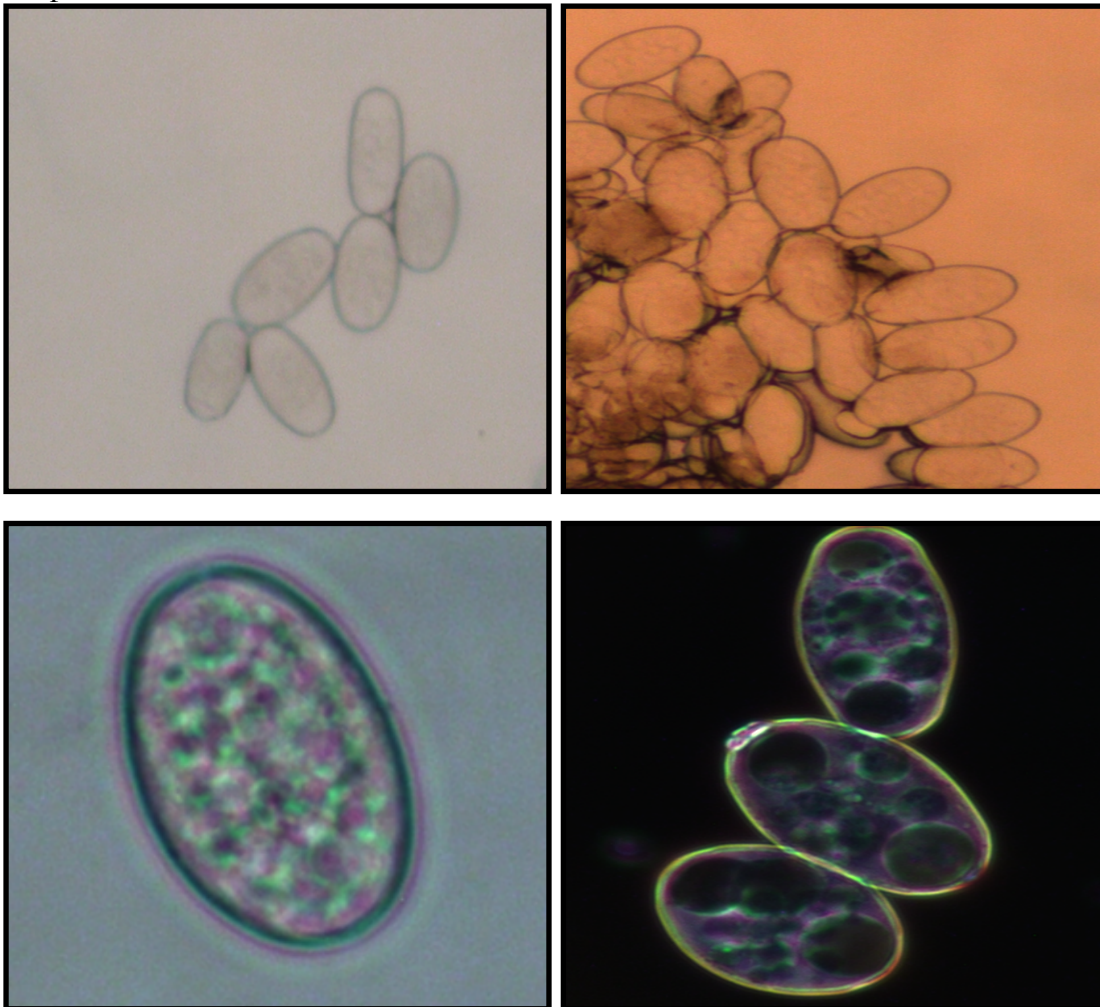
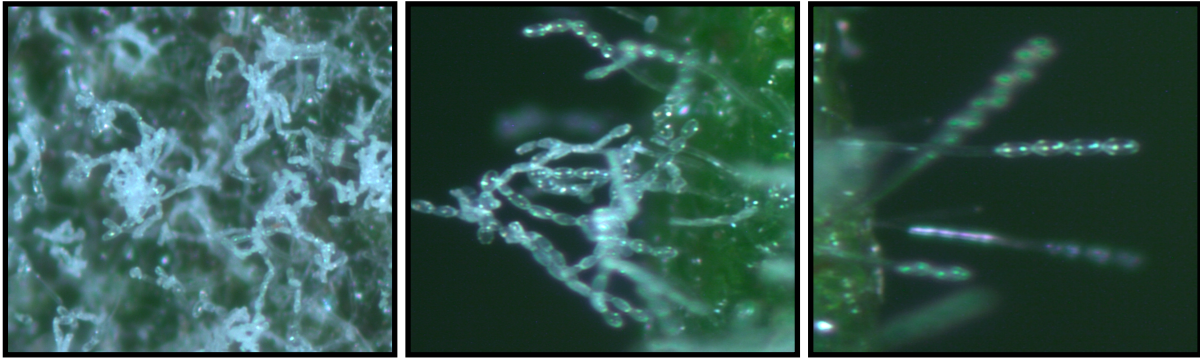


Figure 2: Pictures of conidiophores taken with a Leica Digital Camera hooked onto a dissecting microscope:



Measurements of the length and width of 10 conidia were taken. They were found to have an average width of $19.99 \mu\text{m}$ and an average length of $37.4124 \mu\text{m}$. The average l/w ratio was 1.88.

The following morphological features were noted:

- 1) The conidia are ellipsoid and catenescant. (Figures 1 and 2)
- 2) The mycelium is on leaves, stalks, and flowers and branches at right angles.
- 3) The appressoria is indistinct.

Discussion

The species with the closest DNA sequence were *Euoidium chrysanthemi*, *Golovinomyces artemisiae*, and *Golovinomyces cichoracearum*. Out of 511 nucleotides, *E. chrysanthemi* had one different base, *G. artemisiae* had three different bases and *G. cichoracearum* had 40 different bases.

According to the United States Department of Agriculture Fungal Databases (2015), *C. x morifolium* has been recorded as a host for *E. chrysanthemi* and *G. cichoracearum*. *E. chrysanthemi* has been recorded on *C. x morifolium* in Germany and South Africa. *G. cichoracearum* has been recorded on *C. x morifolium* in Alaska, California, Canada, Florida, North Carolina, New Zealand, Puerto Rico, South Africa, South Dakota, and the Virgin Islands.

Braun and Cook (2012) have described *E. chrysanthemi*, *G. artemisiae*, and *G. cichoracearum* in detail:

E. chrysanthemi have cylindrical, doliform, or ellipsoid-ovoid conidia. They are found in chains and their width ranges from 16-22.5 μm and their length ranges from 35-50 μm . The appressoria is indistinct but can occasionally be nipple shaped. The mycelium is found on stems, leaves and inflorescences branching at right angles.

G. artemisiae have ellipsoid-ovoid to doliiform conidia that are sometimes found to be subcylindric. They are found in chains and their width ranges from 16.0-26.0 μm and their length ranges from 24.0-35.0 μm . The length to width ratio is from 1.1-1.8 μm . The appressoria is nipple shaped.

G. cichoracearum have cylindric-doliiform to ellipsoid-ovoid conidia. They are found in chains and their length ranges from 25.0 μm - 45.0 μm and their width ranges from 14.0 μm - 22.0 μm . The length to width ratio is around 2.0 μm . The appressoria is nipple shaped. Mycelium is found on stems and leaves.

The evidence from the DNA test shows that the fungi is most likely *E. chrysanthemi* due to it having the most similar ITS region sequence. Differences in DNA sequences can be a result of multiple factors such as mutations and crossing over. Characteristic morphology was examined under the microscope to ensure identification accuracy.

The morphology witnessed under the microscope led me to conclude that the species being studied is *E. chrysanthemi*. The most conclusive evidence is the indistinct appressoria. Besides the indistinct appressoria, the high length to width ratio allowed me to eliminate *G. artemisiae*, and the mycelium found on the flowers allowed me to eliminate *G. cichoracearum*. According to

the United States Department of Agriculture Fungal Databases (2015) this is the first case of *E. chrysanthemi* reported on *C. x morifolium* in the United States and the first case of *E. chrysanthemi* on any host in Washington State.

Fungicide Experiments

The second objective of this study was to evaluate four potential fungicides for the control of powdery mildew. If the results do not show promise for any of the potential fungicides, it should not be concluded that the compound lacks fungicidal abilities. Malic acid, ammonium oleate, potassium laurate, and/or sesame oil may have synergistic effects. No conclusion can be made about their synergistic effects in this study. Each compound tested is considered “environmentally friendly” and is currently used in at least one commercial product (EPA 2013).

Materials and Methods

Tests were done to determine if malic acid, ammonium oleate, potassium laurate, and/or sesame oil are able to control or prevent the growth of powdery mildew on *C. x morifolium* in the greenhouse. Two trials were conducted in the Douglas Research Conservatory at the University of Washington. The first trial was conducted in the winter of 2015 and the second trial was conducted in the spring of 2015. For the first trial there were six treatments, each with 12 plant replicates.

(1) Control 1: Distilled water sprayings, *C. x morifolium* not inoculated with powdery mildew

(2) Control 2: Distilled water sprayings, *C. x morifolium* inoculated with powdery mildew

(3) Sulfur at 10.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(4) Ammonium oleate at 2.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(5) Malic acid at 2.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(6) Sesame oil at 5.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

In the second trial, potassium laurate became available. Malic acid was found to be phytotoxic at 2.0% sprayings during the first trial, so a new solution was made containing a 0.5% solution.

(1) Control 1: Distilled water sprayings, *C. x morifolium* not inoculated with powdery mildew

(2) Control 2: Distilled water sprayings, *C. x morifolium* inoculated with powdery mildew

(3) Sulfur at 10.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(4) Ammonium oleate at 2.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(5) Potassium laurate at 20.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(6) Malic acid at 0.5% solution sprayings, *C. x morifolium* inoculated with powdery mildew

(7) Sesame oil at 5.0% solution sprayings, *C. x morifolium* inoculated with powdery mildew

The two experiments done in each trial were:

Experiment 1: Test for preventative qualities of the potential fungicides in the greenhouse.

Spraying were applied at 1 and 7 days before plants were inoculated with powdery mildew.

Hypothesis 1: The potential fungicides will prevent the growth of powdery mildew on *C. x morifolium*.

Experiment 2: Test for curative qualities of the potential fungicides in the greenhouse. Spraying were applied after 75% of the test plants showed a minimum powdery mildew rating of two on the Horsfall-Barratt scale (Table 1).

Hypothesis 2: The potential fungicides will control an existing powdery mildew infection on *C. x morifolium*.

The first control was included to assess if the disease would occur if the plants were only sprayed with water and not inoculated. The second control was identical to the treatments except distilled water was applied to the plants. Distilled water was used to assess the effect of the spray application on the disease. The statistical analysis compared all the treatments to the second control.

Powdery mildew was collected off of *C. x morifolium* plants growing outside at the Center for Urban Horticulture at the University of Washington. In the fall of 2014 five plants were inoculated. This was accomplished by spreading the spores from infected plants onto these five healthy plants using a sterile paintbrush. The plants were then placed into plastic bags and covered in tinfoil for 24 hours to increase humidity and limit light. The five inoculated plants developed signs of powdery mildew and were used to inoculate the plants used in the first trial.

The infected control plants from the first trial were used for the inoculation of the plants in the second trial.

The trials were conducted at the Douglas Research Conservatory. The Conservatory has a “swamp cooler” system. In this system water is pumped through metal filters along the side of the greenhouse and drawn across the zones via fans. In order to heat the greenhouse, hot water is pumped into pipes that run throughout the zones. The first trial was conducted between January 24th and March 14th. In this time period the temperature in the greenhouse ranged from 68.76°-70.27°F with an average of 69.56°F. The humidity ranged from 31.48%-57.57 % with an average of 46.13%. The second trial was conducted between March 15th and April 19th. In this time period the temperature ranged from 68°-72°F with an average of 69.56°F. The humidity ranged from 36.74%-52.04% with an average of 44.61%.

In fall of 2014, cuttings of *C. x morifolium* “Apricot” plants were taken. The cuttings were inspected with a hand lens to confirm that no plants appeared infected by powdery mildew. The cuttings were put into flats containing vermiculite and were placed into a misting chamber in the Douglas Research Conservatory. Within two weeks roots had formed and the non-infected plants were placed in 1 ¾ inch plastic square pots 15 cm apart.

In each trial there were 12 plants per treatment. In the first trial, there were 6 treatments per experiment totaling 144 plants. In the second trial, there were 7 treatments per experiment totaling 168 plants. The greater the number of plants used, the greater the ability of statistical tests’ to detect differences between treatments. The experiments were conducted in a completely randomized design (Figures 3 and 4). Each plant was assigned a 6-digit random number. The plants were then placed in numerical order. The two experiments were integrated together into

this design due to the limited amount of available space in the greenhouse.

The fungicide solutions were created before the onset of the experiments. A 100ml solution of the fungicide and distilled water was added into an Erlenmeyer flask. They were mixed using a stir bar on a stir plate. Each solution was mixed for at least 20 minutes until no sign of the solute remained. The solutions were placed into a 75ml hand sprayer and stored in the dark until applications were applied. The solutions were applied to the plants evenly on all sides until visible droplets could be seen on the foliage. The sesame oil had to be shaken in the hand sprayer for at least one minute before every use in order to emulsify the water and oil.

Common horticultural practices (watering, weeding, etc.) were conducted whenever needed. Nitrogen has been shown to contribute to the growth of powdery mildew (Braun and Cook 2012) so no fertilizer was used in the research. Each plant had its own sub-irrigation system to control for the effect of water on the fungus. All possible symptoms of the disease and other factors such as insect damage were recorded.

The plants were graded on the Horsfall-Barratt Plant Pathology Scale based on a subjective analysis of disease presence (Table 1) (Horsfall and Barratt 1945). Plants were graded every 7 days after initial inoculation. Before the research was completed, photographs were taken of expected outlook of plants under each rating (Figure 5). The same person graded each experiment to reduce variation between graders. The data were collected with a double-blind methodology to eliminate unconscious bias. The experimenter did not know which treatment he was applying and the observer was unaware of which treatment he or she was grading. When grading and treatment applications were completed, the plants were brought into the head house and lined up in random order. This ensured that there were even applications of all the treatments

and that all plants were graded blind on an equal scale regardless of the treatment applied.

Wald linear regression tests (University of California Los Angeles 2015) were used to compare the potential fungicides to the second (inoculated) control. The plants were only compared to the inoculated control to reduce the number of variables. The Wald linear regression model was chosen because of its ability to factor in plants losing leaves throughout the experiment. ANOVA tests aren't able to calculate this variable into the equation. The Wald test was done instead of a One-Way ANOVA because comparing plants with one leaf to plants with 6 leaves can lead to misleading results. A plant with one leaf could have a higher Horsfall-Barratt score than a plant with six leaves even though the plant with one leaf had a less severe outbreak. The null hypothesis of the Wald test is "that a set of parameters is equal to some value" (University of California Los Angeles 2015). In this experiment the null hypothesis is that the treatments powdery mildew scores are equal to the inoculated controls powdery mildew scores. The treatment variables were jointly tested to be equal to the inoculated control at $\alpha=0.05$. The result of the Wald test gives a point estimate, standard error and a confidence interval for the different treatments. The point estimate reports the difference between the control and treatments score. A score of zero corresponds to no difference. The confidence interval reports the range of scores that will be seen in a populations mean. There is a correspondence between the p-values of the individual treatments and the confidence intervals. If the confidence interval does not contain 0, then the p-value is less than 0.05 and the treatment is significantly different from the control. The standard error reports the error, in mean difference (point estimate), between the treatment and the control. The standard error was not reported on the disease growth graphs because it does not report the error in the mean as it does for an ANOVA test. The individual treatments cannot be considered relevant unless there is pooled statistical significance. This is

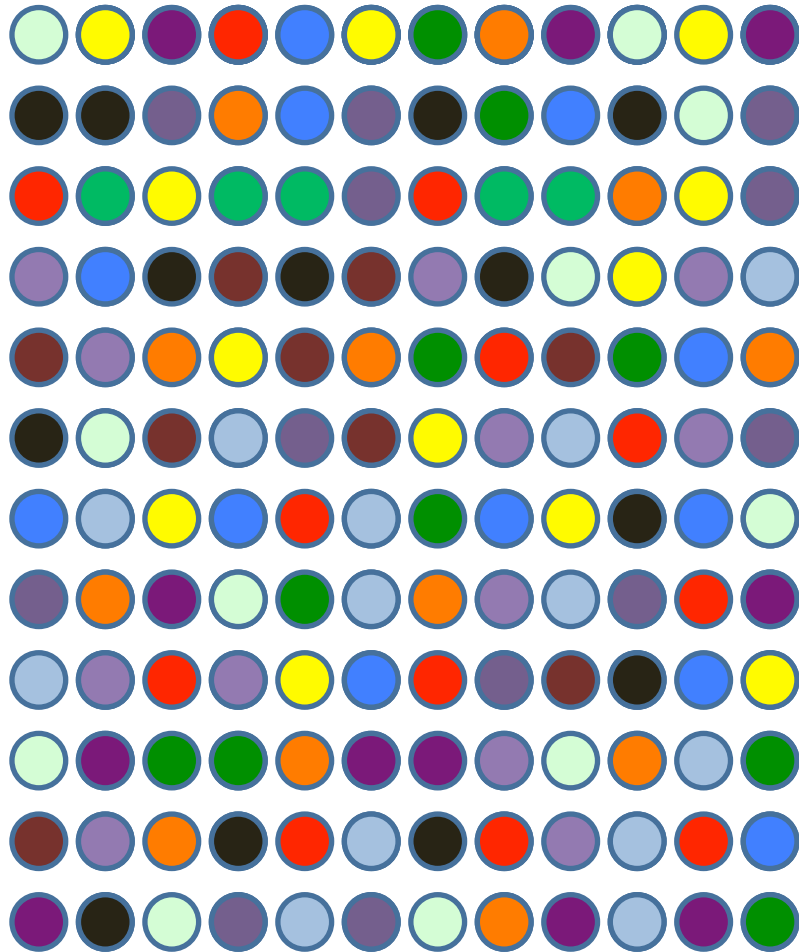
expressed in the p-value below each Wald table. Great disparity among the treatments could imply that there is a problem with the experiment. The pooled statistical significance looks at the overall significance of the data. The tests were computed using R-Commander within R version 3.2.2 (The R Project for Statistical computing). One Way -ANOVA tests were used instead of the Wald test for the biomass calculations. For these calculation there wasn't a need to factor in the number of leaves each plant lost. This could have skewed the biomass data. Plants that lost a similar amount of leaves are more likely to have a similar biomass. If the test was found to be significant, then a Tukey's Honestly Significant Difference test ($P < 0.05$) was conducted to see which treatments had significantly different means.

Leaves were examined and graded on the Horsfall-Barratt Scale for powdery mildew (Horsfall and Barratt 1945). An AUDPC (Area Under the Disease Progress Curve) was created to analyze the change of disease severity over time for the second trial. For the AUDPC the percent of each plant diseased was found. The percentage of each leaf diseased was obtained by calculating the mid point from the range correlated with each Horsfall-Barratt value. The percentage of disease coverage on an entire plant was calculated by averaging all the leaves on a plant.

In the curative experiments, incidence of powdery mildew was noted. This looked at whether there was any sign of powdery mildew on the new growth or old growth. A score of 0 signified that no signs were observed and a score of 1 signified that signs were observed. This was used to evaluate whether the treatments had an effect on the incidence of powdery mildew anywhere on the plant. For the preventative experiments, this evaluation was not conducted. New growth in the preventative experiment was not applied with any treatment so no insightful data could be obtained from this evaluation.

After the second trial was completed, the plants' biomass was measured and compared. The plants used in the first trial were needed for the inoculation of the plants in the second trial. Because of this their biomass could not be obtained. To take the biomass, the plants were initially placed in buckets full of water where their roots were washed of all soil. They were then placed into brown paper bags and brought to the University of Washington herbarium dryer where they sat for four days in 100° Fahrenheit. The plants were weighed in grams with an ADAM digital scale to three decimal places.

Figure 3: Experimental design in the first trial. The preventive and curative experiments were conducted at the same time in a completely randomized design. Treatment applications and inoculations for each experiment were applied on different days.



Preventative Experiment
 ●=Control 1
 ●=Control 2
 ●=Sulfur
 ●= Ammonium Oleate
 ●= Malic Acid
 ●=Sesame Oil

Curative Experiment
 ●=Control 1
 ●=Control 2
 ●=Sulfur
 ●= Ammonium Oleate
 ●= Malic Acid
 ●=Sesame Oil

Figure 4: Picture of the experimental set up in the Douglas Greenhouse. In this picture the curative plants are being inoculated in the first trial.

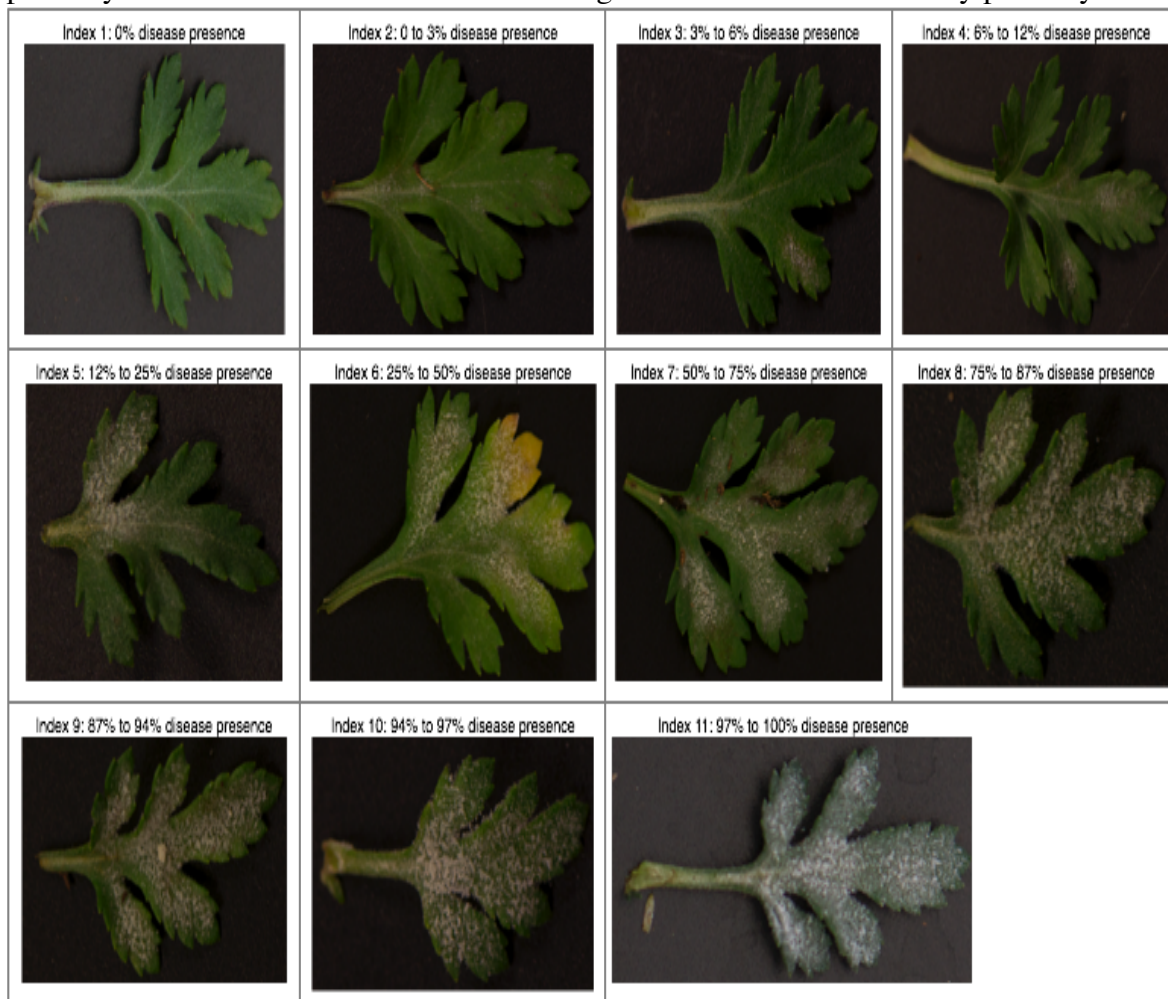


Table 1: Horsfall-Barratt scale used to evaluate disease severity on *C. x morifolium*.

Index	Percent of Leaf Infected
1	0
2	0 to 3
3	3 to 6
4	6 to 12
5	12 to 25
6	25 to 50
7	50 to 75
8	75 to 87
9	87 to 94
10	94 to 97
11	97 to 100

(Horsfall-Barratt 1945)

Figure 5: Horsfall-Barratt scale example created before experiments were conducted for reference. Differences between indexes 9, 10 and 11 were observed by looking at the amount of powdery mildew along the outer margin of the leaves. Index 9 had around 10% of its margin free of powdery mildew. Index 10 had a very small outline of the leaves margin uninfected by powdery mildew. Index 11 had no visible margin of the leaf not infected by powdery mildew.



Results

Preventative Experiments

Multiple tests were done to evaluate the treatments' preventative fungicidal abilities. In each of the following tests a detailed question, hypothesis and null hypothesis were produced to support the overarching hypothesis: The potential fungicides will prevent the growth of powdery mildew on *C. x morifolium*.

Question: In the preventative experiments, is there a difference in powdery mildew severity (expressed through Horsfall-Barratt scores) between the treatments and the control five weeks after inoculation?

Hypothesis: There is a difference in powdery mildew severity between the treatments and the control five weeks after inoculation.

Null Hypothesis: There is no difference in powdery mildew severity between the treatments and the control five weeks after inoculation.

Table 2: Wald linear regressions tests were conducted comparing Horsfall-Barratt scores between the treatments and the inoculated control plants on the third grading day in the preventative experiments. Point estimate scores report the difference between a treatment and the inoculated control populations mean Horsfall-Barratt score. A negative point estimate score reports that the treatment plants population on average has a Horsfall-Barratt score less than the inoculated control. The difference is considered significant if the confidence interval does not contain 0 (0=no difference between the inoculated control and the treatment). *Treatments that are significantly different from the inoculated control at $P < 0.05$.

Preventative:	First Trial			Second Trial		
Treatment	Point Estimates	95% Confidence Intervals	Standard Errors	Point Estimates	95% Confidence Intervals	Standard Errors
Ammonium Oleate	-0.299*	(-0.594, -0.003)	0.151	-1.595*	(-2.361, -0.828)	0.391
Potassium laurate	XXXX	XXXX	XXXX	-1.918	(-2.728, -1.107)	0.414
Malic Acid	0.1389	(-0.3111, 0.589)	0.229	0.628	(-0.514, 1.770)	0.583
Sesame Oil	-0.257	(-0.562, 0.049)	0.156	-1.953*	(-2.713, -1.193)	0.388

First trial: p-value= <0.01

Second trial: p-value= <0.01

First trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis and conclude that at least one treatment application will decrease powdery mildew severity. Ammonium oleate had a point estimate Horsfall-Barratt score 0.299 units less than the inoculated control.

Second trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis and conclude that at least one treatment application will decrease powdery mildew severity. Ammonium oleate had a point estimate Horsfall-Barratt score 1.595 units less than the control. Potassium laurate had a point estimate Horsfall-Barratt score 1.918 units less than the inoculated control. Sesame oil had a point estimate Horsfall-Barratt score 1.953 units less than the inoculated control.

In both trials malic acid was insignificant. This bears evidence that malic acid is the only compound that conclusively has no preventative fungicidal properties.

Analysis of the Biomass of *C. x morifolium* after the Experiments

This analysis was done in the second trial to see if there is a difference in biomass between the treatment groups and the control in the preventative experiment.

Question: Do treatment applications increase or decrease the biomass of the plants?

Hypothesis: Treatment applications will affect the biomass of the plants.

Null Hypothesis: Treatment applications will not affect the biomass of the plants.

Table 3: One-Way ANOVA tests were conducted comparing biomass of the treatments in the preventative experiments. Treatments with the same number next to them showed no statistical difference under Tukey's Honestly Significant Difference test ($P < 0.05$).

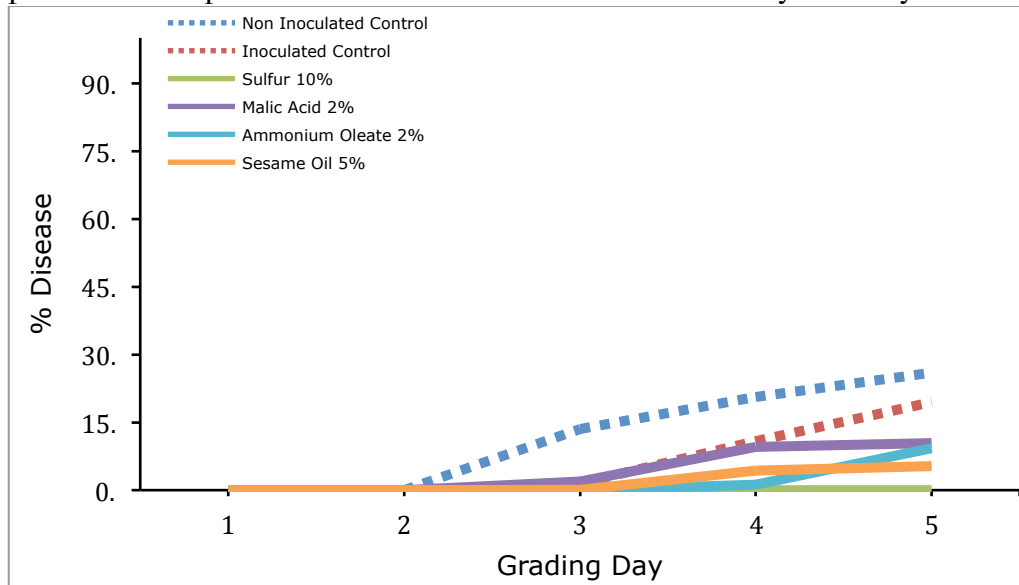
Treatment	Mean	Variance
Not Inoculated Control (1)	0.87833	0.07255
Inoculated Control (1)	0.79733	0.15439
Sulfur (1)	1.02275	0.16513
Ammonium Oleate (1)	0.8795	0.16704
Potassium Laurate (2)	0.25767	0.03325
Malic Acid (1)	0.76942	0.10417
Sesame Oil (1)	0.96017	0.17901

p-value= <0.01

Due to the p-value being less than 0.05, I can reject the null hypothesis and conclude that at least one preventative treatment application will affect the plants' biomass. Plants treated with potassium laurate in the preventative experiment had a statistically lower biomass than all the treatments.

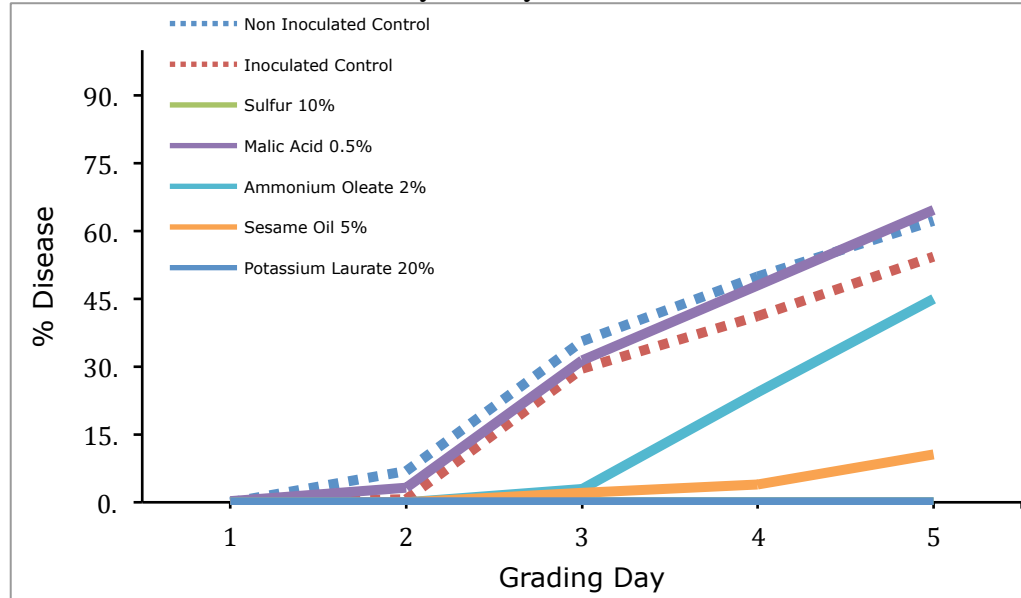
Preventative Experiments Disease Growth Graphs:

Figure 6: Change in disease severity in the preventative experiment (first trial). Grading for the preventative experiment in the first trial was conducted every Saturday for 5 weeks.



In the preventative experiment, plants were inoculated after treatments were applied. This graph shows the growth of powdery mildew for five weeks after treatments were applied. The plants showed no signs of disease until week 2. Sesame oil, sulfur, malic acid and ammonium oleate all have disease levels below the control. Control 2 is below control 1, showing that inoculation had little effect in the first trial. Plants showed similar severity in this trial whether or not inoculation took place.

Figure 7: Change in disease severity in the preventative experiment (second trial). Grading in the second trial was conducted every Sunday for 5 weeks.



In the preventative experiment, plants were inoculated after treatments were applied. This graph shows the growth of powdery mildew for 5 weeks after treatments were applied. Sesame oil, sulfur, and potassium laurate were able to prevent powdery mildew from infecting and colonizing *C. x morifolium*. The disease begins to level off in the controls and malic acid treatment after week 3. This is because the leaves with the most severe disease presence tend to drop off of the plant.

Area Under the Disease Progress Curves for the Preventative Experiments

For the second trial, area under the disease progress curves was created to show the susceptibility of the plants in each treatment to powdery mildew. AUDPC scores were obtained from the graphs to assess the susceptibility of the plants in a given treatment to powdery mildew. No conclusion can be made from these tests because no statistical tests were done comparing the data. This graph was formulated to view the diseases' progress over time under the different

treatments. The AUDPC scores for the preventative experiment are seen in Table 4. The AUDPC graphs for the preventative experiments can be found in the appendix.

Table 4: AUDPC scores of the different treatments in the preventative experiment. The higher the AUDPC value the more susceptible the plants in the given treatment are to powdery mildew.

Treatment	AUDPC Scores
Inoculated Control	98.55
Ammonium Oleate	49.78
Malic Acid	115.06
Sesame Oil	11.26

The scores reveal that the plants in the inoculated control and malic acid treatment groups were the most susceptible to powdery mildew. The sesame oil treatment had the lowest AUDPC score followed by ammonium oleate. No powdery mildew signs were observed in the potassium laurate treatments. Because of this no Area Under the Disease Progress Curve scores could be calculated.

Curative Experiments

Multiple tests were done to evaluate the treatments' curative fungicidal abilities. In each of the following tests a detailed question, hypothesis and null hypothesis were produced to support the overarching hypothesis: The potential fungicides will control an existing powdery mildew infection on *C. x morifolium*.

Question: Are infected plants applied with treatment less likely to have signs of powdery mildew one week after final treatment applications?

Hypothesis: The treatments will decrease the likelihood of powdery mildew signs on infected *C. x morifolium* one week after final treatment applications.

Null Hypothesis: The treatments will not decrease the likelihood of powdery mildew signs on infected *C. x morifolium* one week after final treatment applications.

In this test the whole plant was evaluated for signs of powdery mildew. The plants were given a score of 1 (signs of *E. chrysanthemi*) or 0 (no signs of *E. chrysanthemi*) This test for the preventative experiment did not reveal any insightful results. New growth in the preventative experiment was not applied with any treatment. Because of this all plants had signs of *E. chrysanthemi* and earned a 1 score.

Table 5: Comparison of the incidence of powdery mildew between treatment and control plants one week after final treatment applications in the curative experiments. The point estimate reports the likelihood, as a percent, that a treatment decreases powdery mildew incidence compared to the control. A negative number corresponds to a smaller likelihood that the plants will have signs of powdery mildew. The difference is considered significant if the confidence interval does not contain 0 (0=no difference between the inoculated control and the treatment).
 *Treatments that are significantly different from the inoculated control at P<0.05.

Curative:	First trial			Second trial		
Treatment	Point Estimates	95% Confidence Intervals	Standard Errors	Point Estimates	95% Confidence Intervals	Standard Errors
Ammonium Oleate	-0.865*	(-1.033, -0.696)	0.086	-0.918*	(-1.074, -0.762)	0.08
Potassium laurate	XXXX	XXXX	XXXX	-0.933*	(-1.100, -0.766)	0.085
Malic Acid	-0.100	(-0.462, 0.261)	0.184	-0.175	(-0.392, 0.042)	0.111
Sesame Oil	-0.786*	(-1.055, -0.517)	0.137	-1.015*	(-1.060, -0.969)	0.023

First trial: p-value=0.04

Second trial: p-value= <0.01

First trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis, and conclude that at least one treatment decreased the likelihood of powdery mildew signs on inoculated *C. x morifolium* one week after final applications. Plants treated with sesame oil are 78.6% less likely to have signs of powdery mildew than the inoculated control. Plants treated with ammonium oleate, are 86.5% less likely to have signs of powdery mildew than the inoculated control.

Second trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis, and conclude that at least one treatment decreased the likelihood of powdery mildew signs on *C. x morifolium* one week after final applications. Plants treated with ammonium oleate are 91.8% less likely to have signs of powdery mildew than the control. Plants treated with potassium laurate are 93.3% less likely to have signs of powdery mildew than the control. Plants treated with sesame oil are 100% less likely to have signs of powdery mildew than the control. The point estimates and confidence intervals being over 100% is a consequence of a binary model. None of the plants in the inoculated control was free of powdery mildew signs, which led to this result. A higher sample size would most likely have nullified this problem.

Question: In the curative experiment, do treatment applications decrease powdery mildew severity (expressed through Horsfall-Barratt scores) between the first application and the last day of grading?

Hypothesis: Treatment applications will decrease powdery mildew severity.

Null Hypothesis: Treatment applications will not decrease powdery mildew severity.

Table 6: Wald linear regressions tests were conducted comparing the change in Horsfall-Barratt scores between the treatments and the inoculated control plants. Point estimate scores report the difference between a treatment and the inoculated control populations mean change in Horsfall-Barratt scores before and after the application of a treatment. A negative point estimate score reports that the population on average will have a decrease in Horsfall-Barratt scores after treatment applications compared to the inoculated control. The lower the point estimate score correlates to a higher decline of powdery mildew. The difference is considered significant if the confidence interval does not contain 0 (0=no difference between the inoculated control and the treatment). *Treatments that are significantly different from the inoculated control at $P < 0.05$.

Curative:	First trial			Second trial		
Treatment	Point Estimates	95% Confidence Intervals	Standard Errors	Point Estimates	95% Confidence Intervals	Standard Errors
Ammonium Oleate	-2.989*	(-4.270, -1.708)*	0.653	-7.221*	(-9.046, -5.396)	0.931
Potassium laurate	XXXX	XXXX	XXXX	-6.821*	(-8.967, -4.675)	1.095
Malic Acid	-2.533	(-3.870, -1.196)*	0.682	-5.731*	(-8.333, -3.129)	1.327
Sesame Oil	-4.616	(-6.365, -2.868)*	0.892	-8.195*	(-9.822, -6.567)	0.83

First trial: p-value= <0.01

Second trial: p-value= <0.01

First trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis, and conclude that at least one treatment application will decrease powdery mildew severity. Plants treated with malic acid had point estimate Horsfall-Barratt scores 2.533 units less than the inoculated control. Plants treated with ammonium oleate had point estimate Horsfall-Barratt scores 2.989 units less than the inoculated control. Plants treated with sesame oil had point estimate Horsfall-Barratt scores

4.616 units less than the control.

Second trial:

Due to the p-value being less than 0.05, I can reject the null hypothesis and conclude that at least one treatment application will decrease powdery mildew severity. Plants treated with malic acid had point estimate Horsfall-Barratt scores 5.731 units less than the control. Plants treated with potassium laurate had point estimate Horsfall-Barratt scores 6.821 units less than the control. Plants treated with ammonium oleate had point estimate Horsfall-Barratt scores 7.221 units less than the control. And plants treated with sesame oil had point estimate Horsfall-Barratt scores 8.195 units less than the control.

Analysis of the Biomass of *C. x morifolium* after the Experiments

This analysis was done to see if there was a difference in biomass in the second trial between the treatment groups and the control in the curative experiment.

Question: Do treatment applications increase or decrease the biomass of the plants?

Hypothesis: Treatment applications will affect the biomass of the plants.

Null Hypothesis: Treatment applications will not affect the biomass of the plants.

Table 7: One-Way ANOVA tests were conducted comparing biomass of the treatments in the curative experiments. No statistical difference was observed between any of the treatments and the controls ($P>0.05$).

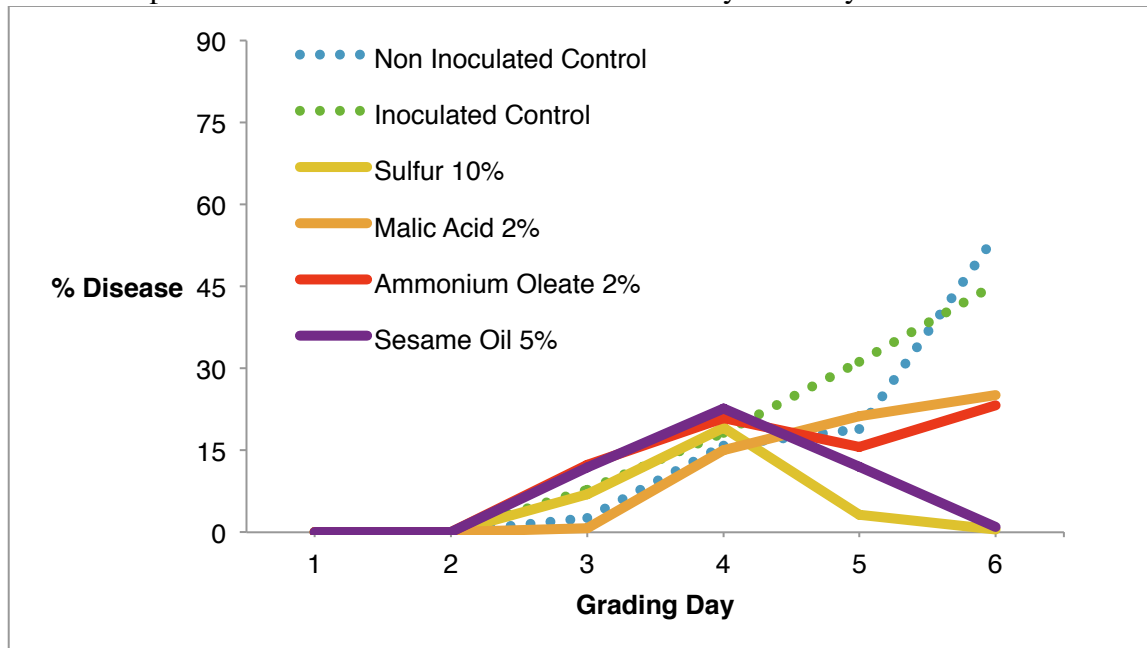
Treatment	Mean	Variance
Not Inoculated Control	0.93225	0.07255
Inoculated Control	0.83508	0.15439
Sulfur	1.05208	0.16513
Ammonium Oleate	0.90017	0.16704
Potassium Laurate	0.7235	0.17901
Malic Acid	0.7235	0.10417
Sesame Oil	0.9375	0.03325

p-value= 6.7

Due to the p-value being greater than 0.05, I can accept the null hypothesis. No statistical difference was observed between any of the treatments or controls biomass.

Curative Experiments Disease Growth Graphs

Figure 8: Change in disease severity in the curative experiment (first trial). Grading for the curative experiment in the first trial was conducted every Saturday for 6 weeks.

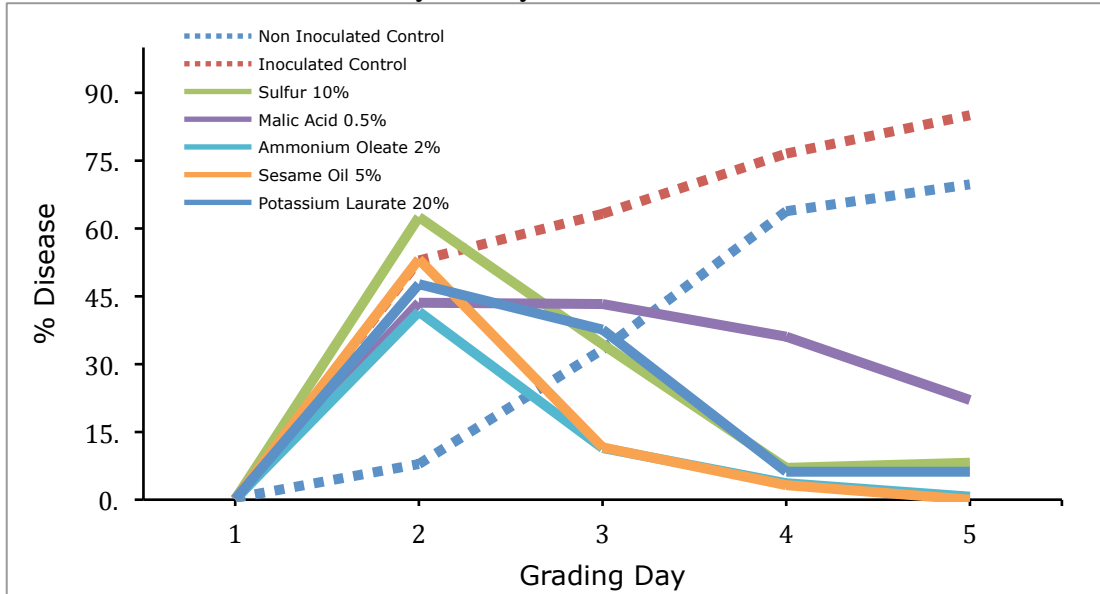


In the first trial of the curative experiment, treatments were applied on the fourth grading day.

This graph shows the growth of powdery mildew for six weeks after inoculation. Powdery mildew grew at a semi-equal rate until week 4 for all of the treatments except malic acid.

Heavily diseased leaves tended to drop after week 4. This could have led to the decline in the treatments during this time. All of the treatments had less incidence of powdery mildew by the end of the experiments than both of the controls.

Figure 9: Change in disease severity in the curative experiment (second trial). Grading in the second trial was conducted every Sunday for 5 weeks.



In the second trial of the curative experiment, treatments were applied on the second grading day. This graph shows the growth of powdery mildew for five weeks after inoculation. Each treatment had a decline in the percent of plant diseased. Heavily diseased leaves tended to drop after week 2, which could have led to the decline. In this experiment inoculating the plants had a major effect on disease severity. This can be seen in the difference between the inoculated control (control 2) and the non-inoculated control (control 1).

Area Under the Disease Progress Curves for the Curative Experiment

AUDPC curves were created to obtain AUDPC scores for the curative treatment (Table 8). No conclusion can be made from these tests because no statistical tests were done comparing the data. This graph was formulated to view the disease's progress over time under the different treatments. The AUDPC graphs for the curative experiment can be found in the appendix.

Table 8: AUDPC scores of the different treatments in the curative experiments. The higher the AUDPC value, the more susceptible the plants in the given treatment are to powdery mildew.

Treatment	AUDPC Scores
Inoculated Control	235.1
Ammonium Oleate	56.88
Potassium Laurate	96.33
Malic Acid	133.94
Sesame Oil	68.09

The AUDPC scores show that all the plants applied with a treatment had much less disease incidence than the control. Ammonium oleate and sesame oil had the lowest scores of all the treatments.

Analysis of the Differences in the Amount of Leaves Lost Between Treatments

Table 9: Average percentage of leaves lost for the curative experiments

Treatment	Average Percentage of Leaves Lost
Non-inoculated Control	38%
Inoculated Control	51%
Sulfur	64%
Ammonium Oleate	65%
Potassium Laurate	85%
Malic Acid (0.5%)	30%
Malic Acid (2%)	70%
Sesame Oil	70%

The control groups had a smaller percentage of leaves lost than all the other groups except for the 0.5% solution of malic acid.

Observable symptoms recorded

In the first trial, 83% of the plants in the curative malic acid treatments had white necrotic spots that turned brown (Figure 10). No spots were recorded on the malic acid preventative treatments.

In the second trial, dead insects were found on eleven plants. Of these eleven plants, eight had dead fungus gnats and three had dead aphids. Eight of the plants were in the sesame oil treatment and three were used in the potassium laurate treatment.

Figure 10: White spots that turned brown within a week were observed on the plants sprayed with 2% malic acid solutions.



Discussion

Differences Between Trials

There were some major differences between the trials that need to be noted. In the first trial, only five plants were used for inoculation. In the second trial, 24 plants were used for inoculation (the plants from the control treatments in the first trial were used for inoculation in the second trial). In the second trial, there were spores in the greenhouse from all the plants used in the first trial as well as from all the spores that were previously in the greenhouse. This could have had some consequences relating to the data. In the first trial, the non-inoculated control (control 1) had a higher severity of *E. chrysanthemi* than the inoculated control (control 2) (Figures 6 and 8). This reveals that inoculation had no effect on outbreak in the first trial. In the first trial, *E. chrysanthemi* signs were not seen until three weeks after initial inoculation (Figures 6 and 8) compared to the second trial, where signs were seen two weeks after inoculation (Figures 7 and 9). In the first trial, the curative treatments were not applied until grading day four because not enough plants exhibited signs of *E. chrysanthemi*. In the second trial, 100% of the plants had signs by grading day two and treatments were applied on that day. Because *E. chrysanthemi* severity was higher in the second trial, it led to more conclusive results. The lower severity in the first trial contributed to lower point estimates in each treatment compared to the second trial (Tables 2, 5, and 6). More detectable differences were observed in the second trial due to these higher point estimate scores. The low *E. chrysanthemi* severity in the first trial caused inconclusive and conflicting data. For example, the preventative experiments in the first trial were insignificant for sesame oil but in the second trial they were significant (Table 2).

Non-Inoculated Control

In the experiments, all of the non-inoculated controls developed signs of *E. chrysanthemi* (Figures 6, 7, 8, and 9). There are a multitude of possible reasons for this result. The number of plants that had infections of powdery mildew in collaboration with the design of the greenhouse caused a mass amount of spores to be blown throughout the zone where the experiments were conducted. It is recommended in future research to conduct a randomized block design. The non-inoculated control needs to be in a separate zone in the greenhouse, preferably within a specialized chamber. Because of this, there were no uninfected plants that could be compared to plants that had *E. chrysanthemi* signs.

Potential Fungicides

Statistical tests were done to evaluate the potential of the treatments studied in this experiment. No statistical analysis was done with the AUDPC scores or line graphs. They are referenced as supporting evidence.

Ammonium Oleate

In the preventative treatments for the first and second trial, 2.0% ammonium oleate applications were found to prevent the growth of *E. chrysanthemi* on *C. x morifolium*. In both trials, the ammonium oleate treatment plants had significantly less point estimate Horsfall-Barratt scores than the control. Plants treated with ammonium oleate had Horsfall-Barratt scores 0.299 units less than the inoculated control (Table 2). In the second trial, the ammonium oleate treatment plants had point estimate Horsfall-Barratt scores 1.595 units less than the inoculated control (Table 2). In the second trial, the plants in the ammonium oleate treatment had an AUDPC score 48.77 units below the inoculated control (Table 4).

From the evidence stated above, it can be concluded that a 2.0% ammonium oleate solution has preventative fungicidal abilities for the control of *E. chrysanthemi*.

In the curative treatments for the first and second trial, ammonium oleate significantly decreased the likelihood of *E. chrysanthemi* signs on *C. x morifolium* compared to the inoculated control. In the first trial, ammonium oleate was found to significantly decrease the likelihood of *E. chrysanthemi* signs. Plants treated with ammonium oleate were found to decrease the likelihood of *E. chrysanthemi* by 86.5% compared to the inoculated control (Table 5). In the second trial, ammonium oleate was found to decrease the likelihood of any *E. chrysanthemi* signs by 91.8% compared to the inoculated control (Table 5). There was also a statistically significant difference between the ammonium oleate curative treatment and the inoculated control in regard to the severity of the disease in both trials. In the first trial, the ammonium oleate curative treatment plants had point estimate Horsfall-Barratt scores 2.989 units less than the inoculated control (Table 6). In the second trial, the ammonium oleate curative treatment plants had point estimate Horsfall-Barratt scores 7.221 units less than the inoculated control (Table 6). In the second trial, ammonium oleate had the lowest AUDPC score of all the treatments (Table 8); it was 178.22 units less than the inoculated control.

The statistical analysis shows that ammonium oleate decreased the likelihood and severity of *E. chrysanthemi* on *C. x morifolium*. From the evidence stated above, it can be concluded that a 2.0% ammonium oleate solution has curative fungicidal properties for the control of *E. chrysanthemi* on *C. x morifolium*. There are some other fungicides on the market that have preventative and curative fungicidal properties. These fungicides have a range of mode of actions such as demethylation inhibitor and quinone outside inhibitor. There are other salt based fungicides, such as potassium bicarbonate, that have preventative and curative fungicidal

properties (University of California Davis 2014b).

Before use applicators should be aware of some negative effects of using ammonium oleate. It should be noted ammonium oleate increased leaf loss compared to the control

(Table 9).

Potassium Laurate

A 20.0% solution of potassium laurate was shown to significantly decrease the growth of *C. x morifolium* compared to the inoculated control. In the preventative experiment, the biomass of the plants, was statistically less than the controls and the treatments (Table 3). The biomass differences in the curative treatment were insignificant (Table 7). The plants growth was stunted by the 20.0% potassium laurate solution. No new growth was observed by the plants in the preventative treatment until after 4 weeks. At this time new shoots started to emerge from the ground. No terminal leaves ever developed on the plants in the preventative potassium laurate experiment. This would explain the difference between the biomass in the curative and preventative treatments.

From this data it can be concluded that a 20.0% potassium laurate solution is phytotoxic to *C. x morifolium* when applied as a foliar spray. Potassium laurate has been found to be toxic to plants containing pubescence. The hair on the plant's leaves can cause the compound to stick to the leaves for long periods of time resulting in burns (Moore et al. 1979).

E. chrysanthemi did not grow on any of the plants in the preventative potassium laurate experiment. The potassium laurate treatment plants had point estimate Horsfall-Barratt scores 1.918 units less than the inoculated control (Table 2). No adequate AUDPC score could be attained because no *E. chrysanthemi* developed on any of the treatment plants.

It can be concluded that a 20.0% solution of potassium laurate has preventative fungicidal properties for the control of *E. chrysanthemi* on *C. x morifolium*. At this percentage of potassium laurate, it is able to completely prevent *E. chrysanthemi* from infecting *C. x morifolium*. Potassium laurate cannot be recommended for use due to its phytotoxic nature.

The curative treatments for potassium laurate significantly decreased the likelihood of *E. chrysanthemi* signs on *C. x morifolium* compared to the inoculated control. Potassium laurate was found to decrease the likelihood of *E. chrysanthemi* signs by 93.3% compared to the inoculated control (Table 5). The potassium laurate curative treatment plants had point estimate Horsfall-Barratt scores 6.821 units less than the inoculated control (Table 6). Potassium laurate had an AUDPC score 138.77 units less than the inoculated control (Table 8).

The statistical analysis shows that potassium laurate decreased the likelihood and severity of *E. chrysanthemi* on *C. x morifolium*. From the evidence stated above, it can be concluded that a 20.0% potassium laurate solution has curative fungicidal properties for the control of *C. x morifolium*. Potassium laurate is able to inhibit the growth of the fungi, yet it cannot be recommended at this percentage due to the solution's phytotoxic nature.

Malic Acid

In the first trial white necrotic marks that turned brown were observed on the new leaves in the malic acid curative treatments. No markings were observed in the preventative experiment. The plants were all taken from cuttings and had fully developed leaves when sprayed in the preventative experiment. Older leaves have a thicker cuticle that can act as a barrier to foliar applications of pesticides (University of Nebraska-Lincoln 2015). This could explain why no necrotic markings were observed on the older leaves. Two percent malic acid treatments were deemed phytotoxic to the plants and the solute percentage was lowered to 0.5% for the second trial.

From the statistical analysis conducted, no statistically significant differences were determined between malic acid and the inoculated control for the preventative experiments

(Table 2). In the second trial, the malic acid preventative treatment had the highest AUDPC scores (Table 4).

From the evidence stated above, it can be concluded that a 0.5% or 2.0% malic acid solution does not have preventative fungicidal properties for the control of *E. chrysanthemi* on *C. x morifolium*.

The statistical analysis for the curative malic acid experiments yielded mixed results. For the first and second trials, there was no statistically significant difference seen in the incidence of *E. chrysanthemi* between the malic acid treatments and the inoculated controls (Table 5). There was a statistically significant difference between the malic acid curative treatment and the inoculated control in regards to the severity of the disease in both trials. In the first trial, the malic acid curative treatment plants had point estimate Horsfall-Barratt scores 2.533 units less than the inoculated control (Table 6). In the first trial, the malic acid curative treatment plants had point estimate Horsfall-Barratt scores 5.731 units less than the inoculated control (Table 6). In the second trial, the malic acid treatment had the highest AUDPC score of all the treatments but had a score 101.16 units less than the inoculated control (Table 8).

The statistical analysis shows that malic acid had no effect on the incidence of *E. chrysanthemi*. There was no difference between the number of plants that had signs of *E. chrysanthemi* in the malic acid treatments and the control. Even though malic acid did not affect incidence of *E. chrysanthemi*, the disease was significantly less severe on the plants treated with malic acid. This suggests that malic acid has the potential to slow down the growth of *E. chrysanthemi* but is unable to inhibit the growth or spread. This leads me to the conclusion that malic acid cannot be used for the control of *E. chrysanthemi*. This research agrees with that done

by Jafari and Hadavi (2012) who found that malic acid did not decrease the incidence of powdery mildew.

Sesame Oil

Dead fungus gnats or aphids were found on 8 out of 24 of the plants sprayed with sesame oil. This is evidence that sesame oil could have insecticidal properties. Sesame oil is used in multiple products as an insecticide (Washington State University 2015).

The statistical analysis done for the preventative sesame oil experiment had mixed results. In the first trial, no statistically significant difference was seen in the severity of *E. chrysanthemi* between the plants in the sesame oil treatment and the inoculated control. In the second trial, there was a statistically significant difference between the sesame oil treatment and the inoculated control in regard to *E. chrysanthemi* severity. In the second trial, the sesame oil treatment plants had point estimate Horsfall-Barratt scores 1.953 units less than the inoculated control (Table 2). In the second trial, sesame oil had the lowest AUDPC score of any of the treatments (Table 8). It was 87.29 units less than the control.

From the line graphs (Figures 6 and 7), the AUDPC scores (Table 4) and the statistical analysis for the second trial (Table 2) it can be concluded that a 5% solution of sesame oil has the potential to prevent the growth of *E. chrysanthemi*. More research needs to be conducted regarding sesame oil's preventative fungicidal properties to obtain more conclusive results. In future research, a surfactant such as Tween-80 should be used in collaboration with sesame oil to improve coverage and uptake.

In the curative treatments for the first and second trial, sesame oil significantly decreased the likelihood of *E. chrysanthemi* signs on *C. x morifolium*. In the first trial, sesame oil was

found to decrease the likelihood of *E. chrysanthemi* signs by 78.6% compared to the inoculated control (Table 5). In the second trial, sesame oil was found to decrease the likelihood of any *E. chrysanthemi* signs by 100% compared to the inoculated control (Table 5). In both trials, there was also a statistically significant difference between the sesame oil curative treatment and the inoculated control in regard to the severity of *E. chrysanthemi*. In the first trial, the sesame oil curative treatment plants had point estimate Horsfall-Barratt scores 4.616 units less than the inoculated control (Table 6). In the second trial, the sesame oil curative treatment plants had point estimate Horsfall-Barratt scores 8.195 units less than the inoculated control (Table 6) and an AUDPC score 167.01 units less than the inoculated control (Table 8).

The statistical analysis shows that sesame oil decreased the likelihood and severity of *E. chrysanthemi* on *C. x morifolium*. From the evidence stated above, it can be concluded that a 5.0% sesame oil solution has curative properties for the control of *E. chrysanthemi* on *C. x morifolium*. This research partially agrees with that done by Keinath and DuBose (2012) who found that Organocide, a fungicide containing 5.0% sesame oil and 92% edible fish oil, has preventative and curative fungicidal properties. It is unclear in their study if it is the sesame oil or edible fish oil that has the fungicidal properties. Some oils are used as curative fungicides for powdery mildew. Organic JMS Stylet Oil used as a curative fungicide has ‘contact fungicide with smothering and barrier effects’ listed as its mode of action. Oils tend to predominately have curative fungicidal properties but have been noted to have preventative properties as well (University of California Davis 2014b). This could explain why sesame oil had significant curative results and mixed preventative results. Before use, applicators should be aware of some negative effects of using sesame oil. It should be noted that sesame oil increased leaf loss compared to the control (Table 9), and dead insects were observed on multiple of the sesame oil

treatment plants. Sesame oil could have damaging effects towards beneficial insects.

Fungicide Comparisons

Sulfur is one of the most used organic fungicides for the control of powdery mildew (Belanger and Labbe 2002), and it proved more successful in this study than any of the potential fungicides being used (Figures 6, 7, 8 and 9). Alternatives to sulfur for the control of powdery mildew are needed for multiple reasons. Sulfur can be phytotoxic in the greenhouse when temperatures exceed 30 C°, it has the potential to negatively affect beneficial organisms (Belanger and Labbe 2002), and it can accelerate the degradation of greenhouse plastic covers (Giotis 2006). It should be noted that oils and fungicidal soaps should never be used in conjugation with sulfur (University of California Davis 2014a). Even though there wasn't statistical evidence to make any conclusions, it can be noted that sulfur had the highest average biomass of any of the treatments. Each of the treatments, except for potassium laurate, that had shown to have fungicidal properties, had a higher average biomass than the controls (Tables 3 and 7).

Leaf Loss Data

It can be observed from Table 9 that plants in the control groups lost a smaller percentage of their leaves than any of the plants in the treatment groups. This could be the reason for the insignificant biomass data in all the treatments except for potassium laurate. Even though plants in the treatment groups were healthier, it was not correlated with a higher biomass. A possibility for the anomaly is that the control groups held onto their diseased leaves longer than the treatment groups. If the experiment was conducted for a longer period of time, significant biomass data probably could have been acquired. *E. chrysanthemi* is acting as a parasite to *C. x morifolium*. Parasitism is when a parasite slowly consumes small amounts of tissue and nutrients

from a host (Freeman et al. 2014). It can be hypothesized that *E. chrysanthemi* is keeping the host leaves alive so that they can be used as a food source for as long as possible. When the fungicides cure *E. chrysanthemi* infections, the leaves drop off the plant. Clear evidence was not obtained in this study to support this claim due to the lack of an uninfected control. An alternative hypothesis is that the compounds were phytotoxic. This can be supported through the leaf loss data. All of the treatments besides malic acid caused the plants to drop leaves at a higher rate than the control (Table 9). In order to test this some changes to the experimental design need to be implemented. Future experiments should be conducted with non-inoculated healthy plants applied with the potential fungicides. This would double the amount of treatments. Another problem with these trials was that there were no, healthy, non-infected, controls. A randomized block design should be implemented with chambers built around each block. This would prevent plants that weren't inoculated from having signs of *E. chrysanthemi*. If this experimental design was used, more conclusive results could be obtained.

Conclusion

In this study, a 5.0% solution of sesame oil revealed mixed results in regards to its fungicidal preventative properties. Because of this, no conclusion can be made on whether it can be adequately used as a preventative fungicide. A 5.0% solution of sesame oil has curative properties for the control of *E. chrysanthemi* on *C. x morifolium*. Sesame oil at a 5.0% solution can cause a plant to lose its leaves and is recommended in severe cases if there are no other options. A 20.0% solution of potassium laurate has preventative and curative properties for the control of *E. chrysanthemi* on *C. x morifolium*. It should be noted that a 20.0% solution of potassium laurate and a 2.0% solution of malic acid shouldn't be considered because of their

phytotoxic effects. A 2.0% solution of ammonium oleate also has preventative and curative properties for the control of *E. chrysanthemi* on *C. x morifolium*. Ammonium oleate at 2.0% solution can cause a plant to lose its leaves and is recommended in severe cases if there are no other options.

Future research needs to be done in relation to this study. The following should be evaluated:

- 1) The synergistic effects of ammonium oleate, malic acid, potassium laurate, and sesame oil.
- 2) The physiological mode of action of ammonium oleate, malic acid, potassium laurate, and sesame oil.
- 3) How long after application does the preventative effect of ammonium oleate last?
- 4) Does sesame oil have preventative fungicidal properties?
- 5) How often curative applications of ammonium oleate, and sesame oil need to be applied?
- 6) At what solution percentage does potassium laurate become phytotoxic to *C. x morifolium* and at these levels does it still have curative and preventative abilities for the control of *E. chrysanthemi*?
- 7) Can sesame oil be used to control aphids and fungus gnats and is it harmful to beneficial insects?
- 8) The reasoning for a plant to lose its leaves at a higher rate than the control when applied with these compounds. Does sesame oil, and ammonium oleate contain phytotoxic properties?
- 9) Can the treatments adequately control *E. chrysanthemi* when applied to plants living outside?

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Appendix

Preventative Experiment AUDPC Graphs

Figure A-1: AUDPC for the preventative inoculated control treatment

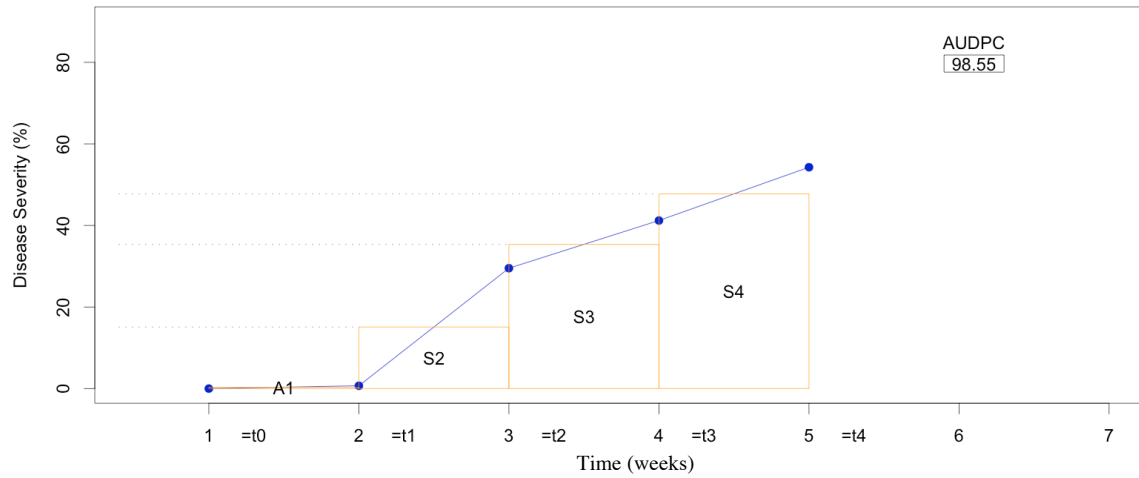


Figure A-2: AUDPC for the preventative ammonium oleate treatment

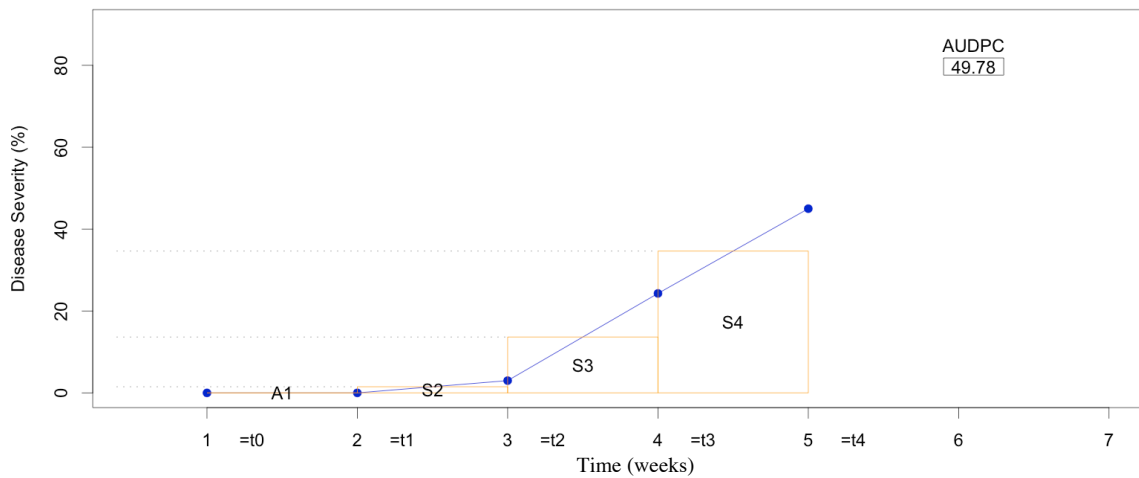


Figure A-3: AUDPC for the preventative malic acid treatment

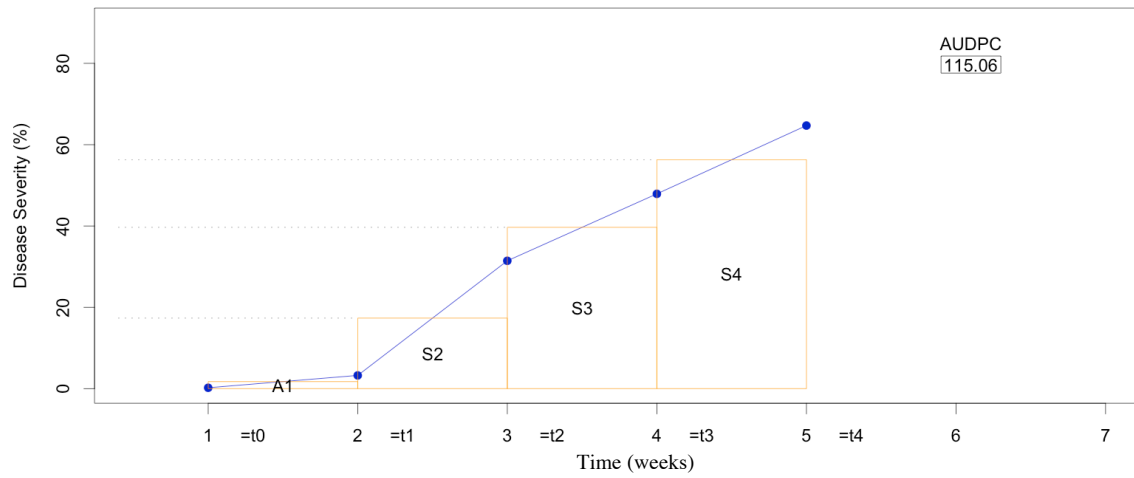
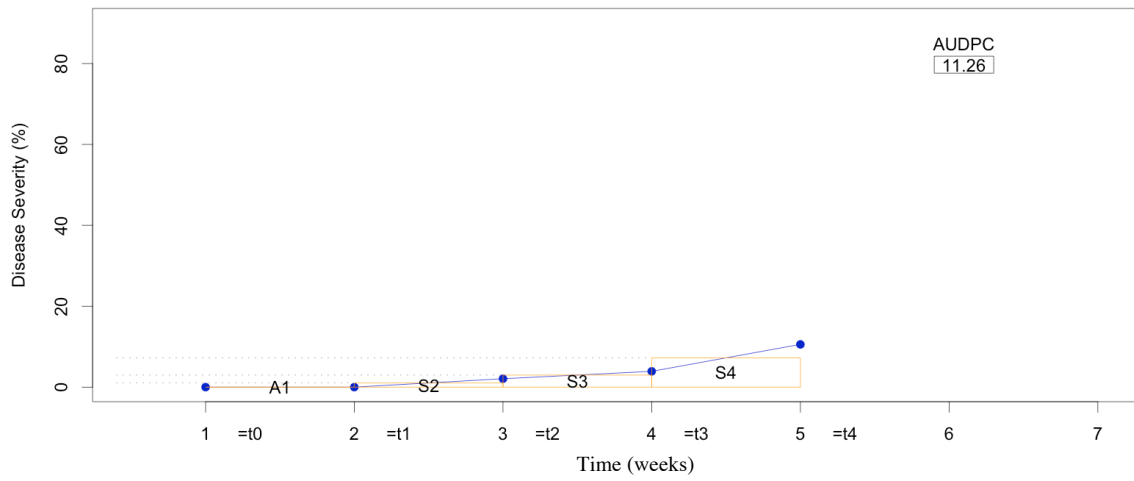


Figure A-4: AUDPC for the preventative sesame oil treatment



Curative Experiment AUDPC Graphs

Figure A-5: AUDPC for the curative inoculated control treatment

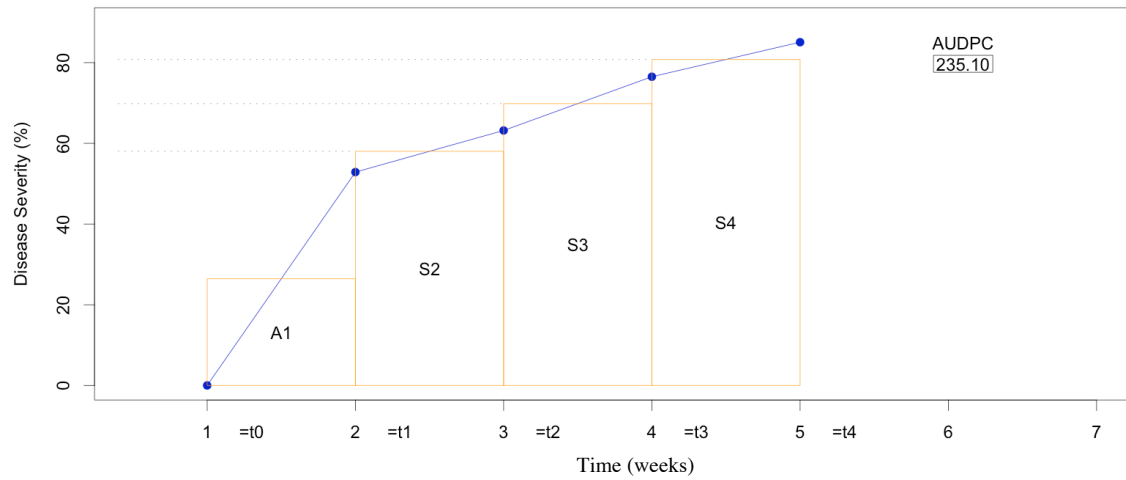


Figure A-6: AUDPC for the curative ammonium oleate treatments

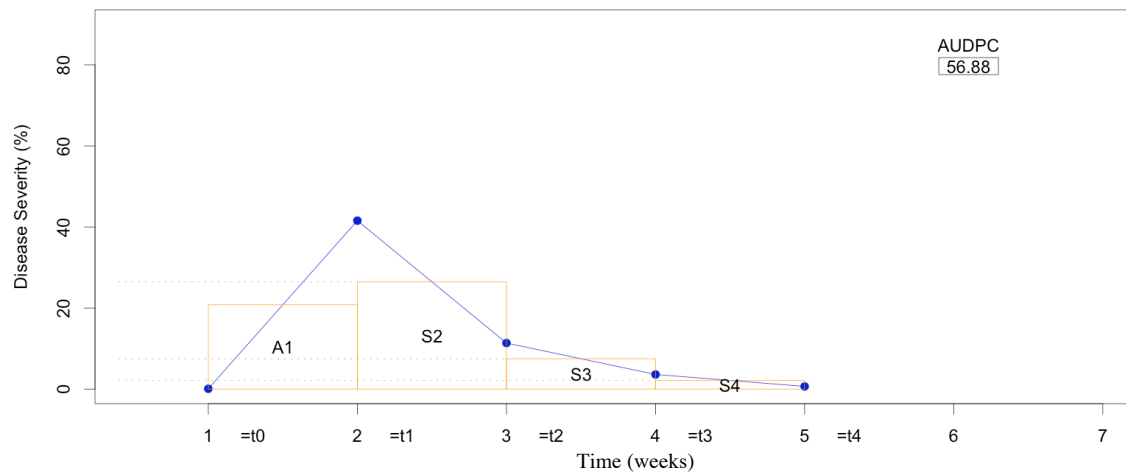


Figure A-7: AUDPC for the curative potassium laurate treatments

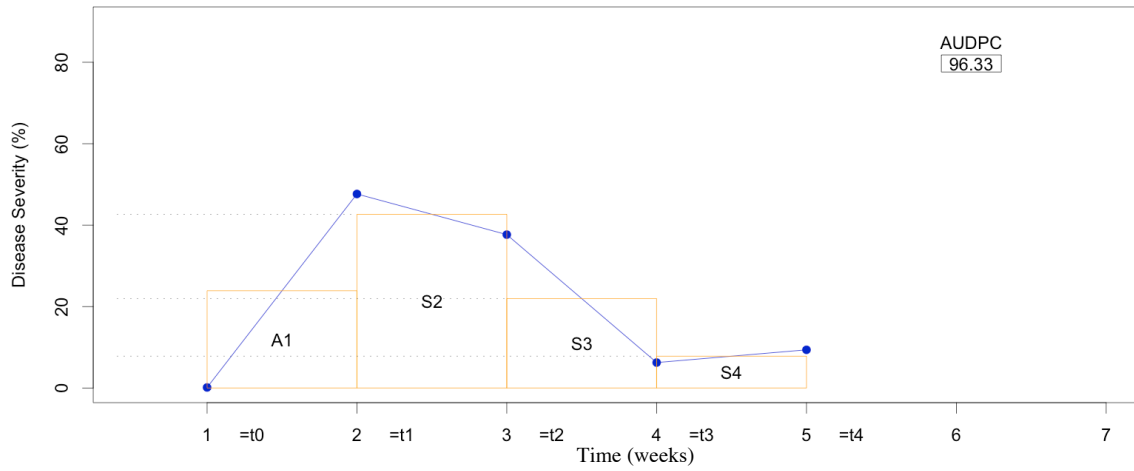


Figure A-8: AUDPC for the curative malic acid treatment

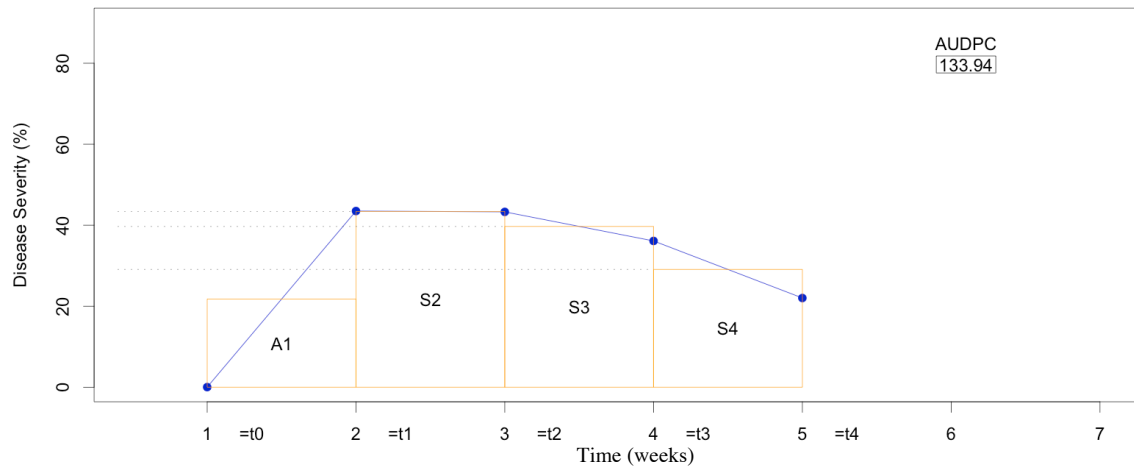


Figure A-9: AUDPC for the curative sesame oil treatment

